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NOTE: Hyperlinks were accurate at the time of manual publication.
1.0 Title: Biology/DNA Detail Overview

In this manual the Biology/DNA Detail defines the technical procedures for examining DNA on forensic evidence and reference samples. In conjunction with the Biology/DNA Quality Manual and LVMPD Forensic Laboratory Quality Manual, this manual also outlines some quality assurance measures that comprise the quality program of the Biology/DNA Detail to ensure the quality and integrity of the data generated by the laboratory. This manual is not designed to be an all-inclusive collection of every possible procedure or variation of procedure which might be used in the recovery and examination of biological samples. Due to the wide variety of evidence received by the analyst, flexibility is sometimes required in the analytical approach. The procedures presented in this manual are intended to provide a sound framework upon which to build.

In accordance with the section 1/100.4 of the LVMPD Department Manual, the following construction and terminology will be followed:

- **“Shall”, “must”, and “will”** are mandatory.
- **“May”** is permissive.
- **“Should”** is used where it is intended that while a procedure is not mandatory, it should be followed in the interest of the Detail.

1.1 General Principles of Forensic DNA Analysis

Forensic DNA analysis is the process of identification and evaluation of biological evidence in criminal matters using DNA technologies. Forensic DNA analysis involves several steps including screening, extraction, quantitation, amplification, and separation, focusing on specific chromosomal locations that vary widely between individuals. These specific locations are used to generate a DNA profile from evidentiary items or from known source samples.

1.2 Overview of the Forensic DNA Testing Process

Evidence and DNA

Law enforcement personnel who submit crime scene evidence for DNA analysis must package and seal the evidence and then arrange for its secure delivery to the Evidence Vault. The LVMPD utilizes the Property Connect Module associated with the LIMS in order to request and initiate the laboratory testing process. All outside jurisdictions must submit a completed Forensic Laboratory Examination Request form (LVMPD 63) to initiate the laboratory testing process. The Forensic Scientist will then request the evidence from the Vault.

Upon receipt of the evidence, Forensic Scientists first determine if the evidence might contain DNA by visually examining the item for indications of body fluid stains, and may perform testing to determine whether specific body fluids that might contain DNA are present. Some items of evidence are analyzed for the purposes of identifying “touch” DNA. The Forensic Scientist collects possible skin cells from areas which may have been handled or come into physical contact with an individual, despite visible staining not being present.
DNA Extraction
When possible, Forensic Scientists analyze only a portion of the stains on the evidence and save the remainder in case future testing is necessary. Generally, stains on fabric are cut out of the item and the DNA is extracted from the cuttings. If the stains are on a hard object, such as a knife, some of the dried body fluid is removed from the object with a moist cotton swab (known as swabbing an item) and the DNA is extracted from the cotton swab. The process used to extract the DNA varies depending on the organic source of the stain and the material containing the stain.

Quantitation
Once the DNA is extracted from the evidence, the amount of DNA must be quantified. This is an important step to estimate how much DNA is present in a sample so that the optimal input can be targeted during the amplification process and for the subsequent detection and analysis.

DNA Amplification
The normalized DNA is subjected to polymerase chain reaction (PCR), which is also referred to as amplification. This process, often analogized as biological photocopying, allows scientists to make copies of specific chromosomal segments. The amplification process gives Forensic Scientists the ability to analyze minute DNA samples. Current technology utilizes PCR amplification with STRs. Short tandem repeat (STR) analysis is a method of determining an individual’s DNA profile by counting the number of times a small DNA sequence (short tandem repeat unit) is repeated at a specific chromosomal location. STR analysis consists of three processes: amplification, electrophoresis, and interpretation.

Amplified DNA Product Separation – Capillary Electrophoresis
After amplification is complete, the DNA is analyzed using an instrument that separates the DNA fragments present in the sample. This process is known as electrophoresis. Capillary electrophoresis is the DNA technology used to separate the small fragments that were amplified during the PCR processing. Capillary electrophoresis is an automated process that analyzes many DNA samples and requires minimal involvement by DNA scientists after the initial set-up procedures are completed. These procedures include cleaning and calibrating the electrophoresis instrument and preparing the amplified DNA for analysis. Special software then measures the length of the DNA fragments, determines the alleles that correspond to the fragments, and compiles a DNA profile for the sample.

1.3 DNA Laboratory Work Streams
The Biology/DNA Detail of the LVMPD Forensic Laboratory has two work streams: Databasing and Casework.

Databasing
Databasing refers to the DNA analysis of database samples for entry into the Local DNA Index System (LDIS) of CODIS and, if eligible, for upload to the State DNA Index System (SDIS) or National DNA Index System (NDIS).

- A database sample is a sample obtained from an individual who is legally required to provide a DNA sample for databasing purposes and whose identity is established at the time of collection of the sample (e.g. felony arrestees, convicted offenders, etc.).
• Database samples are not required to undergo all the processing steps. They may be amplified directly after extraction using a ½ reaction amplification procedure, or amplified directly using sample extracts or FTA sample cards and a direct amplification procedure.

Casework
Casework refers to DNA analysis of evidentiary items submitted by law enforcement personnel. There are several types of casework samples, for example:

• Questioned or unknown samples collected from the crime scene may be any biological sample deposited on virtually any surface.
• Samples collected from unidentified bodies can include: blood, buccal swabs, hairs, bone, teeth, fingernails, tissues from internal organs (including brain), muscle, and skin.
• Reference samples from known individuals including blood, oral/buccal swabs, and/or plucked hairs (e.g. head, pubic).
2.0 Title: Forensic Biology Screening

2.1 Principle
Prior to performing DNA analysis on evidence from casework, examination and reconciliation of evidence package contents must be completed and recorded. Probative areas of the evidence item(s) are identified and recorded as additional evidence and screening tests are performed in order to determine if biological material is suitable for DNA extraction.

While screening, questioned evidence samples and known reference standards shall be processed separately in both time and/or space. With the exception of reference standards contained within a sexual assault kit (SAK) or when additional evidence is submitted/requested after references have been processed, evidence samples will always be screened prior to the known samples.

2.2 Controls and Quality Assurance
Following preparation or receipt of any chemical screening reagent (with the exception of the Christmas Tree Stain), testing must be performed to ensure that the reagents are performing as expected. The results from each lot must be documented in the Resource Manager of the LIMS.

Screening rooms and/or work benches in the primary DNA casework laboratory are utilized for the examination of items of evidence and the collection of samples that may be subjected to PCR analysis. A variety of equipment and supplies are used, including but not limited to:

- Scissors, forceps, scalpels and scalpel blades
- Disposable gloves
- Individually wrapped sterile cotton-tipped applicators
- Racks and sterile tubes
- Presumptive and confirmatory test reagents
- Alternate light source or Crime Scope
- Lab coat (white pre-amp)
- Evidence Tape
- Biosafety cabinet

Special Precautions:
Diluted bleach (10%) or stabilized bleach solution (e.g., Dispatch) shall be used to wash all exposed work areas and any tools/resources used during examination and/or analysis (e.g. re-usable scales or rulers used during photodocumentation). At minimum, decontamination of all exposed work areas should be performed prior to the initiation of screening, between screening of different items of evidence and at the conclusion of all daily screening activities. Disposable bench paper will be placed on top of decontaminated lab benches prior to screening. This bench paper will be changed between the examination of different items of evidence.
Gloves, a mask, and a lab coat will be worn during forensic biology screening. At minimum, gloves should be changed between the handling of different items of evidence and whenever there is the possibility that transfer could occur between different stains and/or areas of interest on a single item.

2.3 Evidence Marking/Specimen Numbering
Evidence examined by members of the Biology/DNA Detail must be marked in some manner so that it can be identified in court. Physical items examined must bear the initials of the examining analyst, an item designator, the event number, and the lab number on the physical item of evidence, if practical. All markings or identifiers will be made in such a manner that the evidentiary value of the item is not compromised. It is not necessary to mark items of evidence which are received, but not examined.

2.3.1 Evidence Package and Sample Designations
Evidence package designations consist of item numbers automatically assigned by the LIMS (e.g. Item 1, Item 2). If there are multiple samples or packages within a package, a sub-item is created with dot numbers assigned as needed (e.g. Item 1.1, Item 1.2 – sample naming will be designated as a subset of the naming applied to the parent item/package).

If multiple areas of an evidentiary item are being tested, each area does not need to be given a unique identifier if numerous attempts yield negative results. It is possible to annotate in the analyst’s laboratory notes and report “multiple areas examined” and cite the results.

All items examined, the areas tested, the types of tests performed and the results of the tests will be documented in the LIMS. Notes should be descriptive and include information such as how much of a swab or clothing item is stained (or if no visible staining is present) when received, the color of the staining present when received and/or transferred to the swab(s) when a swabbing is collected, and how much of each stain was consumed for analysis. Documentation of color and amount of staining present on buccal swabs is not required but amount of sample being cut for analysis must be noted. For items such as hairs and fibers or other miscellaneous items, notes must be included which appropriately document the visual characteristics of the items, including the approximate sizes, colors, etc., as applicable. These notes will be retained within the screening portion of the Biology/DNA worksheet in the LIMS.

The descriptor “as received” should be used in the custom description or in the manual description added to the report of areas swabbed when arbitrarily defining outside vs. inside for an item that has an ambiguous orientation that cannot be readily identified (e.g. latex gloves, condoms, etc.). The “as received” designator will then carry with designated orientation (i.e. “outside, as received”) when describing the location of stains or areas tested.

Paperwork contained in Sexual Assault Kits is not considered biological evidence and does not need to be designated or reflected in the DNA report. Refer to Screening Casework Worksheets in Appendix D for additional information regarding screening and the use of the LIMS.

2.4 Securing Package Contents/Evidence Sealing
Evidence containers and packages (including known reference samples for casework) must be sealed in a manner to preserve the integrity of the evidence and to prevent inadvertent addition or removal of items. All packages must be secured as soon as practicable after examination.
2.5 Photodocumentation

Photodocumentation of physical items of evidence is not required. However, when photodocumentation occurs, the following guidelines should be followed:

1) A scale/ruler must be placed in the field of vision next to each item of evidence being photographed. It is recognized that some close-ups may not be conducive to the use of a scale/ruler. In such situations, an overview photo of the item must include a scale/ruler in the field of vision. Photos intended to document the packaging of items and/or photos taken of serological results (P30, Hematrace, RSID-saliva, and microscopy) do not require the use of a scale/ruler.

2) The Lab Case #, Lab Item #, analyst’s initials and the date must be placed on a placard or otherwise designated in the field of vision for each photo. It is recognized that some close-ups may not be conducive to the required information. In such situations, an overview photo of the item must include all required information in the field of vision.

Note: In the event a photo is being taken of an outer package that contains several other designated Lab Item #s inside, the Lab Item # may be omitted. The outer package itself does not have an associated Lab Item #.

3) Overviews should be taken of each item of evidence to document its overall features.

4) Close-ups of staining and/or areas of interest should be taken if not already visible in the overview photo(s).

5) Each photo file must be made available for discovery by upload into the Object Repository of LIMS. At minimum, photos must be named to include “DNA”, the Lab Case # and Item #. An additional description of the photo is optional (e.g. DNA_14-11953_Item 1 Outside Front Overview).

If the analyst wishes to enhance a photo manually after printing (e.g. adding designations for the areas tested during screening, screening results, etc.), the analyst must initial and date next to any additions made to the printed photo.

2.6 Collection of Touch DNA

Touch DNA commonly refers to the analysis of DNA from skin cells left on an object after it has been touched or casually handled.

2.6.1 Swabbing Touch DNA Samples from Handles, Surfaces, etc.

Collect a DNA sample using one swab moistened with molecular grade water or digest buffer, using the entire surface area of the swab to apply vigorous pressure. Additional swabs may be used for collection depending on the amount of staining transferred to the swab. The color of the staining transferred to the swab, if present, will be documented in the case file.

2.6.2 Swabbing Touch DNA Samples from Firearms

In the absence of a request for latent print analysis or when blood may be present, firearms will be swabbed in their entirety. Avoiding areas which appear to be stained with blood, collect a DNA sample with one swab moistened with molecular grade water or digest buffer, using the entire surface area of the swab to apply vigorous pressure. The swabbing should focus on the following areas while still ensuring the entire surface of the firearm is swabbed:

- Revolvers: trigger, hammer, cylinder release, ridged surfaces on cylinder, and grip
- Semiautomatic handguns: trigger, slide serrations, hammer, and grip
- Weapon magazines: bottom of magazine and the feeding area
• Rifles and shotguns: trigger, stock, and forestock
• Other textured surfaces such as the magazine release, safety, and other buttons present will also be swabbed

Additional swabs may be used for collection depending on the amount of staining transferred to the swab. The color of the staining transferred to the swab, if present, will be documented in the case file.

In the event the firearm is not swabbed in its entirety, a note will be included in the analyst's documentation regarding the specific reasons why this decision was made.

2.7 Y-Screen of Sexual Assault Evidence
A scenario-based approach will be utilized for sampling and processing items within a sexual assault kit. With the exception of deceased victims, items that are documented within the scenario to be of probative nature should be selected for sampling as appropriate. Fingernail swabbings/clippings and underwear will not be sampled unless they are the most probative items within the sexual assault kit or unless the detective specifically requests them to be sampled. Sexual assault kits collected from deceased victims will be sampled in their entirety including fingernail swabbings and underwear.

The sexual assault kit paperwork, P1 reports, and victim interview associated with qualifying sexual assaults, may be reviewed for guidance on which items to sample given the following information:
• The type of penetration that occurred (e.g., vaginal, rectal, oral, digital, etc.)
• Whether ejaculation occurred
• Whether a condom was used
• Other activities (e.g., patient licked, kissed, bitten, etc.)
• Number of offenders
• Location of collection for miscellaneous swabs (e.g., breast, neck) if present in the kit
• If there is an indication that the victim scratched the suspect if fingernail swabbings/clippings are present in the kit
• If there is an indication that semen is expected to be present on the fingernails of the victim if fingernail swabbings/clippings are present in the kit
• Whether unprotected consensual sex is documented within 72 hours of alleged assault

Note: When working a priority zero case, it will be necessary to request buccal swabs from the consensual partner if not received. Documentation of the request will be maintained in LIMS.

Documentation will be made within the LIMS worksheets with the reason why items were and were not selected for sampling. This will include where the information regarding scenario was obtained (i.e. SAK paperwork, LIMS, P1, Victim interviews, etc.).

Sexual assault kits collected from a deceased victim will be sampled in their entirety including any fingernail swabbings/clippings and underwear.

It may also be appropriate to process other items of evidence within a sexual assault kit using traditional serological methods based on the specific case scenario and types of evidence submitted. The reasoning for performing traditional serology will be documented in the case file.
Processing Underwear Contained in SAK
Underwear will not be sampled for processing unless it is the most probative item within the kit or if a detective specifically requests it to be worked. If semen is expected to be present, the underwear should be AP overlaid as appropriate. This may include the entire underwear or only areas of interest indicated through case scenario or observed with oblique lighting or ALS.

The following workflow may be utilized for sampling of underwear within a sexual assault kit:
For AP positive stains, an analyst should sample between ½ cm$^2$ to 1 cm$^2$ of the stain for differential extraction.

- If single assailant and stains are clustered in same area (e.g., inside crotch area), it is permissible to cut and combine the AP positive stains
- If multiple possible contributors (including unprotected consensual, and/or no scenario is provided,), then all stains should be sampled and processed separately as appropriate
- The maximum number of stains sampled should not exceed the number of alleged contributors (e.g., if five alleged contributors based on case information, up to five distinct stains will be sampled, if identified)

If only digital penetration was alleged, a single swabbing may be taken from the area of the underwear where the suspect potentially handled the underwear as dictated by the case scenario. The underwear may also be AP overlaid after touch swabbings have been collected if appropriate.

Additional Items Contained in SAK (e.g., tampons, sanitary pads, diapers, etc.)
Additional items such as tampons, sanitary pads, or diapers will only be sampled for processing when specifically requested by a detective or if they are the most probative items within the sexual assault kit. These items should be screened using AP Overlay to identify areas which may contain possible semen (Refer to Acid Phosphatase Overlay Presumptive Test for Semen). If identified, these areas will be sampled for differential extraction. General swabbings will not be created for alleged digital penetration on tampons and sanitary pads contained in SAKs unless a reason is indicated in the case scenario and documented in the analyst’s notes.

An alternate light source (ALS) may be utilized to identify fluorescence associated with the possible presence of saliva if warranted by the case scenario (Refer to Use of an Alternate Light Source (ALS) in Evidence Examination). If an area of interest is identified, the area may be sampled for non-differential extraction via either a general swabbing or cutting of the top layer.

Note: Diapers and sanitary pads will contain a gel base that should be avoided when sampling

Additional Evidence Items Outside the SAK (e.g., clothing, bedding, condoms, etc.)
With the exception of priority zero cases, invokes, homicides, and when approved by DNA Management, all additional items of evidence submitted outside of the sexual assault kit, regardless of the potential probative nature, will be processed as a separate unit record in LIMS when the results of the SAK are negative (or inconclusive). In the event the SAK yields positive results, the unit record containing the additional items of evidence will be terminated.

Condoms may be processed by collecting a separate swabbing from the inside and outside of the condom, as received. These swabbings will be consumed for differential extraction. Or, the swab(s) that may be contained inside the condom when received may be sampled for differential extraction as appropriate.
For large items of evidence such as clothing, bedding, and other sexual assault evidence outside of the SAK which may contain the presence of semen or saliva, an alternate light source (ALS) may be utilized to identify fluorescence associated (Refer to Use of an Alternate Light Source (ALS) in Evidence Examination). If warranted by the case scenario, fluorescence identified that may be the result of the possible presence of saliva may be sampled for non-differential extraction, whereas fluorescence identified that may be the result of the possible presence of semen should be AP overlaid. (Refer to Acid Phosphatase Overlay Presumptive Test for Semen).

For AP positive stains, sample between ½ cm$^2$ to 1 cm$^2$ of the stain for differential extraction.

- If single assailant and stains are clustered in same area (e.g., inside crotch area), it is permissible to cut and combine the AP positive stains
- If multiple possible contributors (including unprotected consensual, and/or complete loss of consciousness where no scenario is provided), then all stains should be sampled and processed separately
- The maximum number of stains sampled should not exceed the number of alleged contributors (e.g., if five alleged contributors based on case information, up to five distinct stains will be sampled, if identified).

In the event an item of evidence is specifically requested for touch DNA (e.g., lewdness, groping, etc.) and semen and/or saliva is not reasonably expected to be present based on the case scenario, swabbings may be performed in the absence of ALS or AP overlay.

Sampling Guidelines for Swabs Contained in SAK

The below provides guidance for the sampling of swabs contained in sexual assault kits. In the event warranted, the sample-specific or case-specific reasons for deviating from these sampling guidelines will be documented in the analyst's notes. This includes instances in which the qualitative assessment of the sample size/amount of staining present lends to sampling additional or all of the evidence in order to obtain the best opportunity to obtain a DNA typing result.

The following sampling guidelines will be employed based on the number of swabs collected for each sample. Stained portions of swabs will be sampled when available.

<table>
<thead>
<tr>
<th>Number of Swabs</th>
<th>Sampling Guidance</th>
</tr>
</thead>
<tbody>
<tr>
<td>One swab</td>
<td>Sample ½ of swab for extraction</td>
</tr>
<tr>
<td>Two swabs</td>
<td>Sample ½ of each swab and combine for extraction</td>
</tr>
<tr>
<td>Three swabs</td>
<td>Sample ⅓ of each and combine for extraction</td>
</tr>
<tr>
<td>Four swabs</td>
<td>Sample ¼ of each and combine for extraction</td>
</tr>
</tbody>
</table>

Fingernails and swabs taken from the body (e.g., breast swabs, neck swabs, hands, external genitalia, etc.) may be consumed when only one swab is submitted. When more than one swab is submitted, the sampling guidelines above should be employed.

Creation of Microscope Slides for Consumed Swabs

Microscope slides will be created during differential extraction from the pre-digest pellet only when the original evidence was consumed during sampling. These slides will be heat fixed, however, will not be stained nor have coverslips mounted. This slide will not be sub-itemized in LIMS.
Refer to the step 1 of the Method in Microscopic Identification of Spermatozoa for how to create and heat fix the microscope slide.

The slide will be placed in a labeled slide holder and returned to the original sexual assault packaging without searching. The slide holder will be sealed with evidence tape with the P#, initials, and date of the individual creating the seal. A note will be made on the outside of the SAK to indicate that the slide(s) have been added to the package, along with the date the slide(s) were added to the package and the initials of the analyst responsible for doing so. The customer will be notified of the availability of the slide(s) for possible future searching via a report disclaimer (Refer to Evidence Disposition and Case File Disclaimers).

Processing of Reference Standards Associated with Negative SAK Cases:
All requested reference standards will be sampled and extracted during the same time period as the evidence items sampled from the kit. The extraction of reference samples will not wait for the completion of the Y-screen of the associated evidence samples.

Extraction method of sampled items:
Analyst discretion may be used when determining the appropriate extraction type for each sample taken from sexual assault evidence depending on case scenario.

2.8 Traditional Serology for Evidence Which May Contain Semen
The below is intended to provide guidance for the screening of evidence that will not be subjected to the streamlined Y-screen method for sampling of the evidence (Refer to Y-Screen of Sexual Assault Evidence).

Based on the provided case documentation and/or scenario, samples collected directly from a body cavity or genitals that are not contained within a sexual assault kit should be tested for the presence of possible semen (acid phosphatase and P30) and/or using the confirmatory test for sperm. Presumptive testing is not required for these samples.

Note: Based on case scenario, it may not be necessary to test penile swabs collected in male/female sexual assaults for the presence of semen and/or sperm due to the female epithelial cells being the probative fraction. In such situations, a differential extraction may be performed without the completion of serological testing. Documentation of the case scenario and the analyst’s decision to not perform serological testing on the penile swabs will be included in the case file.

Samples collected from clothing that indicate an inconclusive presumptive semen result or a positive presumptive semen result must be confirmed by identifying sperm or indicated with P30 in the absence of sperm. Samples collected from clothing that indicate a negative presumptive semen result do not have to be subjected to further confirmatory semen/sperm tests.

The following guidelines should be followed when screening evidence utilizing P30 and microscopy:
1) If P30 testing is performed first, microscopy must take place regardless of the positive or negative P30 result.
2) If microscopy is performed first and sperm is identified, then P30 testing does not have to be performed on the positive sample.
3) If microscopy is performed first and sperm is not identified, then P30 testing must be performed. If the subsequent P30 testing is positive, the sample should be moved forward for differential extraction and a second microscopic sperm search must be performed as outlined in the differential extraction protocol. Refer to Microscopic Identification of Spermatozoa for additional information.

Note: There may be instances in which a sample may result in a weak positive or inconclusive P30 test and a negative microscopic sperm search. Depending on the case scenario, a regular non-differential extraction of the sample may be warranted, rather than a differential extraction. An explanation for the reason why a sample will be moved forward with a non-differential extraction will be documented in the analyst’s notes and/or the CONFIRMadactyl workbook.

2.9 Requests for Termination of Analysis After Sampling
At times, requests may be received to terminate analysis after screening/sampling has already commenced (e.g. case is adjudicated, etc.). In the event a portion of an item has been sampled into a tube in anticipation of additional analysis when the request for termination is received, the sampled portion will be returned to the original sample packaging. The outside of the package will be physically marked by the Forensic Scientist or Forensic Laboratory Technologist to indicate what has been re-packaged inside (e.g. swabbings, hairs in petri dish, portion of sampled swabs, etc.), and the date/intials of the individual placing the items inside. Alternatively, a split may be performed in ACE with an Officer’s Report to individually book the sampled portion of the item.

2.10 Use of an Alternate Light Source (ALS) in Evidence Examination
Body fluids like semen, saliva, and vaginal fluids naturally fluoresce under certain wavelengths and the use of an alternate light source offers a unique method for locating them. The user can narrow down the specific locations of stains for collection instead of testing entire, large pieces of evidence. It is necessary to tune to visible wavelengths to eliminate any background interference from the article that is examined.

Blood does not fluoresce; it absorbs at 415 nm and reflects at 254 nm. Stain contrast against the substrate background can be observed.

Warning! ALS units can produce bright and intense light. Do not look directly into the light unit.

The Biology/DNA Detail uses the following ALS equipment:
- Mini-CrimeScope MCS-400
- Crime-Lite 80S
- Crime-Lite 82S

2.10.1 Mini-CrimeScope MCS-400
Refer to Maintenance of Mini-Crime Scope MCS-400 for maintenance.

Settings
UV - The UV setting or light, along with clear or yellow goggles, can be used when examining dark colored items or in searching for the presence of saliva. 445/455/CSS/515 - These settings, along with the orange goggles, can be used in searching for the presence of body fluids on items of evidence. The 515 nm setting is the optimal setting for white colored items.
Method
1) Turn on main switch (in the back). Check to make sure there is air circulation on both exhaust top holes and on the fan located above the light guide connection.
2) Aim the wheel towards a wall and turn on the lamp switch (on the front of the unit). Within 1-2 minutes a bright spot will appear. If the spot is weak, verify that the intensity knob on the front of the unit (located below the light guide connection) is open all the way.
3) When finished, turn off the lamp first (front switch). Let the fan run for 3-5 minutes and then turn off the main switch (back switch).
4) Do not restart the lamp when the bulb is still hot. It will shorten the life of both the lamp and the power supply. An arcing noise may be heard when re-starting too early.

2.10.2 Crime-Lite 80S
Refer to Maintenance of Crime-Lite 80S for maintenance.

Settings
The Crime-lite 80S has a fixed nominal wavelength of 485 nm with a 10% bandwidth incorporated (460-510 nm). This setting, along with the orange goggles, can be used in searching for the presence of bodily fluids on items of evidence for longer (over one minute) periods of time. For shorter periods of time (one minute and under), the orange glasses can be used to identify the presence of bodily fluids on items of evidence.

Method
1) Connect the battery adaptor (teal with cord and plug) to the battery (blue and white) by sliding the battery adaptor onto the top of the battery until it “clicks” into place. While the unit is OFF, connect the cord from the battery adaptor into the base of the Crime-lite handle. Note: Inside the storage case there are both a wall adaptor and power cord to use in lieu of the battery. There is a second battery and a carrying case with a shoulder strap in case you would like to mobilize the unit around the room/elsewhere. The battery also has a waist belt clip built in to the teal adaptor portion so that it can be clipped if desired.
2) Ensure that a battery is charged prior to use to guarantee the best output from the light. In order to remove the battery from the charger or the Crime-lite unit, push the white retaining catch down and slide in the opposite direction.
3) In the storage case for the unit is a yellow camera filter lens that screws on to the front of the FujiFilm S100FS camera. The filter can be used on the camera while analyzing evidence in order to photographically document any fluorescence found on the evidence itself. Please handle with care as it is fragile glass and avoid leaving any residue (fingerprints) on the lens. To clean the filter lens, wipe clean with a dry KimWipe.
4) If the unit is used for an extended period of time, allow the unit to cool prior to replacing it back into the storage case.
2.10.3 Crime-lite 82S
Each Crime-lite 82S features sixteen LEDs along with narrow bandwidth light sources, fluorescence viewing filters (anti-glare viewing goggles), and optional attachments to a 5MP Vis-IR camera with optical filters. A tablet PC utilizes image-capture software for visualization and recording of evidence undergoing infrared examination. The Crime-lite 82S can be operated with a compatible rechargeable portable battery or can be AC-powered when connected to the camera and software feature.

IMPORTANT! Parts may only be cleaned with alcohol wipes—do not use bleach or Dispatch on any part of the 82S!

Select the light source you wish to use based on the type of body fluid you are searching for:
- Blood: IR (keep the room lights on)
- Semen and/or saliva: Blue and UV (turn the room lights off)

Safety eyewear must be worn when operating the light source manually or attached to the camera. When operating the camera, the appropriate viewing filter must also be used:
- UV 350-380nm, UV GG420 safety glasses and camera filter II
- Blue 420-470nm, GG495 viewing goggles and camera filter III
- Infrared 800-900nm, IR blocking safety glasses and camera filter IIII

Manual Operation
1) Attach the light to the battery adaptor and then to the rechargeable battery. The light source may also be attached directly to the wall outlet.

To use the portable battery:

To use the wall outlet:
2) When using the UV or IR light source, ensure the key is inserted into the bottom of the unit. If the key is parallel with the 0, the light is locked off; turn it perpendicularly to unlock it:

3) When operating the crime-lite manually and without the use of the camera, use the appropriate goggles to view fluorescent staining (blue and UV lights).

**Note:** IR light source must be connected to the camera in order to see any possible absorption staining.

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**Using the Camera**

Camera assembly:
Using the Camera and Software

1) A set of viewing filters are located on one side of the camera. Depending on the light source being utilized, position the wheel to correspond to the side that holds the descriptions.

2) Turn on the laptop.

3) Click the Crime-lite Cam software. Upon launch of the software, a live display from the camera will appear.
4) When the user is in live mode, the button will appear yellow (default). Clicking on the yellow Live button changes it to green, allowing the user to view select stored images.

5) Use of the Exposure Control enables or disables automatic exposure.
   - Automatic exposure attempts to produce an image of acceptable brightness. When Auto is enabled, the lettering is yellow. The More and Less buttons are manual exposure controls that increase or decrease the image brightness.

6) The camera is equipped with a motorized lens. The Auto button will initiate the auto-focusing procedure. Manual focus can be performed using the Cam controller or by moving the toggle.

7) The Annotate button displays the annotation toolbar, which enables text and graphics to be added to the image.
   - This function may be used to add the Lab Case #, Item number and any other notes directly onto your image.

8) The Grab Image button moves the live image into the image-store located on the C: drive (or desktop shortcut named "Grab Folder") and as a thumbnail on the bottom of the screen in the Crime-lite Cam software. The image-store is temporary storage and will only grab up to 20 images before the older images are deleted to make room for new ones. In order to save images:
• Right-click an image in the image-store area to select options to Delete, Delete All, Delete All But This, Save or Save All.
• To save, highlight your desired image and click Save.
• Click the Save As button to save images directly to a specified location on the computer or thumb drive.
• Alternatively, launch the Grab Folder on your desktop and cut and paste the image to the Working Folder.
  
  **Note:** Unlike the Grab Folder, the Working Folder does not have a capacity for the number of images it can save.

9) The Capture Evidence button will capture the image viewed directly into the Working Folder if all information has been filled out for the Device Settings and Case Details window.

- Enter the information into each field and then select the white box in the upper righthand corner for the information to populate onto the image.

![Device Settings and Case Details](image)

- When using this option, the font size, color, etc. can be adjusted and the text may be edited via Annotate.
- The information entered will be stamped onto the image.

![Image with stamped information](image)

- Click Capture Evidence, and then save.

The captured evidence consists of two files: a .tif image file and a .set text file. If necessary, the .set text file can be opened with a Notepad application.
2.11 Chemical Tests for Blood

After locating a suspected bloodstain, or area in question, the stain should be tested with a preliminary chemical color test to determine if possible blood is present. The results of the test shall be noted. The examiner may choose to conduct general surface rubbings with a swab or filter paper. If the test is positive and blood is indicated, record the results and further DNA testing should be conducted.

Of the several presumptive tests that have been used in the field of Forensic Biology for the detection of blood, phenolphthalein has high sensitivity and relatively low cross reactivity. A commercially available tetramethylbenzidine (TMB) presumptive blood test kit can also be used as an alternative to the phenolphthalein reagents. Use of TMB allows for more visible discernibility of a positive color-change reaction on red and pink substrates than is attainable with the PT reaction.

It is analyst discretion, and dependent upon case scenario, whether to use a test for higher primate blood following the results of the presumptive test. Note: If a sample is going to be reported as “inconclusive” for a chemical test for blood, then an explanation as to why must be detailed in the notes and a statement must be included on the report (refer to reporting Inconclusive Serological Results).

A second test that may be used to detect the presence of hemoglobin is the OneStep ABAcard® HemaTrace® Immunochromatographic Test for the Forensic Identification of Human Blood. This test is optional, however it should be utilized when the case documentation and/or scenario indicates the possibility for a presumptive false positive result.

2.11.1 Phenolphthalein (Kastle-Meyer) Presumptive Test

The basic principle behind the phenolphthalein test is that the heme portion of hemoglobin has peroxidase-like activity which can break down hydrogen peroxide into free hydroxyl radicals. In the presence of an oxidizable compound such as phenolphthalein (Kastle-Meyer reagent), the radicals will oxidize the colorless phenolphthalin into the fuchsia-pink phenolphthalein (PT), thus indicating the presence of blood (heme).

Refer to Appendix A of DNA Quality Manual for Reagent Preparation.

Controls and Quality Assurance Check

A known bloodstain and a negative control must be tested each day before reagents can be used for casework samples. The results must be documented in the exam notes. Should a reagent fail to perform as expected, that lot number must be discarded and the Technical Leader should be notified.

Method and Interpretation

1) A small cutting, swabbing, filter paper rubbing, or extract of the suspected bloodstain is taken.
2) Two drops of PT Step 1 are added to the stain.
   • In the event a color change occurs, the results of the test will be reported as inconclusive (refer to reporting Inconclusive Serological Results).
3) Add two or three drops of 3% hydrogen peroxide.
4) A positive result is indicated by the development of an intense pink color within several seconds. A very weak reaction from a stain which is brown is an indication that the stain is aged or diluted and the heme molecules are less likely to be removed from the substrate.

5) An absence of color change, especially on repeated tests, is an indication that there is no or insufficient hemoglobin present and should be recorded as negative.

**Note:** This is not a confirmatory test for blood.

### 2.11.2 Tetramethylbenzidine (TMB) Presumptive Test

The tetramethylbenzidine (TMB) presumptive test for blood is a catalytic test which is based on the peroxidase-like activity of hemoglobin. Hemoglobin has the ability to cleave oxygen molecules from hydrogen peroxide \( \text{H}_2\text{O}_2 \) and catalyze the reaction from the reduced form of 3,3’,5,5’-tetramethylbenzidine to the oxidized blue-green colored product, thus indicating the presence of blood (heme).

#### Controls and Quality Assurance Check

A known bloodstain and a negative control must be tested each day before the reagents can be used for casework samples. The results must be documented in the exam notes. Should the kit’s reagents fail to perform as expected, immediately discontinue use of the reagents, notify the Technical Leader, and contact the manufacturer.

#### Method and Interpretation

1) A small cutting, swabbing or filter paper rubbing of the suspected bloodstain is taken.

2) A drop of TMB reagent is added to the stain.
   - In the event a color change occurs, the results of the test will be reported as inconclusive (refer to reporting **Inconclusive Serological Results**).

3) Add a drop of 3% \( \text{H}_2\text{O}_2 \).

4) A positive presumptive test result is indicated by the sample turning a deep blue color within several seconds. On a white substrate the developed color appears as a blue-green. On a red substrate the developed color appears more like a deep blue or indigo. This is a presumptive positive indication for the presence of blood. A very weak reaction from a brown stain is an indication that the stain could be aged or diluted and the heme molecules are less likely to be removed from the substrate.

5) An absence of color change, especially on repeated tests, is an indication that there is no or insufficient hemoglobin present and should be recorded as negative.

### 2.11.3 OneStep ABACard® HemaTrace® Immunochromatographic Test for Human (Higher Primate) blood

More specific than the phenolphthalein presumptive test, the OneStep ABACard® HemaTrace® is designed to be used as a screening test for human/higher primate blood in the forensic laboratory and at crime scenes. The OneStep ABACard® HemaTrace® is a rapid immunochromatographic test that is sensitive, capable of detecting trace levels of human (higher primate) hemoglobin, and can be performed on bloodstains that have been stored for many years. Test kits are stored below 82°F (28°C).

#### Principle (from HemaTrace® product literature)

In this test procedure, 200 µL of solution containing the sample is added to the sample well ‘S’, and allowed to soak in. If human (higher primate) hemoglobin (hHb) is present in the specimen, it will react with the mobile monoclonal antihuman (higher primate) Hb antibody, and a mobile
antigen-antibody complex is formed. This mobile antibody-antigen complex migrates through the absorbent membrane device towards the test area ‘T’. In the test area, a polyclonal antihuman (higher primate) Hb antibody is immobilized. This immobilized antibody captures the complex so that an antibody-antigen-antibody sandwich is formed. The conjugated pink dye particles concentrate in a narrow zone on the membrane. When the hHb concentration in the sample exceeds 0.05 µg/µL, the pink dye particles will form a pink-colored band in the test area ‘T’ indicating a positive test result.

As an internal positive control, hHb antibody-dye conjugates cannot bind to the antibody in the test area ‘T’, but are captured by an immobilized anti-immunoglobin antibody present in the control area ‘C’, forming a complex. The captured pink dye particles will form a band in the control area ‘C’, indicating that the test has worked properly.

Controls and Quality Assurance Check
Upon receipt of a new lot, a known bloodstain positive control and a negative control must be tested before the lot can be released for use in casework.

Additionally, a known bloodstain positive control and a negative control must be run with each batch of casework samples. The results must be documented by notes or photos. Should a reagent or device fail to perform as expected more than once, then that lot must be discarded and the Technical Leader should be notified.

Method and Interpretation
1) Remove a small section of the stain or swab (approximately 3-5 mm²), place into a 1.7 mL tube, and soak in 200 µL of Phosphate Buffered Saline (PBS) until heme appears in the supernatant. Label additional tubes for the positive and negative controls and add buffer to the controls. The volume of PBS may be increased if the same sample will be tested for P30 as well.
   **Note**: The supernatant will turn reddish-brown as heme is released from the stain.
2) Allow the stain to soak for 1-5 minutes before testing. Older stains may require longer incubation times. Also, if a sample is being soaked for both P30 and Hematrace testing, the sample may follow the incubation requirements for P30 testing.
3) If retaining the sample for subsequent testing, place the substrate into a spin basket and centrifuge for 3-5 minutes. The substrate may be stored for future use at 2-8º C.
4) If necessary, remove a portion of the supernatant and dilute with additional PBS to obtain a straw-colored or colorless solution. Supernatants which are extremely dark in color may result in the high dose hook effect, thereby yielding a false negative result.
5) Remove the HemaTrace® device from the sealed pouch and label with the lab number and Lab Item #.
6) Pipette approximately 200 µL (4 drops from HemaTrace® dropper) of stain supernatant into the sample well.
7) Read results 10 minutes after adding the sample to the sample well. Positive results may be seen as early as 2 minutes, however a negative result requires a full 10 minutes.
   a. **Positive results**: Indicated by the presence of two pink lines, one each in the test area ‘T’ and in the control area ‘C’. The test is positive and indicates that the human (higher primate) hemoglobin level is at or above 0.05 µg/mL
   b. **Negative results**: Indicated by only one pink line present in the control area ‘C’. This result may indicate that no human (higher primate) hemoglobin is present at detectable
levels or that a very high level of human (higher primate) hemoglobin has resulted in what is termed a “high dose hook effect”. Both of these possibilities can be confirmed by concentration or dilution of the sample followed by a second test with a new device. 

**Note**: In the event blood is the body fluid of interest, samples with negative Hematrace results do not require DNA analysis.

c. **Inconclusive results**: Indicated by the absence of a pink line in the control area ‘C’. The test should be repeated. Make a note of the HemaTrace® device lot number of the inconclusive results.

**Note**: If a sample is going to be reported as “inconclusive” then an explanation as to why must be detailed on the CONFIRMadactyl workbook or in the screening section of the Biology/DNA worksheet in the LIMS. A statement must also be included on the final report (refer to reporting Inconclusive Serological Results).

### 2.12 Chemical Tests for Semen

After locating a suspected semen stain, or area in question, the stain may be tested to determine if possible semen is present.

If a sample is going to be reported as “inconclusive” for a chemical test for semen, then an explanation as to why must be detailed in the notes and a statement must be included on the report (refer to reporting Inconclusive Serological Results).

During the acid phosphatase test, the acid phosphatase present in the semen enzymatically acts on sodium alpha-napthyl phosphate to release alpha-napthyl. The alpha-napthyl can then react with the dye napthanil diazo blue B to form a purple colored diazonium alpha-napthyl compound.

This test is a preliminary color test which, if positive, indicates the possible presence of semen. It is not a confirmatory test since acid phosphatase is found in other substances, including other body fluids. To conserve sample, this test can be optional if the analyst performs microscopic testing of the stain with a positive result or microscopic testing and P30 of the stain when the microscopic results are negative.

**Note**: Due to the age of the evidence being examined, AP tests may result in a negative or inconclusive result. A microscopic sperm search may be necessary to ensure that DNA testing is not warranted.

#### 2.12.1 Acid Phosphatase Overlay Presumptive Test for Semen

Refer to Appendix A of DNA Quality Manual for Reagent Preparation.

**Controls and Quality Assurance Check**

The working solution must be tested against a known semen sample and negative control each day of use before the solution can be used for casework samples. The results must be documented in the exam notes. Should a reagent fail to perform as expected, that lot number must be discarded and the Technical Leader should be notified.

**Method and Interpretation**

1) Place a piece of Whatman No. 3 filter paper over the questioned stain. Put orientation marks on the paper. Spray the paper lightly with distilled water to thoroughly dampen it. Apply pressure to the paper so that it comes in contact with the semen stain for several seconds.
Alternatively, an autoclaved metal rolling pin may be utilized to apply pressure to the paper to ensure thorough contact. When using the rolling pin, apply pressure in sections by rolling over the paper several times and turning the rolling pin in different directions to ensure all grooves of the evidence come into sufficient contact with the paper. Special attention must be paid to apply firm pressure to all areas of interest previously identified visibly or by ALS. Always ensure the rolling pin is coming into direct contact with the paper while applying pressure.

2) Hang the paper in a fume hood so that the side that contacted the stain is facing the front of the hood.

3) Using a spray bottle, or other spraying apparatus, spray the paper thoroughly with the AP overlay working solution.

4) A positive reaction is recorded upon development of a purple color (usually within 3 minutes) which is indicative of possible semen. Old and/or desiccated samples may require closer to 3 minutes of the reaction time for color development. If no color change occurs, the reaction is negative. Color changes which occur after 3 minutes will be reported as inconclusive (refer to reporting Inconclusive Serological Results).

Note: In the event semen is the body fluid of interest, samples with negative AP overlay results do not require DNA analysis.

2.12.2 Microscopic Identification of Spermatozoa

Once a stain has been located, the area can be examined for the presence of spermatozoa. The extraction technique and making of a stained smear slide are the same for identification of epithelial cells and/or spermatozoa.

Once the smear has been dried and heat fixed it can be examined for the presence of cells. Due to the relatively small size of sperm when compared with other cells and cellular debris, the smear must be treated with a differential biological stain. This process allows detection of small numbers of sperm. The specific staining method utilized is the Kernechtrot-Picroindigocarmine stain (KPIC) which is also referred to as the Christmas Tree stain. Specifically, nuclear material stains red and background material stains green so that the sperm head and cell nuclei will be seen as red and other biological material will be seen as green. Definitive identification of spermatozoa is made based on the approximate size and staining pattern in which the acrosomal cap will stain less intensely than the rest of the sperm head while the mid-piece and tail (if present) will stain green.

Reagent Working Solutions:
Solution A: SERI Xmas tree Kernechtrot stain (red).
Solution B: SERI Xmas tree Picroindigocarmine stain (green).

Method
1) Remove 1-3 µL of the re-suspended cell debris pellet sample and heat fix on a microscope slide bearing a thin smear of questioned stain extract for several minutes using a lamp, oven or heat block as a heat source (refer to P30 test instructions).

2) Add a sufficient amount of Solution A (Kernechtrot red reagent) to cover the smear. Allow the slide to stand at room temperature for 15-20 minutes.

3) Wash Solution A off the slide with gentle stream of DI water over an approved disposal container.
4) Add a sufficient amount of Solution B (Picroindigocarmine green reagent) to cover the smear. Allow the slide to stand at room temperature for 5-15 seconds.

5) Wash Solution B off the slide with a gentle stream of ethanol over an approved disposal container. Let the slide air dry at room temperature.

6) After the slide has dried, apply a small drop of mounting medium, such as Permount, to the slide and top with a coverslip.

7) Once dried, the slide is now ready for viewing.

**Estimating the Quantity of Spermatozoa and Nucleated Epithelial Cells**

Refer to Microscope Manuals on the H:drive for instructions on how to utilize the different microscopes available within the Biology/DNA Detail.

The relative amount of spermatozoa present in any evidence sample can be estimated by observing the average number of sperm present in several microscope fields at 40X magnification. A general guideline for this determination will be recorded in the associated notes as follows:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)</td>
<td>no sperm present</td>
</tr>
<tr>
<td>+/-</td>
<td>inconclusive</td>
</tr>
<tr>
<td>+1</td>
<td>one to a few; difficult to locate</td>
</tr>
<tr>
<td>+2</td>
<td>some in several fields</td>
</tr>
<tr>
<td>+3</td>
<td>many in many fields; easy to locate</td>
</tr>
<tr>
<td>+4</td>
<td>many in most fields</td>
</tr>
</tbody>
</table>

**Note**: All “inconclusive” results must include an explanation as to the qualitative reasons for that conclusion. The explanation must be detailed in the CONFIRMadactyl workbook or the screening section of the Biology/DNA worksheet in the LIMS and a statement must be included on the report (refer to reporting **Inconclusive Serological Results**).

An evaluation as to the relative amount of nucleated epithelial cells present in any evidence sample can serve as an aid in determining how a sample will be subsequently processed downstream during the analysis process (e.g. utilizing additional wash steps during differential extraction, etc.). The evaluation can also serve as a general predictor of the potential success of recovering a DNA profile from the sperm cells present in a sample (e.g. extremely high epithelial content present versus +1 sperm).

This evaluation can be estimated by observing the relative amount of nucleated epithelial cells present in several microscope fields at 40X magnification. The nucleus of epithelial cells stains red, whereby cellular debris is green. A general guideline for this determination will be recorded in the microscopy notes of the CONFIRMadactyl workbook as follows:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>neg or (-)</td>
<td>no nucleated epithelial cells present</td>
</tr>
<tr>
<td>low or +1</td>
<td>small amount of nucleated epithelial cells present; little clustering</td>
</tr>
<tr>
<td>mod or +2</td>
<td>moderate amount of nucleated epithelial cells present; some clustering</td>
</tr>
<tr>
<td>high or +3</td>
<td>large amount of nucleated epithelial cells present; cells appear in large clusters making sperm search challenging</td>
</tr>
</tbody>
</table>
extreme or +4 extremely high amount of nucleated epithelial cells present; cells blanketed together making sperm search extremely difficult

In addition, cellular debris, bacteria, and/or yeast may be present which may also stain red. Though the presence of these materials are not expected to affect the human-specific DNA analysis process, they may help to inform why serological results were achieved in the event a DNA profile is not recovered (e.g., the presence of bacteria may result in a false positive AP test). The presence of these items may be noted, in addition to the grading of spermatozoa and nucleated epithelial cells present.

Retention of slides
When a microscopic sperm search is requested for court purposes or is performed post-DNA analysis for the purpose of supplementing the DNA analysis results (e.g., male DNA obtained in the sperm fraction of fingernails from a homicide victim), the slide will be packaged and retained with the original item of evidence. A note will be made on the outside of the package to document that the slide(s) have been sealed inside.

It is not necessary to retain slides created for microscopic sperm searches which occur during routine biological screening (prior to DNA analysis).

2.12.3 SERATEC® PSA SEMIQUANT Test for Seminal Fluid (P30)
The Seratec PSA Semiquant test is a chromatographic immunoassay for the rapid semi-quantitative determination of prostate-specific antigen (PSA) in body fluids. The test was designed to be used clinically as a screening test for prostate-specific antigen, in blood serum, to allow the detection of elevated levels of PSA that might be an indication of prostatic cancer. Relevant to forensic investigations, the test can also be used for the detection of semen, as PSA is normally found in seminal fluid, regardless of cancer activity. The test is sensitive and capable of detecting trace levels of seminal fluid. Test kits are stored at room temperature.

Note: Other body fluids such as blood or urine can also contain PSA. In addition, PSA can be found in very low concentrations in female vaginal fluid. Normally the PSA concentrations in other body fluids are low; therefore p30 test interference with non-seminal fluid samples is not normally expected.

Note: Due to the age of the evidence being examined, p30 tests may result in a negative or inconclusive result. A microscopic sperm search may be necessary to confirm the absence of sperm.

Principle (from Seratec product literature)
In this test procedure, 200 µL of solution containing the sample is added to the test well and allowed to soak in. If prostate-specific antigen (PSA) is present in the specimen, it will react with mobile gold-labeled monoclonal murine anti-PSA antibody in the test well, and a mobile antigen-antibody complex is formed. This mobile antigen-antibody complex migrates through the absorbent membrane device towards the test area ‘T’. In the test area, another anti-PSA antibody is immobilized. This immobilized antibody captures the complex so that an antibody-antigen-antibody sandwich is formed. The sandwich formation is indicated by the appearance of a red line in the test area ‘T’, indicating a positive test result. Additional anti-PSA antibodies will be
bound by immobilized polyclonal goat anti-mouse antibodies in the control area ‘C’ and internal standard area, located in between the control and test areas. The appearance of two red lines in the control and internal standard areas indicate the correct execution of the test.

Controls and Quality Assurance Check
Upon receipt of a new lot, a known semen positive control and a negative control must be tested before the lot can be released for use in casework.

A known semen positive control and a negative control must be run with each batch of casework samples. The results must be documented in the exam notes. Should a reagent or device fail to perform as expected more than one time, then that lot must be discarded and the Technical Leader should be notified.

Method and Interpretation
1) Remove a small section of the stain and place 750 µL of PBS in the tube. Label additional tubes for the positive and negative controls and add buffer to the controls. Incubate at room temperature for a minimum of 10 minutes, up to overnight depending on the age and quantity of the stain. Incubate at 2-8°C, if overnight incubation is used. Place substrate in a spin basket and centrifuge 3-5 minutes.

2) Label the test device with the lab number and Lab Item #. Add 3-5 drops (approximately 200 µL) of the supernatant to the test well.

3) Without disturbing the pellet, remove as much of the supernatant as possible using a sterile pipette tip. In cases involving limited sample size, save the supernatant in a separate tube. Re-suspend the pellet in the remaining liquid.

Note: This pellet contains epithelial cells and sperm cells and is called the cell debris pellet (refer to the Microscopic Identification of Spermatozoa).

Optional: The substrate may be stored and added to the tube for extraction.

4) Read the results 10 minutes after adding the sample to the sample well. Positive results may be seen as early as 1 minute after addition of the sample; however, a negative result requires the full 10 minutes. The internal standard for estimation quantity of PSA cannot be read until 10 minutes after the addition of the sample.

a. **Positive results:** Indicated by the presence of three pink lines, one each in the test ‘T’, internal standard (unmarked on the device), and control ‘C’ areas. The test is positive and indicates that the PSA level is at least 2 ng/mL. The color intensity of the internal standard correlates to a PSA concentration of approximately 4 ng/mL. Therefore, a test result line lighter than the internal standard indicates the sample contains less than 4 ng/mL PSA. If the test result line is darker than the internal standard, the sample contains more than 4 ng/mL PSA.

b. **Negative results:** Indicated by the presence of two pink lines, only in the internal standard (unmarked on the device) and control ‘C’ areas. This result may indicate that no PSA is present at detectable levels.

c. **Inconclusive results:** Indicated by the absence of the two pink lines in the internal standard (unmarked on the device) and control ‘C’ areas. The test should be repeated. Results can also be considered inconclusive when there is a combination of no sperm observed and weak positive results are obtained. Make a note of the Seratec device lot number of the inconclusive results.

   - If a sample is going to be reported as “inconclusive” then an explanation as to why must be detailed in CONFIRMadactyl or in the screening section of the Biology/DNA
worksheet in the LIMS. A statement must be included on the report (refer to reporting Inconclusive Serological Results).

d. **False Negative results:** This can occur when very high levels of PSA are present and there are no lines or only the presence of a strong ‘T’ line, known as the “high dose hook effect”. These results can be confirmed by dilution of the sample followed by a second test with a new device.

### 2.13 Rapid Stain Identification of Human Saliva (RSID™-Saliva)

Alpha-amylases (α-amylases) are present in almost all plants, animals and microorganisms. Different organisms have different chemical and catalytic properties of α-amylase, however each executes the same biochemical reaction in which digestive enzymes break down starches into simpler sugars. Human salivary α-amylase is the major protein component in human saliva which begins the digestion of starch. Human salivary α-amylase is synthesized in salivary gland acinar cells, which are responsible for producing, storing and discharging digestive enzymes. α-amylase is released in large quantities from the salivary cells in response to the detection of food, certain smells and mastication. Studies have shown that α-amylase may vary significantly in concentration between individuals and may at times be at similar concentrations as that found in other body fluids. Therefore, a negative test does not indicate the absence of saliva.

The Rapid Stain Identification of Human Saliva (RSID-Saliva) test is a rapid immunochromatographic test that has been internally demonstrated to detect levels of human α-amylase to a sensitivity of up to 1:500 dilutions (whereas dilutions of up to 1:20,000 have been reported in literature). No cross-reaction has been observed with blood, semen, urine, vaginal secretions, or menstrual blood. Low-level detection of human fecal samples was observed during internal validation studies. Low-level detection of breast milk has been also observed in literature, however this cross-reaction was not observed during internal validation studies. RSID Running Buffer is stored at 2-8°C and then brought to room temperature prior to use. The RSID Extraction Buffer received in the kit is not to be used. The RSID cassettes are stored at room temperature.

**Principle**

The RSID-Saliva test kit uses two mouse monoclonal antibodies specific for human salivary α-amylase. One of these antibodies is conjugated to colloidal gold and is deposited on a conjugate pad beneath the sample window. The other antibody is striped on the “test line” on a membrane attached to the conjugate pad. The “control line” on the membrane consists of anti-mouse IgG antibody and issued as a positive control.

In this test procedure, 100 µL of a solution containing the sample is added to the sample well ‘S’, and allowed to soak into the test strip. If human salivary α-amylase is present in the specimen, an antigen-colloidal gold conjugated antibody complex will form. Sample and antibodies (complexed and free) are transported by bulk fluid flow to the membrane section of the strip test. The immobilized anti-α-amylase antibodies on the test line capture the amylase-antibody-gold-complexes, producing a red line at the test position ‘T’, thereby indicating a positive result. If no human salivary amylase is present in the sample, then gold-conjugated antibody-antigen complexes cannot form, and colloidal gold will not be accumulated at the test line.

As an internal positive control, the anti-mouse IgG on the control line ‘C’ captures any mouse antibodies flowing past the test line, thereby producing a red line at the ‘C’ position and ensuring
that the sample fluid was transported through the length of the test and that the components of
the test strip are working correctly.

With the exception of cases involving loss of consciousness, the suitability of RSID-saliva testing
will be determined on a case-by-case basis utilizing the available case information and scenario.
Rectal swabs, which are known to produce RSID-saliva positive results, may be screened at the
analyst’s discretion as they may not ultimately aid in the determination of which probative samples
to move forward for DNA analysis.

In cases involving loss of consciousness in which semen has not already been identified, RSID-
saliva testing may be suitable for samples collected from the external orifices (e.g. external
genitalia, perianal, etc.), neck, breast, or other external areas of the body. All available case-
related information (including prior and post-alleged assault activities such as showering, etc.)
should be utilized when making the determination of the suitability of RSID-saliva testing and
whether saliva may be possibly present.

**Controls and Quality Assurance Check**

Upon receipt of a new lot, a known saliva positive control and a negative control must be tested
before the lot can be released for use in casework.

A known saliva positive control and a negative control must be run with each batch of casework
samples. The results must be documented by notes or photos. Should a reagent or cassette fail
to perform as expected more than once, then that lot must be discarded and the Technical Leader
should be notified.

**Method and Interpretation**

1) Remove a small section of the stain or swab (about 3-5 mm²), place into a 1.7 mL tube, and
soak in 750 µL of Phosphate Buffered Saline (PBS) for a minimum of one hour, up to
overnight at room temperature. Incubate at 2-8°C, if overnight incubation is used. Vortex the
sample for approximately 10 seconds at the beginning and end of the incubation period.

   **Note**: A note must be made in the analyst’s documentation if a sample may possibly contain
   fecal matter or breast milk (e.g. samples related to an alleged oral or anal assault or breast
   swabs taken from a lactating individual) as this may also influence a positive result.

1) Following incubation, perform a quick spin in order to remove any possible liquid present in
   the cap of the tubes.

2) Remove the sample substrate and place into a spin basket. Centrifuge the sample for 3-5
   minutes at 11,000 to 13,000 RPM. The substrate may be stored for future use at 2-8°C.

3) Remove 20 µL of the supernatant and place into a new 1.7 mL tube containing 80 µL of the
   RSID Running Buffer. Vortex to ensure thorough mixing and then perform a quick spin in
   order to remove any possible liquid present in the cap of the tube.

   **Note**: In cases involving limited sample size, save the supernatant in a separate tube.
   Alternatively, the supernatant may be discarded.

4) Remove the RSID-saliva cassette from the sealed pouch and label with the lab number and
   Lab Item #.

5) Pipette the entire 100 µL of the dilution into the sample well of the RSID-saliva cassette.

6) Read results 10 minutes after adding the sample to the sample well. Positive results may be
   seen as early as 2 minutes, however a negative result requires a full 10 minutes.
a. **Positive results**: Indicated by the presence of two pink lines, one each in the test area ‘T’ and in the control area ‘C’. The test is positive and indicates that the human α-amylase is present in the sample.

b. **Negative results**: Indicated by only one pink line present in the control area ‘C’. This result may indicate that no human α-amylase is present at detectable levels. False negatives due to “high dose hook effect” from the presence of extremely high levels of human α-amylase is not expected to be observed under standard laboratory testing when using the RSID-Saliva test kit, however may be confirmed by diluting the sample in addition RSID Running Buffer and repeating the test using a new RSID-saliva cassette, if necessary.

c. **Inconclusive results**: Indicated by the absence of a pink line in the control area ‘C’. The test should be repeated. Make a note of the RSID-Saliva cassette lot number of the inconclusive results.

**Note**: If a sample is going to be reported as “inconclusive” then an explanation as to why must be detailed on the “Additional Screening Worksheet” and a statement must be included on the report (refer to reporting **Inconclusive Serological Results**).

### 2.14 Microscopic Examination of Hairs

Refer to the Microscope Manuals on the H:drive for instructions on how to utilize the different microscopes available within the Biology/DNA Detail.

**IMPORTANT!** All possible hairs will be placed inside a covered petri dish while awaiting examination. When using a stereo microscope, observe the hair inside the covered petri dish to determine whether root material and/or a skin tag appears to be present. All hairs examined under a compound light microscope will be performed on a wet slide with a coverslip or slide placed on top, thereby securing the hair.

In the event the hair needs to be placed inside a sterile tube for extraction, this transfer will occur at a laboratory bench or in an exam room. During transport from the bench to the microscope and from the microscope to the bench, all hairs must be secured in a covered petri dish or on a wet slide with a coverslip or a second slide on top. This will ensure the hair is secured and not susceptible to loss during transport.

**Previously mounted slides suitable for DNA analysis**

1) If biological material appears to be present and the hairs were previously mounted, return to the laboratory bench or exam room and place several drops of the appropriate mounting media solvent around the perimeter of the coverslip

   **Note**: 100% xylene will dissolve Permount; toluene will dissolve Cytoseal. Nitrile gloves must be worn while working with mounting media solvents. Inspect gloves every 5 minutes for wetness or spots from solvents and change immediately if observed on the glove. It may be necessary for the sample to incubate at room temperature for several minutes to allow the mounting media to dissolve.

2) Carefully remove the coverslip.

3) Using forceps, carefully remove hair(s) and place into a sterile, labeled microcentrifuge tube for extraction. Forceps may be moistened with molecular grade water prior to removing the hair from the petri dish and placing into a sterile microcentrifuge tube.
Unmounted hairs suitable for DNA analysis
1) If biological material appears to be present and the hairs were not previously mounted, return to the laboratory bench or exam room and cut the hair(s) close to the end root and place into a sterile, labeled microcentrifuge tube for extraction. Forceps may be moistened with molecular grade water prior to removing the hair from the petri dish and placing into a sterile microcentrifuge tube.
2) Repackage the remaining hair shaft in glassine paper, a petri dish, or other suitable packaging and return with the original evidence.

Hairs determined to be unsuitable for DNA analysis
If no biological material appears to be present and the hair is determined to be unsuitable for nuclear DNA analysis, return to the laboratory bench or exam room and repackage the hair in glassine paper, a petri dish, or other suitable packaging with the original evidence.

2.15 Sampling Tissue for DNA Analysis
1) Cross section the tissue to access the inner-portion (pink/red) of the tissue
2) Cut approximately a 3-5 mm³ section of this inner-tissue into small pieces and place into a sterile, labeled microcentrifuge tube for extraction.
   Note: If the tissue appears to be highly degraded or of poor quality, larger portions of tissue may be sampled. It may be necessary to cross-section the tissue multiple times to identify a portion that is pink/red. This portion should be sampled for analysis.

2.16 Inconclusive Presumptive and/or Confirmatory Tests
A sample test that indicates an inconclusive presumptive or confirmatory blood or semen/sperm result(s) must have an explanation in the notes as to why it is being called inconclusive and cannot be reported as a positive or negative result. For presumptive tests, this must be documented in the screening section of the Biology/DNA worksheet in the LIMS. For confirmatory tests, this must be documented on the CONFIRMadactyl workbook or screening section of the Biology/DNA worksheet in the LIMS. The reason for the inconclusive will be stated on the report.

2.17 Evidence Return
Once analysis is complete or evidence is no longer needed by lab personnel, it will be returned to the evidence vault via a secure move. This requires two authorized ACE users with passwords.
3.0 Title: Sample Extraction, Purification, and Concentration

Principle
Once samples are identified for DNA testing, they are batched according to extraction methodology.

Quality Assurance

Sample Set-Up and Organization
While extracting, question (Q) and known (K) samples shall be processed separately in both time and/or space. Questioned samples will always be processed prior to the known samples.

When extracting known samples, cases containing multiple individuals of the same sex should be alternated between cases. In addition, male and female samples may be alternated, if possible.

When utilizing the organic extraction fume hood, a maximum of 48 sample tubes (including reagent blanks) may be manually organically extracted at the same time. For samples that must undergo organic differential extractions, a maximum of 24 samples (including reagent blanks) may be manually extracted at the same time (for a total of 48 sample tubes including the epithelial and sperm fractions).

The maximum number of samples that may be processed on a single run of the EZ1 Advanced XL Robot is 14, including reagent blanks.

When necessary, extraction reagents may be doubled or tripled to completely submerge a sample substrate. Reagent blanks will be treated identically as the largest associated sample.

Extraction Sample Designations
Samples processed within the Biology/DNA Detail will follow a specific naming convention. All applicable designations will be retained throughout the duration of processing and reporting.

- Samples will be designated with the Lab Case #, and the Lab Item # (including the use of the word “Item”) with any dot numbers as needed (e.g. 18-12345 Item 1.1).
- If samples are re-extracted, the end of the sample will be appended with RI# to indicate “Re-Isolation” (e.g. 18-12345 Item 1.1-R11).
- When samples are differentially extracted, the end of the sample will be appended with EF to designate “Epithelial Fraction” or SF to designate “Sperm Fraction” (e.g. 18-12345 Item 1.1-SF).

For database samples, a sequential number beginning with the number 1 will be assigned to all re-extracted samples (e.g. 2015-012345-R11).

Prior to extraction, all tubes will be given a sequential tube number that is unique to the batch of samples being processed. This tube number will at least be carried through the duration of the extraction process which requires tube to tube transfers.
IMPORTANT! Tube numbers which include a prefix of “K” or “k” will be identified as a reference standard by the QUANTadillo and AMPalatypus workbooks. Therefore, the use of this prefix must be avoided for questioned samples.

Controls and Standards
Evaluation and monitoring of controls in analytical procedures is essential to the proper interpretation of the test results. For interpretation guidelines that define acceptable results for reagent blank extraction controls, refer to Reagent Blank and Negative Amplification Controls.

Reagent Blanks
Reagent blanks associated with casework, references, and database samples are extracted concurrently with their associated samples and treated identically as their associated samples.

For questioned samples, each Lab Case # being processed will have at least one associated reagent blank per extraction type or run. The reagent blank control(s) shall be the last sample(s) set-up for the extraction and will serve as a control for the preceding samples having the same Lab Case #. Reagent blanks associated with questioned samples are named sequentially per case, as generated, along with the Lab Case # (e.g. 18-01234-RB1, 18-01234-RB2, etc.). The suffixes –EF and –SF are added to the end of the RBs used for epithelial and sperm fractions during differential extractions (e.g. 18-12345-RB1-EF and 18-12345-RB1-SF). In the event it is necessary to perform supplemental analysis for a previously processed case, new reagent blanks will be named with the next sequential number associated with that Lab Case #.

For known samples, at least one reagent blank will be generated for each extraction type or run and may be associated with reference standards from multiple Lab Case #s. Reagent blanks associated with known reference standards are named using the prefix RBK, the date the extraction is started, and the analyst performing the extraction (e.g. RBK-032518-JRC). The reagent blank control(s) associated with known reference standards shall be the last sample(s) set-up within an extraction batch and will serve as a control for all preceding samples.

In databasing, since known reference standards are the only type of extraction performed the prefix used for reagent blanks is only “RB”.
- For manual extraction sets for samples that fail the first time around or are being re-tested for match confirmations, the reagent blank is named according to the date the extraction is started and the analyst performing the extraction

For both casework known reference standards and databasing, extraction reagent blanks must be uniquely named. If more than one extraction is completed by an analyst per day, sequential numbers appended to reagent blank satisfies this requirement. Alternatively, the tube number may be carried through the entire testing process within the sample name due to all tube number/sample name combinations being inherently unique.

The use of dashes between prefix, date, and analyst in reagent blank names are optional.

Extraction, Quantitation and Multiple Reagent Blanks
Multiple reagent blanks may be created during the extraction process, and all reagent blanks created must be subjected to the quantitation process.
In the event multiple reagent blanks are created during the extraction process that control for the same set of samples and all blanks are treated identically, then all reagent blanks will be subjected to the quantitation process and the single reagent blank that demonstrates the greatest signal, if any, will be amplified.

**Clean-up and Concentration of Reagent Blanks**
Reagent blanks must be treated the same as, and parallel to, the forensic and/or casework reference samples being analyzed. The final volume of the blank should always be equal to or less than the lowest associated sample volume before proceeding to amplification.

If a sample needs to undergo clean-up, the associated reagent blank(s) must be cleaned-up as well. Similarly, if a sample needs to be filtered via microcon or CentriVap, the associated reagent blank(s) must also be filtered via microcon or CentriVap. If the reagent blank has already been filtered via microcon or Qiagen cleaned-up using the same reagent and microcon/spin column lot numbers, it is not necessary to re-microcon or re-Qiagen clean-up the associated reagent blank if the blank is already equal to or less than the lowest associated sample volume before proceeding to amplification.

In the event a reagent blank was previously concentrated via Microcon and requires additional processing (e.g., Y-STR analysis), the reagent blank will be reconstituted by adding the same volume of TE or H₂O (whichever utilized during extraction) to both the reagent blank tube and the associated sample(s).

If a reagent blank was previously concentrated via CentriVap, the reagent blank will be reconstituted by adding the same volume of H₂O to both the reagent blank tube and the associated sample(s).

**DNA Extraction Work Area**
This work area should be used for the extraction and isolation of DNA from items of evidence. A variety of equipment and supplies are used, including but not limited to:
- Adjustable pipettes
- Microcentrifuge tube racks
- Sterile microcentrifuge tubes
- Microcon concentrators
- Spin baskets
- Microcentrifuge
- Scissors, forceps, scalpels and sterile wooden sticks
- Sterile aerosol resistant barrier pipette tips
- Disposable gloves: latex/nitrile
- Refrigerator, freezer
- Heating block or incubator
- Vortex
- Lab Coat (white pre-amplification)
- Organic extraction fume hood (casework lab only)
- EZ1 Advanced XL Robots
- UV Crosslinkers
- CentriVap
Special Precautions
It is important that DNA extraction of evidence samples be performed according to accepted laboratory practices in order to prevent cross-contamination between evidence and reference samples and to minimize the potential for sample-to-sample contamination. During extraction, questioned samples are processed separately in time and/or space from known samples.

Diluted bleach (10%) or stabilized bleach solution (e.g., Dispatch) shall be used to wash all exposed work areas. At minimum, decontamination of all exposed work areas should be performed prior to the initiation of DNA extraction and at the conclusion of the daily analysis process.

Gloves, a mask, and a lab coat must be worn during DNA extraction. Gloves should be changed frequently when handling and manipulating different samples. Care should be taken by the analyst to continually monitor the integrity of their gloves and to ensure that transfer does not inadvertently occur between samples due to sample manipulation.

Phenol-Chloroform-Isoamyl Alcohol (PCIA) extractions and xylene processing must be performed in the organic fume hood.

Questioned stains/samples are manually organically extracted or extracted robotically using the Qiagen EZ1 Advanced XL. High-template DNA samples such as reference standards and database samples may be extracted manually using Qiagen, Chelex, or organic extraction chemistries, or robotically using the Qiagen EZ1 Advanced XL.

For manual extractions, only one reagent tube should be opened at a time. Special attention will be paid to the labeled identity and lot number of each reagent on the physical tube. The expiration date is located on the proximal container of small reagent tubes.

3.1 Chelex Extraction
Chelex extractions can be performed on high template DNA samples such as reference and/or database samples.

**Chelex Working Solution (5% weight/volume)**
Total Volume: 10.0 mL
Expiration Date: 5% Chelex working solution must be made fresh on a daily basis as needed.

**Equipment**
1) Balance
2) Pipette
3) Stir bar
4) Autoclaved 20-30 mL Beaker
5) Magnetic stirrer

**Reagents**
1) 0.5 g Chelex 100 resin
2) 10.0 mL Mol. Biol. Grade water
Preparation
1) Add 0.5 g of Chelex 100 Resin to a beaker with a stir bar.
2) Add 10 ml of Mol. Bio. Grade water.
3) Keep the Chelex actively mixing while in use by placing it on a magnetic stirrer.
4) After the extraction is complete, dispose of the remaining solution in the “Chelex Waste” glass bottle.

Note: The recipe may be increased proportionally as necessary. Chelex resin is highly inhibitory to PCR. Care must be taken not to transfer any resin beads into the quantitation or amplification tubes.

3.1.1 Chelex Extraction Sample Preparation

Whole Blood or Bloodstain
1) Add 3 µL whole blood or a bloodstain approximately 3 mm² to a sterile microcentrifuge tube. Label an additional tube as a reagent control. Pipet 1 mL molecular grade H₂O into the tubes. Vortex for approximately 2 seconds.
2) Incubate at room temperature for a minimum of 30 minutes or refrigerate for longer periods of time. Vortex for approximately 5-10 seconds.
3) Place the substrate in a spin basket (optional) and centrifuge for 3-5 minutes at maximum speed.
4) Without disturbing the pellet, carefully remove the supernatant, leaving enough behind to cover the pellet. If the sample is a bloodstain, return the substrate to the tube with the pellet.
5) Continue to Chelex Extraction Procedure

Reference Buccal Swab
1) Add the swab cutting or substrate to a sterile microcentrifuge tube.
2) Label an additional tube as a reagent control.
3) Continue to Chelex Extraction Procedure

3.1.2 Chelex Extraction Procedure
1) Add 200-400 µL 5% Chelex working solution to each tube (sufficient to submerge the sample).
2) Incubate at approximately 56°C from 30 minutes to overnight.
3) Vortex at high speed 5-10 seconds.
4) Incubate at approximately 100°C in a heat block or boil for 8 minutes.
5) Vortex at high speed for 5-10 seconds. Pulse spin.
6) Place the substrate in a spin basket (optional) and centrifuge 3-5 minutes at 11,000-13,000 rpm. Transfer the substrate to a labeled tube.
7) Store the remainder of the extract in the freezer and label accordingly.
3.2 Organic Extraction Procedure

3.2.1 Organic Extraction Sample Preparation

**Whole Blood/Bloodstains, Saliva Stains, Cigarette Butts, “Touch Evidence” Swabs and Buccal Swabs**

*Note:* If a Hematrace test and an extraction are to be performed on the same substrate, to conserve sample, return the substrate to the tube containing the cell pellet after the Hematrace test is completed.

*Note:* If evidence such as envelopes, stamps or tape is being extracted, 10-20 µL of 1M DTT may be added to help break up the adhesive.

1) Add 10-50 µL whole blood, a portion of stained material, cotton swab(s), portion of cigarette butt, or buccal swab into a sterile microcentrifuge tube. Label additional tubes as reagent blank controls, as necessary.

2) Pipette 500 µL of Digest Buffer.

3) Add 10-20 µL of Qiagen Proteinase K solution. Vortex briefly.

4) Incubate samples at 56°C for at least one hour to overnight in a heat block, incubator, or thermomixer.

5) Proceed to **Organic Extraction Procedure**.

**Differential Lysis of Evidence Potentially Containing Spermatozoa**

1) Label additional tubes as reagent blank controls, as necessary.

2) Add 500 µL Digest Buffer and 10-20 µL of Qiagen Proteinase K solution to the tubes containing the cell debris pellet and substrate and reagent blank(s). Vortex. Incubate at approximately 56°C for at least 15 minutes to lyse epithelial cells.

3) Vortex, pulse spin, and then remove the substrate and place into a spin basket insert. Centrifuge for 3 - 5 minutes at 11,000-13,000 rpm. Remove spin basket containing the substrate to a new microcentrifuge tube, label accordingly, and store.

4) Transfer all but 50 µL of the supernatant to a fresh microcentrifuge tube for epithelial DNA analysis being careful not to disturb the pellet. Save for epithelial DNA analysis and proceed to Step 3 of the **Organic Extraction Procedure**.

**Sperm Fraction**

5) To the original tube add 500 µL Digest Buffer and 10-20 µL Qiagen Proteinase K solution. Incubate at 56 °C in a heat block, incubator, or thermomixer for approximately 45 minutes. Spin in microcentrifuge for 3-5 minutes at 11,000-13,000 rpm. Discard all but the last 50 µL of fluid.

6) Wash the pellet as follows: Re-suspend the pellet in 500 µL to 1000 µL Digest Buffer by vortexing briefly. Spin the sample tube in a microcentrifuge for 5 minutes at 11,000-13,000 rpm. Using a sterile 1 mL pipet tip, remove as much of the supernatant as possible without disturbing the pellet.

7) Repeat wash step 5 an additional 1 to 2 times as needed. The number of wash steps can be altered depending on the estimate of the sperm to epithelial cell ratio based on microscopic examinations done during confirmatory testing. The number of wash steps can be increased if a high epithelial cell to sperm cell ratio is observed. The number of wash steps can be decreased if a low epithelial cell to sperm cell ratio is observed.
Note: If sperm have already been identified on the first microscopic exam performed during the initial serological screening, performing an additional microscopic exam is optional. If sperm have not been identified, or the item is associated with a consumed Y-screen sample, refer to Microscopic Identification of Sperm within the Forensic Biology Screening section.

8) Add 500 µL Digest Buffer to the re-suspended sperm pellet. Add 20 µL of 1 M DTT. Add 10-20 µL of Qiagen Proteinase K solution. Vortex.

9) Incubate at approximately 56°C in a heat block, incubator, or thermomixer for at least 1 hour to overnight. Vortex and pulse spin.

10) Proceed to Organic Extraction Procedure Step 3.

Neat Semen Stain
1) Pipette 500 µL of Digest Buffer into a sterile microcentrifuge tube. Add 10-50 µL neat semen, a 3-5 mm² section of stained material, or cotton swab(s).

2) Label sterile microcentrifuge tubes as reagent blank controls, as necessary. Pipette 500 µL of Digest Buffer into each tube.

3) Add 10-20 µL of Qiagen Proteinase K solution.

4) Add 20 µL of 1.0 M DTT and vortex briefly.

5) Proceed to Organic Extraction Procedure.

Note: A stain suspected of being "neat" should be previewed microscopically by use of Christmas Tree staining for determination of a "mixed" origin. Refer to Microscopic Identification of Spermatozoa for additional information.

Cellular Material from Microscope Slides (sperm and nucleated epithelial cells)
1) Microscopically examine the slide to assess the quantity of sperm and other biological material present in the smear.

2) Remove biological material from the slide using one of the following procedures:

Preparation without mounting medium or coverslip
1) Determine whether the slide was prepared with microscopic immersion oil. If so, wash the slide with stream of 95% or absolute ethanol from a pipette or a wash bottle.

2) Place slide in humidity chamber (Petri dish containing a sterile water-moistened filter paper disk). Add sufficient molecular grade water or PBS to the upper surface sufficient to cover the smear.

3) Incubate slide in chamber at 4°C overnight.

4) Swab the moistened biological material from the slide using a sterile cotton-tip swab.

5) Examine slide microscopically to determine whether material has been removed. If so, proceed to Step 2 of the procedure for Whole Blood/Bloodstains, Saliva Stains, Cigarette Butts, “Touch Evidence” Swabs and Buccal Swabs or the procedure for Differential Lysis of Evidence Potentially Containing Spermatozoa to begin the extraction process. Alternatively, the swab may be dried at room temperature if the material is to be stored.

Preparation when mounted with a coverslip
1) Freeze slide at -20°C for approximately 30 minutes.

2) Remove the cover slip by prying it off with a sterile scalpel. Repeat freezing if necessary.

3) Working in the organic fume hood, place the slide in a covered, glass beaker containing enough xylene to immerse the slide.

4) Incubate for 30 minutes at room temperature with intermittent gentle rocking.
5) Remove the slide and rinse with fresh xylene from a glass serological pipette (plastic may not be used) or glass beaker. Discard all xylene into a dedicated hazardous waste receptacle.
6) Allow slide to dry at room temperature.
7) Refer to preparation without mounting medium or coverslip and continue with extraction.

On occasion, the protocols for microscope slides with and without mounting medium or a coverslip do not adequately remove material from the slide and in such cases an alternative method may be employed:
1) Carefully scrape the smeared material from the slide using small quantities of xylene and a sterile, straight-edged scalpel. Elute material from scalpel blade into a microcentrifuge tube using xylene.
2) Centrifuge for 5 minutes. Remove xylene supernatant and discard into dedicated hazardous waste receptacle.
3) Xylene Wash: Wash the cell pellet with 500 µL of fresh xylene. Mix by hand inversion, then centrifuge for 5 minutes at maximum speed. Remove xylene and discard into an dedicated hazardous waste receptacle.
4) Repeat the xylene wash step at least twice for a total of three washes.
5) Wash the pellet with 1.0 mL 95% or absolute ethanol. Mix by inversion; then centrifuge for 5 minutes. Remove ethanol and discard into an organic waste receptacle.
6) Wash the pellet with 1.0 mL of 70% ethanol. Mix by inversion; then centrifuge for 5 minutes in a microcentrifuge. Remove ethanol and discard.
7) Add 500 µL of molecular grade water. Mix by inversion; then centrifuge for 5 minutes. Remove water and discard.
8) Blot the inside of the tube to remove excess liquid with a sterile swab taking care not to disturb the cell pellet. Proceed to proceed to Step 2 of the procedure for Whole Blood/Bloodstains, Saliva Stains, Cigarette Butts, “Touch Evidence” Swabs and Buccal Swabs to begin the extraction process.

**Cellular Material from Microscope Slides (Soft Tissue)**
Examine the slide microscopically to assess the quantity of tissue material present in the smear (i.e., nucleated cells).

**Remove biological material from the slide**
1) Freeze slide at approximately -20°C for approximately 30 minutes.
2) Pry coverslip off using a cold, sterile scalpel.
3) Working in the organic fume hood, place slide in a covered, glass beaker containing sufficient xylene to immerse slide. Incubate at room temperature for at least 30 minutes with agitation (if possible).
4) Remove slide and rinse with fresh xylene followed by a molecular grade water rinse.
5) Using a sterile scalpel, carefully scrape tissue material from the slide and transfer to a sterile microcentrifuge tube.
6) Label additional tubes as reagent blank controls, as necessary.

**Wash the biological material**
1) Perform three 1.0 mL xylene washes of the sample and reagent blank(s) at room temperature for a minimum of 5 minutes on a rocker platform. Centrifuge tissue sample for
2-3 minutes at 11,000-13,000 rpm between washes. Remove xylene supernatant and discard after each wash.

2) Perform one 95% or absolute ethanol wash at room temperature for a minimum of 5 minutes on a rocker. Centrifuge for 2-3 minutes at 11,000-13,000 rpm; then discard ethanol supernatant.

3) Perform one 70% ethanol wash. Centrifuge as above and discard supernatant.

4) Perform one 50% ethanol wash. Centrifuge as above and discard supernatant.

5) Add sterile TE Buffer to the pellet and re-suspend gently by use of a pipette. Draw up and down several times to dislodge pellet. Transfer re-suspended pellet from microcentrifuge to a sterile 15 mL conical polypropylene tube.

6) Perform two TE Buffer washes. Centrifuge for approximately 10 minutes at 2500-3000 rpm between washes. Fill tube to maximum volume with TE Buffer. Place tube on platform rocker for 30 minutes at room temperature for each wash. Following final wash, centrifuge to pellet tissue and discard supernatant.

7) Add 300 µL of TE Buffer to the pellet and vortex briefly to re-suspend pellet.

8) Centrifuge for approximately 3-5 minutes at 11,000-13,000 rpm to pellet. Remove supernatant and discard.

9) Add the following to the sample and reagent blank(s):
   - 500 µL Digest Buffer
   - 10-20 µL of Qiagen Proteinase K solution
   - 20µL 1.0 M DTT (Dithiothreitol)
     a. Vortex briefly. Ensure that tissue material is completely submersed in the solution. Incubate at 56°C in a heat block, incubator, or thermomixer overnight.
     b. If tissue material is still visible following overnight incubation, add 10-20 µL of Qiagen Proteinase K solution and incubate at 56 °C in a heat block, incubator, or thermomixer for an additional 2 hours.
     c. Proceed to Organic Extraction Procedure.

**Hair**

The LVMPD Biology/DNA Detail does not perform microscopic hair comparisons. However, in those instances where hair samples are the only available evidence and/or is deemed critical to the case, the DNA laboratory will perform STR DNA analysis directly on any hairs which exhibit a suitable flesh tag or root material. Alternatively, the intact hairs or the portion of the hairs remaining following STR analysis can be sent to an outside laboratory for mitochondrial DNA analysis when warranted.

1) Cut off about 5 - 10 mm of the proximal (root) end.

2) In the event the hair appears to contain surface dirt or visible contaminants (such as possible blood), wash the hair twice in molecular grade water. If no visible staining or debris is present on the hair, continue to step 4.

3) For mounted hairs freeze the slide in a -20°C freezer for a minimum of 20 minutes. Remove the cover slip by using a scalpel. Alternatively, the coverslip may be removed by soaking the slide in xylene for several hours after cracking the coverslip with a scribe.
   a. Using a pipette, wash away the mounting medium using xylene. Pick up the hair with clean forceps and wash in 100% ethanol: then wash in sterile, deionized water.

4) Label microcentrifuge tubes for samples and reagent blank controls, as necessary.

5) Add 500 µL of Digest Buffer, 20 µL of 1 M Dithiothreitol (DTT) and 20 µL of Qiagen Proteinase K solution to each microcentrifuge tube. Add the hair sample to the appropriate
microcentrifuge tube. Incubate at approximately 56°C for 2 hours to overnight. Hair may soften but not dissolve after incubation. Vortex approximately 30 seconds.

6) If hair root is not completely dissolved, add to the sample an additional 10-20 µL of Qiagen Proteinase K solution.

7) Incubate at approximately 56 °C in a heat block, incubator, or thermomixer for 6 - 8 hours to overnight.

8) Proceed to the Organic Extraction Procedure step 3.

**Material from Paraffin-embedded Tissue**

1) Cut out tissue from paraffin block with a sterile scalpel. Place the tissue in a glass or disposable petri dish and cut away excess paraffin. Cut tissue into small pieces. Transfer tissue to an appropriately labeled microcentrifuge tube.

2) Perform three 1.0 mL xylene washes at room temperature for a minimum of 5 minutes on a rocker platform. Centrifuge tissue sample for 2-3 minutes at 11,000-13,000 rpm between washes. Remove xylene supernatant and discard after each wash.

3) Perform one 95% or absolute ethanol wash at room temperature for a minimum of 5 minutes on a rocker. Centrifuge for 2-3 minutes at 11,000-13,000 rpm; then discard Ethanol supernatant.

4) Perform one 70% ethanol wash. Centrifuge as above and discard supernatant.

5) Perform one 50% ethanol wash. Centrifuge as above and discard supernatant.

6) Add sterile TE Buffer to the pellet and re-suspend gently by use of a pipette. Draw up and down several times to dislodge pellet. Transfer re-suspended pellet from microcentrifuge to a sterile 15 mL conical polypropylene tube.

7) Perform two TE Buffer washes. Centrifuge for approximately 10 minutes at 2500-3000 rpm between washes. Fill tube to maximum volume with TE Buffer. Place tube on platform rocker for 30 minutes at room temperature for each wash. Following final wash, centrifuge to pellet tissue and discard supernatant.

8) Add 300 µL of TE Buffer to the pellet and vortex briefly to re-suspend pellet.

9) Transfer tissue material to a microcentrifuge tube and spin at 11,000-13,000 rpm for 3-5 minutes to pellet. Remove supernatant and discard. Proceed with step 9 of the wash biological material portion of the cellular material from microscope slides (tissue) section.

**Whole/Fresh Tissue**

1) Carefully cut the thawed tissue sample to expose the inner portion of the tissue that contains the most pink/reddish color.

2) Cut a small section or sections of tissue that will be adequate for extraction, and place in a labeled microcentrifuge tube (refer to Sampling Tissue for DNA Analysis in the Forensic Biology Screening Section).

3) Label additional tubes as reagent blank controls, as necessary.

4) Add the following to the sample and reagent blank(s):
   - 500 µL Digest Buffer
   - 10-20 µL of Qiagen Proteinase K solution
   - 20 µL 1.0 M DTT (Dithiothreitol)

5) Vortex briefly. Ensure the tissue material is completely submersed in the solution. Incubate at 56°C until dissolved or overnight.

6) If tissue material is still visible following overnight incubation, add 10-20 µL of Qiagen Proteinase K solution and incubate at 56°C in a heat block, incubator, or thermomixer for an additional two hours.
7) Proceed to Step 3 of Organic Extraction Procedure.

3.2.2 Organic Extraction Procedure

1) Pulse spin microcentrifuge tubes containing lysed samples.
2) Transfer the substrate to a spin basket insert and centrifuge for approximately 3-5 minutes at 11,000-13,000 rpm. Remove the spin basket containing the substrate and store inside a new microcentrifuge tube.
3) In the organic hood add 500 µL buffered phenol-chloroform solution to the lysed cells. The volume may be scaled-up to ensure the phenol-chloroform solution is consistent with the lysis volume (1:1 ratio).
4) Rock at room temperature for 3 - 5 minutes and/or vortex for approximately 15 seconds. Spin in a microcentrifuge for 3 to 5 minutes at 11,000-13,000 rpm. Either transfer the upper aqueous layer to a new sterile microcentrifuge tube or remove and discard the bottom organic layer so a second phenol wash can be performed.
5) Repeat steps 3 and 4 one time for a second phenol wash. Additional phenol washes may be performed by repeating steps 3 and 4 until a clean interphase is obtained.
6) Transfer the upper aqueous phase to a Microcon DNA Fast Flow concentrator tube. Due to volume constraints, only 500 µL can be added to the Microcon tube at one time.
7) Spin the Microcon filter in a centrifuge at 0.5G (approximately 2,500 rpm) for 5-30 minutes, until all or most of the liquid has spun through.
8) Transfer the filter to a new tube or remove the wash from the tube, then add up to 500 µL of TE to the top of the Microcon.
9) Spin the Microcon filter in a centrifuge at 0.5G (approximately 2,500 rpm) for 5-30 minutes, until all or most of the liquid has spun through. Repeat step 8 if necessary.
10) If the filter was spun dry, add a minimum of 20 µL of TE to the filter for elution to ensure enough volume is available for at least a singular quantitation and amplification for either autosomal or Y-STRs.
11) Collect the concentrated DNA by inverting the upper reservoir of the filter into a clean tube and centrifuge for 3 minutes at 1.0G (1000 rcf).
12) If the final volume is less than 20 µL, more TE may be added to the filter and then re-centrifuged as in step 11 or more TE can be added directly to the extract.
13) Store the extract at 2-8°C or frozen and label accordingly.

3.3 Qiagen Extraction Procedure (QIAamp Isolation)

QIAamp extractions can be performed on high template DNA samples such as reference and/or database samples. Before using Qiagen-QIAamp kits, absolute ethanol must be added to Buffers AW1 and AW2 according to the manufacturer's instructions.

1) Cut a portion of stained material or tip of buccal swab, and place into a microcentrifuge tube. Label sterile microcentrifuge tubes for reagent blanks, as necessary.
2) Add the following:
   • 200 µL Buffer ATL warmed to 37°C in an incubator to ensure there is no precipitate in the bottles
   • 20 µL Qiagen Proteinase K solution
     When necessary, the above volumes may be doubled or tripled to ensure that there is enough total volume to complete submerse the substrate.
3) Incubate at 56°C in a heat block, incubator, or thermomixer for at least 1 hour to overnight. Lysis time will vary depending on the size and density of the source material.
4) Briefly centrifuge to remove drops from inside the lid.
**Optional:** Place the substrate in a spin basket and centrifuge at approximately 13,000 rpm for 3 to 5 minutes.

5) Add 200 µL Buffer AL. Vortex gently and incubate at 56°C in a heat block, incubator, or thermomixer for at least 10 minutes. Briefly centrifuge to remove drops from inside the lid. Add 210 µL of ethanol (200 proof). Vortex vigorously.

6) Unpack the QIAamp columns.

7) Briefly centrifuge the sample tube to remove drops from inside the lid. Carefully remove liquid from the microcentrifuge tube and add to the QIAamp column without wetting the rim. Due to volume constraints, only 750 µL of sample can be added to the QIAamp spin column at one time. If there is more than 500 µL of sample to be added, then the remainder can be added after the first 500 µL is spun through the filter and the collection tube is replaced with a new one.

8) Close the cap and centrifuge at approximately 8000 rpm for at least 1 minute. Place the QIAamp spin column in a clean collection tube and discard the tube containing the filtrate.

9) Carefully open the spin column and add the 500 µL Buffer AW1 without wetting the rim.

10) Close the cap and centrifuge at approximately 8000 rpm for at least 1 minute. Place the QIAamp spin column in a clean collection tube and discard the tube containing the filtrate.

11) Carefully open the spin column and add 500 µL Buffer AW2 without wetting the rim.

12) Close the cap and centrifuge at approximately 13,000 rpm for at least 3 minutes.

13) Place the QIAamp spin column in a clean microcentrifuge tube (not provided in kit) and discard the tube with the filtrate. The new tube will be the final collection tube. Carefully open the spin column and add 50-200 µL of Buffer AE.

14) Incubate at room temperature for at least 1 minute.

15) Centrifuge at approximately 8000 rpm for at least 1 minute. Save the extract, label accordingly, and store at 2-8°C or frozen. Discard the spin column.

### 3.4 Qiagen Purification/Clean-up

1) If the samples and blanks being cleaned-up are less than 200 µL, bring the volume of each up to 200 µL with TE.

2) Add 200 µL of AL buffer to the samples and blanks being processed. If the starting volume of a sample or blank is higher than 200 µL, add an equal volume of the AL buffer to the tube (ex: If the volume of a sample is 250 µL, add 250 µL of AL buffer to the sample.) Vortex briefly and incubate at 56°C in a heat block, incubator, or thermomixer for at least 10 minutes. Vortex briefly and pulse spin.

3) Add 200 µL of absolute ethanol to the tube(s), or an amount equal to half the total volume of each sample or blank with the AL buffer added (ex: If the total volume of a sample extract plus AL buffer is 500 µL, add 250 µL of absolute ethanol.) Vortex vigorously and pulse spin.

4) Unpack the QIAamp spin column and collection tube. Add the above mixture to the column. Centrifuge at 8,000 rpm for at least 1 minute. Discard the filtrate and replace the collection tube.

5) Add 500 µL of AW1 buffer to the column. Centrifuge at 8,000 rpm for at least 1 minute. Discard the filtrate and replace the collection tube.

6) Add 500 µL of AW2 buffer and centrifuge at approximately 13,000 rpm for at least 3 minutes. Discard the filtrate and the collection tube.

7) Place the QIAamp spin column in a clean microcentrifuge tube (not provided in kit) and discard the tube with the filtrate. The new tube will be the final collection tube. Carefully open the spin column and add 50-200 µL of AE buffer to the column. Incubate at room temperature for at least 1 minute. Centrifuge at 8,000 rpm for at least 1 minute. Discard the spin column. Save the extract, label accordingly, and store at 2-8°C or frozen.
3.5 Qiagen EZ1 Advanced XL Extraction using the DNA Investigator Kit

3.5.1 EZ1 Extraction Sample Preparation

**Non-Differential Extraction of Questioned Samples**

This protocol is for the EZ1 non-differential extraction processing of questioned samples including but not limited to the following: bloodstains, cigarettes, envelope flaps, swabbings taken of “touch” samples.

1) Add sample cutting to labeled Eppendorf 2.0 mL Safe-Lock or sterile microcentrifuge tube and initiate a reagent blank.
   
   **Note:** At least one reagent blank must be initiated per Lab Case # with each EZ1 run.

2) Add the following to each sample tube and reagent blank:
   - 475 µL of Digest Buffer
   - 25 µL of Qiagen Proteinase K
   
   Vortex the sample for 5-10 seconds to ensure thorough mixing.

3) Incubate tubes at 56°C for at least 60 minutes in a heat block, incubator, or on a thermomixer set to 900 rpm. Perform a quick spin of the tubes in order to remove any liquid from the caps.

4) Place the substrate in a spin basket and spin the sample down for 3-5 minutes at 11,000 to 13,000 rpm.

5) Store spin basket and substrate in a dolphin-nose tube.

6) The lysate may be transferred to a labeled EZ1 flat-bottomed screw-cap tube or may remain in the labeled Eppendorf 2.0 mL Safe-Lock tube based on analyst preference.

**IMPORTANT!** If sample lysates have been stored in the refrigerator prior to extraction, the tubes must be warmed to room temperature or placed in the 37°C incubator prior to the following steps.

Add the following to each sample tube:
   - 400 µL MTL buffer warmed to 37°C in an incubator to ensure there is no precipitate in the bottles
   - 1 µL of thawed hydrated cRNA

7) Vortex for 5 seconds to ensure thorough mixing. Perform a quick spin of the tubes in order to remove any liquid from the caps.

8) Proceed to [Running on the EZ1 Advanced XL Robot](#).

**Differential Extraction of Questioned Samples**

This protocol is for the EZ1 differential extraction of questioned samples containing epithelial and sperm cells.

1) Add sample cutting to labeled Eppendorf 2.0 mL Safe-Lock tube or sterile microcentrifuge tube and initiate a reagent blank.

   **Note:** At least one reagent blank must be initiated per Lab Case # with each EZ1 run.

2) Add the following to each labeled sample tube and reagent blank:
   - 500 µL of Digest Buffer
   - 20 µL of Proteinase K
   
   Vortex the sample for 5-10 seconds to ensure thorough mixing.

3) Incubate tubes at 56°C for at least 15 minutes in a heat block, incubator, or on a thermomixer set to 900 rpm. Perform a quick spin of the tubes in order to remove any liquid from the caps.

4) Place the substrate in a spin basket and spin the sample down for 3-5 minutes at 11,000 to 13,000 rpm.
5) Being careful to not disturb the sperm pellet, transfer all but 50 µL of the supernatant to a labeled Eppendorf 2.0 mL Safe-Lock tube or EZ1 flat-bottomed screw-cap tube. Store spin basket and substrate in a dolphin-nose tube.

**Note:** This is your epithelial fraction. Proceed to step 6 of the non-differential extraction of questioned samples.

### Sperm Fraction:
6) Add the following to each labeled Eppendorf 2.0 mL Safe-Lock tube and reagent blank:
   - 500 µL of Digest Buffer
   - 20 µL of Proteinase K
   - 20 µL of 1 M DTT

Vortex the sample for 5-10 seconds to ensure thorough mixing.

7) Incubate tubes at 56°C for at least 45 minutes in a heat block, incubator, or on a thermomixer set to 900 rpm. Spin the samples for 3-5 minutes at 11,000 to 13,000 rpm.

8) Being careful to not disturb the sperm pellet, remove and discard all but 50 µL of the supernatant.

9) Add 500-1000 µL of Digest Buffer to each of the remaining sperm pellets and vortex for 5-10 seconds to ensure proper resuspension.

10) Spin the samples for 3-5 minutes at 11,000 to 13,000 rpm.

11) Being careful not to disturb the sperm pellet, remove and discard the supernatant.

12) Repeat steps 9 through 11, one to two additional times.

13) If necessary, perform a second microscopic sperm search by referring to [Microscopic Identification of Sperm in the Forensic Biology Screening section](#).

14) Add the following to each labeled Eppendorf 2.0 mL Safe-Lock tube and reagent blank:
   - 500 µL of Digest Buffer
   - 20 µL of Proteinase K
   - 20 µL of 1 M DTT

Vortex the sample for 5-10 seconds to ensure thorough mixing.

15) Incubate tubes at 56°C for at least 1 hour to overnight in a heat block, incubator, or on a thermomixer set to 900 rpm.

16) At the end of the incubation period, the lysate may be transferred to a labeled EZ1 flat-bottomed screw-cap tube or may remain in the labeled Eppendorf 2.0 mL Safe-Lock tube based on analyst preference.

17) Proceed to Step 6 of the **Non-Differential Extraction of Questioned Samples**.

### Hair
1) Cut the root-end of hair to approximately 0.5 cm – 1 cm in length and place in a labeled Eppendorf 2.0 mL Safe-Lock tube or EZ1 flat-bottomed screw-top tube and initiate a reagent blank.

**Note:** At least one reagent blank must be initiated per Lab Case # for each EZ1 run.

2) Add the following to each labeled sample tube and reagent blank:
   - 160 µL of buffer ATL warmed to 37°C in an incubator to ensure there is no precipitate in the bottles
   - 20 µL Qiagen Proteinase K
   - 20 µL of 1M DTT

Vortex the sample for 5-10 seconds to ensure thorough mixing.

3) Incubate tubes at 56°C for at least one hour in a heat block, incubator, or on a thermomixer set to 900 rpm.
- At the end of the incubation period, visually inspect each tube to determine whether the hair has completely dissolved. If necessary, increase the incubation time to ensure complete lysis. An additional 20 µL Qiagen Proteinase K and 20 µL of 1M DTT may be added if necessary.

4) Perform a quick spin of the tubes in order to remove any liquid from the caps.

5) Proceed to **Running on the EZ1 Advanced XL Robot**.

**Known Reference Standards**

This protocol is for the EZ1 extraction processing of reference samples including but not limited to the following: FTA blood cards, buccal swabs, easiCollect FTA cards, easiCollect sponges.

1) Add sample cutting to labeled Eppendorf 2.0 mL Safe-Lock tube or EZ1 flat-bottomed screw-top tube and initiate a reagent blank

   **Note:** At least one reagent blank must be initiated for each EZ1 run

2) Dilute a stock solution of G2 buffer 1:1 using TE in a conical tube.

3) Add the following to each labeled sample tube and reagent blank:
   - 190 µL of diluted G2 buffer stock solution
   - 10 µL of Qiagen Proteinase K

   Vortex the sample for 5-10 seconds to ensure thorough mixing.

4) Incubate tubes at 56°C for at least 15 minutes in a heat block, incubator, or on a thermomixer set to 900 rpm. Perform a quick spin of the tubes in order to remove any liquid from the caps.

   **Note:** The “Trace TD” protocol utilized by the EZ1 instrument is designed to help mitigate the chances of a small substrate being inadvertently picked up by a tip during the isolation process. In the event the reference sample is bulky in nature (e.g. more than ½ of one swab or a large cutting of material), substrates should be removed prior to placing the sample on the instrument. This may be achieved via the use of an autoclaved wooden applicator, a sterile pipette tip or a spin basket spun for 3-5 minutes at 11,000 to 13,000 rpm. Reference standard substrates may be discarded.

5) Proceed to **Running on the EZ1 Advanced XL Robot**.

### 3.5.2 Running the EZ1 Advanced XL Robot

1) Power the instrument on (located in the back left by the power cord).

2) Perform optional daily maintenance:
   - b. Wipe down the inner surfaces of the instrument using 70% ethanol ONLY.
   - c. Perform a UV run as described in the [Daily Maintenance Section](#).

3) Press “Start”.

4) Analyst will be prompted “Create a report file?” Press “ESC” for No.

5) Select Protocol (dependent upon extraction type).
   - a. Press “2” for Trace TD (Tip Dance) for hair extraction or known extraction.

6) Select Elution Buffer – Press “2” for TE.

7) Select Elution Volume – Press the number corresponding with the desired final elution volume. The elution volume is selected per EZ1 run and is analyst discretion.

8) The screen will then show a review of the parameters – Press “ENT”.

9) Analyst will then be prompted to set up the instrument as follows:
   - a. Place Reagent Cartridges warmed to 37°C in an incubator in the cartridge rack located in the back of the instrument; ensure there is no precipitate present in the cartridges prior to placing on the robot
   - b. Place the following in the front rack:
Row 1: Labeled sterile microcentrifuge tubes or EZ1 screw-cap elution tubes  
Row 2: Tips and tip holders  
Row 3: Labeled sample tube containing sample lysate (either Eppendorf 2.0 mL Safe-Lock or EZ1 flat-bottomed screw-cap tube)

10) Press “Start” to begin run.
11) Once run is complete:
   a. Cap all labeled microcentrifuge tubes or screw cap elution tubes containing the extract and remove from the rack for downstream processing.
   b. Discard reagent cartridges and tips/tip holders in the biohazard.
   c. Discard the sample lysate tubes in the biohazard.
   Optional: Sample tubes containing sample lysate may be capped for storage.
   d. Store the labeled extracts at 2-8°C or frozen.
   e. Wipe down inside of instrument with 70% ethanol ONLY.

12) Upon completion of your last run of the day perform maintenance as notated in the Daily Maintenance Section.

3.5.3 Daily Maintenance

The following maintenance protocols only require completion on the days that the instrument is in use.

1) Clean the piercing unit
   a. From the main menu on the EZ1 instrument, Press “2” to select the manual function.
   b. Press “3” to select the cleaning function.
      Note: The door to the EZ1 Advanced XL must be closed in order for the cleaning run to initialize.
   b. Press “Start” to begin the cleaning operation. The instrument will then lower its piercing unit and instruct you to “open door and clean the piercing unit”.
   c. Wipe the piercing unit using a soft laboratory wipe moistened with 70% ethanol.
      Caution! The piercing unit is sharp – wearing two pairs of gloves is recommended.
   d. Once complete, press “ENT”. The piercing unit will return to its original position.
   e. Wipe the O-rings of the tip adapters with a lint-free tissue.
      Note: To maintain good contact between the tip adapters and filter-tips and to prevent liquid from leaking from the tips, the O-rings of the tip adapters should be greased monthly using a small amount of silicon grease.

2) Perform a UV run
   a. From the main menu, press “1” for UV to perform a UV decontamination of the inside of the instrument. The door to the EZ1 must be closed in order to initiate this function.
   b. Set the time for at least 20 minutes and press “ENT”.
   c. Press “Start” to initiate the UV lamp.
      Note: For user safety, the UV lamp cools for approximately 3 minutes. The door to the EZ1 Advanced XL instrument cannot be opened until after the cooling time has elapsed. After cooling, the main menu appears. UV runs may also be performed in between instrument runs if desired.

3) Power off the instrument.

3.6 Concentration of DNA Extracts and Reagent Blanks

DNA extracts may be concentrated before or after quantitation using Microcon DNA Fast Flow Tubes and/or the Labconco CentriVap DNA Concentrator. Sample extracts must contain ≤ 100 µL of EZ1 TE or ≤ 200 µL of Teknova TE (organic extracts) in order to be concentrated using the
CentriVap. Depending on the volume of TE contained in the sample, it may be necessary to concentrate a sample extract via Microcon prior to completing the concentration using the CentriVap.

**IMPORTANT!** In the event an organically extracted sample requires concentration prior to amplification, it is recommended that an additional clean-up step be performed prior to concentration as an added precaution to guard against the presence of inhibition undetected during quantitation. Refer to Qiagen Purification/Clean-up.

Samples which are suggested as “Concentrate” during quantitation will be concentrated prior to amplification. Alternatively, a “No Dil’n/MAX” amplification may be performed if warranted by sample type, the use of replicates, etc. Documentation must be included in the case file regarding the specific reason(s) why a target max amplification is being performed in lieu of concentration.

### 3.6.1 Concentration of DNA Extracts using Microcon DNA Fast Flow Tubes

Microcon DNA Fast Flow Tubes may be used to concentrate database samples extracted in SwabSolution or evidence samples to a lower final volume of extract. Alternatively, evidence sample extracts containing \( \geq 100 \mu L \) of EZ1 TE or \( \geq 200 \mu L \) of Teknova TE (organic extracts) may be first concentrated via Microcon DNA Fast Flow followed by final concentration using the CentriVap.

Samples associated with sexual assault kits and/or samples which will be Y-screened should not be concentrated prior to quantitation.

The suffix -MC will be used to designate when samples have been concentrated using the Microcon in the DNA workbook “makeiTWORK” (refer to Appendix B).

1. Transfer the DNA extract to a Microcon DNA Fast Flow concentrator tube. Due to volume constraints, only 500 \( \mu L \) can be added to the Microcon tube at one time.
2. Spin the Microcon filter in a centrifuge at 0.5G (500 rcf) for 5-30 minutes, until all or most of the liquid has spun through.
3. If the filter was spun dry, add the desired elution volume of TE.
4. Collect the concentrated DNA by inverting the upper reservoir of the filter into a clean tube and centrifuge for 3 minutes at 1.0G (1000 rcf).
5. Store the extract at 2-8°C or frozen and label accordingly.

### 3.6.2 Concentration of DNA Extracts using the CentriVap

Sample extracts must contain \( \leq 100 \mu L \) of EZ1 TE or \( \leq 200 \mu L \) of Teknova TE (organic extracts) in order to be concentrated using the CentriVap. Higher volumes of TE may cause inhibition once concentrated.

Samples associated with sexual assault kits and/or samples which will be Y-screened should not be concentrated prior to quantitation.

The suffix –VAP will be used to designate when samples have been concentrated using the CentriVap in the DNA workbook “makeiTWORK” (refer to Appendix B).

1. Turn on the instrument using the rocker switch on the right side.
2. Open the lid and place samples in the rotor wells with the caps open, ensuring the rotor is balanced. All caps should be placed in the same orientation for all samples. Close the lid.
3) Select from the following list of programs based on the desired run time and temperature:

<table>
<thead>
<tr>
<th>Program Number</th>
<th>Run Time and Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prog 1</td>
<td>35 minutes at 37ºC</td>
</tr>
<tr>
<td>Prog 2</td>
<td>30 minutes at 56ºC</td>
</tr>
<tr>
<td>Prog 3</td>
<td>10 minutes purge</td>
</tr>
<tr>
<td>Prog 4</td>
<td>60 minutes at 21ºC (room temperature)</td>
</tr>
</tbody>
</table>

- Programs 1-3 may be accessed using the quick start button. Program 4 (and Programs 1-3) may be selected by using the up and down arrows to toggle to the desired program on the digital display when the CentriVap is first powered on.
- Press the “Run/Stop” button to begin or discontinue the CentriVap run.

4) The CentriVap will run for the designated amount of time, which should bring the sample to dryness.

**Note:** Additional time may be added to the run if the desired volume has not been reached at the end of the run. Alternatively, the time may be shortened if concentration to dryness is not desired or if dryness is achieved prior to the end of the run.

5) Press the “Run/Stop” button at any time to remove samples.

6) The CentriZap strobe light can be used during the run to monitor the amount of liquid present in the tubes. Point the gun through the glass chamber while the samples are spinning and pull the trigger. Samples will appear to be standing still.

7) Once the run has completed, immediately reconstitute the sample in the desired volume of molecular grade water by pipetting the water up and down over the pellet 5-10 times. The minimum volume for reconstitution is 15 µL if amping only or 17 µL if quanting and amping.

8) The extract may be amplified immediately or stored in the refrigerator until amplification takes place.

**Daily Maintenance**

The following maintenance protocols only require completion on the days that the CentriVap is in use.

1) Open the CentriVap lid and select Prog 3 to perform liquid purge. In 10 seconds, the pump will operate and draw air through the system. The rotor will not turn with the lid open.

2) Wipe inner surfaces with Dispatch.

3) Record completion of maintenance in “CentriVap Maintenance Log”.

**Monthly Maintenance**

1) Check all rubber hoses and gaskets and replace any that show signs of hardening, permanent set, or deterioration.

2) If liquid present, empty and replace the trap.

3) Record completion of maintenance in “CentriVap Maintenance Log”.

**3.7 Microcon Buffer Exchange Clean-up of Qiagen-Extracted Samples**

Microcon DNA Fast Flow Tubes may be used to perform a buffer exchange of samples extracted using either the QIAmp kit or QIAGEN DNA Investigator Kit when inhibition is suspected. In the event a reagent blank is associated with evidence samples which require a buffer exchange, the associated reagent blank must also undergo the same clean-up procedure.

- In the event a reagent blank was previously concentrated via Microcon and buffer exchange is now required, the reagent blank will be reconstituted by adding the same
volume of TE or H₂O (whichever utilized during extraction) to both the reagent blank tube and the associated sample(s).

- If a reagent blank was previously concentrated via CentriVap, the reagent blank will be reconstituted by adding the same volume of H₂O to both the reagent blank tube and the associated sample(s).

The suffix -MC will be used to designate when a buffer exchange clean-up has been performed on samples using the Microcon in the DNA workbook “makeiTWORK” (refer to Appendix B).

1) Transfer the DNA extract to a Microcon DNA Fast Flow concentrator tube. Due to volume constraints, only 500 µL can be added to the Microcon tube at one time.
2) Spin the Microcon filter in a centrifuge at 0.5G (500 rcf) for 5-30 minutes, until all or most of the liquid has spun through.
3) Add a minimum of 100 µL of TE to the filter.
4) Spin the Microcon filter in a centrifuge at 0.5G (500 rcf) for 5-30 minutes, until all or most of the liquid has spun through. Repeat step 3 if necessary.
5) If the filter was spun dry, add the desired elution volume of TE. **Note:** In most cases, the final elution volume should be the same or similar as the original volume of extract as this procedure is intended for buffer exchange and clean-up of potential inhibition, rather than sample concentration.
6) Collect the concentrated DNA by inverting the upper reservoir of the filter into a clean tube and centrifuge for 3 minutes at 1.0G (1000 rcf).
7) Store the extract at 2-8°C or frozen and label accordingly.
Background
The Databasing section is responsible for processing whole blood/bloodstain cards, buccal swab samples and FTA cards submitted from southern Nevada as a result of felony arrests, and felony and most gross misdemeanor convictions. These specimens are collected from several jails, prisons and law enforcement agencies throughout southern Nevada, funneled to the LVMPD for DNA processing, and entered into the Convicted Offender or Arrestee Index of CODIS in accordance with Nevada Revised Statutes.

4.1 Rack-Pack-Label of Databasing Samples
1) Obtain a box of samples for rack/pack/label
2) Count the samples to ensure there is 90/box.
3) Open LIMS. Begin by clicking on the Evidence module > CODIS > New Lab Requests. The list will contain all samples which are ready to be accessioned in LIMS. Highlight each of the desired samples and click on “Receive Evidence” on the top tool bar.

4) Fill out this evidence transfer to ensure that the samples are available for the correct processing in the correct areas. Click “Transfer” and then enter your password on the next screen. When it’s finished transferring, click “OK”. Transferring evidence will automatically trigger the transfer of the Unit Record.
5) Opening only one envelope at a time, remove the bi-fold and two barcode labels. Open the bi-fold and use a letter opener (if needed) to open the inner envelope containing the Whatman EasiCollect Device.
6) Remove the EasiCollect Device. Compare the NV barcode on the back side of the FTA card to the NV barcode on the front side of the bi-fold. The CODIS Administrator must be notified in the event these numbers do not match.
7) Open the device and place the foam applicator-end into a manila coin envelope
8) With the foam applicator in the manila envelope, remove the FTA card and place the 1st barcode label onto the card as shown below. For new applicators, place barcode between the pink areas, NOT where the FTA card says to place the barcode. IMPORTANT! Ensure the barcode is aligned with the top and sides of the card as best as possible and does not hang off of the card. Press down on both ends of the barcode to ensure it does not peel away from the FTA backing card.

9) Place the barcoded FTA card into the rack with 20 slots using the following orientation: Place the card sideways with the perforated edge up and the barcode facing in the direction of the blue arrow
10) While holding the foam applicator end inside the manila envelope, cut the EasiCollect device where it bends and discard the remainder of the device into the trash.

11) Close the manila envelope, tape it shut, and place the second barcode across the top flap of the envelope.

12) Place the bi-fold and manila envelope back into the outer envelope and place into a long brown cardboard box.

13) When all samples are finished, label the brown cardboard box to denote whether the samples are Arrestee or Offender and the sample range. You may also add the date and your initials.
   a. Each of the 5 racks that holds the batch of sample will also be numerically labeled in the order of the samples. A batch of 90 samples will consist of 5 white robot racks.

14) Place the brown cardboard box containing Arrestee/Offender envelopes along with their associated racks on the shelves opposite of the analyst’s lab benches in the Database Lab. The cardboard boxes go on the middle shelf and the racks go on the shelf below. The cards are typically placed in order from oldest to newest (from left to right).

15) The samples are now ready to be electronically transferred in LIMS to the CODIS area:
a. **Step 1**: Under Case Processing > My Work: highlight all Unit Records to be transferred back to the general hold so they may have the database analysis completed. Click “Transfer Unit Record”.

Select the following fields:
- Lab: CODIS
- Examiner: Unassigned
- Reason: Exam
- Include a note in the Comments field that rack, pack, label was completed with the date and initials. When finished, click “OK”.

These unit records can now be found under Case Processing: CODIS > CODIS-Databasing for the next examiner to choose from and assign to themselves. **IMPORTANT!** Must choose the CODIS section again for this transfer.

b. **Step 2**: Under Evidence > My Personal Custody: highlight all of the samples to be transferred out of your custody and click on “Transfer Evidence”.
Select the following fields:

- Transfer type: Returned to Lab Vault
- At Lab: CODIS
- At Section: CODIS – Databasing
- Storage Area: CODIS Section Hold
- Transfer Reason: Other
- Include a note in the Comments field that rack, pack, label was completed with the date and initials.
- Click “Transfer” > enter your password > click “OK”.

The evidence can now be found under Evidence: CODIS > CODIS – Databasing for the next examiner to choose from and assign to themselves. BE SURE you choose the CODIS section again for this transfer.

4.2 Manual Quantitation of Extracted Database Samples using Database TRIOceratops

Below procedures are used to streamline worksheet documentation and interface with 7500 software when performing the manual quantitation of extracted database samples. Refer to the
relevant sections of the Biology/DNA Procedures Manual for Quantifiler Trio quantification and 7500 operation procedures

4.2.1 Creating a lab processing worksheet to document quantification
- Enter the sample names and the plate ID into the designated areas on the Quant list tab—you can copy and DNA paste (or paste special values) samples from other workbooks. You must include “TRIO” within the plate ID.
  Note: the sample names and plate ID will automatically populate the worksheet; the master mix preparation will also automatically calculate for you
- Enter the date on the Quant Setup tab in the format m/d/yy to automatically restrict expired/invalid resources from the drop-down lists; use the drop-down lists to record the resources used for quantification
  Note: the workbook automatically detects diluted extracts when colons appear in sample names to prompt the user to record the diluent used and dilution details; the default diluent can be toggled between TE and H2O
- When the worksheet is complete it should be printed for the case file

4.2.2 Exporting a sample setup file for the 7500
- Enter the “backup drive letter” in the designated area on the Quant list tab that currently corresponds to your pre-formatted and unlocked USB drive
- Click the EXPORT.TXT button on the Quant list tab; you will be prompted to save your quant workbook and the sample setup .txt file will transfer to the backup drive—if no backup drive was entered the workbook will alert you to re-export the .txt file

4.2.3 Importing a sample setup file onto the 7500 for quantification
- Choose the “Quantifier Trio” icon from the home page
- Launch the 7500’s HID software; click File → New
- Enter the experiment name (should match plate ID in TRIOceratops workbook)

![Importing a sample setup file onto the 7500 for quantification](image)

- Click File → Import; Choose the exported .txt file from the USB drive
- Verify that the plate setup is correct (correct samples, wells, tasks, detectors)
- Save the plate setup as an EDS document in the appropriate “DNA lab runs” folder

4.2.4 Exporting results from HID software and importing them into TRIOceratops
- After analyzing data using the HID software, highlight the entire plate (under the View Plate Layout tab with the Analysis drop down) click Export (top toolbar). The only box that should be clicked under Export Properties is Results. Click on the Customize Export tab and ensure the columns to be exported are in the correct orientation as defined in the relevant sections of the Biology/DNA Technical Manual for Quantifiler Trio quantification and 7500 operation procedures. Once everything is correct, define the location you wish to export to
4.2.5 Viewing quantification results in TRIOceratops

- Open the previously created quant workbook and select the Quant list tab; Click the Import .xls button and save the workbook when prompted—choose the .xls results exported from the HID software.
- The workbook automatically summarizes the quantification results from the HID software on the Quant Results tab – the Auto:Y ratio will display “---” if the Y target does not contain a quantification value; the workbook also automatically predicts each sample’s next action using the following logic:
  - Samples with quant values >50 ng/µL are automatically assigned Dilute → Re-Quant
  - Samples (>5 ng/µL) with IPC Ct values > 1.0 cycle above the average IPC Ct are flagged for possible inhibition and automatically assigned QIA → Re-Quant
  - Samples (small target undetermined or <5 ng/µL) with IPC Ct values > 0.5 cycle above the average IPC Ct are flagged for possible inhibition and automatically assigned QIA → Re-Quant
  - Samples (IPC Ct values ≤ average IPC Ct) with quant values <0.125ng/µL for the small target and <0.15ng/µL for the large target are automatically assigned CONC
  - All other samples are automatically assigned a default dilution depending on the chosen amp kit and degradation index.

Note: The average IPC Ct is calculated using standards 2-5 because the standards do not contain inhibitors; standard 1 is omitted from the average because its higher concentration of human DNA competes with the IPC for amplification causing IPC Ct delays; samples with a degradation index greater than 1.5 will automatically be flagged by the workbook in order to alert the analyst.

4.2.6 Assigning actions and automatically calculating amp dilutions

Important: The Quant Results tab must be reviewed carefully to determine the next steps in processing--the workbook predicts next-actions to streamline analysis, however, the analyst must carefully review and update/override the automatically predicted actions as needed/desired using each sample’s drop-down list and/or comment field.

Use the Action drop-downs (listed below) and Comments fields as needed to document the next action for each sample or RB—the DNA and H2O/TE columns update automatically.

<table>
<thead>
<tr>
<th>Samples:</th>
<th>Samples or RB’s:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ng/µL dil’n</td>
<td>Target Max (XXX ng)</td>
</tr>
<tr>
<td>0.1 ng/µL dil’n (XXX ng MAX)</td>
<td>CONC</td>
</tr>
<tr>
<td>0.125 ng/µL dil’n (XXX ng MAX)</td>
<td>QIA → Re-Quant</td>
</tr>
<tr>
<td>0.15 ng/µL dil’n (XXX ng MAX)</td>
<td>QIA + CONC → Re-Quant</td>
</tr>
<tr>
<td>0.2 ng/µL dil’n (XXX ng MAX)</td>
<td>Re-Quant</td>
</tr>
<tr>
<td>0.3 ng/µL dil’n (XXX ng MAX)</td>
<td>No Action Now</td>
</tr>
<tr>
<td>0.4 ng/µL dil’n (XXX ng MAX)</td>
<td>-----</td>
</tr>
<tr>
<td>0.5 ng/µL dil’n (XXX ng MAX)</td>
<td>-----</td>
</tr>
</tbody>
</table>
0.6 ng/µL dil’n (XXX ng MAX)  (dynamically calculated using QT value or selected amp kit)
No Dil’n (XXX ng MAX)
Dilute → Re-Quant

**Note:** The MAX template choices reflect the max volume added to the amplification reaction depending which kit is selected; therefore, the actual MAX varies depending on the volume amplified (the analyst can use each sample’s comment field to note amp volume/target adjustments)

- The analyst is alerted whenever an impossible dilution target is mistakenly chosen (Example: a sample with 0.4ng/µL quant value cannot be diluted to make 0.6ng/µL dil’n (XXXng MAX)
- The analyst can also scale the amp dilutions up or down by simply adjusting the DNA volume—the H2O/TE volume will automatically adjust according to the chosen “Action” and the volume reflected in the DNA column

**Note:** The formulas in the **Action** drop-down and DNA column are replaced by text whenever you modify them—this means that manually modified DNA volumes will no longer automatically adjust for newly chosen actions; however, the diluent volume will still alert the analyst automatically if the new action’s target cannot be achieved (for this reason it’s recommended that analysts avoid adjusting the H2O/TE volumes manually for dilutions)

### 4.2.7 Documenting amp dilutions and printing the quantification results

**IMPORTANT!** The bottom of the **Quant Results** tab is reserved for documenting the resources used to prepare amplification dilutions; refer to the section of the Procedures Manual for the respective STR kit that will be used for amplification to determine which diluent(s) can/should be used

- Enter the date the dilutions are being prepared in the designated field on the **Quant Results** tab in the format m/d/yy—this date will automatically restrict expired/invalid resources from the drop-down lists
- The **Quant Results** tab’s default diluent for making subsequent amp dilutions can be toggled between TE and H2O
- Record the dilution resources on the **Quant Results** tab; IF NO dilutions are being performed, simple enter “N/A” or “----” (or similar) in the designated fields
- When the worksheet is complete it will be printed for the case file

### 4.3 Amplification and 3500xl Set-up using the Zeppelin Fusion 6C Workbook

The Zeppelin workbook will be utilized for interaction with the 3500xl. Refer to the help tab of the workbook for instructions for operation.

### 4.4 PowerPlex Fusion 6C Processing of FTA Samples, Swabs, and Extracts

The PowerPlex Fusion 6C kit allows for direct amplification of samples from FTA storage cards (blood and saliva), swabs processed with SwabSolution and extracted DNA. Quantitation is not required of FTA sample punches or swabs incubated in SwabSolution. Extracted samples will require quantitation prior to amplification (refer to [Extraction of Known Reference Standards on the EZ1](#)).
As a quality check when amplifying full 96-well plates of FTA punches, one Custom Control should also be punched and placed on the amplification plate. There are five custom controls available with DNA known profiles. The Custom Control will ensure that plates have not been switched during processing by providing a means to identify each plate processed on the same day.

### 4.4.1 Amplification Set-up using Extracted DNA

The maximum volume of extract that can be added to the reaction is 7.5 µL. Extracts that have been quantitated using *Quantifiler® Trio* may be diluted with molecular grade water to target approximately 4 ng in a total volume of 7.5 µL OR a dilution can be prepared within the plate so that DNA/H₂O totals 7.5 µL.

1) Prepare the amplification master mix according to the workbook
   - # samples X 2.5 µL  Fusion 6C 5X Master Mix
   - # samples X 2.5 µL  Fusion 6C 5X Primer Pair Mix

2) Pipet 5 µL of prepared mix into each well
3) Add extracts to the appropriate well
4) To the APC well add 7.5 µL H₂O and 1µL of 2800M
5) To the ANC well add 7.5 µL of H₂O

*Note*: Water added to above wells can be water provided in amplification kit or molecular biology grade water that has been QC tested.

6) Cover wells with strip caps
7) Place plate on the thermal cycler and select “6C-24” or “6C-25” using the settings below:

<table>
<thead>
<tr>
<th>Initial incubation step</th>
<th>Cycle 24 or Cycle 25</th>
<th>Final extension</th>
<th>Final hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOLD</td>
<td>CYCLE</td>
<td>HOLD</td>
<td>HOLD</td>
</tr>
<tr>
<td>96 °C 1 min</td>
<td>96 °C 5 seconds</td>
<td>60 °C 1 minute</td>
<td>60 °C 10 minutes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 °C∞</td>
<td></td>
</tr>
</tbody>
</table>

8) To export the plate click **Export 3500 plate record!** and follow the prompts to save the workbook

### 4.4.2 Amplification Set-up using Samples Processed with SwabSolution

1) Add 200 µL to 500 µL of SwabSolution to tubes containing cut swabs or foam applicators. Initiate a reagent blank. The reagent blank should be named “RBSS-<date>-<Analyst’s initials>”. In the event an analyst initiates more than one reagent blank in the same day, a sequential number should be added to the end of the initials in order to distinguish between blanks.

2) Incubate @ 70°C for at least 30 minutes. Substrates may remain in the tubes after incubation.

3) Prepare the amplification master mix according to the workbook
   - # samples X 2.5 µL  Fusion 6C 5X Master Mix
   - # samples X 2.5 µL  Fusion 6C 5X Primer Pair Mix

4) Pipet 5 µL of prepared master mix into each well
5) Add 2 to 7.5 µL of SwabSolution sample liquid to the appropriate well
6) Add water to the SwabSolution samples if necessary to achieve a final volume of 7.5 µL
7) To the APC well add 7.5 µL H₂O and 1 µL of 2800M
8) To the ANC well add 7.5 µL H₂O

4.4.3 Amplification Set-up of Punched FTA Samples
While punching a sample from the dye-indicating saliva FTA paper, it is best to target the white areas as the color change indicates where the DNA is most likely to be deposited. Blood FTA cards should be punched in a visibly saturated area, when available.

**Option:** The analyst may wish to use a 96-well freezer block to hold the plate while setting up so that reagents remain chilled while punching into the plate. Steps 1 and 4 below may be omitted when processing match confirmation samples.

1) Pipette 10 µL of PunchSolution Reagent into each well of a 96-well plate that will contain an FTA punch, APC, or ANC.
2) Punch one 1.2 mm sample from the FTA paper and eject into the appropriate well. Repeat for each sample.
3) Punch one 1.2 mm sample from the custom control into the appropriate well.
4) Incubate the plate at 70°C for 30 minutes or until the wells are completely dry. Do not cover the plate as this will prevent evaporation.
5) Prepare the amplification master mix according to the workbook
   - # samples X 2.5 µL Fusion 6C 5X Master Mix
   - # samples X 2.5 µL Fusion 6C 5X Primer Pair Mix
   - # samples X 2.5 µL 5X AmpSolution™ Reagent
   - # samples X 5.0µL Water, Amplification Grade
6) Pipet 12.5 µL prepared master mix into each well.
7) To the APC well add 1 µL of 2800M.
   **Note:** the ANC well already contains master mix/H₂O
8) Visually check the plate to ensure that the 1.2 mm punches are in the liquid at the bottom of each well.
9) Cover wells with strip caps.
10) Place plate on the thermal cycler and select “6C-24” or “6C-25” using the settings below:

<table>
<thead>
<tr>
<th>Initial incubation step</th>
<th>Cycle 24 or Cycle 25</th>
<th>Final extension</th>
<th>Final hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOLD</td>
<td>CYCLE</td>
<td>HOLD</td>
<td>HOLD</td>
</tr>
<tr>
<td>96 °C</td>
<td>96 °C</td>
<td>60 °C</td>
<td>60 °C</td>
</tr>
<tr>
<td>1 min</td>
<td>5 seconds</td>
<td>1 minute</td>
<td>10 minutes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 °C</td>
<td>∞</td>
</tr>
</tbody>
</table>

11) To export the plate click **Export 3500 plate record!** and follow the prompts to save the workbook

4.4.4 Rehydration of EasiCollect Sponge to Facilitate DNA Transfer
In the event there appears to be an insufficient amount of DNA deposited onto an FTA card due to the lack of white area being present or due to an inconclusive DNA profile being previously generated, sperm wash buffer or SwabSolution may be used to rehydrate the sponge.

1) Pipette 125 µl of sperm wash buffer or SwabSolution onto the sponge portion of the easiCollect device and allow the sponge to rehydrate for 10 minutes.
2) Press the sponge firmly onto the FTA paper for 10 seconds.
3) After 10 seconds, lock the sponge into place within the device. The paper should be allowed to dry overnight while being in contact with the sponge. The FTA card is now ready to be punched either manually or using the robot.

4.5 Direct Amplification Set-up Using the Hamilton easyPunch Robot

**Quality Assurance**

To minimize the risk of contamination within the lab and between samples, as well as maintain the instrument, perform the following precautions:

1) Wipe down work area with 70% ethanol before starting a run.
2) Perform and document maintenance daily, when the Hamilton easyPunch is used. Maintenance is not required on the robot on days it is not in use.

To perform the daily maintenance click on the maintenance icon on the desktop screen. Once the maintenance wizard has begun, select the weekly maintenance wizard to perform the daily maintenance process required. It is not necessary to also run the daily maintenance wizard as all of those steps are included in the weekly maintenance wizard. Follow the prompts given through the maintenance process to complete instrument maintenance with these added instructions:

1) When prompted to clean the punch head, remove it from the instrument and wipe down all surfaces with a kimwipe moistened with 70% ethanol.
2) When prompted to clean the card gripper, use a kimwipe moistened with 70% ethanol to wipe the top and bottom of the gripper as well as in between the gripper arms in a very gentle flossing motion.

4.5.1 Set-up of PunchSolution Protocol using the Hamilton easyPunch Robot

1) Obtain pre-loaded magazines or add sample cards to the magazines. **Note:** Be sure to place the barcoded FTA cards in the card magazine facing to the left so that it can be read by the easyPunch imaging software. When looking at the card magazine, the “Rack #” barcode is on the right (back) side and the arrow faces forward pointing to the left.
2) Pull all carriers out so that they are resting on the instrument loading tray.
3) Ensure the instrument is turned on and proceed to open the easyPunch® Start software.
4) Choose the following settings, including the “HID_easyCollectPlus_PunchSolution.med”. Select the “Play” arrow.

5) On the following screen select ‘Start method using input dialogs with last used settings’.
6) On the following screen, enter the number of FTA cards to be processed in the ‘Number of cards’ field. Then enter the number of card magazines used in the ‘Number of magazines’ field. The next nine (9) fields should default to the correct settings and should not be altered unless they deviate from the image below. Modify the last two (2) fields to indicate ‘Positions of controls per plate’ and ‘Positions of empty wells per plate’ to be used for allelic ladders. The following screen shot is an example for one plate set-up of 90 database samples, one custom control, three allelic ladders, and a positive and negative control.

7) The following screen should default to the correct setting and should not be altered unless it deviates from the image below.
8) Select ‘Columnwise’ as the method setting to be used. Then click ‘Ok’ to continue.

9) Follow the prompt to load the card magazines starting at the top of the leftmost rack, and load racks as you move down the carrier from position 1 to position 10 as shown in the image. For a plate set-up for one batch of 90 database samples, the 5th magazine rack will contain 10 database samples in slots 1-10 and one custom control in slot 11.

Magazines are directional and can only be loaded with the blue arrow facing to the left. Load the carrier racks onto the black loading tray of the easyPunch® and set them directly down into the tray.

If the barcode reader is unable to read the rack barcodes, a barcode error message will pop up on the screen indicating that a barcode could not be read on the rack. Click “Barcode” and enter a name for the rack. Click “Execute” and then ‘OK’ to continue.
10) Load a new 96-well PCR plate onto the plate carrier indicated as position 4 in the window. Place the PunchSolution in position 3.08 – 3.12.

11) Click ‘OK’ to continue. A barcode error message will pop up on the screen indicating that a barcode could not be read on the 96-well plate. Click “Barcode” and enter a name (PCR Run #, e.g. DB15-123) for the plate. Click “Execute”.

12) The tip carrier will be on the instrument. If tips are needed, the protocol will prompt the user to replace the tips during the run.
13) Ensure the plate transporter is empty and in the correct position, as indicated by the solid white border, on the light box. If the transporter is out of position, move it into place until you feel the magnetic stand ‘snap’ into place.

14) Before starting the run, the trough used to collect cleaning punches may be dampened with a few milliliters of water or ethanol to reduce possible static electricity.

15) Click OK. The easyPunch® robot will now perform the PunchSolution setup and distribute the samples into the 96-well plate.

**Note:** Unknown errors may occur during plate processing. Follow prompts on screen if available to attempt to continue the run. If the run cannot be continued after clearing the error the remainder of the set-up may need to be finished manually.

16) Once the run has finished, visually check the plate to ensure that the 1.2 mm punches are in the liquid at the bottom of each well and that no sample card was discarded into the metal recovery trays.
Note: If a sample card was discarded into the recovery tray, check the plate to verify if it was punched prior to being discarded. If a punch was not taken, the sample card will need to be manually punched into the empty well position containing PunchSolution.

17) Add a clear adhesive cover to the plate, spin down in a centrifuge, uncover, and place on 70°C heat block for at least 30 minutes until evaporated.

18) Prepare PowerPlex Fusion 6C amplification master mix according to the “Manual” drop-down under the “Amp Reaction Mix” on the “Lab Worksheet” of the workbook if hand dispensing or the “Calculator” drop-down under the “Amp Reaction Mix” on the “Lab Worksheet” of the workbook if utilizing the robot. Click on the Master Mix calculator tab to enter the number of samples.

# samples X 2.5 µL Fusion 6C 5X Master Mix
# samples X 2.5 µL Fusion 6C 5X Primer Pair Mix
# samples X 2.5 µL 5X AmpSolution™ Reagent
# samples X 5.0 µL Water, Amplification Grade

19) Manually pipette 12.5 µL of prepared master mix to each well, custom control, amplification positive control (APC), and amplification negative control (ANC).

20) Add 1 µL of 2800M positive control to the APC well.

21) Cover wells with strip caps.

22) Place plate on the thermal cycler and choose either the 24 cycle or 25 cycle option for PowerPlex Fusion 6C (“6C-24” or “6C-25”) according to the tables below:

<table>
<thead>
<tr>
<th>Cycle 24 or Cycle 25</th>
<th>Final extension</th>
<th>Final hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denature</td>
<td>Anneal/Extend</td>
<td></td>
</tr>
<tr>
<td>96 °C 5 seconds</td>
<td>60 °C 1 minute</td>
<td>4 °C</td>
</tr>
</tbody>
</table>

4.6 Sample Set-up for 3500xl Instruments

See the table below. The following items are minimally required per initial run.

<table>
<thead>
<tr>
<th>Chemistry</th>
<th>Run Requirement</th>
</tr>
</thead>
</table>
| PowerPlex Fusion 6C | -Can use manually extracted DNA, swabs or FTA punches
| | • Two Allelic Ladders
| | • Positive Control (APC) – 2800M
| | • Negative Control (ANC)
| | • Custom Control for full FTA punched plates only
| | • Reagent Blank (RB) for manual extracts and SwabSolution samples only

4.7 Analysis Run - 3500xl Instruments

Follow the procedures in the Capillary Electrophoresis section of this Manual.

4.8 Data Analysis, Review and Second Reads using GeneMapper ID-X

For an acceptable run, all controls must yield expected results. The Custom Control (FTA punch plate) is not required to yield a full profile; however, a partial profile consistent with the expected results is required. Each Custom Control will be checked in GeneMapper ID-X using the Comparison Tool to ensure that the profile is only consistent with the one staff member it
originated from (refer to GeneMapper ID-X and Data Analysis section). In the event the Comparison Tool identifies multiple individuals that could match to the partial DNA profile from a Custom Control, the Technical Leader must review the Custom Control data and sign off on its use prior to releasing the associated data on the amplification plate.

All homozygous autosomal STR peaks in the amplification positive control for PowerPlex Fusion 6C must achieve the minimum designated analytical and/or stochastic thresholds required for their associated sample’s interpretation as outlined in this manual in order to be considered “passing”.

- For PowerPlex Fusion 6C on the 3500xl, homozygous autosomal STR peaks must achieve a stochastic threshold of 175 RFU for 24-cycle data and 200 RFU for 25-cycle data to be considered “passing”. All typed peaks within a ladder must also reach at least 60 RFU to be included in data analysis. All Y-STR haplotypes must be detected above the 60 RFU analytical threshold to be considered “passing”.

All database samples are reasonably expected to yield single source DNA profiles. In the event a mixture is detected, an investigation will be conducted as to the possible source of the mixture. This may include the consideration of capillary carryover, sample-to-sample contamination, chimerism, etc.). Database samples resulting in mixtures will be re-processed or re-extracted in an attempt to resolve any issues present.

1) Follow GeneMapper ID-X and Data Analysis section of this Manual. Samples that do not yield complete profiles for the CODIS core loci will be re-injected, re-amplified, re-isolated, or microconned along with the reagent blank using an appropriate validated extraction or direct amplification method. Off-ladders will be measured against the allelic ladder and renamed appropriately after confirmation by re-injection, re-amplification, or re-isolation.

**Note:** Off-ladder alleles at the Penta E locus which fall approximately 0.5 bp short of the bin do not require confirmation. These alleles may be manually assigned to the bin without re-injection, re-amplification, or re-isolation.

**Note:** In the event an allele appears as an OMR falling between two loci or as a possible OL falling into a neighboring locus, it may be possible to determine which locus the allele belongs to using the following criteria:

- Evaluate the expected peak heights at the two neighboring loci to determine whether homozygous vs. heterozygous alleles are believed to be present. Homozygotes are expected to have approximately double the peak height as heterozygous alleles.
- Evaluate the number of peaks present at the two neighboring loci. With the exception of rare tri-alleles, no more than two peaks are typically expected to be present within a single source databasing sample.
  - Calculate the number of repeat units the OMR or OL falls from the last allele (physical or virtual) at each locus to determine whether the ambiguous peak is one full repeat unit from either locus’ ladder.
  - In the event the allele is still ambiguous, the sample may be extracted using the EZ1 Advanced XL and amplified using Investigator 24plex QS in order to resolve the allele calls for the neighboring loci. Only the data associated with the Fusion 6C profile will be uploaded to CODIS. Alternatively, both loci may be excluded from CODIS upload.
Documentation for the assignment of the allele call and the reasons why will be maintained in the database packet.

1) Projects in GeneMapper ID-X will be named with analyst initials-date-instrument (e.g., JB-080115-CAL).

2) CODIS will only accept allele designations, and those alleles that are below (designated as "<") and above (designated as ">") the Allelic Ladder range. During import into CODIS, any alleles outside of the CODIS acceptable range of alleles will be converted to a "<" or ">", of the first or last acceptable allele in the range, respectively.

3) **Allele calls**: The first reader will delete all spikes, pull-ups, excess stutter or artifacts.
   - For Fusion 6C on the 3500xl, alleles must be above the analytical threshold of 60 RFU. The stochastic threshold is 175 RFU at 24 cycles or 200 RFU at 25 cycles. Homozygous autosomal peaks must meet the stochastic threshold. Heterozygous peaks between the stochastic and analytical thresholds may be used. A 20% global filter is applied to all samples. Y-STR haplotypes must be above the analytical threshold of 60 RFU.

In the event that Amelogenin only has an “X” below the stochastic threshold, a gender verification step may be performed. The analyst can look at the documentation accompanying the offender/arrestee sample to verify the gender as a female. Further, when using Fusion 6C, the analyst may examine the three Y chromosome markers (DYS391, DYS576 and DYS570) for other indications of a presence/absence of male DNA. The analyst should note which information they used for gender verification. If any ambiguity remains, the analyst will re-process the sample to raise Amelogenin and/or the Y markers above the stochastic threshold.

In the event a sample appears to contain a null X-allele at Amelogenin while a Y-allele is present above analytical threshold, the other Y-STR markers (DYS391, DYS576 and DYS570) should be examined for indications that the sample is originating from a male.

In the event of a null Y-allele (deletion) affecting Amelogenin, it would be expected that DYS576 and DYS570 will also be absent due to their proximity to Amelogenin on the Y chromosome. An allele should still be present at DYS391.

Off-ladder alleles in single source databasing samples can be confirmed through reinjection, reload, or re-amplification.

Single source databasing samples containing suspected tri-alleles and/or duplications/triplications of Y-STR markers can be confirmed through reinjection, reload, or re-amplification with the understanding that if an Offender/Arrestee hit occurs the sample will be re-extracted during the match confirmation process.

4) Reviews are documented using the form “Database Tech Review Form”.

5) The second reader/technical reviewer will assess and confirm each of the allele calls. Any peaks that are not true alleles must be removed before CODIS Export file creation.

6) Repeated samples will be documented and subjected to a second read.

7) The second reader will indicate approval on the Technical Review checklist. Samples that have passed second review can be added to the CODIS Export Table function of GeneMapper ID-X which will create the CMF (Common Message Format) file format required.
4.9 Data - CODIS Export
   1) From the GeneMapper ID-X Project which is opened, change the Table Setting view in the drop-down list to **CODIS Export**.
   2) Looking at the list of samples, under **Specimen Category**, change passing samples to **Convicted Offender** or **Arrestee** as appropriate. Use **Fill Down** to add more, careful to label the ladders and controls as **No Export**. Double check these designations.
   3) For any samples that were re-processed, remove any added suffixes (e.g. -RL, -RI, -CF, etc) and/or tube numbers in the Sample Name column so that only the specimen ID (SN##### or ####-#####) will be exported.
   4) Select **File > Export Table for CODIS**. Select H:\Forensic Data\DNA\1-CODIS\5 - Database CMF's for Upload as the location to save to. The file name is the same as the GeneMapper ID-X Project name, e.g., “JB-080115-CAL”. Select “CMF 3.2(.xml)” from the “Export File As” drop-down menu. Leave the CODIS Laboratory IDs set to “NV0022632.” **Export**.

4.10 CODIS Import
   1) The CODIS Administrator or alternate will complete CODIS Import and searching. Refer to Chapter 13 of this manual
   2) The CODIS Administrator or alternate will verify that each sample has been second read prior to the upload to SDIS.

4.11 Incomplete Profiles
Database samples with fewer than the complete CODIS core loci cannot be uploaded to NDIS, however these samples are allowed at the LDIS and SDIS levels.

4.12 Databasing Processing Forms
   1) Forms in the Data Packet are: Technical Review Checklist-Database Knowns, Extraction Form(s) (if applicable), Quant Set-up Form (if applicable), Quant Run Printouts (if applicable), Amplification Forms (if applicable), 3500xl Run Forms (if applicable), and sample electropherograms when needed to show low samples, artifacts, OL or tri-allele confirmation, etc.
   2) Forms in the data packet generated outside of the LIMS that do not have an electronic signature (e.g. password-protected username printed on electropherograms) must be initialed by the analyst taking ownership of the packet.
5.0 Title: Real-Time Quantitative PCR

Principle
Quantitation involves measuring the quantity and quality of the DNA extract. Prior to nuclear DNA amplification, forensic samples will be quantified to determine the amount of total human DNA and male DNA present. The Biology/DNA Detail of the LVMPD currently uses the Quantifiler® Trio (Trio) quantitation kit in conjunction with a 7500 Sequence Detection System (SDS) and HID software v1.2 for quantitation. The Trio assay utilizes the “QUANTadillo ” workbook to produce laboratory worksheets, interface with instrumentation/analysis software, and summarize quantitation results. These results summaries are interpreted by the analyst to direct and document the next laboratory processing step(s) prior to amplification and serve as documentation for the case file.

Quality Assurance

Quantitation Documentation
Quantitation run names must include the date of the run and the analyst’s initials as well as the identifier “Trio”, which represents the Quantifiler® Trio chemistry used to quantitate samples (e.g. 072115-CDM-TRIO). There should also be additional unique identifiers (such as Q for question samples or K for known samples and/or additional letters or numbers added to the quantitation run name if more than one run is performed in the same day (e.g. 041312-CDM-TRIO-K, 041312-CDM-TRIO2, etc.).

The unique identifier for the quantitative PCR instrument used for each run must be documented on the associated lab-processing worksheets (e.g. Palms, Hoops, Yoyo, Aria, etc.). In the event an unaltered sample extract is re-quantified, the identifier “RQ” and a sequential number (e.g. RQ1, RQ2, etc.) must be placed at the end of the sample name. Samples which have been concentrated and then subsequently re-quantified are not required to include the “RQ” identifier.

Special Precautions
Diluted bleach (10%) or stabilized bleach solution (e.g., Dispatch) shall be used to wash all exposed work areas. At minimum, decontamination of all exposed work areas should be performed prior to the initiation of DNA quantitation and at the conclusion of the daily analysis process.

Gloves, a mask, and a lab coat must be worn during DNA quantitation. Gloves should be changed frequently when handling and manipulating different samples. Care should be taken by the analyst to continually monitor the integrity of their gloves and to ensure that transfer does not inadvertently occur between samples due to sample manipulation.

Only one reagent tube should be opened at a time. Special attention will be paid to the labeled identity and lot number of each reagent on the physical tube and not the color-coded lids. Expiration dates can be found on the proximal containers.
5.1 Quantitation Set-Up
Quantitation set-up for forensic casework samples or casework reference samples occurs in the main DNA lab or in the PCR room. Quantitation set-up for database samples may take place on the benchtop in the DNA Database lab. Questioned samples may be processed on the same optical plate as known samples during quantitation; however, the questioned samples must always be loaded into the plate prior to loading the known samples and should be physically separated by a row/column of the plate when possible.

Inclusion of Standards in Each Run
Five quantitation standards (known DNA concentration samples) are loaded in duplicate in every Quantifiler® Trio quantitation run.

Refer to the Biology/DNA Quality Manual for quality control testing of new lots of Quantifiler Trio and the quality control of the Quantifiler Trio Standard Curve QC.

Dilution of Extracts Prior to Quantitation
An analyst may wish to create dilutions prior to quantitation to avoid obtaining values above the 50 ng of the standard curve for visibly saturated swabs or priority zero and invoke cases (which have expedited turnaround times).

5.2 Quantitation with Quantifiler® Trio

Background
The Quantifiler® Trio (Trio) DNA quantitation kit simultaneously estimates quantities of both total human DNA and human male DNA in a single, highly sensitive real-time PCR reaction. Trio also incorporates the use of a Degradation Index (DI) in order to estimate the overall quality of the DNA extracts through the use of two separate target sequences – one small autosomal (SM) target, whose amplicon is 80 basepairs in length and one large autosomal (LG) target, whose amplicon is 214 basepairs in length. This kit is used in conjunction with the AB 7500 Real-Time PCR System for Human Identification and the HID v1.2 software. The results from using this integrated quantitation system can aid in determining the following:

- If sufficient human and male DNA is present to proceed with short tandem repeat (STR) analysis.
- How much sample to use in STR analysis applications.
- The potential presence of amplification inhibitors.
- The potential presence of sample degradation.
- Based on the results of quantitation, the following values are targeted in the amplification process if possible. Analysts may choose to target more or less template as appropriate.
  - For Investigator 24plex QS, the target amount of human DNA for analysis is between 750 pg and 3.0 ng based on the quantitation value obtained from the large autosomal target.
  - In databasing, the target amount of DNA for analysis with PowerPlex Fusion 6C is between 2.5 and 5 ng based on validation studies.

QPCR Amplification
Quantitation standards are included in every quantitation run. When portions of DNA extracts are diluted prior to quantitation, the diluent used for the dilution, the lot number, and the volumes of DNA extract and diluent utilized will be recorded and maintained in the case file.
5.2.1 Preparing the Quantifiler® Trio Standard Dilution Series

Background
The dilution series utilizes a stock solution which is used to prepare quantitation standards comprising five samples ranging in concentration from 50 ng/µL to 0.005 ng/µL (5 pg/µL). The lowest concentration sample contains on average 1.5 diploid human genome equivalents.

Required Materials
- Thawed Quantifiler® Trio DNA Standard (store at 2-8°C after initial use)
- Adjustable Pipettes
- Thawed DNA dilution buffer (store at 2-8°C after initial use)
- Sterile aerosol-resistant barrier pipette tips
- Low-bind tubes

Note: DNA quantitation standards can be stored for up to one month at 2-8°C.

Procedure
1) Label five low-bind microcentrifuge tubes: Std. 1 through Std. 5. Label an additional low-bind microcentrifuge tube for the negative control.
2) Dispense the required amount of DNA dilution buffer into each standard tube and the negative control tube (at least 90 µL for the negative control).
3) Vortex the Quantifiler Trio DNA Standards for 3-5 seconds. Using a new pipette tip, add the required amount of Quantifiler Trio DNA Standard to the tube labeled Std. 1 and mix thoroughly. Centrifuge briefly.
4) Using a new pipette tip, add the appropriate amount of the prepared standard from the tube labeled Std. 1 to the tube labeled Std. 2 and mix thoroughly. Centrifuge briefly.
5) Repeat Step 4 for the tubes labeled Std. 3-5. See chart below.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Conc. ng/µL</th>
<th>Standard Dilution Amount</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std. 1</td>
<td>50,000</td>
<td>50 µL DNA Standard Stock + 50 µL DNA Dilution Buffer</td>
<td>2X</td>
</tr>
<tr>
<td>Std. 2</td>
<td>5,000</td>
<td>10 µL (Std. 1) + 90 µL DNA Dilution Buffer</td>
<td>10X</td>
</tr>
<tr>
<td>Std. 3</td>
<td>0.500</td>
<td>10 µL (Std. 2) + 90 µL DNA Dilution Buffer</td>
<td>10X</td>
</tr>
<tr>
<td>Std. 4</td>
<td>0.050</td>
<td>10 µL (Std. 3) + 90 µL DNA Dilution Buffer</td>
<td>10X</td>
</tr>
<tr>
<td>Std. 5</td>
<td>0.005</td>
<td>10 µL (Std. 4) + 90 µL DNA Dilution Buffer</td>
<td>10X</td>
</tr>
</tbody>
</table>

Note: The standard dilution amount may be increased or decreased depending on need; however, the relative proportions of DNA solution to buffer should remain the same as described above. There should be a minimal input volume of 10 µL of DNA for dilutions.

5.2.2 Preparing the PCR Reactions for Manual Quantitation

Required Materials
- Thawed Quantifiler® Trio Primer Mix (store at 2-8°C after initial use, protected from light)
- Thawed Quantifiler Trio PCR Reaction Mix (store at 2-8°C after initial use, protected from light)
- Microcentrifuge tubes or low-bind microcentrifuge tubes
- 96-well optical plate (do not UV crosslink plate)
• Extracted DNA samples and appropriate dilutions
• DNA quantitation standard dilution series
• Optical Adhesive Cover
• Adhesive Seal Hand Sealer

**Note:** During plate set-up keep the 96-well optical plate in its base without allowing the optical plate to touch the lab bench.

**Procedure**

1) Calculate the volume of each component needed to prepare the desired number of reactions using the following equations:
   - **Quantifiler® Trio Primer Mix** - 8 µL x # of samples including standards and negative controls
   - **Quantifiler® Trio PCR Reaction Mix** - 10 µL x # of samples including standards and negative controls

   **Note:** The export worksheet will automatically include one reaction plus 10% additional overage. If using a multi-channel pipette, include 7-10 additional reaction volumes per run to ensure there is enough PCR mixture remaining in the trough for the final dispensing.

2) Vortex the thawed primer mix tube for approximately 3 seconds, and centrifuge briefly before opening the tube. Gently vortex the thawed reaction mix tube before using.
3) Pipette the required volumes into a microcentrifuge tube or low-bind microcentrifuge tube. Vortex the PCR mix for 3-5 seconds and centrifuge briefly.
4) Dispense 18 µL of the PCR mixture into each reagent well to be used being careful to prevent bubbles.
5) Add 2 µL of sample, standard, or control to the appropriate wells. All standards will be loaded in duplicate.
6) Seal the reaction plate with the Optical Adhesive Cover using the hand sealer.
7) Centrifuge the plate at 3000 rpm for about 20 seconds in a table-top centrifuge, equipped with plate holders, to remove any bubbles. If necessary, lightly tap the plate to remove bubbles in wells.

5.3 **Quantitation Set-up using the Hamilton STARlet Robot**

If frozen, thaw the Quantifiler Trio Reaction Mix and Primer Mix completely, then vortex 3-5 seconds and centrifuge briefly before opening the tubes.

**Note:** Thawing is required only during first use of the kit. After first use, reagents are stored at 2 to 8 °C and, therefore, do not require subsequent thawing. Do not refreeze the reagents.

Ensure the required daily maintenance has been performed. Refer to [Maintenance of Hamilton STARlet Robot](#) for additional information.

1) Open the **Hamilton ID STARlet** icon on the desktop
2) Select the **Quantification** option on the Method Manager screen

3) Select the desired method: Both methods work identically except **Quant Trio** uses a new pipet tip for each addition of master mix and **Quant Trio MMX Tip Reuse** will reuse pipet tips for each addition of master mix.

4) On the **Protocol Options** window ensure that the **Quant Trio radio button** is selected. Next, **Select Input File** by navigating to the desired file within the analyst-specific **Lab Runs** folder on the desktop or **OS (C;) > Lab Runs**. Finally, select the desired number of master mix tubes to be used (one or four). Press **OK**.

**IMPORTANT!** Do not run the robot using a file which is saved on a flash drive. The file must be manually transferred to the **Lab Runs** folder prior to running the robot.

5) Verify that the correct input file has been selected and press **Accept** to continue or **Cancel** and reselect the correct input file.

6) Verify that the number of samples to be quantified (excluding standards) is correct. Press **Yes** to continue. If the number of samples is incorrect, press **No** and verify that the file is set up correctly.

7) The STARlet will indicate how much master mix will be needed for the quantification run. Master mix is prepared ahead of time according to the QUANTadillo workbook.

**IMPORTANT!** If using the four-tube protocol for master mix, a single master mix must be prepared, vortexed thoroughly, and then distributed to four separate tubes. This is to ensure complete homogeneity of the master mix reagents.

8) Ensure that the specific carriers are pulled out onto the auto load tray and that the deck is set up according to the following:
   - Sufficient amount of 50 µl tips on the carrier occupying spaces 1 to 6
   - The five (5) Quantifier Trio standard tubes are set up in order from 50 ng to 0.005 ng in positions 1 to 5 on the tube rack occupying space 7. Perform a vortex and quick spin of each tube of standard prior to placing in the carriers.
   - Extract tubes are placed in order starting in position 6 on the tube rack occupying space 7. The extract tubes will continue in order onto the tube racks in positions 8 and 9 if necessary. Each tube rack can hold a total of 32 samples. Perform a vortex and spin of each extract tube prior to placing in the carriers.

**IMPORTANT!** Do not skip any positions while placing tubes in the rack.

- A 96-well quantification plate is placed in the spot 1 on the carrier occupying spaces 13 to 18. Orient the plate so that well position A1 is in the upper left corner.
Perform a vortex and quick spin of the master mix tube(s). The master mix tube(s) are placed according to the diagram in spot 5 on the same carrier. See diagram for where to place tube(s) depending on the number of master mix tubes used. 

**Note:** The other carriers on the deck will not be used and should not be pulled forward onto the auto load tray.

\[\text{Diagram showing master mix tube placement}\]

9) Though the previous step instructs how to set up the entire deck, continue through the prompts given by the STARlet to complete the setup. Ensure the tube racks are set up appropriately and press **OK** to continue.

10) The Load Labware screen will prompt to verify how many 50 µl tips are on the deck and the exact position of those tips on the deck. This can be manually adjusted by clicking on or off of each well or by drag-selecting the entire set of 96 samples. Press **OK** to continue.

11) The method will now proceed. All carriers will be automatically pulled onto the robot deck. Close the cover. When the run is complete the **Protocol Complete** window will appear. Press **OK**.

12) After unloading the deck, return to the **Quantification** option on the Method Manager screen and select **Quant Carrier Reload** to reload all used carriers back onto the robot.

13) Seal the reaction plate with the Optical Adhesive Cover using the hand sealer.

14) Centrifuge the plate for 3000 rpm for about 20 seconds in a table-top centrifuge to remove any bubbles. If necessary, lightly tap the plate to remove bubbles in wells.
Common Errors Encountered on the Hamilton STARlet Robot
The following table is meant as a quick reference guide to aid with troubleshooting which may occur while operating the Hamilton STARlet Robot:

<table>
<thead>
<tr>
<th>Observation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No Tip Error:</strong></td>
<td>Choose <strong>Repeat</strong> if tips have been placed in that location, or <strong>Next</strong> to try to pick up a tip at the next location.</td>
</tr>
<tr>
<td>occurs if a channel attempts to pick up a tip and there is no tip in that location. This most often occurs when the tip counter dialogue does not match the actual tips on the deck.</td>
<td>In the event there are multiple empty locations in the tip rack prior to the next useable tip, you will need to click <strong>Next</strong> until the robot is able to locate the next useable tip.</td>
</tr>
</tbody>
</table>

**Insufficient Liquid Error:**
This error occurs when the instrument performs a calculation for the amount of liquid that should be between the bottom of the tip and the bottom of the tube or well, and that calculated volume is less than the amount being aspirated.

The calculation does not include any additional volume in the tube or well, only that above the bottom of the tip. This is greatly influenced by the defined geometry of the tube or well, which is often not entirely accurate.

Choose **Bottom** in order for the tip to go to the bottom of the tube or well and aspirate the specified amount.

In the event there is not enough liquid in the tube or well, the robot will aspirate air to make up the full volume it is trying to obtain. Therefore, if there is a concern that the tube or well truly does not contain enough liquid, it is advisable to verify this prior to selecting the Bottom function.
<table>
<thead>
<tr>
<th>Error Type</th>
<th>Description</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Level Error</td>
<td>No liquid was detected in the tube or well. Possible causes are that the liquid is non-conductive, the plate or well plastic is too thick to allow for conductivity, or there is very little or no liquid in the tube or well.</td>
<td>If certain liquid is present, select <strong>Bottom</strong>. In the event there is not enough liquid in the tube or well, the robot will aspirate air to make up the full volume it is trying to obtain. Therefore, if there is a concern that the tube or well truly does not contain enough liquid, it is advisable to verify this prior to selecting the Bottom function.</td>
</tr>
<tr>
<td>Carrier Already Loaded</td>
<td>The Autoload component is trying to load a carrier but detects that there is already one in that location.</td>
<td>If the carrier in that location is correct, then click <strong>Yes</strong> and it will continue. If it is not, then click <strong>No</strong> which will likely cause you to need to restart the method.</td>
</tr>
<tr>
<td>Carrier Not Detected</td>
<td>The Carrier is not pushed up to the stops on the Load Tray so it cannot be detected by the Autoload.</td>
<td>Gently push the carrier forward until it is resting against the stops and click <strong>Repeat</strong>.</td>
</tr>
</tbody>
</table>
5.4 7500 HID v1.2 Instrument Operation

Creating a Plate Record

Note: Instructions for setting up a plate record using the QUANTadillo workbook is described Appendix B. Instructions for setting up a plate record using a desktop computer is described in Chapter 3 of the “HID Real-Time PCR Analysis Software Version 1.2” user guide.

Note: A plate record can be set-up on the instrument laptop computer or a desktop computer that has the 7500 HID software loaded on it.

5.4.1 Using the Instrument Laptop Computer to Import from the QUANTadillo Workbook

1) Ensure the power is on for the instrument laptop computer and the 7500 SDS instrument. Launch the 7500 HID software from the laptop computer. When prompted, enter the logon ID “DNA” and click ok.

2) Select the Quantifiler® Trio icon from the home page. Alternatively, click on File>New Experiment>New Quantifiler Trio Experiment. The default settings in the Assays tab (on the toolbar) should be default checked to Quantifiler Assay.

Note: The default setting in the Analysis tab (on the toolbar)>Analysis settings>C\T Settings for Threshold, Baseline Start, and Baseline End should be (respectively):

- T. IPC: 0.1, 3, 15
- T. Large Autosomal: 0.2, 3, 15
- T. Small Autosomal: 0.2, 3, 15
- T.Y: 0.2, 3, 15

If everything is correct click cancel.

The default setting in the Tools tab (on the toolbar)>Preferences >Defaults for sample reaction volume should be 20 µL. If everything is correct click cancel.

3) Enter the experiment name (the same name on the QUANTadillo workbook’s plate map) in the appropriate box, click Plate Setup under the Experiment Menu section in the left hand pane.

4) To import a plate set-up which was exported directly to the instrument from QUANTadillo or that was exported to an external thumb drive, select File>Import> and then browse for your plate record and choose import.

5) To save the document, select File>Save As.
   a. If using the instrument laptop computer, save the file under: AB SW&DATA(D) \DNA\Lab Runs\Analyst. Name the file with a minimum of the date and analyst initials in the filename.

5.4.2 Using the Instrument Laptop Computer to Manually Set-up a QPCR plate

1) Follow Steps 1 through 3 of Section 5.4.1

2) Select Define Targets and Samples Tab

3) To assign a sample to the experiment, click Add New Sample and type in the name of the sample. Check that the correct sample type is chosen (Standard or Unknown). Negative controls assigned to the plate should have the sample type listed as Unknown.

4) Repeat Step 3 for remaining samples

5) If there will be any empty wells on the plate, assign a generic sample name to them (e.g. “sample”). This should be done in the event a sample is mis-loaded during plate set-up to

Uncontrolled Copy if not located in Qualtrax  Page 84 of 350
ensure data is collected from that well during the run.

**Note:** If a well does not have any detectors assigned to it at this Step no quantitation data will be collected during the run.

**Note:** If a standard is accidentally removed/unchecked from a well, no matter which well it is assigned to (even if the same one), the quantities (under the Assign target(s) to the selected wells section) must be re-entered for that standard.

6) Select Assign Targets and Samples tab
7) To assign a sample to the plate, highlight the well the sample needs to be added to and select the appropriate check box from the Assign sample(s) to the selected wells window. Scroll down to find the appropriate sample name you entered in Step 3. Repeat for the remaining samples.
8) To save the document, select **File>Save As**.
   a. If using the instrument laptop computer, save the file under: AB SW&DATA(D) \DNA\Lab Runs\Analyst. Name the file with a minimum of the date and analyst initials in the filename. The file can then be copied onto a removable thumb drive to transfer onto the desktop computer.

### 5.4.3 Using a Desktop Computer to Manually Set-up a QPCR plate

1) When manually setting up a plate using a desktop computer, launch the HID software from the virtual desktop portal, choose the appropriate log-on user and click **OK**. Click **Continue without Connection** when an Instrument Connection error window pops up.
2) Select the Quantifiler Trio icon from the home page. Alternatively, click on **File>New Experiment>New Quantifiler Trio Experiment**.
3) Repeat Step 3 from **Section 5.4.1**, and Steps 1 through 7 from **Section 5.4.2**
4) Highlight the entire plate under View Plate Layout tab.
5) Choose Export (on the toolbar). Under the Export Properties tab check the Sample Setup box only. Under the Customize Export tab check the All Sample Setup Fields box.
6) Choose the location the exported file will be saved, the name for the export file, and change the file type to * .txt (Export Properties tab). Click Start Export when complete.
7) Open the newly exported .txt file using Excel. Highlight row 7 and delete. Save as to a removable thumb drive.
8) Repeat Step 2 from **Section 5.4.1** on the instrument computer using the logon “DNA”
9) Select file>Import>Browse and choose the file that corresponds to the plate map that is to be run. Click Start Import then option yes if you are ready to import. Click ok once the message box appears stating the plate setup import was successful.
10) Repeat Step 3 from **Section 5.4.1** and ensure the plate map that was imported is correct. Then repeat Step 5 from **Section 5.4.1** once the plate is ready to run.

### 5.5 Running a Plate on the 7500 Instrument

1) Push the tray door to open it and load the plate into the plate holder. Ensure the plate is properly aligned in the holder.
2) Close the tray door. Apply pressure to the right side of the tray door at an angle.
3) In the HID software, open the experiment that you set up for the run if not already open.
4) Select the Run or Setup tab in the left hand pane>Run Method
5) Verify thermal profile for Quantifiler Trio (should all be default setup based on selecting Quantifiler Trio icon from the homepage)
Stage 1: Reps: 1  95.0 for 2:00
Stage 2: Reps: 40  95.0 for 0:09  60.0 for 0:30

6) Click **Start Run**. The run time is approximately one hour.

5.6 Data Analysis in HID v1.2

5.6.1 Saving the Analyzed Plate Document
It is important that the analyzed plate document be saved prior to turning off the instrument. Transfer the saved run file to a removable thumb drive if performing data analysis on a desktop computer.

**Note:** Analysis can be performed on a desktop computer once the run has been saved. To use a desktop computer, open the HID software, choose the appropriate logon and click ok. Choose Continue without Connection when the instrument connection error message appears. Choose File>Open and select the file you wish to analyze (.eds file).

1) Select **Analysis Settings** (on the toolbar) to open the Analysis Settings dialog box.
2) Verify that the settings are as described in section 5.4.1
3) Highlight all cells that are to be omitted from the analyzed plate. Right click>omit>well.
4) Select **Analyze** or click the green icon at the top of the screen.
5) To save the document, select **File>Save As**. Save the document to the instrument computer: AB SW&DATA(D)\DNA\Lab Runs\Analyst. Click **Yes** to overwrite when a message window pops up.

5.6.2 Analyzing the Plate Document
Viewing analysis results can involve one or more of the following:

**Viewing the Standard Curve**
1) In the Analysis tab in the left hand pane, select **Standard Curve**.
2) Highlight only the wells containing the standard curve.
3) In the Target drop-down list, select the Target that you wish to view. Each target can be chosen separate of the others or all three can be shown at the same time. Use the up/down arrows (below the standard curve plot) to scroll through the standard curve data when all three are selected at the same time.
4) View the $C_T$ values for the quantitation standard reactions and the calculated regression line, slope, Y-intercept, and $R^2$ values for each of the three targets.
5) To omit a standard – highlight the well, right click>omit>choose an option (well, T. large autosomal, T. small autosomal, or T.Y) depending on whether the entire standard needs to be deleted from all curves or just one. Click “analyze” in order to re-analyze the standard curve with the omitted value.

Note: Only one (1) well/standard value may be rejected as an outlier in each individual target standard curve (T. large autosomal, T. small autosomal, T.Y) in order for the standard curve to be deemed passing.

6) To re-include a standard which was previously omitted – highlight the well, right click>include>choose an option (well, T. large autosomal, T. small autosomal, or T.Y) depending on whether the entire standard needs to be re-included for all curves or just one. Click “analyze” in order to re-analyze the standard curve with the included value.

Viewing the Amplification Plot

The amplification plot can display one of the following:

1) Plot of change in normalized reporter signal ($\Delta R_n$) versus cycle number for each reaction.
2) Plot of normalized reporter signal ($R_n$) versus cycle number for each reaction.
3) C$_T$ versus well position on the assay plate.
   a. In the Analysis tab in the left hand pane, select Amplification Plot. Choose the Plot Type, Graph Type, and Plot Color.
   b. Select the appropriate sample(s) in the View Plate Layout tab.

Viewing the Results

1) In the analyzed plate document, select the View Well Table tab.
2) For analyst preference for ease of viewing results on the instrument: click the Show in Table tab and choose which items to be shown or not shown in the table. Click the Group By tab and choose a view.

5.6.3 Printing and Exporting the Results

Print Standard Curve (two options)

Option 1
1) In the View Plate Layout tab within the Analysis tab (left hand pane), select the wells that need to be printed, to include standards, negative controls, and/or samples.

Note: Only the standard curves must be printed from the HID software for the case file. The printing of the negative controls and/or samples is optional.

2) Select Print Report in the top toolbar.
3) Select only Standard Curves (and Results table (By Well) if you would also like to print the sample results). Click Print Report. This .pdf will provide a print out of all standard curves (as well as data for each sample selected).

Note: Only selected wells will be included in the Print Report. Any wells that have been selected which do not contain any detectors cannot be printed using this function and an error box will appear.
Note: The standard curves for only the T. large autosomal, T. small autosomal, and T.Y targets must be included in the case file for casework. At minimum, the T. large autosomal standard curve must be included in the case file for databasing. The HID software also prints a blank standard curve for the IPC, however this may be discarded.

Option 2
1) In the View Plate Layout within the Analysis tab (left hand pane), select only the wells containing the standard curve
2) Click on the icon in the top of the window that has four colored squares and four lines
3) Click on the icon with a pen and four lines to input the name of the plate to show on the standard curve page
4) Click on the print icon to print your standard curve. You can also choose Print to Cute PDF Write to save the file as a .pdf on your H:drive. This setting will allow all three standard curves needed for the file and the plate name to appear on one page for the case file.

Export Results
1) In the View Plate Layout tab within the Analysis tab (left hand pane), highlight the entire plate.
2) To export, select export in the top toolbar and check the box for Results, within the Export Properties tab.
3) Modify the Export file name as needed (should be the quantitation plate name).
4) Choose the location for which the file is to be exported to. This must be done every time a file is to be exported.
5) File type should be .xls
6) Select The Customize Export tab and select the following boxes in this order: Well, Sample Name, Target Name, Quantity, C_T, Degradation Index and Task. Confirm the orientation matches the screen shot below. All samples should be in well order (by row).
7) Return to the Export Properties tab and click Start Export once all fields have been appropriately filled out.

5.7 Interpretation of Quantifiler® Trio Results

About the Standard Curve Results
The standard curve is a graph of the $C_T$ of quantitation standard reactions plotted against the starting quantity of the standards. The software calculates the regression line by calculating the best fit with the quantification standard data points. The regression line formula has the form: $C_T = m \log(Qty) + b$

Where $m$ is the slope, $b$ is the Y-intercept, and Qty is the starting DNA quantity. The values associated with the regression analysis can be interpreted as follows:

$R^2$ value - Measure of the closeness of fit between the standard curve regression line and the individual $C_T$ data points of quantitation standard reactions. A value of 1.00 indicates a perfect fit between the regression line and the data points.

Regression coefficients:
Slope - Indicates the PCR amplification efficiency for the assay. A slope of -3.3 indicates 100% amplification efficiency.

The acceptable range is the following for each target:

<table>
<thead>
<tr>
<th>Target</th>
<th>Small Autosomal</th>
<th>Large Autosomal</th>
<th>Y-Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>-3.0 to -3.6</td>
<td>-3.1 to -3.7</td>
<td>-3.0 to -3.6</td>
</tr>
</tbody>
</table>

If the standard curve in any of these targets has a value outside the acceptable range, use caution when interpreting results. However, it is not necessary to repeat the quantitation procedure. The analyst will circle the slope value to indicate they noticed the value was out of target range.

Y-intercept: Indicates the expected $C_T$ value for a sample with Qty = 1 (for example, 1 ng/µL). Note: The Y-intercept for the large autosomal target is typically lower than the Y-intercept for the small autosomal target or the Y target. This is due to the higher copy number of the large autosomal target relative to the copy number of the small autosomal and Y targets. The Y-intercept value is evaluated during the quality control testing and monitored as each new standard curve is created to ensure it is not drifting over time. Refer to the quality control criteria in
“Quantitative PCR Kit” in the Biology/DNA Quality Manual for additional information.

**R² Value**

An R² value $\geq 0.99$ indicates a close fit between the standard curve regression line and the individual Cₜ data points of quantitation standard reactions.

If the R² value is $\leq 0.98$ check the following:
1) Quantity values entered for the quantitation standards in the plate document setup.
2) Making of serial dilutions of quantitation standards.
3) Loading of reactions for quantitation standards.
4) Failure of reactions containing quantitation standards.

**Note:** It is permissible to delete one “outlier” from the standard curve and re-analyze the data. Should the R² value still not be $\geq 0.98$, the quantitation results must be discarded and the quantitation procedure for all samples on the affected plate must be repeated.

For additional information on potential problems encountered in interpreting the standard curve see the “Quantifiler HP and Trio DNA Quantification Kits User Guide” under “Troubleshooting the Standard Curve”, Chapter 5.

**Using the Internal PCR Control System**

Acceptable IPC Cₜ values for each sample should be between 25.0 and 30.0. Quantitation values with elevated IPC values may indicate that inhibition is present in the sample. Such a sample may need to be diluted and re-quantified or cleaned up using QIAamp or a buffer exchange. Use the Internal PCR Control (IPC) system to distinguish between true negative sample results and reactions affected by:
- The presence of PCR inhibitors
- Assay setup
- A chemistry or instrument failure

Select the Analysis tab (left hand pane) and then Amplification Plot tab of the HID software. Select Plot Type: ΔRn vs Cycle, Graph Type: Log, and Plot Color: Target to observe amplification of the VIC dye (Human Target, small autosomal), ABY dye (Human Target, large autosomal), and FAM (Human Male Target), and the JUN dye (IPC detector). Guidelines for the interpretation of the results are as follows:

<table>
<thead>
<tr>
<th>Quantifier Trio (VIC, ABY, and/or FAM)</th>
<th>IPC (JUN)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No amplification</td>
<td>Amplification</td>
<td>True negative</td>
</tr>
<tr>
<td>No amplification</td>
<td>No amplification</td>
<td>Invalid result</td>
</tr>
<tr>
<td>Amplification, Quantity &gt;5 ng/µL</td>
<td>Amplification appears reduced relative to the average IPC Cₜ value for standards</td>
<td>High sample concentration may contribute to suppression of IPC amplification. This may occur independently or in combination with PCR inhibitors yielding inconclusive IPC results</td>
</tr>
</tbody>
</table>
Quantifiler Trio (VIC, ABY, and/or FAM) | IPC (JUN) | Interpretation
---|---|---
Suppressed Amplification (high C<sub>T</sub> and low ΔR<sub>n</sub>) | No amplification | Possible Inhibitor present

Note: Positive amplification is when the C<sub>T</sub> value for the target is <40. Because samples contain unknown amounts of DNA and inhibitors, a large range of C<sub>T</sub> values is possible. Because the IPC system template DNA is added to the reaction at a fixed concentration, the IPC C<sub>T</sub> should be relatively constant across all the DNA standards.

Evaluation of the IPC:
1) Determining the Normal Range for IPC
To determine the normal range of C<sub>T</sub> values for the IPC, view the JUN dye signal for the quantitation standards under the Analysis tab (left hand pane) in the View Well Table Tab. If the assays were set up properly and the buffer used to dilute the quantitation standards was free of PCR inhibitors, the reactions should show normal IPC amplification across the range of input DNA.

2) True Negative Result
VIC (small autosomal), ABY (large autosomal), and/or FAM (male) dye signal not detected in conjunction with a normal IPC signal.

3) Invalid IPC Results: JUN dye (IPC target) amplification does not occur (C<sub>T</sub> JUN should be consistent with that of the DNA standards)
If the human-specific target, male-specific target, and the IPC target fail to amplify, it is not possible to distinguish between the absence of DNA and inhibition. The sample quantitation must be repeated. This failure to amplify can be observed in the Analysis tab (left hand pane) under the View Well Plate tab or the Amplification Plot tab.

4) Disregarding IPC Results
With high concentrations of human genomic DNA (>5 ng/µL), competition between the human and/or male-specific and IPC PCR reactions may suppress IPC amplification for that sample. In these cases, samples may be diluted and re-quanted in order to confirm a lack of inhibition.

5) Partial PCR Inhibition
Weak amplification (high C<sub>T</sub> and low ΔR<sub>n</sub> value) of the human and/or male-specific targets and no amplification of the IPC may indicate partial or complete PCR inhibition.

Note: For additional information on potential problems when evaluating IPC amplification refer to the Chapter 5 of the “Quantifiler HP and Trio DNA Quantification Kits User Guide” under “Troubleshoot amplification plots”

Inhibition
If the IPC amplification for certain samples appear reduced relative to IPC amplification for quantitation standards, the decreased IPC amplification may be interpreted as partial PCR inhibition.

If the IPC C<sub>T</sub> for a sample is greater than 0.5 cycle from the average IPC C<sub>T</sub> values for Standards 2-5 and the negative controls, this may indicate PCR inhibition.

- All samples which demonstrate the possibility for inhibition after quantitation (elevated IPC C<sub>T</sub> or Undetected IPC C<sub>T</sub> flag) must have an individual note included in the Comments column of the QUANTadillo worksheet which acknowledges the elevated IPC
CT value and the sample-specific action that will be taken in an attempt to overcome the inhibition noted

**Note:** It is not permissible to include a single comment at the worksheet level such as “dilution expected to overcome inhibition” to address all samples at one time. This comment must be included with each individual sample to ensure they are being singularly evaluated and addressed

- All samples which **exhibit an Undetected IPC CT flag** must be re-quantified or subjected to Qiagen clean-up or microcon buffer exchange and then re-quantified in an attempt to cleanse the sample of any inhibitor that may be present. It is not permissible to continue to amplification without these steps. In the event a sample needs to undergo Qiagen clean-up or microcon buffer exchange, the associated reagent blank(s) must undergo the same cleansing procedure as well

- All samples which **exhibit an elevated IPC CT flag that require concentration** must be subjected to Qiagen clean-up or microcon buffer exchange and then re-quantified in an attempt to cleanse the sample of any inhibitor that may be present. It is not permissible to continue to amplification without these steps. In the event a sample needs to undergo Qiagen clean-up or microcon buffer exchange, the associated reagent blank(s) must undergo the same cleansing procedure as well

- Samples which **exhibit an elevated IPC CT flag that will be diluted/normalized prior to amplification or where neat extract will be used in a well dilution** may continue to amplification with the acknowledgement that based on the dilution factor being employed, it is expected to overcome the inhibition present. **IMPORTANT!** The IPC CT value must be evaluated to determine the extent of the inhibition present. In the event it exhibits 3-5 cycles over the average, these samples should be cleaned-up and re-quantitated prior to amplification due to the potential for gross inhibition

**Degradation**

Refers to the data observed when a sample displays a decrease in measured amount for large DNA fragments compared to small DNA fragments. The Degradation Index (DI) is used as a general indicator of whether large DNA fragments may perform poorly relative to small DNA fragments in STR reactions. The DI is calculated by dividing the small autosomal target DNA concentration (ng/µL) by the large autosomal target DNA concentration (ng/µL).

- DI can be affected by both degraded and inhibited DNA.
- QIAGEN Investigator 24plex QS samples that demonstrate a DI greater than 1.5 can be considered to be degraded and may not fully amplify with STR amplification. STR amplification may need to be based on the small autosomal target instead of the large autosomal target depending on both the amount of DNA present in the sample and the degree of degradation.
- Samples which are degraded can be subjected to concentration in order to maximize the template/µL available for amplification. Refer to [Concentration of DNA Extracts](#).

**Determining the Quality Index of a Sample**

- If the IPC CT is not flagged as possibly inhibited and the DI < 1.5 then there is no indication of degradation or inhibition present in the sample
If the IPC $C_T$ is not flagged as possibly inhibited and the DI is > 1.5 then there is an indication of slight to moderate degradation present in the sample. While inhibition is possible, it is not likely.

If the IPC $C_T$ is not flagged as possibly inhibited and the DI is > 3.5 then there is an indication of significant degradation present in the sample. While inhibition is possible, it is not likely.

If the IPC $C_T$ is flagged as possibly inhibited and the DI >1.5 or blank then there is an indication of degradation and/or inhibitors present in the sample.

Evaluating the Reagent Blanks

- Check the reagent blank values. If the small autosomal target yields a quant result > 0.0005 ng/µl, the quantitation may be repeated if sufficient volume is available. A quantitation value should not be obtained for the large autosomal target or the Y-target in the absence of a quantitation value for the small autosomal target for true DNA contamination and therefore instances in which this occurs may be ignored.

  **Note:** All reagent blanks are amplified at target max regardless of quantitation value if the associated sample extracts will be amplified. It is therefore not necessary to troubleshoot the quantitation value ahead of amplification. In the event the associated sample extracts will not be amplified, it is necessary to either repeat the quantitation or amplify the reagent blank at target max in order to troubleshoot.

- In the event the re-quantitation still exhibits values >0.0005 ng/µl in the small autosomal target, the reagent blank will be amplified at target max in an effort to determine the source of the possible contamination.

- If the resultant DNA profile is clean, the analyst must place a note on the electropherogram or on the original quantitation forms documenting that the reagent blank is clean despite having a quant value >0.0005 ng/µl.

- If a Reagent Blank exhibits an elevated IPC result of > 1 cycle from the AVG IPC target, then the quantitation results are considered to be invalid, possibly due to the presence of inhibition. The Reagent Blank must be diluted and re-quantitated in order to assess the presence of inhibition. If the re-quantitation results confirm the lack of inhibition, both the dilution and the neat Reagent Blank must be amplified at maximum template input.

  **Note:** If the Reagent Blank is associated with any samples requiring Qiagen clean-up or microcon buffer exchange, then the Reagent Blank does not require dilution and re-quantitation.

- If a Reagent Blank exhibits an undetermined IPC result and an evaluation of the raw data shows that the JUN dye did not amplify, then the quantitation results are considered to be invalid, possibly due to the presence of inhibition. The Reagent Blank may undergo a Qiagen clean-up or microcon buffer exchange in an attempt to cleanse any inhibition present or will be diluted and re-quantitated in order to assess the presence of inhibition. If the re-quantitation results confirm the lack of inhibition, both the dilution and the neat Reagent Blank must be amplified at maximum template input.

  **Note:** If the Reagent Blank is associated with any samples requiring Qiagen clean-up or microcon buffer exchange, then the Reagent Blank does not require dilution and re-quantitation.
5.8 Non-Sexual Assault Evidence – Dropping Low or No Quantitation Samples Prior to Amplification

Samples which are low template will be subjected to concentration in order to maximize the template/µl available for amplification. Refer to Concentration of DNA Extracts.

After concentration, samples which will achieve a total estimated amplification input of less than approximately 0.035 ng (i.e., 35 pg) of DNA for both the small and large autosomal targets may be terminated and not moved forward for autosomal STR analysis.

IMPORTANT! All swabbings from cartridge casings will be moved forward for DNA analysis regardless of final estimated quantitation value. These samples will not be terminated.

The decision to terminate after quantitation must be clearly notated in the case file. Samples in which all quantitation targets are Undet. will be evaluated on a sample-by-sample basis in order to determine whether the Undet. quantitation value is unexpected given the original sample type, its physical characteristics (i.e., potential good surface area for holding DNA vs. poor surface), the relative condition of the item (e.g., used condition vs. new, freshly collected vs. old, etc.), appearance of sample when collected (e.g., staining present vs. no visible staining), etc.

Collectively, this information will be used to make a decision regarding whether a re-quantitation or additional serological testing will be performed for confirmation of the Undet. result or if the sample may be terminated from further analysis. This evaluation and the decision will be documented in the case file.

The DNA Technical Leader will be consulted when a reagent blank exhibits any quantitation value other than Undet. in the small or Y-targets and all associated samples are eligible to be terminated after quantitation. Based on the review of this data, a decision will be made whether the reagent blank will be amplified. The results of this consultation will be documented in the case file. Samples which exhibit Undet. values in the small and Y-targets, while exhibiting a quantitation value in the large target may be terminated without consultation with the DNA Technical Leader.

5.9 Sexual Assault Cases – Dropping Low or No Quantitation Samples Prior to Amplification

The determination of suitability for autosomal STR amplification of evidence from sexual assault cases which involve at least one female and at least one male assailant will be made based on the results of the quantitation. Cases which involve male-on-male sexual assault, or assaults with female perpetrators are not well characterized by this method.

Samples will be considered for autosomal STR amplification when the total amount of male DNA input is approximately 75 pg or greater when amplifying approximately 2 ng or less of total template. Refer to the Appendix B.5.10 and the Y-screen flow chart for decision-making strategies.

IMPORTANT! Y-STR profiles require direct comparison to a buccal reference standard and are not eligible for CODIS search. Therefore, when utilizing the Y-screen flow chart, samples may be eligible for Y-STR analysis only when a suspect reference standard is available for direct comparison.

Samples in which all quantitation targets are Undet. will be evaluated on a sample-by-sample basis in order to determine whether the Undet. quantitation value is unexpected given the sample.
type, appearance of sample when collected (e.g., staining present vs. no visible staining), etc. Collectively, this information will be used to make a decision regarding whether a re-quantitation or additional serological testing will be performed for confirmation of the Undet. result or if the sample may be terminated from further analysis. This evaluation and the decision will be documented in the case file.

The DNA Technical Leader will be consulted when a reagent blank exhibits any quantitation value other than Undet. in the small or Y-targets and all associated samples are eligible to be terminated after quantitation. Based on the review of this data, a decision will be made whether the reagent blank will be amplified. The results of this consultation will be documented in the case file. Samples which exhibit Undet. values in the small and Y-targets, while exhibiting a quantitation value in the large target may be terminated without consultation with the DNA Technical Leader.

In the event a sample is differentially extracted and one fraction is selected to be moved forward with autosomal STR amplification, the opposing fraction will also be amplified (e.g., the SF fraction is selected based on quantitation results, the EF fraction will also be amplified).

IMPORTANT! Consideration must always be given to the type of evidence submitted and the potential probative nature based on the case scenario. At times, the presence of female DNA is probative, and it is therefore not appropriate to terminate sample analysis even when male DNA is not detected (e.g., penile swabs, condoms, etc.).

5.10 Processing Reference Standards When All Evidence Samples Dropped After Quantitation

In the event all evidence samples are determined to be unsuitable for autosomal STR amplification following quantitation, the processing of the associated reference standards and singularly-associated reagent blank(s) will also be terminated at that time. Volunteer buccal swabs which are CODIS-eligible will be amplified.

Extracts from reference standards will be retained. These extracts may be amplified at a later time in the event additional evidence is requested and processed. Refer to Appendix D for information related to the reporting of reference standard extracts after being originally terminated.

Reagent blanks which are associated with reference standards from other cases requiring amplification will be amplified. It is not necessary to include the reagent blank data in the negative case file unless it is found to be contaminated. In such instances, the data will be placed in the LIMS object repository of all associated reference standards. In the event the terminated references are required for comparison with a supplemental request, the reference standards will be re-extracted as the original (terminated) data is not reportable.
6.0 Title: PCR Amplification

Principle
The amplification process involved in Short Tandem Repeat (STR) analysis makes millions of copies of specific areas on the non-coding region of the DNA strand where small portions of the DNA are repeated multiple times, one right after another. Amplification is a necessary step in DNA analysis to generate enough DNA so that it may be later visualized. In order for a DNA profile to be submitted to the National DNA Index System (NDIS) of CODIS there must be at least 13 core areas identified for convicted offenders and felony arrestees and at least 8 core loci for forensic samples. The amplification run itself does not produce resultant data, but rather is used to prepare for the electrophoresis step which allows the visualization of the DNA profile.

Quality Assurance

PCR Set-up Lab (Pre-Amp PCR Lab)
This lab is used for amplification procedures. All casework STR amplification work is conducted under the hoods. Quantitation procedures may also be conducted in this lab. A variety of equipment and supplies are used including but not limited to:
- Adjustable pipettes
- Sterile aerosol resistant barrier pipette tips
- Microcentrifuge tube and plate racks
- Disposable gloves, latex and nitrile
- Microcentrifuge tubes
- Microcentrifuge(s)
- Vortex mixer(s)
- Lab coat (white pre-amp)
- Refrigerator(s)
- 96-well plates
- Optical caps
- Reservoirs

Special Precautions for Pre-Amp PCR Lab
1) Hoods may be exposed to UV light for 30 minutes before PCR set-up and must be exposed for 30 minutes after PCR set-up.
2) Consumables may be UV cross-linked as described in Biology/DNA Quality Manual.
3) Change pipette tips after each sample or control.
4) Only one reagent tube should be opened at a time. Special attention will be paid to the labeled identity, lot number, and expiration date of each reagent on the physical tube and not the color-coded lids.
5) Diluted bleach (10%) or stabilized bleach solution (e.g., Dispatch) shall be used to decontaminate all work areas. At minimum, decontamination of all exposed work areas should

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be performed prior to the initiation of DNA amplification and at the conclusion of the daily analysis process.

6) Gloves, a mask, and a lab coat must be worn during DNA amplification. Gloves should be changed frequently when handling and manipulating different samples. Care should be taken by the analyst to continually monitor the integrity of their gloves and to ensure that transfer does not inadvertently occur between samples due to sample manipulation.

7) Remove white lab coat before entering amplified DNA work area. Do not re-enter the pre-amp work areas.

**Investigator 24plex QS:** Store tubes of positive control (control DNA 9948), primers, and master mix in the refrigerator in the casework pre-amp lab when in use. Store tubes not yet in use in the freezer in the casework main lab.

**Amplified DNA Work Area (Post-Amp PCR Lab)**

This work area is a physically separate room used only for those activities that involve the handling of amplified DNA. This includes DNA quantitation, DNA amplification, electrophoresis of amplified DNA, waste disposal of amplified DNA solutions and storage of amplified DNA. A variety of equipment and supplies are used including but not limited to:

- Thermal Cyclers (9700s)
- Adjustable pipettes
- Latex/nitrile disposable gloves
- Microcentrifuge tube racks
- Sterile, aerosol-resistant barrier pipette tips
- Genetic Analyzer/CE and supplies
- Refrigerator and freezers
- Lab coat (blue or disposable post-amp)
- Real-time PCR Quantitation systems
- Plate centrifuge

**Special Precautions for Post-Amp PCR Lab**

1) Always remove gloves when leaving the Amplified DNA Work Area to avoid the transfer of amplified DNA into other work areas.

2) Wear only blue or disposable post-amp lab coat while working in amplified DNA work area.

3) Reduce the unnecessary dispersal of DNA around the work area by changing and/or bleaching gloves as needed, particularly if they may have become contaminated with amplified DNA.

4) 10% Diluted bleach or a stabilized bleach solution (e.g., Dispatch) shall be used to wash the exposed work areas.

5) Use the thermal cycler only for amplification.

6) Store tubes and/or trays of amplified DNA in this work area.

7) Store all amplified DNA and associated waste in biohazard bags before removing from the Amplified DNA Work Area through the main hallway.

8) To prevent introduction of amplified DNA into pre-amplification work areas, use one-way passage through the post-amplification work area. Remove coat and gloves. Wash hands. Exit only into the main hallway. Entry into the capillary electrophoresis lab from the main hallway is permissible.

**Investigator 24plex QS:** Freeze tubes of ladder in their foil pouches for long term storage in the casework post-amp lab. Remove the tube of ladder from its foil pouch and refrigerate until consumed in
the casework post-amp lab. Store tubes of BTO size standard in casework post-amp lab freezer.

6.1 Amplification of Reagent Blanks
Reagent blanks are amplified at the maximum concentration conditions (i.e., 15 µL for Investigator 24plex QS) with their associated samples. Reagent blanks are amplified at all loci using the same primers as their associated samples and concurrently in the same instrument. Reagent blanks should be amplified in the same extraction tube number order as their associated samples and in the same batch on the same amplification plate.

It is not necessary to re-amplify the reagent blank when an associated sample is re-amplified provided the following criteria are met:
- The reagent blank produced acceptable results during the first amplification.
- The sample is re-amplified with the same amplification test kit.
- The sample is re-amplified using the same concentration or less as that of the original reagent blank.
- The amplification parameters have not been altered to increase sensitivity.

The new amplification must include an amplification positive and negative control.

Cold Case Reagent Blanks
If a reagent blank cannot be located, the associated samples cannot be re-amplified with a different amplification test kit unless the samples and their associated reagent blank(s) were originally extracted and typed prior to July 1, 2009. While it is not advisable to amplify and type samples using a different technology and/or methodology without an appropriate reagent blank, the Biology/DNA detail recognizes the LVMPD has DNA extracts remaining from older cases where the evidence has been destroyed or all biological material was consumed during the initial extraction process. The pros and cons of such an approach will be discussed with the DNA Technical Leader, the DNA Lab Manager, and the Forensic Lab Director. Approval to proceed will be a last-resort effort to resolve a case. Such approval will be documented by the DNA Technical Leader in the case file prior to amplification.

Depletion of Reagent Blanks
In the event a reagent blank was previously depleted and requires additional processing (e.g., Y-STR analysis), the reagent blank will be reconstituted by adding the same volume of TE or H₂O (whichever utilized during extraction) to both the reagent blank tube and the associated sample(s). Refer to Clean-up and Concentration of Reagent Blanks if the consumption was as a result of being concentrated.

Re-Amplification of Evidence Reagent Blanks Associated with Evidence Samples from Multiple Cases
In the event a reagent blank associated with evidence samples from multiple cases (e.g., RBQ-...) is re-amplified for any reason (e.g., data previously generated in Identifiler Plus and is now being generated in QIAGEN Investigator 24plex QS or being processed for Y-STRs), the resultant data must be placed in the LIMS object repository for each associated case. The data is considered to be associated with forensic samples and therefore must be retained with the case information for all samples originally co-extracted with the blank.
Qiagen Clean-up or Microcon Buffer Exchange of Reagent Blanks
In the event a reagent blank is associated with evidence samples which require a Qiagen clean-up or microcon buffer exchange, the associated reagent blank must also undergo the same clean-up procedure.

6.2 Amplification of Known Reference Standards
Known reference samples may be amplified in the same batch as questioned forensic samples. Questioned forensic samples must be loaded onto the amplification tray prior to the addition of reference standards.

6.3 Amplification of Positive and Negative Controls
A positive and a negative control shall be amplified with each batch. The positive and negative controls shall be amplified concurrently with their associated samples at all loci using the same primers and instrument. The negative control shall be the last reaction added to the plate, regardless of well location.

6.4 Work Product Defined
PCR product is defined as work product, and is stored frozen in the post-PCR laboratory. It may be discarded upon case completion.

6.5 Amplification Run and Sample Naming

Run Names
Assign a run number to each batch from the PCR run log spreadsheet. Run numbers begin with the current year followed by a dash and a sequential batch number, e.g. 18-009. Name the amplification positive control (APC) and amplification negative control (ANC) with this number, e.g. APC 18-009 and ANC 18-009.

Multiple Amplifications and Re-Amplifications
If a sample is amplified multiple times on a plate during the first amplification attempt, the end of the sample will be designated with “AMP” and a sequential number (e.g. Item 1.1.1-AMP1, Item 1.1.1-AMP2). These designations will be retained throughout the duration of processing.

If samples are re-amplified, the end of the sample will be designated with “RA” and a sequential number to indicate “Re-Amplification” (e.g. Item 1.1.1-RA1 or Item 1.1.1-RA2). These designations will be retained throughout the duration of processing. The reason for re-amplification will be included in the case file.

6.6 Amplification Set-up using Qiagen Investigator 24plex QS
The Qiagen Investigator 24plex QS Amplification Kit is a short tandem repeat (STR) multiplex assay that co-amplifies 22 polymorphic STR markers and the Amelogenin gender-determining marker in a single PCR amplification. All twenty of the required CODIS core loci are included in this kit. The loci amplified in this kit are: D1S1656, D2S441, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, CSF1PO, FGA, TH01, TPOX, vWA, SE33, DYS391, and Amelogenin. The kit also contains an internal PCR control (Quality Sensor QS1 and QS2) which provides helpful information about the efficiency of the PCR reaction and the possible presence of PCR inhibitors.
Based upon validation studies for Quantifiler® Trio, the ideal range is 750 pg to 3 ng based on the large autosomal target quantitation value. The Investigator 24plex QS amplification kit accepts up to 15 µL of extract within the amplification reaction. DNA samples may be normalized in molecular grade water or TE to target the desired amount of total DNA input based on the quantitation value obtained. Analysts may target more or less DNA template as case circumstances dictate.

You may calculate dilutions according to V1 x C1 = V2 x C2 where C1 is the concentration obtained from quantitation and C2 is the desired target concentration (total target DNA divided by 10). Select the smallest volume for V1 that will give at least 10 µL for V2. Typically, V1 is 10 µL for casework. Subtract V1 from V2 to calculate the volume of molecular grade water or TE to add to the V1 volume of extract.

Note: The Investigator 24plex amplification kit allows up to 15 µL of extract or normalized extract to be added to the amplification reaction. Therefore 5 µL of molecular grade water or TE must be added to all amplification reactions where the normalizations are made to 10 µL.

Alternatively, you may make your dilution directly in the reaction well. Select 15 µL for V2 and solve for V1. Subtract V1 from V2 to calculate the volume of molecular grade water or TE to add to the well along with the V1 volume of extract.

If your extract is below the target range, you will concentrate it via CentriVap by estimating a concentration factor. This estimated total amplification input will be documented in the case file. Sample extracts must contain ≤ 100 µL of EZ1 TE or ≤ 200 µL of Teknova TE (organic extracts) in order to be concentrated using the CentriVap. Depending on the volume of TE contained in the sample extract, it may be necessary to concentrate a sample extract via Microcon prior to completing the concentration using the CentriVap. Proceed with caution when attempting to concentrate your extract if you have less than 25 µL. Note that the associated reagent blank must be similarly concentrated. Up to 15 µL of concentrated extract may be added to the Investigator 24plex amplification reaction as “Target MAX”.

6.6.1 Manual Amplification Plate Set-up
1) UV- irradiate a 96-well plate, if desired. Label it with the Amp Run Number, the date and your initials.
2) Use the table below to calculate the volume of each component needed to prepare the reaction mix.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast Reaction Mix 2.0</td>
<td>7.5 µL</td>
</tr>
<tr>
<td>Primer Mix</td>
<td>2.5 µL</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>Variable</td>
</tr>
<tr>
<td>Template DNA</td>
<td>Variable</td>
</tr>
<tr>
<td>Total volume DNA</td>
<td>25 µL</td>
</tr>
</tbody>
</table>

Note: Include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers. The AMP and Run Workbook will automatically do this for you. The information from the workbook assumes that a repeat pipettor is used for the
distribution of reaction mix. Add 7-10 volumes of additional master mix if using a multi-channel pipette. Discard excess, unused reaction mix.

3) If frozen, thaw the Investigator 24plex QS Kit Fast Reaction Mix and the Investigator 24plex QS Primer Mix completely, then vortex 3-5 seconds and centrifuge briefly before opening the tubes.

   **Note:** Thawing is required only during first use of the kit. After first use, reagents are stored at 2 to 8 °C and, therefore, do not require subsequent thawing. Do not refreeze the reagents.

4) Pipette the required volumes of components into a microcentrifuge tube.

5) Vortex the reaction mix for approximately 5 seconds, then centrifuge briefly.

6) Dispense 10 μL of the master mix into each reaction well of a 96-well plate that will contain sample, reagent blank, or control.

7) Pipette 10 or 15 μl of normalized sample, concentrated sample, or reagent blank into the corresponding row/column of the 96 well plate. If normalizing directly in the well or if normalizations were performed to 10 μL of input, add sample plus molecular grade water or TE-4 for a total of 15 μL. The final reaction volume (sample, reagent blank, or control plus reaction mix) must be 25 μL.

8) Add 10 μL of control DNA 9948 (approximately 1 ng) to the Amplification Positive Control (APC) well. Add molecular grade water or TE-4 to bring the sample to a total volume of 15 μL.

9) Pipette 15 μL of molecular grade water or TE-4 into the Amplification Negative Control (ANC) well. The ANC shall always be loaded as the last reaction on the plate.

10) Place a strip cap over each column that contains sample and reaction mix. Firmly press down on each cap to ensure the cap is properly seated on the well and there is a tight seal between the cap and the well.

11) When finished in PCR Set-Up, bleach the area and UV irradiate the hood for 30 minutes. Remove white lab coat before entering the post-amp lab.

12) Vortex and briefly centrifuge the plate. Check the wells for consistent volumes.

   **IMPORTANT!** The amplification tray must be vortexed prior to placing it on the thermal cycler. Failure to vortex may be detrimental to the RFU values across the plate due to the heavy salt content associated with the Investigator 24plex QS amplification kit.

13) Turn on the thermal cycler.

14) Select the 28-cycle Investigator 24plex program on the Applied Biosystems 9700 thermal cycler. This kit is validated to perform 28 cycles according to the table below.
15) After the amplification is complete, remove the tray from the thermal cycler and store protected from light. The amplified products can be stored in the CE lab refrigerator for short periods of time. For longer periods, the tray should be frozen. Care should be taken when using amplicons that have been frozen for more than 6 weeks since original amplification. Depending on the quality of the resultant data, it may be necessary to reamplify the samples.

6.6.2 Normalization and Amplification Set-up using the Hamilton STARlet Robot

If frozen, thaw the Investigator 24plex QS Kit Fast Reaction Mix and the Investigator 24plex QS Primer Mix completely, then vortex 3-5 seconds and centrifuge briefly before opening the tubes. 

**Note:** Thawing is required only during first use of the kit. After first use, reagents are stored at 2 to 8 °C and, therefore, do not require subsequent thawing. Do not refreeze the reagents.

Ensure the required daily maintenance has been performed. Refer to Maintenance of Hamilton STARlet Robot for additional information.

Refer to Common Errors Encountered on the Hamilton STARlet Robot for more information and troubleshooting.

1) Open the Hamilton ID STARlet icon on the desktop
2) Select the Normalization & Amplification option on the Method Manager screen
3) Select the desired method: Both methods work identically except Norm_Amp uses a new pipet tip for each addition of master mix and Norm_Amp_MMX_Tip_Reuse will reuse pipet tips for each addition of master mix.

4) On the Amp/Norm Method Inputs window Select Input File by navigating to the desired “Amp & Run Hamilton” workbook in the analyst-specific folder within the Lab Runs folder on the desktop or OS (C:) > Lab Runs. Finally, select the desired number of master mix tubes to be used (one or four). Press Continue.

**IMPORTANT!** Do not run the robot using a file which is saved on a flash drive. The file must be manually transferred to the Lab Runs folder prior to running the robot.

**IMPORTANT!** If using the four-tube protocol for master mix, a single master mix must be prepared, vortexed thoroughly, and then distributed to four separate tubes. This is to ensure complete homogeneity of the master mix reagents.

5) Ensure that the specific carriers are pulled out onto the auto load tray and that the deck is set up according to the following:

- Sufficient amount of 50 µl tips on the carrier occupying spaces 1 to 6
- Extract tubes are placed in order starting in position 1 on the tube rack occupying space 7. The extract tubes will continue in order onto the tube racks in positions 8 and 9 if necessary. Each tube rack can hold a total of 32 samples. Perform a vortex and quick spin of the samples prior to loading in the tube racks.

**IMPORTANT!** Do not skip any positions while placing tubes in the rack.

- Ensure sufficient molecular grade H₂O is in the reagent trough located in spot 5 on the carrier in position 11 to carry out the normalization process and that the lid has been removed.
- A 96-well amplification plate labeled with the Amp ID is placed in the spot 4 on the carrier occupying spaces 13 to 18. Orient the plate so that well position A1 is in the upper left corner.
- Perform a vortex and quick spin of the positive control and master mix tube(s). The positive control tube and master mix tube(s) are placed according to the diagram in spot 5 on the same carrier. See diagram for where to place tube(s) depending on the number of master mix tubes used.
- Sufficient amount of 1000 µl tips in spot 1 on the carrier occupying spaces 19 to 24 and a 96 well deep well plate in spot 4 on the same carrier. Orient the deep well plate so that well position A1 is in the upper left corner.
6) Though the previous step instructs how to set up the entire deck, continue through the prompts given by the STARlet to complete the setup. Ensure the tube racks are set up appropriately and press Continue to proceed.

7) The Edit Tip Count screen will prompt to verify how many 50 µl tips are on the deck and the exact position of those tips on the deck. This can be manually adjusted by clicking on or off of each well or by drag-selecting the entire set of 96 samples. Press OK to continue.

8) The Edit Tip Count screen will prompt to verify how many 1000 µl tips are on the deck and the exact position of those tips on the deck. This can be manually adjusted by clicking on or off of each well or by drag-selecting the entire set of 96 samples. Press OK to continue.

9) The STARlet will indicate how much master mix will be needed for the amplification run. Master mix is prepared ahead of time according to the AMPalatypus & cattyRUNpas workbook.

10) The method will now proceed. All carriers will be automatically pulled onto the robot deck. Close the cover. When the run is complete the Method Complete window will appear. Press OK.

11) After unloading the deck, return to the Normalization & Amplification option on the Method Manager screen and select Norm_Amp Carrier Reload to reload all used carriers back onto the robot.

12) Place a strip cap over each column that contains sample and reaction mix. Firmly press down on each cap to ensure the cap is properly seated on the well and there is a tight seal between the cap and the well.

13) Vortex and briefly centrifuge the plate. Check the wells for consistent volumes. **IMPORTANT!** The amplification tray must be vortexed prior to placing it on the thermal cycler. Failure to vortex may be detrimental to the RFU values across the plate due to the heavy salt content associated with the Investigator 24plex QS amplification kit.

14) Turn on the thermal cycler.

15) Select the 28-cycle Investigator 24plex program on the Applied Biosystems 9700 thermal cycler.
16) After the amplification is complete, remove the tray from the thermal cycler and store protected from light. The amplified products can be stored in the CE lab refrigerator for short periods of time. For longer periods, the tray should be frozen. Care should be taken when using amplicons that have been frozen for more than 6 weeks since original amplification. Depending on the quality of the resultant data, it may be necessary to reamplify the samples.
7.0  Title:  Capillary Electrophoresis

Principle
DNA Electrophoresis is the process which allows for the visualization of the DNA profile by utilizing the amplification product that was generated during the PCR reaction. Allelic ladders, which are a collection of common DNA alleles, are added to the electrophoresis run to be used as standards to identify the unknown alleles contained in the samples. The electrophoresis process separates the DNA by size, which in turn is compared to the ladders to determine the size of the alleles.

The raw data produced through PCR is analyzed using a 3500xl genetic analyzer and GeneMapper ID-X software to produce an electropherogram; which is a graphical representation of the DNA alleles. The run folder and project files produced using GeneMapper ID-X are stored on the instrument or in the analyst folders on the H:drive.

Injection parameters for each 3500xl instrument can be found on each of the 3500xl instruments and in the appropriate validation or performance check notebook. After capillary electrophoresis, injection parameters for each sample can be found in the “Info” tab of the GMID-X project file. The changes in the instrument injection parameters are tracked in a spreadsheet on the H:drive: H:\Forensic Data\DNA\OTHER\Technical Leader\Injection Parameters.

Quality Assurance

Special Precautions
Diluted bleach (10%) or stabilized bleach solution (e.g., Dispatch) shall be used to wash all exposed work areas. At a minimum, decontamination of all exposed work areas should be performed prior to the set-up of DNA electrophoresis plates and at the conclusion of the daily analysis process.

Only one reagent tube should be opened at a time. Special attention will be paid to the labeled identity, lot number, and expiration date of each reagent on the physical tube and not the color-coded lids.

Gloves must be worn during the set-up of DNA electrophoresis plates and should be changed frequently when handling and manipulating different samples. Care should be taken by the analyst to continually monitor the integrity of their gloves and to ensure that transfer does not inadvertently occur between samples during sample manipulation.

7.1  Capillary Electrophoresis Sample and Plate Naming/Information
Genetic analyzer runs must contain the following information: Initials of the examiner, the date the plate is set-up and the abbreviation of the instrument’s name being used (e.g. LMK-081017-HOB).

There should also be additional unique identifiers (such as Q for question samples or K for known samples and/or additional letters or numbers added to the run name if more than one run is performed in the same day (e.g. CDM-041312-K-RIO, CDM-041312-Q-RIO, etc.).
Re-Injection of a Plate
In the event a previously created plate will be re-injected, the plate name will be designated with RJ and a sequential number will be used (e.g. LMK-012917-RJ1-HOB or LMK-012017-RJ2-RIO) to indicate that a “Re-injection” is occurring from the previously loaded/typed plate.

Re-Injection of a Sample
If samples are being re-injected, the end of the sample will be designated with RJ to indicate “Re-injection” and a sequential number (e.g. Item 1.1.1-RJ1 or Item 1.1.1-RJ2). These designations will be retained throughout the duration of processing.

Re-Load of a Sample
If samples are being re-loaded (re-load refers to the creation of a new set of samples from the same amplicon product previously loaded), the end of the sample will be designated with RL to indicate “Re-Load” and a sequential number (e.g. Item 1.1.1-RL1 or Item 1.1.1-RL2). These designations will be retained throughout the duration of processing. The reason the sample is re-loaded will be noted in the case file. The plate containing re-loaded samples must include the initials of the examiner, the date the plate is set-up, and the abbreviation of the instrument’s name being used. In the event more than one run is performed in the same day, the designation -RL may be used as an additional unique identifier, however, is not required. Refer to the requirements for plate naming in Capillary Electrophoresis Sample and Plate Naming/Information above.

When multiple lab numbers are contained in a run, the lab number must be designated on the associated lab worksheets and the electropherogram for each sample.

Typing of Reagent Blanks
Reagent blanks are typed concurrently on the same instrument and are injected at the same injection conditions as their associated samples. Reagent blanks are also typed at the most sensitive volume conditions as their associated samples from extraction.

Note: Assuming the injection/sensitivity requirements are met, the reagent blanks do not have to be re-typed when their associated samples are re-loaded or re-injected.

Internal Size Markers and Allelic Ladders
Internal size standards are included with every sample. At least two ladders must be loaded per plate.

Note: Investigator 24plex data may be collected “by plate” or “by injection”. When selecting the data to be collected “by injection”, at least one ladder must be included within each injection.

7.2 Required Instrument Maintenance for the 3500xl Genetic Analyzers
All maintenance tasks are to be recorded in the individual logbook specific to the instrument each time the maintenance is performed on an instrument. Refer to Appendix C.6 for maintenance instructions.

7.3 Saving Electronic Files
The GeneMapper ID-X files created during analysis of a particular run will be saved by electronic media and are located in the CE run folders and case-specific or batch folders created by the analyst. These electronic media folders will be maintained and stored on the H:drive which are backed up by the Information Technology Bureau (ITB). In addition, CE run folders on the
Instruments are automatically copied to an Instrument Archive folder on the H:drive. Data stored in this folder cannot be modified or deleted because it is write-protected by ITB. However, data contained in this folder may be copied into a different location on the H:drive for data analysis by members of the Biology/DNA Detail.

### 7.4 Using the Genetic Analyzer

#### 7.4.1 Preparing Plate

1. Use the appropriate DNA workbook to create a 3500xl Setup worksheet and to export a 3500xl input .txt file. The workbooks will automatically calculate the necessary reagent quantities for setting up the plate and automatically export the correct 3500xl parameters for electrophoresis.

   **Note:** Investigator 24plex data may be collected “by plate” or “by run”. When selecting the data to be collected “by run”, at least one ladder must be included within each injection.

2. Thaw formamide at room temperature and thaw amplicon plate (if frozen); vortex and then centrifuge amplicon plate briefly to pool all liquid to the bottom of the wells.

3. In a clean microcentrifuge tube, add formamide and size standard according to the setup worksheet, and then distribute this master mix into the appropriate wells of the setup plate according to the setup worksheet.

4. Transfer the amplicons and the allelic ladders into the setup plate as noted on the worksheet.
   - **Investigator 24plex QS master mix formula:** 12 µL Hi-Di formamide and 0.5 µL BTO 550 per sample; pipette 12 µL of master mix into each appropriate well
   - 1 µL of Ladder and 1 µL of sample.
   - **Fusion 6C master mix formula:** 9.5 µL of Hi-Di formamide and 0.5 µL WEN ILS 500 per sample; pipette 10 µL of master mix into each appropriate well
   - 1 µL of Ladder and 1 µL of sample.

5. Cover the plate with a septa and centrifuge the plate briefly to pool all liquid to the bottom of the wells.

6. Denature the setup plate for 3 minutes at approximately 95°C and then chill for 3 minutes using a frozen plate chiller.

7. Assembling the “plate sandwich”
   a. Place the plate in the blue 3500xl base, matching the notched corner orientation.
   b. Place the white retainer on top of assembly.
   c. Line up the retainer holes with the septa holes while pushing downward until the retainer locks into place.
Preparing the 3500xl Instrument

1) **OPTIONAL:** Pre-heat the oven to 60°C. On the Dashboard of the 3500 Series Data Collection Software, set the temperature to 60 (°C). Then click **Start Pre-Heat** button.

2) Open the Maintenance tab and determine if the instrument is due for any maintenance. If it is needed, refer to **Equipment and Instrument Maintenance Appendix**. If it is not needed, proceed to the next step.

3) Select the **Refresh** button to check the status of the consumables on the Dashboard; ensure that they are not expired and that the number of injections remaining in the polymer pouch and buffer levels are adequate.
   a. If new buffer or polymer is needed, refer to the appropriate section in **Appendix C.6**

4) Check the pump assembly for bubbles and run the **Remove Bubbles** wizard if needed.

5) Check for leaks and dried polymer residue around the buffer pin, check valve, and array locking lever. If dried polymer residue is present, remove it with a lab wipe moistened with water.
6) Check the cathode buffer container for condensation. If any is observed, gently tilt the liquid in the container to remove the condensation from the sides.

### 7.4.3 Importing the Plate Record and Mounting Plate

1) On the **Dashboard**, select **Library**, then select **Import**.
2) Navigate to the location where the .txt file is saved. Select the appropriate plate map *.txt file, then select **Open**.
3) With the instrument door closed, push the tray button to bring the autosampler forward. Place any plates to be run on the autosampler with the notched end of the plate base toward the back of the autosampler and the labels facing to the front.
   a. To seat the plate, angle the long-side of the plate assembly that is nearest to the center of the autosampler tray and set that side down first
   b. Snap the plate into place by setting the rest of the plate down onto the autosampler
4) When the door is shut, the autosampler should return to its home position.

### 7.4.4 Link Plate and Verify/Record Injections

1) The plate must be loaded on the autosampler before the plate can be linked. Once the plate is loaded, select the **Workflow** tab and then select **Load Plates for Run** on the left-hand side of the screen
2) In the “Plates on Instrument” section of the main screen, select the **Link Plate** button for the desired plate position (Plate A or Plate B). Locate the desired plate in the Plate Name list and select **Link Plate**

**Note:** You do not need to edit the Run Name at the top of the window.
   a. If there is only one plate to be run, verify the other stacker position is not linked to a plate. If it is, select **Unlink**
   b. To link a second plate if needed, select **Link Plate** for the correct plate position and choose a previously saved plate
IMPORTANT! The plate that is linked first, whether it is in the Plate A plate or in the Plate B position, is the plate that is run first by the 3500xl.

7.4.5 Starting Instrument/Retrieving Completed Data
1) In the Workflow > Load Plates for Run window, select the Start Run button near the bottom of the screen.
2) Wait until the Monitor Run screen is displayed before leaving the instrument. Error messages (ex. expired reagent installed, not enough POP-4 remains for all injections, etc.) may appear before this screen is displayed; the run will not proceed until the messages are acknowledged.
3) If desired, the status of instrument may be monitored. Under the Dashboard, select View Instrument Sensor Details.
4) Previously run plates can be found in the Plates view within the Library.
5) After each run is completed, the raw data is extracted into .hid files that can be analyzed using GeneMapper software.
6) To retrieve the files from the instrument: Open the desktop shortcut to the instrument’s data; depending on how the run was set to collect data it will either be grouped inside subfolders according to injection # within the parent plate folder or all injections inside one parent plate folder.
8.0 Title: GeneMapper ID-X and Data Analysis

GeneMapper ID-X analysis software is used to analyze the raw data collected by the 3500xl Genetic Analyzers. A size curve is created using co-injected labeled DNA fragments of known size and the unknown peaks are assigned a size by interpolation. The sizes are compared to a ladder to assign allele designations.

A single GeneMapper ID-X project will be created which contains all non-STRmix data associated with a case or batch of cases being processed. This includes all original data, re-loads, re-amplifications, reference standards, and controls, as applicable.

When performing STRmix interpretation, separate GeneMapper ID-X projects may be created during the course of case analysis for the non-STRmix and STRmix analyses. Alternatively, evidence samples may be duplicated and analyzed in the single project using two different analysis methods. When duplicating the .hid files within a single project, it is permissible to manually edit the sample name in the GeneMapper ID-X injection list to denote which samples are STRmix.

The injection list(s) for the GeneMapper ID-X project(s) will be printed for the case file and must include the analysis method, panel, and original plate origin in the UD1 column.

Each sample will be edited to remove artifacts:

1) Evidence samples and reference standards will be analyzed using the internally validated locus-specific stutter filters to aid in the determination of the number of contributors for both the analyst and the technical reviewer.

   **Note:** In the event a profile is determined to be unsuitable for STRmix analysis, the electropherogram will be printed for the case file.

   **Note:** Reference standards may be analyzed in the same project as the evidence samples using the locus-specific stutter filters, however, must be exported for STRmix as detailed below.

2) Evidence samples which are eligible for STRmix interpretation will be analyzed a second time using an analysis method which removes the filters for N-2, N-1, and N+1 stutter from all loci and N-½ stutter from the D1S1656 and SE33 loci. These electropherograms will be printed for the case file.

8.1 Creating Projects in GeneMapper ID-X

Refer to Appendix A for GeneMapper ID-X settings.

1) Once the run is completed, transfer a copy of the run folder from the instrument to the appropriate location on the H:drive.

2) Launch the GeneMapper ID-X software using the user-specific username and password (should be the same as CODIS username)
Note: GeneMapper ID-X requires access granted through a server. A user account will be set up by a member of the Biology/DNA Detail with administrative access to the appropriate Security Groups depending upon their detail assignment.

3) A blank project window should appear. If not, select File | New Project. Then select Edit | Add Samples to Project.

4) Separate GeneMapper ID-X projects will be created for evidence samples and reference standards if requiring different analysis parameters. Navigate to the run folder(s) for the data to be analyzed. Highlight the individual evidence or reference files and select Add to List. A ladder must be included in each run folder that is added to the project. Select Clear to remove unwanted files from the project. Select Add.

Note: Once data has been generated by the 3500XL, do not manually modify, delete, or rename .hid files electronically. Any changes required can be done manually by crossing out and initialing the appropriate forms and electropherograms or electronically under the sample name column in the GMID-X software.

5) Click on the first cell in each of the following columns and select the appropriate setting for analysis according to the table below: Analysis Method, Panel, Size Standard

6) Highlight the column(s) and select Edit → Fill Down to apply the setting(s) to all samples in the table.

<table>
<thead>
<tr>
<th>GeneMapper ID-X v1.6 Manual Interpretation and Reference Standards</th>
<th>Settings</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table Setting</strong></td>
<td>Investigator 24plex</td>
<td>35XX Data Analysis</td>
</tr>
<tr>
<td></td>
<td>Fusion 6C</td>
<td>35XX Data Analysis</td>
</tr>
<tr>
<td><strong>Analysis Method</strong></td>
<td>Investigator 24plex</td>
<td>3500INV2PLEX45.55.65.75.45_191107</td>
</tr>
<tr>
<td></td>
<td>3500INV2PLEX45.55.65.75.45_191107_global</td>
<td>REFERENCE Standards Dye-specific analytical thresholds; 20% global filter</td>
</tr>
<tr>
<td></td>
<td>Fusion 6C 3500xl</td>
<td>3500_Fusion6C_24_Filter20_custom_v1.3</td>
</tr>
<tr>
<td></td>
<td>3500_Fusion6C_25_Filter20_custom_v1.3</td>
<td>60 RFU analytical; 25 cycles</td>
</tr>
<tr>
<td><strong>Panel</strong></td>
<td>Investigator 24plex</td>
<td>3500INV2PLEX_191107_panel</td>
</tr>
<tr>
<td></td>
<td>Fusion 6C</td>
<td>PowerPlex_Fusion 6C_PanelsIDX_v1.3_LVMPD_Custom</td>
</tr>
<tr>
<td><strong>Size Standard</strong></td>
<td>Investigator 24plex</td>
<td>3500INV2PLEX-475bp</td>
</tr>
<tr>
<td></td>
<td>Fusion 6C</td>
<td>WEN_ILS_500_CS</td>
</tr>
</tbody>
</table>

The following settings will be used when utilizing STRmix for interpretation:

<table>
<thead>
<tr>
<th>Profile Detail</th>
<th>Analysis Method</th>
<th>Panel</th>
<th>Table Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evidence</td>
<td>STRmix_3500INV2PLEX45.55.65.75.45_191107</td>
<td>3500INV2PLEX_191107_panel</td>
<td>STRmix - EVIDENCE</td>
</tr>
<tr>
<td>Reference</td>
<td>3500INV2PLEX45.55.65.75.45_191107_global</td>
<td></td>
<td>STRmix - REFERENCES</td>
</tr>
</tbody>
</table>
7) In the **Sample Type** column, each sample defaults to sample. For samples that are actually controls or ladders, click on each and select the sample type from the drop-down menu.
   a. Allelic Ladder: **Allelic Ladder** (must be selected for the ladders that will be used in genotyping)
   b. Reagent blanks & ANC: **Negative control**
   c. APC: **Positive control**
   d. Sample: **Sample**

8) Click the **Green Arrow Analyze Button** on the tool bar. The **Save Project** dialog box opens. Name and save the project in the appropriate Security Group in the drop-down menu.

GeneMapper ID-X projects should be named to include reference to the Lab Case number(s) and/or Batch ID that is being analyzed. At minimum, separate GeneMapper ID-X projects being created specifically for STRmix will include “STRmix” in the project name.

The project will be saved in one of the following Security Groups: Casework, Databasing, Practice, Validation, or GeneMapper ID-X.

**Note:** The **GeneMapper ID-X Security Group** is the default security group used when projects need to be accessible to multiple analysts with varied access.

9) An **Analysis Summary** tab will open with Process Quality Value (PQV) summaries of the allelic ladders, controls and samples. Clicking on the hyperlinked numbers in each sample type summary will filter relevant PQV flagged samples. To undo the filter, select the project name in the project navigation pane on the left side of the screen display.

10) Inspect the **SOS** (sample off-scale) and **SQ** (sizing quality) columns on the table. A green square indicates that the sample has passed sizing criteria. A yellow triangle indicates questionable data. A red octagon indicates poor quality data. Samples with these yellow or red flags need to be carefully examined. To examine a sample, ladder, or control, highlight the row and then click on the **Display Plots** icon on the button bar.

11) In the **Plot Setting** drop down menu, select the desired plot setting. If the electropherogram plot displays “No room for labels”, reduce the number of panes displayed or select only one color to view. To view the labels for the ladder, rescale the electropherogram by placing the cursor outside the X or Y axis and moving it until a magnifying glass appears. Drag it to obtain the desired scale. Alternatively, view the allele calls in table format by selecting the **Genotypes Table** tab.

**Note:** Analysts have the option to adjust settings depending upon personal preferences for analyzing data. If this is the case, the examiner needs to create their own plot settings template so the shared settings do not change.
12) Verify that correct allele calls have been made for allelic ladders and controls and that all peaks in the ILS have sized properly. Poor quality ladders will automatically be removed from analysis by the GeneMapper ID-X software. A poor quality ladder can be omitted manually as well by renaming it as a sample and then re-analyzing all samples in the project.

- All typed peaks within a ladder must reach at least 40 RFU for Fusion 6C to be included in data analysis.
- For Investigator 24plex QS, all typed peaks within the ladder must reach at least 50 RFU.

13) Delete and/or rename labels as needed (e.g. spike, pull-up or confirmed off-ladder alleles) by selecting the peak and right-clicking on the label.

- To delete artifacts: Select Delete Label(s).
- To rename artifacts: right-click the peak and select Rename Allele Label and add the appropriate designation or select from the drop down list.

a. When performing STRmix data analysis of evidence samples, remove all non-allelic peaks, with the exception of the N-2, N-1, N+1 stutter peaks. In addition, stutter peaks falling in the N-½ position at the D1S1656 or SE33 loci in Investigator 24plex QS must also remain labeled. These peaks must be exported to STRmix.

**IMPORTANT!** Care must be taken to examine each stutter position for the potential presence of a peak, including those which may be calling OL due to not having a bin or virtual allele present in the ladder. Refer to Off-Ladder Alleles and OMRs Off-Ladder Alleles and OMRs for additional information.

b. During the analysis of reference standards, remove all non-allelic peaks, to include all elevated stutter peaks

**IMPORTANT!** All peaks being interpreted in STRmix must be labeled using completely numerical values. Confirmed off-ladder microvariant peaks and stutter peaks must be renamed prior to export. Refer to Off-Ladder Alleles and OMRs for additional information.

14) Prior to printing, to allow room for the case number and page numbers using the paginator, verify that the bottom right margin is 0.5 inches. In the Samples Plot window, select:

a. File>Page Setup>

b. Click Page Setup

c. Check that bottom print margin is 0.5.

**Note:** This will need to be done once while in “Plot View” (for printing electropherograms) and once while in main project window (for printing injection lists) when setting up GeneMapper ID-X parameters for the first time for each client computer/user.

15) The electropherogram should be rescaled to ensure all loci are visible before printing. It is not necessary to print any passing controls for Investigator 24plex QS (ladder, APC, ANC, or Reagent Blanks).

- Raw data for samples that have failed to size will be printed and included in the case file
- APC, ANC, and RB control samples which do not meet passing criteria (e.g., loss of resolution, dropout of an S and/or Q quality sensor, possible contamination present, etc.) must be printed for the case file

16) The INV24PLEX print (rfu & bp) or Fusion 6C print with BP plot setting can be selected from the drop-down menu to print samples showing only the called alleles (deleted artifacts are...
not shown). This plot setting is the preferred setting for printing sample electropherograms for the case file.

**Note:** It is not necessary to include the orange channel with the BTO size standard when printing Investigator 24plex QS DNA profiles.

17) The **Fusion 6C Ladder for OL** or **INV24PLEX print (rfu & bp)** plot settings can be selected to print Ladder(s), when required. The **INV24PLEX print NEG** plot setting can be selected to print reagent blank and ANC samples, when required. These are the preferred plot settings for printing ladders and negative controls respectively.

**Note:** It is not required to print ladders unless an off-ladder allele is present in the GeneMapper ID-X project. The ladder utilized to size the sample containing the off-ladder allele should be printed. It is permissible to zoom in and print only the locus of interest on the ladder.

18) Print the electropherogram by clicking on **File>Print**.

19) After an entire project has been through technical review it should be removed from the shared project list by exporting to the analyst’s folder on the H:drive.

   In the main project window, select:
   a. Tools>GeneMapper ID-X Manager
   b. Highlight project(s) to be exported and select Export
   c. Navigate to folder on H:drive and select Save
   d. After project(s) is saved, select Delete

20) Projects that were originally analyzed using GeneMapper ID v.3.2 software can be reviewed using GeneMapper ID-X v 1.6 software, but no allele changes can be made. Projects will need to be imported as follows:

   a. In the main project window, select Tools>GeneMapper ID-X Manager
   b. On Projects tab, click Import
   c. Navigate to project and select project .ser file. Click Import.
   d. Close GeneMapper ID-X Manager
   e. File>Open Project
   f. On Projects Tab, select project and click OK
   g. The first time a sample is selected for plot display a message will appear reminding the user that the project samples were analyzed using v. 3.X software and allele editing options are disabled. Click OK and proceed with reviewing data.

### 8.2 Data Analysis

#### 8.2.1 Review of Size Standards

**DNA Size Standard 550 (BTO) in Investigator 24plex QS**

This pre-made, fluorescent tagged standard contains well-characterized fragments of dye-labeled plasmid DNA that are co-injected with the sample to estimate, by interpolation, the sizes of the STR alleles. The sizes of the DNA fragments contained in the size standard mixture in basepairs are: 60, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 250, 260, 280, 300, 320, 340, 360, 380, 400, 425, 450, 475 and 500.
The BTO size standard is sufficient to continue with analysis when DNA fragments up to the 475 peak are present and have sized appropriately, regardless of whether additional pull-up peaks are also present. The detection of the 500 peak is not necessary for analysis. An analytical threshold of 45 RFU will be utilized for the orange dye channel during analysis.

**Size Standard (WEN ILS 500) in Fusion 6C**

This pre-made, fluorescent tagged standard contains well-characterized fragments of dye-labeled plasmid DNA that are co-injected with the sample to estimate, by interpolation, the sizes of the STR alleles. The sizes of the DNA fragments contained in the size standard mixture in basepairs are: 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500.

The WEN ILS 500 size standard is sufficient to continue with analysis when all DNA fragments are present and have sized appropriately, regardless of whether additional pull-up peaks are also present.

**8.2.2 Review of Allelic Ladder**

The allelic ladder provided with the kits is used to determine the genotypes of samples by comparison with the nominal allelic types contained in the pre-made ladder mixture. While the ladder mixture supplied with the kit include the most common alleles, additional alleles do exist and may be detected and noted by the software in "virtual" bins.

In GeneMapper ID-X software, the common alleles are visualized in gray-shaded bins while additional alleles are visualized in pink-shaded “virtual” bins. The allelic ladder(s) in a project must be checked to ensure that the alleles are properly designated. GeneMapper ID-X aids in the performance of this check by verifying that that the specific alleles designated within the panel and bins files are represented within the detected allelic ladder.

- All typed peaks within a ladder must reach at least 60 RFU for Fusion 6C on a 3500xl to be included in data analysis
- For Investigator 24plex QS, all typed peaks within a ladder must reach the dye-specific analytical thresholds (blue: 45 RFU, green: 55 RFU, yellow: 65 RFU, red: 75 RFU, and purple: 45 RFU)
## Investigator 24plex QS Allelic Ladder:

<table>
<thead>
<tr>
<th>Locus</th>
<th>Dye label</th>
<th>Repeat numbers of allelic ladder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amelogenin</td>
<td>6-FAM</td>
<td>X, Y</td>
</tr>
<tr>
<td>TH01</td>
<td>6-FAM</td>
<td>4, 5, 6, 7, 8, 9, 9.3, 10, 10.3, 11, 13, 13.3</td>
</tr>
<tr>
<td>D351358</td>
<td>6-FAM</td>
<td>9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21</td>
</tr>
<tr>
<td>vWA</td>
<td>6-FAM</td>
<td>11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24</td>
</tr>
<tr>
<td>TPOX</td>
<td>BTG</td>
<td>4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15</td>
</tr>
<tr>
<td>DYS391</td>
<td>BTG</td>
<td>7, 8, 9, 10, 11, 12, 13</td>
</tr>
<tr>
<td>D1S1656</td>
<td>BTG</td>
<td>10, 11, 12, 13, 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19.3, 20.3</td>
</tr>
<tr>
<td>D12S391</td>
<td>BTG</td>
<td>14, 15, 16, 17, 17.3, 18, 18.3, 19, 20, 21, 22, 23, 24, 25, 26, 27</td>
</tr>
<tr>
<td>D10S1248</td>
<td>BTY</td>
<td>8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19</td>
</tr>
<tr>
<td>D22S1045</td>
<td>BTY</td>
<td>8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19</td>
</tr>
<tr>
<td>D19S433</td>
<td>BTY</td>
<td>6.2, 8, 9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2</td>
</tr>
<tr>
<td>D8S1179</td>
<td>BTY</td>
<td>7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19</td>
</tr>
<tr>
<td>D2S1338</td>
<td>BTY</td>
<td>12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28</td>
</tr>
<tr>
<td>D2S441</td>
<td>BTY</td>
<td>8, 9, 10, 11, 11.3, 12, 13, 14, 15, 16, 17</td>
</tr>
<tr>
<td>D18S51</td>
<td>BTY</td>
<td>8, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 17.2, 18, 18.2, 19, 20, 21, 21.2, 22, 23, 24, 25, 26, 27, 28</td>
</tr>
<tr>
<td>QS1</td>
<td>BTP</td>
<td>Q, S</td>
</tr>
<tr>
<td>D16S539</td>
<td>BTP</td>
<td>5, 8, 9, 10, 11, 12, 13, 14, 15</td>
</tr>
<tr>
<td>CSF1PO</td>
<td>BTP</td>
<td>5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16</td>
</tr>
<tr>
<td>D13S317</td>
<td>BTP</td>
<td>5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17</td>
</tr>
<tr>
<td>D5S818</td>
<td>BTP</td>
<td>6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18</td>
</tr>
<tr>
<td>D7S820</td>
<td>BTP</td>
<td>5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16</td>
</tr>
<tr>
<td>QS2</td>
<td>BTP</td>
<td>Q, S</td>
</tr>
</tbody>
</table>
Fusion 6C Allelic Ladder:
Additional custom virtual bins added at the following loci:
- D2S441: 14.1
- D12S391: 15.1, 16.1, 16.3, and 19.2
- D1S1656: 16.1
- Penta E: 15.4 and 18.4
- SE33: 15.2, 17.2, 19.1, 19.3, and 27.3

<table>
<thead>
<tr>
<th>STR Locus (dye color)</th>
<th>Ladder Allele</th>
<th>Size Range (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amelogenin</td>
<td>X,Y</td>
<td>80, 89</td>
</tr>
<tr>
<td>D3S1358</td>
<td>9 to 20</td>
<td>90 to 151</td>
</tr>
<tr>
<td>D1S1855</td>
<td>9 to 14, 14.3, 15.3, 16.3, 17.3, 18.3, 19.3, 20.3</td>
<td>152 to 209.5</td>
</tr>
<tr>
<td>D2S441</td>
<td>8.9, 10.1, 11.3, 12 to 17</td>
<td>211 to 252</td>
</tr>
<tr>
<td>D10S1248</td>
<td>8 to 19</td>
<td>254 to 302.5</td>
</tr>
<tr>
<td>D13S317</td>
<td>5 to 17</td>
<td>304.5 to 357</td>
</tr>
<tr>
<td>Penta E</td>
<td>5 to 15, 16, 17, 18.19 to 25</td>
<td>302 to 482</td>
</tr>
<tr>
<td>D16S539</td>
<td>4 to 16</td>
<td>74 to 129.4</td>
</tr>
<tr>
<td>D19S51</td>
<td>7 to 10, 10.2, 11.12, 13, 13.2, 14 to 27</td>
<td>131 to 217.5</td>
</tr>
<tr>
<td>D2S1338</td>
<td>10, 12, 14 to 28</td>
<td>221.5 to 304</td>
</tr>
<tr>
<td>CSF1PO</td>
<td>5 to 16</td>
<td>313 to 366.5</td>
</tr>
<tr>
<td>Penta D</td>
<td>2.2, 3.2, 5 to 17</td>
<td>373.5 to 470</td>
</tr>
<tr>
<td>TH01</td>
<td>3 to 9, 9.3, 10, 11.3.3</td>
<td>55 to 118</td>
</tr>
<tr>
<td>vWA</td>
<td>10 to 24</td>
<td>121 to 192</td>
</tr>
<tr>
<td>D7S820</td>
<td>5 to 16</td>
<td>268 to 315.5</td>
</tr>
<tr>
<td>D5S818</td>
<td>6 to 18</td>
<td>317.5 to 380</td>
</tr>
<tr>
<td>TPOX</td>
<td>4 to 16</td>
<td>396 to 448</td>
</tr>
<tr>
<td>D8S1179</td>
<td>7 to 19</td>
<td>66 to 129.8</td>
</tr>
<tr>
<td>D12S391</td>
<td>14, 15.1, 16, 17, 28.3, 38, 39.3, 19 to 27</td>
<td>130.1 to 190.35</td>
</tr>
<tr>
<td>D19S433</td>
<td>5.2, 2.8, 8.9, 10.11, 12.12, 2, 13, 13.2, 14.2, 15.2, 16.16, 2, 17.2, 18.18.2</td>
<td>192 to 255</td>
</tr>
<tr>
<td>D22S1045</td>
<td>7 to 20</td>
<td>430 to 478</td>
</tr>
<tr>
<td>DYS591</td>
<td>5 to 16</td>
<td>79.5 to 131</td>
</tr>
<tr>
<td>DYS576</td>
<td>11 to 23</td>
<td>302 to 370</td>
</tr>
<tr>
<td>DYS570</td>
<td>10 to 25</td>
<td>380 to 454</td>
</tr>
</tbody>
</table>

8.2.3 Positive Amplification Controls
DNA 9948 and 2800M are controls that serve to evaluate the performance of the amplification and typing procedure.

The DNA 9948 positive control exhibits the following typing results for Investigator 24plex QS:
For DNA 9948 associated with the Investigator 24plex QS kit, the sum total RFU per locus is expected to be approximately 2000 to 7000 RFU based on the 1 ng of total template input for the 3500xl. If the positive control DNA does not exhibit the single source STR typing results listed above, or the peak heights are not appropriate for the amount of template input for DNA 9948, the following steps must be taken:

1) If there appears to be an injection or electrophoretic problem, re-inject or re-load the control (unless attributable to an artifact). In the event a positive control exhibits the expected sum total RFU values prior to a loss of resolution, and all peaks are called appropriately with peak heights above the stochastic threshold at all locations, it is not necessary to perform additional laboratory work. A copy of the electropherogram will be printed for the case file and documentation included that all small molecular weight loci exhibit the expected sum total RFU values prior to the loss of resolution occurring and that the profile yielded the expected DNA types above the stochastic threshold. Refer to Loss of Resolution/Poor Resolution for additional information.

2) If the profile appears low or does not contain a primer peak in the raw data or is missing the Q and S quality sensors in Investigator 24plex QS, re-load the control to ensure an error did not occur during set-up.

3) If the positive control has lower than expected RFU values, but a second positive control sample has the expected peak heights (to include reference standards with a reasonable expectation to yield single source data consistent with peak heights of the target input), this may indicate inaccurate pipetting occurred during sample set-up. The interpretation of the associated samples may be made with caution based on a case-by-case assessment.

4) If the problems are not resolved, DNA samples and reagent blanks co-amplified with the control should be re-amplified, when possible. If the control yields accurate typing results when re-amplified, then the re-amplified sample profiles associated with the passing positive control will be considered acceptable for comparison purposes and statistical calculations. IMPORTANT! Controls must be critically evaluated to determine the potential root cause of the observed issue. When sufficient volume of extract remains of the associated samples and reagent blanks, all samples contained in the original amplification will be considered inconclusive and a re-amplification will be performed.

In the event samples are not available for additional processing due to concentration prior to the initial amplification and consumption during the original sampling, the case file must be annotated with a documented written acknowledgement of a qualitative review of the data. This review will include documentation of the following:

- The amplification positive control yielded all correct allele calls, despite having lower-than-expected overall RFU values.
The Q and S quality sensors are present in each of the samples and their associated reagent blanks.
Documented recognition that the associated evidentiary data may also be lower-than-expected.
Acknowledgement that the data from this first amplification will be reported due to not having the option to re-amplify nor re-isolate the samples.

The 2800M positive control exhibits the following typing results for Fusion 6C:

All autosomal STR peaks in the amplification positive control for Fusion 6C must achieve the minimum designated analytical and stochastic thresholds required in order to be considered “passing”.

8.2.4 Reagent Blank and Negative Amplification Controls
A reagent blank is used as a processing control to represent the conditions on the workbench as the samples are handled and for the extraction chemicals and supplies themselves. A negative amplification control is a test for the possible presence of contamination occurring during amplification set-up. Contamination can be non-amplified DNA or PCR product.

After electrophoresis, the reagent blanks and negative amplification controls are evaluated. If a negative amplification control or reagent blank exhibit any true peaks indicative of a DNA profile above the kit-specific analytical thresholds for Investigator 24plex QS or Fusion 6C not attributable to an artifact (e.g., spikes, baseline, dye blobs, pull-up, spurious peaks), the data generated from analysis of DNA specimens amplified with the reagents contained in the reagent blank or negative amplification control must be further evaluated:

1) The control(s) containing possible contamination will be re-injected, re-loaded or re-amplified to determine whether actual contamination is present. Should the reagent blank or negative control show no evidence of contamination after re-processing the samples associated with that control may be reported.

2) If the control does not contain a primer peak in the raw data or is missing the Q and/or S quality sensors in Investigator 24plex QS, re-load the control to ensure an error did not occur during set-up.

3) In the event a reagent blank exhibits missing Q and/or S quality sensors, the reagent blank will be cleaned up using a microcon buffer exchange and re-amplified when possible in an attempt to clean-up any inhibitors which may be present in the DNA extract. Due to the potential presence of a sample-specific inhibitor, it is not necessary to also clean-up and re-amplify the associated samples. Refer to Microcon Buffer Exchange Clean-up of Qiagen-Extracted
**Samples.** All data associated with an amplification negative control which is missing the Q and/or S quality sensor must be re-amplified, when possible.

In the event samples are not available for additional processing due to concentration prior to the initial amplification and consumption during the original sampling, the case file must be annotated with a documented written acknowledgement of a qualitative review of the data. This review will include documentation of the following:

- The associated amplification positive control yielded all correct allele calls at the appropriate RFU values, and have both the Q and S quality sensors present, despite containing the same molecular grade water used for the ANC
- The Q and S quality sensors are present in each of the samples and their associated reagent blanks
- Acknowledgement that the data from this first amplification will be reported due to not having the option to re-amplify nor re-isolate the samples.

4) DNA samples and reagent blanks co-amplified with a potentially contaminated control should be re-amplified, when possible. If the control does not exhibit contamination when re-amplified, then the re-amplified sample profiles associated with the passing negative control will be considered acceptable for comparison purposes and statistical calculations.

**IMPORTANT!** Controls must be critically evaluated to determine the potential root cause of the observed issue. When sufficient volume of extract remains of the associated samples and reagent blanks, all samples contained in the original amplification will be considered inconclusive and a re-amplification will be performed.

5) If the contamination is still present after additional lab work has been performed, refer to [Interpretation of a Contaminated Control or Sample](#).

**Note:** The GeneMapper ID-X comparison tool can be used to compare possible contamination profiles to staff members and other samples processed in the same run.

8.3 **Allele Designation**

The goal of the evaluation and interpretation of amplified STR data is to determine the DNA profile(s) of the donor(s) of the questioned sample(s) and to allow comparison to the reference sample profile(s). The following guidelines should be applied in making that determination.

- A peak will be defined as a distinct, sharply pointed triangular area of an electropherogram.
- Genotypes are determined from the diagnostic peaks of the appropriate color and size range for a particular locus.

Alleles will be designated as numerical values in accordance with recommendations of the International Society of Forensic Genetics. Allele designation is based on the number of repeat sequences contained within the allele and by comparison to the allelic ladder.

For any off-ladder (OL) labeled peaks that are observed (even if the allele is below threshold or if comparisons are not going to be made), the GeneMapper ID-X sample file is examined to determine whether the peak(s) is attributed to an artifact (e.g., non-specific amplification product, spike or pull-up) or is indicative of a true off-ladder allele microvariant.
Alleles designated as off-ladder by GeneMapper ID-X and not determined to be an artifact must be verified by re-injection, re-loading, or re-amplification. If the microvariant appears in **multiple evidence samples** from the same case otherwise having the same profile, it is not necessary to verify the off-ladder.

Off-ladder peaks which fall into stutter positions of true/virtual parent alleles do not require confirmation prior to export into STRmix.

Because every measure should be taken to interpret evidence samples prior to reference samples, the presence of the microvariant in an associated known sample will not suffice for confirmation, as the evidence should be interpreted before and separate from any associated knowns in the case. As such, off-ladder alleles must always be independently verified in known reference standards and in cases in which only one evidence sample exhibits the microvariant in question.

The NIST STRbase can also be checked at the following website to verify if the allele has been observed by another laboratory: [http://www.cstl.nist.gov/div831/strbase/var_tab.htm](http://www.cstl.nist.gov/div831/strbase/var_tab.htm)

Reference knowns and questioned samples are measured against the Ladder(s) to show consistent base pair sizes for each matching sample injected per case.

Off-ladder (OL) calls that are determined to be true peaks, not artifacts, are compared to the size of the appropriate ladder alleles, and the allelic designation is determined. If the OL allele is between two allelic ladder peaks of the same locus and is not a perfect (i.e., 4 bp for all loci with the exception of D22S1045 which is 3 bp) repeat, but rather varies by 1, 2, or 3 bp from a ladder allele, then it will be designated as an integer of that variation and considered to be a microvariant. For example, if a blue OL peak size is 238.39 bp, and the 36 allele of the D21S11 ladder is 236.32 bp, then the peak will be designated as a 36.2.

At least one (1) ladder printout containing base pair size data from each GeneMapper ID-X project containing the sample(s) with the OL allele(s) should be included in the case file. If the allele is seen to the right (higher molecular weight (MW)) of the largest allelic ladder peak of a locus, it will be assigned the type of the largest physical allele of the allelic ladder at that locus with a greater than sign (>). If the allele is seen to the left of the smallest allelic ladder peak of a locus, the allele will be assigned the type of the smallest physical allele of the allelic ladder at that locus with a less than sign (<). These designations will be made only on the traditional locus-specific stutter filtered electropherogram of questioned samples and **not** those with all stutter products labeled for STRmix. Refer to [Off-Ladder Alleles and OMRs](#) for information regarding labeling of these alleles in reference standards and questioned samples which will be interpreted in STRmix.

In a single-source sample, if an allele is between two loci and either the locus to the right OR left of the OL peak contains two peaks, the allele will be considered to belong with the locus not containing two peaks. The following designations will be made only on the traditional locus-specific stutter filtered electropherogram of questioned samples and **not** those with all stutter products labeled for STRmix. Refer to [Off-Ladder Alleles and OMRs](#) for information regarding labeling of these alleles in reference standards and questioned samples which will be interpreted in STRmix:
• If the allele is to the right of the largest allelic ladder peak of the locus not containing two peaks, it will be assigned the type of the largest physical allele of the allelic ladder with a greater than sign (>).
• If the allele is smaller than the smallest allelic ladder peak of the locus not containing two peaks, the allele will be assigned the type of the smallest physical allele of the allelic ladder with a less than sign (<).
• If an allele is seen between two loci and neither the higher nor lower molecular weight locus contains an allele or both contain two alleles, further evaluation is necessary. First, the base pair size for the OL allele in question will be compared to the base pair values of the largest allelic ladder peak of the lower molecular weight locus and to the smallest allelic ladder peak of the higher molecular weight locus. An evaluation of the physical location and/or peak height ratio of the allele in question with respect to the higher and lower molecular weight loci will be conducted. Generally, the allele in question will be considered to belong to the locus which is closest in proximity and falls within an appropriate size distance from the locus. If the sample is mixed, further evaluation is also necessary.

Note: For non-STRmix samples, the designation of greater than or less than (> or <) will be assigned according to the associated physical allele of the ladder as opposed to any virtual allelic bins at that locus. Refer to Off-Ladder Alleles and OMRs for renaming alleles for STRmix.

8.4 Injection of Controls
Reagent blank controls, negative amplification controls and positive amplification controls must be injected at the same injection conditions as their accompanying samples. Each genetic analyzer has its own instrument-specific injection condition in order to maintain the same relative level of sensitivity across all instruments. In the event of an injection or failure associated with the controls, the reason the data is not being used must be included on the GeneMapper ID-X injection list or electropherogram (e.g. “DNU – injection failure; re-inject”) included in the case file.

8.5 Identification of Non-Allelic Peaks
Non-allelic peaks may be PCR products (e.g., stutter, non-template dependent nucleotide addition, and non-specific amplification product), analytical artifacts (e.g., spikes and raised baseline), instrumental limitations (e.g., incomplete spectral separation resulting in pull-up or bleed-through), or may be introduced into the process (e.g., disassociated primer dye). Generally, non-allelic data such as stutter, non-template dependent nucleotide addition, disassociated dye, and incomplete spectral separation are reproducible; spikes and raised baseline are generally non-reproducible.

It is possible that non-allelic peaks may fall above the analytical threshold or interpretation threshold. The analyst will use their experience and expertise to distinguish non-allelic peaks from true allelic peaks. LVMPD’s analytical threshold was established to filter out some non-allelic peaks but is based primarily on signal-to-noise considerations.

8.6 Stutter
In addition to an allele’s primary peak(s), minor stutter peaks can occur at four base pair intervals (or three base pair intervals at D22S1045). The most common stutter peaks observed in all loci are one full repeat smaller than the primary peak (N-1). It is also possible to see additional N+1 peaks (one full repeat larger) and/or N-2 peaks (two full repeats smaller) when excessive amounts of DNA and/or degraded DNA samples are amplified. In addition, the D1S1656 and SE33 loci in
the Investigator 24plex QS and PowerPlex Fusion 6C amplification kits may exhibit N-½ stutter, which is approximately one-half repeat smaller than the parent allele.

The analyst should be aware of the combined effects of different types of stutter occurring between two alleles (e.g., N+1 and N-1 rockers). Peaks of this type may be seen in both single source and mixed DNA samples, and a notation(s) can be made where appropriate on the electropherogram contained in the case file.

Stutter peaks are evaluated by examining the ratio of the stutter peak height to the height of the appropriate adjacent allele, expressed as a percentage. The height of stutter peaks will vary by locus, and allele-to-allele.

The expected percentage of an N-1 peak as compared to the nominal allele should typically be less than those listed in the tables of locus-specific values. Peaks in the stutter positions greater than this value may indicate the presence of a mixture. However, peaks in the stutter position below the maximum percentage may be stutter or may be a minor allele and should be interpreted with caution in a mixed sample. The allele-specific stutter values may also be evaluated to determine if a peak is stutter or a true allele.

Reference standards (and their associated reagent blanks), and positive controls may utilize a 20% global filter during analysis as they are reasonably expected to originate from single source profiles. Compromised or degraded known reference standards may benefit from being viewed without the use of the 20% global filter.

Stutter peaks may be elevated above established thresholds by the following:
- Overlap is occurring between a stutter peak and true allelic signal thereby resulting in allelic stacking
- Analyzed peak heights above 28,000 RFU may be off-scale in the raw data, meaning that the CCD camera may be saturated. GeneMapper ID-X may not always alert the analyst to the presence of off-scale data. Therefore, the analyst must pay particular attention to data which exceeds the optimal range. When the GeneMapper ID-X software does alert the analyst of off-scale data, the analyzed peak may have been assigned a lower value due to smoothing and baseline functions. Therefore, the observed stutter percentage will be inaccurately high.

In the event a mixture profile exhibits a stutter ratio greater than the maximum allowed and the primary peak is above 28,000 RFU and/or has been labeled off-scale, an attempt should be made to re-amplify the mixture sample using less total template prior to STRmix analysis, when possible. Single source evidence profiles and reference standards which exhibit the above phenomena may be interpreted with caution. Refer to Dealing with Uncharacterized Variants, Allelic and Chromosomal Abnormalities, Allele Resolution and Technical Issues in STRmix for additional information.

- Stutter peaks overlapping an area of elevated baseline or spectral pull-up may exhibit increased percentages. Elevated baselines may indicate an improperly functioning spectral calibration.
- Stutter peaks falling into the rocker position in the overlapping area of stutter products (e.g., N+1/N-1) may result in increased stutter percentages.
8.7 Non-Template Nucleotide Addition (-A)
Amplification conditions have been set to maximize the non-template addition of a 3’ terminal nucleotide by DNA polymerase. Failure to attain complete terminal nucleotide addition results in band splitting (two peaks one base apart). This is most often seen when an excessive amount of DNA is amplified or amplification is performed under sub-optimal PCR conditions. Minus A peaks that are called can be deleted in GeneMapper ID-X or can be indicated on the electropherogram by crossing them out.

The presence of a significant amount of minus A artificially reduces the true peak heights of the alleles due to the signal being split between the minus A peak and the fully adenylated form. Based on the severity, the presence of minus A peaks may affect STRmix modeling.

8.8 Non-Specific Amplification Artifacts
Non-specific amplification products are artifacts that may or may not look like true alleles. These artifacts may occur due to excessive template amplification or the presence of co-extracted bacterial or microbial DNA.

Though the morphology of the peak may be similar to that of a true allele, non-specific amplification artifacts do not exhibit the presence of the characteristic stutter peak that accompanies the amplification of true DNA. Data should be evaluated for the presence of the expected stutter peak when determining whether a peak may be the result of non-specific amplification.

The analyst will evaluate the sample type and the entirety of the profile to distinguish these artifacts from true alleles. If a non-specific amplification artifact is suspected to be present, the sample should be re-amplified when possible. Due to the peak being the result of bacterial or microbial DNA present in the sample extract, re-amplification of the sample may not resolve the issue. However, the peak height of non-specific amplification artifact may not be reproducible when re-amplified as would be expected with true, non-stochastic, human DNA. Documentation will be maintained in the case file when a non-specific amplification artifact is believed to be present. Refer to "Dealing with Uncharacterized Variants, Allelic and Chromosomal Abnormalities, Allele Resolution and Technical Issues in STRmix" for additional information.

Artifacts documented in official notices by Promega or QIAGEN may be deleted without independent confirmation. The presence of the artifact will be documented in the case file or database packet.

8.9 Spikes
Peaks of the same size and similar height (within an order of magnitude of each other) can occur in multiple colors, are not the result of dye-labeled DNA and do not represent a spectral problem. These are spikes typically due to urea crystal in the POP-4 polymer, dust, or particulates in the buffer. Spikes can also occur in a single color and are distinguished by their unique morphology as compared to true peaks. Multi-color spikes can be shown to be artifacts by overlaying all colors. Spikes that fall outside of the kit-specific analysis ranges are not significant.
8.10 Raised Baseline
A raised baseline will appear on an electropherogram as a horizontal line that is higher than the average baseline in the rest of the color channel. It is typically apparent on an electropherogram by noting that the raised baseline appears to be equal to the width between two true allele peaks from an adjacent color channel.

8.11 Migration and Pull-Up
In addition to amplification artifacts described above, the following anomalies can arise during electrophoresis and analysis and notation(s) can be made where appropriate on the electropherograms contained in the case file.

- Significant room temperature fluctuation may result in size variation or migration between injections such that allelic ladder peaks differ by more than approximately ±0.5 bp from allelic peaks in other injections. This will cause GeneMapper ID-X to assign these alleles as off-ladder alleles. Genotyping with a different injection of allelic ladder may alleviate this problem. Data from another amplification, injection, or evidentiary sample may also confirm the migration. If these methods don’t resolve the issue, the sample(s) and an allelic ladder should be re-injected or re-loaded to confirm the typing. Migration at Amelogenin does not need to be confirmed.

- Small artifact peaks can appear in other colors under true peaks. This phenomenon is termed pull-up. Pull-up is a result of spectral overlap between the dyes which is normally corrected for by the spectral. If a pull-up peak is above the minimum peak height detection threshold, it will be sized at the approximate same size as the true peak from the other color channel. Amplification using excess input DNA can lead to off-scale peaks resulting in pull-up. These “pull-up” peaks can be deleted in GeneMapper ID-X or noted on the electropherograms. Typically pull-up peaks are within approximately 10-15 data points of the originating peak.

8.12 Off-Scale Indicators (Saturated Data)
Analyzed peak heights above 28,000 RFU may be off-scale in the raw data, meaning that the CCD camera may be saturated. GeneMapper ID-X may not always alert the analyst to the presence of off-scale data. Therefore, the analyst must pay particular attention to data which exceeds the optimal range. When the GeneMapper ID-X software does alert the analyst of off-scale data, the analyzed peak may have been assigned a lower value due to smoothing and baseline functions.

An off-scale indicator means a data point is saturated. Off-scale data must be interpreted carefully as it can distort mixture ratio and stutter filtering calculations. In addition, saturated data may result in the presence of an increased number of amplification artifacts such as pull-up and raised baseline. It may be difficult to determine whether peaks present in the data are attributable to DNA or artifacts. The type of sample (evidence vs. reference standard) will be considered when assessing the potential presence of ambiguous artifacts in overloaded samples.

In the event a mixture profile exhibits off-scale data, an attempt should be made to re-amplify the mixture sample using less total template prior to STRmix analysis, when possible.

Single source evidence and reference standard profiles which exhibit the above phenomena may be interpreted with caution or diluted in formamide and re-loaded. When diluting in formamide, the description of how each dilution was prepared (e.g., 1:10 dilution prepared using 1 µl of amplicon and 9 µl of formamide; 1 µl of dilution added to load plate) must be included on the load form.
Refer to Dealing with Uncharacterized Variants, Allelic and Chromosomal Abnormalities, Allele Resolution and Technical Issues in STRmix for additional information.

8.13 Evaluation of Quality Sensors QS1 and QS2 in Investigator 24plex QS
The Investigator 24plex QS Kit primer mix contains two (2) internal PCR controls (Quality Sensor QS1 and QS2) to provide helpful information about the efficiency of the PCR and the possible presence of PCR inhibitors. The quality sensors are amplified simultaneously with the STR markers. They are labeled with BTP and appear at fragment sizes of 74 bp (QS1) and 435 bp (QS2).

In general, the successful amplification of the small quality sensor (QS1) indicates that the PCR was set up and conducted correctly, regardless of whether DNA was present or absent in the sample. If no quality sensor is detected in the analysis of the amplification products, this may indicate that pipetting during PCR setup, or the PCR itself, were performed incorrectly or that the sample is suffering from extreme inhibition. The amplification should be repeated for improved results using either the same or different amount of template input.

In addition, the analysis of the two internal control fragments, QS1 and QS2, and of the STR target amplification products allow for the differential identification of the presence of inhibitors or the presence of DNA degradation in an amplification reaction.

In the case of sample degradation, the amplification of smaller target fragments is more efficient than the amplification of larger target fragments. However, degradation of the target template does not hamper amplification of the QS1 and QS2 internal controls. Thus, an equal ratio of QS1 and QS2, together with a ratio in favor of small STR target products suggests the presence of sample degradation.

A peak height ratio of less than 80% between QS1 and QS2 (RFU of S-allele/RFU of Q-allele < 0.80) may indicate inhibition of the PCR reaction. Data should be scrutinized in conjunction with the quantitation data to determine if additional processing may be warranted in the form of sample clean-up. Documentation must be included in the case file regarding this review and the reason(s) why the data was deemed suitable to report.

The following table may serve as a useful reference while examining the quality sensors (QS1 and QS2):

<table>
<thead>
<tr>
<th>Allele Peaks</th>
<th>QS1</th>
<th>QS2</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Successful profile</td>
</tr>
<tr>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>No DNA</td>
</tr>
<tr>
<td>Skislope profile</td>
<td>Present</td>
<td>Dropout</td>
<td>Inhibitors present</td>
</tr>
<tr>
<td>Skislope profile</td>
<td>Present</td>
<td>Degraded DNA</td>
<td></td>
</tr>
</tbody>
</table>

8.14 Loss of Resolution/Poor Resolution
Loss of resolution occurs when there are instrumentation issues or impurities contained within a sample which results in poor sample migration through the capillary. This results in a broadening of peaks and a loss of peak height, typically at the larger sized loci. As loss of resolution typically affects the various-sized loci differently, the relationship between the amount of input DNA and
peak heights is no longer linear. This can be problematic for quantitative-based interpretation methods such as STRmix, which utilizes this relationship during sample deconvolution. The presence of loss of resolution in samples will be notated on the electropherogram or GeneMapper ID-X injection list.

Low-level or mixed samples which exhibit loss of resolution will be re-injected, re-loaded, or re-amplified to resolve the peak broadening issues when the loss of resolution affects the base pair range of the data present in the profile. Heavily degraded partial profiles (single source or mixture) which are consistent with their quantitation data and exhibit complete locus drop-out at base pair ranges prior to the occurrence of loss of resolution (as evidenced by the peak morphology in the BTO), do not require additional laboratory processing. In these instances, the loss of resolution is not expected to affect the limited allelic signal detected at the smaller molecular weight loci in the degraded partial sample. The acknowledgment of the loss of resolution and decision to not perform additional laboratory processing based on the quality of the degraded profile will be documented on the electropherogram.

Robust single-source samples (containing all heterozygous alleles above 225 RFU and homozygous alleles above 400 RFU) which do not exhibit any indications of containing a mixture may be interpreted with caution.

The resolution of peaks must be checked carefully, particularly at loci which contain single base pair microvariants (e.g. D1S1656 and D1S391). Poor resolution may result in shouldering and the loss of minor contributor or stutter peaks, which can affect STRmix deconvolution.

In the event reagent blanks or samples which fail to yield a DNA profile suffer from loss of resolution, documentation must be included in the case file to state that the lack of data is consistent with the expectation based on the item’s quantitation value, despite the loss of resolution.

Amplification negative controls must also include documentation that the lack of data is consistent with expectation, despite the loss of resolution. Alternatively, these items may be re-injected or re-loaded in order to attempt to resolve the loss of resolution. Documentation may be made on either the printed electropherogram or in the GeneMapper ID-X injection list.

8.15 Review of First Pass Data

Due to working in a group setting, plates which are injected on the 3500xl may receive a cursory review of this first pass (FP) data in order to identify samples which may require routine additional processing and to identify plate-level trends in the data which may require troubleshooting or notification of the DNA Technical Leader such as multiple samples with split peaks, etc. This review is optional and may be conducted by a qualified individual other than the interpreting analyst specifically assigned to the case.

At minimum, when this review is performed, it will encompass the following requests:

- Loss of resolution: re-inject or re-load
- Injection failure: re-inject or re-load
- Repeated capillary failures: request capillary change
- No data/quality sensors/primer flash: re-load
Lower than expected reference standard RFU values: may request re-amplification with increased template

The completion of this review will be documented on the amp and load form. The analyst completing the review will handwrite “FP data reviewed” and the date the review took place and their initials.

If specifically documented on the amp and load form, the first pass review may also include requests for re-lads of off-ladder alleles and requests for buffer exchange and re-amplification due to quality sensor drop-out of non-concentrated samples.

Unless otherwise outlined above, the first pass review will not include requests for re-extractions, re-quantitations, concentration, buffer exchange, or re-amplification.

8.16 Required Casework Documentation of Interpretation on Electropherograms
Electropherograms will include documentation regarding the analyst’s observations and suitability for additional interpretation.

At minimum, the following will be documented on casework evidence electropherograms:

- Number of contributors
  - For mixtures, must include the name of at least one locus where the determination for the number of contributors was made
- If an indication of male DNA is present
- Suitability for STRmix interpretation or statistical calculation
- Assumptions, if any, that will be made during interpretation based on the sample type (e.g., intimate, personal, etc.)
- If the maximum degradation setting will be increased during the original interpretation STRmix deconvolution due to the presence of significantly degraded data
- If the data is not being used for additional interpretation (i.e., uninterpretable), the reason why (e.g., DNU due to loss of resolution, due to limited data, no additional conclusions, etc.)

In the event STRmix will be used for interpretation, the required documentation will be captured on the STRmix electropherogram that includes stutter labels. When replicates will be utilized in STRmix, documentation must be included on at least one of the replicate electropherograms. Additional profiles which will be run as replicates will include a reference to the page number or sample ID of the documented electropherogram. If STRmix is not being performed, all documentation will be included on the electropherogram which will be reported.

The following will be documented on reference electropherograms:

- Whether the profile is a full or partial DNA profile
- The sex of the profile

8.17 Exporting GeneMapper ID-X Analysis for STRmix Interpretation and ALLELEigator Table for References
After analysis has been completed, evidence profiles which will be interpreted using STRmix and reference standards are exported to the analyst run folders located within Y:\Forensic Data\STRmix\STRmix Run Files. In the main project window, select the appropriate Table Setting...
noted above. Click “File” then “Export Combined Table”. Select the desired destination and click “Export”.

**Note:** If evidence and reference standards are contained in the same project, select only the injection folder(s) containing the evidence and/or reference profiles from the left-hand project pane. Select the appropriate table setting based on the sample type and then export to STRmix. It is permissible to create additional folders within the STRmix Run Files location (e.g. batch, year, etc.) to house the export files.

ALLELEigator tables will be generated for all reference standards. In the main project window, select the “ALLELEigator” table setting. Click “File” then “Export Combined Table”. Select the desired destination and click “Export”.

### 8.18 GeneMapper ID-X Comparison Tools

The GeneMapper ID-X software has a comparison tool that can be used to compare possible contamination profiles to staff members and other samples processed in the same project. To open the Comparison Tool, go to **Tools>Profile Comparison**.

On the first **Sample Concordance** tab, samples with 100% concordance are listed.

On the **Sample Comparison** tab, after clicking **Compare Profiles** the profiles in the project are compared to each other and possible matches will be listed. The percent match threshold can be adjusted depending on how many possible matches are found.

On the **Lab Reference Comparison** tab, after clicking **Compare Profiles** the profiles in the project are compared to DNA Lab personnel, Custom Controls, and contaminant profiles reported in the forensic DNA community. The percent match threshold can be adjusted depending on how many possible matches are found.

The **Control/QC Comparison** is not currently utilized by the LVMPD Biology/DNA Detail.

**Note:** Samples containing off-ladder (OL) alleles will not be compared so all artifacts should be deleted even if the data isn’t going to be reported if the analyst wants them compared as well. If an Allelic Ladder’s sample type is changed from Allelic Ladder to Sample due to poor sizing quality it will most likely be called a match to every sample in the project.

### 8.19 GeneMapper ID-X Audit Record Maintenance

Only a user with administrator rights or using the administrator login can access the GeneMapper ID-X server. The following procedures can only be performed by a user with administrative access.

**Note:** Currently this feature is turned off, but may be used at a future date when another copy of the server software is installed and the audit triggers can be appropriately applied and managed.

**Note:** An audit record is saved anytime a change (i.e. allele change, delete allele, etc) is made to a project while the audit feature is turned on. The software recommends that the audit records be backed up and deleted when there are over 40,000 records in order to free up space in the database.
To monitor the audit record count, navigate to the following in GMID-X:
**Admin>Audit Manager>Show Audit Record Count**

If the count is close to or exceeds 40,000 records, someone must log on as the administrator (user ID: gmidx) to back up and delete the records by navigating to the following:

To back up audit records:
**Admin>Audit Manager>Backup Audit Records**

The backup should be stored on a CD and stored in the safe in the DNA Lab.

To delete audit records:
**Admin>Audit Manager> Delete Audit Records**

**Investigator 24plex QS locus-specific** stutter values included in GMID-X stutter files

<table>
<thead>
<tr>
<th>Locus</th>
<th>Minus 1 Full Repeat</th>
<th>Plus 1 Full Repeat</th>
<th>Minus 2 Full Repeats</th>
<th>Minus 1/2 Repeat</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>TH01</td>
<td>6.45%</td>
<td>--</td>
<td>1.07%</td>
<td>--</td>
</tr>
<tr>
<td>D3S1358</td>
<td>14.36%</td>
<td>2.68%</td>
<td>1.16%</td>
<td>--</td>
</tr>
<tr>
<td>vWA</td>
<td>13.94%</td>
<td>1.70%</td>
<td>0.56%</td>
<td>--</td>
</tr>
<tr>
<td>D2S1338</td>
<td>12.93%</td>
<td>2.28%</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>TPOX</td>
<td>0.57%</td>
<td>1.08%</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>DYS391</td>
<td>9.41%</td>
<td>1.97%</td>
<td>1.09%</td>
<td>--</td>
</tr>
<tr>
<td>D1S1656</td>
<td>17.12%</td>
<td>2.87%</td>
<td>1.87%</td>
<td>2.41%</td>
</tr>
<tr>
<td>D12S391</td>
<td>17.26%</td>
<td>2.53%</td>
<td>2.53%</td>
<td>--</td>
</tr>
<tr>
<td>SE33</td>
<td>16.90%</td>
<td>2.71%</td>
<td>1.59%</td>
<td>6.00%</td>
</tr>
<tr>
<td>D10S1248</td>
<td>14.54%</td>
<td>1.98%</td>
<td>3.14%</td>
<td>--</td>
</tr>
<tr>
<td>D2S1045</td>
<td>16.87%</td>
<td>9.69%</td>
<td>2.16%</td>
<td>--</td>
</tr>
<tr>
<td>D19S433</td>
<td>13.61%</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>D8S1179</td>
<td>13.54%</td>
<td>5.46%</td>
<td>1.68%</td>
<td>--</td>
</tr>
<tr>
<td>D2S1338</td>
<td>14.28%</td>
<td>1.46%</td>
<td>1.15%</td>
<td>--</td>
</tr>
<tr>
<td>D2S441</td>
<td>9.96%</td>
<td>2.92%</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>D18S51</td>
<td>16.73%</td>
<td>3.52%</td>
<td>2.57%</td>
<td>--</td>
</tr>
<tr>
<td>FGA</td>
<td>14.34%</td>
<td>2.76%</td>
<td>2.54%</td>
<td>--</td>
</tr>
<tr>
<td>D16S539</td>
<td>12.58%</td>
<td>2.16%</td>
<td>1.48%</td>
<td>--</td>
</tr>
<tr>
<td>CSF1PO</td>
<td>12.17%</td>
<td>2.54%</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>D13S317</td>
<td>11.02%</td>
<td>2.95%</td>
<td>0.66%</td>
<td>--</td>
</tr>
<tr>
<td>D9S818</td>
<td>11.05%</td>
<td>2.38%</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>D7S820</td>
<td>10.49%</td>
<td>1.90%</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

**Note:** PowerPlex Fusion 6C utilizes a 20% global stutter filter
**Investigator 24plex QS allele-specific stutter percentages**

The below values are used in the manual calculation of the expected amount of allele-specific stutter. STRmix v2.6 uses intercept and slope values to model generalized stutter products along with the best explanatory variable (longest uninterrupted stretch (LUS), or average stutter).

The best explanatory variable per locus for all generalized stutter products are noted below. Charts reflecting the allele-specific stutter products observed during validation for each type of generalized stutter can be found on the Y:drive at: Y:\DNA\(DNA WORKBOOKS)\CASEWORK\3\ALLELE TABLES ETC. Where noted below, a minimum stutter ratio of 0.001 is used by STRmix.
### N-2 Allele Specific Stutter Percentages

<table>
<thead>
<tr>
<th>Locus</th>
<th>Slope</th>
<th>Intercept</th>
<th>Best Explanatory Variable (STRmix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH01</td>
<td>0</td>
<td>0.00747</td>
<td>Locus Average</td>
</tr>
<tr>
<td>D3S1358</td>
<td>0</td>
<td>0.00861</td>
<td>Locus Average</td>
</tr>
<tr>
<td>vWA</td>
<td>0</td>
<td>0.00719</td>
<td>Locus Average</td>
</tr>
<tr>
<td>D21S11</td>
<td>0</td>
<td>0</td>
<td>Minimum Stutter Ratio</td>
</tr>
<tr>
<td>TPOX</td>
<td>0</td>
<td>0</td>
<td>Minimum Stutter Ratio</td>
</tr>
<tr>
<td>DYS391</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>D1S1655</td>
<td>0</td>
<td>0.01133</td>
<td>Locus Average</td>
</tr>
<tr>
<td>D12S391</td>
<td>0</td>
<td>0.01092</td>
<td>Locus Average</td>
</tr>
<tr>
<td>SE33</td>
<td>0</td>
<td>0.01114</td>
<td>Locus Average</td>
</tr>
<tr>
<td>D10S1248</td>
<td>0</td>
<td>0.01262</td>
<td>Locus Average</td>
</tr>
<tr>
<td>D22S1045</td>
<td>0</td>
<td>0.01094</td>
<td>Locus Average</td>
</tr>
<tr>
<td>D19S433</td>
<td>0</td>
<td>0</td>
<td>Minimum Stutter Ratio</td>
</tr>
<tr>
<td>D8S1179</td>
<td>0</td>
<td>0.00953</td>
<td>Locus Average</td>
</tr>
<tr>
<td>D2S1338</td>
<td>0</td>
<td>0.00862</td>
<td>Locus Average</td>
</tr>
<tr>
<td>D25441</td>
<td>0</td>
<td>0</td>
<td>Minimum Stutter Ratio</td>
</tr>
<tr>
<td>D18S51</td>
<td>0</td>
<td>0.01369</td>
<td>Locus Average</td>
</tr>
<tr>
<td>FGA</td>
<td>0</td>
<td>0.0126</td>
<td>Locus Average</td>
</tr>
<tr>
<td>D16S539</td>
<td>0</td>
<td>0.00862</td>
<td>Locus Average</td>
</tr>
<tr>
<td>CSF1PO</td>
<td>0</td>
<td>0</td>
<td>Minimum Stutter Ratio</td>
</tr>
<tr>
<td>D13S317</td>
<td>0</td>
<td>0</td>
<td>Minimum Stutter Ratio</td>
</tr>
<tr>
<td>D5S818</td>
<td>0</td>
<td>0</td>
<td>Minimum Stutter Ratio</td>
</tr>
<tr>
<td>D7S820</td>
<td>0</td>
<td>0</td>
<td>Minimum Stutter Ratio</td>
</tr>
</tbody>
</table>

### N-1/2 Allele Specific Stutter Percentages

<table>
<thead>
<tr>
<th>Locus</th>
<th>Slope</th>
<th>Intercept</th>
<th>Best Explanatory Variable (STRmix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH01</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D3S1358</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>vWA</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D21S11</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TPOX</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>DYS391</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>D1S1655</td>
<td>0</td>
<td>0.01137</td>
<td>Locus Average</td>
</tr>
<tr>
<td>D12S391</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SE33</td>
<td>0</td>
<td>0.02682</td>
<td>Locus Average</td>
</tr>
<tr>
<td>D10S1248</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D22S1045</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D19S433</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D8S1179</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D2S1338</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D25441</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D18S51</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>FGA</td>
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<tr>
<td>D16S539</td>
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</tr>
<tr>
<td>D5S818</td>
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</tr>
<tr>
<td>D7S820</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
9.0 Title: Interpretation Guidelines and STRmix

The interpretation of results generated from casework samples is a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-set rule. The Biology/DNA Detail will use appropriate procedures for test data interpretation. The laboratory has developed and adheres to general guidelines and minimum criteria for the interpretation and reporting of analytical results which are generally accepted in the scientific community. These criteria are based on validation studies, published scientific literature, and the experience of the analyst. It is to be expected that these interpretation guidelines will evolve as the collective experiences of the laboratory and forensic community in general continues to grow.

9.1 Sample Types

Samples are grouped into questioned samples which have an unknown origin and reference (known) standards. Questioned samples may yield single source, mixture profiles, or no results.

Types of Reference Standards:

Primary Standards are samples known to originate from a particular individual and are usually in the form of buccal swabs or blood. Pulled hair, tissue or bone may also be submitted as a primary standard if the originating individual is known. Primary standards are taken directly from the body for the purpose of being used as a reference standard, rather than as a questioned evidence sample.

Secondary standards are samples removed from the victim or a personal item known to belong to the victim (i.e., bloody clothing, toothbrush). Secondary standards may be used if a primary standard is unavailable or unusable. If used, the secondary standard will be noted in the report. Based on the circumstances of the case, a secondary standard may be processed as either a questioned or a reference sample.

A secondary standard submitted from a suspect may be used for comparison but not for statistical calculations. When it is necessary to use a secondary standard from a deceased victim, the secondary standard may be used for comparison and statistics.

Presumed standards are samples which are known to originate from a particular individual, however are typically processed as an evidence sample in order to first determine whether it contains probative evidential value (e.g. epithelial fraction of a vaginal swab, breast swab, etc.).

Under certain documented circumstances, a resultant single source DNA profile (or distinguishable component of a mixture) may be assumed to belong to the individual from whom it originated for comparison purposes. A presumed standard may be used if a primary standard is unavailable or unusable. The use of a presumed standard will be notated in the analyst's notes and in the report.
Surreptitious standards are samples which are collected from physical items known to have been used or discarded by a person of interest (e.g. cigarette butts, drinking containers, etc.). Surreptitious standards will be processed in the laboratory as questioned samples.

Under certain documented circumstances, a resultant single source DNA profile may be assumed to belong to the person of interest who is known to have used or discarded the item for purposes of comparison. The DNA profile will be used as a reference standard in STRmix for comparison to other evidence items. Refer to the section for Manual creation of .txt files for unidentified contributors, DNA profiles of reference standards processed from outside vendors, and surreptitious standards.

Items submitted as surreptitious standards which yield mixture DNA profiles will be considered as unsuitable for comparison.

A surreptitious standard may be used if a primary standard is unavailable or unusable; however the DNA profile is not eligible for CODIS entry. The use of a surreptitious standard will be notated in the analyst’s notes and in the report. All reported comparisons will reference only the donor of the DNA profile to the surreptitious standard and will not make a direct association with an individual.

Note: An official reference standard buccal swab is required for confirmation that the surreptitious standard has the same DNA profile as the person of interest.

9.2 Preliminary Evaluation of Data and Application of Peak Height Thresholds
The analytical thresholds (ATs) were established through internal validation studies. The analytical thresholds are set to 45 RFU for blue, 55 RFU for green, 65 RFU for yellow, 75 RFU for red, 45 RFU for purple, and 45 RFU for orange dye channels. Any peak detected less than the dye-specific analytical threshold does not reliably represent DNA template.

The interpretation (stochastic) threshold (ST) is set at 400 RFU for each 3500xl validated for use with Investigator 24plex QS. Peaks which are detected between the dye-specific analytical thresholds and 399 RFU are authentic signal and should be evaluated to discern alleles from artifacts. This threshold will be used as a qualitative assessment only and does not impact the ability to interpret or report evidence data analyzed using STRmix.

Reference standards must have all homozygous loci above the 400 RFU interpretation threshold and all heterozygotes above the dye-specific analytical thresholds to be considered complete.

IMPORTANT! Due to the use of a 20% global filter during analysis of reference standards, peaks below 80 RFU will be filtered when the highest peak at a locus is 400 RFU. Therefore, if a reference standard locus is believed to be homozygous and the single peak is between 225 and 400 RFU, it must be manually verified that the sister allele was not erroneously removed during the application of the global filter.

9.3 Single Source Profiles
A sample is consistent with originating from a single source if one or two alleles are present at all loci for which typing results were obtained (although tri-allelic loci may occur). Loci with a single
homozygous peak called below the 400 RFU interpretation threshold may indicate the possibility of allelic drop-out.

The sex of a male profile donor may be reported if the Y-allele at Amelogenin is between the analytical and stochastic threshold or if an allele is present at the DYS391 locus. In the absence of a Y-allele at Amelogenin, the DYS391 marker should be examined to confirm a null allele is not occurring. Reference standards may be amplified in PowerPlex Fusion 6C in an attempt to determine the presence of a null allele.

**Note:** Evidence samples may not be amplified using PowerPlex Fusion 6C.

### 9.4 Tri-Alleles and Duplications/Triplications of Y-STR Markers

A locus is consistent with being tri-allelic if three alleles are exhibited and there are no other indications of the presence of a possible mixture.

The Y-STR markers are haplotypes with the expectation of only one allele being present when there are no other indications of the presence of a possible mixture. It has been characterized that duplications and triplications of these markers may occur within male individuals.

#### Confirmation of Tri-Alleles

Questioned samples suspected of containing a tri-allele will be re-amplified to confirm the presence, when possible. If there is not sufficient extract remaining for re-amplification, the locus will not be used for interpretation.

If the suspected tri-allele exhibits a balanced PHR with each allele being greater than the expected 50% PHR for Investigator 24plex with one another and the tri-allele appears in **multiple evidence samples** from the same case otherwise having the same profile, it is not necessary to verify the tri-allele by re-amplification. All other non-balanced indications of a tri-allele must be independently re-amplified for verification purposes.

Because every measure should be taken to interpret evidence samples prior to reference samples, the presence of the tri-allele in an associated known reference sample will not suffice for confirmation. Suspected tri-alleles must always be independently verified in known reference standards and in cases in which only one evidence sample exhibits the tri-allele in question.

Currently, statistical calculations cannot be performed on loci containing tri-alleles using the Popstats or STRmix software.

#### Confirmation of Duplications/Triplications of Y-STR Markers

Questioned samples suspected of containing a duplication/triplication of a Y-STR marker will be re-amplified to confirm the presence, when possible. If there is not sufficient extract remaining for re-amplification, the locus will not be used for interpretation.

Re-amplification is not required when the duplication/triplication appears in multiple evidence samples otherwise having the same DNA profile and one of the following criteria are met:

- The duplication/triplication alleles do not fall into stutter positions
- The peak in the N-4 stutter position is at least 20% or greater than the peak height of the other peak and the peak in N+4 stutter position is at least 15% or greater than the peak of height of the other peak
If a possible duplication/triplication peak falls into the N-4 stutter position of a second peak and may possibly be the result of high stutter rather than true DNA, the sample will be re-amplified to confirm the presence of a true duplication/triplication.

Because every measure should be taken to interpret evidence samples prior to reference samples, the presence of the duplication/triplication in an associated known reference sample will not suffice for confirmation.

9.5 Mixture Profiles
A mixture is a DNA profile which originates from two or more individuals. An individual’s contribution to a mixed biological sample is generally proportional to their quantitative representation within the DNA typing results.

The determination of a mixture is made by a collective evaluation of the electropherogram and all data which is present. Generally, the following characteristics observed in a sample may be indicative of a mixture when taking into consideration the totality of the electropherogram observed:

- 4 peak pattern observed at a locus
- 3 peak pattern observed at a locus
- 2 or more peaks observed at the DYS391 locus
- Significant peak height imbalances of alleles for a heterozygous genotype at a locus with the exception of low template amplifications, which should be interpreted with caution
- The presence of elevated stutter at a locus
- Possible additional peaks falling into allelic bins below the analytical threshold

9.6 Manual Comparison of Reference Standards to Outsourcing and Legacy Data
A known individual is manually determined to be included in a single source sample or mixture sample if their alleles are present at all loci where DNA typing results were deemed interpretable. Though rare, it is possible that one allelic difference may exist due to mutation (in even rarer instances, two mutation events have been observed). The analyst must thoroughly document if the possibility of mutation is suspected in their case notes.

A known individual is excluded from a single source or mixture sample if their alleles are missing at any loci where the DNA typing results were deemed complete. Alleles that fall within a stutter position may be evaluated on a sample-by-sample basis and may affect the conclusions in mixed samples.

9.7 STRmix Interpretation
STRmix is a fully continuous probabilistic genotyping software for the interpretation of autosomal STR DNA profiling results. STRmix can deconvolute a mixture into the individual contributor(s)/component(s) in the absence of reference standards. STRmix is also used to provide a statistical weight for comparisons of reference profile(s) to evidence profile(s).

The LVMPD Biology/DNA Detail is using STRmix v2.6 for the interpretation of Investigator 24plex QS profiles and Identifiler Plus profiles. STRmix is not available for use with data generated from other amplification kits or with data generated by an outsourcing vendor.
Interpretation protocols are not intended to be applied retroactively. The Biology/DNA Detail will re-evaluate previously reported DNA data and conclusions based on three scenarios:

1) Anytime a forensic DNA expert performs an evaluation of their previously reported data (preparing for court, writing a supplemental report, etc.), the expert has a right to retract or re-analyze data using STRmix that is no longer supported by significant evolution in technology or fundamental practices.
   - The original requestor will be notified via email that the Biology/DNA Detail will be issuing a supplemental report based on the re-evaluation of the case file. Documentation of this email communication will be maintained in the case file.

2) When a written request is received for STRmix analysis of an item which was previously reported as inconclusive (or portion thereof). Requests will only be considered when submitted from a Lieutenant or a person of equivalent or higher rank and must be approved by the DNA Laboratory Manager, Supervisor, or DNA Technical Leader.
   - The original requestor will be notified via email if additional DNA profiles are identified that may be suitable for STRmix re-interpretation outside of those specifically requested.

3) When issued a court order.

All requests for STRmix analysis must be within the capability and validated application(s) of the laboratory. In the event an item of evidence which was previously amplified and reported using Identifiler Plus will be re-amplified using QIAGEN Investigator 24plex QS for purposes of potential STRmix interpretation, the associated reference standards previously processed using Identifiler Plus will also be re-amplified using QIAGEN Investigator 24plex QS.

Information pertaining to the settings for Identifiler Plus DNA profiles may be found at the end of this protocol. All other information regarding the determination of the number of contributors, profile suitability, review of diagnostics, and reporting of Identifiler Plus data will be the same as outlined below.

9.7.1 Determination of the Number of Contributors to the Evidence Profile

The assignment of the number of contributors to a profile must be made by the analyst prior to the comparison of the profile to any reference samples. The potential of peaks to be stutter should be considered. The number of contributors chosen for the analysis should be the most likely number required to reasonably explain the observed profile(s) which will be analyzed in STRmix.

Sometimes the number of contributors may be unclear. This could be because the profile is complex and may contain low-level indications of additional contributors, is too limited, or because case circumstance suggests that the possibility of additional contributors exists such as when it may be reasonable to assume the presence of a contributor. The analyst will use their professional judgment when assessing the number of contributors, and may take into account sub-analytical threshold (AT) peaks and high stutter.

If ambiguity exists in determining the number of contributors (e.g. three vs. four contributors), provision is made for an analyst to perform multiple interpretations of the DNA profile using the assignment of differing numbers of contributors without the use of reference standards (unless being used for conditioning). After review of the STRmix output, and prior to comparison with the reference profiles, it may then be determined that a
deconvolution does not conform to scientific expectation. The review of the STRmix output and course of action must be thoroughly documented in the case file. The STRmix output of any deconvolution not being reported will be retained in the case file along with documentation as to why it will not be used for reporting. Alternatively, an analyst may choose to report multiple interpretations if they produce viable deconvolutions and diagnostics.

Additional amplifications may be performed to confirm the presence or absence of drop-in/drop-out peaks to aid in the determination of the number of contributors.

**Method for Assigning the Number of Contributors for a Profile**

When assigning the number of contributors to a profile, the profile in its entirety will be reviewed. If replicate amplifications have been undertaken, all qualifying replicate profiles will be reviewed when determining the number of contributors (refer to *Replicates* section below).

The following steps will be followed in assigning the number of contributors to a profile:

1) Review the profile as a whole, assessing the level of degradation, presence of low-level peaks, noisy or clean baseline and general quality (template) of the profile.

2) Find the locus with the highest number of unambiguous allelic peaks. If the number of allelic peaks present is an odd number, add 1. Divide this final number by 2 to obtain the initial proposal of the number of contributors to the profile.

3) Review peak height imbalances at the most informative locus (typically having the greatest number of alleles). Taking into account allele sharing/stacking, visually attempt to “pair” alleles using the peak height ratio expectations (weaker sister allele). In the event there is too much imbalance between alleles, this may indicate the presence of an additional contributor above the value obtained by allele count alone.

4) If data is in the stochastic range, review the electropherogram for sub-analytical threshold (AT) peaks and high stutter, which may indicate the presence of additional contributor(s).

5) If one or more contributors at this locus is either trace or a clear major, check that this pattern is represented at other loci.

6) Apply the general pattern of contributors (number and proportion) to other loci in the profile. If it holds, assign this number of contributors to the profile. Otherwise consider the addition or subtraction of one contributor.

The assumed number of contributors will be documented on the STRmix electropherogram, to include whether there is an indication of male DNA being present. Documentation will include reference to the specific loci used to determine the number of contributors when applying steps #2, #3, and #4 above for mixture profiles. These same loci will be utilized to manually evaluate the mixture proportions, weights, and genotype combinations (to include the expectation for genotypes containing allelic drop-out) after the STRmix run.

All DNA profiles interpreted in STRmix must contain at least one locus which may be manually evaluated for mixture proportion, weights, and genotype combination expectations. Stochastic DNA profiles containing loci with sub-analytical threshold data indicative of a mixture may be evaluated for mixture proportions, when possible. For these
stochastic profiles, the weights and genotype combinations are expected to demonstrate an increased uncertainty in the genotypes of the contributors.

Notes:
- Discriminating loci such as FGA, D2S1338, D1S1656, and D12S391, and SE33 (where applicable) are likely to be the most informative when determining the number of likely contributors to a profile. Amelogenin and DYS391 may also be used.
- DYS391 will be used to increase the number of male contributors above “at least one” under the following conditions:
  - Two (or more) peaks are present which do not fall into stutter positions.
  - Two (or more) peaks are present and the peak in the N-4 stutter position is at least 20% or greater than the peak height of the other peak and the peak in N+4 stutter position is at least 15% or greater than the peak of height of the other peak.
  - If a peak falls into the N-4 stutter position of a second peak and may possibly be the result of high stutter rather than true DNA, the number of males will not be increased from “at least one”.
  - In the event it is believed that a DYS391 duplication may be present due to the case scenario and the entirety of the profile data, the analyst must document this observation as the reason why the number of male contributors is not being increased for that particular profile.
- The presence of one or two trace peaks below 225 RFU may indicate the presence of drop-in and may not always require the consideration of an additional contributor.
- Peaks falling within bins below the AT may be useful to indicate the presence of low-level or trace additional contributors. When using the presence of peaks below AT to infer an extra contributor, there must also be low-level peaks below the ST in the profile to support this conclusion.
- Mixtures of DNA with four contributors should be interpreted with care as the possibility of misinterpreting the true number of contributors increases with each additional contributor. Mixtures which appear to contain five or more contributors will not be interpreted.
9.7.2 Replicates (Within Same Kit or Inter-Kit using ID Plus and Investigator 24plex QS)
A replicate amplification may help in determining the number of contributors. A replicate is defined as a repeat amplification of the same extract. This may occur within the same amplification kit (i.e., two or more amplifications using Investigator 24plex QS) or using two different amplification chemistries (i.e., ID Plus and Investigator 24plex QS). The replicate may be amplified with different amounts of template DNA or the same amount of template.

The use of replicates during STRmix deconvolution have been demonstrated during validation to improve the sensitivity and specificity of the interpretation. Replicates increase the LR when comparing to known contributors and lower the LR when comparing to non-contributors.

Not all replicate profiles are required to be included in the STRmix interpretation. For example, if a full profile was obtained from one amplification and no further information was obtained from previous or subsequent amplifications, it is not required to be included in the STRmix interpretation. In the event different replicate strategies are utilized within the same case file (e.g., some evidence profiles utilize replicates during interpretation, while others do not even though replicate profiles are available), a note will be made regarding the specific reason for choosing (or not choosing) to use the replicate amplifications for STRmix.

When analyzing replicates in STRmix, the highest likely number of contributors will be used for deconvolution if the replicate amplifications indicate a differing number of contributors being present. The analyst will document on the electropherogram if replicates will be used for deconvolution.

9.7.3 Qualitative Requirements for STRmix Interpretation
STRmix will be used to deconvolute and interpret single source and mixture evidence profiles from two, three, or four contributors.

When multiple consistent single source evidence samples are obtained from the same case with all homozygous loci above the 400 RFU stochastic threshold and all alleles at heterozygous loci above 225 RFU (the drop-in cap used for 3500xl data in STRmix), at least one of the profiles must be interpreted in STRmix. A note must be included which references the Lab Case # and item number of all other items with the same interpretation. The least robust profile containing the most possible uncertainty should be used for STRmix interpretation.

At minimum, STRmix interpretation will be attempted on the following sample types:

- **Single source DNA profiles**: contain at least one allele above the dye-specific analytical threshold at 6 or more loci (not to include Amelogenin or the DYS391 locus)
- **Conditioned mixture DNA profiles**: contain at least one minor/foreign allele above the dye-specific analytical threshold at 6 or more loci (not to include Amelogenin or the DYS391 locus)
- **Non-conditioned mixture DNA profiles**: contain at least one allele above the dye-specific analytical threshold at 8 or more loci (not to include Amelogenin or the DYS391 locus)
Profiles which are determined to be eligible for STRmix interpretation will be documented on the electropherogram.

Exceptions for when STRmix interpretation may not take place or be necessary:
- If there are indications of five or more contributors to the evidence profile
- The data does not meet the qualitative requirements as outlined above for single source, conditioned mixtures, and non-conditioned mixtures
- If the inclusion of a reference standard does not provide probative value in the context of the case (i.e., an individual’s own single source DNA on their intimate body swabs or clothing)
- If carryover is determined to be present between the epithelial and sperm fractions of differentially extracted samples

9.7.4 Manual Comparison of References to Single Source Profiles
With the exception of reasonably assumed contributors, manual comparison of reference standards from the case may be made between evidentiary single source profiles where all homozygous loci are above the 400 RFU stochastic threshold and all alleles at heterozygous loci are above 225 RFU (the drop-in cap used for STRmix 3500xl data). When making a manual comparison, a deconvolution and likelihood ratio calculation may be performed for potentially included individuals in a single STRmix run.

All other comparisons of evidentiary profiles to reference standards will be based on the deconvolution of the item in STRmix. Refer to Determination of Inclusion, Exclusion, and Uninformative with STRmix for additional information.

Single source profiles which are resolved to a weight of 1.00 (or 100%) at all loci will be reported as full single source profiles.

9.7.5 Determination of Inclusion, Exclusion, and Uninformative with STRmix
Only qualifying single source profiles (as noted above) which have been manually compared to a reference standard may be analyzed and have a likelihood ratio calculated within a single STRmix run. In the event a single source profile is determined to be from an unknown contributor, STRmix deconvolution must still be performed, even in the absence of a reference standard.

With the exception of qualifying single source profiles and the use of assumed contributors, STRmix deconvolution will take place prior to, and independently from, the comparison to reference standards and calculation of a likelihood ratio. After deconvolution, STRmix statistical analysis will be used to support the inclusion, exclusion, or uninformative comparison for each individual reference standard.

Alternatively, if after STRmix deconvolution two or more loci have been fully resolved to a weight of 1.00 (or 100%), the genotypes for each contributor at the fully resolved loci may be used to perform a manual comparison to the reference standards for purposes of exclusion. Exclusions are required at two or more of the resolved loci. A note must be made on the STRmix deconvolution to document that the results will be used for manual exclusion.
If the deconvolution contains only one unknown due to either being single source or the use of conditioning, the genotype resolution for the unknown can be found in the “Component Interpretation” section of the report.

When more than one unknown is being considered during deconvolution, the “Weights” section of the STRmix Summary Report may be used to document the resolution of complete genotypes. When applicable, a copy of the relevant pages of the “Weights” exhibiting the full genotype resolutions will be retained as part of the case file.

If a reference standard is manually excluded, a LR will not be calculated. The reference standard will be reported as an exclusion.

In the event a manual comparison of a single locus results in an unexpected exclusion, additional troubleshooting may be required. Refer to the troubleshooting section of Reviewing a STRmix Output.

The following conclusions will be drawn based on the exponents of the calculated LR values:

<table>
<thead>
<tr>
<th>Calculated LR Value</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^4$ or greater</td>
<td>Inclusion</td>
</tr>
<tr>
<td>$10^3$ to $10^{-3}$</td>
<td>Uninformative</td>
</tr>
<tr>
<td>$10^{-4}$ or lower</td>
<td>Exclusion</td>
</tr>
</tbody>
</table>

If a profile is determined to be unsuitable for interpretation before or following STRmix deconvolution, an explanation as to why the DNA evidence is uninterpretable must be documented (too limited or too complex) in the case file and in the report.

Any mixture which exhibits signs that it is from greater than four contributors based on maximum allele count, inconsistencies in peak height balance or percent contribution will be deemed as uninterpretable due to the complexity of the data. No portion of these mixtures will be considered suitable for comparison.

### 9.7.6 Assumed Contributors

In certain circumstances, it may be reasonable to assume the presence of a contributor in a DNA mixture. Examples include:

- Intimate samples
- Reference standards received from consensual partners
- Items documented to have been removed directly from someone’s person
- Clothing items documented to have been provided directly to investigators (e.g., detectives, SA nurses, etc.) by victims
- Environmental samples in which individuals may have habitually touched or handled an item (e.g. bedding, steering wheel of car, items recovered from a domestic location where an individual is documented to reside, etc.)
- Contamination events having a known source (e.g. staff involved in evidence handling, collection, or testing)
- When there is written documentation that the defense has conceded the presence of an individual on an item of evidence
In these circumstances, the DNA profile from the assumed contributor(s) will be used to assist interpretation of the additional contributors’ DNA profile during the deconvolution, when available. Assuming that someone is present in both Hp (prosecution hypothesis) and Hd (defense hypothesis) fixes their genotype during the deconvolution process (essentially forces an LR of 1 at every locus for their portion of the genotype set). This is known as conditioning the deconvolution.

The assumption of any contributor to a mixture must be supported by the data, regardless of case scenario. The assumption of the presence of one or more specific contributors may require the analyst to adjust a previously assumed number of contributors to the mixture once evaluating the data using the assumption.

It is analyst discretion to determine whether an assumption will be made during the interpretation of items documented to have been removed directly from someone’s person when staining is present (e.g. bloodstain on a victim’s shirt removed at autopsy) based on whether there is a potential probative association with that specific item. This determination will be made using the case information available. The reason(s) for the final determination regarding conditioning will be documented within the case file.

**Conditioning the deconvolution for non-environmental samples (to include clothing items documented to have been provided directly to investigators (e.g., detectives, SA nurses, etc.) by victims) and calculating LRs**

If an analyst determines that the data clearly supports the presence of a reasonably assumed contributor, this will be documented and can be used as a basis for using an assumption during STRmix deconvolution. This evaluation will take into account the alleles of the assumed donor being present, LVMPD-validated stratified peak height ratio expectations, apparent mixture ratios, and kit-specific stutter ratio expectations. In addition, information from associated fractions (i.e., epithelial and sperm fractions) of the same sample can be used in this evaluation as a means to assess the possibility of carryover. When the assumed contributor contains low-level alleles with the potential of allelic drop-out/masking, the assumed donor’s alleles will be designated on the electropherogram as a means to demonstrate that the data supports the presence of the assumed contributor.

If, based on the review of the profile data, it is ambiguous as to whether a reasonably assumed contributor may be present (contributor is low-level, etc.), a deconvolution will be performed using STRmix to statistically support whether an individual may be conditioned. The component interpretation contained in the STRmix output will be used to justify the assumption that a specific individual is a contributor to the mixture. Alternatively, an LR supporting the assumption may be performed to the contributor in question and included in the case file. The subsequent LR must be $10^4$ or greater (the conclusive zone) to justify the use of the assumption to re-run the STRmix deconvolution using conditioning. It is analyst discretion as to whether to re-condition a deconvolution using the assumption of a contributor based on an inclusionary LR.

**Conditioning the deconvolution of environmental samples and calculating LRs**

Environmental samples in which individuals may have habitually touched or handled an item (e.g., bedding, steering wheel of car, items recovered from a domestic location where an individual is documented to reside, etc.), a deconvolution will be performed using
STRmix to statistically support whether an individual may be conditioned. An LR supporting the assumption will be performed to the contributor in question and included in the case file. The subsequent unified LR must be $10^4$ or greater (the conclusive zone) to justify the use of the assumption to re-run the STRmix deconvolution using conditioning. It is analyst discretion as to whether to re-condition a deconvolution using the assumption of a contributor based on an inclusionary unified LR.

In the event a unified LR cannot be calculated due to having a difference of greater than one unknown contributor between the Hp and Hd propositions, the environmental sample will not be eligible for conditioning.

If an LR is performed to determine the potential inclusions of non-probative individuals (e.g., victims or consensual partners) for the purposes of conditioning a STRmix analysis, the individual likelihood calculations do not need to be included in the report, but must be available in the case file along with a note that the calculation was used to support conditioning the deconvolution.

### 9.7.7 Setting-up Propositions for the Calculation of the Likelihood Ratio (LR)

The likelihood ratio assesses the probability of the evidence given two alternate propositions or hypotheses; one that aligns with the prosecution (Hp) and one that aligns with the defense (Hd). Hp is typically inclusionary of the person of interest. The defense proposition typically aligns with the person of interest not being a donor to the DNA profile.

Analysis should be set up with the first proposition (Hp in STRmix) comprising the person of interest, known individuals who may be assumed or conditioned, or unknown individuals, up to the number of contributors. The second proposition (Hd in STRmix) should consist of any assumed (or conditioned) individuals and then all unknown individuals up to the number of contributors.

#### Examples

Two-person mixture from intimate swab collected from victim. DNA profile corresponds to victim and suspect.

- Hp: DNA originates from victim and suspect
- Hd: DNA originates from victim and one unknown

Three-person mixture from firearm located at scene. One component corresponds to suspect.

- Hp: DNA originates from suspect and two unknowns
- Hd: DNA originates from three unknowns

Three-person mixture from firearm located at scene. One component corresponds to suspect 1 and another to suspect 2. Three LRs may be calculated:

- Hp1: DNA originates from suspect 1 and two unknowns
- Hp2: DNA originates from suspect 2 and two unknowns
- Hp3: DNA originates from suspect 1, suspect 2, and one unknown
- Hd: DNA originates from three unknowns
9.7.8 Deconvolution of DNA Profiles in v2.6 of STRmix

With the exception of conditioning a reasonably assumed contributor, all qualifying DNA profiles will be deconvoluted in STRmix prior to the input of reference standards and the calculation of a LR statistic. Only single source profiles where all homozygous alleles are above 400 RFU and heterozygous alleles are above 225 RFU may undergo an LR calculation with the included reference standard during a single STRmix run.

IMPORTANT! If using a reference standard that was previously typed using Identifiler Plus to condition a deconvolution of Investigator 24plex QS data, all non-Identifiler Plus loci must be omitted prior to performing the deconvolution. Without this omission, the deconvolution will not be performed in the software. Refer to Dealing with Uncharacterized Variants, Allelic and Chromosomal Abnormalities, Allele Resolution and Technical Issues in STRmix 2.6.

1) Log-on to the STRmix terminals from H:\Forensic Data\STRmix and launch STRmix from the Start Menu in the lower left-hand corner.

2) To begin a new STRmix deconvolution and analysis, click “Interpretation” from the Main Page.

3) Enter the Lab Case # and evidence item number into the “Case Number” and/or “Sample ID” field. Additional notes may be added to the “Sample ID” field such as “DECON” or into the free text “Case Notes” field, including whether conditioning is being utilized during the deconvolution.

   Note: STRmix results folders are prefixed with the information entered into the Case Number field, followed by sample ID, then the date and time in the format yyyy-mm-dd-hh-mm-ss. The Analyst’s initials may also be added prior to the case number to assist in more quickly locating the results of the STRmix run.

4) Enter the “Number of Contributors” corresponding with the profile to be interpreted and click “Next”.

ARCHIVED
5) Select the appropriate kit from the drop-down list next to “Profiling Kit”

<table>
<thead>
<tr>
<th>Name of Kit</th>
<th>Qualifying Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>3500_INV24 (STRmix v2.6)</td>
<td>3500xl QIAGEN Investigator 24plex QS data interpreted in STRmix v2.6</td>
</tr>
<tr>
<td>3130_INV24 (STRmix v2.6)</td>
<td>3130XL QIAGEN Investigator 24plex QS data interpreted only in STRmix v2.6</td>
</tr>
<tr>
<td>IDPlus_3130 (STRmix v2.6)</td>
<td>3130XL Identifiler Plus data interpreted only in STRmix v2.6</td>
</tr>
<tr>
<td>LVMPD Qiagen_Investigator_24plex I</td>
<td>QIAGEN Investigator 24plex QS data previously interpreted in STRmix v2.4</td>
</tr>
<tr>
<td>Identifiler Plus</td>
<td>Identifiler Plus data previously interpreted in STRmix v2.3</td>
</tr>
</tbody>
</table>

6) Evidence input files may be entered by ‘drag and drop’ into the “Evidence profile data window or by navigation to the file path using the “+” button. Multiple profiles may be added at the same time by ticking the selection box to the left of each sample you wish to add.

   a. If adding profiles by navigation, select the “+” button and then choose “Select” to navigate to the input file. Select “Add Profile Data” to add the input file and “Confirm”.
b. To remove a profile, highlight the selected input file and select the (-) button. 

Note: Evidence input files will automatically be appended with “_EV” in STRmix.

c. Refer to Dealing with Uncharacterized Variants, Allelic and Chromosomal
Abnormalities, Allele Resolution and Technical Issues in STRmix 2.6 in the event 
loci will be ignored from the deconvolution.

7) If adding DNA profiles from multiple amplification kits, select the “+ Use Multiple 
Kits” button from the right-hand side of the “Add Profile Data” window and choose a 
second kit from the drop-down menu and “Select”

a. Add the evidence files by drag and drop or navigation as described above.

8) In the “Reference Profile Data” box, add reference input files by drag and drop or using 
the navigate buttons as described above. 

Note: Reference input files will automatically be appended with “_REF”

a. Within STRmix v2.6, reference files are not associated with a kit. If you select a 
.csv input file (where loci are defined generically by locus numbers and not by 
names), the software will prompt you to select the relevant amplification kit. 
Highlight the desired amplification kit and choose “Select”.

9) Reference input files will automatically be assigned as known contributors under Hp. 
Should conditioning be required, select the reference of interest and then tick the Hd 
box.

10) Click the check mark for “Perform Database Search”
11) Select “Start” to proceed with the interpretation

![STRmix Interpretation](image)

12) When “Start” is selected, STRmix will start the calculation. A Calculation Progress window will open and the progress may be visualized in the progress bar. When the calculation is finished, a summary of the findings of the analysis will automatically open (unless using Batch Mode). The results of the run are saved automatically. The results folder may be opened by selecting the “Open Interpretation Results Directory” icon: at the upper right-hand corner

![Open Interpretation Results Directory](image)

The results folder will contain the following information:
- AlleleFreq folder containing each allele frequency used with the analysis, where applicable
- Extended output folder containing size regression information and any extended outputs, where appropriate
- Inputs folder containing all input files saved in the STRmix format (appended with _EV or _REF)
- Kits folder containing all kit files used with the analysis
- Log folder containing the Interpretation output which details all information printed within the calculation progress window
- Reports folder containing the .pdf report and reporting elements
- Stutters folder containing all stutter and size regression files used within the analysis
- A config.xml and config_input.xml file containing all run information
• The Component Interpretation containing all accepted genotypes and weights per contributor
• A Results.txt file containing result information including the ten genotype combinations at each locus with the highest weights
• A results.xml file containing result information

Refer to STRmix Summary Reports for additional explanation of the report.

A second “DBSearch” folder will be created at the same location, which contains the following information:
• AlleleFreq folder containing each allele frequency used with the analysis
• Inputs folder containing all input files saved in the STRmix format (appended with _EV)
• Log folder containing the Database Search output which details all information printed within the calculation progress window
• Populations folder containing the population file used in the database search
• Reports folder containing the .pdf report and reporting settings
• A config.xml and config_input.xml file containing all run information
• A DBSearchResults.txt file containing database search information and any matches identified above the cut-off
• A results.xml file containing result information

13) Select “Finish” to return to the main window.

9.7.9 Calculating an LR from a Previous Analysis

Note: Refer to the section for Manual creation of .txt files for unidentified contributors, DNA profiles of reference standards processed from outside vendors, and surreptitious standards, if applicable.

1) Select “Investigation” from the Main Page and then select “LR from Previous”

2) Select “Browse” to navigate to the config.xml file from the desired interpretation results folder or drag and drop the folder directly into the “Previous Interpretation” field

3) The Sample ID will automatically be appended with “-LRPrev”. Add the Item number which will be compared during the LR calculation. Select “Next” to proceed
4) Add reference input files by drag and drop or using the navigate buttons as described above

**Note:** Reference input files will automatically be appended with “_REF”

a. Within STRmix v2.6, reference files are not associated with a kit. If you select a .csv input file (where loci are defined generically by locus numbers and not by names), the software will prompt you to select the relevant amplification kit. Highlight the desired amplification kit and choose “Select”

b. Refer to Dealing with Uncharacterized Variants, Allelic and Chromosomal Abnormalities, Allele Resolution and Technical Issues in STRmix 2.6 in the event loci will be ignored from the LR calculation

5) Select “Start” to begin the LR calculation. The report will automatically be generated at the completion of the run.

### 9.7.10 Batch Mode

Within Batch Mode, a number of different STRmix analyses may be queued to run sequentially. There is no need for the interpretation to utilize the same kits, settings, or number of contributors. A single batch may contain interpretations and LR from Previous calculations

4.12.1 Select “Batch Mode” from the Main Page

4.12.2 Select “Add to Batch” drop-down list and select the desired interpretation strategy (Interpretation, LR from Previous, or LR Batch)
• **Add Interpretation**: Performs the deconvolution of a single evidence sample

• **Add LR from Previous**: Performs the comparison and LR calculation to a single evidence sample

• **Add LR Batch**: Allows the user to calculate multiple likelihood ratios from multiple reference inputs to a previously run deconvolution and vice versa. The kits do not need to be the same for each deconvolution and reference sample (Refer to **Add LR Batch** below)

4.12.3 Complete the analysis set-up. On completion, you will return to the Batch Mode window and the case details will be entered in the Calculations in Batch window.

4.12.4 Select “Add to Batch” to enter the next workflow. Repeat for as many analyses as needed. **Note**: Selecting the STRmix logo or the back arrow (<) will return you to the main directory window. The batch details will be saved in the queue, even upon exiting STRmix as long as you do not log off of the STRmix terminal.

5) Select “Start” to begin the batch run. Selecting “Stop” at any time will stop the current interpretation and return the user to the Batch Mode window. The batch details are saved and the batch may be started again at a later date.

**Add LR Batch**

1) Select “Add LR Batch” from the dropdown

2) Drag and drop the run folder or folders of interest into the “Interpretations” box on the left side of the screen or navigate to the config.xml file of the deconvolution you wish to add using the “+” button
3) Drag and drop the reference standard(s) you wish to compare into the “Reference Profiles” box on the right side of the screen or navigate to the reference input file by using the “+” button.

4) Select “Start”. The Batch Mode screen will re-appear. Select “Start” to begin analysis. An LR will be calculated for each reference sample entered to each deconvolution entered. The results will be saved as the case name (of the deconvolution)–sample name–LRPrev_reference profile sample name.
5) Results for each deconvolution comparison with the reference standard will include Comments details the samples being compared

---

**LR From Previous Report**

<table>
<thead>
<tr>
<th>DETAILS</th>
<th>RUN PARAMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>STDMIX VERSION: STRmix 2.2.3</td>
<td>CONTRIBUTORS:</td>
</tr>
<tr>
<td>USER: jj1418k</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL RUN TIME: Seconds</td>
<td>SAMPLE FILE: P119 2646 Item 3.06, REF.csv</td>
</tr>
<tr>
<td>REPORT RUN: Sep 24 2019 15:55:36</td>
<td>KNOWN CONTRIBUTORS UNDER MR:</td>
</tr>
<tr>
<td>CASE NUMBER: P119 2564</td>
<td></td>
</tr>
<tr>
<td>SAMPLE NAME: Item 3-LRrev_P119 2646 Item 1, 09</td>
<td></td>
</tr>
<tr>
<td>COMMENTS: LR calculated comparing to REF P119 2646 Item 1, 09, REF.csv</td>
<td></td>
</tr>
<tr>
<td>LR calculated from previous Interpretation in Y:\STRmix\STRmix_Results\Folder STRmix_1.1.3277 P119 2646 Item 1, 09, REF.csv</td>
<td></td>
</tr>
<tr>
<td>SR ID: 3232087</td>
<td></td>
</tr>
</tbody>
</table>

**9.7.11 Review of STRmix Summary Reports**

Upon completion of each STRmix analysis, a summary report is automatically generated and saved in .pdf format within the portal-specific subfolder at Y:\STRmix\STRmix_Results. The Interpretation Report and/or LR from Previous Report includes the following default sections, as applicable:
## Section Heading | Function
---|---
Details | STRmix version, user, date and time of analysis, total run time, report version, date report run, case number, sample ID, comments, and seed
Run Parameters | Number of contributors and profiling kit used for deconvolution, name of sample file, known contributors utilized under Hp and Hd (if applicable)
Summary of Contributors | Template, mixture proportions, degradation, and contributor order giving the highest LR, if applicable
Post Burn-In Summary | Run diagnostics associated with deconvolution
Variance Charts | Graphical representation of the allele variance and individual stutter variances utilized during the deconvolution
Locus Efficiencies | Graphical and numerical representation of the locus-specific amplification efficiencies utilized during deconvolution at the AT (Detection Threshold)
Evidence Input File(s) | List of the alleles, heights, and sizes per locus contained in the STRmix input file for evidentiary samples used during deconvolution
Reference Input File(s) | List of genotypes per locus contained in the STRmix input file for reference samples using during the deconvolution and/or calculation of an LR
Component Interpretation | Summary of the most likely genotype combinations for each individual contributor, whether the contributor is an Assumed or Questioned contributor, the weight of the genotypes, and alleles which achieved a weighting of ≥ 100%
Contributor Interpretation Summary ≥ 100% | Summary of the obligate alleles and/or genotypes which achieved a weighting of ≥ 100% for each contributor during deconvolution
Summary of LR | Displays the summary of LR table, including the LR settings applied, and the unrelated (99% 1-sided lower HPD interval), biological relationship, and unified LRs
Per Locus Likelihood Ratios | Breakdown of LR calculations per locus and population group for unrelated individuals and reported as sub-source (Factor of NI), sub-sub source (previously the point estimate), and 99% 1-sided lower HPD interval

**Retroactive creation of STRmix Summary Reports**
In addition to the default report settings noted above, the following additional sections are available to be included in a report which is created retroactively:

| Section Heading | Function |
---|---|
Settings | Records all run settings used in analysis, including all settings associated with the case, MCMC, kit, profile, LR, and performance
Additional Kit Details | Records all kit settings used in the analysis including stutter and regression files, if applicable
Weights | List of all genotype combinations considered for each contributor and their weight per locus as determined during deconvolution
Interpretation Details
Details pertaining to the previous interpretation if undertaking an LR from previous

In order to create a new summary reports for a previously run deconvolution or LR calculation:
1) Select “Reports” from the Main Page

2) Drag and drop the run folder of interest in the “Previous Calculation” window or navigate to the config.xml file of the analysis you wish to add using the “Select File” button

3) Where appropriate, select the components to be included in the report by checking them on or off. Only components relevant to the type of STRmix analysis will be available for configuration in the report

4) In the “Output File” field, modify the name the report will be saved under, if desired. If this field is not modified, the software will prompt the user regarding whether they wish to save over the previously generated default report

5) Select “Run” to generate the report

Primary Run Diagnostics
The review and assessment of the primary run diagnostics will provide an indication as to how well STRmix has been able to describe the observed evidentiary data during deconvolution. Each of the following diagnostics must be evaluated using the locations within the profile with the most
information (i.e., the greatest number of alleles present or where a known contributor has been conditioned). In the event that the quality of the profile is such that these evaluations cannot be made and compared against the STRmix output, the profile will be deemed unsuitable for STRmix analysis.

- **Review of Mixture Proportions**
  Where possible, the mixture proportions generated by STRmix must be assessed for their intuitiveness in comparison to the observed electropherogram. The percent contribution of the contributors can be calculated by applying the following formula:

  \[
  \text{Contributor percent contribution} = \frac{\sum \text{RFU values of the contributor alleles at the locus}}{\sum \text{RFU values of all alleles at the locus}}
  \]

  There may be times when stochastic DNA profiles contain sub-threshold data that is indicative of a possible mixture. These loci may be assessed to ensure the mixture proportions are appropriate given the quality and expected variability of the data present. It is important to maintain perspective of the variability in peak heights/peak height ratios associated with stochastic data when utilizing loci with sub-threshold peaks for the evaluation of mixture proportions.

- **Review of Weights and Genotype Combinations**
  The weights and genotype combinations generated by STRmix must be assessed for their intuitiveness, where possible. The distribution of weights across the genotype combinations should be similar as to what the analyst would assign manually based on pairing together alleles using the LVMPD-validated stratified peak height ratio expectations and mixture proportions.

**Secondary Run Diagnostics**
There are numerous diagnostics in the summary output of STRmix that may indicate that an interpretation has not completed as expected. These are found in the “Post Burn-in Summary” in the Interpretation Report. An example is provided below. This information will be reviewed by the analyst to ensure the results of the deconvolution are conforming to expectation.

**POST BURN-IN SUMMARY**

<table>
<thead>
<tr>
<th>Total iterations</th>
<th>Effective sample size</th>
<th>Acceptance rate</th>
<th>log(likelihood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,472,927</td>
<td>19,252,35</td>
<td>1 in 11.18</td>
<td>23.69</td>
</tr>
<tr>
<td>1.14</td>
<td>3.992</td>
<td>10.251</td>
<td>16.778</td>
</tr>
<tr>
<td>8.652</td>
<td>6.999</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **log(likelihood)**
  This value shows the average log(likelihood) for the entire post burn-in during deconvolution. This is the log of the average likelihood (or probability) value created at each of the post burn-in iterations. The larger this value, the better STRmix has been able to describe the observed data. A negative value suggests that STRmix has not been able
to describe the data very well given the information it has been provided (input file, number of contributors, stutter files, etc.).

Reasons why this value may be low or negative include:
- The profile is low-level and there is very little data making up the likelihood.
- The number of contributors is incorrect and therefore STRmix must consider forced stochastic events to explain the profile (e.g., large heterozygote peak imbalances or variation in mixture proportions across the profile).
- True data has been accidentally removed during GeneMapper ID-X analysis, particularly stutter peaks, and must now be described in STRmix by drop-out.
- Artifactual peaks have been accidentally left labeled and must now be accounted for in STRmix by drop-in.

A low or negative value for the average log(likelihood) may indicate that the analysis requires additional scrutiny.

Note: High quality mixture DNA profiles are likely to give higher average log(likelihood) values than high quality single source profiles. Therefore, low average log(likelihood) values alone are not necessarily an indicator of an issue.

- Gelman-Rubin Convergence Diagnostic
  This diagnostic informs the user whether the MCMC analysis has likely converged. STRmix uses eight (8) independent chains to carry out the MCMC analysis and ideally each chain will be sampling in the same space after burn-in.

  The following are reasons why the chains may have not converged during analysis:
  - Problematic GeneMapper ID-X analysis: an artifactual peak was accidentally left clicked on, a true allelic peak was accidentally removed, or smoothing/poor single bp resolution has resulted in the omission of a peak that STRmix expected to find in the input file.
  - The assumed number of contributors is incorrect.
  - The assumed contributor was incorrectly assigned.
  - The complexity of the profile exceeds the capacity for STRmix to effectively describe the data.
  - The analysis has not run long enough for the chains to converge in the same space.

  Whether or not the chains have spent time in the same space can be gauged by the within-chain and between-chain variances. These two variances are used to calculate the variance of what is called the 'stationary distribution'. If all chains have sampled the same space, then the within-chain variance and the variance of the stationary distribution will be approximately equal. If chains have spent time in different spaces, then the variation between the chains is likely to be larger than the variation within the chains. This is calculated by the Gelman-Rubin convergence diagnostic (GR), which is a ratio of the stationary distribution and within-chain variances.

  For a converged analysis, the GR will be 1. If the GR is > 1.2, then there exists the possibility that the analysis hasn’t converged. If the GR value is > 1.2, the results of the analysis will be closely scrutinized as noted above in an attempt to determine if an issue with the data is resulting in the non-convergence.
GR values may be affected by run-to-run MCMC variability and therefore may differ slightly if the interpretation is re-run using the same parameters. The STRmix software is set to “Auto-continue” the interpretation for 50,000 additional post burn-in accepts when the threshold is not met at the end of interpretation. This allows additional time for convergence without user intervention. The analysis will terminate following these extra iterations, regardless of the GR value at that point.

When all other possible reasons for non-convergence have been eliminated, the analysis may be re-run increasing the number of required accepts for both the MCMC and burn-in by a factor of 10 (Burn-in Accepts = 1,000,000 and Post Burn-in Accepts = 500,000) within the “Run Settings” of the “Interpretation” module. Alternatively, there may be times when the Auto-continue GR value will be higher than the GR value following the initial post-burnin period. This may indicate the presence of wandering chain(s) during the MCMC. As such, it is permissible to repeat the MCMC using the original default number of accepts.

Documentation will be maintained in the case file that the data was re-scrutinized to ensure that the above-referenced issues are not present which may have resulted in the non-convergence. This re-examination of the data must take place prior to re-running STRmix using the same parameters or for extended iterations. The original run demonstrating the elevated GR value(s), in its entirety, will be maintained in the case file.

When running using extended iterations, a report must be manually re-generated which includes the “Settings” portion of the STRmix Summary Report. Refer to Retroactive creation of STRmix Summary Reports.
- **Allele Variance and Stutter Variance Constants**
  Each of these values represent the average value for allele variance and individual stutter variance constants across the entire post burn-in period. These values can be used as a guide as to the level of stochastic variation in peak heights that is present in the profile.
  - If the variance constant has increased markedly from the mode of the prior distribution (see below), then this may indicate that the DNA profile is sub-optimal or that the number of contributors is incorrect. Used in conjunction with the average log(likelihood), a large variance or stutter variance constant can indicate poor amplification.
  - If the sample is simply low-level, this may result in a low average log(likelihood) and an average variance constant.
  - If some data has been omitted, left on, or misinterpreted, this may result in a low average log(likelihood) and high variances.
**Total Iterations**

The value displayed in this section indicates the total number of post burn-in iterations that ran during deconvolution. This value, along with the number of accepts chosen for the analysis, can inform the user as to how often a new proposed set of parameters was accepted by STRmix. This is referred to as the acceptance rate.

- **Acceptance Rate**
  The acceptance rate is calculated by dividing the total number of iterations by the required 50,000 post burn-in accepts.
A very low acceptance rate (e.g. 1 in thousands to millions) may, in combination with the other diagnostics, indicate that the analysis needs to be run for additional iterations. However, on its own (and without any other indication of sub-optimal results), a low acceptance rate is not an indication that additional or re-work is required.

- **Effective Sample Size**
  Effective sample size (ESS) is the number of independent samples the MCMC has taken from the posterior distribution of all parameters. A low ESS in relation to the total number of iterations suggests that the MCMC has not moved very far with each step or has had a low acceptance rate. A low absolute value of ESS (e.g. 10s or 100s) will mean that there is potential for a large difference in weights if the analysis were to be run again in STRmix. This will be taken into account during HPD interval generation in any LR calculations (unless the genotype sets are completely resolved on a single combination, in which case there will be no effect of ESS on the HPD interval). A low ESS on its own is not an indication that additional or re-work is required.

**Reviewing the Run Diagnostics**
Each diagnostic will be reviewed in conjunction with the evidentiary profile, weights, and the other diagnostics. The review of the run diagnostics is an important part of the interpretation process. The values in the plots below give an indication of the range of values expected for some of the diagnostics for different contributors based on the LVMPDs internal Investigator 24plex QS validation data.
The LVMPD will ensure that the interpretation of evidence samples is completed, and the results authorized, prior to the comparison to reference samples. This includes a thorough review of the STRmix output file to ensure the results are conforming to expectation and were set-up appropriately. Single source profiles where all homozygous loci are above the 400 RFU stochastic threshold and all alleles at heterozygous loci are above 225 RFU (the drop-in cap used for STRmix) may be deconvoluted and have a likelihood ratio calculated within a single STRmix run. The results of this run will be similarly reviewed and documented to ensure the results conform to expectation and that the run was set-up appropriately.

The analyst will individually place their handwritten initials next to each of the following fields in the deconvolution output file to signify their review and acceptance of the information:

- Run Parameters heading or margin to take ownership of section
- Mixture proportions
- Gelman-Rubin convergence diagnostic(s) – original and Auto-continue, if applicable
- log(likelihood)
- Variance charts to take ownership of section when only single kit used during interpretation; must separately authorize the sets of variance charts when performing multi-kit interpretation
- Settings (if applicable) – authorize bolded changes to default settings (e.g., extended iterations, ignored loci, etc.)
- Evidence Input File(s) to take ownership of section when single kit used for interpretation; must authorize sets of samples separately when performing multi-kit
- Reference Input File(s), if present for conditioning to take ownership of section
• The component interpretation for each contributor (e.g., Contributor 1 (97.00%) and Contributor 2 (3.00%))
  o Ignored loci must be acknowledged and authorized as they will appear blank on component interpretation. Alternatively, it is permissible to manually re-generate a STRmix Summary Report which includes the “Settings”. See above for required authorizations when the Settings section is included in the report.

Documentation will be included in the STRmix output which denotes that the deconvolution has conformed to expectation and is thereby authorized to be used for the comparison of reference standards, and the calculation of likelihood ratios, using the LR from Previous method. In the event a single run was performed for the deconvolution and likelihood ratio calculation from a qualifying single source profile to a reference standard, documentation will be included that the STRmix run and likelihood ratio calculation conformed to expectation.

After review of the STRmix output, and prior to comparison with the reference profiles, it may be determined that the deconvolution does not conform to scientific expectation. This may necessitate additional STRmix runs with adjustments made to the run parameters (e.g., number of contributors, extended iterations, use of conditioning, etc.) or result in the DNA profile being reported as inconclusive. Documentation must be included on the STRmix output regarding the qualitative reasons a profile will be reported as inconclusive after STRmix has been performed.

Upon performing an “LR from Previous”, the analyst will once again document the review of the LR calculation in the STRmix output file to ensure the results are conforming to expectation and were set-up appropriately. The analyst will individually place their handwritten initials next to each of the following fields in the “LR from Previous” output file to signify their review and acceptance of the information:

• Run Parameters heading or margin to take ownership of section
• Settings (if applicable) – authorize bolded changes to default settings (e.g., ignored loci, etc.)
• Reference Input File(s) to take ownership of section
• Most conservative unified LR, if utilizing to establish justification for conditioning an environmental sample
• Review of the locus-level likelihood ratio calculations to take ownership of section

The analyst will designate (e.g., via highlighting, use of asterisks, etc.) the final likelihood ratio value which will be used to populate the report based on the sample type and circumstances (i.e., sub-sub source, 99% 1-sided lower HPD interval, or unified). Likelihood ratios which fall into the inconclusive range or which will be reported as exclusions will be highlighted and documented.

Troubleshooting
It is important when a STRmix analysis is carried out, that the results are interpreted by examining the weightings of various genotypes and the DNA profile(s) observed. There are instances when the results obtained do not intuitively seem correct. Examples of this are:

• Large LRs (greater than 1) are obtained for all loci, except one locus where the LR is 0, and the reference standard is consistent with the evidence profile
• The mixture proportions do not reflect what is observed
• The degradation does not reflect what is observed
• The interpreted contributor genotypes are not intuitively correct
The following may have caused the observed issues:
- The incorrect input file was selected for analysis
- The GeneMapper ID-X project was edited incorrectly
- The number of contributors is incorrect
- The assumed contributor was incorrectly designated
- The PCR has been affected by inhibition
- The MCMC has not run for enough iterations

Should the weights and/or the diagnostics highlight to the analyst that further scrutiny is required, then a number of re-work options may be considered. A review will be conducted of the proposed number of contributors. Additional analytical work such as a re-run or a re-amplification may be performed to strengthen the assumption regarding the number of contributors or to assist with allele designation/sub-optimal amplification performance.

The total MCMC iterations and burn-in may be increased by a factor of 10 if the acceptance rate is low, the ESS is low, and/or the GR value is significantly above 1.2. This should only be performed after all potential sources of the problems have been eliminated by verifying the input file used during analysis, the selected reference standards used for assumptions, the designated number of contributors, etc.

The following table is meant as a quick reference guide to aid with troubleshooting:

<table>
<thead>
<tr>
<th>Observation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>The mixture proportions do not reflect what is observed in the electropherogram AND/OR the degradation does not reflect what is observed in the electropherogram AND/OR the interpreted contributor genotypes are not reasonably intuitive.</td>
<td>Verify that the correct table setting was used to export the samples from GeneMapper ID-X by reviewing the evidence input file(s) to ensure they contain allele, height, and size information.</td>
</tr>
<tr>
<td>A low or negative average log(likelihood).</td>
<td>Re-evaluate the number of contributors used during deconvolution. Consider re-running the STRmix deconvolution using one more or one fewer number of contributors.</td>
</tr>
<tr>
<td></td>
<td>Assess the original DNA profile and Q and S markers to determine if inhibition may be present. Perform a microcon clean-up of the sample and re-amplify.</td>
</tr>
<tr>
<td></td>
<td>Consider re-running the STRmix deconvolution using an increased number of iterations.</td>
</tr>
<tr>
<td></td>
<td>Data has been removed from the GeneMapper ID-X project that is truly allelic and/or stutter. The data</td>
</tr>
<tr>
<td>Issue Description</td>
<td>Resolution</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Artifact peaks have been left labeled in the GeneMapper ID-X project. The peaks must be removed and re-imported into STRmix.</td>
<td></td>
</tr>
<tr>
<td>Data has been removed from the GeneMapper ID-X project that is truly allelic and/or stutter. The data must be re-inserted and re-imported into STRmix.</td>
<td></td>
</tr>
<tr>
<td>The 3500XL and/or GeneMapper ID-X has failed to resolve a single bp peak (either allelic or stutter). Re-amplify or re-load the sample on the 3500XL to attempt to resolve the smoothing. If unresolved, re-run the STRmix deconvolution ignoring the locus with the failed single bp resolution.</td>
<td></td>
</tr>
<tr>
<td>Gelman-Rubin value is &gt; 1.2.</td>
<td>Consider re-running the STRmix deconvolution using an increased number of iterations.</td>
</tr>
<tr>
<td>The 3500XL and/or GeneMapper ID-X has failed to resolve a single bp peak (either allelic or stutter). Re-amplify or re-load the sample on the 3500XL to attempt to resolve the smoothing. If unresolved, re-run the STRmix deconvolution ignoring the locus with the failed single bp resolution.</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td></td>
</tr>
<tr>
<td>The evidence profile contains saturated data that is not being modeled appropriately in STRmix. Re-amplify or re-load the sample on the 3500XL using less template to attempt to resolve the saturation. If unresolved, re-run the STRmix deconvolution ignoring the locus with the problematic saturation.</td>
<td></td>
</tr>
<tr>
<td>Multiple diagnostics display sub-optimal results.</td>
<td></td>
</tr>
<tr>
<td>Once all other actions have been exhausted, consider deeming the profile inconclusive due to the complexity of the data.</td>
<td></td>
</tr>
<tr>
<td>The STRmix run does not progress at the MCMC burn-in stage and the chains do not move. Error message also received that states Degradation started at “0”.</td>
<td></td>
</tr>
<tr>
<td>Check “allele per locus” setting for both the evidence and reference input files is set to 20.</td>
<td></td>
</tr>
<tr>
<td>The STRmix run does not progress past the MCMC burn-in stage and the log likelihood are listed as Infinity and/or NaN.</td>
<td></td>
</tr>
<tr>
<td>The number of contributors designated is too few and must be increased.</td>
<td></td>
</tr>
</tbody>
</table>

**Retention of Unreported STRmix Runs**

STRmix output files will be maintained in the case file when the diagnostics indicate the interpretation ran appropriately, however the review of the output results in the analyst deciding to perform additional STRmix analyses or lab work. Examples include when review of the STRmix output indicates the necessity to run for extended iterations, the originally assigned number of contributors may be incorrect, the decision to perform a replicate amplification, or the decision to perform an additional deconvolution with the use of conditioning. The original STRmix output file, in its entirety, will be maintained in the case file. The first page of the output file will be marked as data not used, along with the reason the run is not being reported, and the date and initials of the analyst rejecting the run. These files will be designated as “DNR” within the STRmix Run Files. This designation may be made by adding DNR to the file name or by segregating these files into a case-specific DNR folder.
9.7.12 Dealing with Uncharacterized Variants, Allelic and Chromosomal Abnormalities, Allele Resolution and Technical Issues in STRmix

The basis for STRmix deconvolution is that the expected height of a peak of a certain size, at a given locus, can be determined given a set of mass parameters (to include the amount of DNA template, degradation, and locus-specific amplification efficiency). In the event biological factors impact the peak height of an allele in an evidence sample, or if there are issues with the designation of the appropriate allele call in GeneMapper ID-X of an evidence sample, STRmix is unable to appropriately model the data. Examples include:

- Primer binding site mutations
- Tri-alleles
- Somatic mutations
- Null alleles
- Saturation
- Technical issues such as loci compromised by potential pull-up or poor single bp resolution. The poor single bp resolution may occur between two true peaks (ex. 9.3,10 at TH01), or between a stutter peak and a true peak.

If it is suspected that the evidence data is affected by one of the above examples, the locus should be ignored while adding the STRmix input files to an “Interpretation” or “Investigation”

**Note:** Observed peak heights within an electropherogram may be saturated if they are above the 28,000 RFU saturation threshold denoted in STRmix. When saturated, the peak’s height is not accurately captured and therefore the observed stutter peak height calculated from the observed allele will be smaller than its expected value. STRmix can implement an alternative model for expected stutter when the corresponding expected allelic peaks are saturated, however the locus may also be ignored.

1) Select the kit associated with the evidence profile from “Profiling Kit”
2) Select “Kit Settings” and then the tab for “Loci”
3) Select the loci that you wish to ignore for the particular run by ticking the box in the “Ignore?” column and then select “Apply”
4) Continue with the rest of the profile set up as described above
When using the ignore locus function, all reference profiles must be manually compared to the omitted loci. A record will be made within the “Case Notes” box of the STRmix run (i.e. the “Comments” field of the STRmix output file) specifying the locus omitted and the reason for the omission. Alternatively, documentation regarding why a locus was omitted may be included on the electropherogram. In addition, the omitted locus will be acknowledged and documented in the Component Interpretation of the deconvolution or in the “Settings” section of the STRmix Summary Report.

**IMPORTANT!** If using a reference standard that was previously typed using Identifiler Plus to condition a deconvolution of Investigator 24plex QS data, all non-Identifiler Plus loci must be omitted prior to performing the deconvolution. Without this omission, the deconvolution will not be performed in the software.

**Note:** In the event a sample is single source, it is not necessary to ignore a locus when an unresolved stutter peak is present on the shoulder of a true allele peak. The stutter variance may be elevated for the STRmix run, however it should not affect the ability for STRmix to appropriately assign the true genotype at the affected locus. This will be verified by the analyst in the review of the STRmix output.

**Note:** Mixture profiles with poor single bp resolution at a locus must be evaluated to determine whether the peak height of the unresolved allele may be considered as allelic within the profile and whether the weights of the deconvoluted genotypes are appropriately considering the possibility of a Q-allele. If the STRmix deconvolution at the affected locus does not conform to expectation, the deconvolution must be re-run omitting the locus.

**Note:** Mixed samples which exhibit saturated data may be re-amplified using less input DNA or may be diluted with formamide and re-loaded in an attempt to resolve the
saturation issues. Alternatively, loci exhibiting saturated data may be omitted from the STRmix analysis utilizing the procedure below. No more than one locus per color channel may be omitted due to saturation. Saturated single source samples which do not exhibit any indications of containing a mixture may be interpreted with caution. The STRmix output file will be critically evaluated to ensure the deconvolution conforms to expectations.

There may be situations where it is not possible to identify the above abnormalities in the evidentiary profile prior to deconvolution and the unmasking of reference profile data (e.g. failure to recognize possible tri-allelic patterns or primer binding site mutations in mixture DNA profiles). If, after unmasking information contained within a reference standard, it appears that the original deconvolution may be affected by the presence of these abnormalities, the number of contributors used for interpretation will be re-evaluated to ensure it is still appropriate given the new information. If yes, the STRmix output will be re-verified to ensure the results are intuitively correct given the expected mixture proportions and genotypes.

Once verified, the affected locus may be ignored during the “LR from Previous” calculation. Documentation must be included in the case file as to why the locus is being omitted from the LR calculation, to include documentation that a manual comparison of the reference standard to the evidence at the omitted locus does not result in an exclusion.

In the event the number of contributors changes as a result of the unmasking, the STRmix deconvolution will be re-run using the appropriate number of contributors and the affected locus will be omitted from interpretation. Documentation must be included in the case file as to why the locus is being omitted from deconvolution. The results of the original interpretation will be retained in the case file, along with documentation as to the reason it is being rejected.

### 9.7.13 Off-Ladder Alleles and OMRs

Non-numeric values such as OL, < or >, or OMR (outside marker range) are not permitted within the STRmix input files. Unambiguous alleles (to include confirmed OLs) must appear in the corresponding input file as their actual allelic size designation.

**Note:** Peaks in stutter positions of unambiguous alleles which do not fall into bins must be manually designated as their actual allelic size designation prior to export from GeneMapper ID-X. These OL peaks do not require confirmation.

Alleles labeled OMR must be manually assigned in GeneMapper ID-X to the locus where they belong, when possible. OMRs will be treated as off-ladder alleles and require confirmation. To assign an OMR to a marker in GeneMapper ID-X:

- Click on the locus header on the electropherogram to select the locus where the OMR is to be assigned.
- Click on the OMR peak to select it and then right click and select “Add allele label”.
- Manually add the allele call to the box. The marker name will be displayed where the peak will be assigned.
In the event a < or > allele or OMR falls more than two repeat units from the end of the ladder, the locus must be removed from the deconvolution using the “Ignore Locus” option above due to the possibility that the alleles are not being sized accurately. In the event it cannot be determined which locus an OMR belongs to, both loci will be removed from the deconvolution using the “Ignore Locus” function.

If a LR is calculated, the reference standard must be manually compared at all ignored loci to ensure the locus does not result in an exclusion.

Note: In certain circumstances, the stutter product for an OMR allele may appear as an allele assigned to the neighboring locus. Due to limitations of the GeneMapper ID-X software, it is not possible to re-assign the locus for the stutter peak to accompany its parent allele when the OMR allele ultimately is not associated with this same locus. Seek assistance from the DNA Technical Leader how to best address this rare occurrence for STRmix analysis.

- Example: The 13-allele at the D12S391 locus of QIAGEN Investigator 24plex QS profile will appear as OMR allele between D1S1656 and D12S391 while its stutter will appear as 19.3-allele at D1S1656. The OMR allele can be assigned as 13-allele of the D12S391 locus, however, the 19.3 cannot be reassigned. Moreover, a similar OMR allele determined to be 20.3 at D1S1656 can be assigned manually to accompany its stutter.
- Due to stutter filters not being applied to OMR peaks, the user must manually determine whether the stutter peak can be deleted from the locus-specific stutter filtered GeneMapper ID-X project based upon the stutter filter settings of the assigned locus.

### 9.7.14 Increasing the Maximum Degradation Setting in STRmix v2.6

The default maximum degradation setting in STRmix is set to 0.01. This setting constrains the amount of degradation that may be proposed per contributor during the MCMC process as a means to ensure the deconvolution can progress in an efficient manner.

Some samples which exhibit extreme degradation may benefit from the increase of the maximum degradation setting to 0.1. This may occur following a deconvolution with unintuitive results when utilizing the default setting or prior to the initial deconvolution when authorized by the second read technical reviewer.

1) After adding an evidence sample to the STRmix deconvolution, select “Kit Settings"
2) From the “General” tab, manually change the “Maximum Degradation” value from 0.01 to 0.1 and select “Apply”. This change will only apply to the current deconvolution run.
9.7.15 Manual Database Search in STRmix v2.6

1) Click on the “Investigation” tab and then “Database Search”
2) Drag and drop the entire deconvolution results folder into the “Previous Interpretation” field or manually browse to the config.xml file from the desired interpretation results folder
3) The Database file and all associated settings will automatically load. In the event a database is not already loaded, select “Browse” and link the .csv file contained in the folder that opens.
4) Click “Start” to begin the search

5) A warning will appear that allele frequencies for DYS391, QS1, and QS2 are 0.0 in the NIST_Investigator_Cauc.csv file. Click OK.

6) The database search will progress and the results will be saved in a new DBSearch folder as described below.
9.7.16 Review of Database Search Reports in STRmix

The LVMPD utilizes a database of known elimination standards loaded into STRmix which are routinely searched with every deconvolution performed. All DNA profiles contained in this database include complete information at all expanded CODIS core loci. The DNA profiles have been broken into four general categories:

- LVMPD: Active LVMPD employees
- OJ (<location>): Employees of a specified outside jurisdiction
- VENDOR: An individual who proved a service of some kind to the DNA Laboratory
- RETIRED: Retired or former LVMPD employees

When available, employees will be identified in the database using their P# and initials. Vendors or individuals who do not work for the LVMPD will be identified using their initials and an identifying characteristic (e.g. MT_QIAGEN). Following deconvolution, every DNA profile is compared with the active database and likelihood ratios are calculated for every person in the database using the following propositions:

- \( H_p \): The DNA originated from the database individual and \( N-1 \) unknown individuals
- \( H_d \): The DNA originated from \( N \) unknown individuals

The database search utilizes a theta value of 0.01, the sub source correction, and the NIST Caucasian allele frequencies. The LR calculations do not take into consideration sampling uncertainty.

Upon completion of each STRmix analysis and database search, a DBSearch report is automatically generated and saved in .pdf format within the portal-specific DBSearch subfolder at Y:\STRmix(STRmix Results). The Database Search Report includes the following default sections, as applicable:

<table>
<thead>
<tr>
<th>Section Heading</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Details</td>
<td>STRmix version, user, date and time of analysis, and date report run</td>
</tr>
<tr>
<td>Database Search Setup</td>
<td>Interpretation chosen for search, profiling kit, sample file, name of database searched, number of individuals in the database, minimum LR, population file used, type of search, the theta value, whether sub-source LRs used, and the final search ID</td>
</tr>
<tr>
<td>Previous Interpretation Details</td>
<td>All information related to the original deconvolution used for the database search</td>
</tr>
<tr>
<td>Data Validation</td>
<td>Number and list of loci not included in the database search</td>
</tr>
<tr>
<td>LR Results</td>
<td>Case number, sample ID, gender, and LR associated with any database hits above the defined threshold</td>
</tr>
</tbody>
</table>

The threshold associated with the LVMPD database is set to 10,000 which corresponds with the bottom of the conclusive range. Only LR results \( \geq \) this value will be returned in the DBSearch report.
In the event a database search returns an individual with an LR above the threshold, the analyst will perform the following investigation:

1) Utilize the “Current P Number list” which can be found on the Criminalistics Bureau’s SharePoint site under “Shared Documents” to determine the identity of the individual
   • In the event the individual does not have a P#/initials, a written request will be made to DNA Management for the identity of the individual

2) OnBase and/or P1 will be utilized to determine whether the individual had reasonable access to the evidence during the course of their routine duties. This information will be utilized to determine whether the LR is potentially adventitious or contamination based on the type of evidence, case processing, quality of the DNA profile, number of contributors, and the strength of the LR

3) The disposition of all potential matches (contamination or adventitious) must be reviewed and approved by DNA Management

**Adventitious matches:**
If a match is determined to be adventitious, the following statement will be handwritten, dated, and initialed, on the DBSearch report by a member of DNA Management:
   “Adventitious match due to <high order mixture or low template amounts>. Data ok to report.”

The DBSearch report will be retained with the sample deconvolution and paginated in the case file. The sample will be reported as normal without additional documentation.

**Potential contamination:**
In the event it is believed that contamination may exist, the DNA profile of the individual identified by the database search will be utilized to perform an “LR from Previous” calculation to verify that the LR is still greater than the database threshold when taking into consideration the additional MCMC and sample uncertainty. Refer to Manual creation of .txt files for unidentified contributors, DNA profiles of reference standards processed from outside vendors, and surreptitious standards.
   • If the LR is still above the database threshold, the analyst will attempt to troubleshoot the origin of the contamination by working backwards through the DNA analysis process. Samples will be re-extracted, as necessary
   • In the event re-extraction is not possible or the contamination is confirmed to be present on the evidence item itself, the DNA profile of the identified contaminant will be utilized to condition a new deconvolution. Refer to Attributable Contamination for additional information.

DBSearch reports with negative results will be retained electronically in the STRmix Runs Folder with all other samples processed in the case. It is not necessary to include a hard copy in the paginated case file.
9.7.17 Backwards Compatibility and Supplemental Analysis

When receiving new reference standard data which requires comparison to evidence profiles previously interpreted using STRmix v2.3 or v2.4.06, the original settings.ini file will be used to pull the previous interpretation into STRmix.

If comparing Investigator 24plex QS reference data to evidence profile data analyzed using Identifiler Plus, STRmix will automatically re-order the Investigator 24plex QS loci in the reference standard to be compatible with Identifiler Plus. LRs will only be provided for those loci in common between the two kits. In the event multiple reference standards are being considered in a single proposition, the calculated LR will be provided for only the loci in common between all of the references and the evidence profile.

9.7.18 Use of DNA Profile Searcher for Single Source Unknown Profiles Generated during Outsourcing or Prior to the Use of STRmix

The DNA profiles of all members of the Forensic Laboratory, crime scene analysts, sexual assault nurse examiners, detectives, and investigating agents of the LVMPD and other law enforcement agencies who have provided their DNA sample on a voluntary basis are contained within the “DNA Profile Searcher” on the H:drive for comparison to non-STRmix casework, outsourcing, and database samples. In addition, profiles from unattributable contamination events and samples voluntarily provided by visitors to and vendors of the Forensic Laboratory are also contained within this searchable spreadsheet. These profiles may include information at only the original CODIS core 13 loci or the expanded loci.

All unknown single source profiles and distinguishable mixtures from non-STRmix casework which contain component(s) with complete genotypes resolved at least six (6) of the core CODIS loci will be searched in the “DNA Profile Searcher” prior to being reported. Loci containing obligate alleles may be utilized to further refine the search to ensure the identified individual is a possible match. This search will help to ensure that the unknown profile does not belong to a staff member or other attributable contaminant. The completion of the search and the date will be tracked on the processing checklist.

Using the DNA Profile Searcher

- Launch to “++DNA Profile Searcher++” file located at H:\Forensic Data\DNA\OTHER\Staff Index.
- The Searcher defaults to opening with a view of all of the DNA profiles contained within the spreadsheet in Identifiler Plus locus order. To begin searching a possible contaminant profile, choose the first locus where contamination may be occurring and select the first drop-down arrow on the left for that locus.
- Click “(Select All)” to uncheck the box which automatically shows all DNA profiles containing any allele at that locus.
- Click both the box denoting the smallest allele present in the possible contaminant profile and click the box denoting “(Blanks)”. Then Click “OK”.
- Select the second drop-down arrow on the right for the locus to enter the second allele and “(Blanks)”. In the event the contaminant profile is homozygous and thus only contains one allele, only the box for “(Blanks)” should be selected.

Note: Do not search any partial loci where there is a possibility of dropout. Only complete loci should be searched.
• Continue to add the possible contaminant DNA profile information at the additional loci where contamination may have been detected. The “DNA Profile Searcher” will automatically filter out any profile which does not meet the user designated allelic criteria.

**Note:** If more than one profile remains after searching all complete loci, manually compare the alleles at each locus from each of the remaining profiles to the partial loci in the searched profile to further eliminate/narrow down possible sources.

• When closing the “DNA Profile Searcher” program, the user will be asked if they wish to save the changes made to the program. Click “Don’t Save” to exit the program.

• The use of the “DNA Profile Searcher” must be documented on the analyst checklist contained on the casework review form along with the date the search was performed.

9.8 Interpretation of a Contaminated Control or Sample

Acceptable results for the reagent blank, negative amplification control and positive amplification control have been previously described in this manual. There are several types of contamination which can be categorized as either attributable or unattributable. Attributable contamination describes a contamination event where the source of the contamination is known and can be identified. Unattributable contamination describes a contamination event where the source of the contamination is unknown and cannot be identified.

All DNA profiles interpreted by the DNA Laboratory are searched in either the STRmix staff elimination database of DNA Profile Searcher as described above (Refer to Review of Database Search Reports in STRmix and Use of DNA Profile Searcher for Single Source Unknown Profiles Generated during Outsourcing or Prior to the Use of STRmix). In addition, all profiles entered into LDIS are searched against the Elimination/Staff Index prior to upload to SDIS.

The Biology/DNA Detail makes the distinction between three types of attributable contamination:

1) Contamination that occurs within the DNA lab as a result of the DNA examiner’s direct handling of the sample (analyst profile in control or sample and sample-to-sample contamination)

2) Contamination that occurs prior to being processed by the DNA examiner in the DNA lab by someone with reasonable access to the evidence

3) Contamination detected using the “DNA Profile Searcher” and/or Staff Elimination Database for all other individuals

9.8.1 Attributable Contamination

*Contamination by the DNA examiner(s) in the case*

If contamination of any sample or any negative control can be attributed to a DNA examiner’s (e.g. Forensic Scientist, Forensic Laboratory Technologist, etc.) handling of the evidence or samples, the contamination will be documented in a case note and the sample will be re-processed through re-injection, re-amplification, re-load and/or re-extraction if possible. If resolved, the data may be used and the contamination does not need to be mentioned in the report. If the contamination cannot be resolved (i.e., no extract remaining, cannot be re-extracted), a Corrective Action Report will be documented.

If the contaminated sample is a mixture, a new buccal swab will be requested and collected from the DNA examiner for confirmation with the profile in the Staff/Elimination Index. The
known DNA profile of the DNA examiner may be used to condition the STRmix deconvolution in an attempt to further resolve the mixture DNA profile. All data associated with the buccal swab confirmation will be included in the case file. The name of the DNA examiner will not be used in the naming of the Sample ID or listed with the associated with the worksheet documentation. Refer to Assumed Contributors for additional information for conditioning deconvolutions from environmental samples.

A case note will be included in the case file to thoroughly document the circumstances of the case and the course of action being taken. The title of the DNA examiner will be generically identified in the report as an assumed contributor (e.g. Forensic Scientist, Forensic Laboratory Technologist, etc.).

If the entire DNA profile can be attributed to the examiner, the sample will be reported as inconclusive and the source of the contamination will be identified in the report.

Refer to “Contamination Attributable to an Internal DNA Laboratory Processing Event” for reporting statements.

Contamination by an individual with documented reasonable access to the evidence

If contamination of a sample (to include outsourcing samples not previously searched in the “DNA Profile Searcher”) can be attributed to a member in the Elimination/Staff Index who had reasonable access to the evidence outside the control of the DNA analyst (e.g., CSA, authorized individual on scene, etc.), the contamination will be documented in a case note. The individual attributable to the exogenous DNA will be notified that the source of the contamination will be referenced in the report.

When applicable and the contaminated sample is a mixture, a new buccal swab will be requested and collected from the individual for confirmation with the profile in the Staff/Elimination Index. The known DNA profile of the individual may be used to condition the STRmix deconvolution in an attempt to further resolve the mixture DNA profile. All data associated with the buccal swab confirmation will be included in the case file. The name of the individual will not be used in the naming of the Sample ID or listed with the associated with the worksheet documentation. Refer to Assumed Contributors for additional information for conditioning deconvolutions from environmental samples.

The case note will thoroughly document the circumstances of the case and the course of action being taken. The title of the individual will be generically identified (e.g. Crime Scene Analyst, etc.) in the report as an assumed contributor.

If the entire DNA profile can be attributed to the individual, the sample will be reported as inconclusive and the source of the contamination will be identified in the report.

In the event, the individual refuses to provide a new buccal swab, then the contaminated evidentiary profile will be reported as inconclusive.

Refer to “Contamination Attributable to an Individual External to the Laboratory Processing Events” for reporting statements.
All other attributable contamination
If contamination of a sample can be attributed to a member in the Elimination/Staff Index who did not have direct access to the evidence (e.g. maintenance workers, etc.), the contamination will be documented in a case note.

When the contaminated sample is a mixture, an attempt will be made to confirm the DNA profile is correct by reprocessing the original voluntary database sample. The known DNA profile of the individual may be used to condition the STRmix deconvolution in an attempt to further resolve the mixture DNA profile. All data associated with the buccal swab confirmation will be included in the case file. The name of the individual will not be used in the naming of the Sample ID or listed with the associated with the worksheet documentation. Refer to Assumed Contributors for additional information for conditioning deconvolutions from environmental samples.

Refer to “Contamination Attributable to an Individual External to the Laboratory Processing Events” for reporting statements.

Elimination standards submitted for direct comparison
In the event an elimination standard is received from a detective or officer with a request for the direct comparison to evidence profiles developed from the case, the sample will be reported using the results (and statistics, if applicable) of the direct comparison. A case note is not required to be included in the case file.

9.8.2 Unattributable Contamination
Evidentiary profiles processed in-house which are suspected of unattributable contamination should be entered into and compared against profiles contained in the “DNA Profile Searcher” located on the H:drive. If the profile is consistent with a profile in the spreadsheet and/or suspected of being a contaminant, the profile will not be used for case comparisons or entered into CODIS.

Reagent blanks and negative amplification controls will be examined for the presence of any true allelic peaks indicative of a profile above the kit-specific analytical threshold. An attempt should be made to remediate the contamination through re-injection, re-load or re-amplification. If resolved, the data may be used and the contamination does not need to be mentioned in the report, but must be reflected in the analyst’s laboratory notes. If the peaks are not resolved the associated samples will be reported as inconclusive and the contamination will be mentioned in the report. In extreme circumstances, the data may be reported with the documented approval of the Technical Leader. Refer to “Contamination Report Statements” for reporting statements.

The DNA Technical Leader will be notified of all unattributable contamination and the contaminant profile will be added to the “DNA Profile Searcher”. If unattributable contamination in the “DNA Profile Searcher” shows a repeat pattern, the Technical Leader will determine the next warranted action. Actions may include notifying the manufacturer of a consumable or posting such information on a DNA information website to see if other laboratories have identified the same issue. If the contamination stems from something within the control of the DNA Laboratory, a “Corrective Action Report” will be completed.
In any case of unattributable contamination, a case note will be included in the case file to document its discovery and the workflow employed during troubleshooting.

Electronic copies of affected electropherograms and any resultant case notes generated will be maintained within the “Exogenous DNA Log” (E-DNA) folder (H:\Forensic Data\DNA\OTHER\Exogenous DNA Log). A hard copy binder of E-DNA entries prior to December 2014 has been archived and will be maintained in Qualtrax for reference.
10.0 Title:  Creation of Allele Tables and Use of Ribbon Accessories

The Biology/DNA Detail has developed an Excel workbook named “ALLELEigator” to summarize STR DNA typing results in a standard format, known as an allele table, to serve as documentation for case files. After using GeneMapper ID-X v1.6 to analyze electrophoresis data, the analyst will export a text file from GeneMapper that is imported into ALLELEigator to create the allele tables.

For cases interpreted using STRmix, only reference standard profiles will be contained in the allele tables included in the case files.

Note:  The code and templates in ALLELEigator make several assumptions in order to automatically generate allele tables; the following considerations should be used in order to most efficiently use this workbook:

- Samples must be named consistently; ALLELEigator uses a parsing key, such as analyst initials or the word "Item" (case sensitive) to locate both sample names and event or lab numbers from exported GeneMapper .txt files
- ALLELEigator assumes that samples are named by Event # followed by sample name with a place holder in between, such as a dash "-" or underscore "_"
- ALLELEigator is only adapted to handle a maximum of 96 samples from a single event or lab number from a single GeneMapper ID-X project
- The default page setup in ALLELEigator is set to print 1 page for each allele table, although the template contains up to 12 pages of tables (reset print area as needed)

10.1 Preparing to use ALLELEigator

Refer to Appendix B to set up Excel for DNA workbook use.

10.2 Exporting GeneMapper ID-X .txt files for ALLELEigator

Ensure that the Sample Type for all APC samples is set to Positive Control and that all ANC samples and reagent blanks are set to Negative Control in the main GeneMapper ID-X application window; all ladders must be named LADDER_INV24 or LADDER_6C for the macro to work properly.
10.3 Using ALLELEigator to Automatically Generate Allele Tables

All alleles above the Investigator 24plex QS dye-specific analytical thresholds are represented on the allele table.

1) Open ALLELEigator and select the tiffCAKE tab on the ribbon to access ALLELEigator’s controls.
2) Enter the parsing key (analyst initials or Item) where indicated on the ribbon; the parsing key must match up with sample names (case-sensitive).
3) Enter the GeneMapper project name where indicated on the ribbon; the name should match the name of GeneMapper ID-X project that the technical reviewer will use to review data.
4) Click the Make my Allele Tables! Button on the ribbon; you will be prompted to choose the exported GeneMapper ID-X .txt file—choose the correct file and click Open (macro will automatically generate allele tables for each event or lab number on a separate tab or prompt you to correct your GeneMapper file as needed).

You will be prompted to save the workbook—choose the destination and enter the workbook name and Click Save (the user can now modify each allele table as needed for case files).
In forensic DNA testing, statistical calculations are performed on evidentiary DNA profiles compared to a known reference standard of the case to aid in the assessment of the significance of an inclusion. Intimate samples that are collected directly from the individual’s body, body cavity, or genitals do not require statistical calculations. Similarly, single source profiles obtained from items which are documented to have been removed from a person and/or from consensual partners do not require statistical calculations if the assumption of their DNA being present is made during interpretation. Refer to exceptions regarding the issuance of supplemental reports.

DNA profiles from evidence samples which were generated by an outsourcing lab will continue to be reported using random match probabilities (RMP) and binary likelihood ratios. The outsourcing data is not eligible for statistical calculations in STRmix. All DNA statistics reported by the LVMPD will be truncated to three significant figures.

11.1 Statistical Guidelines using STRmix

All evidence profiles interpreted using STRmix, whether single source or mixed, will be reported using a likelihood ratio statistic.

11.1.1 Database

When statistics are calculated using STRmix, the NIST Database will be used for Identifiler Plus and Investigator 24plex. The NIST Database includes allele frequency data of African Americans (AA), Caucasians (CAU), and Hispanics (HSP).

11.1.2 Calculation of Likelihood Ratios in STRmix

Likelihood Ratios (LRs) provide the probability ratio of the evidence, given two competing hypotheses, typically referred to as the prosecution (Hp) and defense (Hd) hypotheses.

The following is an example of competing hypotheses:
Hp = the victim and suspect are the two contributors in the mixture versus,
Hd = the victim and an unknown individual are the two contributors in the mixture.

For the above example, the LR statistic answers the question:
“How many times more (or less) likely is it to observe the mixture DNA profile if it originated from a mixture of the victim and the suspect than if it originated from the victim and one unknown contributor?”

Note: The LR statement must be modified to reflect the sample-specific Hp and Hd hypotheses.

Various alternate hypotheses can be considered as deemed appropriate, and based on case scenario, it may be necessary to calculate and report the LR for multiple competing hypotheses in a single case.
Likelihood ratios require a fixed number of assumed contributors due to the development of the specific alternative hypotheses. In STRmix, likelihood ratios are calculated by multiplying the weight of a genotype (as determined during the MCMC process) at a locus by the probability of observing the genotypes given the proposed hypotheses.

The joint probability across all of the loci in the DNA profile is calculated by multiplying their respective frequencies together using the product rule.

\[
LR = \frac{Pr(E|H_1)}{Pr(E|H_2)} = LR_C = \sum w_j Pr(S_j|H_1) - \sum w_u Pr(S_u|H_2)
\]

**LR**: Likelihood ratio for the continuous model (STRmix)

**Pr**: Probability of the evidence given the hypothesis, i.e. \( Pr(E|H) \)

**\( W_j \)**: Probability weight of genotype “\( j \)”

**\( W_u \)**: Probability weight of unknown genotype “\( u \)”

**\( S_j \)**: Genotype “\( j \)”

**\( S_u \)**: Unknown genotype “\( u \)”

**\( H_1 \)**: Hypothesis 1 or the prosecution hypothesis (\( H_p \))

**\( H_2 \)**: Hypothesis 2 or the defense hypothesis (\( H_d \))

### 11.1.3 Formulae for Likelihood Ratios in STRmix

STRmix utilizes the Balding and Nichols formulae (equations 4.10 from NRCII) for the calculation of genotype frequencies. For single source profiles:

For heterozygote loci (equation 1):

\[
\frac{2[\theta + (1-\theta)p_j]}{(1+\theta)(1+2\theta)} \quad \text{For heterozygote loci (equation 1)}
\]

For homozygote loci (equation 2):

\[
\frac{3\theta + (1-\theta)p_j}{(1+\theta)(1+2\theta)} \quad \text{For homozygote loci (equation 2)}
\]

Where \( p_i \) is the allele frequency for allele \( i \), \( p_j \) the allele frequency for allele \( j \) and \( \theta \) is the \( F_{ST} \) value. The allele frequencies used within equations 1 and 2 are posterior mean frequencies. These are calculated using the following equation:

\[
\frac{x_i + \frac{1}{2}}{N_a + 1}
\]
Where for the given locus, $x_i$ is the number of observations of allele $i$ in a database, $N_a$ is the number of alleles in that database and $k$ is the number of allele designations with non-zero observations in the database at that locus.

11.2 Statistical Guidelines for Outsourced and Legacy Cases

11.2.1 Databases for Outsourced and Legacy Cases

The LVMPD laboratory will utilize the FBI Amended Popstats Database for **Profiler Plus/COFiler, Identifiler, and Identifiler Plus** data which includes allele frequency data of African Americans (BLK), Caucasians (CAU) and Southwest Hispanics (SWH). The LVMPD laboratory will utilize the NIST Popstats Database for **Investigator 24plex** data which includes allele frequency data of African Americans (AA), Caucasians (CAU), and Hispanics (HSP). A combined (COMB) NIST population group may also be calculated for use in CODIS entry but will not be reported. Other populations may be added to the Popstats file, or can be calculated manually. The allele frequency data are in the Popstats Data Files and also on file in the laboratory.

11.2.2 Random Match Probability (RMP): Calculation of Single Source Genotype Frequency for Outsourced and Legacy Cases

Random match probability (RMP) calculations should be used for single-source profiles or if a single major contributor or a single minor contributor can be deduced within a mixed profile. The RMP statistic assesses the overall rarity of the DNA profile obtained from the evidence sample. The statistic answers the question: “What is the probability of randomly selecting an unrelated individual from the general population having a DNA profile that is consistent with the DNA profile obtained from the evidence sample?”

11.2.2.1 If an individual is homozygous for a particular locus, the genotype frequency is calculated as $p^2 + p (1-p) \theta$, where $p$ is the frequency of the allele and $\theta$ (theta) is used as a measure of the effects of population subdivision (inbreeding coefficient). Theta is utilized to account for population substructure: 0.01 can be used for large populations (e.g., Las Vegas) and 0.03 for small, isolated populations (e.g., Native Americans and Amish).

**Example:** An individual has a genotype of 13,13. The frequency of the 13 allele = 0.130.

\[
\text{Genotype frequency} = (0.130)^2 + 0.130(1-0.130)(0.01) \\
\text{Genotype frequency} = 0.0169 + 0.130(0.87)(0.01) \\
\text{Genotype frequency} = 0.0169 + 0.001131 \\
\text{Genotype frequency} = 0.018031
\]

11.2.2.2 If an individual is heterozygous at a particular locus, the genotype frequency is calculated as $2pq$, where $p$ and $q$ are the frequencies of the respective alleles.

**Example:** An individual has a genotype of 9, 11. The frequency of the 9 allele = 0.135 and the frequency of the 11 allele = 0.213.

\[
\text{Genotype frequency} = 2 (0.135 \times 0.213) \\
\text{Genotype frequency} = 2 (0.028755) \\
\text{Genotype frequency} = 0.05751
\]
11.2.2.3 The joint probability across multiple loci can be calculated by multiplying their respective frequencies together using the product rule, assuming independence of loci.

**Example:** An individual has the following types as determined by results from STR profiling:

- **Locus 1:** 12, 13 (allele frequency of 0.145 and 0.192)
- **Locus 2:** 17, 25 (allele frequency of 0.002 and 0.046)
- **Locus 3:** 17, 18 (allele frequency of 0.159 and 0.110)

**Frequency** = (Freq. of Locus 1) \( \times \) (Freq. of Locus 2) \( \times \) (Freq. of Locus 3)

**Frequency** = (0.05568) \( \times \) (0.000184) \( \times \) (0.03498)

**Frequency** = 0.000000358

**Probability** = 1 in 2,793,296.089 \( (= 1/0.000000358) \)

Probability of observing the given genotype is reported as rarer than 1 in 2.79 million.

**Note:** Statistical information is not utilized regarding estimated frequencies for the Amelogenin locus.

**Note:** Effective March 24, 2015, the Biology/DNA Detail stopped reporting identity statements when an RMP statistic exceeded the threshold of approximately 1 in 100 times the world’s estimated population. Therefore, reports disseminated prior to this date may exhibit identity statements for individuals who cannot be excluded from single source DNA profiles (to include major, minor and deduced components of mixtures).

11.2.3 **Binary Likelihood Ratio (LR) for Mixtures for Outsourced Cases and Previously Interpreted Legacy Data**

Binary likelihood ratios are typically calculated for mixtures, however, single source profiles may also be calculated using the equation below:

\[
L = \frac{P(E \mid C_x)}{P(E \mid C_y)}
\]

The numerator in a likelihood calculation represents the prosecution’s hypothesis and the denominator represents the opposing (typically the defense’s) hypothesis.

The above equation describes:

How many times more (or less) likely it is to observe the evidence profile given that the prosecution’s hypothesis is true than if the defense’s hypothesis is true?

Likelihood ratios require a fixed number of assumed contributors due to the development of the specific alternative hypotheses. Therefore special attention must be paid to potential allelic dropout and stutter, as these two occurrences cannot be factored into the calculation when using Popstats. Loci exhibiting signs of the possibility of allelic drop-out cannot be utilized when performing unrestricted likelihood ratio calculations.
11.2.4 Minimum Allele Frequency in Popstats for Outsourced Cases

A minimum allele frequency estimation is calculated for STR loci which demonstrate alleles with a low frequency of occurrence. The intent of this application is to set a lower limit for the frequency for such rare alleles and, consequently, produce a conservative allele frequency estimation that does not underestimate the allele’s frequency of occurrence. The approach utilized is a basic procedure described previously by Budowle et al, 1991 and National Research Council, 1996. The minimum allele frequency is calculated using the following expression:

$$ P_{\text{min}} = \frac{5}{2n} $$

where $n$ represents the sample size (individuals).

The minimum allele frequency estimation is automatically utilized by the Popstats software when an allele is entered with a low frequency of occurrence.

11.2.5 Use of Popstats for Outsourced and Legacy Cases

Popstats currently resides on all three of the remote CODIS Workstations. Instructions for utilizing the Popstats software can be found in the reference section on the CODIS website (also accessible through one of the remote CODIS workstations), on the “Help” menu in the Popstats window, or by utilizing the following directions:

1) After logging into a remote CODIS workstation, launch the Analyst Workbench, and open Popstats (on the lower left of Workbench Explorer) and select the type of calculation to be performed from these choices:
   - **Forensic Single Sample**: allows you to calculate a random match probability (RMP) on a single forensic sample profile. Note: A single source major, minor, or deduced profile from a mixture is data entered as a “Forensic Single Sample”.
   - **Forensic Mixture**: the likelihood ratio allows you to determine how many times more (or less) likely it is to observe the mixture DNA profile given two different hypotheses. Indistinguishable mixtures or restricted mixture profiles from mixtures with multiple significant contributors will be data entered using “Forensic Mixture”.
   - **Parentage**: allows you to perform parentage and reverse parentage calculations.
   - **Kinship**: allows you to perform single-parent calculations and evaluates the likelihood that the pair of given DNA profiles are associated by kinship vs. by chance.
   - The **Match Estimation and Partial Match** options are used to evaluate the eligibility of entering specimens into CODIS and are not reported out as statistics in reports.
   - For Forensic Single Samples, the Probability Formula under the Configuration Summary should be set to the default NRC ‘96 for the 1996 National Research Council formulae.

2) Type a valid Specimen ID (to include the event or Lab Case # and sample name) in the Specimen ID field (or names of the Biological Parent, Child and Alleged Parent, if you want the names printed on the report). If the profile is a major, minor, deduced, etc. type of profile this must be noted here or in the Comments section. In the event multiple samples possess the same DNA profile and therefore the same associated statistic, each individual sample name must be listed in the Comments section. **Optional**: Click “Import” and choose the .xml file exported from ALLELEigator to automatically populate the Specimen ID and Target Profile grid; modify as needed.

3) Type valid alleles in the Target Profile grid(s). **Optional**: Refer to above optional import function using exported ALLELEigator .xml file.
4) Click the Calculate button.

Note: Examples of each of these types of calculations are included in the references on the CODIS website accessed from a remote CODIS workstation.

11.2.6 Popstats Mixture Calculations for Outsourced and Legacy Cases

Likelihood ratios can be calculated on the probative sample when the parties involved cannot be excluded as being a contributor to the mixture profile. Only loci where all alleles present are above the interpretation threshold can be used for statistics. The data printed for the file should include the “Likelihood Ratio for Mixtures” report.

As previously discussed, Popstats utilizes the Likelihood Ratio to compare two alternative explanations for a mixture profile, and is described by the following equation:

\[
L = \frac{P(E | C_x)}{P(E | C_y)}
\]

Where:
- \(C_x\) is the first explanation (the alleles of the mixed DNA profile attributed to unknown contributors under explanation \(C_x\)).
- \(C_y\) is the second explanation (the alleles of the mixed DNA profile attributed to unknown contributors under explanation \(C_y\)).
- \(x\) is the number of unknown contributors under explanation \(C_x\).
- \(y\) is the number of unknown contributors under explanation \(C_y\).
- \(P(E | C_x)\) is the probability of the DNA profile (E) using explanation \(C_x\).
- \(P(E | C_y)\) is the probability of the DNA profile (E) using explanation \(C_y\).

Likelihood ratios require a fixed number of assumed contributors due to the development of the specific alternative hypotheses. Therefore only loci that do not exhibit signs of allelic drop-out are suitable for likelihood ratio calculation.

11.2.6.1 The Likelihood Ratio Target Profile window has three grids which allow you to enter:
- The mixed DNA profile
- The alleles of the mixed DNA profile attributed to \(x\) unknown contributors under explanation \(C_x\)
- The alleles of the mixed DNA profile attributed to \(y\) unknown contributors under explanation \(C_y\)
- You must also specify the number of unknown contributors, \(x\) and \(y\), for explanations \(C_x\) and \(C_y\) respectively. Compute the minimum number of unknown contributors for each explanation as follows:

11.2.6.1.1 For the profile described by each hypothesis, divide the number of alleles at each locus by two and round up to the nearest integer.

11.2.6.1.2 The maximum computed value across all loci represents the minimum number of unknown contributors.
11.2.6.1.3 For example, if there are three alleles at locus CSF1PO, four alleles at locus D13S317, and two alleles at locus D16S539 from x contributors, then x must be equal to 2.

11.2.6.2 Select Forensic Mixture from the Popstats Calculations on the Popstats toolbar.

11.2.6.3 In the Mixture target profile grid, enter the sample name in the Specimen ID field.

11.2.6.4 On the first line in the Comments field, type in the total number of assumed contributors (TNAC) (may also use the abbreviation for assumed total number of contributors – ATNC). Press Enter to move to the second line in the Comments field and then type the hypothesis being assumed under Hp, to include the names and/or sample item numbers of the assumed contributors. Press Enter to move the third line in the Comments field and then type the hypothesis being assumed under H2, to include the names and/or sample item numbers of any assumed contributors.

Example: TNAC: 2
H1: Mixture of Summer Day (Item 1) and Daniel Day (Item 3)
H2: Mixture of Summer Day (Item 1) and 1 Unknown

11.2.6.5 Enter the mixture profile into the target profile grid. Un-check any loci that you will not use in the statistic due to possible allelic drop-out.

11.2.6.6 In the “# unknowns for H1” box, enter the number of unknown profiles in the mixture profile under the first hypothesis and in the target profile grid, enter the alleles of the unknown profile(s):

11.2.6.6.1 If the profile is consistent with a mixture of a victim and suspect, this value would be “0” since there are no unknown profiles. No alleles would be entered into the grid.

11.2.6.6.2 If the profile is consistent with a mixture of a victim, suspect, and a third unknown individual, this value would be “1” accounting for the unknown individual and you would ONLY type in the alleles unaccounted for by both the victim and suspect. Do not deduce a profile.

Example: the profile obtained at vWA is 14, 15, 16, 17, and 19. Suspect is 14,15, victim is 17,19. The 16 allele is unknown and would be entered into the grid.

11.2.6.7 In the “# unknowns for H2” box, enter the number of unknown profiles in the mixture profile under the second hypothesis and in the target profile grid, enter the alleles of the unknown profile(s):

11.2.6.7.1 If the profile is consistent with a mixture of a victim and suspect, this value would be “1” because the alternate hypothesis is that the profile is actually a mixture of the victim and an unknown individual. Only the unknown alleles are entered into the grid (any alleles that are not consistent with the victim’s alleles).
11.2.6.7.2 If the profile is consistent with a mixture of a victim, suspect, and a third unknown individual, this value would be “2” because the alternate hypothesis is that the profile is actually a mixture of the victim and two unknown individuals. The unknown alleles are entered into the grid (any alleles that are not consistent with the victim’s alleles).

Example: the profile obtained at vWA is 14, 15, 16, 17, and 19. The victim is 17, 19. The 14, 15, and 16 alleles are unknown and would be entered into the grid regardless of the suspects known profile.

Example: Below is an entry made of a mixture profile which includes the victim and suspect.

<table>
<thead>
<tr>
<th>Mixture Profile</th>
<th>Victim</th>
<th>Suspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>vWA</td>
<td>14, 15, 16</td>
<td>14, 15, 16</td>
</tr>
<tr>
<td>h261</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>h262</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>h263</td>
<td>8, 9</td>
<td>30, 31, 32</td>
</tr>
<tr>
<td>h264</td>
<td>9, 10, 11</td>
<td>9, 11</td>
</tr>
<tr>
<td>h265</td>
<td>10, 11, 12</td>
<td>10, 11, 12</td>
</tr>
</tbody>
</table>

**Note:**Allele 4 entries for Mixture Profile are cut-off in screen capture, however may be viewed on screen by scrolling to the right.
11.2.6.8 Once entry is complete, click the “Calculate” button on the tool bar. Print the “Likelihood Ratio for Mixtures” report. Report the most conservative LR statistic of the three population groups (CAU, AA, and SWH) calculated. The strength of the evidence is based upon the final calculated likelihood ratio.

11.3 Number Conversions

<table>
<thead>
<tr>
<th>exponent</th>
<th>conversion</th>
<th>commas</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>million</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>billion</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>trillion</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>quadrillion</td>
<td>5</td>
</tr>
<tr>
<td>18</td>
<td>quintillion</td>
<td>6</td>
</tr>
<tr>
<td>21</td>
<td>sextillion</td>
<td>7</td>
</tr>
<tr>
<td>24</td>
<td>septillion</td>
<td>8</td>
</tr>
<tr>
<td>27</td>
<td>octillion</td>
<td>9</td>
</tr>
<tr>
<td>30</td>
<td>nonillion</td>
<td>10</td>
</tr>
<tr>
<td>33</td>
<td>decillion</td>
<td>11</td>
</tr>
<tr>
<td>36</td>
<td>undecillion</td>
<td>12</td>
</tr>
<tr>
<td>39</td>
<td>duodecillion</td>
<td>13</td>
</tr>
<tr>
<td>42</td>
<td>tredecillion</td>
<td>14</td>
</tr>
<tr>
<td>45</td>
<td>quattuordecillion</td>
<td>15</td>
</tr>
<tr>
<td>48</td>
<td>quindecillion</td>
<td>16</td>
</tr>
<tr>
<td>51</td>
<td>sexdecillion</td>
<td>17</td>
</tr>
<tr>
<td>54</td>
<td>septendecillion</td>
<td>18</td>
</tr>
<tr>
<td>57</td>
<td>octodecillion</td>
<td>19</td>
</tr>
<tr>
<td>60</td>
<td>novemdecillion</td>
<td>20</td>
</tr>
<tr>
<td>63</td>
<td>vigintillion</td>
<td>21</td>
</tr>
</tbody>
</table>

11.4 Popstats Parentage Calculations

The LVMPD may be involved in paternity and relatedness cases. In these cases, DNA evidence is generally interpreted using likelihood ratios, comparing probabilities of the evidence under alternative propositions. STRmix cannot be used to perform these types of calculations and therefore all such calculations will be performed in Popstats.

To determine if the alleged father is the true biological father, the DNA profiles of the child, mother, and alleged father are compared. A child inherits two different alleles at each genetic locus—one from the mother and one from the father.

If a child has an allele that the mother does not have, this obligate allele has to come from the biological father. The results are either an exclusion—the alleged father is not the biological father—or an inclusion.

The parentage index utilizes a likelihood ratio to calculate a standard paternity trio where the mother and child are known and the father is unknown.
The reverse parentage index is utilized in situations where the DNA profile of the child is known, however both parents are in question (e.g. abandoned baby). The reverse parentage statistics function under the “Parentage” tab in Popstats should be used to evaluate the possibility that the abandoned baby is the biological child of the suspected couple. Single parentage statistics may subsequently be used to independently evaluate the evidence against each of the suspected parents.

A single parent comparison is used to evaluate each parent independently in a reverse parentage scenario or when only one alleged parent is available for testing. This calculation is often used in cases of missing persons or potential homicides in which a body has not been located and a secondary standard is not available from the victim. The single parent comparison may be used to infer whether a single source evidence sample may be biologically related to a single alleged parent. Utilize the “Kinship” tab in Popstats to calculate the single parent statistics for parent/offspring.

When determining possible parentage based on STR profiles, an individual cannot be excluded based on a single locus difference. Exclusions are required at two or more loci. In the event a possible mutation is present, exclusions are required at three or more loci. The majority of mutations are observed as a single repeat shift in either direction, thereby resulting in an allele one repeat above or below the expected obligate allele. Double allele shifts have been observed, however are rare.

11.4.1 Calculation of Standard Parentage Trio and Reverse Parentage

To access the parentage calculations, select the “Popstats Parentage Calculation” from the Workbench Explorer pane. For Trio calculations, select the “Trio” button. The Parentage Trio calculation requires three DNA profiles: biological parent, child, and alleged parent. No more than two alleles are allowed at each locus. Because the known parent and child relationship is assumed to be certain, at least one allele of the known parent and child must match at every locus.

For Reverse Parentage Calculations, select the “Reverse button”. The Reverse Parentage calculation requires three DNA profiles: biological mother, biological father, and alleged child.

1) Enter the profile using any one of the methods listed above.
2) Repeat set 1 for the child (or biological father for Reverse Parentage) window and the alleged parent (or alleged child for Reverse Parentage) window.
3) Verify that all of the STR loci have checks in the checkboxes to the left of the locus names in the first column. Deselect the checkboxes for STR loci which will not be included in the calculation.
4) Click Calculate.
5) Print the “Parentage Trio Calculations” (parentage trio), or “Reverse Parentage Statistics” (reverse parentage) report.
11.4.3 Calculation of Single Parentage Statistics

1) To access the single parentage calculations, select the “Kinship Popstats Calculations” from the Workbench Explorer pane. The Single Parentage Statistics window is available only when the Parent-Offspring (PO) kinship relationship has been selected in the pane on the right side of the screen.
2) Enter the DNA profiles of the reference and evidence samples to be considered and select “Calculate”.
3) Select the “Single Parentage Statistics” tab under “Kinship” at the top of the screen.

This window displays the following:

For each valid combination of loci and population group:
- Probability of exclusion
- Parent offspring index
- Consistency status

For each population group:
- Combined probability of exclusion
- Combined parentage index
- Combined consistency status

Single parentage labels the per locus consistency between the genotypes of the reference and the evidence profiles using “Yes” for a locus that is consistent, and “No” for a locus that is inconsistent. This appears in “Match” column.

Popstats labels the “Combined Profile Statistics” consistency status using “Yes” when all selected loci are consistent; “Mutation” when one or two selected loci require mutation to be consistent; and “No” when three or more selected loci are inconsistent. The consistency status is labeled “Inconclusive” when one or more selected loci have no population data for a population group.
4) The Single Parentage Statistics report is only available when selecting the Parent-Offspring (PO) kinship relationship. Select “Print” and then “Popstats Single Parentage Statistics DNA Testing Results”. The report contains the following:
   - The first page includes the reference and evidence profiles along with the consistency status of each locus.
   - Subsequent pages display by population group and locus, and provide the PE and PI, and the combined PE and PI.

11.5 Mutation rates
The following corresponds with the mutation rates of common autosomal STR loci observed during paternity testing:

<table>
<thead>
<tr>
<th>STR System</th>
<th>Maternal Meloses (%)</th>
<th>Paternal Meloses (%)</th>
<th>Number from Either</th>
<th>Total Number of Mutations</th>
<th>Mutation Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1PO</td>
<td>95/304,307 (0.001)</td>
<td>982/643,118 (0.15)</td>
<td>410</td>
<td>1,467</td>
<td>1.016%</td>
</tr>
<tr>
<td>FGA</td>
<td>205/405,230 (0.050)</td>
<td>2,210/492,776 (0.32)</td>
<td>210</td>
<td>2,125</td>
<td>0.28%</td>
</tr>
<tr>
<td>TH01</td>
<td>31/327,172 (0.009)</td>
<td>41/452,362 (0.009)</td>
<td>28</td>
<td>100/779,354</td>
<td>0.013%</td>
</tr>
<tr>
<td>TPOX</td>
<td>18/406,661 (0.0045)</td>
<td>54/457,420 (0.012)</td>
<td>28</td>
<td>100/857,481</td>
<td>0.012%</td>
</tr>
<tr>
<td>vWA</td>
<td>104/564,989 (0.003)</td>
<td>1,482/675,517 (0.17)</td>
<td>814</td>
<td>2,480/1,437,945</td>
<td>0.17%</td>
</tr>
<tr>
<td>D3S1358</td>
<td>60/405,452 (0.015)</td>
<td>713/559,636 (0.13)</td>
<td>979</td>
<td>1,182/964,288</td>
<td>0.12%</td>
</tr>
<tr>
<td>D8S112</td>
<td>111/451,736 (0.025)</td>
<td>763/659,600 (0.12)</td>
<td>385</td>
<td>1,269</td>
<td>0.11%</td>
</tr>
<tr>
<td>D7S820</td>
<td>59/440,562 (0.013)</td>
<td>745/644,243 (0.12)</td>
<td>285</td>
<td>1,089/1,085,305</td>
<td>0.10%</td>
</tr>
<tr>
<td>D8S1179</td>
<td>96/409,269 (0.023)</td>
<td>779/489,968 (0.16)</td>
<td>364</td>
<td>1,239/899,837</td>
<td>0.14%</td>
</tr>
<tr>
<td>D13S317</td>
<td>192/482,136 (0.040)</td>
<td>881/623,146 (0.19)</td>
<td>485</td>
<td>1,795/1,103,282</td>
<td>0.14%</td>
</tr>
<tr>
<td>D16S530</td>
<td>129/467,774 (0.028)</td>
<td>540/404,485 (0.11)</td>
<td>372</td>
<td>1,041/962,239</td>
<td>0.11%</td>
</tr>
<tr>
<td>D18S51</td>
<td>186/296,244 (0.057)</td>
<td>1,084/494,098 (0.22)</td>
<td>466</td>
<td>1,746/790,342</td>
<td>0.22%</td>
</tr>
<tr>
<td>D21S11</td>
<td>464/145,398 (0.11)</td>
<td>772/326,708 (0.15)</td>
<td>580</td>
<td>1,816/662,096</td>
<td>0.19%</td>
</tr>
<tr>
<td>Penta D</td>
<td>12/45,762 (0.003)</td>
<td>21,112,501 (0.035)</td>
<td>24</td>
<td>574/41,202</td>
<td>0.14%</td>
</tr>
<tr>
<td>Penta E</td>
<td>29/41,311 (0.006)</td>
<td>255/58,719 (0.13)</td>
<td>59</td>
<td>163/100,030</td>
<td>0.16%</td>
</tr>
<tr>
<td>D2S1338</td>
<td>15/32,830 (0.023)</td>
<td>157/152,310 (0.10)</td>
<td>90</td>
<td>262/225,140</td>
<td>0.12%</td>
</tr>
<tr>
<td>D19S433</td>
<td>38/730,403 (0.043)</td>
<td>783/103,489 (0.079)</td>
<td>71</td>
<td>187/173,490</td>
<td>0.11%</td>
</tr>
<tr>
<td>SE35 (ACTBP2)</td>
<td>0.330 (0.030)</td>
<td>0.330/91,940 (0.64)</td>
<td>None reported</td>
<td>330/91,940</td>
<td>0.64%</td>
</tr>
</tbody>
</table>

See also [http://www.cstl.nist.gov/strbase/mutations.htm](http://www.cstl.nist.gov/strbase/mutations.htm). Information derived from AABB 2003 annual report provided from over three dozen paternity testing laboratories. The reported mutations are divided into maternal or paternal sources or from either when the source of the mutation observed in a child could not be determined. Very little mutation rate data has been published to-date on the newer STR loci. References have been included below the locus where there are data available.
12.0 Title: Reporting Guidelines

12.1 Lab Reports
All DNA Laboratory Reports should be as clear and complete as possible. The laboratory reports will include the following criteria when applicable:

- Case identifier
- Description of evidence examined
- Methodology
- Locus or amplification system
- Results and/or conclusions
- Interpretive statement (either quantitative or qualitative)
- Date Issued
- Disposition of evidence
- Electronic signature and title of the case analyst

The issue date of a report is the date the report is published in LIMS. This is the date that is printed on the report. The address of the DNA Annex will be placed at the bottom of the first page of the lab report.

12.2 Release of Case Report Information
DNA Laboratory reports are distributed to the requesting officer and/or the Deputy District Attorney who submitted the Forensic Laboratory Examination Request. Reports generated from CSA Evidence Impound reports for certain property crime evidence types will be distributed to the Sergeant of the area command section or investigating detail of the incident. Reports generated from the submission of Volunteer Consent forms will be distributed to the officer assigned to the Event #. Upon receipt of a discovery subpoena or subpoena duces tecum, copies of the case file contents and subpoenaed records will be provided. Refer to the LVMPD Forensic Quality Manual for policies that govern the release of information.

12.3 Design of LVMPD Biology/DNA Forensic Casework Report of Examination
The Forensic Casework Report of Examination is divided into five (5) main sections:

1) A header which contains all of the case-pertinent information to include the report distribution date, agency, location, primary case # (and additional linked case #’s, if applicable), incident, requester, Lab Case #, and subject(s).

2) A results table which contains all of the evidence items examined in the case, their corresponding Lab Item #, associated impound information (package and item numbers), descriptions, and a summary of the examination and/or screening results or other case-specific circumstances (if applicable).

3) A “Results, Opinions, and Interpretations” section which contains the individual results of each Lab Item # subjected to the DNA analysis process.

4) If applicable, a table which details items of evidence received, but not examined.
5) A “Notes” section which includes disclaimers, defines terms used in the report, and provides additional information to aid in the understanding of the reported results and statistics.

12.4 Violence Against Women Act (VAWA)
In the event a sexual assault kit was collected from a victim who originally invoked their right to privacy under the VAWA law, but then later filed an official report with a law enforcement agency under their own name, the issued DNA analysis report will utilize the victim’s true name, rather than John or Jane Doe.

12.5 Serology Reporting Statements
Based on case documentation from investigating agents and visual examination, items will be screened for blood and/or semen accordingly. The results of all screening tests conducted will be reported. These results will appear in the “Results, Opinions, and Interpretations” column in the results table.

12.5.1 Presumptive Screening Tests
The following tests are each considered to be presumptive tests:
- Phenolphthalein (PT)
- Tetramethylbenzidine (TMB)
- Hematrace
- Acid Phosphatase (AP)
- P30
- RSID-saliva

The following statement will be placed below the results table when the results of these presumptive screening tests are being reported: “Tests for blood, semen, and/or saliva are presumptive in nature and therefore provide an indication, but not confirmation, of the presence of a body fluid.”

The following statements will be used to populate the “Results, Opinions, and Interpretations” results field in the table for presumptive screening tests:
- Phenolphthalein (PT) or Tetramethylbenzidine (TMB) Testing:
  1) Positive presumptive blood test(s)
  2) Negative presumptive blood test(s)
  3) Inconclusive presumptive blood test(s)**
- Hematrace:
  1) Blood indicated
  2) Human blood negative
  3) Blood inconclusive**
- Acid Phosphatase (AP) Testing
  1) Positive presumptive semen test(s)
  2) Negative presumptive semen test(s)
  3) Inconclusive presumptive semen test(s)**
- P30 Testing
1) Semen indicated
2) Semen negative
3) Semen inconclusive**

In certain instances additional body fluids (saliva and vaginal) have been demonstrated to yield weak P30 results which may be false positives. Based on the totality of a case (e.g. case scenario, no evidence of sperm, etc.), the analyst may choose to report the P30 results as “semen inconclusive” instead of “semen indicated”. Documentation must be included in the analyst’s notes and on the Report of Examination as to why the results are being reported as inconclusive.

- RSID-Saliva Testing
  1) Positive presumptive saliva test
  2) Negative presumptive saliva test
  3) Inconclusive presumptive saliva test**

12.5.2 Confirmatory Microscopic Sperm Search
The microscopic sperm search is considered a confirmatory test and will be reported using the following statements in the “Results, Opinions, and Interpretations”:
1) Sperm positive
2) Sperm negative
3) Sperm inconclusive**

In the event a sample yields a P30 positive result, however no sperm were identified and no foreign DNA was obtained, the following statement should be placed below the results table:
“A positive P30 result in the absence of spermatozoa and foreign DNA cannot be conclusively attributed to the presence of semen. P30 can be detected in other body fluids.”

12.5.3 Inconclusive Serological Results
All inconclusive serological test(s) must include documentation of a qualitative reason for the inconclusive result. Inconclusive results should be marked with an **asterisk or other symbol to indicate that additional information/explanation will be noted below the results table (e.g. invalid color test result, sperm head morphology, only one or two sperm heads noted etc.).

The following statement(s) are examples which may be included at the bottom of the table in order to satisfy this requirement:
1) Inconclusive PT, Hematrace, AP, P30 test(s) and/or RSID-Saliva:
The possible presence or absence of <blood/semen/saliva> could not be determined due to insufficient information obtained from the chemical analysis.
2) Inconclusive microscopic sperm search:
The presence or absence of sperm could not be confirmed due to insufficient information obtained from the microscopic analysis.

12.5.4 Results of Visual Examinations
- Negative examination using Alternate Light Source (ALS):
  “No apparent semen <or saliva/blood> stain(s) observed”
• Negative visual examination for staining using the naked eye:
  “No apparent stain(s) observed”

12.6 Additional Summary Result Table Statements
The following table provides an overview of additional case-specific circumstances and the
 corresponding statements which should be placed in the “Results, Opinions, and Interpretations”
 column of the table, if applicable:

<table>
<thead>
<tr>
<th>Circumstance</th>
<th>Summary Result Table Statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>An item of evidence was requested, but not examined</td>
<td>Not examined</td>
</tr>
<tr>
<td>An item of evidence was received, but not examined</td>
<td>Received, not examined</td>
</tr>
<tr>
<td>Additional areas of staining are identified, hairs are removed, etc. during the examination of an evidence item, which may be suitable for future testing</td>
<td>Not tested at this time</td>
</tr>
<tr>
<td>Insufficient root material present for STR analysis based on visual and/or microscopic examination of a hair</td>
<td>Not suitable for STR Testing</td>
</tr>
<tr>
<td>Item of evidence opened, however, no serological testing or DNA analysis performed</td>
<td>No further &lt;examination/testing&gt; at this time</td>
</tr>
<tr>
<td>Serological testing completed, however, no DNA analysis performed</td>
<td>No further testing at this time</td>
</tr>
</tbody>
</table>

12.7 Referencing Additional Information Within and/or Below the Results Table
At times it may be necessary to reference additional case-specific information, such as to cross-
reference to another case file, previously issued reports, or name disparities. Such instances may
warrant additional statements below the results table.

The following table provides an overview of case-specific circumstances in which it may be
necessary to include an additional statement of explanation. In the event the statement only refers
to specific sample(s), an asterisk (*) or other symbol should be used in the results table and/or in
the “Results, Opinions, and Interpretations” to identify the sample(s) to which the statement
applies:

<table>
<thead>
<tr>
<th>Circumstance</th>
<th>Additional Statement Included Below Results Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Referencing another analyst’s case file for data</td>
<td><strong>“Refer to the case file for Event # &lt;XX XXXX-XXXX &gt; issued by &lt;Generic Title, Name, P#&gt; for information related to &lt;Subject&gt;.”</strong></td>
</tr>
<tr>
<td>Referencing previously issued reports</td>
<td><strong>“Refer to the original/supplemental report issued by &lt;Generic Title, Name, P#&gt; dated &lt;XXX&gt; for related information.”</strong></td>
</tr>
<tr>
<td>Referencing evidence with multiple Event #s worked under one Event #</td>
<td><strong>“Evidence was booked under Event # &lt;XX XXXX-XXXX&gt;.”</strong></td>
</tr>
<tr>
<td>Referencing name disparities</td>
<td><strong>“&lt;Last/First name&gt; spelled differently than on request.”</strong></td>
</tr>
<tr>
<td>Referencing items being examined while in another analyst’s custody</td>
<td><strong>“Evidence was examined while in the custody of &lt;Generic Title, Name, P#&gt;.</strong></td>
</tr>
</tbody>
</table>
### 12.8 Y-Screen Report Statements

For cases in which Y-screening was performed, a header entitled **“Male DNA Screening”** will be added in bold as the first section of the Results, Opinions, and Interpretations section of the report. The following verbiage will be used to detail the results of the Y-screen process:

“The following evidence samples were screened to identify samples containing male-specific DNA:"

Samples will be grouped together based on their Y-screen results. Differentially extracted samples will be reported as the parent item number (without reference to the epithelial or sperm fractions) using the Y-screen result containing the highest male quantitation value. It may be necessary to modify the below statements to singular/plural based on the number of samples falling into each of the below categories.

- **Samples with male DNA detected and autosomal STR analysis was performed (green and no color):**
  
  “Male DNA was detected in the following sample. This sample was selected for further testing.”

- **Male DNA detected, however no autosomal STR analysis performed (green or no color):**
  
  “Male DNA was detected in the following sample; however, this sample was not selected for further testing at this time.”

- **Samples with male DNA < 50 pg and AUTO:Y ≤ 55 (no color):**
  
  “Due to an insufficient amount of male DNA, this sample was not selected for further testing at this time.”

- **Samples with male DNA < 50 pg and AUTO:Y > 55 (pink):**
  
  “Due to an insufficient amount of male DNA compared to the amount of total human DNA, this sample was not selected for further testing at this time.”
• No male DNA detected (i.e., Y-target is Undet.) (pink):
  “Male DNA was not detected in the following sample. This sample was not processed further.”

Example:

Results, Opinions, and Interpretations

Male DNA Screening
The following evidence items were screened to identify samples containing male-specific DNA:

Male DNA was detected in the following sample. This sample was selected for further testing:
  • Lab Item 2: Breast swabs

Male DNA was detected in the following sample; however, these items were not selected for further testing at this time:
  • Lab Item 5: Miscellaneous swab from left flank
  • Lab Item 6: Neck swabs

Male DNA was not detected in the following sample. This sample was not processed further:
  • Lab Item 3: Oral swabs

Due to an insufficient amount of male DNA compared to the amount of total human DNA, this item was not selected for further testing at this time:
  • Lab Item 4.1: Right hand fingernail swabs

Due to an insufficient amount of male DNA, this item was not selected for further testing at this time:
  • Lab Item 4.2: Left hand fingernail swabs

It is permissible to group together the statements for the samples with male DNA < 50 pg and AUTO:Y ≤ 55 (no color) and samples with male DNA < 50 pg and AUTO:Y > 55 (pink) noted above. These samples may also indicate the necessity of the Y-STR report disclaimer if no other samples were amplified in the case and a male reference standard is available for comparison (Refer to criteria for inclusion of Y-STR report disclaimer):
“Due to an insufficient amount of male DNA and/or insufficient amount of male DNA compared to the amount of total human DNA, the following items were not selected for further testing at this time:"

Example:

Results, Opinions and Interpretations:

Male DNA Screening
The evidence items in this section were screened to identify samples containing male-specific DNA:

Due to an insufficient amount of male DNA and/or insufficient amount of male DNA compared to the amount of total human DNA, the following items were not selected for further testing at this time:
  • Lab Item 2: Vaginal swabs
  • Lab Item 2.2: Cervical swabs
  • Lab Item 5.1: Right hand fingernails swab
  • Lab Item 5.2: Left hand fingernails swab
  • Lab Item 6: Right breast swabs

Termination at Quantitation for Non-Sexual Assault Evidence
For cases in which a non-sexual assault sample will be terminated based on the quantitation results, a header entitled “DNA Screening” will be added in bold in the Results, Opinions, and Interpretations section of the report. In the event Y-screen has also been performed, this section will immediately follow those results.
The following verbiage will be used to detail the results of the quantitation process:
“The following evidence items were screened to identify samples containing insufficient DNA for autosomal STR amplification:"

Samples will be grouped together as a list to denote those which will not be moved forward for autosomal amplification. It may be necessary to modify the statements to singular/plural based on the number of samples being terminated.
“Due to an insufficient amount of DNA being detected during quantitation, PCR amplification was not performed on these items:"

Example:

<table>
<thead>
<tr>
<th>Results, Opinions, and Interpretations</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Screening</td>
</tr>
<tr>
<td>The following evidence items were screened to identify samples containing insufficient DNA for autosomal STR amplification:</td>
</tr>
<tr>
<td>Due to an insufficient amount of DNA being detected during quantitation, PCR amplification was not performed on these items:</td>
</tr>
<tr>
<td>- Lab Item 1: Left hand fingernail swabs</td>
</tr>
<tr>
<td>- Lab Item 2: Right hand fingernail swabs</td>
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<tr>
<td>- Lab Item 5: Swab from door handle</td>
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</tbody>
</table>

12.9 Report Statement When All Reference Standards Terminated After Quantitation
In the event all evidence samples are determined to be unsuitable for autosomal STR amplification following quantitation and the processing of the associated reference standards were terminated, the following report statement will be placed below the final Male DNA Screening or DNA Screening result reported. This statement will be the last statement in the Results, Opinions, and Interpretations section:
“At this time, all evidence items are deemed unsuitable for autosomal STR amplification. As a result, PCR amplification was not performed on the following reference standards. Extracts from these reference standards will be retained and may be amplified at a later time, if necessary:"

At this time, all evidence items are deemed unsuitable for autosomal STR amplification. As a result, PCR amplification was not performed on the following reference standard. Extracts from this reference standard will be retained and may be amplified at a later time, if necessary:
- Lab Item 1: Reference standard from Victim

12.10 Autosomal STR Analysis Report Conclusions
General reporting guidelines are provided throughout this procedure and most reporting scenarios will fall within the recommendations of this protocol. The following sections are meant to provide guidance when writing reports and are not intended to encompass all situations. Ultimately, all statements included in the final report must accurately reflect the data observed. Unless otherwise noted, the styles in which the items appear on the report (e.g. bold, italics, etc.) are analyst discretion, however must be consistently applied within a report.

When autosomal STR analysis has occurred, a header entitled “DNA STR Processing” will be added in bold in Results, Opinions, and Interpretations section of the report. In the event Y-screening or termination at quant has also taken place, the DNA STR Processing section will be placed immediately below the Male DNA Screening or DNA Screening results.
**Note:** It is permissible to use slight variations of the **lead-ins** to the below statements such as “All items listed below...”, however the remainder of the PCR amplification statements must remain as stated.
12.10.1 When autosomal STR amplification is performed using the **Investigator 24plex QS** amplification kit, the following statement is the first statement in the “Results, Opinions, and Interpretations” section of the DNA report:

“The following items were subjected to PCR amplification at the following STR genetic loci: TH01, D3S1358, vWA, D21S11, TPOX, DYS391, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. The sex-determining Amelogenin locus was also examined. <Where applicable, STRmix was used for interpretation.>”

12.10.2 When autosomal STR amplification is performed using the **PowerPlex Fusion 6C** amplification kit, the following statement is the first statement in the “Results, Opinions, and Interpretations” section of the DNA report:

“The following items were subjected to PCR amplification at the following STR genetic loci: D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, SE33, D22S1045, DYS391, FGA, DYS576, and DYS570. The sex-determining Amelogenin locus was also examined.”

12.10.3 When STR amplification was previously performed using the **Identifiler** or **Identifiler Plus** amplification kit, the following statement is the first statement in the “Results, Opinions, and Interpretations” section of the DNA report:

“The following items were subjected to PCR amplification at the following STR genetic loci: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA. The sex-determining Amelogenin locus was also examined. <Where applicable, STRmix was used for interpretation.>”

12.10.4 If comparisons are being made between questioned samples and reference standards that were amplified in two different amplification kits, the following statement will be contained just below the designation of the loci:

“Only the genetic loci contained in both the DNA profiles obtained from the evidence sample(s) and the reference standard(s) will be used for comparison.”

12.10.5 If applicable, each sample conclusion statement should include the following descriptive elements in the report language. Reporting of sample conclusions associated with evidence profiles generated from outsourcing laboratories will mimic the language as originally reported by the outsourcing laboratory.

1) A statement as to whether there is an indication of male DNA being present and the number of males indicated

2) The assumption as to the number of contributors used during interpretation

3) Whether the DNA profile is suitable for comparison

4) If the DNA profile is not suitable for comparison, whether it is due to the profile being too limited in nature or too complex

12.10.6 Unless an assumption has been made, each conclusion statement must include individual comparisons of each of the reference profile(s) to the DNA sample results. Alternatively, the following report statement may be utilized:
“All other submitted reference standards <and designated unknown profiles> are excluded as possible contributors to the DNA profile obtained.”

**12.10.7 Statements for assumptions made during interpretation regarding intimate samples or personal conditional knowns**

During interpretation, it is reasonable to assume the presence of the donor’s DNA profile when an item of evidence is intimate, personal in nature, or if they are a documented consensual partner. When an assumption has been made, the following notes will be included in the DNA report, as applicable:

**Note 1:** “Evidence collected directly from the body or personal items removed directly from the body are intimate samples; therefore, the donor may be reasonably assumed to be present should the item produce a DNA profile that is suitable for comparison. <DNA profiles from <environmental samples which are habitually touched or handled by an individual><documented consensual partners> may also be reasonably assumed to be present.>

**Note 2:** “In instances in which contributors can be assumed, no statistical calculations will be reported for the assumed contributors.”

**12.10.7.1** If the profile does not show signs of additional foreign contributor(s), the following report statement may be used:

Assuming Michelle Monday (Item 3) is a contributor to the DNA profile obtained <from the epithelial fraction of the vaginal swab (Item 1-EF)>, no foreign DNA results were obtained.

The DNA profile obtained <from the epithelial fraction of the vaginal swab (Item 1-EF)> is consistent with Michelle Monday (Item 3).

**Note:** In these instances, it is permissible to use the word “partial” as a qualitative indicator of the DNA profile.

**12.10.7.2** In the event a sample qualifies for the assumption of contributors due to its intimate nature, etc., however the foreign component does not contain allelic data above analytical threshold at 6 or more loci (not to include Amelogenin or the DYS391 locus), the following report statement may be used:

Assuming Victim (Item XXX), the DNA profile is consistent with a mixture, <including at least one male contributor>. Due to the limited nature of this profile, the foreign component is unsuitable for interpretation and therefore no additional conclusions can be made.

**12.10.7.3** In the event a sample qualifies for the assumption of contributors due to its intimate nature, etc., however the assumed DNA profile is low-level and there are indications of a mixture based only on data below the analytical threshold, the following report statement may be used:
Assuming Victim (Item XXX) is present, a mixture is indicated in the DNA profile. Due to <the limited nature of the data foreign to the victim / there being no unambiguous DNA foreign to the victim>, it is unsuitable for interpretation and therefore no additional conclusions can be made.

12.10.8 Observation of Carryover in Differentially Extracted Samples
It is reasonable to assume that samples which were differentially extracted (regardless of whether intimate, personal, or environmental in nature) may include carryover between the epithelial and sperm fractions. In instances in which carryover is determined to be present in a two-person mixture, and the opposing fraction yields a single source profile, this may be notated in the report.

Statistical calculations are not required when the assumption of both the donor and carryover can be made.

The DNA profile obtained from the epithelial fraction of the vaginal swab (Item 3.1-EF) is consistent with a mixture of two individuals with at least one being male. Assuming Viola Vegas (Item 6) is a contributor to this mixture, a foreign DNA profile was obtained. The foreign DNA profile is consistent with carryover from the sperm fraction of the vaginal swab (refer to Item 3.1-SF).

The DNA profile obtained from the epithelial fraction of the stain from the seat of the taxi cab (Item 3-EF) is consistent with a mixture of two individuals with at least one being male. Assuming Sallie Mae (Item 1) is a contributor to this mixture, the remainder of the DNA profile is consistent with carryover from the sperm fraction of the stain from the seat of the taxicab (refer to Item 3-SF).

12.10.9 Statements for Reference Standard Typing Results
A qualitative statement describing the DNA profile results will be included in the Results, Opinions, and Interpretations Section for all reference standards. This statement will include the gender of the reference standard and whether it yielded a full or partial profile.

Example:

**Lab Item 1: Reference standard from Victim**
A full female profile was obtained.

12.10.10 Statements for the Use of Presumed Standards
In the event the DNA profile obtained from an evidence item is being utilized as a presumed standard, the following statement must be placed at the beginning of the “Results, Opinions, and Interpretations”:

“The DNA profile obtained from the <evidence item> yielded a <male/female> profile. Due to the intimate nature of this sample, it can be assumed that this DNA profile is that of the <name of the sample donor>. For comparison purposes, this DNA profile will be utilized as the presumed standard for <name of the sample donor>.”
All subsequent comparisons made to the presumed standard will utilize a variation of the following language when making inclusions/exclusions:
“The DNA profile obtained from <evidence item> is consistent with the presumed standard for <subject name>.”

12.10.11 Identical Twins
In the event a case involves confirmed identical twins, the following statement will be added as a caveat to the DNA report prior to the first reported item conclusion:

“The DNA profiles from <name of twin #1> and <name of twin #2> are confirmed as being genetically identical twins. It is therefore not possible to distinguish which individual is the contributor of the DNA profile(s) obtained. All genetic matches will be associated to both individuals.”

The DNA Technical Leader should be consulted for all cases involving confirmed identical twins. This language may be modified and tailored to the case-specific information.

12.11 Reporting of STRmix Interpretations
The following information is described when reporting STRmix results:
- The number of contributors used for STRmix interpretation, including whether there is an indication of male DNA being present
- The names of individuals compared to the evidence profiles which are assumed, included, excluded, and identified in the uninformative range
- The likelihood ratio value associated with all included individuals, with the exception of assumed contributors
- A statement of propositions and likelihood ratio(s) which can explain the evidence

The likelihood ratio in STRmix is calculating the probability of obtaining the evidence given the person of interest is a contributor against the probability of obtaining the evidence if the person of interest is not a contributor. The calculation result reflects the probability as it relates to the DNA profile as a whole (whether single source or a mixture).

Conclusions for each item will be present in the “Results, Opinions, and Interpretations” section of the report. It is acceptable to group similar conclusions for multiple items together such as with single source samples associated with the same individual. Differentially extracted samples containing an epithelial (EF) and sperm fraction (SF) originating from a single item may be placed under a single heading.

If a case involves multiple persons of interest (POI) and multiple inclusionary likelihood ratio calculations have been performed for an item of evidence, the individual likelihood ratio calculations as well as the likelihood ratio calculation(s) considering the multiple persons of interest in combination will be included in the report. As examples, if two POIs have likelihood ratios calculated individually that both support their inclusion and a likelihood ratio is also calculated for the two POIs together in the mixture that supports their inclusion, all three calculations will be reported.

If likelihood ratios are performed to determine the potential inclusions of non-probative individuals (e.g., victims or consensual partners) for the purposes of conditioning a STRmix analysis, the
individual likelihood ratio calculations do not need to be included in the report, but must be available in the case file.

If conversely, two individual likelihood ratios independently support inclusion, but the likelihood ratio calculated for their presence together in the mixture suggests this cannot occur (LR with an exponent of $10^{-4}$ or less), the two individual inclusionary LRs will be reported along with the information that they both cannot be contributing DNA together to the mixture.

It is recognized that different situations may require different reporting strategies, and in general, what is reported will be the most informative for the case.

If an evidence profile results in an unknown component (or components) that are searchable and uploadable to CODIS, a statement will be included in the report to notify the reader that the component(s) will be searched in CODIS.

**Assumptions**

All assumptions that are made as part of the interpretation will be stated within the report. This includes the assigned number of contributors and any assumed contributors utilized during interpretation of the evidence sample.

**Likelihood Ratio**

- The point estimate “Sub-sub-source” LR found below the “Per Locus Likelihood Ratio” table will be reported for full single source Identifiler Plus and Investigator 24plex QS profiles which have been resolved in STRmix with 100% weight at every locus. This calculation includes an estimation for which a deviation of approximately ± 10-fold may exist
- The 99% 1-sided lower HPD interval (Higher Posterior Density) LR found below the “Per Locus Likelihood Ratio” table will be reported for partial single source (where one or more loci exhibit genotype combinations with weights less than 100%), two, three, and four-person Identifiler Plus and Investigator 24plex QS profiles. This same value is also noted as the “Unrelated” LR in the “Summary of LR” table.
- Where applicable, the unified LR found in the “Summary of LR” table will be calculated and maintained in the case file.

**Exceptions:**

- Likelihood ratios are truncated to three (3) significant figures (without rounding). The most conservative (lowest) population result will be reported.
Reporting Statements
The following is the example format that will be used for reporting statements containing fully resolved (100% weight at all loci) single source profile with the *unrelated likelihood ratio*:

**Lab Item 1.1: Swab of something**
Number of contributors: 1 female
Individually included: Jane Doe (Item 1; LR = approximately <insert most conservative sub-sub-source LR from STRmix>)
Excluded: Peter Jones (Item 5), Jimmy Dean (Item 8)
The probability of observing this DNA profile is approximately \(\text{<insert most conservative sub-sub-source LR from STRmix>}\) times more likely if it originated from Jane Doe (Item 1) than if it originated from an unknown random contributor.

The following is the example format that will be used for reporting statements containing mixtures or partial single source profiles (one or more loci exhibit genotype combinations with weights less than 100%) with \textit{99\% 1-sided lower HPD interval}:

\textbf{Lab Item 2.2: Stain from something}

Number of contributors: 3 (at least two males)
Assumed contributor(s): Jane Doe (Item 3)
Individually included:
  - John Smith (Item 1; LR = at least \text{<insert most conservative 99\% 1-sided lower HPD interval from STRmix>})
  - Susie Logan (Item 5; LR = at least \text{<insert most conservative 99\% 1-sided lower HPD interval from STRmix>})
Excluded:
  - Mark Johnson (Item 6)

\textit{Combination of Included Individuals:}
The probability of observing the mixture DNA profile is at least \(\text{<insert most conservative 99\% 1-sided lower HPD interval from STRmix>}\) times more likely if it originated from Jane Doe (Item 3), John Smith (Item 1), and Susie Logan (Item 5) than if it originated from Jane Doe (Item 3) and two unknown random contributors.

\textbf{Lab Item 3.3: Swab of something}

Number of contributors: 1 male
Individually included:
  - Peter Jones (Item 5; LR = at least \text{<insert most conservative 99\% 1-sided lower HPD interval from STRmix>})
Excluded:
  - Jane Doe (Item 6)
  - Jimmy Dean (Item 8)

The probability of observing this DNA profile is at least \(\text{<insert most conservative 99\% 1-sided lower HPD interval from STRmix>}\) times more likely if it originated from Peter Jones (Item 5) than if it originated from an unknown random contributor.

The following is the example format that will be used for reporting statements using the \textit{unified} likelihood ratio:

\textbf{Lab Item 4.4: Stain from something}

Number of contributors: 3 (at least one male)
Assumed contributor(s): Jane Doe (Item 6)
Individually included:
  - Joe Smith (Item 3; LR = at least \text{<insert most conservative unified value from STRmix>})
Excluded:
  - Jimmy Dean (Item 8)

\textit{With Consideration of Untested Biological Relatives:}
The probability of observing this mixture DNA profile is at least \textit{<insert most conservative unified value from STRmix> times more likely if it originated from Jane Doe (Item 6), Joe Smith (Item 3) and one unknown random contributor than if it originated from Jane Doe (Item 6) and two unknown random contributors, to include the possibility of untested biological relatives of Joe Smith (Item 3).}

The following is the example format that will be used for reporting likelihood statements associated with a \textit{defined relative of interest}:

\textbf{Lab Item 4.6: Stain from something}

Number of contributors: 2 (at least one male)
Assumed contributor(s): Jane Doe (Item 6)
Individually included: Joe Smith (Item 3; LR = at least \textit{<insert most conservative LR value of untested biological relative of interest from STRmix>})
Excluded: Jimmy Dean (Item 8)

The probability of observing this mixture DNA profile is at least \textit{<insert LR value of relative of interest>} times more likely if it originated from Jane Doe (Item 6) and Joe Smith (Item 3) than if it originated from Jane Doe (Item 6) and an untested <insert biological familial relationship> of Joe Smith (Item 3).

\textbf{Reporting Deconvolutions without inclusionary LR Calculations}

The following are example formats that will be used for reporting profiles which have been deconvoluted only and have not had a likelihood ratio calculation:

\textbf{Lab Item 5.5: Swab from something}

Number of contributors: 2 (at least one male)
Assumed contributor(s): Monica Jackson (Item 9)

Assuming Monica Jackson (Item 9) is a contributor to the DNA profile obtained, a foreign contributor was detected.

\textbf{Lab Item 5.9: Swab from something}

Number of contributors: 2 (at least one male)
Excluded: John Smith (Item 5)

No additional conclusions can be made regarding the contributor(s) to this DNA profile at this time.

\textbf{Reporting Uninformative LRs 10^{3} to 10^{-3}}

Propositions which result in likelihood ratio values with exponents between 10^{3} to 10^{-3} will be considered uninformative and will be reported using the following statement:

“The likelihood ratio value noted above for <name and Item #> is within the uninformative range. Therefore, this does not provide sufficient support whether <name and Item #> is
included or excluded as a contributor to this sample. Internal validations and published studies help inform the limits of STRmix to where a false positive or false negative result may possibly arise. Likelihood ratios with exponents between $10^{-1}$ and $10^3$ have the potential to support a false inclusion or exclusion based on LVMPD internal validation studies.”

Individuals who are independently determined to be uninformative will not be considered in additional propositions in combination with included contributors.

The following is the example format that will be used for uninformative reporting statements:

**Lab Item 6.6: Swab of something**

Number of contributors: 2 (at least one male)
Individually included: Peter Jones (Item 5; LR = at least <insert most conservative 99% 1-sided lower HPD interval from STRmix>)

Uninformative: John Deere (Item 7; LR = at least <insert most conservative 99% 1-sided lower HPD interval from STRmix>)

Excluded: Jane Doe (Item 9)

The probability of observing this mixture DNA profile is at least <insert 99% 1-sided lower HPD interval from STRmix> times more likely if it originated from Peter Jones (Item 5) and one unknown random contributor than if it originated from two unknown random contributors.

“The likelihood ratio value noted above for John Deere (Item 7) is within the uninformative range. Therefore, this does not provide sufficient support whether John Deere (Item 7) is included or excluded as a contributor to this sample. Internal validations and published studies help inform the limits of STRmix to where a false positive or false negative result may possibly arise. Likelihood ratios with exponents between $10^{-3}$ and $10^3$ have the potential to support a false inclusion or exclusion based on LVMPD internal validation studies.”

**Reporting Exclusionary LRs $10^{-4}$ or less**

Propositions considering individual contributors which result in likelihood ratio values with exponents of $10^{-4}$ or lower will be reported as exclusions.

**Exclusion of Multiple Individuals to the same DNA profile**

In the event that multiple individuals are excluded as contributors to the same DNA profile, it is permissible to state “All other submitted reference standards” rather than individually list the excluded individuals and their item numbers.

**Profiles deemed unsuitable for STRmix analysis due to not meeting the required qualitative criteria for interpretation**

In the event a profile is determined to be unsuitable for STRmix interpretation due to not meeting the required qualitative criteria for interpretation, the following descriptive elements will be stated and the following report wording will be used:
• Whether the sample yielded a DNA profile or a mixture DNA profile.
• A statement as to whether there is an indication of at least one (1) male individual being present
• The report statement: “Due to the <limited or complex> nature of this profile, it is unsuitable for interpretation. <The likely number of contributors to this profile cannot be determined.>”

The following is the example report statement that will be used for profiles deemed unsuitable for STRmix due to not meeting the qualitative criteria for interpretation:

**Lab Item 7.7: Swab of something**
“The DNA profile obtained is consistent with a mixture, including at least one male contributor. Due to the limited nature of this profile, it is unsuitable for interpretation.”

*Mixture profiles of five or more contributors deemed unsuitable for STRmix analysis*
In the event a profile exhibits characteristics of five or more contributors, the following descriptive elements will be stated and the following report wording will be used:
• A statement that the sample yielded a mixture DNA profile.
• A statement that there are at least <insert number of contributors greater than or equal to five> contributors present.
• A statement as to whether there is an indication of at least one (1) male individual being present
• The report statement: “Due to the complexity of the data, no additional conclusions can be made regarding this mixture DNA profile.”

The following is the example report statement that will be used for profiles deemed unsuitable for STRmix due to having five or more contributors:

**Lab Item 8.1: Swab of something**
“The DNA profile obtained is consistent with a mixture of at least five contributors with at least one being male. Due to the complexity of the data, no additional conclusions can be made regarding this mixture DNA profile.”

*No DNA profile obtained*
The following report statement will be used when DNA typing results were not obtained:

**Lab Item 8.5: Swab of something**
“A DNA profile was not obtained.”

*Presence of single source contributor with up to two additional alleles*
The following report statement may be used in the event a DNA profile is consistent with a single source contributor, however there are one or two additional alleles present which meet the following criteria:
• Non-stutter position
• Unambiguous allele
• Less than 225 RFU drop-in cap
• Cannot reasonably pair with the weaker/stronger sister allele present
This statement may also be adjusted for use with single source assumed contributors that have one or two minor alleles present, even when not interpreting the profile in STRmix:

“<One additional allele/Two additional alleles> <was/were> detected in this sample, however due to the limited data available, no additional conclusions can be made regarding the source of <this allele/these alleles>.”

Reporting two interpretations differing in the number of contributors or the use of conditioning

In the event an analyst chooses to report two viable STRmix interpretations differing in the assumed number of contributors for the same item of evidence or if they wish to report an interpretation with and without conditioning, the number of contributors and individual reference comparisons for each STRmix analysis will be reported.

The following is the example format that will be used when reporting two interpretations differing in the number of contributors:

**Lab Item 10: Swab of something (differing in number of contributors)**

**Number of contributors:** 3 (at least one male)

Individually included: Donald Baker (Item 5; LR = at least <insert most conservative 99% 1-sided lower HPD interval from STRmix>)

Excluded: Stacy Monroe (Item 3)

The probability of observing this mixture DNA profile is at least <insert 99% 1-sided lower HPD interval from STRmix> times more likely if it originated from Donald Baker (Item 5) and two unknown random contributors than if it originated from three unknown random contributors.

**Number of contributors:** 4 (at least one male)

Individually included: Donald Baker (Item 5; LR = at least <insert most conservative 99% 1-sided lower HPD interval from STRmix>)

Excluded: Stacy Monroe (Item 3)

The probability of observing this mixture DNA profile is at least <insert 99% 1-sided lower HPD interval from STRmix> times more likely if it originated from Donald Baker (Item 5) and three unknown random contributors than if it originated from four unknown random contributors.

The following is the example format that will be used when reporting two interpretations differing in the conditioning of the item:

**Lab Item 10.1: Swab of something (with and without assumptions)**

**Interpretation with Assumptions:**

**Number of contributors:** 2 (at least one male)

**Assumed contributor(s):** Marcy Thompson (Item 1)
Individually included: Donald Baker (Item 5; LR = at least
<insert most conservative 99% 1-sided lower HPD interval from STRmix>)

Excluded: Stacy Monroe (Item 3)

The probability of observing this mixture DNA profile is at least <insert 99% 1-sided lower HPD interval from STRmix> times more likely if it originated from Marcy Thompson (Item 1) and Donald Baker (Item 5) than if it originated from Marcy Thompson (Item 1) and one unknown random contributor.

**Interpretation without Assumptions:**
Number of contributors: 2 (at least one male)
Individually included: Donald Baker (Item 5; LR = at least <insert most conservative 99% 1-sided lower HPD interval from STRmix>)

Excluded: Stacy Monroe (Item 3)

The probability of observing this mixture DNA profile is at least <insert 99% 1-sided lower HPD interval from STRmix> times more likely if it originated from Marcy Thompson (Item 1) and Donald Baker (Item 5) than if it originated from two unknown random contributors.

**Reporting multiple independently included individuals, but a combined exclusion**
In the event two (or more) individual likelihood ratios support inclusion with an item of evidence, but the likelihood ratio calculated for the presence of the individuals together in the mixture suggests that this cannot occur (exponent of LR is $10^{-4}$ or less), the following report wording will be included in the report:

“Though there is support for the individual inclusions of <name (Item #)> and <name (Item #)> to the mixture DNA profile, these individuals cannot be present in the mixture together. Refer to the independent inclusionary statistics above.”

The following is the example format that will be used when multiple individuals are independently included, but result in a combined exclusion:

**Lab Item 11.1: Swab of something**
Number of contributors: 4 (at least one male)
Assumed contributor(s): Monica Jones (Item 9)
Individually included: Thomas Parson (Item 12; LR = at least <insert most conservative 99% 1-sided lower HPD interval from STRmix>)

Benjamin Bell (Item 13; LR = at least <insert most conservative 99% 1-sided lower HPD interval from STRmix>)

Excluded: Laura Fink (Item 2)

Though there is support for the individual inclusions of Thomas Parson (Item 12) and Benjamin Bell (Item 13) to the mixture DNA profile, these individuals cannot be present in the mixture together. Refer to the independent inclusionary statistics reported above.
If a combined exclusion is identified for all individually included contributors to a 3 or 4-person mixture, the different combinations of included contributors will be reported.

The following is the example format that will be used when reporting multiple combinations of inclusionary likelihood ratios, but a combined exclusion of all contributors:

**Lab Item 9.1: Swab of something**

Number of contributors: 4 (at least two males)

Individually included:
- Peter Jones (Item 3; LR = at least \(<\text{insert most conservative 99% 1-sided lower HPD interval from STRmix}>\))
- Annie Barf (Item 9; LR = at least \(<\text{insert most conservative 99% 1-sided lower HPD interval from STRmix}>\))
- Joe Smith (Item 4; LR = at least \(<\text{insert most conservative 99% 1-sided lower HPD interval from STRmix}>\))

Excluded:
- Jimmy Dean (Item 8)

Though there is support for the individual inclusions of Peter Jones (Item 3), Annie Barf (Item 9), and Joe Smith (Item 4) to the mixture DNA profile, these individuals cannot be present in the mixture together. Refer to the different combinations of contributors which may explain the mixture below.

**Combinations of Included Individuals:**

The probability of observing this mixture DNA profile is at least \(<\text{insert 99% 1-sided lower HPD interval from STRmix}>\) times more likely if it originated from Peter Jones (Item 5), Annie Barf (Item 9) and two unknown random contributors than if it originated from four unknown random contributors.

The probability of observing the mixture DNA profile it is at least \(<\text{insert 99% 1-sided lower HPD interval from STRmix}>\) times more likely if it originated from Peter Jones (Item 3), Joe Smith (Item 4) and two unknown random contributors than if it originated from four unknown random contributors.

The probability of observing the mixture DNA profile it is at least \(<\text{insert 99% 1-sided lower HPD interval from STRmix}>\) times more likely if it originated from Annie Barf (Item 9), Joe Smith (Item 4) and two unknown random contributors than if it originated from four unknown random contributors.

**Reporting multiple independently included individuals who are biologically related**

In situations in which documentation exists regarding the biological relatedness between the two individually included contributors, the following disclaimer will be included in the report:

“**In DNA mixtures of closely related individuals** (such as parents, offspring, and siblings), false inclusions of other closely-related family members can occur due to the elevated sharing of genetic information between relatives.”
Reporting full single source unidentified contributors

If a full single source DNA profile does not match a known reference standard, this unknown profile will be assigned a sequential number based on gender (Male #1/Female #1). Amelogenin and/or the DYS391 locus may be utilized for gender determination. In the event Amelogenin and the DYS391 locus do not yield interpretable results above the analytical threshold, an item may be simply referred to as an "unknown individual".

The following is the example format that will be used when reporting full single source unidentified contributors:

**Lab Item 12: Swab of something**

Number of contributors: 1 male
Excluded: Laura Fink (Item 2)

The DNA profile obtained is consistent with a single unknown male contributor (Male #1).

The following report statement will be included at the end of the report to document that the unknown individual was not included in the likelihood ratio calculation(s) for mixture profiles due to not having an official reference standard for this individual or was not compared to a partial single source profile that did not deconvolute to 100% at all loci:

“No conclusions can be reached at this time regarding whether <insert unknown profile and number (refer to Item #)> can be considered a possible DNA contributor to the other DNA profile(s) reported in this case. Should this individual be identified, please submit reference buccal swabs and a new Laboratory Request <in Property Connect> to perform comparisons.”

Surreptitious standards

In the event a single source DNA profile obtained from an evidence item is being utilized as a surreptitious standard, the following statement must be placed at the beginning of the "Results, Opinions, and Interpretations":

“The DNA profile obtained from the <evidence item> yielded a <male/female> profile. For comparison purposes, this DNA profile will be utilized as the surreptitious standard for <subject name>.”

All subsequent comparisons made to the surreptitious standard will utilize a variation of the following language when making inclusions/exclusions:

The probability of observing this DNA profile is <approximately/at least> <insert sub-sub-source LR/99% 1-sided lower HPD interval from STRmix> times more likely if it originated from the donor to the <evidence item> than if it originated from...

Report disclaimers included at the end of reports which contain STRmix interpretations

The following statements will be included at the end of reports, as applicable:

When likelihood ratios have been reported for known reference standards, add the following three (3) statements:

“Where applicable, likelihood ratios (LR) were calculated to assess whether each submitted reference standard is statistically included or excluded, individually, as a contributor to the
reported DNA profile(s). The reported LR value for an “Individually Included” or “Uninformative” reference standard is reflective of the likelihood ratio calculation associated with the listed individual, without being considered in combination with other reference standards, except where an “Assumed Contributor” is denoted. The LR value answers the question “How many times more (or less) likely is it to observe the evidence DNA profile if the individual listed is a contributor to the DNA profile, than if the person listed is not a contributor?”

“The number of contributors utilized during STRmix interpretation is the most likely number required to reasonably explain the observed evidence DNA profile. This assessment was made during analysis with consideration of the quality of the DNA profile data.”

“The likelihood ratios are based upon propositions that can explain the evidence. This includes assumptions as to the number of contributors present in the DNA profile and, unless otherwise noted, that each unknown contributor is unrelated to the named reference standards. Since a range of propositions might explain the evidence, either interested party to this case, prosecution and/or defense, may request an additional likelihood ratio that incorporates an additional proposition more accurately representing their position. All requests must be submitted in a timely manner, must be reasonable given the test results, and must be within the capability and validated application of the program used.”

Request for buccal swabs
When STRmix analysis has yielded unidentified components or a surreptitious standard has been used for comparison purposes, the following statement will be added to the end of the report:
“For comparison purposes, please collect reference buccal swab(s) from <the consensual partner or> individuals believed to be involved in (or who have had reasonable access to) this incident. When a reference buccal swab is obtained, please submit a Forensic Laboratory Request <in Property Connect> to complete the case.”

Statistical statements
For all situations in which statistics were calculated and reported using STRmix v2.6, the following statement must be included at the end of the report:

NIST Population Database
“Statistical probabilities were calculated using the recommendations of the National Research Council (NRC II) utilizing the NIST database (Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) U.S. population data for 29 autosomal STR loci. Forensic Sci. Int. Genet. 7: e82-e83 and Steffen, C., Coble, M., Gettings, K., Vallone, P. Corrigendum to ‘U.S. Population Data for 29 Autosomal STR Loci’ [Forensic Sci. Int. Genet. 7 (2013) e82-83]. Forensic Sci Int. Genet. 31 (2017) e36-e40). The probability that has been reported is the most conservative value obtained from the US Caucasian (CAU), African American (AA), and Hispanic (HSP) population databases. All likelihood ratios calculated by the LVMPD are truncated to three significant figures.”

12.12 Statistical Report Statements Previously Reported using STRmix v2.3 for Identifiler Plus
For situations in which statistics were previously calculated and reported using STRmix v2.3, the following statement must be included at the end of the report:

Expanded FBI Population Database
“Statistical probabilities were calculated using the recommendations of the National Research Council (NRC II) utilizing the FBI database (Moretti, T.R., Moreno, L.I., Smerick, J.B., Pignone, M.L., Hizon, R., Buckleton, J.S., Bright, J.A., Onorato, A.J. (2016) Population data on the expanded CODIS core STR loci for eleven populations of significance for forensic DNA analyses in the United States. Forensic Sci. Int. Genet. 25: 175-181). The probability that has been reported is the most conservative value obtained from the US Caucasian (CAU), African American (BLK), and Southwest Hispanic (SWH) population databases. All likelihood ratios calculated by the LVMPD are truncated to three significant figures.”

12.13 Statistical Report Statements using Popstats for Outsourced Cases and Legacy Cases

12.13.7 Random Match Probability (RMP)
The following statement should be utilized when a Random Match Probability has been calculated for a sample:

“The probability of randomly selecting an unrelated individual from the general population having a DNA profile that is consistent with the <full/partial/major/minor/deduced> DNA profile obtained from the evidence sample is approximately 1 in <insert most conservative Popstats calculation truncated to three (3) significant figures (without rounding)>.”

12.13.8 Likelihood Ratio (LR) for Outsourced Cases
The following statement should be utilized when a Likelihood Ratio (LR) has been calculated for a sample.

“The mixture DNA profile obtained is consistent with originating from the known DNA profiles of <name #1> and <name #2>. The probability of observing this mixture DNA profile is <insert most conservative Popstats calculation truncated to three (3) significant figures (without rounding)> times more likely if the mixture originated from <name #1> and <name #2> than if it originated from <name #1> and an unrelated individual randomly selected from the general population.”

12.13.8.3 The LR statement above must be modified to reflect the sample-specific H1 and H2 hypotheses.

12.13.9 For all situations in which statistics were calculated and reported utilizing the Popstats software, the following statement must be included at the end of the report depending on the population database utilized:

FBI Amended Population Database:
“Statistical probabilities were calculated using the recommendations of the National Research Council (NRC II) utilizing the FBI database (J Forensic Sci 44 (6) (1999): 1277-1286 and J Forensic Sci doi: 10.1111/1556-4029.12806; J Forensic Sci 46 (3) (2001) 453-489 and Forensic Science Communications 3 (3) (2001)). The probability that has been reported is the most conservative value obtained from the US Caucasian (CAU), African American (BLK), and Southwest Hispanic (SWH) population databases. These numbers are an estimation for which a deviation of approximately +/- 10-fold may exist. All random match probabilities, combined probability of inclusions/exclusions, and likelihood ratios calculated by the LVMPD are truncated to three significant figures.”
NIST Population Database:
Statistical probabilities were calculated using the recommendations of the National Research Council (NRC II) utilizing the NIST database (Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) U.S. population data for 29 autosomal STR loci. Forensic Sci. Int. Genet. 7: e82-e83 and Steffen, C., Coble, M., Gettings, K. Vallone, P. Corrigendum to ‘U.S. Population Data for 29 Autosomal STR Loci’ [Forensic Sci. Int. Genet. 7 (2013) e82-83], Forensic Sci Int. Genet. 31 (2017) e36-e40). The probability that has been reported is the most conservative value obtained from the US Caucasian (CAU), African American (AA), and Hispanic (HSP) population databases. These numbers are an estimation for which a deviation of approximately +/- 10-fold may exist. All random match probabilities and likelihood ratios calculated by the LVMPD are truncated to three significant figures.”

12.14 Amended Reports
Amended reports will be issued when an error is identified associated with a previous report. These errors may be either administrative or technical in nature. The purpose is to retract the information contained in the original report and supersede it with the information contained in the amended report.

12.14.7 Amended Reports due to Re-Evaluation of Previously Reported Data
A Forensic Scientist has the right to retract data previously reported data that is no longer supported by significant evolution in technology or fundamental practices. An amended report will be issued which details the specific item numbers which were re-evaluated and the amended results.

The following disclaimer will be used:
“This lab report amends the previous lab report dated <original report date>. The originally generated data associated with <insert item #> was subjected to re-evaluation on <insert date>. The results of this re-evaluation are reported below.”

12.14.8 Amended Reports due to FBI Amended Allele Frequency Data
Due to the June 2015 release of amended allele frequencies associated with the FBI population databases utilized in the Popstats software for Identifiler Plus profiles, the LVMPD will re-calculate DNA statistics for all potentially affected DNA cases with upcoming court dates, discovery requests, or if a case is otherwise requested by legal counsel. The amended reports fall into one of the following two categories and will utilize the following reporting disclaimers and statistical citations:

12.14.8.3 Report Disclaimer and statistical statement for amended reports with statistics that change from that previously reported:
“This lab report amends the previous lab report dated <original report date> due to the release of amended allele frequencies associated with the FBI population database used for the previously reported statistical calculations.

On June 3, 2015, the FBI provided a pre-publication copy of the erratum regarding the original publication “Population data on the thirteen CODIS core short tandem repeat loci in African Americans, US Caucasians, Hispanics,
Bahamians, Jamaicans and Trinidadians” (J Forensic Sci 1999;44(6):1277-86). The erratum notes and amends several errors made in the original allele frequency data published in 1999 and utilized within the Popstats software. This software program is used by the LVMPD to calculate reported statistics. The underlying data that created the allele frequencies employed by the Popstats software is provided from and maintained by the FBI.

The below amended report utilizes the amended allele frequency data for all statistical calculations. These amended allele frequencies have been internally performance checked by the LVMPD to demonstrate that they have been successfully updated in the Popstats software. All reported statistical calculations will be reported using the same number of significant figures <and/or identity thresholds> as originally reported on <insert date of original report>.

Statistical probabilities were calculated using the recommendations of the National Research Council (NRC II) utilizing the FBI database (J Forensic Sci 44 (6) (1999): 1277-1286 and J Forensic Sci doi: 10.1111/1556-4029.12806; J Forensic Sci 46 (3) (2001) 453-489 and Forensic Science Communications 3 (3) (2001)). The probability that has been reported is the most conservative value obtained from the US Caucasian (CAU), African American (BLK), and Southwest Hispanic (SWH) population databases. These numbers are an estimation for which a deviation of approximately +/- 10-fold may exist."

12.14.8.4 Report Disclaimer and statistical statement for amended reports with statistics that do not change from that previously reported:"
“This lab report amends the previous lab report dated <original report date> due to the release of amended allele frequencies associated with the FBI population database used for the previously reported statistical calculations.

On June 3, 2015, the FBI provided a pre-publication copy of the erratum regarding the original publication “Population data on the thirteen CODIS core short tandem repeat loci in African Americans, US Caucasians, Hispanics, Bahamians, Jamaicans and Trinidadians” (J Forensic Sci 1999;44(6):1277-86). The erratum notes and amends several errors made in the original allele frequency data published in 1999 and utilized within the Popstats software. This software program is used by the LVMPD to calculate reported statistics. The underlying data that created the allele frequencies employed by the Popstats software is provided from and maintained by the FBI.

On <insert date of new calculations>, the originally reported data associated with this case file was subjected to recalculation using the amended allele frequency data for all previously performed statistical calculations. Based upon this recalculation event, the statistical values did not change from those previously reported by the LVMPD on <insert date of original report>.

Statistical probabilities were calculated using the recommendations of the National Research Council (NRC II) utilizing the FBI database (J Forensic Sci
12.14.9 Amended Reports due to NIST Amended Allele Frequency Data

The LVMPD will only re-calculate DNA statistics upon request for Qiagen Investigator 24plex QS profiles generated prior to August 29, 2017. The amended reports fall into one of two categories and will utilize the following reporting disclaimers:

**12.14.9.3 Report Disclaimer and statistical statement for amended reports with statistics that change from that previously reported:**

“This lab report amends the previous lab report dated <original report date> due to the release of amended allele frequencies associated with the NIST population database used for the previously reported statistical calculations.

The below amended report utilizes the amended allele frequency data for all statistical calculations. These amended allele frequencies have been internally performance checked by the LVMPD to demonstrate that they have been successfully updated in the Popstats software. All reported statistical calculations will be reported using the same number of significant figures <and/or identity thresholds> as originally reported on <insert date of original report>.

Statistical probabilities were calculated using the recommendations of the National Research Council (NRC II) utilizing the NIST database (Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) U.S. population data for 29 autosomal STR loci. Forensic Sci. Int. Genet. 7: e82-e83 and Steffen, C., Coble, M., Gettings, K. Vallone, P. Corrigendum to ‘U.S. Population Data for 29 Autosomal STR Loci’ [Forensic Sci. Int. Genet. 7 (2013) e82-83]. Forensic Sci Int. Genet. 31: e36-e40). The probability that has been reported is the most conservative value obtained from the US Caucasian (CAU), African American (AA), and Hispanic (HSP) population databases. All likelihood ratios calculated by the LVMPD are truncated to three significant figures. These numbers are an estimation for which a deviation of approximately +/- 10-fold may exist. All likelihood ratios calculated by the LVMPD are truncated to three significant figures.”

**12.14.9.4 Report Disclaimer and statistical statement for amended reports with statistics that do not change from that previously reported:**

“This lab report amends the previous lab report dated <original report date> due to the release of amended allele frequencies associated with the NIST population database used for the previously reported statistical calculations.

On <insert date of new calculations>, the originally reported data associated with this case file was subjected to recalculation using the amended allele frequency data for all previously performed statistical calculations. Based
upon this recalculation event, the statistical values did not change from those previously reported by the LVMPD on <insert date of original report>.

Statistical probabilities were calculated using the recommendations of the National Research Council (NRC II) utilizing the NIST database (Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) U.S. population data for 29 autosomal STR loci. Forensic Sci. Int. Genet. 7: e82-e83 and Steffen, C., Coble, M., Gettings, K. Vallone, P. Corrigendum to ‘U.S. Population Data for 29 Autosomal STR Loci’ [Forensic Sci. Int. Genet. 7 (2013) e82-83]. Forensic Sci Int. Genet. 31: e36-e40). The probability that has been reported is the most conservative value obtained from the US Caucasian (CAU), African American (AA), and Hispanic (HSP) population databases. All likelihood ratios calculated by the LVMPD are truncated to three significant figures. These numbers are an estimation for which a deviation of approximately +/- 10-fold may exist. All likelihood ratios calculated by the LVMPD are truncated to three significant figures."

12.15 Supplemental Reports

Supplemental reports represent additional information (new evidence, new comparisons to reference standards, additional interpretation) as it pertains to a previously reported case. With the exception of supplemental reports associated with legacy data, supplemental reports typically do not retract previously reported data or supersede previously reported results. Refer to Supplemental Report Disclaimer for Reinterpretation of Legacy Data (Profiler Plus/COfiler, Identifiler, or Identifiler Plus) for additional information.

Required Changes When Writing Supplemental Reports

In the event a supplemental report is required due to the submission of additional evidence items and/or reference standards for analysis/comparison for a case, changes are not required to be made to the original evidence interpretations or reporting style with the exception of the following items:

1) Where applicable, previously reported RMP statements will be updated to reflect non-identity statements, truncated to three significant figures.
2) Previously issued CPE statistics will be converted to CPI using the originally generated Popstats data.
3) Uninterpretable report statements must be updated to include the qualitative reason for deeming the profile (or a portion thereof) as uninterpretable.
4) Inconclusive contributors identified in STRmix will be updated to “Uninformative” and the originally calculated LR will be included in the supplemental report, along with the updated uninformative report statement.
5) Previously reported and designated unknown profiles (e.g. Unknown Male #1, Unknown Female #1, etc.) must be searched in the “DNA Profile Searcher” and documented in the Analyst Checklist.
6) All previously issued statistics calculated prior to the June 2015 allele frequency update will be re-calculated utilizing the updated FBI amended allele frequencies.
7) If a DNA profile was entered into CODIS the CODIS statement will be repeated with the following modifications:
“The <major/partial/minor/deduced/a portion of the mixture> DNA profile was searched against the Local DNA Index System (CODIS) and was uploaded to the <State/National> DNA Index System (CODIS) for comparison. You will be notified of any match(es).”

12.15.7 Supplemental Reports and STRmix

If a reference standard is received to be compared to an item which was previously interpreted prior to the use of STRmix and determined to be eligible for single source or mixture-level statistics, however the statistic was not calculated (RMP, CPI or LR) or an unknown component was determined to be present, the item may be re-analyzed and compared to the reference standard in STRmix based on analyst discretion. Alternatively, the evidence may utilize the interpretation guidelines that were adopted in the Technical Manual at the time of the original analysis (to include the use of Popstats for statistical calculations). Analysts may refer to these archived Technical Manuals for guidance.

Items of evidence which were previously reported as uninterpretable (or a portion thereof) or where a CPI was calculated, but resulted in a low statistic may be re-analyzed in STRmix based on analyst discretion. These items will be reported using the results of STRmix analysis.

Whenever STRmix is used for reinterpretation, the injection list and electropherograms for data re-analyzed in GeneMapper ID-X and exported into STRmix will be printed for the supplemental case file.

When a reference standard is received for comparison to a previously reported unknown single source evidence profile and a STRmix deconvolution has not yet been performed, the GeneMapper ID-X STRmix injection list from the original analysis will be printed for the supplemental case file, along with a copy of the previously reported evidence electropherogram. This documentation will be re-paginated in the supplemental case file in support of the new STRmix run and comparison to the reference standard.

If the evidence sample was not previously analyzed using the necessary STRmix analysis parameters, the sample will be re-analyzed in GeneMapper ID-X and exported to STRmix in accordance with Chapter 8. The new STRmix injection list and electropherogram will be printed for the supplemental case file.

Supplemental Report Disclaimer for Reinterpretation in STRmix based on Analyst Discretion

When an analyst re-evaluates their previously reported data and determines it to be suitable for STRmix reinterpretation, the following disclaimer will be used:

“The data originally generated in <insert year of original analysis> associated with <insert item #> was subjected to re-evaluation and re-analysis using the STRmix software adopted by the Biology/DNA Detail on September 18, 2017. The results of this re-analysis are reported below.”

STRmix Re-Analysis Due to Lieutenant Written Request or Legal Court Order

Only items which were previously reported as uninterpretable (or a portion thereof) will be considered for re-analysis using STRmix. If statistics were previously reported (RMP, CPI, or LR using Popstats), the item will not be re-analyzed in STRmix.

- The supplemental report will only include the results of the specific items re-analyzed and not all items contained within the original report.
• The following disclaimer will be used when STRmix analysis is performed:
  “A <request or court order> for re-analysis of <insert item #> was received on <insert date>. The data originally generated in <insert year of original analysis> associated with this item # was subjected to re-analysis using the STRmix software. The results of this re-analysis are reported below.”

• The following disclaimer will be used when re-evaluation of the original data does not support the use of STRmix:
  “A <request or court order> for re-analysis of <insert item #> was received on <insert date>. Based on a re-evaluation of the quality of the data originally generated and the laboratory’s policies and procedures associated with the validated use of the STRmix software, it was determined that <insert item #> is not suitable for re-analysis.”

12.15.8 Supplemental Report Disclaimer for Reinterpretation of Legacy Data (Profiler Plus/COfiler, Identifiler, or Identifiler Plus)

Based on the SWGDAM “Clarification on the Reinterpretation of Data Typed with Legacy Amplification Test Kits” released in June 2016, the following will be used as a definition for reinterpretation: “Assessing/evaluating allele calls, genotype calls (to include potential allelic drop-out), a change in the assumptions used, or removing alleles (or entire loci) from statistical estimates from legacy amplification test kit data, are all considered reinterpretation.”

If a supplemental report is being issued in which comparisons are made to legacy data that has had the original interpretation modified in any way (whether manually or with STRmix), the following statement must be included as a disclaimer in the report:

“Due to the receipt of a supplemental request, this report supersedes all previously issued DNA results and conclusions. The below reinterpretation is based on guidance set forth by the Scientific Working Group on DNA Analysis Methods (SWGDAM) and a review of the validations and standard operating procedures in place at the time the original/supplemental report was issued on <XXX>.”

12.15.9 Supplemental Report Disclaimer for Reinterpretation using a Requested Alternative Set of Propositions

The LVMPD allows either interested party to a case, the prosecution and/or defense, to request an additional likelihood ratio calculation that incorporates an additional proposition which more accurately represents their position. In cases in which such a request is received, the following statement will be included as a disclaimer on the report:

"A request for an additional likelihood ratio calculation for <insert Lab Case # and Item #> was received on <insert date>. The data originally generated in <insert year of original analysis> associated with this item # was subjected to re-evaluation to determine whether the requested alternative likelihood ratio is reasonable given the previously reported test results. The results of the two alternative evaluations of the <insert Lab Case # and Item #> are reported below. These evaluations differ in <insert the difference between the originally reported proposition and the requested proposition (e.g. the number of contributors which can explain the observed mixture DNA profile)>.”
12.16 Modification of Identity Statements

When issuing a supplemental or amended report for reports issued prior to March 24, 2015 in which additional comparisons/interpretations are taking place AND identity statements were previously reported, the following statement will be added to the DNA report prior to the first reported item conclusion in order to explain the modification from identity statements:

“This report supersedes all previously issued DNA results and conclusions based on modifications to the Biology/DNA policies and procedures effective March 24, 2015.

On March 24, 2015, the Biology/DNA Detail ceased reporting identity statements when an individual could not be excluded from a DNA profile that was rarer than approximately 1 in 100 times the world’s estimated population. Where applicable, the below reported conclusions represent the original statistical calculations performed on <insert date from Popstats print-out> without being truncated for identity purposes. Modified statistical statements will be delineated using an <asterisk (*)/ other symbol>.

The modification to the new RMP report statement will also extend to supplemental or amended reports in which additional comparisons/interpretations take place and non-identity RMP statements were previously reported. The language will be modified from “…rarer to 1 in XXX” to the updated RMP statement “The probability of randomly selecting an unrelated individual from the general population having a DNA profile that is consistent with…”

12.17 Evidence Disposition and Case File Disclaimers

The following statements will be added above the signature line at the end of the report in the following order:

1) “The evidence is returned to secure storage.”
2) “Dates of laboratory testing: <insert date Unit Record was assigned in LIMS> to <insert date when laboratory work completed on analyst checklist>.”
3) “This report does not constitute the entire case file. The case file may be comprised of worksheets, images, analytical data and other documents.”

The following statements will be added to the end of each report, as applicable:

- “DNA extracts generated during the analysis of this case and/or cuttings taken from the evidence may be available for future testing.”

For reports in which STR processing was conducted, the following statement may be included in the report, based on analyst discretion and/or specific recommendation by the DNA Management:

- “The reported DNA profile results can aid in answering questions regarding who may have deposited DNA on an item of evidence and where this DNA was deposited. However, the presence or absence of a DNA profile cannot answer questions with regards to the timeframe and/or circumstances in which the DNA was deposited on an item of evidence.”

For reports containing differentially extracted samples that were subjected to DNA STR Processing:

- “Items with the EF/SF fraction results were extracted using a differential extraction technique. This technique attempts to separate non-sperm cell DNA (EF fraction) from
potential sperm cell DNA (SF fraction). This terminology does not imply the presence or absence of spermatozoa in this case.”

For reports which contain assumed contributors:
- “In instances in which contributors can be assumed, no statistical calculations will be reported for the assumed contributors.”

For reports containing samples where microscope slide(s) were created and are available for future searching:
- “As part of the analytical process, slides were prepared from Item XXX for possible sperm identification. These slides were re-packaged within the sexual assault kit <and/or outer packaging>; however, were not examined at this time.”

The following will be placed in the notes section of negative case report when all evidence samples were terminated prior to amplification, however the associated reagent blank was amplified to confirm whether contamination exists and is found to be clean:
- “As a quality control measure, a reagent blank negative control associated with samples extracted in this case (<insert RB name>) was subjected to PCR amplification at the following genetic loci: TH01, D3S1358, vWA, D21S11, TPOX, DYS391, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D21S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. The sex-determining Amelogenin locus was also examined. This reagent blank negative control did not exhibit any DNA data.”

When all samples in a case yield negative or inconclusive results, however a sample has male DNA detected (no color or pink), and there is a male reference standard available for comparison:
- “Lab Item XXX may be suitable for male-specific Y-STR testing by an outside laboratory. This item may be requested for Y-STR testing if accompanied by reference buccal swab(s) from male individuals believed to be involved in this incident (to include consensual partner(s)).”

12.18 Parentage Report Statements

12.18.7 Parentage Index
If the alleged father cannot be excluded as the biological father, the following statement should be utilized:

"The DNA profiles obtained from <Child XXX> and <Mother XXX> were compared with that of <Alleged Father XXX>. <Alleged Father XXX> cannot be excluded as the biological father of Child (Item 2) as determined by the presence of the obligate paternal allele at all of the loci tested.

It is <XXX (insert parentage index)> times more likely to observe the DNA profile obtained from <Child> if <Alleged Father> is the biological father than if a random, untested man in the general population who is unrelated to <Alleged Father> is the biological father."
The probability of paternity is <XX.XXXXX% (insert probability of parentage)> (assuming a prior probability of 0.50) as compared to a random, untested individual from the general population.”

If the alleged father can be excluded as the biological father, the following statement should be utilized:

“The DNA profiles obtained from <Child XXX> and <Mother XXX> were compared with that of <Alleged Father XXX>. <Alleged Father XXX> is excluded as the biological father of <Child XXX>.”

12.18.8 Reverse Parentage Index and Reverse Probability of Paternity

If the alleged couple cannot be excluded as the biological parents of the child, the following statement should be utilized:

“The DNA profile of <Alleged Mother XXX> was compared with that of <Child XXX>. <Alleged Mother XXX> cannot be excluded as the biological mother of <Child XXX>.

It is <XXXXX (insert reverse parentage index)> times more likely to observe the DNA profile obtained from <Child> if they are the biological child of <Alleged Mother> and <Alleged Father> than if the DNA profile originated from a random, untested individual from the general population.

The probability of paternity is <XX.XXXXX% (insert probability of parentage)> (assuming a prior probability of 0.50) as compared to a random, untested individual from the general population.”

If the alleged couple can be excluded as the biological parents of the child, the following statement should be utilized:

“The DNA profile of <Child XXX> was compared with that of <Alleged Mother XXX> and <Alleged Father XXX>. <Child XXX> is excluded as the biological child of <Alleged Father XXX> and <Alleged Mother XXX>.”

12.18.9 Single Parent Comparison

In cases of missing persons or potential homicides in which a body has not been located and a secondary standard is not available, the single parent comparison may be used to infer whether a single source evidence sample may be biologically related to a single alleged parent.

If the alleged parent cannot be excluded as the biological parent of the child, the following statement should be utilized:

“The DNA profile of <Alleged Parent> was compared with that of <Child>. <Alleged Parent> cannot be excluded as the biological <mother/father> of <Child>.

It is <XXXXX (insert single parentage index)> times more likely to observe the DNA profile obtained from <Child> if <Alleged Parent> is the biological <mother/father> of <Child> than if a random, untested <woman/man> from the general population is the biological <mother/father>. The probability of excluding a random <woman/man> from the general population as the biological <mother/father> given the alleles of the child is greater than <XX.X (insert single parentage probability of exclusion)>%.”
If the alleged parent can be excluded as the biological parent of the child, the following statement should be utilized:

“The DNA profile of <Alleged Parent> was compared with that of <Child>. <Alleged Parent> can be excluded as the biological <mother/father> of <Child>.

12.19 Mutation Statements
Refer to mutation rates observed in the Popstats program.

If two (2) or fewer possible mutations are observed, in the conclusions state “The presence of a possible mutation was observed in the DNA profile of <XXX>. This was taken into consideration when performing statistical calculations.”

12.20 Contamination Report Statements
There are several types of contamination which can be categorized as either attributable or unattributable. Attributable contamination describes a contamination event where the source of the contamination is known and can be identified. Unattributable contamination describes a contamination event where the source of the contamination is unknown and cannot be identified.

12.20.7 Contamination Attributable to an Internal DNA Laboratory Processing Event
- If contaminated reagent blanks and/or controls cannot be resolved through re-analysis, the following report statement will be used:
  “Due to an associated control failure, no conclusions will be made with regard to <XX>.”

- If contaminated samples cannot be resolved through re-analysis (whether due to internal sample-to-sample contamination, sample switch, etc.), the following report statement will be used:
  “Due to a sample contamination issue, no conclusions will be made in regard to <XX>.”

- If a buccal swab is not available for confirmation from a previously employed DNA examiner to be used to attempt to resolve mixture DNA profiles, the following statement will be used:
  “A <insert job title> previously employed with the LVMPD Forensic Laboratory cannot be excluded as a contributor to the DNA profile obtained from <XXXX>. No additional conclusions can be made regarding this mixture.”

- If a DNA examiner (Forensic Scientist, Forensic Laboratory Technologist, etc.) in the Biology/DNA Detail contaminated a sample and their known DNA profile was subsequently confirmed and used to condition the deconvolution, the following disclaimer will precede the reporting of the STRmix results:
  "During a routine search of the LVMPD staff elimination database, the known DNA profile of the <insert job title> <screening/processing> the evidence in this case was identified as a DNA contributor to the mixture DNA profile obtained from <insert Lab Item #>. The original evidence was re-processed, however the same <insert job title>’s DNA profile was present in the resultant DNA mixture. It therefore appears the original evidence item itself was inadvertently contaminated during the examination process."
In an effort to further resolve the DNA contributors who may be present in combination with the contamination, the known DNA profile of the <insert job title> was utilized in the interpretation of the mixture DNA profile obtained from <insert Lab Item #>. The results of this interpretation, which assumes the presence of the contaminating DNA profile, are reported below:

The name of the DNA examiner will not be listed in the body of the report. Only the title of their position (e.g. Forensic Scientist, Forensic Laboratory Technologist, etc.) will be reported when identifying that an assumed contributor was used for interpretation.

12.20.8 Contamination Attributable to a Sample Switch Occurring within the Laboratory

“Due to a sample contamination issue, no conclusions will be made in regard to <XX>.”

12.20.9 Contamination Attributable to an Individual External to the Laboratory Processing Events

- If contamination is identified by a search of the elimination database with any individual other than the DNA examiner and a buccal swab is not available for confirmation from the individual identified as the potential source of the contamination, the following report statement will be used:
  “An individual contained within the LVMPD elimination database cannot be excluded as a contributor to the DNA profile obtained from <XXXX>. No additional conclusions can be made regarding this mixture.”

- If an individual had documented reasonable access to the evidence and their known DNA profile was subsequently confirmed and used to condition the deconvolution, the following disclaimer will precede the reporting of the STRmix results:
  “An individual associated with the LVMPD elimination database cannot be excluded as a contributor to the DNA profile obtained from <XXXX>. This individual’s DNA profile was utilized to further interpret the evidence DNA profile. The results of this interpretation are reported below.”

The name of the individual will not be listed in the report. Only the title of their position (e.g. Crime Scene Analyst, etc.) will be reported when identifying that an assumed contributor was used for interpretation.

- In the event a sample is entered into CODIS and later hits on an elimination standard, an additional report will be issued to reflect the detection of the contamination. The affected sample(s) and/or case(s) will be re-analyzed when possible. The contaminated profile will be removed from CODIS.

12.20.10 Unattributable Contamination

Examples include contaminated reagent blanks and/or controls which cannot be resolved through re-analysis. In the “Results, Opinions, and Interpretations” state: “Due to an associated control failure, no conclusions will be made with regard to <XX>.”
12.21 CODIS Report Statements

Anytime a DNA profile is searched in the CODIS database a statement to that effect must be included in the report. The following statements will be utilized when a DNA profile will be entered into CODIS. Reference standards requiring the below statements will be added to the report using the Lab Item # as a heading:

12.21.7 Regular or Supplemental Search
“The <major/partial/minor/deduced/a portion of the mixture> DNA profile <will be/was> searched against the Local DNA Index System (CODIS) and <then/was> uploaded to the <State/National> DNA Index System (CODIS) for comparison. You will be notified of any match(es).”

12.21.8 Volunteer Sample
“The full <male/female> DNA profile obtained from the volunteer sample submitted from <XXX> will be searched against the Local DNA Index System (CODIS) and then uploaded to the State DNA Index System (CODIS) for comparison. You will be notified of any match(es).”

12.21.9 Juvenile Volunteer Sample
“The full <male/female> DNA profile obtained from the volunteer sample submitted from <XXX> cannot be entered into CODIS because <he/she> was a juvenile at the time of consent.”

Note: In the event the above statement is not utilized, documentation must be retained in the case file regarding the notification of the Detective and/or investigating agent.

12.21.10 Sample Removal Statements
“The DNA profile was removed from the <National DNA Index System (CODIS) and/or State DNA Index System (CODIS)> and will be retained at the Local DNA Index System (CODIS) because <insert reason>.”

“The <major/partial/minor/deduced/a portion of the mixture> DNA profile obtained from <XX> was removed from the <Local/State/National> DNA Index System (CODIS) because<insert reason>.”

12.21.11 Sample Update Statement Following Additional Analysis
“A DNA profile was previously obtained from this item by <insert analyst/vendor name or refer to previous report>, which was <searched in/uploaded to> the <Local/State> DNA Index System (CODIS) for comparison. Additional genetic information obtained during this analysis will be used to update the profile and upload it to the <State/National> DNA Index System (CODIS) for comparison. You will be notified if there are any match(es).”

12.21.12 Sample Update Statement When CODIS Level Found To Be Elevated Since Original Entry
“The DNA profile obtained from <XXX> was previously searched in the Local DNA Index System (CODIS) <and uploaded to the State DNA Index System (CODIS)> for comparison. Due to changes in CODIS search procedures since then the profile has been uploaded to the <State/National> DNA Index System (CODIS) for comparison. You will be notified if there are any match(es)”
LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
BIOLOGY/DNA DETAIL

13.0 Title:  CODIS Policies and Procedures

13.1 CODIS Policies:
LVMPD and CODIS Responsibilities

- Receive and store DNA samples from convicted persons pursuant to NRS 176.0913, convicted offenders from out-of-state on probation or parole pursuant to NRS 176.0916, sex registrants pursuant to NRS 179D.240, 179D.443, and 179D.460, and persons arrested for a felony pursuant to NRS 176.09123.

- Provide a repository of DNA profiles from convicted persons, persons required to register as sex offenders, and persons arrested for a felony, for entry into CODIS at the Local level for upload to the State and National levels.

- Provide a repository of DNA profiles from forensic cases, unidentified human remains, relatives of missing persons, and voluntary samples for comparison in the Local CODIS system and possible upload to the State and National CODIS systems, if eligible for comparison.

- Ensure that the laboratory is in compliance with National DNA Index System (NDIS) regulations.

- Provide information to investigators to assist in the investigation of crimes and identification of recovered human remains.

- Access to the CODIS program is limited to the CODIS Administrator, alternate administrators, and other approved CODIS users to include the analysts, Biology/DNA Detail Laboratory Manager, Biology/DNA Detail Supervisor and Technical Leader. No instrument or software may be linked or interfaced to the CODIS PC or software.

- Court orders may also be received for expungement of database, voluntary, or forensic samples from the database.

- The only persons permitted to provide information to outside agencies are the Laboratory Director, the CODIS Administrator, alternate administrators, and other CODIS users to include the analysts, Biology/DNA Detail Laboratory Manager, Biology/DNA Detail Supervisor and Technical Leader:
  a. The identity of any person/agency making a request for information should be verified prior to the release of any information.
  b. The release of information is restricted to appropriate criminal justice agencies.

- All DNA records and DNA samples submitted to the LVMPD shall be exempt from the public records law (NRS239.010-239.030). DNA records and DNA samples submitted in association with CODIS to the LVMPD may only be released for the following authorized purposes:
  a. For law enforcement identification purposes, including the identification of human remains;
  b. For criminal defense and appeal purposes, to a defendant, who shall have access through proper legal proceedings to samples and analyses performed in connection with the case in which such defendant is charged or was convicted; and
  c. To criminal justice agencies, if personally identifiable information is removed, for forensic validation studies, forensic protocol development or quality control purposes and establishment or maintenance of a population statistics database.
Rules of Authority

- The CODIS program and DNA Databasing Laboratory are part of the Biology/DNA Detail of the LVMPD Forensic Laboratory. Each DNA analyst has casework and/or database duties depending on competency and proficiency status for the STR amplification kit used in each workflow.
- To further the goals and objectives of the CODIS program the Laboratory Director has delegated the authority for running and maintaining the program to the CODIS Administrator.
- If two analysts are in disagreement over a problem, a third qualified DNA analyst, the Technical Leader or the CODIS Administrator may be requested to resolve a technical issue.
- The Nevada CODIS State Administrator at the WCSO Forensic Sciences Division will also be the "Designated State Official" (DSO) for communications with NDIS. The responsibilities of this position include: uploading data from the local LVMPD and WCSO databases to the state and national databases, performing auto searches of the state database, and keeping the computer systems functional and secure. The State Administrator communicates information from national CODIS State Administrator meetings and is available by phone or email for technical assistance.
- Requests for searches will be directed to the Biology/DNA Detail of the LVMPD and the WCSO laboratory.

LVMPD Forensic Lab Workflow

LVMPD is a local user of CODIS. LVMPD participates at the LDIS (Local DNA Index System) level and eligible profiles are then uploaded to the SDIS (State DNA Index System) level which is maintained by the Washoe County Sheriff's Office (WCSO). The SDIS level, WCSO, then uploads the profiles up to the NDIS (National DNA Index System) level which is maintained by the Federal Bureau of Investigation (FBI) if they meet the appropriate criteria.

The Biology/DNA Detail processes several types of samples for entry into CODIS, each categorized into one of two groups: casework or database (includes convicted offenders and felony arrestees). The database samples are entered into the Local DNA Index System (LDIS) through a direct electronic import by the CODIS Administrator (or alternate) using the Convicted Offender or Arrestee specimen categories assigned during technical review. The casework samples are entered by keyboard STR Data Entry or direct electronic import by the analyst who owns the sample.

Further classification occurs when a sample ‘hits’ in CODIS. An “Offender Hit” occurs when an unsolved casework DNA profile is connected to a convicted offender DNA profile. An “Arrestee Hit” occurs when an unsolved casework DNA profile is connected to an arrestee DNA profile. Typically these two kinds of hits come from database samples. “Forensic Hits” occur when casework DNA profiles match from one case to another.

Elimination Database

The LVMPD Forensic Lab maintains a database of employee profiles to identify any biological contamination that may occur. The Elimination Index includes the DNA profiles of all members of the Forensic Laboratory and Crime Scene Analysts of the Criminalistics Bureau of the LVMPD required to provide a sample, as well as DNA profiles of lab vendors, detectives, investigating agents of the LVMPD, nurse examiners and other Law Enforcement personnel from outside jurisdictions who have provided their DNA sample on a voluntary basis for entry into the index. The Elimination Index is used for screening purposes only. The index is not used for investigative purposes, and profiles that reside in the Elimination Index of CODIS cannot be uploaded to SDIS or NDIS.
13.2 Casework Samples

This group consists of forensic known and unknown samples, as well as volunteer samples, that may originate from evidence and reference standards collected during any law enforcement agency investigation.

Samples will be analyzed using the DNA procedures developed for casework. Analysis will be attempted at the original STR core CODIS loci: D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, CSF1PO, TPOX, TH01 and D16S539 and the Amelogenin sex indicator. The Qiagen Investigator 24plex QS kit also tests at SE33, D1S1656, D2S441, D2S1338, D10S1248, D19S433 and D22S1045, as well as a Y-marker DYS391.

Analysis results will be maintained in the original laboratory case file or scanned into the Object Repository of the LIMS, and in electronic format in the DNA laboratory.

A CODIS STR Data Entry (SDE) packet is created for those samples that are eligible for keyboard STR Data Entry or direct electronic import.

No CODIS entries may be made until the case file has been technically reviewed by a qualified DNA Analyst. Casework samples are not uploaded to the State DNA database (SDIS) until after administrative review and the report has been released. The DNA section uses these criteria and also checklists on review forms to ensure proper review of CODIS eligible samples.

Volunteer Index

Known samples, also called Voluntary or Volunteer samples, can originate from any law enforcement agency or CSI and are considered evidentiary. Typically these are buccal swabs and require an accompanying signed consent form from the donor. Volunteer samples are processed as part of a case and are tracked as evidence; they are not processed as Database samples. Juveniles are not allowed to be entered as a volunteer sample at this time. If the consent form is signed by a juvenile or with the parents’ consent the sample will NOT be entered into the Volunteer Index.

All reference standards with a signed volunteer consent will be entered into CODIS with the exception of the following:

1) The individual signing the volunteer consent form also signed a victim consent form.

2) If a Volunteer Consent Form is received from the listed victim in the case, the Detective will be contacted to determine if the individual should be entered. In the event a response is not received by the time the case is completed, the profile will not be entered into CODIS. The profile may be entered at a later date if a response is received from the Detective.

3) If a Volunteer Consent Form is received from an elimination or consensual partner in the case, the Detective may be contacted to determine if the individual should be entered. In the event a response is not received by the time the case is completed, the profile will be entered into CODIS. The profile may be deleted at a later date if a response is received from the Detective.

Documentation of all discussions with the Detective regarding the Volunteer Consent Form will be included in the case file or OR.

Samples that consist of single source DNA profiles from volunteer reference standard samples that contain complete results for D2S1338, D19S433 and all 13 core CODIS loci for upload only to SDIS
and will NOT be uploaded to NDIS. If a case has both a CODIS eligible volunteer sample and a forensic sample that is a benchwork match to the volunteer, the sample that will go to the higher level of CODIS for searching will be entered and uploaded.

**Forensic Indexes**
Evidence collected at crime scenes during any law enforcement agency investigation may be suitable for DNA analysis and entry into CODIS. These samples are typically forensic unknowns and are run against the offender, arrestee, volunteer, and all forensic unknown indexes in CODIS if they meet specific eligibility criteria. Not all samples will qualify as CODIS has strict requirements for what is allowed for entry into the indexes.

Some samples may match a known buccal swab in the case but the evidence profile may still be eligible to be entered in the Forensic indexes.

- **Forensic Unknown Index**
  Consists of single source DNA profiles from forensic samples that contain complete results for all 13 original CODIS core loci.

- **Forensic Partial Index**
  Consists of single source DNA profiles from forensic samples that do not contain complete results for all 13 original CODIS core loci and/or that may indicate a possibility of allelic dropout (e.g. the +). In order to search/upload a forensic partial profile at LDIS, SDIS or NDIS the moderate match estimation (MME) must be calculated first.

- **State Only Forensic Partial Index**
  Consists of samples as described above that do not meet the NDIS MME calculation requirements.

- **Local Only Forensic Partial Index**
  Consists of samples as described above that do not meet the SDIS or NDIS MME calculation requirements.

- **Forensic Mixture Index**
  Consists of DNA profiles from forensic samples that contain DNA contributed from more than one source, typically two individuals. In order to search/upload a forensic mixture profile at LDIS, SDIS or NDIS the moderate match estimation (MME) must be calculated first.

- **State Only Forensic Mixture Index**
  Consists of samples as described above that do not meet the NDIS MME requirements.

- **Local Only Forensic Mixture Index**
  Consists of samples as described above that do not meet the SDIS or NDIS MME calculation requirements.

- **Forensic Targeted Index**
  Consists of forensic partial and mixture specimens that do not meet the NDIS MME calculation requirements but will meet a match rarity estimate (MRE) calculation with stringency assigned by locus.

**Other Casework CODIS Indexes**
- **Unidentified Human** - samples originating from a deceased person’s tissue, bone, tooth or hair sample
- **Relatives of Missing Persons** - samples originating from primary reference standards such as buccal swabs or blood cards; these are from 1st degree relatives such as a biological mother, biological father, sibling, or biological child
• **Missing Persons** - samples originating from an alleged missing person’s property such as a hair brush, toothbrush, saved teeth, etc

The following table lists the minimum number of required loci needed for entry into each successive level of CODIS from LDIS to SDIS and finally to NDIS.

**Note:** References made to D2 and D19 are specifically for D2S1338 and D19S433, respectively.

<table>
<thead>
<tr>
<th>Category/Indexes</th>
<th>LDIS (Local)</th>
<th>SDIS (State)</th>
<th>NDIS (National)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forensic</td>
<td>Minimum of 13 complete original CODIS core loci</td>
<td>Minimum of 13 complete original CODIS core loci</td>
<td>Minimum of 13 complete original CODIS core loci</td>
</tr>
<tr>
<td>Forensic Partial</td>
<td>N/A (See Local Only Forensic Partial)</td>
<td>N/A (See State Only Forensic Partial)</td>
<td>Minimum of 8 original CODIS core loci and MME value greater than 1.0000E+007</td>
</tr>
<tr>
<td>State Only Forensic Partial</td>
<td>N/A (See Local Only Forensic Partial)</td>
<td>Minimum of 6 original CODIS core loci and MME value greater than 1.0000E+004</td>
<td>N/A (See Forensic Partial)</td>
</tr>
<tr>
<td>Local Only Forensic Partial</td>
<td>Minimum of 6 original CODIS core loci and/or D2 and D19, and MME value greater than 1.0000E+003</td>
<td>N/A (See State Only Forensic Partial)</td>
<td>N/A (See Forensic Partial)</td>
</tr>
<tr>
<td>Forensic Mixture</td>
<td>N/A (See Local Only Forensic Mixture)</td>
<td>N/A (See State Only Forensic Mixture)</td>
<td>Minimum of 8 original CODIS core loci and MME value greater than 1.0000E+007</td>
</tr>
<tr>
<td>State Only Forensic Mixture</td>
<td>N/A (See Local Only Forensic Mixture)</td>
<td>Minimum of 6 original CODIS core loci and MME value greater than 1.0000E+004</td>
<td>N/A (See Forensic Mixture)</td>
</tr>
<tr>
<td>Local Only Forensic Mixture</td>
<td>Minimum of 6 original CODIS core loci and/or D2 and D19, and MME value greater than 1.0000E+003</td>
<td>N/A (See State Only Forensic Mixture)</td>
<td>N/A (See Forensic Mixture)</td>
</tr>
<tr>
<td>Forensic Targeted</td>
<td>N/A</td>
<td>N/A</td>
<td>Minimum of 8 original CODIS core loci and MRE value greater than 1.0000E+07</td>
</tr>
<tr>
<td>Volunteer</td>
<td>Minimum of 8 complete original CODIS core loci and/or D2 and D19</td>
<td>Minimum of 8 complete original CODIS core loci and Amelogenin</td>
<td>N/A (not eligible for NDIS)</td>
</tr>
</tbody>
</table>
Nominating Forensic Unknown Profiles to the DNA Index of Special Concern (DISC)

Eligible forensic profiles can be marked as DNA Index of Special Concern (DISC) profiles in CODIS if they meet certain criteria listed below. These profiles will be searched at high stringency against rapid DNA arrestee profiles and potentially result in a hit while the arrestee is still in custody. Decisions on which profiles will be marked as DISC profiles will be made upon discussions with investigators.

- Serious crime with significant public safety concern (e.g. priority zero) to include: sexual assault, homicide, kidnapping or terrorism
- Probative single source profile with complete results at the original CODIS core 13 loci (i.e. Forensic Unknown)
- Source ID is No
- Has been searched at NDIS and SDIS at least once

All DISC-enabled profiles must be recertified on an annual basis that they still meet the criteria listed above. A profile may be DISC-enabled in STR/Y-STR Data Entry by changing the “Rapid-enabled” drop-down from “NA” to “Yes” which will allow Casework metadata fields to be completed with the following required case/investigator information: Investigative Agency ID (ORI), Investigative Case Tracking ID, Investigator Phone number, Statute of Limitation, Offense Description, Extradition Information, and Investigative Agency Contact Information.

13.2.1 Assessment of Data for CODIS Eligibility – In-House and Outsourced

The analyst, CODIS Administrator, or DNA Lab Manager may assess the eligibility of profile entry into CODIS with assistance from a LEST or Forensic Lab Technologist. In the event CODIS eligibility has not already been documented by the submitting officer, a note with initials/P# and the date will be added to the “Comments” section of the LIMS Unit Record regarding where eligibility documentation can be found for each item being entered (e.g., CODIS eligibility documented on: CSA impound report and email in RFLE scan, under Profile Detail on worksheet, in email from Detective in Object Repository, on CODIS SDEs, etc.).

Analysts are bound by the requirements of the “Annual Review of DNA Records Acceptable at NDIS”.

- The CODIS Decision Tree was developed to establish rules for what is eligible for upload.
  - This is a summary of the general principles:
    a. Evidence profile must come from a crime investigation
    b. Benchwork match samples are allowed
c. Profiles, especially from mixtures, are assessed and only those alleles that must have come from the perpetrator are allowed.
d. Suspect profiles on suspect’s clothing (probative) are NOT allowed.
e. Suspect’s profile on suspect’s belongings found in suspect’s possession are NOT allowed unless additional details may be provided [example: through a sexual assault victim’s statement that the assault occurred in defendant’s vehicle (scene of the crime) and the semen stain recovered from the vehicle contains a mixture of the suspect and the victim].
f. Forensic unknown DNA records awaiting elimination samples/standards may be entered as long as the laboratory has requested the elimination samples/standards and the request is documented.
g. Crime scene reports stamped with “BTB from suspect” are not sufficient documentation to satisfy CODIS eligibility. Additional information as to why the item is believed to be from the suspect must be documented.

Outsourced Cases

Outsourced cases are assessed for CODIS-eligible profiles. An Outsourcing Laboratory Technical and Administrative Review Form is used to document the review of CODIS eligibility and placed on the right-hand side of the file or scanned into the Object Repository of the LIMS with other administrative paperwork.

Once the outsourced case has been technically reviewed, a Keyboard CODIS STR Data Entry packet will be created for those samples that are eligible for keyboard STR data entry. The technical reviewer will sign off and enter the profile into CODIS, assigning it to themself. A CODIS Entry report will be written to be sent out for all outsourced cases that have an eligible CODIS profile to notify the investigators that a profile will be searched in the local/state/national DNA Index System (CODIS); this report is generated outside of the LIMS and scanned into the Object Repository after distribution. The CODIS STR Data Entry packet with the Specimen Details report print-out attached and CODIS entry report will be administratively reviewed prior to upload to SDIS/NDIS if eligible.

13.2.2 Casework Sample Entry

A CODIS STR Data Entry packet is created for those samples eligible for entry into CODIS. After technical review, the profile is entered into the CODIS software and a copy of the LDIS Specimen Details Reports is attached to the packet for administrative review.

Volunteer, outsourced casework or legacy data

Prior to technical review, the packet for samples eligible for keyboard STR data entry includes:
- Keyboard CODIS SDE form
- Copy of the electropherogram for all partial profiles, mixture profiles, or deduced profiles
- Copy of Match-Estimation Report for partial and mixture profiles (if applicable)

Printed electropherograms are not required for full single source evidence sample profiles or volunteer samples. The first page of the packet (the SDE form) must be initialed by the analyst.

Samples interpreted with STRmix

For samples interpreted with STRmix, a COSTaR CODIS SDE form without any additional attached print-outs is created prior to technical review. The analyst must initial and date on the designated line.
13.2.2.1 Naming the Casework Specimen

The forensic specimen number will be entered into the Specimen ID field and will consist of the following if applicable:

- The case type prefix, according to the list below:
  - **AS**: Assault (other than sex assault), **AH**: Attempted Homicide, **AUTO**: Auto Theft, **B**: Burglary,
  - **FA**: Firearms, **GL**: Grand Larceny, **H**: Homicide
  - **HCC**: Homicide Cold Case, **HI**: Home Invasion, **KD**: Kidnapping, **M**: Miscellaneous Other,
  - **R**: Robbery, **SA**: Sex assault, **SC**: Sex Crimes (other than sex assault), **V**: Volunteer, **UHR**: Unidentified Human Remains, **MP**: Missing Person

  **Note**: Samples entered prior to October 2007 have the analyst’s initials as the prefix. Samples processed outside of the LIMS will have the Event # in the specimen name, while samples processed in the LIMS will have the LAB #. Samples entered prior to 2001 may have the LVMPD ACE sample number rather than the Event #.

- If the specimen has been matched to a suspect, (“Benchwork” match), then the Specimen ID will have a “K” after the case type (e.g. SAK). It is not necessary to enter “VK” for volunteer samples.

- Cases from outside jurisdictions such as North Las Vegas or Henderson will include the abbreviation of the submitting agency in the Specimen ID (e.g. SAKHPD).

  **Note**: A complete list of OJ abbreviations can be found in the Shared Documents folder on the Criminalistics Bureau SharePoint site

- The lab # without any dashes, will follow next (e.g. SAKHPD1316643).

- Then a dash followed by the item # will be next (e.g. SAK1316643-1B). This includes if it is a sperm fraction or epithelial fraction (e.g. SAKHPD1316643-1B-SF or 1B-EF).

- If the sample was booked under one lab number but processed under a different lab number, then an “-X” will be added to the item # (e.g. SAKHPD1316643-2-X). The lab number used in the specimen name should be the one it’s being processed/reported under. The lab number the sample is booked under will then be added to the comments field for cross-reference.

- If needed, add additional information such as: minor profile (-MIN), major profile (-MAJ), deduced profile (-DED), contributor 1 (-C1).

13.2.2.2 Determining DNA Profile for Keyboard STR Data Entry (Refer to 13.2.2.3 if using STRmix for Entry)

*For partial single source evidence sample profiles*: When a locus has only one peak called below the kit-specific interpretation threshold, the single allele may be entered with the addition of a “+” symbol indicating there is the possibility of an additional allele not being called. A printed electropherogram should be included with the packet.

If a profile has complete data at the original 13 CODIS core loci but has drop-out at other non-core loci it should still be entered in the Forensic Unknown specimen category, rather than the Forensic Partial or State Only Forensic Partial specimen categories. A moderate match estimation (MME) calculation, described further below, does not need to be calculated for these profiles.

*For distinguishable mixture evidence sample profiles where a partial single source major profile can be deconvoluted*: When one or both of the major contributor peaks are interpretable, the allele(s) may be entered into CODIS. A printed electropherogram and any relevant mixture deconvolution worksheets should be included with the packet.
A single major allele may be entered with the addition of a “+” symbol if there is an assumed minor contributor that determines the single allele is an obligate allele of the foreign contributor.

For 2-person distinguishable mixture evidence sample profiles where a partial single source minor profile can be deconvoluted: When one or both of the minor contributor peaks are called below the kit-specific interpretation threshold, the allele(s) may be entered into CODIS. If there is only one minor peak detected below the interpretation threshold in combination with the presence of 1 or 2 major peaks, the minor allele may be entered one of two ways:

- The minor allele may be entered with the addition of a “+” symbol thereby indicating the possibility of an additional allele not being called or being shared with the major contributor or,
- In the event it is possible to deconvolute a genotype for the minor component, both minor alleles may be entered. A printed electropherogram and any relevant mixture deconvolution worksheets should be included with the packet.

For 2-person indistinguishable mixture evidence sample profiles with an assumed contributor where a partial single source foreign profile can be deduced:

When one or both of the foreign contributor peaks are called below the interpretation threshold the allele(s) may be entered into CODIS. If there is one foreign contributor allele detected below the interpretation threshold in combination with the presence of 1 or 2 assumed contributor peaks, the foreign allele may be entered one of two ways:

- The foreign contributor allele may be entered with the addition of a “+” symbol indicating there is the possibility of an additional allele not being called or being shared with the assumed contributor or,
- In the event it is possible to deconvolute a genotype for the foreign contributor, both foreign alleles may be entered. A printed electropherogram and any relevant mixture deconvolution worksheets should be included with the packet.

For mixture samples being entered into CODIS in the Forensic Mixture, State Only Forensic Mixture, or Local Only Forensic Mixture specimen categories, the “+” symbol may be used to designate certain alleles to be required to match in the event only an obligate allele based on an assumed contributor and not the complete unknown genotype can be deconvoluted from the rest of the mixture. The “+” symbol should not be used to indicate possible drop-out in mixtures unless only a single allele is being entered at the locus where it is used.

For partial and mixture samples being entered into CODIS in the Forensic Targeted specimen category, the partial locus indicator must be used to designate certain loci with one or two alleles to be evaluated at moderate stringency. All loci will be searched at high stringency with the exception of designated partial loci and loci with more than two alleles. Loci with a “+” required allele must be marked partial or have more than 2 alleles for the required allele to have an effect as CODIS ignores the required allele in a high stringency search.

13.2.2.3 Using STRmix and the COSTaR Workbook to Determine DNA Profile for CODIS Entry

Casework profiles interpreted with STRmix and being considered for CODIS entry will have a STRmix deconvolution performed in order to use the COSTaR workbook to attempt to generate a CODIS eligible profile. The workbook can be used to target specific contributors or all contributors of a mixture. It may not be necessary to run the COSTaR workbook on all profiles obtained in a case (e.g., sexual assault with a single assailant where only the victim and the same unknown contributor are observed in multiple samples). In the event a full NDIS-eligible profile (Forensic
Unknown (i.e., X > 200 RFU at Amelogenin, X,Y at Amelogenin and/or peak present at DYS391) allele entries for the Amelogenin (AM) locus must be entered, if available. This can be done directly on the tab or by clicking the “Print ARChived” button.

<table>
<thead>
<tr>
<th>CODIS Level</th>
<th>Maximum Trimming Threshold</th>
<th>Maximum Alleles per Locus</th>
<th>Number of Loci Required</th>
<th>MME Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local</td>
<td>10%</td>
<td>6</td>
<td>6 original CODIS core 13 and/or D2S1338 or D19S433</td>
<td>1 in 1,000</td>
</tr>
<tr>
<td>State</td>
<td>5%</td>
<td>4</td>
<td>6 original CODIS core 13</td>
<td>1 in 10,000</td>
</tr>
<tr>
<td>National</td>
<td>5%</td>
<td>4</td>
<td>8 original CODIS core 13</td>
<td>1 in 10,000,000</td>
</tr>
</tbody>
</table>

**Using the COSTaR Workbook**

1. On the **STRmix** tab of the COSTaR workbook, click on the “Import” button and navigate to the desired STRmix Run Files folder and double click on the folder for the previously deconvoluted run. Select the “Results.txt” file contained in the run folder and click Open. Alternatively, the “Import Data & Create CODIS Sheet” button may be clicked instead (see second bullet of step 7 for further guidance).
2. On the **Contributors** tab, click on the desired “Contributor #” button.
3. On the **Allele Weights Summary** tab, verify there are no allele weights greater than 100%. If there are, close the workbook and start over with step #1.
4. On the **MME** tab, review obligate alleles selected by COSTaR and if any partial locus boxes have been checked. No changes should be made to this tab as it would affect the CODIS SDE form and CODIS import.
5. On the **CODIS** (CODIS SDE form) tab, update any fields highlighted in yellow to include: Spec ID, Source ID drop-down, Case ID, Comments, and Benchwork Match (if applicable).

**Note:** For single source samples, the Sample and Spec ID fields will both populate blank. The yellow highlight on these fields will disappear once the Spec ID field is edited and nothing should be added to the Sample field.

**Note:** Avoid using a “&” symbol in the specimen ID name or Comments box as these can cause errors when importing into CODIS. A “+” symbol or the word “and” may be used instead.

- For COSTaR profiles originating from single source full and partial profiles where the gender is clear (i.e. X > 200 RFU at Amelogenin, X,Y at Amelogenin and/or peak present at DYS391) allele entries for the Amelogenin (AM) locus must be entered, if available. This can be done directly on the tab or by clicking the “Print ARChived” button.
CODIS Sheet" button and selecting the appropriate radio button. Allele entries made on the form will be expected to be manually entered in CODIS after import.

- For COSTaR contributor profiles originating from mixture profiles where the gender appears to be clear for the given contributor, it is analyst discretion whether to update the allele entries for the Amelogenin (AM) locus. This can be done directly on the tab or by clicking the “Print CODIS Sheet" button and selecting the appropriate radio button. Allele entries made on the form will be expected to be manually entered in CODIS after import.

- If the gender will not be chosen, click the “Print CODIS Sheet button” and select the “Unknown” radio button.

- The following should be considered when determining whether a profile or target component of a mixture profile is Forensic Unknown, Forensic Partial or Forensic Mixture, and whether the Partial Profile drop-down should be Yes or No for Forensic Unknown or Forensic Mixture profiles.

<table>
<thead>
<tr>
<th>Forensic Unknown</th>
<th>Partial Profile – No</th>
<th>All loci in profile or component of mixture decon’d to 100% w/ no complete locus drop-out. No other genotypes or Qs considered during deconvolution.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forensic Unknown</td>
<td>Partial Profile – Yes</td>
<td>All original core 13 loci in profile or component of a mixture decon’d to 100% but some non-core loci being entered are decon’d &lt;100% with Qs OR complete drop-out at any of these non-core loci. No other genotypes considered during deconvolution aside from Qs at all loci being entered.</td>
</tr>
<tr>
<td>Forensic Partial</td>
<td>Partial Profile – Yes</td>
<td>At least one original core 13 locus being entered decon’d &lt;100% with Qs OR complete drop-out at any original core 13 locus. No other genotypes considered during deconvolution aside from Qs at all loci being entered.</td>
</tr>
<tr>
<td>Forensic Mixture</td>
<td>Partial Profile – No</td>
<td>More than two alleles to be entered at any locus OR multiple genotypes considered in profile/component decon at any locus entered. Data entered for all loci and no Qs considered during deconvolution.</td>
</tr>
<tr>
<td>Forensic Mixture</td>
<td>Partial Profile – Yes</td>
<td>More than two alleles to be entered at any locus OR multiple genotypes considered in profile/component decon at any locus entered. Data not entered at all loci or Qs considered during deconvolution for loci being entered.</td>
</tr>
</tbody>
</table>

6) Hand initial and date the printed COSTaR CODIS SDE form on the Analyst line. The form can be submitted for technical review by itself without any additional documents attached (e.g. other COSTaR tabs, electropherograms, portions of STRmix report, etc). However, if assumptions are made during a STRmix deconvolution for CODIS purposes only then the STR Interpretation Report must be attached to the COSTaR CODIS SDE form for review.
7) Save the workbook within the deconvoluted run folder named with the CODIS specimen name.

- If the profile is not able to be uploaded due to the MME or insufficient number of core loci, save the file with "DNU" in front of the filename with at least the case #, item #, and contributor # (e.g. what specimen ID would have been). The COSTaR CODIS SDE form will be printed, dated and initialed, and scanned into the FRED Unit Record Object Repository to show that an attempt was made. Additional edits to the yellow-highlighted fields are not required for these.

- If the “Import Data & CODIS Sheets” button was clicked in step 1, then each contributor will have a separate workbook saved in this location. Workbook filenames may include “Do Not Enter” if they will not be uploaded due to the MME.
  
  i. If the contributor profile met CODIS scenario eligibility but is not able to be uploaded due to the MME or insufficient number of core loci, save the file with “DNU” in front of the filename with at least the case #, item #, and contributor # (e.g. what specimen ID would have been). The COSTaR CODIS SDE form will be printed, dated and initialed, and scanned into the FRED Unit Record Object Repository to show that an attempt was made. Additional edits to the yellow-highlighted fields are not required for these.

  ii. If the contributor profile meets the MME but not CODIS scenario eligibility (e.g. consistent with victim/elimination included in mixture), open the Workbook and add the reason for no CODIS entry to the Comments box on the CODIS (CODIS SDE form) tab with your initials/P# and the date. Additional edits to the yellow-highlighted fields are not required for these. Save the workbook with “DNU” in front of the existing filename. The CODIS SDE form does not need to be printed for the OR.

  iii. A single contributor profile may be chosen for CODIS entry when each of the following criteria are met:

  - The item met CODIS scenario eligibility
  - The contributor profile(s) met the MME
  - Based on the case information available, it is determined that a single contributor profile is reasonably expected to be the suspect. This will be also verified in comparison with other profiles of the case (e.g. only looking for one suspect and the same contributor profile appears on multiple items)

The reasoning for why one COSTaR contributor profile was chosen for CODIS entry must be documented. For the contributor profile(s) not chosen, open the Workbook and add the reason for no CODIS entry to the Comments box on the CODIS (CODIS SDE form) tab with your initials/P# and the date. Additional edits to the yellow-highlighted fields are not required for these. Save the workbook(s) with “DNU” in front of the existing filename. The CODIS SDE form(s) does not need to be printed for the OR.

Note: When “Forensic Targeted” is chosen as the specimen category the “Core 13 MME” value listed on the COSTaR CODIS SDE worksheet is actually the MRE value which is what CODIS calculates and uses to evaluate the eligibility (must be greater than 1.00E+07) for Forensic Targeted profiles. No additional calculations are necessary outside of what is already done in the COSTaR workbook to show these profiles are eligible but the MRE
Refining contributor genotypes using conditioning for searching purposes

It is possible to assume that an interpreted profile from a victim, POI, or an unidentified person is a contributor to a sample in order to resolve additional unidentified contributors for the purposes of searching the CODIS databases. For example, if the victim is present in a DNA mixture, the mixture may be conditioned on the victim’s profile in order to refine the genotypes of the other non-victim contributor(s). Additionally, if the component interpretation of a DNA mixture yields a single genotype with a weight greater than or equal to 100% at all or most loci, one may condition the MCMC on the genotypes for this contributor to refine the genotypes for the other contributor(s). This is for CODIS upload purposes only. The analyst will document the steps performed to generate the searchable profile in the CODIS SDE packet. All STRmix runs performed for CODIS purposes only will be reviewed to ensure the results conform with expectation prior to entering any deconvolution results into CODIS. The review of the STRmix output will be documented in accordance with Reviewing the Run Diagnostics in Chapter 9.

For instances in which the ≥ 100% is an unidentified contributor, the analyst must manually create a .txt reference file for this contributor so that it may be used as an assumed contributor in a regular STRmix deconvolution. The .txt file will contain the marker, allele, and size of information for the ≥ 100% contributor.

- Reference standards or interpreted single contributor in a case which contain complete genotypes for at least 8 of the CODIS core 13 loci can be used as an assumed contributor to an evidence profile for the purposes of interpreting another profile for searching CODIS
- A printout of the STRmix deconvolution including the component interpretation supporting the assumption will be included in the case file if it is an unambiguous inclusion based on the comparison of the deconvoluted ≥ 100% genotypes to the contributor-specific >=100% genotypes
- If this is completed for a mixture where the component deconvolution is not clear based on a comparison of the deconvoluted and contributor-specific genotypes, an LR supporting the assumption of the individual contributor will be performed and included in the case file. The value of the LR must be 10^4 (10,000) or greater to justify the use of the assumption
- The sample name for the STRmix deconvolution will include the naming convention: “_CODIS upload purposes only”. This deconvolution will be searched against the staff database prior to upload

Manual creation of .txt files for unidentified contributors, DNA profiles of reference standards processed from outside vendors, and surreptitious standards

1) Open the “.txt Template for Unidentified Contributors” using Microsoft Excel located in the DNA Workbooks folder on the H:drive
2) Enter the Sample Name for the unidentified contributor <ex. Male 1/Female 1> in well A2. Copy and paste the name so that is listed in each cell from A2 to A26.
3) Enter the allele(s) for each fully resolved genotype of the unknown contributor at the appropriate locus by entering a single allele in the “Allele 1” column and the second allele in the Allele 2 column. Do not enter any alleles for loci which contain only obligates.
**Note:** Homozygotes may be entered by using either a single allele in the “Allele 1” column or by entered the same allele into both the “Allele 1” and “Allele 2” columns. If entering only a single allele, the <Enter allele> must be deleted in the “Allele 2” column.

4) Repeat steps 2 and 3 for each unidentified contributor associated with the case. In the event there are not any additional contributors to create, delete the unused rows in their entirety by highlighting the entire row, right clicking, and selecting Delete.

5) Save the file as a text tab delineated (.txt) file in the appropriate location in the “STRmix Run Files” folder on the H:drive. The file will be named “Unidentified Contributors for Conditioning_<Lab Case #>.”

6) The file is now ready to be used in STRmix for conditioning. Refer to Chapter 9.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Marker</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Allele 3</th>
<th>Allele 4</th>
<th>Allele 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male 1</td>
<td>struction</td>
<td>3</td>
<td>10</td>
<td>12</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Male 2</td>
<td>construction</td>
<td>5</td>
<td>2</td>
<td>15</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Male 3</td>
<td>construction</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Male 4</td>
<td>construction</td>
<td>9</td>
<td>1</td>
<td>19</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Male 5</td>
<td>construction</td>
<td>11</td>
<td>3</td>
<td>17</td>
<td>12</td>
<td>7</td>
</tr>
</tbody>
</table>

### 13.2.2.4 Match Estimation for Forensic Partial and Forensic Mixture Profiles from Outsourced Casework and Legacy Data Samples

For forensic partial or forensic mixture profiles (including Local Only and State Only specimen categories) a moderate match estimation (MME) must be calculated prior to technical review to determine the correct specimen category (i.e. LDIS, SDIS or NDIS). The MME is automatically calculated within the COSTaR workbook and prints on the COSTaR CODIS SDE form. For outsourced casework and legacy data profiles the MME can be calculated in the Popstats module of CODIS. In CODIS, click on Popstats on the bottom left side and then click on the Match Estimation on the top left-hand side. Enter the sample name in the Specimen ID field, leave the population size default in the Database Specimen Count field set by CODIS. Only data from the original CODIS core 13 loci being searched in each database should be entered for SDIS and NDIS calculations. Only data from the original CODIS core 13 loci and/or D2S1338 or D19S433 should be entered for LDIS calculations.

The required minimum number of loci for each database must be entered to meet the completeness definition for each database regardless of what the match estimation calculation is (see table below). The CODIS Administrator will notify DNA analysts via email if the completeness definition change in between manual revisions.

A moderate match estimation (MME) threshold will be used to determine whether a partial or mixture profile is eligible for LDIS, SDIS or NDIS. The MME is automatically calculated based on NDIS core loci requirements during STR Data Entry using the combined (COMB) population group of the NIST population database. However, it can be calculated prior to entry to determine the
correct specimen category using the Match Estimation tool in Popstats which also uses the set NIST population database to calculate the average inverse match rarity estimate (MRE), which is the same result as a MME. Obtaining the same result for the COMB population group is dependent on the analyst to only enter data for the original 13 CODIS core loci.

<table>
<thead>
<tr>
<th>Database</th>
<th># of Loci Allowed to Miss</th>
<th># of Alleles Allowed (Mixtures)</th>
<th># of Loci Required</th>
<th>MME (COMB Inverse MRE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDIS</td>
<td>0</td>
<td>4</td>
<td>8 original core (no D2S1338/D19S433)</td>
<td>&gt; 10,000,000 (1.000E+007)</td>
</tr>
<tr>
<td>SDIS</td>
<td>0</td>
<td>4</td>
<td>6 original core (no D2S1338/D19S433)</td>
<td>&gt; 10,000 (1.0000E+004)</td>
</tr>
<tr>
<td>LDIS</td>
<td>0</td>
<td>6</td>
<td>Any 6 original core and/or D2S1338/D19S433</td>
<td>&gt; 1,000 (1.0000E+003)</td>
</tr>
</tbody>
</table>

After entering the profile, click Calculate and print the Match-Estimation Report to attach to the keyboard SDE packet for technical review. **Note:** On the Match-Estimation Report print-out, the number listed for the “total number of loci in target specimen” refers to the number of loci with allele entries in the Popstats calculation regardless of whether they’ve been unchecked for the calculation (i.e. D2S1338, D19S433 and Amelogenin). It is not necessary for analysts to enter allele calls into the Match Estimation tool in Popstats for loci other than the original CODIS core loci simply for the purpose of making this number accurate, nor hand correct the print-out. This number has no effect on the calculation.

The official MME will be calculated automatically during STR data entry. This calculation automatically excludes any loci besides the original CODIS core 13, so the complete profile as it will be uploaded should be entered. If a sample is being considered for only LDIS entry with data at D2S1338 or D19S433 the Combined (COMB) Inverse MRE calculated in the Match Estimation tool should be used for determining whether the profile will be entered into CODIS because D2S1338 and D19S433 would not be included in the MME calculation done during STR Data Entry.

For samples being considered for the Forensic Targeted specimen category, the MME value calculated must be less than 1 in 10 million for at least 8 of the original CODIS core 13 loci. In the Match Estimation tool in Popstats the locus stringency for each locus with one or two alleles that can be searched at high stringency must be changed from the default “M” (moderate) to “H” (high). The resulting MRE value must be greater than 1 in 10 million to be allowed for entry and upload. If the MRE value is not met the sample can be considered for entry in a State Only or Local Only Forensic Partial or Mixture specimen category.

If a partial or mixture profile is obtained from an item of evidence that would be CODIS eligible based on the case scenario, however it does not meet the required MME for entry into at least the LDIS database then a print-out of the match estimation report must be included within the casefile or scanned into the O.R. to show that CODIS entry was considered but not allowed because the profile was not suitable for entry.

**13.2.2.5 STR Data Entry of Casework Samples in CODIS Software**

Samples will be entered into LDIS after technical review through keyboard STR Data Entry or direct electronic import using the COSTaR workbook.
Keyboard STR Data Entry
Volunteer, outsourced casework and legacy data samples will be entered into LDIS after technical review through the “STR Data Entry” module in CODIS.

- The Specimen ID field will be the specimen name including prefix, case number and sample item number.
- The Specimen Category can be picked from the drop-down menu.
- The source ID and partial profile fields can be picked from a drop-down menu of either “Yes” or “No”. A profile being entered in the Forensic Unknown specimen category with complete data at the original 13 CODIS core loci but possible drop-out at other loci should have “Yes” selected in this drop-down. This selection will not affect how the profile is searched.
- The case ID field will consist of the actual event/agency case number (e.g. 091104-1604).
- The comments field should be filled out with where the profile came from (example: “Major profile from sperm fraction of vaginal swabs” or “volunteer sample”). No names should be used in any field.
- Click on the second blank next to Amelogenin, type in the results with a comma in between the alleles of a heterozygote. For a homozygote, type a single allele. Fill in the first vertical column, then the next column is a data entry double-check. You will be prompted if there is a disagreement between the readings, if the alleles do not match those accepted for the locus, or if an allele entry is known to be a rare allele. Data entry can be double-checked by using the arrow keys to go backwards and then forward again through the column.
- If the profile is from a male and the Qiagen Investigator 24plex kit was used for analysis, the Y-STR result can be entered below the list of autosomal loci.
- For partial profiles, the Partial Profile indicator drop-down should be changed to Yes for applicable loci to allow for moderate stringency searching at these loci.

Note: On a Match Detail Report a partial locus entry will be indicated with a “{P}” symbol.

- The forensic DNA profile will be entered into LDIS according to the CODIS operating procedures and the "Annual Review of DNA Records Acceptable at NDIS". Once entered the STR data will be saved and a LDIS Specimen Detail Report will be printed and placed with the packet for review in the case file.

Note: After clicking Save, the moderate match estimate (MME) will be calculated for partial and mixture DNA profiles only and the match rarity estimate (MRE) will be calculated for targeted forensic profiles. Verify that the appropriate threshold estimated using Match Estimation is still met for the selected category. If it does not, the specimen category will have to be corrected which will require a change on the LIMS worksheet and in the lab report.

Direct Electronic Import into CODIS Using the COSTaR Workbook
1) Open the COSTaR workbook saved in the deconvoluted run folder in the STRmix Run Files folder. On the CODIS tab, click on the “Export” button with the CODIS logo. Click Yes on the subsequent pop-up confirming that technical review has been completed. Click OK on the subsequent pop-up confirming that the export was successful.
2) Log into a CODIS workstation and launch the Analyst Workbench program.
3) Click on the Specimen Manager module.
4) Click on the Import icon or select Specimen Manager>Import Specimens from top toolbar.
5) Navigate to: H:\Forensic Data\DNA\1-CODIS\Casework CMFs for Upload
6) Select your file named as “CODIS username_CODIS specimen ID”. More than one file may be imported at the same time by holding the CTRL button and selecting multiple files. Click Open.

7) In the Select Import Type pop-up, ensure the Data Import radio button is selected and your username is selected from the Assign To User drop-down. Click OK.

8) In the Import Confirmation pop-up, click OK.

9) Click on the Message Center module.

10) Double-click on the bolded Import STR Files in the CODIS Message Center.

11) Double-click on the bolded DAT file named as “CODIS username_CODIS specimen ID” to execute the file. An import status bar will pop-up momentarily as the file loads. The DAT file will un-bold when it is finished. This step can be repeated for additional DAT files if multiple files were imported in step #6.

12) Double-click on the bolded Import Reports in the CODIS Message Center.

13) Double-click on the bolded IMP file names as “CODIS username_CODIS specimen ID” to execute the file.

14) A new LDIS Import Reconciliation Report window will open. Verify “1” appears under the New Specimens Total column and a “0” appears under all other columns. If a “1” appears under the New Specimens Problem, Specimen Update Total/Problem or Unchanged Specimens Total columns, then additional troubleshooting will need to be performed in the next step. Close the window.

15) A second LDIS Import Reconciliation Report window will open with each locus listed for the specimen imported. Pay attention to any symbols or codes next to the loci and consult the key/legend at the bottom of the page. A ‘+’ (presence of an obligate allele) or ‘>’ (off-ladder allele conversion occurred) is informative and does not signify a problem that needs to be addressed further. Guidance from the CODIS Administrator or designee should be sought if any other code appears. Close the window and the IMP file will un-bold. Steps 12-15 can be repeated if multiple DAT files were executed in step 11.

16) Click on the Specimen Manager module.

17) Double-click on the “_Recent Casework Specimens-not marked” in the Specimen View.

18) Click on your newly added specimen. Click on the red Edit STR/Y-STR Data icon or select Tools>STR/Y-STR Entry from top toolbar.

19) Review entries and confirm MME is still consistent with the Specimen Category chosen. If an X or X,Y was added to the CODIS SDE form, entries for Amelogenin must be keyboard entered at this time. It is analyst discretion to also add entries for DYS391.

20) Click Save (if applicable), Print and Close.

21) Attach the printed LDIS Specimen Details Report to the COSTaR CODIS SDE form for administrative review.

### 13.2.2.6 Upload of DNA profile to SDIS and/or NDIS

After STR data entry, the CODIS STR Data Entry packet will be administratively reviewed for typographical accuracy.

- For volunteer samples, the packet will consist of a Keyboard CODIS SDE form and LDIS Specimen Details Report
For outsourced casework and legacy data samples, the packet will consist of a Keyboard CODIS SDE form, printed electropherograms and mixture deconvolution worksheets (if applicable), Match Estimation report (if applicable), and LDIS Specimen Details Report.

For samples interpreted with STRmix, the packet will consist of a COSTaR CODIS SDE form and LDIS Specimen Details Report.

After confirmation that the report has been released, the CODIS STR Data Entry packet will be given to the CODIS Administrator at which time it will be “Marked for Upload” in CODIS, if applicable. After being marked for upload the packet is stored on the left side of the case file or scanned into the Object Repository of the LIMS. Forensic samples will be uploaded into the State (SDIS) and National (NDIS) database at least monthly.

13.2.3 Administrative Sample Removal
A forensic or volunteer sample may be removed when both the DNA analyst and the CODIS administrator have evaluated the sample and agree upon its removal. A sample may also be removed if the investigator submits a CODIS Hit Disposition Form after receiving a CODIS hit report and determines that the individual identified is not a suspect. A “CODIS Deletion Approval” form will be filled out and approved by the CODIS Administrator, Technical Leader and DNA Lab Manager. The approval form, CODIS Hit Disposition Form (if applicable), and CODIS Deletion Report print-out then become a part of the case file along with the technical and administrative review forms when a supplemental report is issued indicating the disposition of the CODIS entry. The CODIS Deletion Report will also be archived in the CODIS computer.

A volunteer sample may also be removed at the written request of the volunteer, the volunteer’s legal counsel or a court order.

13.3 Database Samples
The majority of known samples, also called database samples, come from convicted offenders, sexual registrants, and felony arrestees collected throughout southern Nevada’s jails, prisons, courts, parole and probation offices, and law enforcement agencies. Individuals who provide a DNA sample for inclusion in the DNA database are selected pursuant to NRS 176.0913 (convicted offenders), NRS 176.0916 (convicted offenders from out-of-state on parole/probation in NV), NRS 179D.240, 179D.443, and 179D.460 (sex registrants), and NRS 176.09123 (arrestees). These samples are buccal swabs, FTA card collection devices, or blood samples collected by the various agencies and retrieved by or sent to the LVMPD Forensic Lab on a regular basis.

The specimen biographical information submitted with each collection kit for all database samples is maintained in the Sample Tracking and Control Software (STaCS) which is a state-wide database that is accessed through a virtual private network (VPN) to the Washoe County Sheriff’s Office where the server is stored. Prior to 2013, the information was stored in an Access Database; however, all information in the Access Database has been transferred over to the STaCS database and LIMS.

Samples will be analyzed using any of the validated extraction DNA procedures developed for databasing. Analysis of the original CODIS core STR loci will be completed for each sample: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, vWA, TPOX, D18S51, D5S818, FGA and the Amelogenin sex indicator. Additional loci provided by the PowerPlex Fusion 6C kit: D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433,
D22S1045, Penta D, Penta E, DYS391, DYS570, and DYS576. Results at all loci tested will be exported from GeneMapper ID-X and imported into CODIS. Data analysis results will be maintained in electronic format.

DNA results will be technically reviewed by a second qualified DNA Analyst. If the two analysts do not agree on the interpretation, the sample can be re-injected, re-amplified, or re-isolated. For the “Technical Review Checklist-Database Knowns”, the technical reviewer will sign and date the review form where indicated.

**Note**: The technical review of database-specific proficiency tests will be documented utilizing the “Database Proficiency Technical Review Checklist” instead of the “Technical Review Checklist-Database Knowns”.

After technical review, the GeneMapper ID-X (GMID-X) CODIS Export Table is converted to a Common Message Format (CMF) file and saved onto the H:drive (refer to Data – CODIS Export). The CODIS Administrator (or alternate) imports the CMF files into the CODIS workstation.

The Convicted Offender/Registrant/Arrestee samples are considered to be “reference materials”.

The Database laboratory currently does not process Casework reference samples.

**Database Sample Indexes**

- **Offender Index**
  Consists of single source DNA profiles from convicted offenders or sex registrants.

- **Arrestee Index**
  Consists of single source DNA profiles from persons arrested for a felony offense.

- **Multi-Allelic Offender Index**
  Consists of DNA profiles from offenders (arrestee or convicted offender) with three or more alleles at two or more loci.

The following table lists the minimum number of required loci needed for entry into each successive level of CODIS from LDIS to SDIS and finally to NDIS. (Note: References made to D2 and D19 are specifically for D2S1338 and D19S433, respectively)

<table>
<thead>
<tr>
<th>Category/Indexes</th>
<th>LDIS (Local)</th>
<th>SDIS (State)</th>
<th>NDIS (National)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Convicted Offender/Arrestee/ Multi-Allelic Offender</td>
<td>Minimum of 10 complete original CODIS core loci and/or D2 and D19</td>
<td>13 complete original CODIS core loci and Amelogenin</td>
<td>13 complete original CODIS core loci</td>
</tr>
</tbody>
</table>

**13.3.1 Receipt of Arrestee Samples into the Laboratory**

1) DNA samples on FTA cards will be collected using the DNA collection kit approved by the laboratory. The appropriate information will be filled out on the collection kit bi-fold or printed and attached to the collection kit at the time of collection. A pre-printed NV seven-digit barcode number (e.g. NV1234567) is on the bi-fold and collection device.

2) DNA samples may be submitted in whole blood collection kits if the subject refuses to provide a sample on an FTA card. This blood collection kit will contain a purple-topped tube(s) and have
the NV seven-digit barcode number associated with the collection kit bi-fold written on the outside of the box at the time of collection. A collection kit bi-fold will be submitted with the appropriate information filled out or attached.

3) Samples accepted at the laboratory will fall into two categories:
   a. Samples to be destroyed within 5 business days after notice because probable cause (PC) could not be established and no other felony arrests have occurred that require sample collection (NRS 176.09123 2d). The biographical information entered into STaCS at the time of collection associated with an NV number printed on the collection kit will have to be rejected from the STaCS database. If an outside agency takes care of destroying the sample themselves, they will notify the LVMPD Forensic Lab that the information needs to be rejected from STaCS.
   b. Samples to be processed awaiting an administrative message (AM) from the State Repository that the fingerprints using the LiveScan have been confirmed to the individual. If LiveScan is used during collection, there may not be inked prints collected at the same time on the bi-fold. Collection kits will have PC paperwork attached or available in OnBase for verification, or if the person is arrested pursuant to a warrant, then the warrant will be attached or available in OnBase. Upon receipt into the lab these samples will be counted.
      • If PC cannot be established because proper documentation is not available for verification, then the collecting agency will be contacted to submit paperwork or give approval for destruction and rejection in STaCS.
      • The AM message received will contain the state identification (SID) number, process control number (PCN), date of arrest, originating agency case (or identification) number (OCA), and name of the individual. This information will be used to match it up with one of the collection kits received which will have at least the PCN and name of the individual written on the outside. The AM message will be retained in OnBase or imported into the Object Repository of the LIMS following receipt into STaCS at the lab. If an AM message is not automatically received, then the PCN can be queried through J-LINK to see if an SID was returned. Due to fingerprint processing errors, the SID may also be provided by LVMPD AFIS after the error is researched and cleared by them.

4) Upon receipt of the AM messages (or manual verification via J-LINK), the kits associated with the AM will be received in STaCS and assigned a received date.
   • Databasing: The specimen information from the collection bi-fold in the kit or the attached paperwork will be accepted/entered into STaCS by first scanning the unique NV barcode pre-printed on the collection bi-fold which is also present on the FTA card. After verifying that all of the information is correct and accepting the sample, an additional STaCS number (e.g. 2014-012345) will be assigned and five new barcode labels will be printed. One will be attached to the paperwork attached to the outer envelope to assist in scanning into the LIMS, one will be affixed as a label on the upper right hand corner of the outer envelope, one will be affixed on the outside top left of the collection bi-fold, and the other two will be placed inside of the envelope to be applied to the FTA card and a coin envelope added later to package the sponge applicator when the kit is opened for sampling, or to the bloodstain card prepared and a coin envelope added to the package to store it.

   The STaCS number becomes the unique specimen number that will be used in the LIMS as the “LAB #” and used to identify the specimen in CODIS. The information from STaCS can be imported directly into the LIMS via Excel. Any paperwork attached to the outside of the kit upon receipt will be scanned into the specimen’s Object Repository in the LIMS if it is not already in OnBase.
• Accessioning: The additional STaCS barcode labels will be affixed to the FTA card and a coin envelope that is added to package the blue collection device containing the sponge applicator for long-term storage. If the FTA card is sampled at this time, a sample punch will be made into a labeled, sterile microcentrifuge tube or plate.

For whole blood samples, a bloodstain card will be spotted using blood from one of the tubes from which a punch can be made into a labeled, sterile microcentrifuge tube for manual extraction. The additional STaCS barcode labels will be affixed to the bloodstain card and a coin envelope added to package the bloodstain card for long-term storage. The blood tube(s) will be stored in a refrigerator temporarily until after passing data is obtained, at which time it may be destroyed.

5) The collection envelope containing the bi-fold, FTA card, sponge applicator, and any other paperwork will then be placed in secured storage, arranged in ascending numerical order. The samples will be available for database match confirmation and retained in storage indefinitely.

• For whole blood samples, the bi-fold, coin envelope containing the bloodstain card, and any other paperwork will be re-packaged in the white envelope with a “Biohazard” sticker label and then placed in secured storage, arranged in ascending numerical order with the FTA collection envelopes. The samples will be available for database match confirmation and retained in storage indefinitely.

6) Incidents relating to problematic collection of database samples will be documented in the “Database Problem Sample Log” kept in the CODIS Administrator’s office or in the “STaCS Rejections & Destructions” spreadsheet maintained by the CODIS Administrator on the H:drive.

13.3.2 Sample Removal – Expungement of Arrestee Samples

Expungement of the DNA record, profile, and specimen will occur when:

Pursuant to NRS 176.09125, a person whose DNA record or DNA profile has been included in the state DNA database in accordance with NRS 176.09123 requests the expungement of their DNA profile through the official form maintained by the Central Repository of Nevada Records of Criminal History, a division of the Nevada Department of Public Safety.

OR, a certified copy of a court order to expunge a person’s DNA record or profile that has been included in the state DNA database is received by the Forensic Lab indicating that a felony arrest no longer qualifies or the person has not been convicted pursuant to the excerpt from the amendments made by the DNA Fingerprint Act of 2005 detailed below. All documentation received by the Forensic Lab will be forwarded to the Central Repository for their record maintenance.

Amendments made by the DNA Fingerprint Act of 2005 (P.L. 109-162) require expungement if “the person has not been convicted of an offense on the basis of which that analysis was or could have been included in the index, and the responsible agency or official of that State receives, for each charge against the person on the basis of which the analysis was or could have been included in the index, a certified copy of a final court order establishing that such charge has been dismissed or has resulted in an acquittal or that no charge was filed within the applicable time period.” (see 42 U.S.C.§14132(d)(2)(A)(ii))
• Per NRS 176.09125 subsection 3, the forensic laboratory shall not destroy a biological specimen or purge the DNA profile of a person if the forensic laboratory is notified by a law enforcement agency that the person has a prior felony, a new felony arrest or a pending felony charge for which collection of a biological specimen is authorized.

• If the Central Repository determines that expungement is necessary, a copy of the expungement form will be forwarded to the LVMPD Forensic Lab within six weeks of the written request with the identifying details of the sample to expunge and the reason for its expungement.

• Within thirty business days of the receipt of the expungement form from the Central Repository or a certified court order received directly at the Forensic Lab, the LVMPD Forensic Lab will run an NCIC report and research court records to see if the subject has another qualifying offense that would allow the state to keep the DNA profile and sample, in accordance with NRS 176.09125 subsection 3.
  ➢ If an alternative qualifying offense exists, the Central Repository will be notified within the same thirty business days by returning the form with “Denied” circled or forwarding the court order with an expungement form completed by the Forensic Lab with “Denied” circled. The sample and DNA profile will be retained by authority of the alternate offense. The Central Repository will notify the arrestee that the request for expungement is denied and the sample will not be destroyed.
  ➢ If no alternative qualifying offense exists, within the same thirty business days the laboratory will expunge from CODIS (LDIS, SDIS, and NDIS), LIMS, and STaCS databases all pertinent records associated with the request in accordance with local, state, and national requirements (NDIS Expunge a DNA profile – Operational Procedure).

A copy of the expungement form or court order and a print-out of CODIS generated delete report will be retained in the DNA section and the Central Repository will be notified that expungement has been completed by returning the form with “Granted” circled or forwarding the court order with an expungement form completed by the Forensic Lab with “Granted” circled. The Central Repository will notify the arrestee the request for expungement has been granted. The collection kit and sample will be destroyed.

In LIMS, the name of the arrestee will be changed to “Arrestee (first name) EXPUNGEMENT (last name)”. All other identifying information including the date of birth, State ID number, social security number, and CS number, will be manually deleted. The date of expungement will be entered in the “Date of Death” field.

Pursuant to NDIS procedures, if the LVMPD expunges a DNA record, other than a forensic unknown, forensic mixture or forensic partial that has generated an interstate candidate match, the LVMPD will notify the any other laboratory involved in the candidate match that an expungement is being performed. The LVMPD will notify the State CODIS Administrator of any such expungements so that the NDIS custodian is notified of any candidate matches associated with the expunged record that need to be deleted from NDIS.

13.3.3 Receipt of Convicted Offender/Registrant Samples into the Laboratory
1) DNA samples (swabs or FTA cards) will be collected using the DNA collection kits approved by the laboratory. The appropriate forms will also be filled out or printed and attached to the collection kit at the time of collection.

- The contents of a kit received with swabs are stored in the original envelope that has the offender’s information written on the outside and a SN or NN barcode affixed to the envelope, as well as the fingerprint form and swab box.
- The contents of a kit received with an FTA card are stored in an envelope that contains a bi-fold with the offender’s information written on it or with attached paperwork. A pre-printed NV number is on the bi-fold and FTA card.

2) Upon receipt, the kits will be counted.

3) Databasing: The specimen information submitted with the collection kit will be accepted/entered into STaCS by first scanning the unique NV barcode pre-printed on the outer envelope which is also present on the FTA card; this assigns a received date to the kit. After verifying that all of the information is correct, or entering the information if it was not entered electronically at the collection site, and accepting the sample, an additional STaCS number (e.g. 2014-012345) will be assigned and four new barcode labels will be printed. One will be affixed as a label on the upper right hand corner of the outer envelope, one will be affixed on the outside top left of the collection bi-fold, and the other two will be placed inside of the envelope to be applied to the FTA card and a coin envelope added later to package the sponge applicator when the kit is opening for sampling. The STaCS number becomes the unique specimen number that will be used in the LIMS as the “LAB #” and used to identify the specimen in CODIS.

For older collection kits received containing swabs, the information filled out on the outside of the envelope will be manually entered into STaCS to assign the STaCS number. Barcode labels will be printed to affix to the outside of the envelope, fingerprint form, and swab box. The STaCS number becomes the unique specimen number that will be used in the LIMS as the “LAB #” and used to identify the specimen in CODIS.

STaCS specimen information can be imported directly into the LIMS via Excel or manually entered into the LIMS.

Note: For swab samples received prior to 2014, the unique specimen number consists of “SN”, for Southern Nevada or “NN” for Northern Nevada, followed by a five-digit sequential number (e.g. SN12345 or NN12345). The SN barcodes are affixed as labels to the upper right-hand corner of the collection envelope and the swab box. All of the information from these samples was entered into an Access Database which has been transferred over to the LIMS and STaCS databases.

4) Accessioning:
   a) During accessioning for the kits with swabs the additional barcode labels are affixed to the fingerprint form and swab box. The two swabs contained in the kit are retained in the original swab box. If swabs are sampled at this time, one of the two swabs is cut with a disposable scalpel into a labeled, sterile microcentrifuge tube or plate. The other swab can be re-tested later during the confirmation procedure should a match occur.
   b) During accessioning for the kits with the STaCS number the additional STaCS barcode labels will be affixed to the FTA card and a coin envelope that is added to package the blue collection device containing the sponge applicator for long-term storage. If the FTA card is
sampled at this time, a sample punch will be made into a labeled, sterile microcentrifuge tube or plate.

5) The DNA collection kit containing the swabs/FTA card and any other paperwork will then be placed in secured storage, arranged in ascending numerical order. The samples will be available for database match confirmation and retained in storage indefinitely.

6) Incidents relating to problematic collection of database samples will be documented in the “Database Problem Sample Log” kept in the CODIS Administrator’s office or in the “STaCS Rejections & Destructions” spreadsheet maintained by the CODIS Administrator on the H:drive.

13.3.4 Sample Removal – Expungement of Offender Samples
The LVMPD Forensic Lab routinely receives lists from the Nevada State Administration of persons whose personal information for DNA work entered into CODIS must be completely removed (expunged) and any related database samples destroyed. The LVMPD Forensic Lab still tracks the statistics and accounts for the work performed but does not allow personnel to see anything that relates to the biographical or genetic information to a particular person. A mandatory reason for expungement is recorded.

A person whose DNA records or DNA profile has been included in the State DNA database in accordance with NRS 176.0913, NRS 179D.240, NRS179D.443, and NRS 179D.460 may request a court order for expungement of their sample and DNA profile.

- Upon receipt of a valid court order or letter from Nevada State Administration, the LVMPD Forensic Lab will run an NCIC report and research court records to see if the offender/registrant has another qualifying offense that would allow the state to keep the DNA profile and sample.
  - If an alternative qualifying offense exists, the Court issuing the expungement order will be notified by letter that another qualifying offense exists and the sample and DNA profile are being retained by authority of the alternate offense.
  - If no alternative qualifying offense exists, the laboratory will expunge from the CODIS (LDIS, SDIS, and NDIS), LIMS, and STaCS databases all pertinent records associated with the request in accordance with local, state, and national requirements (NDIS Expunge a DNA Profile – Operational Procedure).

A copy of the court order or letter, and a print-out of the deletion report will be retained in the DNA section. The collection kit and sample will be destroyed.

In LIMS, the name of the arrestee will be changed to “Offender [or Registrant] (first name) EXPUNGEMENT (last name)”. All other identifying information including the date of birth, State ID number, social security number, will be manually deleted. The date of expungement will be entered in the “Date of Death” field.

Pursuant to NDIS procedures, if the LVMPD expunges a DNA record, other than a forensic unknown, forensic mixture or forensic partial that has generated an interstate candidate match, the LVMPD will notify the any other laboratory involved in the candidate match that an expungement is being performed. The LVMPD will notify the State CODIS Administrator of any such
expungements so that the NDIS custodian is notified of any candidate matches associated with the expunged record that need to be deleted from NDIS.

13.3.5 Database Sample Entry

1) Import Specimens: On the CODIS workstation - Open the Analyst Workbench. Click on the Specimen Manager on the left bottom side of the screen. The default screen appears with a number of specimens filling the screen. Select Specimen Manager > Import Specimens from the top menu. In the pop-up window, navigate to the CMF file(s) on the H:drive. Highlight the file(s) and select Import CMF Files. In the Assign file to: pop-up window, scroll down to the user name of the analyst who processed the samples and click OK. The import process will complete and a message will be sent to the Message Center. Close the Specimen Manager window.

Open Message Center and click on Import STR Files. The messages that have not been executed will be in bold. Double-click on each file, starting from the bottom, to execute the messages. The new import will load to populate the next step. Click on Import Reports and again double-click each file to execute. The file will then load (this step is the one that loads the samples into Specimen Manager). Print the first page of the “LDIS Import Reconciliation Report”. The Import Report print-out is attached to the data packet that then goes into a folder labeled for the week showing imported database samples and auto searches that is kept indefinitely.

Note: if there are any error messages on the Import Report. Most of the time the error will be an FGA-extended ladder allele that was not converted to “>30” or other loci in which your allele is “< or >” the ladder. If there are error messages, the time to correct them is now by going back to GeneMapper ID-X and ascertaining the error. Then open the sample in Specimen Manager and select “Edit STR/YSTR Specimen” to manually make the correction to the locus before performing any searches or marking the sample for upload. When the import is done, close Message Center.

2) Verify Imported Specimens: Open Specimen Manager. Select File>New and then click on the DNA Typing System tab to create a view that is for samples assigned to the analyst on the date of the import. Click OK. The screen fills with the samples in numerical order. Scroll down and confirm that the appropriate username and specimen category is assigned to each specimen. Also verify that all re-processing suffixes (e.g. -RL, -RI, etc) have been removed. If they have not, open the sample in Specimen Manager and select “Edit STR/YSTR Specimen” and then rename the specimen. Close Specimen Manager when finished.

After the new samples are imported into CODIS, the CODIS Administrator (or alternate) will perform an AutoSearch named “Std. to Std.”, to check for “Offender Duplicates” (example: offender sample to an offender or arrestee sample; there is not an “Arrestee Duplicate” disposition) and conviction matches (example: volunteer sample to an offender/arrestee sample). Refer to Database Autosearch.

After database samples have been imported into CODIS the sample information in the STaCS database needs to be updated using the “CODIS upload query” in the “Offender Dynamic Search” menu option. A range of numbers can be queried to list them in an Excel worksheet where the upload date can be added and then imported into the “CODIS Update” menu option.
13.3.6 Sample Sealing

Database samples are not collected with a chain of custody and are not considered evidentiary in nature. Therefore, they do not require the evidence sealing provision outlined in the LVMPD Forensic Quality Manual. Database samples are collected individually in gum-sealed envelopes. After the samples have been returned to the envelopes after processing, the envelopes are resealed with clear tape.

13.3.7 Destruction or Administrative Removal of Ineligible Database Samples

Upon verification of the fact that a DNA record is not eligible for inclusion in CODIS, the sample will be subjected to an administrative removal of the sample from CODIS and destruction of the DNA sample collection kit.

Documentation associated with why the sample is not eligible for inclusion in CODIS, and when the sample was administratively removed and destroyed will be retained by the Biology/DNA Detail.

A list of expungements/ineligible database samples will be maintained by the CODIS administrator. The collection kit and sample will be destroyed. CODIS Deletion Reports will be maintained in the weekly upload folder.

13.4 CODIS Searches

Entry of newly developed profiles will be conducted as soon as possible after Technical Review. Both Database and Casework Searches are conducted by the CODIS Administrator (or alternate) at least monthly using AutoSearcher for all new profiles entered. Searches are only performed at the local level.

13.4.1 Database Autosearch

All database sample batches will be searched using AutoSearcher, “Std. to Std.”. The search for duplicates within the batch serves two purposes: a within-run check for inadvertent duplications and a random re-analysis since recidivists’ samples occur within almost every batch of samples tested.

In AutoSearcher, double-click on the file called “Std. to Std.” and then AutoSearcher>Perform Search from the top menu. This will search all new data from offender, arrestee, multi-allelic offender, volunteer or elimination standards that have been entered since the previous auto search against the Offender, Arrestee, Multi-Allelic Offender, Volunteer and Elimination indexes. Only profiles that are considered complete will be auto searched. A profile is considered complete for LDIS searches in the Offender, Arrestee, Volunteer and Elimination Indexes if it includes at least 10 loci (including D2S1338 and D19S433).

After the search is complete, go to the Message Center and select AutoSearcher Reports. Double-click on the recent search to execute the message and load the matches into Match Manager. A copy of the Std. to Std. AutoSearcher Report should be printed to verify name/IDs and filed in a folder for the week showing imported database samples and auto searches that is kept indefinitely. It is mandatory that a name/ID check is performed before a specimen is “Marked for Upload” by the CODIS Administrator (or alternate).
A Match Detail Report is printed for conviction matches involving volunteer samples. The verified name is written on the print-out and filed on the left side of the case file or scanned into the Object Repository in the LIMS.

After the name/ID check is performed, the convicted offender and arrestee DNA profiles will be marked for upload to SDIS according to the CODIS operating procedures and the "CODIS Standards for Acceptance of DNA Data at NDIS". This is done by highlighting all of the new samples in Specimen Manager, then right-click and select Mark Specimen For Upload.

### 13.4.2 Casework Autosearch

All casework profiles entered into the CODIS local database will be searched against the local database, including the Elimination Index, using the following search parameters:

<table>
<thead>
<tr>
<th>Autosearch Name</th>
<th>Target Specimens</th>
<th>Searched Against</th>
<th>Search Stringency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate w 1 MM_date</td>
<td>All NDIS Forensic Unknowns/Mixtures/Partials, Forensic Targeted, &amp; Missing Person/UHR-related categories</td>
<td>Offenders, Arrestees, Eliminations, Volunteers, &amp; NDIS Forensic Unknowns/Mixtures/Partials</td>
<td>Moderate stringency with one locus mismatch</td>
</tr>
<tr>
<td>Moderate_date</td>
<td>All SDIS/LDIS Forensic Mixtures Partials</td>
<td>Offenders, Arrestees, Eliminations, Volunteers, &amp; All Forensic Unknowns/Mixtures/Partials</td>
<td>Moderate stringency</td>
</tr>
</tbody>
</table>

In AutoSearcher, double-click on the autosearch name and then select AutoSearcher>Perform Search from the top menu. This will search all new data that has been entered (after Technical Review is complete) since the previous auto search against the local database. Only profiles that are considered complete will be auto searched. A profile is considered complete for LDIS searches in the Forensic Partial, Forensic or Forensic Mixture Indexes if it includes at least 6 loci (may include D2S1433 or D19S433). A profile is considered complete for searches in the Missing Persons and Unidentified Humans Remains Indexes if it includes at least 8 loci (excluding D2S1338 and D19S433).

After the search is complete, go to the Message Center and select AutoSearcher Reports. Double-click on the recent search to execute the message and load the matches into Match Manager. No one will use the Remote Search on any data that has not been through Technical Review. (CODIS-NDIS #BT110806).

### 13.4.3 Keyboard Searches

A keyboard search is a manual search of a forensic DNA profile in the Nevada CODIS state database or other state database. A request for a keyboard search may be made when the investigation warrants an immediate search of the database. This search allows the Forensic Lab flexibility of searching a DNA profile in which a threat to the community substantiates an immediate search. CODIS eligibility rules still apply. An Emergency Upload and Search Request (EUSR) for eligible profiles at SDIS and/or NDIS may be performed with CODIS 9.0 in lieu of a keyboard search request.

- The agency must submit the request for a keyboard search through the Local CODIS Administrator via email or letter to the CODIS State Administrator. This request must include...
the reason for the keyboard search, origin of the DNA profile, and verification the DNA profile has been technically reviewed.

- Keyboard searches are only performed after technical review and if a profile will ultimately be entered into CODIS. Therefore, while the initial search is a “one time event”, ultimately the sample will be entered into the database and automatically searched against the database on some routine basis.
- In the event a keyboard search is performed, a printout of the matches (if any) will be printed and retained in the case file as a record. In the event there are no matches, this record would serve as a negative database search.
- In special circumstances, SDIS labs can be sent a technically reviewed DNA profile and request the profile to be keyboard searched at NDIS or in other states. This request is conducted through the NV State CODIS Administrator in writing and if approved, the NV state administrator submits the request to all the other labs and informs the LVMPD of any hits.
13.5 CODIS Hits and Match Confirmation Procedures

When a match, or hit, occurs in CODIS, there are several steps that must be followed to confirm and report out the DNA match. These procedures differ based upon the source of the DNA profile, database or casework, and whether the case(s) are solved or unsolved. In the event a submitted buccal swab is associated with CODIS investigative leads for multiple cases, the requestor for each affected case will be notified via email that the Biology/DNA Detail will be issuing a supplemental report. A copy of this email will be maintained in the Object Repository of each affected case in LIMS.

For high stringency matches or matches involving most forensic unknown and/or forensic partial profiles and some forensic mixture profiles, the determination of a match may be made by visual side-by-side comparison of the target and candidate profiles. However, for mixtures entered using the COSTaR workbook the determination of a match may be made using a STRmix database search of CODIS candidate profiles against the target profile’s STRmix deconvolution settings file to calculate a likelihood ratio to determine the best candidate(s).

Dispositioning Matches Using the STRmix Database Creator and Reference File Maker

In CODIS, click on the Match Manager module.
- Then click on the “STRmix DB Creator” saved view. Add an additional filter for the date(s) of the matches you need to disposition.
- Highlight all matches and select Print, Report Type: Match Details Report (Short), and Print Preview.
- Click the first icon “Export Report”. Change the save as file type to a .csv file and save file as “date_STRmix database” (e.g. “040119_STRmix database”) in the folder H\Forensic Data\DNA\1-CODIS\Casework STRmix Databases. An “Export completed” message will pop up when done. Click OK.

Open the STRmix DB Creator workbook.
- Click the “Select File and Create Database” button.
- Navigate to the STRmix database file created from CODIS. Click Open. Click OK in pop-up. Change the save as file type to a .csv file and save file as “date_Candidates” (e.g. “040119_Candidates”) in the folder H\Forensic Data\DNA\1-CODIS\Casework STRmix Databases. Click OK in pop-up.

In STRmix, click on the “Investigation” module. Then click on the “Database Search” module.
- Click “Browse” button for Previous Interpretation and navigate to the STRmix deconvolution folder of the target specimen and select the “settings.ini” file if deconvoluted in STRmix v2.4 or the “config.xml” file if deconvoluted in STRmix v2.6. Click open. Alternatively, you may drag and drop the entire deconvolution folder into the Previous Interpretation box.
- The default staff elimination database must be updated to the Candidate database created above with the STRmix DB Creator workbook. Click the “Browse” button for Database File and navigate to the Candidates.csv file. Click Open. Alternatively, you may drag and drop the Candidates.csv file into the Database File box.
- The default Minimum LR must be updated to 50,000
- Verify the other following settings:
  - Set the Population for Search to “NIST_Investigator_Cauc”
  - Leave Extended Output box unchecked
Set Type of Search to “Standard”
Enter “0.01b(1.0,1.0)” for FST for Search. This sets the theta value to 0.01.
Check the Assign Sub-Source LR box
If deconvoluted in STRmix v2.4, enter “CODIS_DBSearch_Case # Item #” for Search ID (e.g. CODIS_DBSearch_19-012345 Item 1). If deconvoluted in STRmix v2.6 it will auto-populate with info entered during the deconvolution. Add “CODIS_” to the front of the name: “CODIS_Analyst initials_Case #_Item #-DBSearch” (e.g. CODIS_JD_19-012345_Item 1 decon-DBSearch).
Click “Start” button. Click OK if you get a warning about DYS391 or QS1/QS2 allele frequencies.

A “Database Search Report” window will open. Scroll-down to the “LR RESULTS” section to see if any candidate samples are listed.

Note: For mixtures, it is important to remember that this search is a comparison to the entire mixture and all of its contributors, not just the contributor targeted in the CODIS search (e.g. “-C2”). If any comparisons were done to this sample with a reference standard, the candidate names should be compared to any listed reference standards to determine if the match should be dispositioned as a Conviction Match instead.

- If there are no candidates with LR > 50,000, “No matches found at specified Minimum LR cutoff level” will be listed.
  - In the DATABASE SEARCH SETUP section of the report, verify the correct deconvolution file and candidate database were chosen for the search.
  - Navigate to STRmix Results folder. Add “DNU” in front of the DBsearch folder name. Copy the folder into the Item sub-folder in the analyst’s STRmix Run Files folder. Nothing needs to be printed.
  - Close the report window. Click “Finish” button in the STRmix RESULTS window.
- If there are candidates with LR > 50,000, they will be listed with the Case Number (always “CODIS”), Sample (CODIS specimen ID), Gender (if available), and LR. (e.g. “CODIS...2014-012345...X,Y...1.0000E10”).
  - For candidates with an LR > 100,000: Print the report and highlight the case and item number listed for the Sample file name in the DATABASE SEARCH SETUP section and matched candidate specimen ID listed under Sample in the LR RESULTS section. This report will be kept for the CODIS hit packet during the match confirmation process of the candidate sample. If the match will be dispositioned as a Conviction Match instead, this report should be scanned into the LIMS with a copy of the dispositioned Match Details Report.
    ▪ If multiple candidates are returned from the database search for an item because it is a mixture, an LR from previous calculation may be performed with all of the candidates to show it is possible for all of them to be contributors together (see below).
  - For candidates with an LR between 50,000-100,000 a separate LR from Previous calculation must be performed in STRmix using the Reference File Maker to create a reference .csv file from the candidate profile (see below). Print the report and highlight the case and item number listed for the Sample file name in the DATABASE SEARCH SETUP section and matched candidate specimen ID listed under Sample in the LR RESULTS section. This report will be kept for the CODIS hit packet during the match confirmation process of the
candidate sample. If the match will be dispositioned as a Conviction Match instead, this report should be saved to scan into the LIMS along with any STRmix paperwork created below and a copy of the dispositioned Match Details Report.

- Navigate to STRmix Results folder. Copy the folder into the Item sub-folder in the analyst’s STRmix Run Files folder.
- Close the report window. Click “Finish” button in the STRmix RESULTS window.

If needed (i.e. candidate LR between 50,000-100,000 or multiple candidates/contributors to a mixture), open the Reference File Maker workbook.

- Type the candidate Sample CODIS specimen ID listed on the Database Search Report in the first cell under the Sample File column (cell A2). Hit Enter.
- Click the “Choose Database” button and navigate to the appropriate Candidates.csv file in the folder: H:\Forensic Data\DNA\1-CODIS\Casework STRmix Databases. Click Open.
- Change the save as file type to Text (Tab delimited).txt file and save as the candidate specimen ID in the DBSearch folder (e.g. “2014-012345”). Click OK in the pop-up.

In STRmix, click on the “Investigation” module. Then click on the “LR From Previous” module.

- Navigate to the STRmix deconvolution folder of the target specimen. Select the settings file and click Open. Click “Select” button. Alternatively, you may drag and drop the entire deconvolution folder into the box.
- In the CASE DETAILS window, make the following changes:
  - Case Number: “CODIS_target CODIS specimen ID” (e.g. “CODIS_R1901234-1-C1”). Sample ID: Add the Candidate Sample CODIS specimen ID to what is already populated (e.g. “Item 1-decon-LRPrev 2014-012345”).
  - Click “Next” button.
- In the Profile Data window:
  - Drag and drop the text file into the Reference Profile Data box. “_REF.csv” will be appended to the name.
  - Click “Start” button.

An “LR From Previous Report” window will open. Scroll down to the PER LOCUS LIKELIHOOD RATIOS section of the report and review the 99% 1-sided lower HPD interval values.

- If the lowest value is less than 50,000:
  - Navigate to STRmix Results folder. Add “DNU” in front of the LRPrev folder name. Copy the folder into the Item sub-folder in the analyst’s STRmix Run Files folder. Nothing needs to be printed.
  - Close the report window. Click Finish in the STRmix RESULTS window.
- If the lowest value is greater than 50,000
  - Print the LR From Previous Report for the CODIS hit packet created during the match confirmation process of the candidate sample. If the match will be dispositioned as a Conviction Match instead, this report should be scanned into the LIMS with copies of the Database Search Report and dispositioned Match Details Report. The LR From Previous Report will be marked “For CODIS purposes only” prior to it being included in a CODIS hit packet or scanned into the LIMS.
Navigate to STRmix Results folder. Copy the LRPRev results folder to inside of the DBSearch results folder in the Item sub-folder in the analyst’s STRmix Run Files folder.

- Close the report window. Click Finish in the STRmix RESULTS window.

For any matches not captured in the initial filtering of matches using the STRmix DB Creator view in CODIS due to the candidate profile being a partial or mixture profile, the matches will have to be manually dispositioned by visual comparison. Look for matches between samples of the same case that can be dispositioned as Benchwork Matches. The Contributor Genotype Summary of the original deconvolution STRmix report may be consulted if any loci deconvoluted >=100% for the targeted contributor that can be visually compared to the candidate specimens, or vice versa. Alternatively, it’s possible a Reference File Maker profile could be manually created for one of the candidate partial or mixture profiles if the profile is close to a complete single source profile except for a few mixture or partial loci that could be removed leaving a single source profile that could be compared in the “LR from Previous Analysis” step. If neither of these are viable options then the match will have to be dispositioned as No Match.

13.5.1 Offender/Arrestee to Unsolved Case(s)

If a LDIS match occurs between an offender or arrestee and an unsolved forensic case, the following steps will be followed:

1) STR technology- the allele designations will be confirmed through the comparison of analyzed data.

2) The forensic specimen case file is pulled and a determination is made whether a match confirmation is necessary.
   a. If the sample hits against duplicate database samples from the same person, only a fingerprint match and biographical information check between the multiple collection kits is required. The specimen details for all samples involved must also be updated.
   b. If a sample hits against a database sample that has already been confirmed, the prior match data is used and the specimen details for all samples involved must be updated.

3) If a match confirmation is necessary, a lab member will retrieve the envelope containing the DNA sample swab box or FTA card and the fingerprint form, if collected. A copy of the biographical information will be printed from STaCS or photocopied from the envelope and fingerprint form (after comparison by a member of the Latent Print detail, if applicable) to include in the hit report file. Re-analysis of the database DNA sample will be conducted as soon as possible. Any of the validated extraction and STR amplification methods for reference standards can be used. A full profile is not required but there must be enough data to show concordance with the original profile in CODIS. The DNA match confirmation data is subject to technical review, signed and dated. The match confirmation sample data packet will be stored in the weekly folders containing database sample data packets and autosearches maintained by the CODIS Administrator.
   a. A national criminal history check will be performed using the National Crime Information Center (NCIC) or Interstate Identification Index (III) to query for the individual’s FBI #.
      Documentation of this check will be performed as follows:
      - Arrestee: FBI # and initials/date added to Comments field in StaCS prior to printing for CODIS hit packet.
      - Convicted Offender: FBI# with initials/date added to outer envelope of SN samples or back of bi-fold for StaCS samples, prior to photocopying for inclusion in CODIS hit packet.
4) Fingerprint comparison is used to help with the confirmation process when possible. If the fingerprint is not comparable due to quality, the match is still valid. Fingerprints confirmed via LiveScan at the time of collection do not need to be re-confirmed.

5) An administrative CODIS hit data packet is prepared by the CODIS Administrator for the analyst who will write the report and includes the following:
   a. Database Hit Review Form (CODIS Administrator completes Database Task Checklist on top portion). This form lists the applicable LIMS lab numbers involved in the hit(s) and serves as a coversheet for the documentation being scanned into the LIMS under each lab number listed.
   b. Copy of Local Match Detail Report(s) with updated Source ID and Disposition
   c. Copy of outer envelope and fingerprint form (after comparison by member of the Latent Print Detail or Nevada DPS) for swab samples, or copy of both sides of bi-fold for FTA card samples (after comparison by member of the Latent Print Detail or Nevada DPS, if applicable), or copy of StaCS print-out.
      **Note:** For arrestee samples, fingerprints are being verified via Livescan and therefore there may not be any additional verification by the Latent Print Detail. In these instances, only the updated StaCS print-out is needed.
   d. Copy of District Court print-out showing sentencing requirement to provide a DNA sample, if applicable
      **Note:** For arrestee samples because sample eligibility is being determined up front with probable cause paperwork, no print-outs of this verification will be required in the hit data packet.
   e. Copy of Technical Review Checklist-Database Knowns form used for technical review of sample when it was re-tested
   f. Electropherogram of sample when it was re-tested
   g. Copies of the STRmix DBSearch Results report (page 1) and STRmix LR from Previous Analysis report, if applicable
   h. Copies of LDIS Specimen Details Report(s) with updated Source ID and STR/Y-STR Comments fields

6) A CODIS Hit Notification report will be prepared and sent by a member of the Biology/DNA Detail to the appropriate investigator informing them that a match has been detected. The following biographical information will be relayed, if available:
   a. The name of the convicted offender or arrestee, state identification (SID) number, social security number (SSN), qualifying offense and date of birth.
   b. This information is to be used as investigative information and may be used as probable cause to obtain a search warrant for a DNA sample for the purposes of comparison.
   c. The investigator will be informed that confirmation of the match for future legal proceedings will require a suspect reference standard (buccal swab collection), which can be submitted to LVMPD via standard evidence submission procedures.

The hit data packet should be page numbered and initialed with the total number of pages written on the first page. If being scanned into the LIMS, the lab # only needs to appear on the front page. All paperwork (report and data packet) associated with the match confirmation will be administratively reviewed prior to the report being distributed. The reviewer will complete the Database Administrative Review Checklist on the bottom part of the Database Hit Review Form. The paperwork will be scanned into the Object Repository of the LIMS for the case(s) involved in the hit(s). These are approved by the CODIS Administrator or by the analyst writing the hit report.
This paperwork will be retained utilizing the same document retention policies set forth for the case file in which it resides.

**Note:** For cases worked outside of the LIMS this paperwork is filed on the left side of the case file. If any of the paperwork does not include the unique case/lab identifier somewhere on the page then it should be written on the bottom right hand corner of the page.

After the CODIS Hit Notification report is released, a follow-up e-mail with a CODIS Hit Disposition form will be sent to the investigator to determine the disposition of the investigative lead. The CODIS Hit Disposition form returned to the CODIS Administrator will be scanned into the Object Repository in the LIMS and approved by the CODIS Administrator (or designee).

### 13.5.2 Unsolved Cases to Solved Cases

If a LDIS match occurs between an unsolved case and a solved case, either a benchwork match to a known reference sample (“Forensic Hit”) or a volunteer sample (“User Defined #1” or “Volunteer Hit”), the following procedure is used:

1. The case data from both cases are compared and reviewed.
2. The original requestor for the unsolved case will be notified via email that based on a CODIS investigative lead, the Biology/DNA Detail will be issuing a supplemental report for the case. Documentation of this email communication will be maintained in the case file or the case’s Object Repository.
3. The allele table of the reference standard from the solved case is placed within the unsolved case’s file and statistics are performed if necessary. Copies of the DBSearch Results report (page 1) and STRmix LR from Previous Analysis report (if applicable), Local Match Detail Report(s) and LDIS Specimen Details Report(s) with updated Source ID, Disposition, and Comments fields are also included within the case file or placed in the unsolved case’s Object Repository.
4. A supplemental report is prepared and then technical and administrative review takes place. The submitting investigators of all cases associated with the hit(s) will be contacted, via report or electronically by the CODIS Administrator or casework analyst. Information from each case to be included: event #, crime type, sample name, and sample description. Documentation of this contact will be retained in each file or scanned into the Object Repository of the LIMS.

### 13.5.3 Solved Cases to Solved Cases

If a LDIS match occurs between two or more solved cases (each already includes a statistical comparison to an evidentiary reference standard), the following procedure is used:

1. The case data from all cases are compared and reviewed.
2. The original requestors for each case are notified via email that the cases have hit to each other and have already been compared to buccal swabs. Information from each case to be included: event #, crime type, sample name, and sample description.
3. A copy of the email, DBSearch Results report (page 1) and STRmix LR from Previous Analysis report (if applicable), CODIS Match Details Report, and updated LDIS Specimen Details Report(s) will be included within the case file or placed in each case’s Object Repository.
13.5.4 Unsolved Case to Unsolved Case

If a LDIS match occurs between two unsolved cases ("Forensic Hit") the following procedure is used:

1) The case data from both cases are compared and reviewed.

2) An administrative CODIS hit data packet is prepared by the CODIS Administrator for the analyst who will write the report and includes the following:
   a. Database Hit Review Form (CODIS administrator completes Database Task Checklist on top portion). This form lists the applicable LIMS lab numbers involved in the hit(s) and serves as a coversheet for the documentation being scanned into the LIMS under each lab number listed.
   b. Copy of Local Match Detail Report(s) with updated Disposition
   c. Copies of the DBSearch Results report (page 1) and STRmix LR from Previous Analysis report (if applicable), Copies of LDIS Specimen Details Report(s) with updated STR/Y-STR Comments field

3) A CODIS Hit Notification report will be prepared and sent by a member of the Biology/DNA Detail to the appropriate investigators informing them that a match has been detected between the unsolved cases.

The hit data packet should be page numbered and initialed with the total number of pages written on the first page. If being scanned into the LIMS, the lab # only needs to appear on the front page. All paperwork (report and data packet) associated with the forensic hit will be administratively reviewed prior to the report being distributed. The reviewer will complete the Database Administrative Review Checklist on the bottom part of the Database Hit Review Form. The paperwork will be scanned into the Object Repository of the LIMS for the case(s) involved in the hit(s). These are approved by the CODIS Administrator or by the analyst writing the hit report. This paperwork will be retained utilizing the same document retention policies set forth for the case file in which it resides.

Note: For cases worked outside of the LIMS this paperwork is filed on the left side of the case file. If any of the paperwork does not include the unique case/lab identifier somewhere on the page then it should be written on the bottom right hand corner of the page.

13.5.5 Forensic or Offender/Arrestee – Interstate Matches

If the match involves either a forensic or offender/arrestee profile developed by another forensic lab the verification procedures will be similar to those above, except that the two agencies will share portions of the procedures. The DNA case analysts or the CODIS Administrators of the respective labs will communicate using the format described in the NDIS Manual, "Confirm an Interstate Candidate Match". Match Data Requests and Match Data Responses are typically sent via electronic mail, faxed and/or sent by regular mail.

- If the LVMPD receives a match request from an outside agency to confirm a database sample, an administrative CODIS hit data packet similar to those generated for LDIS matches will be prepared by the CODIS Administrator, including updated State or National Match Detail Report(s) and LDIS Specimen Details Report(s). A letter will be sent to the requesting laboratory with all biographical information available on the database sample collection kit. The hit data packet should be page numbered and initialed with the total number of pages written on the first page. The letter and paperwork associated with the hit will be administratively reviewed prior to the letter being sent. The reviewer will complete the Database Administrative
Review Checklist on the bottom part of the Database Hit Review Form. The letter stamped with a distribution date and CODIS hit data packet will be scanned into the Object Repository of the LIMS for the database sample.

- If the LVMPD receives a request to exchange case information for a match between two unsolved cases, an administrative CODIS hit data packet will be prepared by the CODIS Administrator including updated State or National Match Detail Report(s) and LDIS Specimen Details Report(s). Case information may be exchanged electronically via email or a letter may be sent to the requesting laboratory with the case and investigating agency information available. Additionally, a CODIS Hit Notification report is written to notify the case investigator of the LDIS case of the other agency’s case information. The hit data packet should be page numbered and initialed with the total number of pages written on the first page. If being scanned into the LIMS, the lab # only needs to appear on the front page. All paperwork (report and data packet) associated with the forensic hit will be administratively reviewed prior to the report being distributed. The reviewer will complete the Database Administrative Review Checklist on the bottom part of the Database Hit Review Form. The paperwork will be scanned into the Object Repository of the LIMS for the case(s) involved in the hit(s). These are approved by the CODIS Administrator or by the analyst writing the hit report. This paperwork will be retained utilizing the same document retention policies set forth for the case file in which it resides.

13.5.6 Solved Cases to Offender/Arrestee/Volunteer and Intrastate Offender/Arrestee to Offender/Arrestee Hits

**Benchwork Match or Previous Hit** - If the match involves any previously solved case to an offender, arrestee or volunteer, all involved cases and samples will be researched to ensure the provided biographical information matches (e.g. name, DOB, SSN, etc.). The verification of the biographical information will be noted on the Match Detail Report by the CODIS Administrator or the DNA analyst. The Match Detail Report generated in CODIS will be placed on the left hand side of the appropriate case file or scanned into the Object Repository of the LIMS. These matches are dispositioned as a “Conviction Match” in the Match Manager program in CODIS.

If there is a discrepancy in the biographical information, the reason for the discrepancy will be evaluated and parties involved (e.g. detectives, P&P, Prisons) will be notified as appropriate.

**Offender/Arrestee to Offender/Arrestee Match** – If the match involves an offender/arrestee to offender/arrestee match within Nevada, the SN, NN (for Northern Nevada) or STaCS number will be checked in the respective database to ensure the biographical information provided on the envelope matches (e.g. name, DOB, SSN, etc.). These matches are dispositioned as an “Offender Duplicate” in the Match Manager program in CODIS; there is no “arrestee duplicate” disposition option in CODIS.

If there is a discrepancy in the biographical information, fingerprint forms will be checked for offenders or the administrative message (AM) will be checked for arrestees. If needed, all parties involved (e.g. P&P, Prisons) will be notified and re-collection will take place, if possible.

13.5.7 Interstate Offender/Arrestee to Solved Case(s)

**LVMPD Solved Case** – If the match involves a solved case worked by the LVMPD, the other state will be informed of the name of the person from the solved case (e.g. benchwork match or
confirmed database sample). This is done via electronic mail. The other state will then confirm if that name matches their offender/arrestee. These matches are dispositioned as a “Conviction Match” in the Match Manager program in CODIS. The Match Detail Report generated in CODIS will be placed on the left-hand side of the appropriate case file or scanned into the Object Repository of the LIMS.

If there is a discrepancy (the other state informs us the biographical information does not match), the other state would be asked to perform a confirmation match on the offender/arrestee. All parties involved will be notified and further action will be taken.

**LVMPD Database Sample** – If the match involves a LVMPD database sample with another state’s solved case, the other state will inform us of the biographical information via electronic mail. The LVMPD will confirm if the provided name matches our offender/arrestee via electronic mail. These correspondences are kept indefinitely in a folder maintained by the CODIS Administrator and archived electronically by year. These matches are dispositioned as a “Conviction Match” in the Match Manager program in CODIS.

If there is a discrepancy (the biographical information does not match), the other state will notify the LVMPD and relay what further action they require (e.g. confirm the sample, send report, etc.).

### 13.5.8 Hits to Ineligible Samples
In the event a hit occurs to a sample that is deemed ineligible to be retained in CODIS, a hit notification report will be issued to the investigating agent advising them of the hit. In addition, the sample will be deleted and a supplemental DNA report will be issued stating the sample has been removed from CODIS because it is no longer eligible. Refer to Administrative Sample Removal (casework samples) or Destruction or Administrative Removal of Database Samples (database samples).

### 13.5.9 Record Keeping, Hit Reporting and Administrative Review of CODIS Hit Reports
Copies of match documents are kept indefinitely in electronic formats, and in the file maintained by the CODIS Administrator or in the case files or Object Repository of the LIMS.

An NDIS required tally of hits is sent to the State Administrator each month.

A list of conviction matches is recorded each month for LDIS database and forensic samples that occur with other LDIS, SDIS, or NDIS samples.

The technical review of database samples is recorded on the “Technical Review Checklist-Database Knowns” form.

The administrative review of CODIS hit reports and associated paperwork is documented on the “Database Hit Review” form and will include a review of the eligibility of the CODIS profile, CODIS Hit Notification report or letter for clerical errors, a review of the individual’s biographical data, qualifying offense and the DNA profile generated.
Match documentation is filed in the case file or LIMS Object Repository, or if involving a database sample only within the LIMS Object Repository of the database sample. Electronic copies of hit notification reports prior to the LIMS are stored by case Event # and letters are stored by Match ID.

A candidate match shall be dispositioned as soon as possible in the Match Manager program in CODIS. A good faith effort shall be made to perform internal match confirmation processing, data review and completion of a CODIS Hit Notification report within 30 days.

13.5.10 Elimination (Staff) Index Hits

The Forensic Laboratory is committed to being proactive and taking the necessary steps to reduce the possibility of biological contamination of evidence or reference samples by field and laboratory staff. The Laboratory will use a database of employee profiles to help identify any biological contamination that does occur. Currently all Forensic Laboratory employees and CSI employees who routinely handle evidence are required to provide a sample. Other members of the Law Enforcement community may provide samples on a voluntary basis. Contamination events will be documented on a “Corrective Action Report” or on a “case note” in the case file.

The Biology/DNA Detail will make every effort to protect the identity of the staff’s personal genetic information and will refrain from turning over the entire index at the request of an agent.

The Elimination Index is used for screening purposes only. The index is not used for investigative purposes and profiles that reside in the Elimination Index of CODIS cannot be uploaded to SDIS or NDIS. In addition, the Biology/DNA Detail is prohibited from releasing DNA profiles contained in the index unless a court order directs the lab to do so.

In the event there is a match between an evidentiary sample and the Elimination Index, the following will take place:

a. It will be determined if the staff member handled the evidence at any time: either at the scene of the crime or in the laboratory. The profile will be deleted from CODIS. The case will be re-worked, if possible, and a supplemental DNA report will be issued if it is not caught prior to the original report being released.

b. If a forensic profile already resides in CODIS and is found to match an employee staff member, it will be determined if the staff member handled the evidence at any time: either at the scene of the crime or in the laboratory. The case will be re-worked, if possible, and the DNA profile will be deleted from CODIS. A supplemental DNA report will be issued.

Refer to Interpretation of a Contaminated Control or Sample and Contamination Report Statements for interpretation criteria and report wording associated with Elimination Index hits.
Appendix A  Title: GeneMapper ID-X and STRmix Settings

ALLELEigator Table Setting: (Investigator 24plex QS or Fusion 6C)

Samples Tab

Genotypes Tab
Investigator 24plex QS Size Standard Settings

Investigator 24plex QS 3500INV24PLEX45.55.65.75.45_191107 Analysis Method

Allele Tab

Peak Detector Tab
Investigator 24plex QS 3500INV24PLEX45.55.65.75.45_191107_global Analysis Method

**Allele Tab**

**Peak Detector Tab**
### Investigator 24plex QS Panel Settings for 3500INV24PLEX_191107_panel

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<th>Molec Size</th>
<th>DNA Size</th>
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<th>Y DNA Size</th>
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**ARCHIVED**
Investigator 24plex QS Plot Settings
There are custom INV24PLEX print plot settings: (rfu only) and (rfu & bp)

**Sample Header** tab: Sample File, Sample Name, Size Quality Overridden (SQO), Sample Off-scale (SOS), Sizing Quality (SQ), and Outside Marker Range (OMR) check marked

**Genotype Header** tab, all but Sample File check-marked

**Sizing Table** tab: Sample File Name, Marker, Allele, Size, and Height check marked

**Labels** tab:

![Image of Inv24plex QS Plot Settings](archived.png)

**Display Settings** tab:

![Image of Display Settings](archived.png)
Fusion 6C Size Standard Settings

![Image of Fusion 6C Size Standard Settings](image-url)
Fusion 6C Analysis Method

Allele Tab

Peak Detector Tab 3500xI
Peak Quality Tab 3500xl

Fusion 6C Panel Settings

Plot Setting: Data Interpretation
There is a custom Data Interpretation plot setting: Fusion 6C Review with BP.

Under the Sample Header tab, the following items should be check-marked: Sample File, Sample Name, Sample Off-scale (SOS), Sizing Quality (SQ), Sample Spike (SSPK), Mixed Source (MIX), Outside Marker Range (OMR) and Composite GQ (CGQ).

Under the Genotype Header tab, all but Sample File should be check-marked.
Under the **Sizing Table** tab, the following items should be check-marked: Sample File Name, Marker, Allele, Size and Height.

Under the **Labels** tab, the following should be selected from the different drop down menus: Assigned Allele: Allele Call, Size, and Height; Custom Allele: Allele Call, Size, and Height; Allelic Ladder: Allele Call and Height; and Artifact: Artifact Label, Size, and Height. Dye Color-Border should be selected from the Label Color drop down menu.

The **Display Settings** tab should look like the following:

![Display Settings tab](image)

**Plot Setting:** Fusion 6C Print with BP (used to print electropherograms with no artifacts shown)

Under the **Sample Header Tab**, the following items should be check-marked: Sample File, Sample Name, Sizing Quality Overridden (SQO) and Sizing Quality (SQ).

Under the **Genotype Header** tab, all but Sample File should be check-marked.

Under the **Sizing Table** tab, the following items should be check-marked: Sample File Name, Marker, Allele, Size, and Height.
The **Labels** tab should look like the following:

![Labels Tab](image1)

The **Display Settings** tab should look like the following:

![Display Settings Tab](image2)

**Plot Setting**: Fusion 6C Ladder for OL (used to print Ladder electropherograms for off-ladder alleles)

Under the **Sample Header Tab**, the following items should be check-marked: Sample File, Sizing Quality Overridden (SQO) and Sizing Quality (SQ).

Under the **Genotype Header** tab, Sample File, Sample Name, Sizing Quality Overridden, Sample Off-Scale, Sizing Quality, Outside Marker Range should be check-marked.
Under the **Sizing Table** tab, all items should be check-marked.

The **Labels** tab should look like the following:
The **Display Settings** tab should look like the following:

---

**Settings for STRmix v2.6**

**Kit Settings**

The following kit settings will be used for the *new interpretation of 3500xl Investigator 24plex QS profiles in STRmix v2.6*. The stutter values are published in the Biology/DNA Detail's STRmix Parameter document.
### GENERAL

#### DOUBLE BACK STUTTER
- **Stutter Enabled**: ✓
- **Position Relative to Parent**: -2, 0
- **Inversely Proportional To**
  - Expected Height of Stutter...

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<thead>
<tr>
<th>Maximum Stutter Ratio</th>
<th>Variance</th>
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</thead>
<tbody>
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</tbody>
</table>

- **Stutter Regression File**: LVMPD INV24_Double Back Stutter Regression.txt
- **Stutter Exceptions File**: Select a value

#### MINUS 2 BP STUTTER
- **Stutter Enabled**: ✓
- **Position Relative to Parent**: 0.2
- **Inversely Proportional To**
  - Expected Height of Stutter...

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<thead>
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<th>Maximum Stutter Ratio</th>
<th>Variance</th>
</tr>
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<tr>
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<td>1.808, 1.738</td>
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- **Stutter Regression File**: LVMPD INV24_Minus 2 BP Stutter Regression II.txt
- **Stutter Exceptions File**: Select a value

[Add New Stutter]
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The following kit settings will be used for the new interpretation of 3130xl Investigator 24plex QS profiles in STRmix v2.6. The stutter values are published in the Biology/DNA Detail's STRmix Parameter document.
### BACK STUTTER

- **Stutter Enabled**: Yes
- **Position Relative to Parent**: -1, 0
- **Maximum Stutter Ratio**:
  - No Maximum: 0.3
- **Variance**: 1.647, 3.484

**Stutter Regression File**
- LVMPD INV24_Back Stutter Regression.txt

**Stutter Exceptions File**
- LVMPD INV24_Back Stutter Exceptions.csv

### FORWARD STUTTER

- **Stutter Enabled**: Yes
- **Position Relative to Parent**: 1, 0
- **Inversely Proportional To**
  - Expected Height of Stutter

**Stutter Regression File**
- LVMPD INV24_Forward Stutter Regression.txt

**Stutter Exceptions File**
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Uncontrolled Copy if not located in Qualtrax
The following kit settings will be used for the new interpretation of Identifier Plus profiles in STRmix v2.6. The stutter values are published in the Biology/DNA Detail’s STRmix Parameter document.
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**Stutter Regression File**
LVMPD_IDPlus_GS_back_stutter_file.txt

**Stutter Exceptions File**
LVMPD_IDPlus_GS_Back Stutter Exceptions file.csv

## FORWARD STUTTER

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**Stutter Regression File**
LVMPD_IDPlus_GS_forward_stutter_file.txt

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**Stutter Regression File**
LVMPD_IDPlus_GS_double_back_stutter_file.txt

**Stutter Exceptions File**
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**IMPORTANT!** The settings associated with the previously validated “Identifiler Plus” kit for STRmix v2.3 and “LVMPD Qiagen_Investigator_24plex I” kit for STRmix v2.4.06 are also loaded into STRmix v2.6 so that the software may be utilized to calculate new LRs from previously interpreted evidence samples. These two kits will not be utilized for any new STRmix deconvolutions.
**STRmix Default Settings**

The following default settings will be used for the *new interpretation of all new Investigator 24plex QS or Identifiler Plus profiles in STRmix v2.6*

![STRmix Default Settings](image_url)

**Results Directory**

Y:\STRmix\v2.6\STRmix Results\v1.0.37

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**Gelman-Rubin**

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**Contributor Range**

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The following populations will be used in STRmix v2.6:

![STRmix screenshot]

**ARCHIVED**
The following default report settings will be used for STRmix v2.6

**IMPORTANT!** STRmix Summary Reports may be generated retroactively to include the additional sections, as needed.
Appendix B: Title: USE OF DNA WORKBOOKS

The Biology/DNA Detail has developed several Excel workbooks to assure quality and enhance efficiency through automation of laboratory processing documentation and calculation assistance. The master templates of all such workbooks are electronically secured on the forensic lab’s H:drive with read-only access restricting workbook users from making unauthorized changes to the templates. Password-protected and/or full read-write access is granted only to the CODIS Administrator, the DNA Lab Supervisor, the Technical Leader, the DNA Lab Manager, the Forensic Lab’s Quality Manager, and/or designee(s) responsible for updating the workbooks. The workbooks currently used by the Biology/DNA Detail are briefly outlined below.

**Resource Management/Distribution & Performance Check Workbooks**
- **huLOTapai**: centralized utility that manages DNA resources (reagents, instruments and settings, and their associated expiration and/or maintenance dates); distributes resources to respective laboratory processing workbooks and restricts analysts from using expired resources or resources that haven’t yet been approved for usage
- **LADDERpillar**: collection of integrated workbooks monitoring 3500xl performance using allelic ladder measurements
- **KoalaManderCuda**: collection of integrated workbooks normalizing 3500xl injection conditions for DNA interpretation using amplified DNA dilution series

**DNA Databasing & Forensic Casework Workbooks**
- **CONFIRMadactyl**: documents p30/microscopic sperm/RSID/hematrace screening
- **EXTRACTasaurus** or **DB EXTRACTasaurus**: documents DNA extraction
- **QUANTadillo**: documents Quantifiler Trio DNA quantification
- **makeiTWORK**: documents Qiagen re-purification/clean-up and/or DNA concentration
- **AMPalatypus & cattyRUNpas**: documents Investigator 24plex amplification/electrophoresis
- **Zeppelin**: documents Fusion 6C amplification/electrophoresis on the 3500xl
- **ALLELEigator**: converts GeneMapper ID-X data into allele table summaries

**Important**: Within the DNA workbooks, never “cut” and/or “paste,” never “delete” and/or “insert” rows/columns/cells, never “sort,” and never “fill-down” unless specifically noted these actions are permissible… always instead use “copy” and “paste special values” (or “copy” and “DNA paste” from the customized ribbon) to move data and only in the specific locations designated for user-input (deleting data inside these user-input fields is ok as long as it does not cause a “shift” event to the cells/columns/rows of the workbook or any linked formulas)

**Important**: DNA workbooks utilize a protective “printblocker” feature ensuring that only completed worksheets are printed for case files—any incomplete worksheets are automatically printed/marked **DRAFT** to clearly identify incomplete worksheets… if any resources or results are not applicable, the user must still enter something (such as “N/A” or “--”) to satisfy the printblocker’s protective logic so that the worksheet no longer prints as a draft
B.1 Preparing to use DNA workbooks and DNA workbook maintenance

B.1.1 Update Excel security settings for DNA workbook use

\textbf{Note:} security settings must be modified before using DNA workbooks; each user will need to do this once per each computer

- Open Excel; Click the \textit{File} tab on the top navigation ribbon then \textit{Options} from the navigation menu on the left; Choose \textit{Trust Center} from the navigation menu on the left side and then click the \textit{Trust Center Settings} button on the right side (another smaller window will open)
- Choose \textit{Macro Settings} from the navigation menu on the left side of the new window and then \textit{Enable all macros} on the right side
- Choose \textit{External Content} from the navigation menu on the left side of this same window and then \textit{Enable all Data Connections} and \textit{Enable automatic update for all Workbook Links} on the right side
- Click the \textit{OK} button twice to close both windows and then exit/close Microsoft Excel—the updates will take effect the next time you open Microsoft Excel

B.1.2 Maintaining protective drop-down list selection features of DNA workbooks

\textbf{Note:} DNA workbooks retrieve resources (pipets, reagents, instruments etc.) from huLOTapai found on the H:drive, requiring network access to leverage its protective management of drop-down lists

- When opening DNA workbooks you will be prompted whether to update links…
  - If you will be selecting resources click \textit{Update}
  - If you don’t need to select any resources, you can click \textit{Don’t Update}

B.1.3 Setting up an external USB drive for DNA workbook use

\textbf{Note:} DNA workbooks may require access to E:\QUANTS\do not delete.txt and E:\RUNS\do not delete.txt if network instrument access is blocked (the drive may be “E” or a different letter for each user/computer/USB combination)

- Copy the \textit{QUANTS} and \textit{RUNS} folders from \texttt{H:\Forensic Data\DNA\(DNA WORKBOOKS)\(RESOURCES)\USB setup} directly onto your USB drive
- Do not move/delete these folders or the “do not delete.txt” files inside them (workbooks will not be able to utilize your backup USB drive)

B.1.4 Optional: Importing a customized ribbon to simplify workbook transcription

\textbf{Note:} These customization steps add a \texttt{tifflCAKE} menu to the \textit{Home}” tab of the ribbon featuring \texttt{DNA paste} and \texttt{ALLELEigator paste} buttons—DNA paste is used in all DNA workbooks except ALLELEigator while ALLELEigator paste should only be used within the ALLELEigator workbook; each user will need to do this one time per computer (as desired)

- Open Excel; Click the \textit{File} tab on the top navigation ribbon and then click \textit{Options} from the navigation menu on the left (a new window will open)
- Choose \textit{Customize Ribbon} from the new window’s navigation menu on the left side, select \textit{Main Tabs} under the top right ribbon customization drop-down list, select \textit{Home} from the ribbon navigation tree located immediately below, then click the \textit{Import/Export} button further below, and select \textit{Import Customization File} (a window will open to select customization file)
B.2 Using DNA workbooks to Print Labels for Tubes, Slides, or P30/Hematrace/RSID

Note: for convenience, multiple workbooks include automatic label makers utilized according to the same steps outlined below

- Select the LABELS tab and designate first on the label template in the position on the label sheet from which labels should start—if using a new label sheet no changes are needed since the template is initialized to generate labels from the first label position
- Click the Make Labels button—verify/modifity the sample names on your labels as needed then load the printer with label sheets and print

Note: if labels fit on one page (second page isn’t needed) DO NOT REDESIGNATE the print area or your samples will not line up properly with the labels; instead adjust the settings in the print dialog window to print Pages 1 to 1

B.3 CONFIRMadactyl

Note: Appendix B.3 procedures are simply used to streamline worksheet documentation; refer to the relevant sections of Biology/DNA Procedures Manual for Forensic Biology Screening procedures
B.3.1 Creating a lab processing worksheet to document forensic screening

- Enter sample ID’s and descriptions (or Event #s, item numbers, and descriptions) in the designated area on the INPUT tab—the information will be automatically transferred to the lab worksheet on the WORKSHEET tab
- Pick the screening types from the drop-down menus on the WORKSHEET tab—the worksheet will automatically update itself to match the screening types so that you are prompted to record the respective resources and complete the respective result fields
- Enter the date on the WORKSHEET tab in the format m/d/yy to automatically restrict expired/invalid resources from the drop-down lists; use the drop-down lists to record the resources used for screening on the WORKSHEET tab
- Record screening results and extraction info for each sample in the designated area on the WORKSHEET tab—when the worksheet is complete it should be printed for the case file
- Refer to B.2 for label printing instructions

B.3.3 Using CONFIRMadactyl’s utility tab to prepare sample lists for extraction

- Sample and extraction info is automatically transferred from the WORKSHEET tab to the UTILITY tab for you—you can select the list in the designated area as instructed on the UTILITY tab then choose Sort from the ribbon’s Data tab to sort using headers by the EXTRACTasaurus tool column as shown below

- use copy and DNA paste (or copy and paste special values) to transfer samples from the UTILITY tab of CONFIRMadactyl to the INPUT tab of EXTRACTasaurus

B.4 EXTRACTasaurus and DB EXTRACTasaurus

Note: Appendix B.4 procedures are simply used to streamline worksheet documentation; refer to the relevant sections of the Biology/DNA Procedures Manual for Extraction procedures

B.4.1 Creating a lab processing worksheet to document DNA extraction

- Pick the extraction type from the drop-down menu on the INPUT tab—the worksheet will automatically update itself to match the extraction type’s required documentation fields
- Enter sample ID and description (or Event #, item number, and description as applicable) in the designated area on the INPUT tab—the information will be automatically transferred to the lab worksheet on the WORKSHEET tab and create the reagent blank for you

Note: only enter the sample names one time for differential sets—the worksheet will automatically duplicate the set and append “-EF” and “-SF” accordingly

Note: re-isolates should be appended with RI# (use 1, 2, etc. in place of #)
• Enter the date on the WORKSHEET tab in the format m/d/yy to automatically restrict expired/invalid resources from the drop-down lists; use the drop-downs to record resources used as well as incubation times, tube numbers, and elution volume in the designated fields
• When the worksheet is complete it should be printed for the case file

B.4.2 Using EXTRACTasurus’s utility tab to prepare sample lists
• Tube numbers and sample names are automatically transferred to the UTILITY tab for you—choose tube number/dilution factor naming preferences from the drop-down
• Copy and DNA paste (or copy and paste special values) from the UTILITY tab into other DNA workbooks

B.4.3 Using EXTRACTasaurus to print storage logs and/or tube labels
Note: This function is not applicable to DB EXTRACTasaurus
• Use each sample’s drop-down list on the UTILITY tab to specify whether you need to store/label extract vs. substrate and extract
• Select the FREEZER TEMPLATE tab, designate first on the template position matching the starting position inside the box then click the Add samples to box(es) button and verify/modify the samples as needed—print the log(s) and place inside the lab’s binder (remember to record the storage box’s label on the worksheet)
• Refer to B.2 for label printing instructions; labels will print in duplicate for samples marked “SUB+EXT” on the utility tab

B.5 QUANTadillo for the Quantitation of Casework Samples
Note: Appendix B.5 procedures are used to streamline worksheet documentation and interface with 7500 software for the quantitation of casework samples. Refer to the relevant sections of the Biology/DNA Procedures Manual for Quantifiler Trio quantification and 7500 operation procedures

B.5.1 Creating Quantitation Lab Processing Worksheets:
• Click on the “tiffCAKE” tab at the top of the workbook.
• Enter the Quant name in the box in the ribbon
• Select the Plate Map Orientation based on whether the quantitation tray will be set up in columns or rows
  IMPORTANT! The orientation must be set to columns in order to utilize the Hamilton robot
• Enter the letter associated with the unlocked USB drive which will be used to manually transfer the files to the 7500 and/or Hamilton robot
• On the “QT list” tab, enter the sample names which will be quanted. In the event the sample names are being copied and pasted into the tab as a list, the Paste Special Values function must be utilized. The “DNA paste” button may be used from the “Home” tab for this purpose
IMPORTANT! Do not rename or move the “Standard” samples from the list. The Standards will automatically sort into the appropriate wells based on the selected plate map orientation. The workbook must contain two of each Standard 1 to 5 in order to export appropriately.

- Select the “LAB worksheet” tab to view the 96-well plate map
- Enter the date the quantitation plate will be set-up and run in the Date field. Press Enter to complete the entry and ensure the workbook remains in active form
- Select the individual who is setting up the plate from the Analyst dropdown
- Select the resources (reaction mix, primer mix, standard curve, and pipets) used to set-up the quantitation plate

IMPORTANT! In the event the Hamilton robot will be utilized to set-up the quantitation, the first pipet selected in the Pipets dropdown must correspond with a Hamilton robot. The master mix calculator will update its calculations based on whether the pipets correspond with a robotic set-up or manual. All robotic set-ups assume one tube of master mix will be created for the robot deck

- Select the 7500 instrument which will be used to analyze the data
- If dilutions are being made, enter the description of how each dilution was prepared in the “Dilution Prep Details” (e.g., 1:10 dilution prepared using 1 µl of extract and 9 µl of H2O). Select the lot of H2O utilized to make the dilutions. Press Enter to complete the entry and ensure the workbook remains in active form
- Once all information has been entered, save the workbook at the desired location as the plate name

B.5.2 Exporting to the Hamilton and/or the 7500

- If the quantitation plate will be set-up robotically, select the “Export Hamilton” button from the “tiffCAKE” ribbon
- The workbook will prompt the user to save the workbook again. Navigate to the desired location and enter the workbook name
- A Hamilton Export file will be automatically generated in the “QUANTS” folder of the unlocked USB drive designated on the tiffCAKE ribbon. This file will be named “Hamilton QT <plate name entered on ribbon>”
- Select the “Export 7500” from the “tiffCAKE” ribbon
- The workbook will prompt the user to save the workbook again. Navigate to the desired location and enter the workbook name
- A .txt file will be automatically generated in the “QUANTS” folder of the unlocked USB drive designated on the tiffCAKE ribbon. This .txt file will have the same name as the plate name entered on the ribbon
- These files are ready for use for the 7500 and/or Hamilton Robot

B.5.3 Importing a Sample Setup File onto the 7500 for Quantitation

- Launch the 7500’s HID software; click File → New
- Choose the “Quantifiler Trio” icon from the home page
- Enter the experiment name (should match plate ID in QUANTadillo workbook)
• Click File → Import; Choose the exported .txt file from B.5.2
  Note: the file can be found on the desktop on the instrument laptop, within the “Import” folder
• Verify that the plate setup is correct (correct samples, wells, tasks, detectors)
• Save the plate setup as an EDS document in the appropriate “DNA lab runs” folder

B.5.4 Exporting Results from the HID Software
• After analyzing data using the HID software, highlight the entire plate (under the View Plate Layout tab with the Analysis drop down) click Export (top toolbar). The only box that should be clicked under Export Properties is Results.
• Click on the Customize Export tab and ensure the columns to be exported are in the correct orientation as defined in the relevant sections of the Biology/DNA Procedures Manual for Quantifiler Trio quantification and 7500 operation procedures.
• Once everything is correct, define the location you wish to export to.

B.5.5 Importing Data from the 7500 into QUANTadillo
• Open the quant workbook created in B.5.2 and click on the “tiffCAKE” tab at the top of the workbook
• Select the Results Sort based on whether the quantitation data will be sorted by questioned sample name, columns, or rows
  Note: In the event a different orientation is desired after import, the data must be re-imported into the workbook. The same workbook can be used for this re-import function.
• Select the “Import 7500” button from the “tiffCAKE” ribbon
• The workbook will prompt the user to save the workbook again. Navigate to the desired location and enter the workbook name
• Select the appropriate _data.xls file containing the data associated with the plate
  IMPORTANT! In the event the selected _data.xls file does not match the sample names on the “QT list” tab, the user will be notified upon importing the data. Select “Cancel” on the notification in order to view the samples which are mismatched or “OK” to accept the mismatching data and continue to import the data
B.5.6 Reviewing the Standard Curves

- Click on the “Standards” tab to review the Ct values for the IPC, SM target, LG target, and Y target for each of the standards and the slopes, Y-intercepts, and $R^2$ values associated with each standard curve
  - Slopes which are outside of the acceptable ranges will be flagged for review
  - $R^2$ values which are < 0.98 will be flagged as failing criteria. A standard must be omitted in the HID software and the data re-imported in an attempt to produce a passing standard curve. Alternatively, the entire set of samples must be re-quanted with a passing standard curve in order to view the quantitation results
- Once all standard curve data has been reviewed, print the “Standards” tab for the case file.
  
  **Note:** It is not necessary to print the standard curves from the HID software

B.5.7 Reviewing the Results

- Click on the “Results” tab to view the quantitation data for the sample results
- On the “tiffCAKE” ribbon, select the STR kit corresponding with the data type
- Select whether the majority of the samples on the plate will be normalized manually or by the robot
- Select the desired concentration method as either the CentriVap or microcon. Enter the default starting volume associated with the majority of the samples into the “CONC Extract Vol” box and the default final desired volume for concentration of the majority of the samples into the “CONC Final Vol” box
  
  **Note:** Alternatively, these volumes can be modified on a sample-by-sample basis
- Review the quantitation data for each sample:
  - The Auto:Y ratio will display “---” if the SM or Y target does not contain a quantification value
  - If the degradation index is > the selected kit’s associated DI value (e.g., 1.5 for INV24), the Auto:Y will automatically be re-calculated using the LG and Y targets
- The workbook automatically predicts each sample’s next action using the following logic:
  - Samples with quant values >50 ng/µL are automatically assigned **ReQuant**
  - The average IPC Ct is calculated using Standards 2-5 because the Standards do not contain inhibitors; Standard 1 is omitted from the average because its higher concentration of human DNA competes with the IPC for amplification causing IPC Ct delays.
    - Samples (>5 ng/µL) with IPC Ct values > 1.0 cycle above the average IPC Ct are flagged for possible inhibition and automatically assigned **QIAgen**
    - Samples (small target undetermined or <5 ng/µL) with IPC Ct values > 0.5 cycle above the average IPC Ct are flagged for possible inhibition and automatically assigned **QIAgen**
  - Samples (IPC Ct values ≤ average IPC Ct) with quant values < the selected kit’s associated default normalization for ideal large target (e.g., 0.07 ng/µl for INV24 on 3500) are automatically assigned **Concentrate**
  - Samples with a degradation index > the selected kit’s associated DI value will automatically be flagged by the workbook in order to alert the analyst.
  - All other samples are automatically assigned a default dilution depending on the STR kit selected in the tiffCAKE ribbon
B.5.8 Assigning Actions and Automatically Calculating Amplification Dilutions and Concentrated Values

**IMPORTANT!** The “Results” tab must be reviewed carefully to determine the next steps in processing—the workbook predicts next actions to streamline analysis, but the analyst must carefully review and update/override the automatically predicted actions as needed/desired using each sample’s drop-down list and/or comment field

- The following Actions are available in the dropdown:

```
---
QIAgen
ReQuant
Concentrate
No Dil’n/MAX
DIL 0.05 ng/ul
DIL 0.07 ng/ul
DIL 0.085 ng/ul
DIL 0.1 ng/ul
DIL 0.125 ng/ul
DIL 0.2 ng/ul
DIL 0.3 ng/ul
DIL 0.4 ng/ul
DIL 0.5 ng/ul
DIL 0.6 ng/ul
```

- All samples which **demonstrate a flag in the IPC C\text{\textsubscript{T}} (elevated IPC C\text{\textsubscript{T}} or Undetected IPC C\text{\textsubscript{T}} flag)** must have an individual note included in the Comments column which acknowledges the elevated IPC C\text{\textsubscript{T}} value and the sample-specific action that will be taken in an attempt to overcome the inhibition noted

  **Note:** It is not permissible to include a single comment at the worksheet level such as “dilution expected to overcome inhibition” to address all samples at one time. This comment must be included with each individual sample to ensure they are being singularly evaluated and addressed

  - All samples which **exhibit an Undetected IPC C\text{\textsubscript{T}} flag** must be re-quanted or subjected to Qiagen clean-up or microcon buffer exchange and then re-quanted in an attempt to cleanse the sample of any inhibitor that may be present. It is not permissible to continue to amplification without these steps. In the event a sample needs to undergo Qiagen clean-up or microcon buffer exchange, the associated reagent blank(s) must undergo the same cleansing procedure as well

  - All samples which **exhibit an elevated IPC C\text{\textsubscript{T}} flag that require concentration** must be subjected to Qiagen clean-up or microcon buffer exchange and then re-quanted in an attempt to cleanse the sample of any inhibitor that may be present. It is not permissible to continue to amplification without these steps. In the event a sample needs to undergo Qiagen clean-up or microcon buffer exchange, the associated reagent blank(s) must undergo the same cleansing procedure as well

  - Samples which **exhibit an elevated IPC C\text{\textsubscript{T}} flag that will be diluted/normalized prior to amplification or where neat extract will be used in a well dilution** may
continue to amplification with the acknowledgment that based on the dilution factor being employed, it is expected to overcome the inhibition present.  

**IMPORTANT!** The IPC C_T value must be evaluated to determine the extent of the inhibition present.  In the event it exhibits 3-5 cycles over the average, these samples should be cleaned-up and re-quanted prior to amplification due to the potential for gross inhibition.

- Use the Action dropdowns as needed to document the next action for each sample or reagent blank:
  - The DNA and H2O columns will update automatically to reflect the volumes required for normalization or concentration based on the action selected. When Concentrate is selected, the DNA and H2O volumes will default to the extract and final volumes previously designated in the “tiffCAKE” ribbon.
  - The volumes associated with the dilutions and/or Amp Vol may be scaled up or down by manually adjusting the DNA volume—the H2O volume will automatically adjust according to the chosen “Action” and the volume reflected in the DNA column. Press Enter to complete the entry and ensure the workbook remains in active form.

  **Note:** The formulas in the Action drop-down and DNA column are replaced by text whenever modified—this means that manually modified DNA volumes will no longer automatically adjust for newly chosen actions; however, the diluent volume will still alert the analyst automatically if the new action’s target cannot be achieved.
  - The “Amp Vol” column will default to the maximum volume associated with the STR amplification kit designated in the “tiffCAKE” ribbon. This value may be manually modified if well dilutions will be made during amplification. Press Enter to complete the entry and ensure the workbook remains in active form.
  - The “Final Target ng / Alt. Workflow” column will update with the total amount of template which will be amplified based on the sample-specific quantitation values, the normalization or concentration factors based on the Action selected, and the amplification volume.
  - The analyst is alerted whenever an impossible dilution target is mistakenly chosen (e.g., a sample with 0.4 ng/µL quant value cannot be diluted to make 0.6 ng/µL dilution).

**B.5.9 Dropping Low Quanting Non-Sexual Assault Samples**
Non-sexual assault samples are eligible to be dropped when the final target input after concentration is approximately 0.035 ng or less for both the small and large targets. These samples may be manually designated as “DROP” using the dropdown in the “Final Target ng / Alt. Workflow” column. These samples must have the Action selected as Concentrate.

- When all associated evidence will be dropped, the reference standards and reagent blanks associated with the case will also be designated as “DROP”.
- Reagent blanks which exhibit any quantitation value other than Undet. in the small or Y-targets must remain marked as Concentrate when all associated samples qualify to be discontinued from testing. The decision whether to also discontinue the processing of the reagent blank will be made in consultation with the DNA Technical Leader and documented in the case file.

Samples designated as “DROP” will not be exported to downstream worklists.
B.5.10 Evaluation of Sexual Assault Samples using the Y-Screen Criteria

The evaluation of the Y-screen criteria will be used for sexual assault evidence containing at least one female and at least one male assailant. The Y target of all evidence samples will be color-coded based on the following two-prong assessment:

<table>
<thead>
<tr>
<th>Quantitation Results</th>
<th>Suggested Amplification Action</th>
</tr>
</thead>
</table>
| Male DNA ≥ 75 pg     | • Expected to yield interpretable autosomal STR male profile  
                      | • Autosomal amplification recommended based on case-specific circumstances |
| Male DNA Undet.      | • No further examination recommended |
| Male DNA < 75 pg     | • Evaluate for amplification on a case-specific basis  
                      | • Samples may be alternatively considered for Y-STR analysis |

IMPORTANT! The workbook automatically recognizes reagent blanks and does not apply the color-code criteria. In addition, samples which include tube numbers with “(K…” or “(k…” are also recognized by the workbook as reference standards and therefore do not contain the color-code criteria. All other samples will be color-coded.

Sexual assault samples which will be dropped due to the application of the above criteria will be designated as “DROP” using the dropdown in the “Final Target ng / Alt. Workflow” column. Change the action to increase the targets (if possible) to determine if non-colored samples turn green, while still maintaining autosomal targets within the dynamic range (e.g., up to 2 ng large target input for INV24 on 3500xl). Refer to the flow chart for sample-by-sample decision-making for Y-screened samples

- When all associated evidence will be dropped, the reference standards and reagent blanks associated with the case will also be designated as “DROP”
- Reagent blanks which exhibit any quantitation value other than Undet. in the small or Y-targets must remain marked as Concentrate when all associated samples qualify to be discontinued from testing. The decision whether to also discontinue the processing of the reagent blank will be made in consultation with the DNA Technical Leader and documented in the case file

B.5.11 Performing Multiple Amplifications or Re-Amplifications

The dropdown in the “Final Target ng / Alt. Workflow” may be utilized when performing multiple amplifications or re-amplifications. This will automatically append the suffix AMP1, AMP2, etc. or RA1, RA2, etc. to the sample name when creating the downstream sample worklists.

- Copy the rows by selecting the three row numbers on the left side, corresponding to the sample
- Right click and select copy
- Click the first row of the next sample on the side then right click and select “Insert copies cells”
- Designate the samples as AMP1, AMP2, etc. in the “Final Target ng / Alt. Workflow” column, as appropriate

B.5.12 Creation of Worklists

Once all sample actions have been selected and dropped samples designated, click the “Make Lists” button on the “tiffCAKE” ribbon. Tabs containing the “MANUAL DILN”, “QIA & CONC”, and/or “AMP LIST” will be automatically created based upon the sample order in the Results tab, and the actions and normalization methods selected.

These lists may be utilized to copy and paste special values into the appropriate downstream workbooks

- These lists may be manipulated to relocate samples prior to being pasted into downstream workbooks to include re-arranging results sorted by sample names back into tube number order (if desired)

**IMPORTANT!** When rearranging samples on this list, the word "end" must always be present in Column A after the last sample on the list. This is required for the functionality of this workbook to ensure the ability to re-import data and re-generate lists, when necessary

**IMPORTANT!** Do not include the “end” when copying and pasting special values into downstream workbooks. This is required only for QUANTadillo functionality

- The term “GET” will be present on the AMP LIST when the Action currently selected or the sample does not contain the necessary information for normalization and amplification (i.e., QIagen or ReQuant). The “GET” serves as a placeholder and error handler to alert the user that additional information is required to set up the normalization and amplification. This information must be updated in the Amplification workbook prior to being able to export to the Hamilton

B.5.13 Termination Verification

Quantitation workbooks which include at least one sample designated as “DROP” will require a documented verification that the samples are being designated for termination appropriately in accordance with Sections B.5.9 Dropping Low Quanting Non-Sexual Assault Samples and B.5.14 Flow chart for Sample-by-Sample Decision-Making for Y-Screened Samples.

With the exception of priority zero rush cases, this verification must take place prior to the amplification of any associated samples, when applicable. In the event all samples are being dropped and the case will be reported as negative, this documented verification must be completed prior to the case file being entered into technical review. The individual completing the verification must record their handwritten initials and P#, along with the date the verification was performed.
B.5.14 Flow chart for Sample-by-Sample Decision-Making for Y-Screened Samples

This flow chart is applied after all actions have been selected and final color-coding of samples has occurred.

IMPORTANT! Y-STR profiles require direct comparison to a buccal reference standard and are not eligible for CODIS search. Therefore, samples may be eligible for Y-STR analysis only when a suspect reference standard is available for direct comparison.

^It will be necessary to re-assess the workflow employed when cases initially believed to be single contributor are determined to potentially contain multiple contributors based on the initial DNA analysis results. If applicable, thought processes regarding why additional amplifications are not performed will be documented when presented with this information.

*In the event a sample is differentially extracted and one fraction (EF or SF) is selected for autosomal STR amplification, the opposing fraction will also be amplified, regardless of color-coding.
B.6 makeiTWORK

Appendix B.6 procedures are simply used to streamline worksheet documentation; refer to the relevant sections of the Biology/DNA Procedures Manual for Qiagen Cleanup and Microcon/Centrivap concentration.

B.6.1 Creating a lab processing worksheet for Qiagen cleanup and/or DNA concentration

- Enter the sample names in the designated area on the “input” tab using the following rules:
  a. Qiagen cleanup samples must include QIA in their names
  b. Microcon concentration samples must include MC in their names
  c. Centrivap concentrations samples must include VAP in their names

  **Note:** The QIA & MC & VAP tab automatically updates itself to reflect each sample’s relevant documentation fields and required resource documentation.

- Enter the date in the format m/d/yy to automatically restrict expired/invalid resources and complete the worksheet using the drop-down lists.
- Refer to B.2 for label printing instructions.

B.7 AMPalatypus & cattyRUNpas

**Note:** Appendix B.7 procedures are used to streamline worksheet documentation and interface with 3500XL Data Collection software; refer to the relevant sections of the Biology/DNA Procedures Manual for amplification and electrophoresis procedures.

- Click on the “tiffCAKE” tab at the top of the workbook.
- Select the STRkit which will be utilized for amplification.
- Enter the letter associated with the unlocked USB drive which will be used to manually transfer the files to the Genetic Analyzer and/or Hamilton robot.

B.7.1 Creating a Lab Worksheet

- Click on the “tiffCAKE” tab at the top of the workbook.
- Select the STRkit which will be utilized for amplification and/or electrophoresis.
- Enter the letter associated with the unlocked USB drive which will be used to manually transfer the files to the Genetic Analyzer and/or Hamilton robot.

  **If performing an amplification set-up:**
  - Ensure the “Amp Setup” box is selected.
  - Enter the Amp ID# associated with the amplification. A link to the Amp# Log is provided in the ribbon.
  - Check the “Use the Matrix or P10?” checkbox if manually loading amp wells using Matrix or P10 pipets.
  
  **Note:** This may be overridden at the sample level, if needed.

  **If performing electrophoresis:**
  - Ensure the “CE Setup” box is selected in the “tiffCAKE” tab.
  - Enter the Run name associated with the CE plate. If the run is a reinjection of a previously setup CE plate, include RJ# in the run name.
  - Select whether the data will be collected by injection or by plate in the dropdown.

Uncontrolled Copy if not located in Qualtrax
On the “Amp & Inj list” tab, enter the sample names which will be amplified and/or loaded and the volumes necessary for the dilution and amplification if using the Hamilton robot for normalization. In the event the sample names and volumes are being copied and pasted into the tab as a list from the QUANTadillo workbook the Paste Special Values function must be utilized. The “DNA paste” button may be used from the “Home” tab for this purpose.

**IMPORTANT!** Do not include the word “end” when copying and pasting from QUANTadillo.

- Add an APC, ANC, and/or LADDER as applicable into the sample list. 
  **Note:** If collecting data by injection, one ladder must be included per injection; if collecting data by plate, at least two ladders must be included in the list.

- When reinjecting a sample, append the sample name with RJ#.
- When reloading a sample, use the “Reload?” column to select the reload number in the list position where the sample will be loaded. Copy the amp well and sample name fields from the amplicon’s associated amp workbook, then paste special values into the new list over the Amp Well and Sample Name fields associated with the reload sample.
  **IMPORTANT!** If/when rearranging the sample list, ensure that all values (normalization/amp volumes, RL well information, etc.) remain associated with their respective samples. Copy and paste special values or DNA paste must be used to move information to ensure that linked formulas do not become corrupted. Also keep in mind that some fields that are automatically populated by formulas may have been replaced by text and will no longer update accordingly. Users may wish to use another text-only workbook tab to rearrange samples and their associated values prior to copying into the Amp & Inj list (QUANTadillo’s AMP tab may be used for this purpose).

- Select the “LAB worksheet” tab to view the 96-well plate map. The lab worksheet is automatically formatted depending on the ribbon’s AMP Setup and CE Setup or whether the run name indicates reinjection.
- Enter the amplification/load/reinjection date(s) as applicable to populate resource dropdown lists accordingly.
- Select the resources from dropdown lists. For CE Setup/Reinjection, the plate/run names(s) will be appending according to selected instruments and adopted injection conditions will be automatically updated.
  **IMPORTANT!** In the event the Hamilton robot will be utilized to set-up the normalization and amplification, the first pipet selected in the Pipets dropdown must correspond with a Hamilton robot. The master mix calculator will update its calculations based on whether the pipets correspond with a robotic set-up or manual. All robotic set-ups assume one tube of master mix will be created for the robot deck.

- If applicable, enter the Run #s which will be performed. A link to the Run# Log is provided in the ribbon. Press Enter to complete the entry and ensure the workbook remains in active form.
- Once all information has been entered, save the workbook at the desired location as the plate name.
B.7.2 Exporting to the Hamilton

- If the normalization and amplification plate will be set-up robotically, select the “Export Hamilton” button from the “tiffCAKE” ribbon
- The workbook will prompt the user to save the workbook. Navigate to the desired location and enter the workbook name
- A Hamilton Export file will be automatically generated in the “AMPS” folder of the unlocked USB drive designated on the tiffCAKE ribbon. This file will be named “Hamilton AMP <Amp ID>”
- Transfer this file to the robot’s input folder; refer to step 4 of Section 6.6.2 for Hamilton import procedures.

B.7.3 Exporting to the Genetic Analyzer

- Select the “Export CE!” from the “tiffCAKE” ribbon
- The workbook will prompt the user to save the workbook. Navigate to the desired location and enter the workbook name
- A .txt file will be automatically generated in the “RUNS” folder of the unlocked USB drive designated on the tiffCAKE ribbon. This .txt file will have the same name as the plate name
- These files are ready for use for the Genetic Analyzer. Refer to step 1 of Section 7.4.3 for CE import procedures.
Appendix C  Title: Instrument and Equipment Maintenance

C.1 7500 Maintenance using HID v1.2 Software

The following table displays the recommended 7500 instrument and laptop maintenance schedule when using the HID v1.2 software. Monthly, quarterly, semi-annual, and annual maintenance tasks should be performed using the listed steps/reference information at the frequencies indicated by the table. Documentation including Field Service/Maintenance Reports, maintenance checklists, and Corrective Action Reports (if required) will be maintained in the "ABI 7500 HID Maintenance Logbook".

<table>
<thead>
<tr>
<th>Frequency</th>
<th>HID Maintenance Task</th>
<th>See Step:</th>
<th>Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monthly</td>
<td>Hardware/function test</td>
<td>C.1.1</td>
<td>56-57</td>
</tr>
<tr>
<td></td>
<td>Check lamp status (replace lamp if needed)</td>
<td>C.1.2</td>
<td>58 / 63-65</td>
</tr>
</tbody>
</table>
|               | **Note:** lamp replacement must be followed by ROI  
calibration/optical calibration/dye calibration---steps/references are noted in semi-annual task list of this maintenance table |
|               | Decontaminate block                             | C.1.3     | 59-62      |
|               | Background calibration                          | C.1.5     | 19-24 / 30 |
|               | Reboot laptop and wipe surface of 7500 with lint-free cloth | --- | --- |
| Quarterly     | Disk cleanup/defragment disks/archive data     | C.1.10    | 6          |
| Semi-Annually | 1) Hardware/function test                       | C.1.1     | 30-34      |
| (In noted order) | Check lamp status (replace if needed)       | C.1.2     | 121-125    |
|               | **Note:** lamp replacement must be followed by ROI  
calibration/optical calibration/dye calibration---steps/references are noted in semi-annual task list of this maintenance table |
|               | 3) Decontaminate block                          | C.1.3     | 59-62      |
|               | 4) ROI calibration**                            | C.1.4     | 7-17       |
|               | 5) Background calibration                       | C.1.5     | 19-24 / 30 |
|               | 6) Optical calibration**                        | C.1.6     | 19 / 25-29 |
|               | 7) Dye calibration: ABY, JUN, MP, FAM, VIC (Quantifiler Trio)** | C.1.7     | 31-44      |
|               | 8) Reboot laptop and wipe surface of 7500 with lint-free cloth | --- | --- |
| Annually      | Performed by AB technician; includes all semi-annual procedures | --- | --- |
| As needed     | Decontaminate block                             | C.1.3     | 59-62      |
|               | Check lamp status (replace lamp if needed)    | C.1.2     | 58 / 63-65 |
|               | **Note:** lamp replacement must be followed by ROI  
calibration/optical calibration/dye calibration---steps/references are noted in semi-annual task list of this maintenance table |
### Frequency

<table>
<thead>
<tr>
<th>Frequency</th>
<th>HID Maintenance Task</th>
<th>See Step</th>
<th>Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Replace 7500 fuses</td>
<td>---</td>
<td>66-67</td>
</tr>
<tr>
<td></td>
<td>RNase P verification</td>
<td>---</td>
<td>66-67</td>
</tr>
<tr>
<td>(Note: RNase P verification is typically only performed at the request of AB Technical Support for troubleshooting purposes)</td>
<td></td>
<td>C.1.9</td>
<td>45-54</td>
</tr>
<tr>
<td></td>
<td>Update Windows operating system</td>
<td>call AB</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Update 7500 software</td>
<td>call AB</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>After fatal error alert - reboot laptop/7500</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

^ Reference page numbers refer to the “Applied Biosystems 75010/7500 Fast Real-Time PCR Systems Maintenance Guide” (PN# 4387777 Rev. D)

** The ROI calibration plate is used to perform both ROI and Optical calibration procedures. This plate, as well as the Quantifiler dye calibration plates, can be used to calibrate up to three freeze/thaw cycles and must be stored frozen, although they can remain at room temperature up to 8 hours between calibrations if protected from light exposure.

### C.1.1 HID Function Test

In the 7500 Software, Select Instrument ➤ Function Test from the top navigation bar

Click the All Tests button; the software will indicate whether each function tested has passed or failed; refer as needed to the “Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Maintenance Guide” (PN# 4387777 Revision D) to address any failures

Click CLOSE when finished to close the function test dialog box.

### C.1.2 HID Lamp Status and Replacement

Note: Gloves must be worn at all times while handling the halogen bulb; oil/lotion transferred from the hands to the bulb may cause the bulb to burst during operation HID Check Lamp Status

In the 7500 Software, select Instrument ➤ Lamp Status/Replacement from the top navigation bar to determine the status of the halogen bulb—a dialog box will open displaying the following:

- **Condition** (indicating one of the following):
  - Good: the lamp is functioning well and does not need to be replaced
  - Failed: the lamp bulb must be replaced; see step C.1.2 to replace the lamp
  - Change Soon: the lamp usage exceeds the 2000-hour replacement recommendation
  - Usage: total hours of lamp illumination

- **Date Last Replaced**: date of last lamp replacement

Click Close when finished to close the lamp status dialog box; you can close the plate document without saving OR you can keep the plate document open to perform additional maintenance tasks

### HID Lamp Removal

1) Power off/cool the 7500 instrument then open the main access door by inserting a swab stick into the
pinhole on the edge of the access door and pushing until the mechanism unlashes
2) Remove the bulb by opening the access door, moving the lamp release lever downward then sliding the bulb up/out of the slotted mount

HID Lamp Installation
1) Return the lamp release lever to the upward position–place the new bulb in the slotted mount and carefully slide the bulb down into place
2) Close the main access door and power on the 7500; launch 7500 software.

HID Verifying Lamp Installation
1) Select Instrument ► Instrument Maintenance Manager from the top navigation bar ► ROI (left hand pane) to launch the ROI Inspector dialog box; in the dialog box select Lamp Control ► Idle then look through the vents at the top of the access door and verify whether the lamp illuminated (light is visible) then click OK

If the lamp did NOT illuminate:
1) Repeat lamp replacement procedures above using a second new halogen bulb to determine whether the first replacement bulb is defective
Note: If the second bulb also doesn’t illuminate check instrument fuses for failures and replace as needed before repeating the lamp replacement procedures again (see pages 66-67 of the “Applied Biosystems7500/7500 Fast Real-Time PCR System Maintenance Guide” PN# 4387777 Revision D); if all attempts to achieve illumination are unsuccessful, call Applied Biosystems Technical Support
2) Once the lamp successfully illuminates, select Instrument ► Lamp Status/Replacement from the top navigation bar; click Reset Lamp Timer in the dialog box, then OK; close the plate document without saving and perform the following calibrations (in order)
  ▪ Regions of Interest (ROI) Calibration
  ▪ Background Calibration
  ▪ Optical Calibration
  ▪ Dye Calibration
OPTIONAL: RNase P Verification (omitted unless requested by AB Technical Support)

C.1.3 HID Decontaminate the Sample Block
Identify the contaminated wells of the sample block
1) In the 7500 software, click Instrument ► Instrument Maintenance Manager from the top navigation bar ► ROI (left hand pane) ► Start Manual Calibration.
2) Select “Move block up” button
3) Select Filter A then click Snapshot in the dialog box.
4) Repeat for each filter
Note: Keep track of the wells exhibiting significantly higher signal compared to majority of wells’ signal (contamination) and then perform decontamination steps below
Decontaminate the instrument block

1) Open the main access door by inserting a swab stick into the pinhole on the edge of the access door and pushing until the mechanism unlatches
2) Lift the silver latch knob on the heated cover then push the heated cover door toward the back of the 7500
3) Clean the contaminated wells by swabbing with molecular grade water or pipetting a small volume of molecular grade water up and down (remove any excess)

4) Pull the heated cover door to the front of the instrument then lift the latch and secure the heated cover door to the cross bar; close the main access door to the instrument

Verify that contamination was eliminated from affected wells

In the ROI Inspector dialog box, change the lamp control setting drop-down menu from Off to Idle; click Snapshot.

If new wells exhibit contamination:
1) Repeat decontamination using deionized water
2) Verify that contamination was eliminated

If the contamination remains in the same wells after deionized water decontamination:
1) Repeat decontamination using 95% ethanol
2) Verify that contamination was eliminated

If the contamination remains in the same wells after 95% ethanol decontamination steps:
1) Repeat decontamination using 10% bleach solution (followed by deionized water rinses)
2) Verify that contamination was eliminated
After successful decontamination, ensure that the heated cover door and main access door are both completely closed and latched (otherwise the 7500 will display an error message)

Click Done to close the ROI Inspector dialog box; you can close the plate document without saving or you can keep the plate document open to perform additional maintenance tasks

C.1.4 HID ROI Calibration

Note: Do not remove the ROI calibration plate from its packaging until you are ready to load the instrument – light exposure affects the plate’s performance; this plate will be used for both ROI and Optical calibrations for up to a maximum of 3 instruments

1) Obtain the ROI calibration plate from the freezer and warm to room temperature; vortex then centrifuge 2 minutes at less than 1500rpm

2) In the 7500 software, select Instrument ▶ Instrument Maintenance Manager from the top navigation bar ▶ ROI (left hand pane) to launch the ROI screen.
   a. Right click on the black area of the dialog box and choose “Display Properties” to launch the Viewer Control Properties dialog box
   b. Check Auto Contrast, Zoom, Show Saturation and enter “4000” in the “Sat. Threshold” field; click OK to close the Viewer Control Properties dialog box

3) In the ROI Inspector screen, click Start Calibration and follow the wizard in the software system for automatic instrument ROI Calibration. Click Start Run when prompted.
   • When the calibration is complete check the calibration status (Passed/Failed). If the calibration is not passing refer to page 17 of the “Applied Biosystems7500/7500 Fast Real-Time PCR System Maintenance Guide” PN# 4387777 Revision D); if all attempts to achieve illumination are unsuccessful, call Applied Biosystems Technical Support


C.1.5 HID Background Calibration

Note: Background plates are included in the calibration kit. Alternatively, 50 µL of molecular grade water may be pipetted into each well of a 96-well plate and sealed with optical adhesive.

Frozen kit plates – Can be used multiple times prior to expiration of the Spectral kit; warm to room temperature before using. Check to ensure there is not any evaporation in the wells.

1) Centrifuge the background plate 2 minutes at < 1500g; load the plate onto the instrument

2) In the 7500 software, select Instrument ▶ Instrument Maintenance Manager from the top navigation bar ▶ Background (left hand pane) to launch the background screen.

3) Click Start Calibration and follow the wizard in the software system for automatic instrument Background Calibration.
   • When the calibration is complete check the calibration status (Passed/Failed). If the calibration is not passing refer to page 30 of the “Applied Biosystems7500/7500 Fast Real-Time PCR System Maintenance Guide” PN# 4387777 Revision D); if all attempts to achieve illumination are unsuccessful, call Applied Biosystems Technical Support

Note: DO NOT complete the background plate wizard. Once the run is complete and the window appears stating the background plate passed, close out the window using the “x” at the top right corner.
C.1.6 HID Optical Calibration

**Note:** Since the ROI plate is used for both ROI and Optical calibration, the C.1.4 step 1 ROI plate does not have to be re-frozen between each calibration if less than 8 hours has passed since thawing

1) Load the ROI calibration plate into the instrument; see ROI calibration for instructions related to plate preparation
2) In the 7500 software, select **Instrument ► Instrument Maintenance Manager** from the top navigation bar ► **Optical (left hand pane)** to launch the optical screen.
3) Click Start Calibration and follow the wizard in the software system for automatic instrument Optical Calibration.
   - When the calibration is complete check the calibration status (Passed/Failed). If the calibration is not passing refer to page 30 of the “Applied Biosystems7500/7500 Fast Real-Time PCR System Maintenance Guide” PN# 4387777 Revision D); if all attempts to achieve illumination are unsuccessful, call Applied Biosystems Technical Support

C.1.7 HID Dye Calibration

**Note:** Do not remove any dye calibration plates from their packages until ready to be loaded on the instrument – light exposure affects each plate’s performance; Quantifiler-associated dye plates can be used for a maximum of 3 freeze/thaw cycles

1) Remove the ABY, JUN, MP, VIC, and FAM dye plates from the calibration kits in the freezer and allow them to warm to room temperature; vortex and centrifuge for 2 minutes at less than 1500 rpm just before use
2) In the 7500 software, select **Instrument ► Instrument Maintenance Manager** from the top navigation bar ► **Dye (left hand pane)** to launch the dye screen.
3) Select Custom Dye Calibration and choose ABY, JUN, or MP dye plate and load onto the instrument. Check the box labeled “The custom dye plate is loaded into the instrument” and then click finished
4) Click Start Calibration and follow the wizard in the software system for automatic instrument Dye Calibration.
   • When the calibration is complete check the calibration status (Passed/Failed). If the calibration is not passing refer to page 43 of the “Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide” PN# 4387777 Revision D); if all attempts to achieve illumination are unsuccessful, call Applied Biosystems Technical Support

5) Repeat steps 1 through 4 for the other two dyes

6) In the 7500 software, select Instrument ► Instrument Maintenance Manager from the top navigation bar ► Dye (left hand pane) to launch the dye screen.

7) Select System Dye Calibration and only select FAM and VIC from the list of options.

8) Click Start Calibration and follow the wizard in the software system for automatic Dye Calibration of FAM and VIC.

Note: FAM and VIC will be performed back to back within the software system calibration (in this order).

C.1.8HID Reference Dye Spectra
C.1.9 HID RNase P Verification

**Note:** RNaseP Verification is not associated with regular maintenance procedures; it is only typically performed when directed by AB Technical Support for troubleshooting purposes

a. Prepare the RNaseP plate by centrifuging 2 minutes < 1500 rpm; load the plate onto the 7500

b. In the 7500 software, select **Instrument ▶ Instrument Maintenance Manager** from the top navigation bar ▶ **RNase P (left hand pane)** to launch the dye screen.

c. Complete the calibration as instructed by the wizard.

d. When the calibration is complete check the calibration status (Passed/Failed). If the calibration failed refer to page 51 of the “Applied Biosystems7500/7500 Fast Real-Time PCR System Maintenance Guide” PN# 4387777 Revision D); if all attempts to achieve illumination are unsuccessful, call Applied Biosystems Technical Support.

e. For a passing calibration, analyze the plate

f. Select the Standard Curve tab. Click the upper-left corner of the Plate Layout to select all wells. Verify the R2 value is greater than or equal to 0.990.

**Note:** If the R2 value is less than 0.990, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Applied Biosystems Technical Services.

g. Click Next, remove the calibration plate, and discard.

h. Click Finish, then Yes when prompted to save the experiment.

C.1.10 HID Laptop Maintenance - Disk Cleanup/Defragmentation/Data Archival

**HID Disk Cleanup**

1) From the Windows start menu, select **All Programs ▶ Accessories ▶ System Tools ▶ Disk Cleanup**

2) Select the first drive/partition from the drop-down list and click OK (click OK for any additional prompts); ensure that all item checkboxes are selected, then click OK

3) Repeat for each additional drive/partition until all have been cleaned up

**HID Defragment drives**

1) On the laptop's desktop view, right click on **All Programs ▶ Accessories ▶ System Tools ▶ Disk Defragmenter**

2) Select the first drive/partition in the upper right volume pane then click **Defragment** (repeat for each additional drive/partition displayed in the volume pane until all have been defragmented)
HID Archive data
1) Create a new folder within the archive folder named according to the date in YYMMDD format
2) Move all files (since last archival) from the import folder and each analyst’s folder to the newly created folder in the archive folder
3) Reboot the computer.

C.2 Thermal Cycler Maintenance
Hardware diagnostics tests and system performance tests are conducted quarterly for all validated thermal cyclers. Temperature tests using the Driftcon® Temperature Verification System are also conducted quarterly. The Driftcon® Temperature Verification System must be calibrated annually (when in use) through an external vendor. Thermal cycler maintenance is documented using “9700 TC Quarterly Maintenance” forms.

C.2.1 Hardware Diagnostics
Display Test
1) Using the thermalcycler’s on-screen menus and corresponding function buttons (top row buttons labeled F1 through F5) select Util ► Diag ► Hard ► Disp from the main menu
2) Follow the thermalcycler’s displayed instructions to complete the display test and record the result on the maintenance form (PASS indicates no dead pixels)
3) Select Exit ► Exit ► Exit to go back to the thermalcycler’s main menu

Keypad Test
1) Using the thermalcycler’s on-screen menus and corresponding function buttons (top row buttons labeled F1 through F5) select Util ► Diag ► Hard ► Keypad from the main menu
2) Follow the thermalcycler’s displayed instructions to complete the keypad test; record the result on the maintenance form (PASS indicates all keys detected)
3) Select Exit ► Exit ► Exit to go back to the thermalcycler’s main menu

C.2.2 System Performance Tests
Cycle Test
1) Place an empty 96-well plate onto the sample block and close/latch the cover if possible
2) Using the thermalcycler’s on-screen menus and corresponding function buttons (top row buttons labeled F1 through F5) select Util ► Diag ► System ► Cycle ► Cont
3) The thermal cycler will automatically display the results; record them on the maintenance form and evaluate the status (PASS indicates results within the form’s noted “Acceptable Range”)
4) Select Cancel ► Exit ► Exit ► Exit to go back to the thermalcycler’s main menu

C.2.3 Driftcon® Temperature Verification System
IMPORTANT: the multi-probe must rest in its rack while not in use to prevent damage; only when necessary, gently clean individual probes using a kimwipe dampened with distilled water

Driftcon® Set-Up
1) Obtain current temperature (convert from Fahrenheit to Celsius) and % humidity from the thermostat in the room containing the thermal cyclers (may use same values for all thermal cyclers in a single day)
2) Use the off-white cable to connect the multi-probe to the black box, and use the black serial cable to connect the black box to the laptop
3) Prepare thermal cycler for Driftcon® run
   a. Turn on thermal cycler and push back lid
   b. Level the multi-probe on the thermal cycler block (connector on right side) ensuring each
      individual spring-loaded probe isn’t “sticking” and that all probes are resting evenly
   c. Press F5 and change user to drift and press F1 to accept
   d. Wait to start run until software is launched

4) Launch the Driftcon® software
   a. Click the Driftcon® icon for the Mr. A. Admin account to display the password field; enter
      the password driftcon
   b. Press Synchronize on main menu; make sure both boxes for Application Data and User
      Data are checked, and then press Sync
   c. Click the bottom login button to launch the main Driftcon® software

Performing Driftcon® Temperature Verification Tests
1) Click the Start button on the Driftcon® main menu
2) Choose qPCR then click next
3) “Hardware box” information (informs user of measurements or credits left); click next
4) Select TC# using dropdown then click next
5) Choose Driftcon (default) then click next
6) “Layout” information will appear; click next
7) Enter the “instrument settings” as Standard/Max Mode from the dropdown menu; enter
   humidity and °C room temperature; set “heated lid” to off or on depending on type of probe;
   then click next
8) Enter any notes/comments (optional) then click next
9) Click finish to begin
10) Start the Driftcon program on the thermal cycler (the Driftcon® software automatically starts
    capturing/displaying data once the program begins)
    a. The Driftcon® software automatically generates a report when testing is complete; however,
       the thermal cycler program will continue and begin another cycle that isn’t needed—you
       can stop the thermal cycler program early as soon as the software opens the results
       window

OPTIONAL: the user may choose to monitor the measurements in real-time during data collection
but it is not required—tips and hints for viewing:

Driftcon Information
1) Step Info: Indicates the current protocol stage of data collection
2) Fixed Info: General test/probe information
3) Surface View: Pictorial representation of block temperatures
4) Actual View: Quantitative representation of block temperatures
5) Measurement Locations: each probe’s well assignment

Probe Information
Displays exact temperature readings for every probe at every measurement
6) Click the Print icon button on the bottom-right of the results window; choose Cute PDF Writer,
   then Apply and Print to launch a SaveAs window allowing you to enter a filename (use TC# in
   name) and choose its file path to save in appropriate location
Reviewing Driftcon® Results Reports
Pages 1 through 3 identify/summarizes:
- Testing date/time, thermal cycler, and manufacturer/model
- Driftcon® Verification System, software version, and calibration information
- Protocol, probe locations, environmental conditions, and analysis specifications

Pages 4 through 9 summarizes:
- Measurements/results for 6 different temperature targets (one per page)
  **Note:** Passing results are indicated by green checkmark -- exclamation point and/or question mark icons are also ok; failing results are indicated by red “x” icons
- **Values after 30 seconds:** Verify that the status for all probes on every page is “Active”
- **Step results:** Verify that pages 4 through 9 display passing results for “Hold time” and “Max. overshoot”, “Avg. overshoot”, “Max. undershoot”, and “Avg. undershoot” items; also verify that page 4 displays a passing result for “Heat rate” and page 5 displays a passing result for “Cool rate” items
  **Note:** The Driftcon® software may report a red “x” for the “overshoot” parameters at 95°C, 90°C, and 70°C when the measured temperature is greater than the upper limit of the specification range. Similarly, a red “x” may be reported for the “undershoot” parameters at 30°C, 50°C, and 60°C when the measured temperature is less than the lower limit of the specification range. These results are acceptable as the measured value is better than the specification.
- **Accuracy results:** Verify that pages 4 through 9 only display passing results
- **Uniformity results:** Verify that pages 4 through 9 only display passing results

Driftcon® Temperature Verification System References
CYCLERtest BV and GENO-Tronics BV. The Netherlands. Manual may be found at the following website: https://www.cyclertest.com/documentation/driftcon/manual.aspx

C.3 Maintenance of Mini-CrimeScope MCS-400

**Replacing the 400W metal halide bulb**
1) The 400W bulb should be replaced after 3-3.5 years of use.
2) Make sure unit is completely cooled before replacing the bulb. Disconnect the power cord. Open the side door.
3) Unplug the power cable for the lamp. Unscrew the top right-hand thumbscrew by hand or by using small flat blade screwdriver.
4) Pull the lamp assembly up and out of the lamp compartment and lay flat on a table.
5) Use the screwdriver if needed and remove the last two thumbscrews. Pull the mounting ring up and away from the lamp and power cable.
6) Remove the old lamp from the frame and replace with the new unit.
7) Seat and rotate the new lamp with the return lead in the upper right-hand corner.
8) Replace the lamp mounting ring and align the lamp return lead with the notch in the ring. Tighten the thumbscrews by hand and then tighten until snug with the screwdriver.
9) Slide the unit back into the holder in the lamp compartment and tighten the last thumbscrew.
10) Reconnect the lamp power cable, close the door, and lock with the screwdriver.
Cleaning the filters and optics
Both ends of the light guide should be cleaned on a regular basis or when needed (spots in the beam image) with lens paper (or tissue) with ethanol

C.4 Maintenance of Crime-Lite 80S
Remove dust and deposits from external surfaces using a soft dry cloth or paper tissue. When necessary, use a soft cloth or paper tissue dampened with a mild cleaning solution. Allow all surfaces to dry thoroughly before resuming use. Water-based solutions should contain only a little liquid soap, detergent or mild bleach. Alcohol-based cleaning agents may eventually degrade the surface of some types of plastic and should be used sparingly on such materials.

The unit contains no user serviceable parts. Do not drop, handle with care and allow for ventilation of the unit. There is an internal mechanism that will power the unit off in case of overheating.

C.5 Maintenance of Hamilton STARlet Robot
To minimize the risk of contamination within the lab and between samples, as well as maintain the instrument, perform the following precautions:
- Wipe down work area with 70% ethanol before starting a run.
- Perform and document maintenance daily, when the Hamilton STARlet is used. Maintenance is not required on the robot on days it is not in use.

Daily Maintenance
The following daily duties are required each day the robot is in use:
- Perform daily maintenance wizard
- Check level of molecular grade water trough used for normalization and add additional, if needed
- Wipe instrument with 70% ETOH
- Run the UV decontamination wizard for 15 minutes prior to first run of the day

Daily Maintenance Wizard
1) Open the Microlab STAR Maintenance icon on the desktop
2) Select the Daily Maintenance box
3) Click the green arrow to start the program
4) Select Yes when asked if you want to execute the daily maintenance for deck and waste
5) Select OK to proceed when asked if the deck is clean
6) Check the tip waste and empty if necessary. Select OK
7) Select Yes when asked to execute the 1000 µl Channel tightness check
8) Select Yes when asked to execute the 1000 µl Channel cLLD check.
9) The program will indicate whether the daily maintenance was executed successfully. Select OK to close the program.

UV Decontamination
Performed daily for 15 minutes prior to the first run of the day. Additional UV runs are optional thereafter.
1) Open the StarUVLight Software icon on the desktop
2) With the cover in the up position, install the UV protection shield in the correct position on the auto load tray. The feet on the shield feed into positions 2 and 30 on the upper track of the auto load tray. Close the cover over the shield.
3) Ensure the STARlet/STARplus radio button is selected
4) Enter the desired time (15 minutes) to run the UV light.
5) Press Start. Do not adjust the settings

Weekly Maintenance
The following daily duties are required monthly in addition to the daily maintenance duties noted above:
- Perform monthly maintenance wizard
- Replace and fill water reservoir with molecular grade water
- Perform a shutdown and reboot of the robot and computer

Weekly Maintenance Wizard
1) Open on the Microlab STAR Maintenance icon on the desktop
2) Select the Weekly Maintenance box
3) Click the green arrow to start the program
4) Select Yes when asked if you want to execute the weekly maintenance for deck and waste
5) Ensure there are no carriers on the auto load tray. Select OK to continue
6) Remove and clean the carriers with 70% ETOH. Select OK to continue
7) Clean the deck with 70% ETOH. Select OK to proceed
8) Check the tip waste and empty if necessary. Click OK
9) Wipe the laser scanner window with 70% ETOH. Select OK to continue
10) Select Yes when asked to execute the 1000µl Channel tightness check
11) Select Yes when asked to execute the 1000µl Channel cLLD check.
12) The program will indicate whether the weekly maintenance was executed successfully. Select OK to close the program.

Quarterly Computer Maintenance: Disk Cleanup/Defragmentation/Data Archival

Disk Cleanup
1) From the Windows start menu, select Windows Administrative Tools > Disk Cleanup. Alternatively, you can select the Disk Cleanup tile from the start menu or the task bar.
2) Ensure that all item checkboxes are selected, then press OK to continue
3) Press Delete Files to run the disk cleanup.

Defragment Drives
1) From the Windows start menu, select Windows Administrative Tools > Defragment and Optimize Drives. Alternatively, you can select the Defragment and Optimize Drives tile from the start menu or the task bar.
2) Select the first drive and press Optimize (repeat for each additional drive displayed until all have been defragmented)
Archive Data
1) The individual lab personnel folders and an archive folder will be located here: **OS (C:) > Lab Runs**
2) Create a new folder within the archive folder named according to the date in YYMMDD format
3) Move all folders & files (since the last archival) to the newly created folder in the archive folder
4) Reboot the computer and STARlet

C.6 3500xl Instrument Maintenance
The 3500 Series Data Collection Software employs wizards to assist the user in performing maintenance procedures. Access the appropriate wizard from the **Wizards** button on the dashboard or from the **Maintenance** tab. Follow the prompts to complete any required tasks. The **Maintenance Calendar** on the Dashboard may also be used to remind users when tasks are due.

Interior

Polymer Delivery Pump
C.6.1 Weekly Maintenance

C.6.1.1 Computer Maintenance

1) Close the 3500 Series Data Collection software
2) Power off the computer
3) Make sure the instrument door is closed then power off the 3500xl using the button on the front left of the instrument
4) When the computer is completely powered off, wait 60 seconds, then power on.
5) Wait until the Windows™ login screen is displayed, do not log-in yet
6) Power on the 3500xl and wait until the green status light on the front panel is on and not flashing before proceeding
7) Log-in to the computer and wait for the green checkmark to appear in the lower corner of the screen
8) Start the 3500 Series Data Collection software

C.6.1.2 Clean Anode Buffer Container Valve Pin

Use a kimwipe to clean the anode buffer container valve pin assembly on the polymer delivery pump.

C.6.1.3 Wash Pump and Channels Wizard

IMPORTANT! The 3500xl used solely for databasing may go up to 14 days between washing the pump and channels wizard. All 3500xls utilized for casework must have the wash pump and channels wizard performed on a weekly basis.

1) Select the Maintenance tab or Wizards from the Dashboard.
2) Select the Wash Pump and Channels button.
3) Follow the on-screen prompts.
   a. DO NOT throw away the Anode Buffer Container on the instrument as directed by the wizard unless it’s been on the instrument longer than desired, otherwise just set aside to place back onto instrument once cleaning is done.
   b. When using the Conditioning Reagent, load an empty Anode Buffer Container on to the instrument to collect waste.
4) Remove adhesive covering on top of any pouches before placing onto the instrument.
   a. Ensure the black O-ring on the post for the Pouch Connection remains on the post.
   b. Ensure the RFID tags face the inside of the instrument.
   Note: Cap the polymer pouch after removal from the instrument until it’s placed back on the instrument.

C.6.1.4 Flush the Water Trap (Pump Trap)

1) Fill the supplied 20 mL plastic syringe with approximately 5-10 mL Molecular Grade Water (ambient temperature). Expel any bubbles from the syringe.
   a. DO NOT use a syringe smaller than 20 mL because doing so may generate excessive pressure within the trap.
2) Attach the syringe to the forward-facing Luer fitting at the top of the pump block.
a. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.

3) Open the Luer fitting by grasping the body of the fitting and turning it and the attached syringe approximately one-half turn counterclockwise.

4) Flush the trap by pushing slowly and steadily on the syringe plunger. Take approximately 30 seconds to flush 5 mL of water through the trap. Do not use excessive force.
   a. To avoid damaging the trap seals, flush less than 5 mL of water in a 30 sec period.

5) Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counter-clockwise with the other hand.

6) Close the Luer fitting by lightly turning it clockwise until the fitting seals against the block.

7) Wipe down the inside of the instrument. Clean off any debris including dried polymer and buffer.
   a. Sterile water and lint free wipes may be used; however the instrument must be dry before use.

C.6.2 Other Maintenance as Directed By Dashboard

C.6.2.1 Polymer Pouch Replacement

Polymer should not remain on the instrument for more than 14 days.

1) Remove the polymer from storage 4 °C. Allow refrigerated polymer to equilibrate to ambient temperature before use

2) Select the Maintenance tab or select Wizards on the Dashboard

3) Select the Replenish Polymer button
4) Remove the seal from the top of the pouch.
   a. There may be a tiny droplet of polymer inside the fitment (residual from the pouch filling process). This is not expected to cause any performance issues.
5) Follow the on-screen prompts. This wizard takes 10-20 minutes to complete.
6) When removing the polymer, push the lever down and continue pushing after the first resistance to completely unlock the assembly.
7) When installing the polymer pouch, the RFID label faces the instrument. Slide the pouch fitment onto the slot of the lever assembly. Push the lever up to snap the pouch into the connector end of the instrument pump.

8) Select the **Refresh** button to update the consumable information on the dashboard.

**C.6.2.2 Buffer Replacement**

Anode and Cathode Buffers should not remain on the instrument for more than 14 days.

1) Allow the refrigerated Cathode Buffer Container (CBC) and Anode Buffer Container (ABC) to equilibrate to ambient temperature before use.
2) Before removing the seal from the ABC, tilt the container so that the buffer collects in the larger of the two compartments and the smaller compartment is empty. Verify that buffer level is at or above the fill line.
a. Remove and discard the old ABC from the instrument into a post-amp waste container. Empty ABCs may be saved to use as waste containers during Wash Pump and Channels Wizard.

b. With the RFID label toward the instrument, place the new ABC into the anode-end of the instrument.

c. Position the anode in the large chamber of the ABC, then push the ABC up and back to install.

3) Gently squeeze the center of the Cathode Buffer Container to remove from the instrument.

4) Verify the buffer levels of the new container are at or above the fill line and that the seal is intact.

   a. Tilt the CBC back and forth gently to equally distribute the buffer across the top of the baffles. If you do not tilt it, the buffer sticks to the baffles due to surface tension.

5) Remove the seal from the new CBC and wipe off any buffer across the top of the CBC to prevent arcing during the run.

6) Remove septa from the current CBC container and place on the new CBC container.

   a. Align the appropriate septum on each side of the container

   Note: Replace with new septa at least once per month

   b. Discard the old CBC container in a post-amp waste container

7) With the tab facing to the front and the RFID tab to the right, install the new CBC on the autosampler tray.

   a. When properly installed, the CBC tabs will click as they are snapped into place.

8) Select the Refresh button to update the consumable information on the dashboard.

C.6.2.3 Cathode Buffer Container Septa Replacement

Note: This may have already been performed during replacement of the buffers above.

1) Present the sample tray by pressing the Tray button on the instrument.

2) Remove the old septa from the cathode buffer container.

3) Insert the new septa into the cathode buffer container.

4) Close the instrument door and discard the old septa.

C.6.3.2 Capillary Replacement

Replacement of the capillary array is dependent on data quality and may be performed on an as needed basis. Capillaries are guaranteed for 160 injections. Direct amplification can reduce the life
of the capillaries. It might be necessary to periodically run an injection of amplification negatives to prolong the life of the array.

1) Select the **Maintenance** tab or **Wizards** from the Dashboard.
2) Select the **Install Capillary Array** button.

3) Follow the on-screen prompts. This wizard takes 15-45 minutes to complete.

**IMPORTANT!** A spatial calibration must always be performed after removal or replacement of the capillary array, after opening the laser detection cell door, or after moving the instrument.

**C.6.3.3 Spatial Calibration**

The 3500 Series Data Collection software uses the images collected during the spatial calibration to establish a relationship between the signal emitted by each capillary and the position where the signal falls and is detected by the CCD camera. A spatial calibration must be performed every time a capillary array is removed or replaced, the detector door is opened, or the instrument is moved.

1) Select the **Maintenance** tab.
2) In the left navigation pane under the **Calibrate** menu, select **Spatial**.
3) Select either **Fill** or **No Fill** to fill the array with polymer before starting the calibration. The “Perform QC Checks” box should remain checked. Select **Start Calibration**.

4) Evaluate the calibration results to ensure that there is:
   a. A sharp peak for each capillary (small shoulders are acceptable).
   b. A ‘+’ marker at the apex of each peak and no off-apex markers
   c. An even peak profile (all peaks about the same height).
   d. Capillary spacing values are expected to be near 15 or 16 pixels between capillaries.

5) Accept the results if the criteria above are met.
6) If the calibration failed: Reject Results. Any of the following may be attempted:
a. Repeat spatial with a polymer fill
b. Pre-heat the oven prior to running a spatial.
c. Reposition the array and window and repeat spatial
d. Detection cell window can be cleaned with Methanol only if needed
e. If the spatial still fails, then see the 3500XL User Guide for instrument maintenance and troubleshooting. Service may be required.

Note: The software does not save historical calibration results. Only the most recent spatial calibration is maintained in the software.

C.6.3.4 Spectral Calibration
A spectral calibration creates a matrix that corrects for the overlapping fluorescence emission spectra of the dies. Perform a spectral calibration when a new capillary array is installed, after any service on the optics, or if there is an increase in pull-up observed.

*Investigator 24plex QS*
1) At first use, thaw the Matrix Standard BT6 completely. Before opening the tubes, vortex and then centrifuge briefly to collect the contents
2) Create a master mix containing 225 µl of formamide and 25 µl of Matrix Standard BT6. Vortex to mix thoroughly and perform quick spin
3) Add 10 µl of master mix to each of the wells in columns 1 thru 3 of a 96-well optical plate and cover with a plate septae
4) Centrifuge briefly
5) Denature the plate for 3 minutes at 95°C
6) Snap freeze by placing the plate in the freezer for 3 minutes
7) Briefly centrifuge the plate
8) Place the assembled plate on the instrument
9) Select the Maintenance tab
10) In the left navigation pane under the Calibrate menu, select Spectral, and then Calibration Run
11) The number of wells in the spectral calibration plate and the position in the instrument must be specified
12) Under Chemistry Standard select Matrix Standard and dye set BT6
13) Enable Allow Borrowing
14) Click Start Run
15) Once the run has completed, click a capillary in the table to display the results for each capillary below the run results table
   • The quality value (Q value) of each capillary must be greater than 0.95 and the condition number range (C value) must be between 1 and 13.5
   • Check the matrix samples for a flat baseline. There should be 6 peaks with peak heights of about 1000-6000 RFU for each matrix sample with the optimal range being between 3000-5000 RFU
   • When a spectral calibration is successfully completed, the Overall row displays green results. If the Overall row displays red results, refer to the “Spectral calibration troubleshooting” section of the Applied Biosystems 3500/3500xL Genetic Analyzers User Guide
16) For each capillary, select and display the spectral and raw data. Check that the data meet the following criteria:
   - The order of the peaks in the spectral profile from left to right should read orange-red-yellow-green-blue-purple
   - No extraneous peaks should appear in the raw data profile
   - Peak morphology in the spectral profile should show no gross overlaps, dips or other irregularities. Separate and distinct peaks should be visible

17) If the data for all capillaries meet the criteria above, click Accept. If any capillary data do not meet the criteria, click Reject and refer to “Spectral calibration troubleshooting” section of the Applied Biosystems 3500/3500xL Genetic Analyzers User Guide

**PowerPlex Fusion 6C**
1) Pre-heat the oven to 60°C, ideally 30 minutes before the first injection
2) At first use, thaw the 6C Matrix Mix and Matrix Dilution Buffer completely
   a. After the first use, store the reagents at 2-10°C
   a. Label the tube with the date of dilution. The diluted 6C Matrix Mix can be stored for up to 1 week at 2-10°C
4) Add 10 µL of the diluted 6C Matrix Mix to 500 µL of Hi-Di Formamide. Vortex for 10-15 seconds
5) Add 15 µL of the 6C Matrix Mix with formamide to each well of columns 1-3 of a 96-well plate. 
   Cover with a plate septum and centrifuge briefly to remove bubbles. DO NOT heat denature.
6) Place the assembled plate onto the instrument.
7) Select the Maintenance tab.
8) In the left navigation pane under the Calibrate menu, select Spectral.

9) Select the Calibration Run tab. Under Calibration Settings, select Matrix Standard in the 
   Chemistry Standard pull-down menu.
10) Select Promega J6 in the Dye Set pull-down menu. Leave the “Allow Borrowing” box checked. 
    The software is configured to allow up to 3 borrowing events
11) Select A1 as the starting well for the standards.
    a. A plate does not have to be created in the software for calibration
12) Once the instrument has pre-heated to 60°C, press the Start Run button
13) Evaluate the spectral calibration run
   a. Passing and failing capillaries are shown in green and red, respectively, and borrowed capillaries are shown in yellow with an arrow indicating the capillary from which the results were borrowed.

   

   b. For dye set J6, the Quality Value minimum is .95 and the Condition Number maximum is 8.0.
   c. To determine the reason the capillary failed, view the Spectral Calibration Report.

14) If the calibration passed: Accept the results.
15) If the calibration failed: Reject the results.
   a. Verify plate was set up correctly.
   b. Fill array will polymer in case of blocked capillary.
   c. Check for bubbles in the system and use bubble remove wizard if needed.
   d. See the 3500 User Guide spectral calibration troubleshooting. Also, refer to the PowerPlex 6C Matrix Standard Technical Manual. Service may be required.

C.6.4 Quarterly Computer Maintenance

C.6.4.1 Archive Library Items
1) Navigate to Archive Screen.

2) Specify the date category and specify a date range. Click OK.

3) Specify a location and file name for the archive (typically two-digit year followed by two digit month and two digit day).
4) Click Save. A message is displayed when the archive is complete.
C.6.4.2 Purge Library Entries
1) Access the Purge function.

2) Click Yes in the Purge warning message stating that you are about to permanently delete all the files in the library.
3) Specify the date category and range, then click OK.
4) Click Yes in the Purge warning message.
   a. A message is displayed when all records are deleted.

C.6.4.3 Archive Data Files
1) Access the Windows™ backup function: Start > Control Panel > Backup and Restore Center.
2) Copy the archive to a network location.

C.6.4.4 Defragment the Hard Drive
1) Go to Start > Programs > Accessories > System Tools > Disk Defragmenter and then follow the prompts.

C.6.5 Annual Maintenance
Annual preventive maintenance will be performed by a qualified technician.
Appendix D: Title: USE OF LIMS

The Laboratory Information Management System (LIMS) will be used by the Biology/DNA Detail for creation of worksheets and for generating the final report of analysis.

D.1 Casework Worksheets
D.1.1 Screening
- Be consistent with the custom description in the worksheets and report. Throughout the rest of the case file descriptions can be truncated.
- Only use items (Item 1) and sub-items (Item 1.2) within the evidence exams section of the worksheet.
  - Custom designators (e.g. KG1A) may only be used for case/items previously worked outside of LIMS.
- Use "swab from" in the custom description when referring to a swab received in-house that has been collected elsewhere by a CSA, detective or other lab personnel and "swabbing from" in the custom description when a DNA analyst performs the swabbing of an item for touch/non-visible DNA.
  - Do not use dashes in item custom descriptions as it will interrupt the grammatical flow in the body of the report.
  - Use "from" or "of" in either of these phrases as long as it is consistently used within the same case.
  - It is permissible to refer to an item as "stain from" or "swabbing from stain" when visible staining is present regardless of whether collected via cutting or swabbing.
- It is analyst discretion whether or not to imbed the link to their photos directly into the worksheet. As long as the raw images are in the Object Repository, they do not need to be imbedded.
  - Do not attach PowerPoint presentations containing item photos in the Object Repository.
  - If the print outs of the PowerPoint are in the printed case file and the raw images are in the Object Repository, no additional effort is required.
- If any change (edit custom description or change in the dash of an Event #) is made within the Evidence Exams section, hit Refresh and manually toggle on/off the drop down for each sample a change was made for.
- It is analyst discretion whether or not they want to use the Item notes field in the outer packaging section (below where the ACE item description is automatically populated).
- DNA detail is NOT to be used for the parent item if sub-items are being created.
  - Use "Add Item Note/Photos" and click "No further examination at this time", utilize the screening/sampling detail and click "No further testing at this time", or use the "Not examined at this time" check box next to the parent drop down and document the "reason" in the box that appears.
  - The Note/Photos field allows for documentation of any ALS/visual examinations that do not coincide with additional screening tests and/or screening dates.
• **All WinAce descriptions** will be edited with a custom description for consistency. There should be no descriptions changed and some left all uppercase.

• **Re-isolate a sample**: report out the sample being used for reporting purposes and make a separate note in the open notes field with accompanying dates for all of the initial testing. Note everything that would have been reported for the first isolation (with date) and note a reason for the re-isolate (e.g. “RI due to unexpected results”).

• **“No further analysis”** needs to be recorded in Batching comments and manually added to the table, after P30, RSID-saliva and microscopic sperm search exams, if the sample is being dropped.

**When to Make Sub-Items:**

**Note:** For all examples shown below dashes are used, however indentation, bullets, two dashes, etc. can be used for formatting the description; this is analyst discretion.

If a section of an evidence item or a specific stain(s) on an item are screened [whether or not the screening results are positive or negative] a sub-item is required (e.g. only the crotch on a pair of shorts is overlaid; PT test a RBS on a t-shirt, etc.). That testing is isolated to one specific area of the entire item and must be documented as a subset of that item.

If an entire item is screened with negative results, a sub-item is **not required** (e.g. AP overlay on an entire pair of underwear/shorts, but the results were negative). This can be recorded under the parent because the entire parent item is what is being examined. In this instance only, do not click “no further testing” on the worksheet because there is further testing and the presumptive test will trigger the item to appear on the report table. If a swabbing for touch/wearer is done after screening, the swabbing will require a sub-item.

If an item is screened (e.g. water bottle, sock/shirt) for saliva/wearer/touch only and there are no additional stains that could be tested in the future, sub-items are **not required**, but the swabbing of the item must be added to the report to include the locations of the item specifically swabbed (e.g. swabbing taken from mouth area of water bottle, swabbing taken from inside neck/collar-area of shirt, swabbing taken from grips, trigger, slide serrations of gun, etc.).
If a t-shirt is swabbed for wearer only, but there is a bloodstain that was not requested, a sub-item is required in the event the blood stain is requested at a later date.

All negative stains, using the same presumptive tests, can be clumped together into one negative sub-item. This also applies to multiple screening tests. All stain(s) taken on for DNA will then be a separate sub-item(s).

Multiple positive stains, using the same presumptive test, can be clumped together into one sub-item. However, the analyst must describe where each of the additional stains is located on the item. In the event one (or more) of those stains is requested at a later date, it will become a sub-item of the original sub-item. This information must be listed on the worksheet as well as the report.
It is analyst discretion if they wish to create a sub-item for everything, if not required as stated above.

**Making Sub-Items:**

**Note:** When making the sub-items in the evidence exams, an asterisk (*) may be placed in the five required fields (Event #, impound officer, impound package, impound item, and WinAce number) instead of the actual impound information.

**Option 1:**
1) From within the worksheet tab in the Unit Record, click on the particular worksheet containing the item that needs to be sub-itemized.
2) Click on the clipboard icon near the top of the window for "Assign Exams to Worksheet." Another window will appear that contains the entire list of evidence items that have been entered for this event. This is also the same window that is used to assign/unassign evidence to the worksheet. Be careful NOT to check/uncheck the boxes at this time. Check marks only appear next to the items that have been assigned for DNA analysis.
3) Click on the item which needs a sub-item and click on the little box icon near the top with the green + sign to add a sub-item. A "New Evidence" window will appear.
4) Enter the appropriate information to make the sub-item and then click “Add”.
5) Once finished with all of the sub-items, close the "New Evidence" window.
6) Mark the box next to the sub-item(s) added and close the "Assign Exams" window.
7) The sub-items will appear under "All Exams" in the worksheet tab. Right click on the sub-item(s) that were added (under the "All Exams") and transfer them to the same worksheet the parent item(s) is on by clicking "Add items to worksheet". The sub-items will now appear with the parent item under the worksheet (# X).
8) Open the worksheet the sub-item(s) were added to. All sub-item(s) added are now available in the drop down lists to add for examination.

**Option 2:**
1) From within any worksheet click “Evidence Exams”.
2) Click on the item which needs a sub-item and click on the little box icon near the top with the green + sign to add a sub-item. A "New Evidence" window will appear.
3) Enter the appropriate information to make the sub-item.
4) Once finished with all of the sub-items, close the "New Evidence" window.
5) Mark the box next to the sub-item(s) added and close the "Assign Exams" window.
6) Save and Close the worksheet and re-open before selecting the sub-item(s) from any drop down within the worksheet. Once re-opened, in the worksheet toggle off/on in order for the evidence information to appear on the report.

**D.1.2 Reagents**

The worksheet must contain all lot numbers associated with screening methods that are not otherwise captured in the CONFIRMadactyl workbook (e.g. Phenolphthalein, AP Overlay, molecular grade water, etc.)
- If two different lot numbers of the same reagent are used on the same day, click "Add Screening/Test Reagent" and enter the additional lot numbers.
- If screening reagents were not added at the time of screening and they have expired, hand enter them in the comments field to the right of the reagent.
• If the reagent is visible and correct on the printed version of the worksheet, DO NOT add the lot number to the comments field. The reviewer can open the resource repository to verify the correct lot, if necessary.

D.1.3 Results

Note: Sections of the LIMS worksheet used to document the reported profile results may be used to automatically populate the report. Alternatively, Forensic Scientists may manually populate report statements using those “Reporting Guidelines”.

• Use Generate Results and Review Results prior to drafting the reports.

• Exclusions: exclude everyone from everything (regardless of gender) with the only exception being single source intimate body samples that are consistent with the person they were collected from and no indication of the presence of a foreign contributor(s).

• When an item of evidence will be terminated following quantitation (for both sexual assault and non-sexual assault-related samples), the selection of “No DNA profile obtained” will be selected from the Reported dropdown in LIMS in order to populate the report.

D.1.4 Sexual Assault Kits

The LIMS worksheet for sexual assault kits does not include a field to specifically capture the items of evidence received, but not examined. It is necessary to capture this information as it will be added to a table in the report to detail these items.

The information may be captured one of two ways:

1) Documenting Items Not Examined in Worksheet as Sub-Items or Lab Items:

• If not already done so in ACE, the contents of the sexual assault kit may be designated in the worksheet by making sub-items for all items received in the kit, including those items that will not be tested. Only those items deemed appropriate for work based on the SAK workflow will be screened and analyzed. For those items not being tested, check the box “No further examination at this time” in the worksheet.

• Sexual assault kits which have their contents designated on the outside of the kit have already been entered into ACE and each item has been assigned a Lab Item number. While processing, each Lab Item number may be added to the worksheet, including those items that will not be examined. For those items not being examined, check the box “No further examination at this time” in the worksheet.

Each of these items will ultimately populate in the report. These items will be moved from the main results table to the table which denotes items of evidence received, but not examined (Refer to Table of Items Received, Not Examined).

2) Documenting Items Not Examined in Worksheet without Making Sub-Items:

Items received, but not examined may be documented in the notes section of the sexual assault kit worksheet, without being sub-itemized. The information for each of these items will then be used to manually populate the report table which denotes items of evidence received, but not examined (Refer to Table of Items Received, Not Examined).

For all sexual assault kits:
• Place copies of the nurse’s notes/SAK paperwork on the left-hand side of the file folder for review. Due to privacy laws, these notes must be shredded and discarded (do not paginate or add to object repository) after the report has been released.

• With the exception of SAKs collected by Crime Scene Analysts at autopsy, when entering the booking information for a SAK in the Evidence Exams section, the officer is the SANE nurse (e.g. Dermanelian) and list the package as SAK. The report will show “Dermanelian – SAK”.

The following evidence item(s) were received and examined:

<table>
<thead>
<tr>
<th>Lab Item #</th>
<th>Impound Pkg #</th>
<th>Impound Item #</th>
<th>Description</th>
<th>Results, Opinion, and Interpretations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item 1</td>
<td>Dermanelian - SAK</td>
<td></td>
<td>Sexual Assault Kit from Aysa Castro</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-- Reference standard</td>
<td>Full female profile</td>
<td></td>
</tr>
<tr>
<td>Item 2</td>
<td></td>
<td>-- Breast swabs</td>
<td>Negative presumptive saliva test</td>
<td></td>
</tr>
<tr>
<td>Item 3</td>
<td></td>
<td>-- Oral swabs</td>
<td>Sperm negative</td>
<td></td>
</tr>
<tr>
<td>Item 3.1</td>
<td></td>
<td>-- Fingernail swabs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Item 3.2</td>
<td></td>
<td></td>
<td>Right hand fingernails swab</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Left hand fingernails swab</td>
<td></td>
</tr>
</tbody>
</table>

Tests for blood, semen, and/or saliva are presumptive in nature and therefore provide an indication, but not confirmation, of the presence of a body fluid.

In the event items are inadvertently received that will not be examined, it is not necessary to create a LIMS worksheet. These items will be designated on the ACE secure move receipt to show that they were taken into possession by the analyst and will be manually added to the report table which denotes items of evidence received, but not examined.

D.1.5 Packages Containing Un-inventoried Items Without Impound Item Numbers (Backpacks, Bedding, Clothing, Gun/Magazine/Ammunition, etc.)

All items contained in packages without unique impound item numbers (e.g. items contained in backpacks, bedding, clothing, gun/magazine/ammunition, etc.) must be inventoried to include items not examined. This may be achieved one of two ways:

- Item may be designated in the worksheet by making sub-items, for all items received, including those items that will not be examined. It is permissible to make one sub-item to encapsulate all items that are not being examined, rather than making separate sub-items per item. For those items not being tested, check the box “No further examination at this time” in the worksheet. Each of these items will ultimately populate in the report. These items will be moved from the main results table to the table which denotes items of evidence received, but not examined (Refer to Table of Items Received, Not Examined).

- Alternatively, the items received, but not examined may be documented in the notes. The information for each of these items will then be used to manually populate the report table which denotes items of evidence received, but not examined (Refer to Table of Items Received, Not Examined).

- Items not requested will be notated in the worksheet.

D.1.6 Packages Containing Multiple Impound Items Not Requested

All impound items contained within a package, but not requested will be notated in the worksheet in the “Insert Items Not Requested” field (with the exception of sexual assault kit worksheets, which
does not include this field). Manually enter the Impound Item # and Description for these items contained within the package, but were not requested.

- The information for each of these items will then be used to manually populate the report table which denotes items of evidence received, but not examined (Refer to Table of Items Received, Not Examined).

D.1.7 Buccal Swab Kits and Reference Standards
- Sub-items DO NOT need to be created for the buccal swab kits where there are multiple swab boxes (usually seen with OJs). The check boxes within the template reflect multiple boxes and this is adequate.
- Regardless of cellular type (buccal, blood, etc.), all standards must be renamed to “reference standard” for all custom descriptions
- The “Name” box in BSK should only be typed in if the volunteer statement is going to appear on the report.

D.1.8 Cross-Referencing Items from Different Cases
When an item of evidence is booked under an additional Event #, it will appear in the worksheet as "Item # (lab case it is booked under)” (e.g., Item 1 (13-06123)). In order to cross-reference, samples must be delineated in some manner in the extraction workbook and forward: "Lab case worked under - Item # (Lab case booked under)” (e.g., 14-00017 - Item 1 {14-00018} where 14-00017 is what it is worked under and 14-00018 is what it was booked under). The dash before “Item” can be a dash, space or underline.

Note: Brackets [ ] will not import into the HID software on the 7500 and therefore should be avoided. The use of parentheses may result in an error with the allele table macro when also utilizing tube numbers at the end of the sample names.

D.1.9 OJ’s
- Ensure the OJ Event # formatting found within the auto-populated fields of the worksheet have been corrected, using or deleting zeros if necessary (usually within outer packaging description), to reflect the appropriate event for the lab case.
- OJ packages/evidence: the evidence info fields (e.g. booking officer, pkg #, item #) should correspond with the item of evidence. Change within the worksheet and Evidence Exams if they do not match.
  - Be aware if there are no package or item number(s) on the evidence. If there is no package number, the report and chain of custody form should look like: 9288/1. If there is no item number, the report and chain of custody should look like: 9288-1. The worksheet may be overwritten once populated for outer packaging.
D.1.10 Printing or Saving
Remember to **expand all collapsed fields** in the worksheet before printing or saving. If a field is left collapsed it will not be seen when the worksheet is printed and published upon release of the report.

D.1.11 Additional Notes
- **DO NOT delete any drop-down sections** in the worksheet that control for logic (e.g. the profile detail section when reporting out a buccal swab outside of the buccal swab template for a SAK). If deleted an error will be generated and will not allow "Generate Results/Review Results" to be viewed. If not readily identified and rectified, it's possible the worksheet will have to be recreated from scratch as the error will be saved in the logic.
- **DO NOT close the entire system if an error is encountered** within the worksheet. Close the worksheet, re-launch, and attempt the recovery option, if necessary. If LIMS is closed the recovery option is no longer possible within the worksheet.
- If an **item is not being worked** that has already been assigned to the Unit Record, un-assign it from the Unit Record. It is not appropriate to assign an item to a worksheet, without any analysis taking place. Remove it from the assigned analyses by clicking on the “Assign Evidence” and unchecking the box of the item not being worked. Make a note (with date/initials) in the details tab of the Unit Record as to why it isn't being worked, if there isn't a note there already.
- If a worksheet is opened after a draft has already been generated to make changes to any of the conclusions, custom description or evidence information in “Evidence Exams” (profile or screening sections), click “Generate Results” even if another version of the draft is being checked in. The “Review Results” view (which is submitted for discovery) will more accurately reflect what will be on the draft. If only the notes are being fixed it is not necessary to click review results as those fields do not populate on the report when re-drafted.

D.2 Reports

D.2.1 Headers
- For cases involving OJ events, manually correct the Event # in the header of the report to include any dashes.
  **Note**: Subsequent report pages cannot be changed.
- In the event an Event # is not showing up in the header **DO NOT** manually enter it.
- If two related Event #s are being worked together as one, simply adding a “Related Lab Case” will **NOT** suffice for getting the additional Event # on the report.
  - Under Lab Case Details click on the tab for Lab Request and open it. In the Lab Request, click on the “+” button next to the Agency.
  - The Manage Agencies box will pop up and click "Add." Lab Request Agency box will appear. Fill out as shown below.
  - Click OK. Verify the primary Event # is still marked as primary.
  - Save and close all the way out. The other Event #(s) will now appear on the report.
- If the requestor (and requestor only) is listed incorrectly in the header of the report, change manually on the report to accurately reflect the correct requestor. The system will always pull the first requestor listed in the first lab request and places that on the report even though exams could have been requested in the 2nd lab request. The requestor will be notified of the completed exams (as long as they are entered as a requestor or CC) so the name on the actual report is not a cause for concern.

- **Victim and Suspect Names** – if neither is listed in LIMS, no additional research is necessary. *State of Nevada and Business Names CANNOT be added as victims in LIMS.* If the names are not there, do not add them. If they are incorrect based on the evidence or other documents, check OnBase, or contact the detective to verify the details. **Note:** For any supplemental work where the original report listed a business as the victim in the header of the report (previously done outside of LIMS) the analyst can manually add a business into the header of the LIMS report, however this is not required.
D.2.2 Results Table

- Table header will state: “The following evidence item(s) was (were) received and examined.” It is permissible to change this header statement to be singular, rather than plural, based on the case-specific circumstances.
  
  **Note:** In the event a report is only comparing previously generated DNA profiles with a reference standard processed with a different case, the header statement will be modified to: “The following results, opinions, and interpretations are reported below.”

- **Items will be left in the order** that the LIMS system places them on the results table of the report, with the exception of buccal swabs. Buccal swabs should be reported at the end of the table even if the buccal swabs were analyzed in the middle of the worksheet.

- **Do not hand type rows into the table** on the report. If a draft report is created and something is missing from the table, cancel, evaluate and re-draft.

- Custom descriptions created in “Evidence Exams” in worksheets will match descriptions in report table.

- **Bullets** will be put in the short results table in the “Results, Opinions, and Interpretations” column.

- LIMS automatically reports "presumptive test(s)" in the short results table on the report. The "(s)" may be left regardless of how many presumptive tests were actually performed. It is analyst discretion if they wish to remove the "(s)" if only one test was performed.

- If any of the "Additional Report Statements" are chosen, they will auto-populate below the evidence disposition statement in the body of the report. Any statement that shows in the body of the report should be moved above the evidence disposition statement or to the table depending on where it is appropriate.

- When referring to any additional statement included below the table use a * or other symbol to reflect this (e.g. * - Evidence was booked under Event # <XX XXXX-XXXX>). A symbol should be used whenever an additional statement is used to show what is being referenced.

- If another Event # needs to be referenced within the report, use the real Event # and not the Lab Case # so it is clear to the client what is being referenced. It is analyst discretion if they wish to include the Lab Case # in addition to the Event #, however this is not required.

- Bold lines will be placed around each package on the table.

- **Merge the repetitive package information** so it is not listed more than once.

- Merge the repetitive item information together (e.g. multiple sub-items from one parent item)

- If the results table is longer than one page on the report, add the **table header** to each additional page.

- Items which were received, but not examined which were either designated with a sub-item or with a Lab Item # (to include items not examined from a sexual assault kit) will be manually moved from the Results table to the table documenting items of evidence which were received, but not examined (Refer to Table of Items Received, Not Examined).
Example:

The following evidence item(s) were received and examined:

<table>
<thead>
<tr>
<th>Lab Item #</th>
<th>Impound Pkg #</th>
<th>Impound Item #</th>
<th>Description</th>
<th>Results, Opinion, and Interpretations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item 1</td>
<td>Demanelian - SAK</td>
<td></td>
<td>Sexual Assault Kit from Ayse Castro</td>
<td>Reference standard</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Full female profile</td>
</tr>
<tr>
<td>Item 2</td>
<td></td>
<td></td>
<td>Breast swabs</td>
<td>Negative presumptive saliva test</td>
</tr>
<tr>
<td>Item 3</td>
<td></td>
<td></td>
<td>Oral swabs</td>
<td>Semen negative</td>
</tr>
<tr>
<td>Item 3.1</td>
<td></td>
<td></td>
<td>Fingernail swab</td>
<td></td>
</tr>
<tr>
<td>Item 3.2</td>
<td></td>
<td></td>
<td>Left hand fingernail swab</td>
<td></td>
</tr>
<tr>
<td>Item 4</td>
<td></td>
<td>Underpants</td>
<td></td>
<td>Not examined</td>
</tr>
</tbody>
</table>

Tests for blood, semen, and/or saliva are presumptive in nature and therefore provide an indication, but not confirmation, of the presence of a body fluid.

<table>
<thead>
<tr>
<th>Lab Item #</th>
<th>Impound Pkg #</th>
<th>Impound Item #</th>
<th>Description</th>
<th>Results, Opinion, and Interpretations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item 4</td>
<td>Demanelian - SAK</td>
<td>Underpants</td>
<td></td>
<td>Not examined</td>
</tr>
</tbody>
</table>

- Supplemental reports containing reference standard extracts which are being amplified for the first time due to being previously terminated from analysis will be listed in the report using their original Lab Item #. The Impound Pkg # and Impound Item # will be updated to state “DNA Freezer”. The description will be edited to state “Extract from <insert originally reported description>”. The report will also include a reference to the original report for related information.

Example:

<table>
<thead>
<tr>
<th>Lab Item #</th>
<th>Impound Pkg #</th>
<th>Impound Item #</th>
<th>Description</th>
<th>Results, Opinion, and Interpretations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item 1*</td>
<td>DNA Freezer</td>
<td></td>
<td>Extraction from the swabs from the interior door handle of the east facing front door</td>
<td></td>
</tr>
<tr>
<td>Item 3.1*</td>
<td>DNA Freezer</td>
<td></td>
<td>Extract from the cutting from the “Pro Club” t-shirt</td>
<td></td>
</tr>
<tr>
<td>Item 5*</td>
<td>008368-1</td>
<td>1</td>
<td>Extract from the reference standard from Willie Carter*</td>
<td></td>
</tr>
</tbody>
</table>

*These items were re-analyzed. Refer to the original report issued by FS II Kimberly Dannenberger/P# 13772 dated 11/26/2013 for information and previous interpretation results.

- When extracts are pulled from long term storage for additional testing that were previously generated before LIMS, the items will be reported with the new Lab Item #, Previous Lab #, Storage Location, and Description.
Example:

<table>
<thead>
<tr>
<th>Lab Item #</th>
<th>Previous Lab Item #</th>
<th>Storage Location</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item 1</td>
<td>KG1</td>
<td>DNA Freezer</td>
<td>Extract from wristwatch</td>
</tr>
<tr>
<td>Item 2</td>
<td>KG2</td>
<td>DNA Freezer</td>
<td>Extract from red knit glove</td>
</tr>
<tr>
<td>Item 3</td>
<td>KG3</td>
<td>DNA Freezer</td>
<td>Extract from Nike baseball glove</td>
</tr>
<tr>
<td>Item 4</td>
<td>KG4</td>
<td>DNA Freezer</td>
<td>Extract from buccal swabs from mercey Williams</td>
</tr>
<tr>
<td>Item 5</td>
<td>KG5</td>
<td>DNA Freezer</td>
<td>Extract from buccal swabs from Jamier Matthews</td>
</tr>
<tr>
<td>Item 6</td>
<td>KG6</td>
<td>DNA Freezer</td>
<td>Extract from buccal swabs from Pierre Joshlin</td>
</tr>
</tbody>
</table>

Refer to original and supplemental case files dated April 5, 2007 and May 7, 2007 for information related to screening and extraction of all listed items above.

D.2.3 Results, Opinions, and Interpretations

- Add **Lab Item X: and the description of the item** as a header for each paragraph describing the conclusions for that item.
- With the exception of the use of presumed or surreptitious samples, **Items will be left in the order** that the LIMS system places them in the body of the report which should be the same as the above table. Presumed or surreptitious samples will be listed first. **Note:** The results associated with reference standards may also be listed prior to the evidence samples based on analyst discretion.
- Where applicable, manually spell “Epithelial Fraction” or “Sperm Fraction” to the body of the report for each sample that underwent a differential extraction (e.g. Item 3.1-EF).
- Custom descriptions created in “Evidence Exams” in worksheets can be truncated within the body of the report.
- Volunteer samples can be combined into one statement in the body of the report.
- The evidence disposition statement is set for the entire laboratory and may not be modified.
- While drafting new reports, add the following statement to every reported generated with the exception of CODIS reports, reports issued by outsourcing laboratories, and CODIS entry reports performed outside of LIMS by clicking the box:

  ---This report does not constitute the entire case file. The case file may be comprised of worksheets, images, analytical data and other documents---

- When referring to samples previously worked from a different case, cross referenced lab case # or report (e.g. original report) using a * or other symbol to reflect this (e.g. Item 1*). This should point the reader to the table which will summarize what the symbol is referring to.

D.2.4 Table of Items Received, Not Examined

When physical custody is taken of items of evidence which were not specifically requested for examination (e.g., one sealed brown paper bag containing five individually sealed manila envelopes, each containing an item of evidence, or a single package containing a gun, magazine, and ammunition where only the gun is requested, etc.) or were not tested due to workflow (e.g., evidence items contained in a sexual assault kit not tested due to scenario), a separate table will be **manually created** in the report below the “Results, Opinions, and Interpretations” section. This table will document those items which were received but were not examined at this time.

The table will appear as follows, to include the use of the disclaimer “The following evidence item(s) was (were) received, but not examined for the purposes of this report.”
This table will include the following information, as applicable: Impound package #, Impound Item #, Lab Item #, Description (may be generic ACE description), and the “Results, Opinions, and Interpretations” of “Received, not examined”

The symbol “---” may be utilized in the table when an item of evidence does not have a Impound Package #, Impound Item #, or Lab Item # associated with it.

Previously tested sexual assault kits which are re-opened in order to retrieve the victim reference standard and packages containing items which were previously tested will be included in the table of items received, but not examined with a reference that these items were previously examined and reported. The date of the original report for the testing results of these items will be included below the table.

Example:

<table>
<thead>
<tr>
<th>Lab Item #</th>
<th>Impound Pkg #</th>
<th>Impound Item #</th>
<th>Description</th>
<th>Results, Opinions, and Interpretations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item 123</td>
<td>Demanellian - SAK</td>
<td>Cervical swabs</td>
<td></td>
<td>Received, not examined</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>Envelopes reported to contain fingernail snippings and oral swabs***</td>
<td></td>
<td>Received, not examined</td>
</tr>
</tbody>
</table>

**Items previously examined by Bode Cellmark Forensics. Refer to the original report issued by Bode Cellmark Forensics dated 10/9/2019 for related information.

***Items previously received but not examined by Bode Cellmark Forensics.

Note: Items which were removed from an item of evidence during examination (e.g. hairs removed from a shirt and re-packaged in a petri dish, etc.) or areas of possible staining identified on an item of evidence during examination that could be tested in the future will remain in the main results table under the entry for the examined item. These items will not be included in the table of items received, not examined.

D.3 Technical and Administrative Reviews

If the technical reviewer needs to re-sign off on the review in LIMS because the administrative reviewer flagged something to be changed in the report or worksheet, save a copy of the old draft report and/or notes so the technical reviewer can verify the changes were necessary.