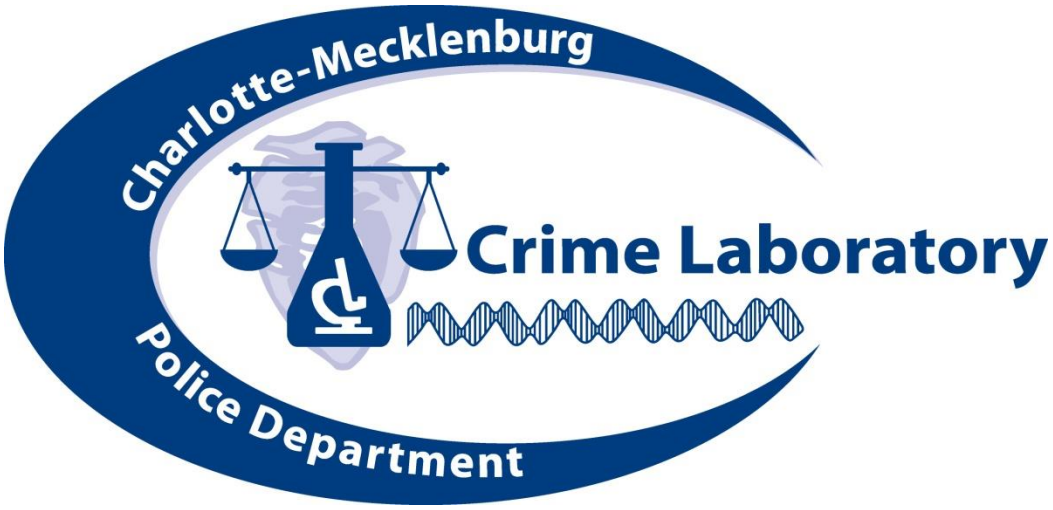

CMPD Crime Laboratory Biology

Standard Operating Procedures

Foreword

General

This manual is designed to ensure that the CMPD Biology Laboratory produces scientifically valid and professionally acceptable casework analysis. It is designed to be used in conjunction with other safety, standard operating procedure, and training manuals to achieve this goal. The CMPD Crime Laboratory Quality Manual and Policy Manuals are our parent manuals and will be followed along with this manual.

The CMPD Biology SOP Manual is a compilation primarily of guidelines provided by Applied Biosystems, Promega, Qiagen, professional publications and presentations, and CMPD validation studies.

Throughout this document, references are made to other CMPD manuals in the laboratory and anyone consulting this manual for information regarding the function of the lab should have access to the other manuals. The other manuals include but are not limited to:

1. Biology QA Manual
2. Biology Training Manual
3. CMPD Crime Laboratory Safety Manual
4. CMPD Crime Laboratory Quality Manual (QM)
5. CMPD Policy and Procedures and Field Volumes

If there are any questions regarding the contents of this or any Biology manual, they may be directed to the Chief Criminalist.

Non-Routine Procedures

This manual covers a wide variety of situations that will be encountered frequently in the analysis of forensic Biology evidence. However, not every situation that will be encountered can be addressed in this manual. Therefore, non-routine procedures will need to be performed occasionally. In the event this occurs only published protocols will be used. A small validation study may be required in some circumstances. The DNA analyst, the DNA Technical Leader, and the Chief Criminalist will discuss the situation and develop an appropriate approach. All non-routine procedures will be documented in the case file and, following the validation, will be added to the manual. Any deviations from approved SOP's must be approved by the DNA Technical Leader prior to moving on to the next step in the process.

Critical Reagents

The analyst must perform quality control on the reagents that are critical to the process (see Biology Quality Assurance Manual Section 8 for instructions).

References

References to the basic publications or validation studies are given at the end of each section. The reference list is maintained separately in the section. The articles listed at the end of each section are presented as the fundamental articles to the procedure.

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1. Evidence Screening and Stain Identification Procedures and Techniques

1.1 Visual Examination and Evidence Evaluation

1.1.1 Decontamination:

Understanding how contamination occurs and taking steps to prevent contamination are two of the crucial components of evaluating and performing DNA collection on evidence items. Every time evidence is handled, proper steps must be taken to ensure that the results of any testing are of the highest quality.

Gloves, laboratory coat, and a face mask will always be worn by the analyst when handling and collecting evidence. Prior to collecting any evidence from an item, new gloves will be worn. Gloves will be changed frequently and periodically, and must be changed between evidence items, or when they become contaminated or damaged. When donning gloves, they should always be inspected for holes or tears before continuing to work.

Two common ways analyst introduced contamination can occur are either directly or indirectly.

Direct contamination can occur when biological material such saliva, sweat or skin cells from an analyst comes into contact with an item of evidence. To ensure the evidence is not contaminated in this way, avoid talking, sneezing, and coughing over evidence.

Indirect contamination can occur when biological material present on the outside of packaging, on equipment, benches, or utensils are transferred onto the gloves of the analyst and the gloves are not changed before handling the item of evidence. To ensure that the evidence is not contaminated in this way, avoid the touching of equipment, utensils, hair, face, door handles, light switches, pens, paper, rulers, etc. without subsequently changing gloves.

Cross contamination of evidence can be introduced by the workspace. To prevent this type of contamination, it is required to clean between evidence items the bench or table area with 10% bleach solution or DNA surface decontaminant (DNA Away) followed by rinsing with water and drying with ethanol, and any reusable utensils that you are intending to use (scissors, forceps, rulers etc.) utilizing a 10% bleach solution or DNA surface decontaminant followed by a rinse of ethanol. After use, pipettes and centrifuges should be wiped cleaned with DNA Away, di water and ethanol. It is also required to place a clean new piece of butcher paper on your bench or table. Additionally, it is always good laboratory practice to organize your workspace to clearly delineate the clean space.

Anyone assisting in the collection of evidence must also wear clean gloves, a lab coat, and a face mask.

Each piece of evidence will be opened and swabbed separately. This may not be necessary for individual evidence items packaged together; however, practices will be employed to mitigate the possibility of cross contamination. When collecting swabs, always handle carefully and avoid touching the cotton tipped end to any unintended surface. All evidence should be handled as little as possible while still ensuring a complete examination and collection.

1.1.2 Notes:

Before taking evidence into custody for testing in the Biology Section, the outer packaging and seal must be examined and intact. The initials, date and CMPD code number of the individual who packaged the evidence are also required to be present. The analyst who breaks the initial seal is responsible for preservation and documentation of not only biological stains, but any possibly probative evidence present on an item. The examiner must rely on their expertise, training, experience and knowledge of the case to determine which evidence is potentially probative. Consultation with other analysts is encouraged. Such consultations are required when a potential analysis is outside the analyst's own expertise. The results of consultations with experts outside the Biology section are to be included in the case notes.

An accurate inventory of the evidence that includes notes as to labeling and packaging along with documentation of observations and descriptions are integral to the work performed by the Criminalists in the Biology Section. Attention and care should be taken with each evidence item to consistently ensure the highest quality results. The laboratory notes for an item of evidence are the opportunity to record detailed descriptions, staining, observations, tests performed, swabs collected, and any consumption required for testing. If the entirety of a stain is collected for testing this should also be noted. Written notes should also include the collection and retention of samples (e.g. swabs collected as Item 1.1 → To DNA / remaining portion of swabs packaged and retained).

It is acceptable to mark around observed staining to be tested during visual examination or to document results of testing next to each stain. This may assist the analyst in distinguishing areas to be tested. Caution must be taken to not mark through additional stains or areas that may be swabbed for testing and/or collection for additional examination(s). Any inadvertent alterations of the evidence item (such as markings or cutting through multiple layers) should also be documented.

Prior to the evaluation of a firearm for biological evidence and collection of swabs, special care will be given to safety. All firearms turned in to Property Control should be unloaded; however, it is laboratory practice and best practice for firearm safety,

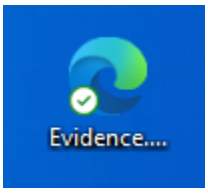
that every firearm is doublechecked. In addition to all evidence packaging requirements, all firearms received in the laboratory after May 2023 are required to have a stamp on the exterior of the box with the wording “Firearm is unloaded” with the CMPD code number of the individual that was responsible for the unloading. Additionally, a zip tie or hard plastic string through the barrel of the firearm is also now required to be included by the packager of the evidence. Notation of the presence of these requirements should be included in the Biology notes. If the exterior packaging does not include the “Firearm is unloaded” stamp with the CMPD code number, stop the examination and notify the Biology Section Supervisor, DNA Team Leader or designee. Property Control will be notified, and the firearm will be returned to Property Control. Documentation should be included in the case file and once the issue is remedied, the evidence may be recalled from Property Control for examination.

Prior to turning a sexual assault case file in for technical review, there will be an evaluation of the need for a consensual partner elimination standard to be requested. This evaluation must be performed by a DNA analyst trained in CODIS profile eligibility. An email notification to the detective must be printed and contained in the case file administrative paperwork. Refer to Biology QA 20 for specific guidance.

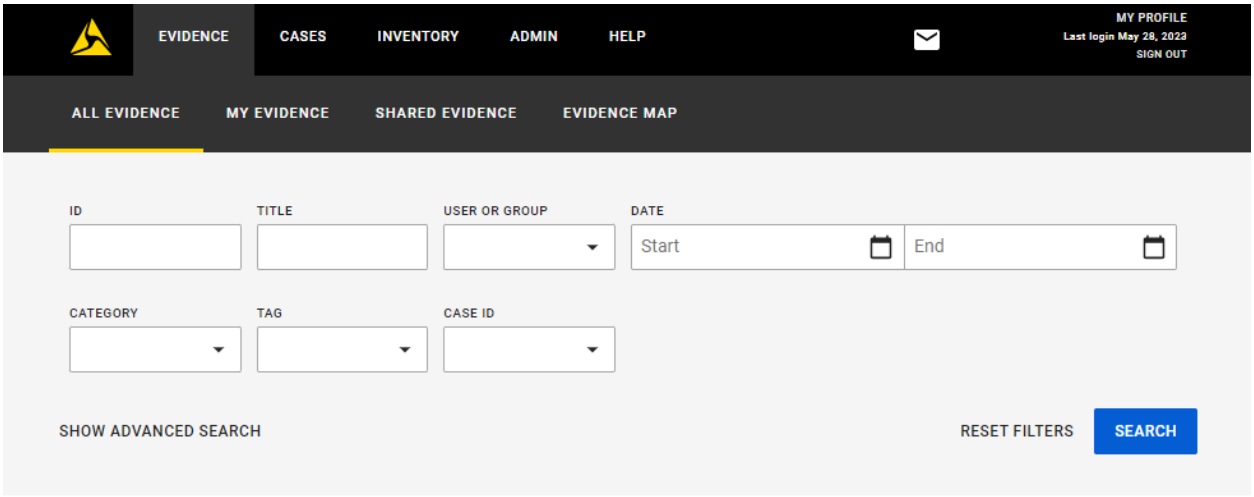
1.1.3 Photographs:

Photographs of evidence taken as part of the examination process are uploaded and retained using the secure digital evidence software provided through Axon Evidence (Evidence.com).

To upload photos to Axon Evidence (Evidence.com):
Double click on the desktop Evidence.com icon to open the software. This should automatically log you into the software. If this doesn’t automatically log you in, contact the Section Supervisor or a DNA Team Leader.



From the My Dashboard screen Click on the Evidence Tab

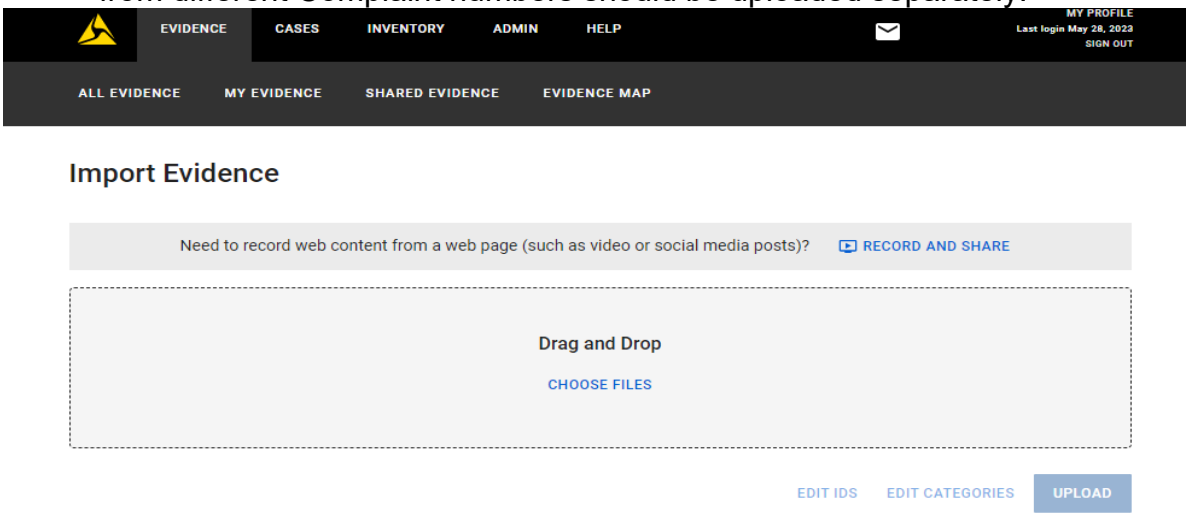


From this screen you can Upload or Review photos

To Upload Case File Photos:

1) Click on Import Evidence

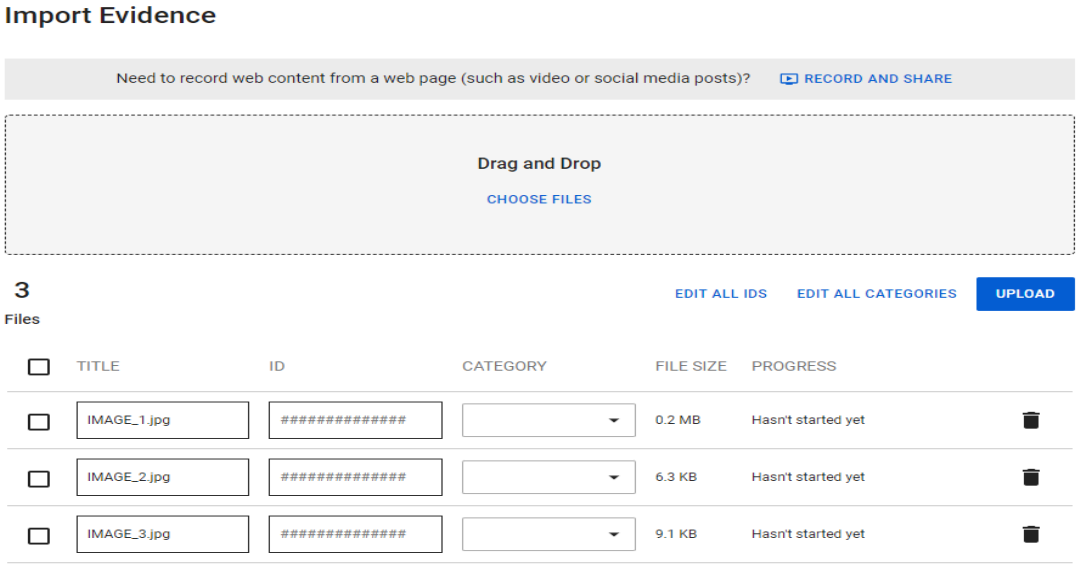
2) Click on Choose Files (you may also use the Drag and Drop feature but open the folder and select all so that the files are loaded individually) – multiple files from the same Complaint number can be selected and uploaded together, but images from different Complaint numbers should be uploaded separately.



3) Click on Edit All IDs
Type in Complaint Number (no dashes or spaces)

Click Update

4) Click on Edit All Categories
   Select Case File from the dropdown menu
   Click Update



5) Verify that all the photos are present, the correct Complaint number is listed, and the correct Category is selected.

6) Click Upload

7) Once you have verified that the photos have been uploaded, then remove them from your temporary storage (desktop/thumb drive etc.)

To Search for Photos to Review:

1) From the Evidence tab:
   Use the ID field to Search using Complaint Number
   Use the User or Group field to search by the name of the individual whom uploaded the photos
   Use both the ID and User together to narrow the search

2) Click the box next to the ID number to use the Review function to view multiple photos at once or simply click on the image name to view individually

To Correct or Delete Photos (example – photos were accidentally uploaded twice and the duplicates need to be deleted):

1) Select the duplicate photos in your Evidence tab – ensure that you are picking the correct photos
2) Change the ID from the Complaint # to “Accidental”
3) Change the Category to “Accidental” Be sure to remove Case File from Category list. It takes two steps to complete this. First step – Add “Accidental” Second step – Remove “Case File”
4) Make note of the correction or deletion of photos in your case file.
5) Once you receive the automated email stating the “The system will delete the files in 45 days.”, either include this in your case file or attach the email to the Case Info tab in PLIMS.

Nurse’s Photographs in SAK:

Photographs taken by the nurse and received as part of the sexual assault evidence collection kit will be designated by a new item number in PLIMS and transferred to Property Control. These photos are not uploaded to Evidence.com.

1) Make a PLIMS sub-item from the SAK

2) Fill out the PLIMS fields as follows:
    - Qty: 1 (all uploads will be Qty = 1 regardless of number of photos)
    - Category: Evidence
    - Packaging: CD/DVD envelope (Do not label as Digital Upload)
    - Item Type: Media
    - Attribute Type Media: Photographs
    - Description: Photo CD removed from Sexual Assault Kit (Item X)
    - The collecting officer of the SAK will be listed as the Collector

3) Place the blue envelope into a larger 10x13 evidence envelope, seal and label properly, and transfer to GBC.

4) Make sure that your notes reflect that you have removed the CD and created a new PLIMS sub-Item. In the report add the new sub-Item to the Items Received and report as not examined.

1.2 Alternate Light Source (ALS) and Microscopic Examination

High quality lighting is required for the examination of all items of evidence. It is recognized that some evidence, such as spermatozoa and hair, is too small to be studied with the naked eye; therefore, a magnifying glass, stereoscope, and microscope with magnification of at least 400 X should be readily available to the analyst.

The initial examination of any item of evidence for stains is a visual one. A thorough visual examination is the primary method used to locate suspected blood stains on most items. However, since it is often difficult to visualize blood stains on red, dark, or textured surfaces, additional examinations should be considered Presumptive testing of the area where blood might be hidden may be conducted when sample size or patterns are not considerations or when the substrate causes the light sources to be ineffective.

Bodily fluids such as semen and saliva are not easily detected by visual examination. Location of these stains on clothing or other items may be aided by the use of an alternate light source (ALS), such as the Mini-Crimescope. When semen or saliva stains fluoresce under the ALS it is due to the presence of the bacteria *Pseudomonas fluorescens* in those stains. Because the level of bacteria varies with each stain, an absence of fluorescence does not necessarily indicate that no stain is present. It is also important to note that certain synthetic fibers are highly reflective in the presence of an alternate light source and this reflectivity may mask a potential stain. Evaluating the evidence utilizing differing wavelengths, changing the lens color of the protective glasses and using different distances and angles are essential for best results when using the ALS. Therefore, presumptive testing for the presence of semen or saliva or microscopic testing for the presence of spermatozoa may follow negative visual and ALS examinations.

Caution should be used when using an alternate light source to locate stains. Since the light is in the UV part of the spectrum it will break down DNA. Any searches for biological material with the light should be of very short duration. It is also important to note that blood stains, even when mixed with other fluids, do not fluoresce under UV light. Unless it is set on “white light” the alternate light source should not be used to search for blood stains.

1.3 Evidence Collection

1.3.1 Collection Utilizing the M-Vac System

The M-Vac System is a wet-vacuum based forensic DNA collection device that sprays a sterile, DNA free solution onto the surface and simultaneously applies vacuum pressure to collect target DNA material. In the User Manual the overall instrument is referred to as the Support Equipment Case (SEC) and the M-Vac refers to the separation unit and the sampling head together. For ease of description and consistency of language, in the following protocol the instrument will be referred to as the M-Vac System.

A. Purpose and Introduction

Due to its design as a wet-vacuum, the M-Vac System may be effective at recovering trace amounts of biological material from touch DNA. As an

alternative approach to traditional evidence collection through swabbing, the M-Vac System may be particularly effective on rough porous surfaces such as rock, brick, or concrete. The M-Vac System may also be used on evidence items with large surface areas and as a secondary approach to evidence collection from items after the original collection methods have been performed.

B. Materials

- SEC (Support Equipment Case) – M-Vac System
- Separation Unit and Sampling Head – M-Vac100
- Extension Tubing 10ft or longer – TB10
- Surface Rinse Solution Bag – SRS-1000
- Sterile Pre-Filter – PF040
- Disposable Filter Unit – 168-0045

Separation units, sampling heads, sterile pre-filters, and disposable filter units are one time use and should not be reused. The surface rinse solution and extension tubing may be used on more than one item of evidence; however, no attempt should be made to sterilize or refill the surface rinse solution bag once empty. The extension tubing should be replaced if liquid overfills the collection bottle and infiltrates the tubing.

C. Procedures

Setup

1. Turn the M-Vac System power switch on.

2. Place a Surface Rinse Solution bag (" solution bag") in the pressure chamber. Make sure that the port for the bag is through the notch at the bottom of the pressure chamber door.

3. Remove a new separation unit and sampling head from its packaging. Tighten the clear, top portion of the separation unit by tightening, releasing, and tightening again. Pull the switch on the sampling head back to turn it off. Place the separation unit and sampling head in their respective holders on the M-Vac System. Avoid contaminating the solution line and vacuum port on the separation unit.

4. Attach extension tubing and solution line to the separation unit. The solution line twists onto the solution line connected to the separation unit. Push the extension tubing slightly past the raised ring on the vacuum port. Avoid contaminating either end of the solution line and extension tubing respectively. The solution line attached to the extension tubing has a "check valve" attached. Do not remove this valve when changing separation units.

5. Break the plastic tip off of the solution bag port and connect the opposite end of the solution line attached to the extension tubing by pushing and twisting the fittings together. Do not contaminate either of these fittings while connecting.

6. Connect the extension tubing to the M-Vac System by pushing it into the port labeled "to M-VAC"

7. Turn the Solution Pressure switch on. The solution bag is pressurized when the low pressure light below the Solution Pressure switch turns off.

Sample Collection

1. Turn the Vacuum switch on.

2. Ensure the top portion of the separation unit is tight and that neither the low nor high “Flow" lights are illuminated. If either light is illuminated, consult the “Troubleshooting" section of the user manual. The separation unit must remain upright during sampling. Airflow will be affected.
3. Place sampling head on evidence in a flat orientation with light contact. Avoid pushing the sampling head firmly against the evidence. The sampling head should glide along the evidence while sampling. Also, avoid tilting the sampling head from side to side or up and down because this will affect the vacuum efficiency. Evidence should be sampled top to bottom not side to side due to the orientation in which the solution is applied.

4. Push the switch on the sampling head up to begin applying the solution to the evidence. At the same time, begin moving the sampling head along the evidence at a rate of approximately 2.5 - 5 in/sec (this will vary depending on substrate). The solution may be turned on and off as needed. The vacuum will remain on; therefore, an area where solution was previously applied can be further sampled without applying additional solution. Due to the construction of the sampling head, the previous sampling head path should be overlapped slightly to ensure that the entirety of the desired area is sampled.

5. Do not fill the sample collection bottle to the top. This will compromise the separation unit and extension tubing. The sample collection bottle should only be filled to approximately 80%. If further sampling of the evidence is necessary the sample collection bottle should be replaced or the contents of the sample collection bottle should be emptied into a vacuum filter unit before sampling resumes (only after the vacuum is turned off). Do not exceed five collection bottles per area of collection.

6. Allow the evidence item on which the M-Vac System has been applied to dry before repackaging.

Filtering

If the contents of the sample collection bottle are full of debris, a pre-filtering step should be performed. This is done using a PF 040 which is a sterile, 40 micron pre-filter. The pre-filter will remove large debris. Depending on the amount of solution collected, the filtrate may need to be emptied into the vacuum filter unit during the pre-filtering process.

For liquid sample collection performed with the M-Vac System by Crime Scene Search personnel, filtering must occur within 7 days from the original collection date. If there is currently no DNA request on the Item or if the request is unassigned, the M-Vac Solution Transfer Worksheet will be completed by Biology Section personnel and retained with the case file. Prior to filing in the case file the worksheet will be administratively reviewed and then dated and initialed by the reviewer. The collected filter, once dry, will be retained as evidence in the walk-in freezer of General Biology Storage. Original Crime Scene Search packaging that has a seal with a date, initial, and code number will be retained as evidence (returned to Property Control) and not discarded.

1. Open a vacuum filter unit and attach the extension tubing.

2. Turn the Vacuum switch on and maintain the filter unit in an upright position.

3. Swirl the contents of the sample collection bottle to get the contents into solution and begin to slowly pour the solution onto the filter. Intermittently swirl the bottle throughout the process. Filtering should not take a long time. If the process is taking longer than 5 minutes, the vacuum switch should be turned off while the power switch remains on. This will allow the cooling fan to remain running. Even with the vacuum off, the vacuum pressure should remain, allowing the filtering process to continue. After an appropriate time for cooling, the vacuum may be turned on again but only after the vacuum pressure is released by disconnecting the hose.

4. It is recommended to rinse the bottle and vacuum filter funnel walls once. This can be done by dumping the contents of the filtrate bottle attached to the vacuum filter unit into the sample collection bottle or by rinsing the sample collection bottle with DI water. The vacuum filter funnel walls are rinsed using DI water.

5. The vacuum filter unit should be set aside for the filter to dry before sampling occurs.

6. Once dry, the filter should be cut out and half of it sampled for DNA with the other half retained as evidence in the walk-in freezer of General Biology Storage. The half used for DNA should be cut into smaller pieces so that it will

fit in the extraction tube. If apparent loose debris is noticed upon cutting the filter, it should be retained with the remaining portion of the filter.

Shutdown

1. Ensure that the vacuum, solution pressure, and power switches are off. There is no specific order; however, to ensure that each is turned off shutdown the vacuum, then the solution, followed by the power.

2. To maintain sterility of the solution line while not in use, leave the last used separation unit and sampling head connected to the extension tubing and solution line.

3. Wipe down the M-Vac System with DNA Away after use including the extension tubing since the exterior surface may have come into contact with an evidence item during collection.

4. Inspect the liquid trap after use. If liquid is present, the trap should be emptied per the instructions in the manufacturer's user manual.

D. Safety Considerations

The vacuum pump in the Support Equipment Case (SEC) is only intended to be used with items supplied or recommended by M-Vac Systems, Inc. Do not attempt to use the vacuum created by the SEC for any other purpose.

Prior to opening the back panel of the SEC, make sure to unplug the power cord on the SEC from the power outlet.

Do not open the solution pressure chamber door while the chamber is pressurized.

Do not run the vacuum pump for extended periods of time. This will cause the system to overheat.

Annual maintenance will be performed by the manufacturer to include calibration, inspection, ventilation filter replacement, and exhaust filter replacement.

E. Limitations

- The M-Vac System collection technique is not suitable for body fluid stained evidence items due to the size of the collection head and the pressurized solution that may spread the stain to non-stained areas.

- The M-Vac System was originally developed for collection of samples to test for microbes (bacteria and fungus, mold) and is also used for sampling meat and produce for pathogen detection. These microbes and pathogens are deleterious to nucleic acids, therefore; it's important for the filter to be dried (in a secure and clean environment) soon after collection.

- Due to the wet-vacuum design of the M-Vac System, collection from non-porous substrates is not as effective as a traditional two swab collection method.

F. Validation

This procedure is generally accepted in the forensic community and was validated internally at CMPD.

1.3.2 Collection Utilizing Swabbing and Cutting

A. Purpose and Introduction

Evaluating evidence items for the collection of biological material is a process that requires diligence on the part of the analyst to ensure the evidence item and the samples collected are kept free of contamination. It is important that the process and the results are well-documented and that the resulting sample is collected and stored appropriately. Documentation of an item is done prior to sample collection and must include a written description and may include drawings and/or photographs.

Collection of biological material occurs in two main ways 1) swabbing the surface of the evidence item to collect the material or 2) by removing a portion of the material by taking a small cutting.

Swabbing is the preferred collection method for hard non-porous items and with items that require collection across a large area with diffuse biological material.

Cutting is the preferred collection method for items with discrete staining.

Analysts may use their discretion on the collection method but should consider the substrate material, the biological material being targeted for collection, the size of the surface area, and the diffuseness of the biological material.

Before collecting a sample from an evidence item, it is necessary to know what information is being sought by the requesting officer. The information may appear in the laboratory request but there are times when additional clarification is needed from the officer.

Information gathering should be performed to the extent necessary and may include:

- If the item needs to be sent to another section of the laboratory as this may determine what portion of an item can be swabbed.
- If the item belongs to the suspect or victim or someone else.
- How the evidence item is related to the crime.
- How the evidence item may have been touched, handled, or worn by the individual.
- Where the item was located since environmental conditions can affect results and sometimes will preclude swabbing an item (e.g. fire debris containing solvents).

B. Materials

- Sterile Cotton Swab(s)
- Deionized water

C. Procedures

Swabbing:

The evidence item should first be examined for the presence of possible ridge detail and if apparent, the analyst should consult with a Latent Fingerprint Examiner prior to swabbing. This should be done regardless of a service request existing for fingerprint testing.

Once the evidence item has been thoroughly documented and visually examined, the targeted areas will be swabbed utilizing deionized water and cotton tipped sterile swabs.

Possible blood stains that have been identified on porous and/or fabric evidence through presumptive testing may be swabbed and collected with a moistened sterile swab ensuring that adequate transfer of the stain occurs (covering the majority of the swab).

Body fluid stains of semen or saliva that have been identified on the evidence through presumptive and/or confirmatory testing will not routinely be collected with swabs unless the surface is hard, non-porous, and not conducive for collection by cutting the stain from the material.

Evidence items that are being swabbed for the collection of biological material associated with possible trace DNA will utilize a two-swab method. This method entails first moistening one sterile cotton tipped swab with deionized water, swabbing over the targeted area, and then following over the same area with a second dry cotton tipped swab.

A single swab may be utilized for the collection of biological material associated with possible trace DNA when the targeted surface area is small. A moist and dry approach should still be employed. This may be accomplished by moistening a small portion of a single swab and rolling the swab from moist to dry along the surface.

Both the moist and dry swabs are to be packaged together with the outer packaging properly labeled.

The outer packaging will be sealed once the swabs are dry. The seal includes the analyst's initials, CMPD code # and the date the package is being sealed.

Cutting:

Possible blood stains on porous and/or fabric materials that do not adequately transfer by swabbing the surface may be collected by cutting the stain (or a portion of the stain) from the evidence item.

Semen and saliva stains do not visibly transfer to the swab and will be collected by cutting the stain (or a portion of the stain) from the evidence item.

D. Considerations

For firearms swabbed in possession cases, the swab collection will be limited to grips and the base of the magazine if applicable (the magazine must be the one removed from the firearm).

Additional swabs should be collected from the "switch" if it is present on the firearm or as associated evidence packaged with the firearm. Information regarding the presence of the switch may be on the service request or on the assignment page or alternatively the switch may be visually observed to be present by the examining analyst.

A firearm modified with a switch essentially makes the firearm a weapon of mass destruction.

Items that are in the possession of an individual and are collected directly from that person will not be swabbed or sent for DNA testing to establish that possession.

1.3.3 Collection from Discharged Cartridge Casings and Live Rounds

A. Purpose and Introduction

The modified ATF single swab protocol uses a rinse solution of Buffer G2 and a mixture (BTMix) of bovine serum albumin (BSA) and Glycine-Glycine-Histidine

tripeptide (GGH) dissolved in nuclease-free water. The rinse solution was optimized to moisten the surface, loosen possible cellular material on the surface, chelate metal ions, inactivate nucleases and proteases, and complement the EZ1 DNA Investigator kit chemistry. For this method, the rinse solution is dispensed on the exterior surface of the Discharged Cartridge Casing (DCC) several times, the DCC exterior surface is swabbed with one swab, and the rinse solution is added to the tube containing the swab head. The swab and rinse solution are incubated and then prepared for purification on the EZ1.

The swab and freeze procedure may be employed based on case scenario or operational necessity.

Crime Scene Search collects the DCCs found at homicide scenes using Loci tubes. These tubes allow the Firearms section to screen the DCCs while still allowing DNA processing to occur. If there are multiple DCCs that are determined to be from one gun, one of those DCCs will be selected by the Firearms section for IBIS/NIBIN entry. The DCC(s) entered into IBIS/NIBIN are not suitable for DNA testing. IBIS/NIBIN entry forms are attached under the Case Info tab in PLIMS for reference as to which DCCs were entered into IBIS/NIBIN and which DCCs were grouped as likely being fired from the same gun. If this form is not already included with the service request, the analyst should refer to the Case Info tab before processing and/or combining extracts.

DCCs collected at crime scenes other than homicides will be packaged in manilla envelopes. In the rare event DCCs from non-homicide scenes are accepted for testing, processing for DNA must occur before the DCCs are screened by the Firearms section. The analyst should not combine any extracts until receiving guidance from the Firearms section regarding results of screening.

Swabbing of DCCs may occur prior to examination by the Fingerprint Section of the laboratory. With specialized equipment latent fingerprints can be developed on the surface of the DCC even post swabbing for DNA.

Further reference to materials, procedures, and guidance for this protocol is found in SOP 2.1.2.

1.3.4 References

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- CMPD M-Vac Validation Study – 2018
- CMPD DCC (Discharged Cartridge Casing) Validation Study – 2020
- CMPD DCC Swab Material Modification Study - 2023

1.4 Processing and Retention for DNA testing of Sexual Assault Evidence Collection Kit Samples

1.4.1 Purpose and Introduction

In accordance with the 2019 North Carolina Survivor Act (enacted into law September 19, 2019) every sexual assault kit (SAK) submitted to the CMPD laboratory from a reported sexual assault will be analyzed to develop DNA profiles that may be eligible for entry into CODIS. This legislation impacts all SAKs collected on or after July 1, 2019, including those associated with non-criminal cases. All SAKs submitted to the laboratory will be completely inventoried and processed using a Direct to DNA approach. Swabs collected in the SAK as part of the SANE examination will account for the required testing; however, every swab collected, may not need to be sampled for DNA analysis.

This following guidance is provided to inform the selection of samples from the SAK for Direct to DNA testing from which a CODIS eligible DNA profile may be obtained. CODIS eligibility requirements are further detailed in the Biology QA Manual Section 20. All casework scenarios cannot be anticipated; however, this information and an understanding of the case should always be used to make the best decisions concerning sample selection and sample processing.

1.4.2 Guidance (SAK swabs – Direct to DNA)

Cutting and Inventory – Sample Selection

It is impossible to include all possible scenarios that may be encountered in forensic casework. The following guidelines are to assist the analyst in making informed decisions regarding each case they examine. There is no requirement to go back to additional swabs unless requested in a subsequent service request.

For cases where the victim can recount the details of the assault only the applicable orifice and/or body swabs need to be sampled.

> Example: For a vaginal assault, vaginal, external genitalia and anal swabs should be sampled. For an anal assault, both anal and external genitalia swabs should be sampled.

For those victims that cannot recount or convey the details of the assault and for multiple perpetrator cases, all components of the kit should be sampled. Since juveniles encompass a wide range of developmental stages, this may include younger/non-verbal juveniles along with persons with cognitive disabilities or impairment. Each case should be assessed individually.

Fingernail swabs will only be sampled in stranger cases and cases with documentation of scratching, fighting, strangulation, or potential body fluid.

Guidance for extraction type:

Differential extraction:

- Possible ejaculation (orifice swabs, underwear, body swabs)
- Penile penetration or attempted penile penetration
- Condom used
- Consensual intercourse within 120 hours

Non-differential extraction:

- Digital penetration
- Fondling
- Oral assault by perpetrator
- Fingernail swabs (unless scenario details indicate otherwise)
- Strangulation
- Kissing/sucking/licking
- Reference sample(s)

A juvenile is defined as a person 17 years old or younger and encompasses a wide range of developmental stages.

All samples cut and extracted will be quantified. Decisions on amplification will be made based on case scenario and quantitation results and may be found in Biology SOP 3.

1.4.3 Guidance (SAK underwear – Direct to DNA)

SAK underwear that is collected by the nurse from the victim as part of the SANE exam (packaged in the kit box or as a separate item) does not require DNA testing unless specific circumstances deem it to be necessary, such as underwear worn during the incident, but the SAK was collected several days later. If the kit swabs fail to provide a DNA profile for comparison the underwear may be requested by the investigator for DNA analysis through a separate service request.

In general, exceptions to the policy will be approved and documented prior to being assigned to an analyst and may occur when 1) the case is a priority, court, or expedite due to the condensed timeline associated with those cases or 2) the underwear is the only evidence collected in the SAK.

Underwear packaged in the kit box that is untested, will be inventoried, sub-itemized and repackaged separately for possible future DNA testing.

When performing testing, dependent on case scenario, the underwear should be swabbed for trace DNA collection. For example, children's underwear or underwear from a forcible fondling or underwear from an attempted sexual assault from a stranger should be swabbed. These swabs will be collected after viewing with the ALS and prior to AP press out. It may not be necessary to perform DNA testing on the collected swabs depending on the results of the AP press out and DNA testing of stains. It is not necessary to collect trace DNA swabs in all cases; however, all tested SAK underwear will at least be visibly screened for staining, viewed using an ALS, and AP screened (press out) to select an area for DNA testing or to establish the absence of stains for DNA testing. Testing of SAK underwear may be concluded when negative AP screening results are obtained. If a differential extraction is performed, a slide will be made during differential extraction but does not need to be examined.

1.4.4 Guidance (Additional Collections – Pubic Combings, Microscopic Slides, and other collections (non-swabs) – Direct to DNA)

The pubic comb and pubic combings are not required to be tested, and the envelope containing these items is not required to be opened if not being tested.

Cases with no external genitalia swabs collected may be an exception depending on case scenario. Cases in which an oral assault was alleged, no semen was indicated, and no external genitalia swabs were collected should have the comb swabbed due to the possible transfer of saliva or skin cells to the pubic hair as part of the assault. If the

pubic combings envelope is opened to swab the pubic combings comb, possible/apparent hairs should be noted and reported. If no possible/apparent hairs are observed, it should be noted and reported.

If the pubic combings are the only collection in the SAK, the comb will be swabbed and any apparent hairs with root material may be DNA tested.

The microscopic slides prepared as part of the SANE exam and typically packaged with the orifice swabs are not required to be examined or tested unless the microscopic slides are the only collection in the SAK. Testing of the microscopic slides will consist of a swabbing of the slide surface with a moistened swab followed by a dry swab. The collected swabs will then be DNA tested. Differential extractions should be performed.

Tampons, sanitary napkins, and other various non-swab collections will be inventoried, sub-itemized and repackaged separately for possible future DNA testing.

1.4.5 Guidance (Serology testing – SAK Direct to DNA)

Serology testing of SAK swabs is only performed if it is specifically requested and will only be performed after DNA testing is completed. Typically, serological testing will be performed on those swabs from which comparable and probative DNA profiles are obtained. When serological testing is requested, the slides that were made during the differential extraction process from the swabs that produced comparable and probative DNA profiles will be examined. Additional serological testing may be performed if specifically requested.

1.4.6 SAK Retention

After the kit has been inventoried and cut, it should be returned to Property Control unless the assignment specifies that serology testing was requested. If serology testing is requested, the kit will be transferred to GBS in case further testing is required. Once all testing is complete, the DNA analyst working the case will return the kit to Property Control.

At times, a DNA analyst may choose not to extract a sample that has been cut by the individual who has inventoried the kit. These cuttings are not required to be repackaged in the original sexual assault evidence kit. The chain of custody and documentation must be clear and the PLIMS label must be accurate. If the cutting was placed into a PrepFiler Express Lysep Column, it will be transferred to a new 1.5mL tube, labeled appropriately and packaged in an inner manila envelope followed by an outer manila envelope. The PLIMS Item Type will be changed to “Biological Other” and the PLIMS Description will be changed to “Cuttings of [swab type]”.

1.4.7 Reporting

Sexual assault kits that are inventoried and cut for Direct to DNA processing, do not require a report to be written. The accompanying inventory worksheets and any laboratory information worksheets will be included in the DNA case file. If the underwear is swabbed or tested or pubic hair combs are swabbed/hairs examined, those examinations will be performed by a qualified individual. There is no requirement for a separate report to be written by the serologist for any testing described above as part of the Direct to DNA processing of sexual assault kits. All testing performed will be reported in the DNA report. Underwear that is repackaged will be noted in the results. The DNA Analyst is responsible for ensuring all inventoried evidence is reported and returned to Property Control. All items that are part of the inventory must be reflected in the report.

When the inventory and cutting of a SAK is performed by a DNA analyst, the Group Processing information on the TR/AR form will be completed. This is to prevent a DNA analyst from receiving a case file for TR that they performed the inventory/cutting of the SAK.

1.4.8 STIMS workflow - https://www.sexualassaultkittracking.ncdoj.gov

- The individual accepting the service request shall receive the kit and add the complaint # in the format XXXXXXXXXXXXX (no dashes). If the kit is not found in STIMS, the SAKI coordinator needs to be notified.

- The DNA analyst will check the box for CODIS entry (This is only for unknown profiles of items in the kit; including items taken from the SAK, repackaged and later analyzed.)

- If there is a CODIS entry, the technical reviewer shall enter the date for CODIS entry as the TR completion date.

- The administrative reviewer shall verify all entries are correct, add the AR completion date, and send the kit back to the agency.

- If a CODIS hit is received, then the CODIS admin/backup will add the hit information.

1.5 Bloodstain Identification

A list of the procedures available to perform analysis on blood evidence is provided. If more than one type of test is performed on the same stain, each result must be recorded. Bloodstain testing is not required on known standards. Stains that test presumptively negative for the presence of blood may not require DNA testing. Case scenario and the probative nature of the collection in the

absence of a positive presumptive test should be used to decide when to proceed to DNA testing.

1.5.1 Phenolphthalin - (KM)

A. Purpose and Introduction

Phenolphthalin testing is a chemical test that is used as a presumptive test for the presence of blood. It is a very sensitive test and will detect small traces of blood. If this test is negative, the testing on that stain generally ceases. However, it is acceptable to perform further testing if the analyst has a reason to believe that a substance may be interfering with the peroxidase-like reaction.

Due to its sensitivity, the phenolphthalin test is an acceptable method for determining that blood is not likely present on a given item.

B. Materials

- Phenolphthalin or Kastle-Meyer Reagent
- 3% Hydrogen Peroxide
- Deionized water

C. Procedures

1. Test controls prior to performing tests on case samples. The positive control will be a known blood standard, while the negative control will be the reagents only. The passing of the controls will be noted on the worksheet by the date. If multiple dates are needed for analysis, all dates will be noted in the control blank on the worksheet.

2. Acceptable methods of sample collection for testing:

   i. Swab-to-swab (for use with visible staining) --Dampen a swab(s) with deionized water. The number of swabs prepared will depend on the number of sample swabs. Press one of the sterile swab(s) against each sample swab. Hold for a minimum of 10 seconds or until an obvious transfer can be seen.

   ii. Filter paper (for use with visible or non-visible staining)--Dampen the filter paper then either rub the sample swab(s) against the filter paper for a minimum of 10 seconds or until a visible transfer of stain is seen (for visible staining) or for non-visible staining press the filter paper around the sample swab(s) for a minimum of 10 seconds.

iii. Cutting (visible or non-visible staining)--A small cutting of sample may be obtained. Place the cutting on a piece of filter paper and place a drop of deionized water on the cutting.

3. Apply one or two drops of phenolphthalin (KM) reagent to each test sample.

4. Count 10 seconds then apply an equal number of drops of 3% hydrogen peroxide solution to each test sample.

5. ***Interpretation:*** A visible change of color (bright pink) within 10 seconds is considered positive. No change of color to pink within the 10 seconds *or* any change of color that is not consistent with that on the controls is considered negative.

6. If the remaining (original) stain appears to contain sufficient sample for DNA testing, dispose of the test swab. If little or no original stain remains, dry and package the test swab as a sub item.

7. Dry the evidence swabs before returning them to their original package.

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents:
Phenolphthalin is a known carcinogen if ingested in large amounts. It may cause irritation of the lungs if inhaled. If inhaled move the subject to fresh air. Avoid contact with the skin. If exposed wash with soap and water.

Zinc should not be exposed to an open flame, or heated with sulfur. Zinc waste should always be disposed of in a sealed glass container.

E. Limitations

- This test gives a chemical indication for the presence of blood. It is not a confirmatory test for blood.

- Only an immediate pink color change should be deemed a positive reaction. The reagents will turn pink in time even in the absence of blood.

- Color change following the addition of the phenolphthalin but prior to the addition of the hydrogen peroxide suggests the presence of a chemical oxidant.

- Plant peroxidases may cause a false positive reaction. These can be deactivated with heat or time. The pH of the phenolphthalin reagent however is not optimal for plant peroxidases.

- For items that are pink or may leach a pink color when swabbed, leucomalachite green (LMG) may be used.

F. Validation

This procedure is generally accepted in the forensic community. See blood references (Section 1.5.4)

1.5.2 Leucomalachite Green - (LMG) - Two Step Method

A. Purpose and Introduction

Leucomalachite green testing is a chemical test that is used as a presumptive test for the presence of blood. It is a very sensitive test and will detect small traces of blood. If this test is negative, the testing on that stain generally ceases. However, it is acceptable to perform further testing if the analyst has a reason to believe that a substance may be interfering with the peroxidase-like reaction.

Though phenolphthalin testing is the primary test used for presumptive testing for the presence of blood, LMG is useful when a red or pink color on the test substrate would make accurate reading of the phenolphthalin test impossible.

The LMG test is an acceptable method for determining that blood is not likely to be present on a given item.

B. Materials

- Leucomalachite Green Reagent (LMG)
- Glacial Acetic Acid
- Deionized water

C. Procedure

1. Test controls prior to performing tests on case sample. The positive control will be a known blood standard, while the negative control will be the reagents. The passing of the controls will be noted on the worksheet by the date. If multiple dates are needed for analysis, all dates will be noted in the control blank on the worksheet.

2. Acceptable methods of sample collection:

i. Swab-to-swab (for use with visible staining) --Dampen a swab(s) with deionized water. The number of swabs prepared will depend on the number of sample swabs. Press one of the sterile swabs against each sample swab. Hold the swab for a minimum of 10 seconds or until an obvious transfer can be seen.

ii. Filter paper (for use with visible or non-visible staining)--Dampen the filter paper then either rub the sample swab(s) against the filter paper for a minimum of 10 seconds or until a visible transfer of stain is seen (for visible staining) or for non-visible staining press the filter paper around the sample swab(s) for a minimum of 10 seconds.

iii. Cutting (visible or non-visible staining) --A small cutting of sample may be obtained. Place the cutting on a piece of filter paper and place a drop of deionized water on the cutting.

3. Apply one or two drops of leucomalachite green (LMG) reagent to each test sample.

4. Count 10 seconds then apply an equal number of drops of 3% hydrogen peroxide solution to each test sample.

5. ***Interpretation:*** A visible change of color (blue-green) within 10 seconds is considered positive. No change of color to green within the 10 seconds **or** any change of color that is not consistent with that on the controls is considered negative.

6. If the remaining (original) stain appears to contain sufficient sample for DNA testing, dispose of the test swab. If little or no original stain remains, dry and package the test swab as a sub item.

7. Dry the evidence swabs before returning them to their original package.

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents:

Leucomalachite green may cause skin, eye and lung irritation. If exposed, wash with soap and copious amounts of water.

Glacial acetic acid may cause severe irritation or burns to the eyes, skin and lungs. Wear proper protective garments and handle the reagent in a

ventilated hood. Though it is stored over zinc, the mixed reagent produces a gas that may explode. Always provide proper ventilation and refrigerate at 4°C when not in use.

Zinc should not be exposed to an open flame or heated with sulfur. Zinc waste should always be disposed of in a sealed glass container.

E. Limitations

- This test gives a chemical indication for the presence of blood. It is not a confirmatory test for blood.
- Only an immediate blue-green color change should be deemed a positive reaction.
- Color change following the addition of the leucomalachite green but prior to the addition of the hydrogen peroxide suggests the presence of a chemical oxidant.

F. Validation

This procedure is generally accepted in the forensic community. See blood references (Section 1.5.4).

1.5.3 Hematrace - ABAcard®

A. Purpose and Introduction

This procedure provides a method for the probable identification of human blood using a commercially prepared hemoglobin detection card. The ABAcard® HemaTrace test strips are manufactured by Abacus Diagnostics, Inc. The human hemoglobin present in the extract will combine with a monoclonal antihuman hemoglobin antibody that is labeled with a dye. Any antibody-antigen formed then migrates through an absorbent membrane to the test area of the strip. The test area has an immobilized polyclonal antihuman hemoglobin that will capture the Ag-Ab complex to form an Ab-Ag-Ab sandwich. The pink dye becomes visible as a band in the test region at concentrations of human hemoglobin above about 0.05 µg/ml. An internal control consisting of human hemoglobin antibody–dye conjugate cannot bind to the antibody in the test area but is captured by an antibody in the control area. A correctly functioning positive test will therefore show two pink bands, one in the test area and one in the control area. A correctly functioning negative test will show only one pink band, in the control area. If there is any problem with the test there will be no visible bands.

This test may be performed to confirm blood of probable human origin, for questions of non-human origin, or when an unexpected negative quantitation value is obtained on a presumptive positive blood sample. Such testing may be required based on the facts of the case and/or as listed on the Service Request.

B. Materials

- Extraction Buffer (included in the kit)
- OneStep ABAcard® Hematrace Test Strips

C. Procedures

1. Test controls prior to performing tests on case samples. The positive control will be a known blood standard, while the negative control will be the buffer. Negative results for controls must wait a full 10 minutes to ensure that the reagent is completely clean and there is no introduction into the test of anything that would cause non-specific reactions. The positive control must be positive within 5 minutes for a passing result. The passing of the controls will be noted on the worksheet by the date. If multiple dates are needed for analysis, all dates will be noted in the control blank on the worksheet.

2. Cut a portion from each swab present. The cutting(s) in total should amount to approximately ¼ of a swab or a small portion of a stain (approx. 2mm^2) in the buffer extraction vial. The size of the cutting depends on the stain intensity; the darker the staining the less sample needed. Incubate at room temperature for 5 minutes.

3. Add approximately 80 µl (or 2 drops with the dropper) of the extract to the "S" well of the ABAcard and allow the liquid to migrate to the top of the strip.

4. Read the sample results at 5 minutes. Positive results may be seen as soon as 1 minute. Non-specific reactions may occur after 5 minutes and may result in false positives, so all test results for samples will not exceed 5 minutes.

5. ***Interpretation:*** Examine the strip for a pink band at the test "T" and control "C" locations. For a positive result, both locations must exhibit a visible band. If only a "C" band is present, the test is negative. If only the "T" value is present, the test is invalid. False negative results may occur when the Hb concentration is extremely high, and therefore; when this is a possibility, re-test taking a smaller cutting. A test result cannot be reported unless the "C" band is present.

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

E. Limitations

- ABAcard HemaTrace is only for in-vitro detection of human hemoglobin (primates) for forensic use. It may be used for medical research but is not for diagnostic use. The test must be performed in strict accordance with these instructions to obtain accurate and reproducible results.

- Old and encrusted bloodstains should be properly extracted before use. Storage temperature of the stain may also affect the results.

- Positive results may be obtained from whole blood from the domestic ferret. However in forensic casework, the practical implications of this cross reactivity is minimal, since one can assume that the number of cases where ferret blood and higher primates may be found is low and crime scene investigation can determine if a pet ferret was possibly at the scene. The test results should be interpreted in conjunction with other information.

- Due to the extreme sensitivity of the test, trace levels of hemoglobin might be detected occasionally in body fluid samples other than blood (e.g. urine, semen stool, saliva, vaginal fluid, perspiration). However, knowing this fact, this limitation has no practical impact in the vast majority of cases.

F. Validation

This procedure is generally accepted in the forensic community and was validated by the CMPD laboratory.

1.5.4 Blood References:

- Gaensslen, R.E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*; U.S. Government Printing Office: Washington, DC, 1983.

- Lee, H.C., Identification and Grouping of Bloodstains, *Forensic Science Handbook, Vol. I*, Ed. R. Saferstein, Prentice Hall, Englewood Cliffs, NJ, 1982.

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- Tobe, S., Watson, N. & N. Daeid. Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular weight DNA, *J Forensic Sci*, 52 (1), pp. 102-109, 2007.

- Laux, D. Effects of luminol on the subsequent analysis of bloodstains. *J Forensic Sci*, 36 (5), pp 1512-1520, 1991.

- Higaki, RS and WMS Philp. A Study of the Sensitivity, Stability and Specificity of Phenolphthalein as an Indicator Test for Blood. *Can Soc Foren Sci J*. 9:97-102. 1976.

- Atkinson, C., Silenieks, E., Pearman, C.Validation of ABAcard® HemaTrace® Kits to Determine the Presence of Higher Primate Hemoglobin in Bloodstains

- Swander, C.J., Stites, J.G.Evaluation of the ABAcard® HemaTrace® for the forensic identification of human blood. MAFS Annual Meeting.

- Kristaly, A., Smith, D.A.S. Validation of the ABAcard® HemaTrace® for the rapid forensic identification of human blood.

- CMPD Internal Validation of ABAcard® Hematrace and IR

- CMPD Internal Material Modification - 2024

1.6 Seminal Stain Identification

A list of the procedures available to perform analysis on semen evidence is provided. If more than one type of test is performed on the same stain, each result must be recorded.

- Clothing, bedding, and other non-SAK items (not including underwear that is collected by the nurse from the victim as part of the SANE exam) will have presumptive testing for semen (AP testing) performed prior to sample collection for DNA testing. Sample selection for DNA testing can be benefitted by confirmatory testing. Stains with a positive AP test that are sampled for DNA testing will have the confirmation of semen attempted first through microscopic examination and then through p30 testing if the microscopic slide examination is negative.

- Penile swabs and condom swabs do not require AP testing prior to DNA processing. Differential extractions should be performed regardless of AP testing results and depending on the probative nature of the results, microscopic slides made during extraction may be examined.

- For SAK evidence in Direct to DNA cases that have serology requested, the serological tests requested will be performed if a comparable non-victim profile is

obtained. The microscopic slides created during the differential extraction may be examined first if semen serology is requested. If the extraction slide is negative, then the SAK components will be examined for the presence of semen. If both swabs and slides are present in the SAK, then both the nurse's slides and the prepared slides (from the swabs) must both be negative to be reported as negative.

- Positive AP stains may be retained in the laboratory for DNA analysis even in the absence of confirmation of semen.

- P30 is not considered a confirmatory test for semen; however, a combination of a positive AP result and a positive p30 test may be used to indicate the presence of semen. A p30 test will not be performed on samples with negative AP results, as a positive result would only indicate human proteins.

Swabs	AP+	AP+	AP+	AP +	AP-	AP-	AP-
Smear slide (if present)	Micro +	Micro – or Not tested	Micro -	Micro -	Micro +	Micro – or Not tested	Micro -
Analyst Slide	Micro – or Not prepared	Micro +	Micro -	Micro -	Micro – or Not prepared	Micro +	Micro -
P30	Not tested	Not tested	P30 +	P30 -	Not tested	Not tested	Not tested
Conclusion:	Semen detected	Semen detected	Semen indicated	No Semen detected	Semen detected	Semen detected	No semen detected

1.6.1 Acid Phosphatase – AP Spot Test (SERI)

A. Purpose and Introduction

If a stain is identified as possible semen by either visual or ALS examination, additional presumptive testing will be performed.

An acid phosphatase (AP) test is a biochemical test that is sensitive but not specific for seminal acid phosphatase (SAP), a constituent of semen. Acid phosphatase is a relatively stable enzyme found in most body fluids. Its activity in human semen is 500-1000 times greater than in any other body fluid. The presence of acid phosphatase is detected when an appropriate enzyme substrate, like alpha-naphthylphosphate, is dissolved in an acid buffer and reacts with acid phosphatase to hydrolyze to a colored product.

Because of the increased activity in human semen, a positive AP test is considered indicative of semen. The test is used as a screening tool for the

location of semen stains. As with all presumptive tests, this test is non-specific. Vaginal secretions, bacteria, fungi, and certain plants can also catalyze the reaction resulting in a positive result.

Since SAP degrades with heat and over time, and the level of initial SAP varies with the individual donor, negative results can still occur while spermatozoa and/or p30 activity may still be present. Based on experience and results of the visual and UV exams the analyst may choose to do further testing even in the absence of a positive AP result.

B. Materials

- AP Spot Test Reagent (prepared daily)
- Deionized water

C. Procedures

1. Prepare reagent and add to the reagent preparation log. Add 0.26 grams of the AP Spot Test to 10 ml of deionized water. The reagent volume may be reduced or increased as long as the ratio of AP Spot Test to deionized water remains constant. Mix the solution thoroughly. Place the AP working solution in a light protected container to minimize light exposure. Cap the AP working solution when not in use to minimize oxidation (darkening of the solution).

2. Test controls prior to performing tests on case samples. Record the passing QC both in the reagent preparation log and on the case notes worksheet. The positive control will be a known semen standard, while the negative control will be the reagents only. The passing of the controls will be noted on the worksheet by the date.

3. Acceptable methods of sample collection:

   i. Swab to Filter paper--Dampen the filter paper, then wrap around the swab and press for a minimum of 30 seconds. Each swab should be tested and recorded individually; however, will be reported collectively as positive if at least one swab tests as positive.

   ii. A combination of ALS and pressout may be used with underpants & large items. For the pressout obtain sterile filter paper large enough to cover the stain. Dampen the filter paper with deionized water and lay it over the surface to be tested. On the filter paper, mark the location of any landmarks such as seams or leg holes. Press the filter paper onto the sample for a minimum of 30 seconds. Additional time should be considered based on the evidence age and type. Flip the filter paper over onto a clean piece of paper before applying reagent.

4. Add 1 to 2 drops of AP Spot Test reagent to the filter paper. Pressouts will require sufficient reagent to cover the test surface of the filter paper.

5. Interpretation: Examine the filter paper for the development of a purple color within 3 minutes. A pink, tan, or any other color change other than purple is not a positive result and should be recorded as negative. The grading scale is explained as follows:

   Negative: no purple color change observed within 3 minutes

   Positive: a purple color change observed within 3 minutes

6. Dry the evidence swabs or clothing items before returning them to their original package.

D. Safety Considerations

Observe standard laboratory practices.

E. Limitations

- This is a presumptive test for the presence of semen. Confirmation requires further testing such as sperm identification or p30 detection.

- Allowing the reaction to proceed beyond 3 minutes may elicit a purple color in the absence of seminal fluid. This is a normal catalytic reaction that may occur without the presence of seminal fluid. Therefore, the test will not be read after 3 minutes.

- Vaginal acid phosphatase may be present which will result in a more pink than purple color change. This reaction is usually much slower (>120 seconds) than that seen with seminal acid phosphatase except in pregnant women and young pre-pubescent girls who may exhibit increased vaginal acid phosphatase levels.

- Fecal stains and certain plants may give false positive reactions.

- Since a negative AP result does not necessarily preclude the possibility that a stain contains semen, further serological testing or DNA profiling may still be attempted at the discretion of the serologist or analyst. Consideration to the age of stain and the evidence type should be given. Negative AP tests from older stains and condom swabs are two instances where microscopic slides should always be made and evaluated.

- Seminal acid phosphatase is also lost from body cavities due to drainage of fluid, swallowing, spitting or other activities.

F. Validation

This procedure is generally accepted in the forensic community. See semen references (Section 1.6.4).

1.6.2 Microscopic Examination using SERI R540 Xmas Tree Stain

A. Purpose and Introduction

After the presumptive test is performed, tests that confirm the presence of semen are available. Semen may be confirmed by microscopic identification of spermatozoa. Microscopic identification is the ideal test to confirm semen since the likelihood of obtaining genetic information is much greater if sperm are present.

B. Materials

- Kernechtrot stain (Stain A)
- Picroindigocarmine stain (Stain B)
- Microscope with 400X
- Centrifuge
- Microscopic slides
- Pipettes
- 1.5mL microcentrifuge tubes

C. Procedures

1. Stained Christmas Tree Stain – Kernechtrot Stain (Stain A) and Picroindigocarmine Stain (Stain B)

A. A small portion of the suspected stain or cuttings of approximately 5-10% of each swab(s) should be immersed in 500ul of deionized water for 15-60 minutes at room temperature and centrifuged at max speed for 5 minutes to collect the sperm cell pellet. Remove approximately 450ul of the supernatant to be discarded or retained for additional testing (p30). Re-suspend the cell pellet in no more than 50ul and place at least a representative portion of the liquid on a slide (no less than 15ul).

If the AP test is positive and the microscopic test is negative, p30 testing will be conducted.

B. Fix the stain of interest to a glass slide using heat (i.e. candle flame or hot plate).

C. Cover the fixed sample with Stain A for at least 5 minutes and then rinse

the slide with DI water.

D. Cover the fixed sample with Stain B for 15-60 seconds and then rinse the slide with 95% ethanol. Allow to dry.

E. Examine the slide at 200 X or 400 X with a light microscope and search for the presence of spermatozoa. Xylene or Xylene substitute may be used under the cover slip to minimize the viewing of background.

F. ***Interpretation:*** Spermatozoa are identified by the presence of the acrosomal cap and indicated by a red stained body and dark-outlined head. The presence of tails is not necessary to confirm spermatozoa; however, their presence should be recorded in the notes by writing out “tails”. The amount of spermatozoa present should be confirmed at 400 X and graded from few to 4+ using the following guidelines (scale refers to whole slide):

Few: fewer than 5 sperm
1+: hard to find
2+: some in some fields, easy to find
3+: many or some in most fields
4+: too numerous to count (TNTC)

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents:

E. Limitations

- This is a confirmatory test for the presence of semen. However, the absence of sperm cells does not mean semen is not present.
- Cellular material, including epithelial cells as well as yeast and bacteria, may also stain. These, however, may be easily distinguished from sperm due to the characteristic shape of the sperm cell and differential staining of the sperm heads.
- This stain is not specific for human sperm. Sperm from other species will also be stained. However, sperm from different species are relatively distinct from human sperm.

F. Validation

This procedure is generally accepted in the forensic community. See semen references (Section 1.6.4).

1.6.3 p30 Analysis - ABAcard®

A. Purpose and Introduction

A p30 test will be conducted if the presumptive test for AP is positive and the microscopic test for the presence of sperm cells is negative. A combination of a positive AP result and a positive p30 test may be used to indicate the presence of semen. A p30 test will not be performed on samples with negative AP results.

For medical or behavioral reasons, some individuals have a low sperm count (azoospermic) while others produce no sperm (aspermic). A test for the prostate specific antigen also known as p30, is a viable means of indicating semen in cases where no spermatozoa were located upon microscopic examination and presumptive testing for AP was positive.

B. Materials

- OneStep ABAcard® p30 Test Strips
- Extraction Buffer
- Deionized water
- Spin baskets
- Centrifuge

C. Procedures

1. Test controls prior to performing tests on case samples. The positive control will be a known semen standard, while the negative control will be the extraction buffer included in the p30 kit. Negative results for controls must wait a full 10 minutes to ensure that the reagent is completely clean and there is no introduction into the test of anything that would cause non-specific reactions. The positive control must be positive within 5 minutes for a passing result. The passing of the controls will be noted on the worksheet by the date. If multiple dates are needed for analysis, all dates will be noted in the control blank on the worksheet.

2. Cut a portion from each swab present. The cutting(s) in total should amount to approximately ¼ of a swab or a small portion of a stain in 500-750 µl extraction buffer in a microcentrifuge tube. Nuclease free water may be substituted for the extraction buffer if preparing a microscope slide for sperm search.

3. Allow the sample(s) to incubate at room temperature for a minimum of 30 minutes and no longer than 2 hours. Samples being incubated >2 hours should be refrigerated (2-8°C) for up to 24 hours. If refrigeration is used, the sample must be brought back to room temperature prior to proceeding with the test.

5. Place the sample in a spin basket and centrifuge for 5 minutes at 13,000 RPM.

6. Add ~80 µl (or 2 drops with the dropper) of the extract to the "S" well of the ABAcard and allow the liquid to migrate to the top of the strip.

7. Read the sample results at 5 minutes. Positive results may be seen as soon as 1 minute. Non-specific reactions may occur after 5 minutes and may result in false positives, so all test results for samples will not exceed 5 minutes.

8. ***Interpretation:*** Examine the strip for a pink band at the test "T" and control "C" locations. For a positive result, both locations must exhibit a visible band. If only a "C" band is present, the test is negative. If only the "T" value is present, the test is invalid and may need to be repeated using dilutions of the original extract. A test result cannot be reported unless the "C" band is present.

D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

E. Limitations

- p30 degrades with heat and over time

- If the concentration of the extract is extremely high, a high dose hook effect may be observed thus yielding a false negative. Extracts of stains that yield high acid phosphatase activity and negative p30 results, should be diluted approximately 1:100 and re-tested.

- There have been reports of PSA at low levels in some female body fluids. However, these reported levels are significantly lower than those observed in semen (less than 1 ng/mL versus 200,000 – 5.5 million ng/mL).

- Positive results may be obtained with male urine, which has a reported p30 mean value of 260 ng/mL. Test results should be interpreted in conjunction with AP test results and any additional information.

- This test will not be performed on samples with AP negative results, as a positive result would only indicate human proteins.

F. Validation

This procedure is generally accepted in the forensic community. See semen references (Section 1.6.4).

1.6.4 Semen References

- Gaensslen, R.E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*; U.S. Government Printing Office: Washington, DC, 1983

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- Vennemann M et al. Sensitivity and specificity of presumptive tests for blood, saliva and semen. Forensic Sci Med Pathol (2014) 10:69-75.
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- Seri Methods Manual Brentamine Reaction February 11, 2015.
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- Kristaly, A et al., *Validation of the OneStep ABACard® PSA Test for the Rapid Forensic Identification of Semen*, presented at the Spring 1999 Meeting of the Southern Association of Forensic Scientists, Decatur, GA.
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- Schmidt, S et al., Prostate-specific antigen in female urine: A prospective study involving 217 women. *Elsevier Science* pp 717-720, 2001
- CMPD Internal Procedure ABAcard® p30 Material Modification Study - 2024
- CMPD Internal Procedure p30 Modification Study – 2014
- CMPD Internal Validation – AP Spot Test (SERI) - 2020
- CMPD Internal Validation – R540 Christmas Tree Stain (SERI) - 2023

1.7 Saliva Stain Examination

A procedure is available to perform analysis on suspected saliva evidence.

1.7.1 Phadebas® test

A. Purpose and Introduction

If a potential saliva stain is visualized or located with the alternate light source or if saliva is suspected to be present on a sample based on case information, a presumptive test for the presence of saliva should be performed. If a positive result is obtained the stains will be forwarded to DNA analysis.

Amylase testing may be performed on swabs after being forwarded directly to DNA analysis if serology is specifically requested and a comparable non-victim DNA profile was obtained.

The comb of the pubic hair combings does not need to be routinely swabbed. The comb only needs to be swabbed if the case scenario dictates. Older cases in which an oral assault was alleged, no semen was indicated, and no external genitalia swabs were collected should have the comb swabbed due to the possible transfer of saliva or skin cells to the pubic hair combings as part of the assault.

α-amylase is a component of the saliva of certain mammals, including humans. Because it is present in much higher quantities in human saliva than any other bodily fluids and is rarely detectable in domestic dogs and cats, testing for α-amylase may be used as a presumptive test for the presence of saliva on crime scene samples.

The Phadebas test is a specific test for α-amylase; however, since it is not exclusively found in saliva. It is considered a presumptive test for saliva. If the results of the testing are graded as 1+ through 4+, the sample(s) is reported as presumptively positive for saliva. A result of <1 is still indicative of α-amylase activity; however, due to the low concentration is not considered presumptively positive for saliva.

B. Materials

- Phadebas® tablets
- 0.5 M NaOH
- Sterile deionized water
- Oven
- Centrifuge

C. Procedure

1. Test controls in conjunction with case samples. The positive control will be a known saliva standard while the negative control will be the reagents only.

2. Label clear test tubes with separate identifiers for each questioned sample to be tested. In addition, label one tube as the negative control and one tube as the positive control.

3. Place a 5 mm^2 piece of the stained fabric (or total equivalent size if testing multiple cuttings) or 1/3 of a stained swab (or equivalent total amount from several swabs) into a test tube.

4. Place a 5 mm^2 piece of filter paper (or $^1/_3$ of a swab) saturated with human saliva from a donor known to have high amylase activity in the tube labeled positive control.

5. Place ¼ of a Phadebas® tablet into each tube.

6. Add 1 mL of deionized water to each tube.

7. The negative control will contain only the ¼ Phadebas® tablet and deionized water.

8. If blood is suspected, Saliva+/Blood+ and Saliva-/Blood+ controls should be included.

9. Vortex all samples and place in a 37°C incubator.

10. After 30 minutes, add 1 mL of 0.5 M NaOH to the tubes and vortex.

11. Centrifuge the tubes for 5 minutes.

12. Read result immediately using the color chart.

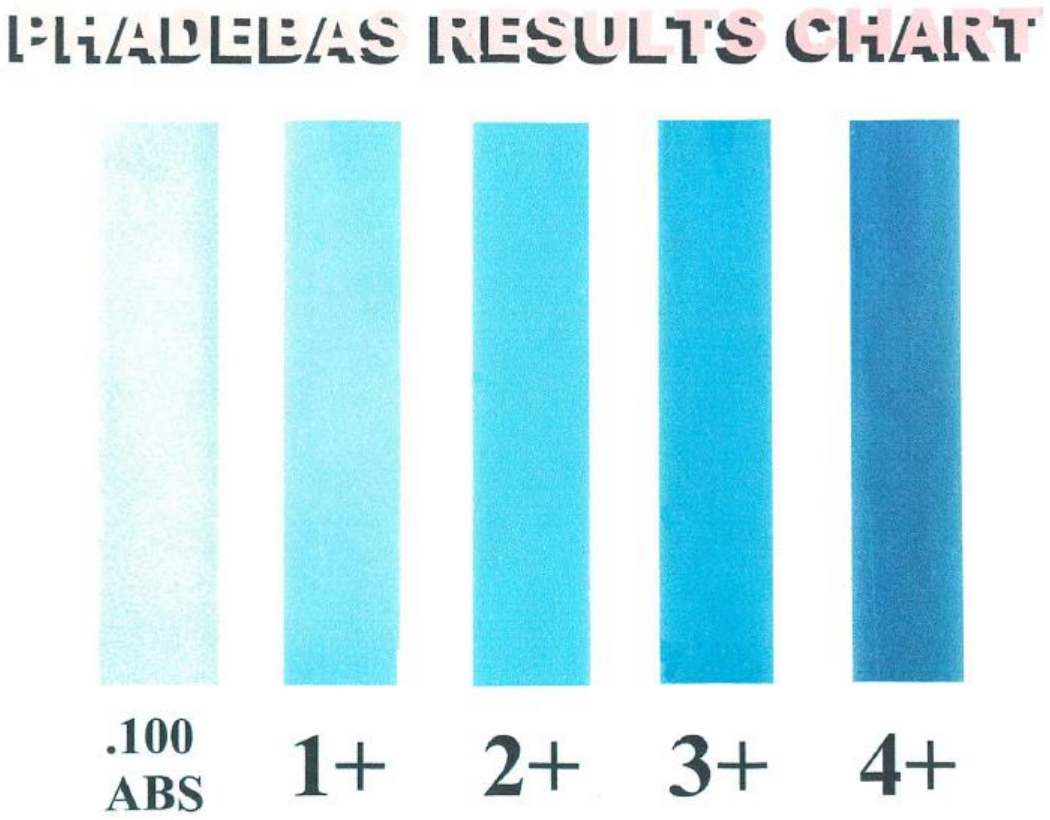
13. ***Interpretation (color chart is found below):***
    Sample and control results will be compared to an absorbance chart (ranging from 0.100 ABS to 4+). 0.100 ABS will be recorded as <1.

    A positive control passes if the supernatant intensity meets or exceeds the intensity depicted by the 1+ example on the color chart. The positive control serves to test the function of the Phadebas tablets and does not serve as a basis of comparison for any test sample. A negative control passes if the supernatant is colorless. The passing of the controls will be noted on the worksheet by the date. If multiple dates are needed for analysis, all dates will be noted in the control blank on the worksheet.

Samples with a blue supernatant that meets or exceeds the intensity depicted by the 1+ example on the color chart is indicative of a presumptive positive reaction for the presence of saliva.

Samples with a colorless supernatant (as compared to the negative control) are considered presumptively negative for the presence of saliva.

Since the Phadebas test is specific for α-amylase, a <1 result is indicative of the presence of α-amylase; however, not in a concentration sufficient to be presumptively positive for saliva. Samples that are <1 may be brought forward for DNA analysis.



D. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

Sodium Hydroxide is a corrosive chemical, which may be fatal if absorbed through the skin. It causes severe eye and skin burns, gastrointestinal burns if ingested and severe irritation if inhaled. Accidental skin or eye contact will be

treated with routine laboratory safety practices. Do not induce vomiting if ingested, seek immediate medical aid. Wear appropriate personal protective equipment and use the fume hood when using this chemical. It generates a lot of heat when put into solution so add this chemical slowly and carefully.

E. Limitations

- The Phadebas® tablet test, or other amylase tests, are sensitive, but not specific, tests for saliva. The theoretical basis relies on the fact that alpha amylase is present in much higher quantities in saliva than other bodily fluids.
- While it is often a good indicator of saliva, other bodily fluids including feces and urine contain a significantly lesser amount of amylase and may react positively with the test.

- This test gives a chemical indication for the presence of amylase. It is not confirmatory for the presence of saliva.

- When testing for amylase in the presence of semen, positive results should be interpreted with caution.

- This test is not specific for human amylase. It can also be found in higher primates, pigs, rodents, and elephants.

F. Validation

This procedure is generally accepted in the forensic community and was validated internally at CMPD.

The Phadebas interpretation chart was developed by the NC State Crime Laboratory (formerly the SBI Lab) when that laboratory validated Phadebas for case work. A spectral analysis was performed to establish a grading scale and that chart (as referenced above) was shared with the CMPD Crime Laboratory.

A scale may be applied to the Phadebas test results because according to the manufacturer (currently Magle Scientific) Phadebas testing is quantitative when spectrophotometry is employed. A standard curve of absorbance is supplied in each box of Phadebas tablets. The darker the color of the supernatant, the more concentrated the amount of amylase activity. The test is considered semi-quantitative when used to test forensic samples.

1.7.2 References:

- Gaensslen, R.E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*; U.S. Government Printing Office: Washington, DC, 1983

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- Magle Life Sciences (Magle AB), 2007. Phadebas amylase test. Directions for use. Package insert.
- Keating, S.M., & D.F. Higgs. The detection of amylase on swabs from sexual assault cases. *J Forensic Sc. Soc.*, 34 (2), pp. 89-93, 1993
- CMPD Internal Procedure Modification Study

1.8 Hair Collection and Examination

Possible/apparent hair(s) submitted or found on items of evidence submitted to the laboratory may be a source of nuclear DNA. When evidence is examined for biological substances, hairs/fibers may be collected from the item and/or left on the item for possible future examination. The observation of possible hairs or trace material will be documented in the notes and included in the analyst's report.

If there is a potential that the trace material may be lost, possible hair(s) should be collected, packaged, appropriately labeled and returned to the original container. The evidence item may also be wrapped in butcher paper to prevent loss of possible trace material and returned to the original packaging.

The screening of hairs for DNA testing is not routinely conducted; however, it may be performed in specific cases in which the only evidence in the case is hair evidence or the probative value is established.

A qualified analyst may examine a possible hair visually or microscopically for the presence of a root for nuclear DNA analysis. Microscopic analysis of possible/apparent hair(s) is only performed to determine presence of a root for suitability DNA testing. No hair comparisons, determinations of somatic origin, or race of apparent human hairs are performed.

- https://archives.fbi.gov/archives/about-us/lab/forensic-science-ommunications/fsc/july2000/deedric1.htm
- FBI — Deedrick - Forensic Science Communications - January 2004

- FBI — Forensic Science Communications - July 2004
- BASIC HAIR SCREENING – Forensic Testing Services (forensic-testing.net)

2. Extraction and Purification of DNA

A. Purpose and Introduction:

The goal of DNA extraction and purification is to recover/extract high molecular weight DNA in sufficient quantities. Using standard procedures, DNA is from various biological specimens.

B. Materials and Instrumentation:

- PrepFiler® Express Forensic DNA Extraction Kit
- PrepFiler® Express BTA Forensic DNA Extraction Kit
- EZ1/EZ2® Investigator Kit
- QIAcube
- Sardstedt/QIAcube 1.5ml tubes
- DNA-free Polyester Flock Swabs (DCC process)
- 15mL Disposable Beaker (DCC process)
- EZ1® Advanced XL with standard or low volume elution card
- Digest Buffer
- QIAgen Proteinase K
- DTT
- Thermal mixer
- Microcentrifuge
- SpeedVac™ DNA130
- Automate Express™ with variable elution volume card
- Hoods
- Pipettes (2, 10, 20, 100, 200 and 1000 µl)

C. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents:

Sodium Dodecyl Sulfate (SDS) potentially causes burns when in contact with the skin or eyes. It causes irritation if inhaled and may cause severe digestive tract irritation if ingested. Accidental skin or eye contact will be treated according to routine laboratory safety procedures (give milk or water if ingested and seek medical assistance). When preparing solutions using this chemical, proper personal protective equipment, including a dust mask or respirator, should be employed.

D. Minimum Standards and Controls

A reagent blank should be processed as the last sample of a set for each extraction protocol followed. The purpose of this control is to ensure that systemic contamination has not occurred due to the reagents used in the procedure. All reagent blanks will be processed under the same conditions as required for the sample with the least amount of DNA.

For samples extracted with Prepfiler Express:

- Evidence sample(s): the Lysep column lot number and expiration date will be recorded on the examination worksheet(s)
- Reagent blank: the Lysep column lot number and expiration date will be recorded on the extraction worksheet

The reagent blank Lysep column lot is not required to be the same lot as the evidence sample(s). All lots used will be recorded in the case file and must be QC'd prior to use.

For samples extracted with Prepfiler Express BTA kits, the lot number and expiration date of the kit used will be recorded on the examination worksheet(s) and the contents used as a discrete entity.

E. Suggested Sampling Amounts

Every effort should be made to consume no more than 1/2 of the total staining available including staining on swabs if visible. If the entire staining on swabs is cut, the sample is considered consumed and must have approval of the DNA Technical Leader or designee. The amounts listed are suggestions and the amount will depend on the type of substrate, age of the evidence, diffuseness of the staining, and the size of the staining. Multiple cuttings may be taken from larger stains. For swabs, ~1/2 of each swab will be sampled up to 4 swabs (for a total equivalent of 2 swabs). When more than 4 swabs are collected/submitted, the amount of each swab should be decreased to not exceed a total of equivalent of 2 swabs. The amount sampled must fit in the extraction tube and the material completely covered by the extraction reagents.

Liquid blood/saliva	up to 5 µl
Blood on substrate	up to 10 mm^2
Saliva on fabric	up to 10 mm^2
BF on swabs	at least an 1/8 of each swab – up to total of 2 swabs
Touch DNA swabs	½ of each swab- up to total of 2 swabs
M-Vac Collection Filter	half of the filter
Hair root	up to 5 mm
Shaft control	up to 1.0 cm

Blood standard	10 mm^2 cutting
Buccal swab	up to total of 1 swab (if more than 1 swab submitted)
Buccal scrubber	up to 3 teeth or 5mm
Buccal foam	1/8 sponge
Envelope	up to 15 mm^2
Chewing gum	1 swab
Cigarette Butt	up to 25 mm^2 (paper only, no filters)
Bone	up to 50 mg powdered
Teeth	up to 50 mg powdered

F. Other Considerations

DNA extracts will be eluted in sufficient volume to perform both an autosomal and Y-STR PCR analysis if necessary. The minimum elution volume for all extraction types except the EZ1 Large Volume protocol is 40 µl. For samples extracted with the EZ1 Large Volume protocol (samples collected from DCCs and live rounds), the minimum elution volume is 20 µl.

Alternate standards will be treated as known standards so they may be run in conjunction with other known standards. The standard elution volume associated with knowns being extracted on the Automate is 100 µl. When extracting an alternate standard, the elution volume for the extraction batch should be lowered to 40ul.

DNA extracts may be consumed during DNA testing; however, original samples (swabs, fabric cuttings, stains) will not routinely be consumed. Approximately half of the available sample will be used for DNA analysis with the remaining portion being retained. If a portion of a stain remains from the original evidence item of which a small sample was taken for DNA processing, the stain is not considered consumed, and the DNA extract material does not need to be retained.

If consumption of a sample is approved by the DNA Technical Leader or designee, the cutting will be kept in a tube labeled with the DNA # and M and created as a sub item in PLIMS. This will be noted with an asterisk (*) on the extraction sheet by the quantity sampled. The (*) indicates sample saved so no further notation needs to be made. For purposes of quantity, a tip is approximately $^1/_8$ of a swab.

Re-extractions may require consumption of the remaining portion of the swabs or stain and if consumed will be labeled as DNA # -M.

Consumption of the root portion of the hair and the collection swab from the DCC protocol do not require approval as they are consumed as part of the normal DNA processing. Additionally, due to the size of the collection material and the type of evidence, chisel tip swabs collected from fingernails may be consumed without approval. DNA extract tubes will be retained even if the volume of liquid has been

consumed.

A DNA extract sub-item will be created for each item in PLIMS that is sampled for extraction. For samples that are consumed, a DNA Extract and Material sub-item will be created for the retained consumed swab material and DNA extract. The original empty DNA extract tube must be retained.

Under most circumstances, separate DNA extracts are processed independently of one another with individually reported results; however, two DNA extracts associated with the collection of biological material from the surfaces of discharged cartridge casings (DCC) or live rounds may be combined prior to concentration and quantitation. A DNA extract from a DCC will not be combined with a DNA extract of a live round. This combining will require information from the investigator (e.g. known number of shooters, location and proximity of casings, and/or association provided by Firearm examination) as it will need to be determined prior to the SpeedVac concentration step.

DNA extracts that are combined will have a new Item created in PLIMS (not a sub-item) with the item description listing the source items from which it is created.

Hoods must be cleaned after each use with DNA Away or similar, di water and ethanol, followed by a 15 minute UV cycle. Pipettes and centrifuges are wiped cleaned with DNA Away or similar, di water and ethanol.

G. Validation

These procedures are generally accepted in the forensic community and have undergone internal validation at the CMPD.

2.1 EZ1/EZ2 DNA Investigator Kit on the EZ1 Advanced XL

The EZ1 Advanced XL utilizing either the standard trace protocol or the large-volume protocol provides fully automated purification of genomic DNA using silica-coated magnetic particles. Magnetic-particle technology eliminates tedious centrifugation steps, and the easy-to-use instrument allows purification of 1-14 samples in a single run.

The large-volume protocol allows for efficient DNA purification from dilute samples with low concentrations of DNA but also enables purification from samples that require larger volumes for thorough lysis with the ability to apply the same elution volume as the standard trace protocol.

2.1.1 EZ1 Differential Extraction with the QIAcube

The QIAcube uses advanced technology to automate the repetitive steps of a manual differential extraction enabling seamless integration of automated, low-throughput

sample prep into the laboratory workflow. Up to 12 samples can be processed per run. An 11 sample run will require a deionized water balance tube.

Collection from Sexual Assault Slides (If applicable)

1. Gently slide the coverslip off the slide. Moisten a sterile swab with di water and swab the coverslip. Using a clean surface for each sample, cut (or pull) the swab material from the applicator stick, and place it into a 1.5 mL labeled centrifuge tube.

2. Moisten a sterile swab with di water and remove any material on the slide with the swab. Using a clean surface for each sample, cut (or pull) the swab material from the applicator stick and place it into the same 1.5 mL tube as the swab of the coverslip.

Differential Procedure

3. Place the swab/cutting into a 1.5 mL micro-centrifuge tube. Prepare fresh DNA IQ Digest buffer/Proteinase K master mix. Each sample requires 490µl DNA IQ Digest buffer and 10µl of Proteinase K. Add 500µl DNA IQ Digest buffer/ Proteinase K master mix to each sample tube. Ensure that the swab /cutting is completely soaked in the buffer. Tightly close the lid.

Lysis procedure:

1. Vortex for 10 seconds, then place sample in thermal shaker. Incubate at 56°C for 1 hour at 250-900 rpm.

2. Remove from the thermal shaker and spin briefly to collect liquid at the bottom of the tube.

3. Carefully transfer the swab/cutting into the spin basket using a wood applicator stick, tweezers or pipette tip.

4. Centrifuge at maximum speed for 5 minutes.

5. Remove the spin basket and discard.

QIAcube® instrument set-up

6. Ensure waste the drawer is empty, otherwise, empty waste drawer.

7. Open instrument door.

8. Refer to *Protocol sheet* for the rotor adapter loading diagram and reagent volumes needed.

9. Refill DNA IQ Digest buffer and water jars, if necessary. DNA IQ Digest buffer should be in position 1 and water should be in position 2 in the reagent bottle rack on the instrument deck.

10. Load combined SF/NSF samples into rotor adapters in position L3 and secure the lid in the rotor adapter. (Use gloves, gloves/kimwipes, or sticks)

11. Place loaded rotor adapters into centrifuge. Rotor adapters only fit in centrifuge buckets one way. Refer to *Shaker and Rotor Loading sheet* for proper rotor adapter placement on the instrument deck centrifuge.

12. Load empty 2.0 ml QIAGEN screw-cap tubes into shaker. Refer to *Shaker and Rotor Loading sheet* for proper tube placement on the instrument deck. Remember to insert the shaker rack plugs into the positions that correspond to the tube placement.

13. Ensure all tubes are seated completely in their positions and all caps are secured either in the rotor adapter or shaker lid holder.

14. Verify 1000µl wide bore tips are loaded, caps are removed from tubes, and all screwcap tops are removed from reagent bottles. Close instrument door.

15. Turn instrument on. From the Menu, select DNA→Pipetting→Epithelial and Sperm Cell.

    a. Select:

        i. Separate and Lyse 6 mod for 6 or fewer samples.

        ii. Separate and Lyse 12A mod for greater than 6 samples.

16. Start Run.

17. At the end of the Separate and Lyse 6 mod:

    a. Remove the tubes from the shaker, capping them as they are removed. These are your epithelial cell fractions. Set aside for epithelial cell purification on the EZ-1.

    b. Remove the rotor adapters. Remove tubes from rotor adapters, capping them as they are removed, to prevent splashing. These are your sperm cell fractions.

    c. Proceed to step 20.

18. After completion of Separate and Lyse 12A mod:

a. Remove the tubes from the shaker, capping them as they are removed. These are your epithelial cell fractions. Set aside for epithelial cell purification on the EZ-1.

b. Refill DNA IQ Digest buffer and water jars, if necessary.

c. Restock 1000µl wide bore tips, as needed.

d. Select and run Separate and Lyse 12B mod to complete the wash steps of sperm cell fraction. Follow prompts on screen.

e. At the end of the Separate and Lyse 12B mod, remove the rotor adapters. Remove tubes from the rotor adapters, capping them as they are removed, to prevent splashing.

19. Following completion of the run:

a. Re-cap reagent bottles.

b. Empty waste tray.

c. Wipe down deck with kimwipe pre-moistened with deionized water followed by a kimwipe pre-moistened with ethanol.

d. Wipe down instrument door with kimwipe pre-moistened with deionized water ONLY. Do not use ethanol on the door of the instrument!

e. Turn off QIAcube®.

Epithelial Cell Preparation

20. Briefly centrifuge tube to collect liquid at the bottom of the tube.

21. The epithelial cell fraction(s) do not require pre-treatment prior to loading onto the EZ1 XL Advanced instrument. Proceed to Trace Protocol.

Slide Preparation/Sperm Cell Digestion

22. Re-suspend the pellet in the remaining 50µl of solution.

a. For all cases where sperm was not previously confirmed - spot 2-5µl of the resuspended pellet on a microscope slide and heat fix. The slide will be retained and may be examined at a later date. Christmas Tree staining does not have to be performed at this time; however, will be done if the examination of the slide is requested

Note: *The creation of a microscopic slide may be skipped if an examiner has previously identified sperm. If spermatozoa were not confirmed in the screener's report, then it will be reported in the DNA report.*

23. Prepare DNA IQ Digest buffer solution for the sperm cell fraction by mixing 160µl of DNA IQ Digest buffer and 40µl of 1M DTT per sample. If processing multiple samples a larger volume can be prepared.

24. Add 200µl of freshly prepared DNA IQ Digest buffer solution (containing DTT) to the sperm cell fraction.

25. Incubate for 10 minutes at 750 rpm at 70°C.

26. Briefly centrifuge tube to collect liquid at the bottom of the tube.

27. Cut the hinge of each 1.5ml sperm cell fraction tube prior to loading onto the instrument. Remove each cap as each sample is loaded into the rack.

28. Proceed to Trace Protocol instrument set-up

Trace Protocol- instrument set up:

1. Switch on the EZ1 instrument.

2. From the Main Menu, press 2 to display the "Manual" menu. Press 3 for Clean, and then press Start to lower the piercing unit. Open the instrument door. Wipe all prongs with a Kimwipe moistened with ethanol. Press ENT, then ESC to return to Main Menu. Repeat piercing unit cleaning between fraction runs on the instrument.

3. Press Start, select "No" for "Create a Report File

4. Select 1 for Trace protocol.

5. Choose the elution buffer and volume. Select 2 to elute in TE buffer. Select 1 to elute in 40µl or 4 to elute in 200µl.

    a. For all samples except the non-sperm cell fraction of the differential extraction the elution volume is 40µl

    b. The elution volume of the non-sperm cell fraction of the differential extraction is 200µl*
        i. * 40µl elution volume may be used for non-body and intimate non-orifice samples (e.g. breast swabs, clothing articles or bedding, etc.)

6. Press any key to continue through the text on the display to begin worktable setup

7. Open the instrument door by pushing up, then remove the cartridge rack and tip and tube rack from the instrument.

8. Invert the reagent cartridges twice to resuspend the magnetic particles and then tap to deposit any particles or liquid from the foil to the bottom of the wells.

9. Load the cartridges into the rack by sliding each reagent cartridge along the groove towards the back of the rack until it clicks into place. Make sure that the notches in the cartridges align with those in the rack. You may have to put slight pressure on the front tab of the cartridge once completely loaded to secure into rack.

10. Insert the loaded cartridge rack into the instrument.

11. Load the tip & tube rack:

    a. Fourth row: Load with sample tubes containing the lysate. This tube must be placed in the rack gently. Avoid splashing of the contents.

    b. Third row: Leave empty.

    c. Second row: Load with tips inserted into tip holders.

    d. First row: Load with labeled 1.5ml QIAGEN screw cap elution tubes.

12. Insert the loaded tip & tube rack into the front of the instrument and close the door. (Do NOT slam the door—this may harm the UV lamp filament.)

13. Press Start to begin purification.

DO NOT OPEN THE DOORS ONCE THE RUN HAS STARTED.

14. Once run is completed, press ENT to unlock door. Open the instrument door.

15. Remove and cap the elution tubes containing your purified DNA.

16. Remove the cartridge rack and the tip & tube rack and discard sample preparation waste.

17. After each run clean the cartridge rack and the tip & tube rack with di water followed by ethanol on a Kimwipe.

18. Close the instrument door.

19. Follow onscreen instructions to perform UV decontamination of worktable surfaces for 20 minutes (Selecting less than 20-minute lamp cycle may lead to reduction in lamp's lifetime). If performing UV decontamination between the non-sperm cell and sperm cell fractions, you may have to wait ~ 5 min. for the bulb to cool.

Store the samples at 2-8°C (short term storage) or freeze at -15 to -25°C (long term storage). Vortex briefly and spin in a microcentrifuge for 5 seconds prior to quantification or amplification.

2.1.2 Collection from Discharged Cartridge Casings and Live Rounds with EZ1 Extraction

The collection protocol uses a rinse solution of Buffer G2 and a mixture (BTMix) of bovine serum albumin (BSA) and Glycine-Glycine-Histidine tripeptide (GGH) dissolved in nuclease-free water. The rinse solution was optimized to moisten the surface, loosen possible cellular material on the surface, chelate metal ions, inactivate nucleases and proteases, and complement the EZ1 DNA Investigator kit chemistry.

If red-brown staining consistent with being a possible blood stain is observed on the surface of the DCC or live round, it will be presumptively tested for the presence of blood. If the presumptive test is positive, the stain will be collected. Due to the extremely sensitive nature of the DCC and live round collection process, no further processing of the DCC or live round will be done. The swab with the stain collection may be tested for DNA based on the case scenario. If the presumptive test is negative, then the DCC or live round will be processed following the steps below.

All swabs collected from DCCs or live rounds will be extracted; however, there are several points at which the analyst may pause during processing to best accommodate their workflow:

- After swabbing (swab and freeze)—these samples may be stored frozen for up to two weeks
- After extraction prior to combining of extracts. Store frozen.

Solution preparation

Prepare rinse solution as follows for each sample to be collected:

- 600µl Qiagen buffer G2
- 18µl of BTMix

A master mix in the ratio above may be created if sampling multiple casings.

Sample collection

1. UV all the 15mL disposable beakers that will be used in the collection.

2. Pick up the casing with a pair of rubber tipped forceps with tips inside of casing and pressure exerted outwards. If processing a live round, use rubber tipped forceps to grip the exterior surface.

3. Hold the casing or live round at an angle over 15mL beaker.

4. Using pipette – slowly dispense 600µl of rinse solution on one side of the casing or live round and collect the run-off in the beaker.

5. Pipette 300µl of the collected rinse solution to rinse the remaining sides of the casing or live round collecting the run-off in the beaker. Repeat rinse 8x so that entire exterior surface of the casing or live round and headstamp have been rinsed at least twice.

6. Tap casing or live round gently on side of beaker to release excess liquid.

7. Swab the exterior surface of the casing or live round with a sterile foam swab.

8. Cut swab from the handle and place the entire foam swab into a Qiagen Investigator Lyse&Spin basket-tube assembly.

9. Add all rinse solution to the Lyse&Spin basket-tube assembly containing the swab.

10. For swab and freeze (only if applicable):

    i. Create two reagent blanks (designated DNA#-A or B) by adding 600µl of the remaining G2+BTMix solution to a new Qiagen Investigator Lyse&Spin basket-tube assembly.
    ii. Place Qiagen Lyse&Spin basket-tube assembly containing the swab and solution in Freezer at -20℃.
    iii. When ready to purify samples previously swabbed and held, remove samples from freezer and thaw.
    iv. Proceed to Pre-treatment lysis procedure.

11. Proceed to pre-treatment prior to purification on the EZ1® Advanced XL instrument using the Large-Volume protocol.

12. Rinse casing with diH2O, followed by 95% ethanol to dry, prior to returning item to packaging. Ensure that the DCC is completely rinsed

with no residual diH2O and completely dry to mitigate the corrosion and possible obliteration of detail used by the Firearm Section for examination and comparison.

Pre-treatment lysis procedure:

1. Create two reagent blanks (designated DNA#-A or B) by adding 600µl of the remaining unused G2+BTMix solution to a Qiagen Investigator Lyse&Spin basket-tube assembly
    a. This step may be skipped if samples are “Swab and Freeze”. Reagent blanks should have been created prior to freezing.

2. Add 20µl Qiagen Proteinase K (20mg/ml) to each basket-tube assembly.

3. Using the thermal mixer, incubate ~3-24 hours at 56℃ at 750 rpm.

4. Centrifuge for 1.5 minutes at max speed to transfer liquid from basket to tube.
    a. Ensure no liquid remains in the basket. If liquid remains, repeat the centrifugation step until all liquid has passed through the membrane of the basket.
    b. Retain material and place in new microcentrifuge tube (or store basket in 2nd 2ml tube from Lyse&Spin package).

5. Add 300µl Buffer MTL and 1.0µl (1.0 µg/µl) carrier RNA to each tube.

6. Vortex and centrifuge briefly.

7. Cut the hinge of each Lyse&Spin tube prior to loading onto the instrument. Remove each cap as each sample is loaded into the rack.

8. Proceed to Large-Volume protocol.

Large Volume Protocol with Low Elution Volume Card

Instrument Set-up for Purification

1. Switch on the EZ1 instrument (ensure that correct low elution card is inserted)

2. From the Main Menu, press 2 to display the “Manual” menu. Press 3 for Clean, and then press Start to lower the piercing unit. Open the instrument door. Wipe all prongs with a Kimwipe moistened with ethanol. Press ENT, then ESC to return to Main Menu.

3. Press Start, select “No” for “Create a Report File”

4. Select 3 for Large Volume protocol.

5. Choose the elution buffer and volume. Select 2 to elute in TE buffer. Select 1 to elute in 20µl.

6. Press any key to continue through the text on the display to begin worktable setup

7. Open the instrument door by pushing up, then remove the cartridge rack and tip and tube rack from the instrument.

8. Invert the reagent cartridges twice to resuspend the magnetic particles and then tap to deposit any particles or liquid from the foil to the bottom of the wells.

9. Load the cartridges into the rack by sliding each reagent cartridge along the groove towards the back of the rack until it clicks into place. Make sure that the notches in the cartridges align with those in the rack. You may have to put slight pressure on the front tab of the cartridge once completely loaded to secure into rack.

10. Insert the loaded cartridge rack into the instrument.

11. Load the tip & tube rack:

    a. Fourth row: Load with sample tubes containing the lysate. These tubes must be placed in the rack gently. Avoid splashing of the contents.

    b. Third row: Leave empty.

    c. Second row: Load with tips inserted into tip holders.

    d. First row: Load with 1.5ml screw cap elution tubes. Remove caps prior to run start.

12. Insert the loaded tip & tube rack into the front of the instrument and close the door. (Do NOT slam the door—this may harm the UV lamp filament.)

13. Press Start to begin purification.

DO NOT OPEN THE DOORS ONCE THE RUN HAS STARTED.

14. Once run is completed, press ENT to unlock door. Open the instrument door.

15. Remove and cap the elution tubes containing your purified DNA.

16. Remove the cartridge rack and the tip & tube rack and discard sample preparation waste.

17. After each run clean the tip and tube rack and cartridge rack with di water followed by ethanol on a Kimwipe.

18. Close the instrument door.

19. Follow onscreen instructions to perform UV decontamination of worktable surfaces for 20 minutes (Selecting less than 20 minute lamp cycle may lead to reduction in lamp's lifetime).

20. If combining samples after purification on EZ1, proceed to Speed Vac protocol.
    i. Combine extracts (up to 2) in a new 1.5ml screw cap tube
    ii. Combine reagent blanks A and B
        1. The combined reagent blank will be designated DNA #-C
    iii. Briefly vortex and centrifuge
    iv. Remove caps and proceed to Speed Vac protocol- reconstitute to 20µl.

2.1.3 EZ1 Advanced XL References:

- EZ1 DNA Investigator Handbook
- QIAcube User Manual

- Automated differential wash protocols – Qiagen Sample and Assay Technologies

- Timken, Mark; Klein, Sonja; and Buoncristiani, Martin; Improving the efficacy of the standard DNA differential extraction method for sexual assault evidence.

- CMPD Internal Validation Study QIAcube (2019)

- CMPD Internal Validation Study QIAcube-EZ1 Advanced XL (2020)

- CMPD Internal Validation Study EZ1 Advanced – DCC (2020)

- CMPD Internal Performance Check – Low Elution Volume (2021)

2.2 PrepFiler Express™ with the AutoMate – variable elution card v1.1

Introduction: The PrepFiler Express method involves the lysis of DNA followed by the automated extraction and wash using the Automate Express. The cartridge provides the magnetic particles for DNA binding, wash buffers to purify and buffer for elution. This extraction involves the lysis of samples using the PrepFiler Express™ Forensic DNA Extraction Kit or the PrepFiler Express™ BTA Forensic DNA Extraction Kit and the extraction and purification of those samples using the AutoMate Express ™.

2.2.1 Preparation of DNA samples using PrepFiler Express™ Forensic DNA Extraction Kit

Hair - Examine the hair for the presence of sheath material. Note the possible presence of body fluids on the hair.

Wash the hairs:
For unmounted hairs: (this step is optional)

i. Fill a clean 50 mL beaker with 10-25 mL of 95-100% ethanol.

ii. Pick up a single hair and immerse the hair in the ethanol (minimum of 5 seconds).

iii. Fill a clean 50 mL beaker with 10-25 mL of sterile deionized (di) water.

iv. Transfer the hair and immerse it in the di water (minimum of 5 seconds).

v. Repeat steps i-iv for each hair to be analyzed. Clean or use new beakers for each hair.

For mounted hairs:

i. Freeze the slide in a –15° C to –20° C freezer for 20 minutes.

ii. Remove the cover slip by prying it off using a scalpel. Alternatively, the cover slip may be removed by soaking the slide in xylene for several hours after cracking the cover slip with a diamond scribe.

iii. Using a Pasteur pipette, wash away the mounting medium by squirting the slide with xylene.

iv. Pick up the hair and immerse in 10-25 mL of 95-100% ethanol.

v. Wash the hair in 10-25 mL of sterile di water.

Note: *Because hair may contain cellular material on the surface which may or may not originate from the hair donor; cut off about 0.5 – 1.0 cm of the shaft adjacent to the root portion for separate analysis as a control. It should be noted that this shaft is a substrate control and is used as an interpretation aid, not a negative control. If the hairs are not long enough, then no shaft control needs to be run, but this should be noted in the notes.*

Extraction Procedure:

1. If buffer contains precipitate, heat the solution to 37˚C, then vortex the bottle for 5 seconds.

2. Bring the thermal shaker temperature to 70˚C.

3. Prepare a fresh PrepFiler lysis solution. Each sample requires: 500 µl PrepFiler Lysis buffer and 5 µl 1 M DTT.

4. Insert a PrepFiler LySep column into a hingeless PrepFiler sample tube, then carefully transfer the sample into the PrepFiler LySep column.

5. Add 500 µl of freshly prepared PrepFiler lysis solution to the column/tube assembly.

6. Tightly close the lid of the column/tube assembly.

7. Place the column/tube assembly in a thermal shaker, then incubate it at 70˚C and 750 rpm for 40 minutes.

Note: *Do not exceed the 40 minute incubation time as this may result in salt precipitation from the lysis buffer.*

8. Centrifuge the column/tube assembly for 2 minutes at 10,000 x g to transfer the lysate to the sample tube.

9. If the volume of sample lysate collected in the sample tube is less than 300 µl:

   a. Centrifuge the column/tube assembly for an additional 5 minutes.

   b. If the volume is still less than 300 µl, then add enough PrepFiler Lysis Buffer to bring the lysate volume up to 300 µl.

   Note: *a 300 µl lysate volume is necessary for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated extraction run. Lower lysate volume may cause liquid handling problems.*

10. Complete substrate removal as follows:

    a. Carefully remove the PrepFiler LySep column from the samples tube. If there is clear lysate remaining in the PrepFiler LySep column, transfer the lysate to the sample tube.

    b. Properly dispose of the PrepFiler LySep column. Used LySep columns are potentially hazardous.

    c. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler Sample Tube (sediment may cause liquid handling problems during the automate run).

d. If you observe any salt precipitation, heat the lysate to 37 °C until the precipitate goes back into solution, then use a pipette to mix the sample lysate. Do not load any sample tube that contains precipitate onto the AutoMate Express.

1. Proceed to SOP 2.2.4.

2.2.2 Preparation of DNA samples using PrepFiler Express™ BTA Forensic DNA Extraction Kit *(for bone, teeth and adhesives)*

Sample Preparation:

Bone specimens should be stored at –20°C to –70°C until processed. Steps 1-4 of the bone processing procedure should be performed in a vented hood.

To clean: Clean Dremel bits, cutting disc and Micro-Mill grinding chamber thoroughly using 10% bleach, followed by a di water rinse and then 95% ethanol. Allow the surfaces of the Micro-Mill to dry thoroughly before use. Use a new sanding disc (if needed) for each sample.

Bone:

1. Using a separating disc affixed to a Dremel tool, or a Stryker saw, cut the bone specimen to a size of approximately 2 cm x 5 cm.

2. Using the Dremel tool with an emery disk affixed, sand the outer surface of the bone specimen such that the outer surface of the bone appears visibly free of dirt, blood, marrow, and debris (this step may be skipped if no debris is present).

3. Place the sanded bone specimen in a Micro-Mill Grinder, set the timer for two one-minute increments and grind until powder.

Tooth:

1a. Using a separating disc affixed to a Dremel tool, or a Stryker saw, cut out any filling material present on the teeth (if applicable).

2a. Rinse the teeth with sterile di water, soak in sterile di water for 20 minutes, then rinse with 95% ethanol and air dry.

3a. Place the specimen in a Micro-Mill Grinder, set the timer for two one-minute increments and grind until powder.

Extraction Procedure:

1. Insert a PrepFiler LySep column into a hingeless PrepFiler sample tube, then carefully transfer the sample (~50 mg of ground bone or tooth) into the PrepFiler LySep column.

2. Bring the thermal shaker temperature to 56˚C.

3. Prepare a fresh PrepFiler BTA lysis solution. Each sample requires: 220 µl PrepFiler BTA Lysis buffer, 3 µl 1 M DTT, and 7 µl Proteinase K.

4. Add 230 µl of freshly prepared PrepFiler BTA lysis solution to the column/tube assembly.

5. Tightly close the lid of the column/tube assembly, and vortex briefly.

6. Place the column/tube assembly in a thermal shaker, then incubate it according to sample type:

- For bone and teeth: at 56˚C, 1100 rpm for at least 2 hours up to 18 hours.
- For adhesive substrates: at 56˚C, 750 rpm for 40 minutes.

7. Centrifuge the column/tube assembly according to sample type:

- For bone and teeth: 90 seconds at 10,000 x g to transfer the lysate to the sample tube.
- For adhesive substrates: 2 minutes at 10,000 x g to transfer the lysate to the sample tube.

8. If the volume of sample lysate collected in the sample tube is less than 150 µl:
- Centrifuge the column/tube assembly for an additional 5 minutes.

- If the volume is still less than 150 µl, then add enough PrepFiler BTA Lysis Buffer to bring the lysate volume up to 150 µl.

Note: *A 150 µl lysate volume is necessary for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated extraction run. Lower lysate volume may cause liquid handling problems.*

9. Prepare sample to load:

a. Carefully remove the PrepFiler LySep column from the samples tube. If there is clear lysate remaining in the PrepFiler LySep column, transfer the lysate to the sample tube.

b. Properly dispose of the PrepFiler LySep column. Used LySep columns are potentially hazardous.

c. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler Sample Tube (sediment may cause liquid handling problems during the automate run).

d. If you observe any salt precipitation, heat the lysate to 37 °C until the precipitate goes back into solution, then use a pipette to mix the sample lysate. Do not load any sample tube that contains precipitate on the AutoMate Express.

10. Proceed to SOP 2.2.4.

2.2.3 Preparation of differential DNA samples using PrepFiler Express™ Forensic DNA Extraction Kit

Collection from Sexual Assault Slides (If applicable)

1. Gently slide the coverslip off the slide. Moisten a sterile swab with di water and swab the coverslip. Using a clean surface for each sample, cut (or pull) the swab material from the applicator stick, and place it into a 1.5 mL labeled centrifuge tube.

2. Moisten a sterile swab with di water and remove any material on the slide with the swab. Using a clean surface for each sample, cut (or pull) the swab material from the applicator stick and place it into the same 1.5 mL tube as the swab of the coverslip.

Differential Extraction Procedure:

1. Place the swab/cutting into a 1.5 mL micro-centrifuge tube. Prepare fresh Digest Buffer/Proteinase K master mix. Each sample requires 490 µl DNA IQ Digest Buffer and 10 µL of Proteinase K (20 mg/mL). Add 500 µl Digest Buffer/Proteinase K master mix to each sample tube. Ensure that the swab/cutting is completely soaked in the buffer. Tightly close the lid.

2. Vortex for 10 seconds, then place sample in thermal shaker. Incubate at 56°C for 1 hour at 250-900 rpm.

3. Remove from the thermal shaker and spin briefly to collect liquid at the bottom of the tube.

4. Carefully transfer the swab/µL into the spin basket using a wood applicator stick, tweezers or pipette tip.

5. Centrifuge at maximum speed for 5 minutes.

6. Remove the spin basket and discard.

7. Insert a PrepFiler LySep column into a hinge-less PrepFiler sample tube, then carefully transfer 350 µL of the supernatant (non-sperm cell fraction) into the PrepFiler LySep column. Store this tube for non-sperm cell DNA extraction.

8. Remove and discard all but 50 µl from the 1.5 mL micro-centrifuge tube without disturbing the sperm pellet. Set aside for the sperm cell fraction wash steps.

9. Prepare Prep Filer lysis solution for the non-sperm cell fraction by mixing 200 µL of PrepFiler lysis buffer and 2 µl of 1M DTT per sample. If processing multiple samples a larger volume can be prepared. Add 150 µl of freshly prepared PrepFiler lysis solution (containing DTT) to the non-sperm cell fraction.

10. Incubate for 10 minutes at 70°C at 750 rpm.

11. Centrifuge the column/tube assembly for 2 minutes at 10,000 x g to transfer the lysate to the sample tube.

12. If the volume of sample lysate collected in the sample tube is less than 300 µL:
    a. Centrifuge the column/tube assembly for an additional 5 minutes.

    b. If the volume is still less than 300 µL, then add enough PrepFiler Lysis Buffer to bring the lysate volume up to 300 µL.

Note: *A 300 µL lysate volume is necessary for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated extraction run. Lower lysate volume may cause liquid handling problems.*

13. Complete substrate removal as follows:

    a. Carefully remove the PrepFiler LySep column from the samples tube. If there is clear lysate remaining in the PrepFiler LySep column, transfer the lysate to the sample tube.

    b. Properly dispose of the PrepFiler LySep column. Used LySep columns are potentially hazardous.

    c. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler Sample Tube (sediment may cause liquid handling problems during the automate run).

    d. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the sample lysate. Do not load any sample tube that contains precipitate onto the AutoMate Express.

14. Proceed to SOP 2.2.4.

While non-sperm fraction is running on the Automate instrument, proceed to sperm cell fraction wash steps.

Sperm cell fraction-- Wash Steps:

15. Wash with Digest Buffer as follows:

    a. Add 500 µl Digest Buffer (not Digest buffer/Pro K solution).

    b. Vortex for 10 seconds.

    c. Spin at maximum speed for 4 minutes.

    d. Without disturbing the pellet, remove all but 50 µl of the supernatant.

    e. Repeat step **a-d** for a total of three Digest Buffer washes.

    f. After the final wash step, add 500 µl of sterile H_2O, vortex for 10 seconds and centrifuge for 5 minutes at maximum speed.

    g. Remove all but 50 µl of the supernatant and re-suspend the pellet in the remaining 50 µl of solution.

    h. For all cases where sperm was not previously confirmed - spot 2-5µl of the resuspended pellet on a microscope slide and heat fix. The slide will be retained and may be examined at a later date. Christmas Tree staining does not have to be performed at this time; however, will be done if the examination of the slide is requested.

Note: *The creation of a microscopic slide may be skipped if an examiner has previously identified sperm on this sample. If spermatozoa were not confirmed in the screener's report, then it will be reported in the DNA report.*

16. Insert a PrepFiler LySep column into a hinge-less PrepFiler sample tube, then carefully transfer the remaining 50 µl sample into the PrepFiler LySep column.

17. Prepare Prep Filer lysis solution for the sperm cell fraction by mixing 500 µl of PrepFiler lysis buffer and 8 µl of 1M DTT per sample. If processing multiple samples a larger volume can be prepared.

18. Add 500 µl of freshly prepared PrepFiler lysis solution (containing DTT) to the sperm cell fraction.

19. Incubate for 10 minutes at 750 rpm at 70°C.

20. Centrifuge the column/tube assembly for 2 minutes at 10,000 × g to transfer the lysate to the sample tube (for Automate Express).

21. If the sample lysate volume collected in the sample tube is < 300 µl:
    a. Centrifuge the column/tube assembly for an additional 5 minutes.

    b. If the volume is still less than 300 µl, then add enough PrepFiler Lysis Buffer to bring the lysate volume up to 300 µl.

Note: *A 300 µl lysate volume is necessary for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated extraction run. Lower lysate volume may cause liquid handling problems.*

22. Complete substrate removal as follows:

    a. Carefully remove the PrepFiler LySep column from the sample tube. If there is clear lysate remaining in the PrepFiler LySep column, transfer the lysate to the sample tube.

    b. Properly dispose of the PrepFiler LySep column. Used LySep olumns are potentially hazardous.

    c. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler Sample Tube (sediment may cause liquid handling problems during the automate run).

    d. If you observe any salt precipitation, heat the lysate to 37˚C until the precipitate goes back into solution, then use a pipette to mix the sample lysate. Do not load any sample tube that contains precipitate onto the AutoMate Express.

23. Proceed to SOP 2.2.4.

2.2.4 AutoMate Express Set up and Sample Loading

1. Open the instrument door by pushing up, then remove the cartridge rack and tip and tube rack from the instrument.

2. From the main menu, press **1** to display the “manual” menu. Press **3** for Clean, and then press **1** to lower the piercing unit. Open the instrument door. Wipe all prongs with ethanol on a Kimwipe. Attention should be given to avoid getting ethanol on the O-rings. Press **Esc** to get back to the main menu.

3. Shake and tap the reagent cartridges to resuspend the magnetic particles and to deposit any particles or liquid from the foil.

Note: *Check to ensure that there is no apparent crystallization in any of the cartridge wells. If observed, heat the cartridge to 37°C until the precipitate goes back into solution.*

4. Load the cartridges into the rack by sliding each reagent cartridge along the groove towards the back of the rack until it clicks into place. Make sure that the notches in the cartridges align with those in the rack.

5. Insert the loaded cartridge rack into the instrument.

6. Load the tip & tube rack:

    - Row S (fourth row): Load with PrepFiler sample tubes containing the lysate. This tube must be placed in the rack gently, or the Lysep column left on until the tube is in place. Avoid splashing of the contents.

    - Row T2 (third row): Load with AutoMate express Tips inserted into tip holders.

    - Row T1 (second row): Leave empty

    - Row E (first row): Load with labeled PrepFiler elution tubes with the caps open and secured.

7. Insert the loaded tip & tube rack into the front of the instrument and close the door.

8. Press Start, then go sequentially through the prompts pressing “enter” as each step is verified.

    - For PrepFiler Express kit, press 1
    - For PrepFiler Express BTA kit, press 2
    - Note – You must select the appropriate elution volume
    - The standard elution volume for questioned samples is **40 µl**
    - The standard elution volume of the non-sperm cell fraction of the differential extraction is **200 µl** - a 40µl elution volume may be used for non-body and intimate non-orifice samples (e.g. breast swabs, clothing articles or bedding, etc.)
    - The standard elution volume for known samples is **100 µl** – older samples that are suspected to be degraded or samples run in conjunction with an alternate standard may be eluted in **40 µl**

9. Press “START”. *A screen should show the steps and the approximate run time remaining.*

DO NOT OPEN THE DOORS ONCE THE RUN HAS STARTED.

10. At the end of the run, press the “enter” button to continue and to return to the main menu and open the instrument door.

11. Remove the cartridge rack and the tip & tube rack.

12. Remove and cap the elution tubes containing your purified DNA.

13. Close the instrument door. After each run clean the tip and tube rack with di water followed by ethanol on a Kimwipe.

Store the samples at 2-8˚C (short term storage) or freeze at -15 to -25˚C (long term storage). Vortex briefly and spin in a microcentrifuge for 5 seconds prior to quantification or amplification. If desired, the sample may be concentrated with the Microcon-100 unit.

2.2.5 PrepFiler/AutoMate References:

- JFS, 7/2012, Vol. 57 No. 4 pp.1022-1030 Liu, et al.
- Applied Biosystems by life technologies, PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits, User Guide
- Applied Biosystems by life technologies, AutoMate Express™ Instrument, User Guide
- CMPD Internal Validation Study (2012)
- CMPD Internal Material Modification – Automate Differential Extraction (2016)
- CMPD Internal Validation – Automate Variable Elution (2017)
- CMPD Internal Performance Check – Knowns (2020)

2.3 DNA Extract Cleanup and Concentration

2.3.1 Microcon Cleanup/Concentration

Introduction: The Microcon unit can be used to remove salts and other PCR inhibitors from the DNA extract through multiple washings and elutions. In addition, the Microcon unit may be used to concentrate DNA extracts. The corresponding

reagent blank has to be concentrated/cleaned up at the same time. Elution in TE at the last step should be no less than 40 µl.

1. Assemble a Microcon unit. Irradiate the assembled unit and the filtrate tubes with ultraviolet light for a minimum of 20 minutes in the extraction hood or for a minimum of 2 minutes in the UV crosslinker, then add 200 µl TE to the top of the microconcentrator. Spin at maximum speed for 3 minutes. Discard the filtrate and place the microconcentrator into a new pre-sterilized filtrate tube.

2. Approximate the amount of eluant remaining from your extract and record on the worksheet.

3. Transfer the DNA eluant to the top of the microconcentrator. If the entire volume will not fit into the microconcentrator, this step may be repeated. Retain the original extraction tube.

4. Place a spin cap on the microconcentrator and spin in a microcentrifuge at 500 x g (2475 rpm) for 5 minutes. If liquid is still visible in the concentrator, spin for additional time. Caution: Do not spin to dryness. (**Note**: *If used for concentration, spin until concentrated to the desired volume, then proceed to step 8*).

5. Carefully remove the microconcentrator unit from the assembly and discard the filtrate fluid from the filtrate cup. Return the microconcentrator to the top of the filtrate cup.

6. Remove the spin cap and add 400 µl TE to the microconcentrator. Replace the spin cap and spin the assembly in a microcentrifuge at 500 x g (2475 rpm) for 10 minutes. If liquid is still visible in the concentrator, spin for additional time. Caution: Do not spin to dryness.

7. Remove the spin cap and add a measured volume of TE or appropriate solution that is between 40 µl and 200 µl to the microconcentrator.

8. Remove the microconcentrator from the filtrate cup and carefully invert the microconcentrator onto a labeled retentate cup (the microcon 1.5 mL tube) or a 1.5 mL tube. Discard the filtrate cup.

9. Spin the assembly in a microcentrifuge at 500 x g (2475 rpm) for 5 minutes.

10. Discard the microconcentrator. Make sure that the volume is no less than 40 µl. The retentate cup (the microcon 1.5 mL tube) or a 1.5 mL tube may be used for long term storage.

11. The samples are now ready for DNA quantitation and PCR. Due to the tube transfer step, these new samples will be labeled DNA # -C.

Store the samples at 2-8˚C (short term storage) or freeze at –15 to –25˚C (long term storage). Prior to the use of samples after storage, vortex briefly and spin in a microcentrifuge for 5 seconds.

2.3.2 Microcon References:

- Anderson T, Comey CT, Fisher DL, Medintz I, Scherczinger 1997, Greenspoon S; VS Extraction, VS Mock Samples, VS Bone Extraction, VS Buccal FTA, VS PowerPlex 16, VS DNA IQ

- Microcon Operators Manual, Millipore 2000.

- Forensic DNA Typing: Biology and Technology behind the STR Markers. John M. Butler. Academic Press 2001.

2.3.3 SpeedVac Concentration

1. Select samples for SpeedVac, briefly vortex and centrifuge.

2. Turn on SpeedVac. - lid remains locked until instrument is turned on.

3. Load samples into rotor. As each sample is loaded, remove cap. Ensure the tubes are balanced to prevent damaging instrument.

4. Close lid.

5. Select program for run.
   - For 6 or fewer samples with volumes ~40µl, use Program 3.
   - For 7 or more samples, or samples with volumes >40µl, use Program 2.

   a. Press **Program 3** and verify the following parameters:
      1. Time is set to 1:00 hour.
      2. Temperature is set to “no”.

   b. Press **Program 2** and verify the following parameters:
      1. Time is set to 1:30 hours.
      2. Temperature is set to “no”.

6. Press “Auto Run”.

7. SpeedVac will beep when run is complete. Press “Stop” to silence.

8. After rotor has stopped spinning, lid will unlock. Check samples to ensure completely dry. If not dry, spin for additional time.

9. Once all samples are dry, remove each sample tube from the rotor, recapping as removing.

10. Add 20µl nuclease-free water to each tube to bring up to volume. The concentrated DNA extracts do not require the DNA # -C label since it is the same DNA extract tube originally eluted into.

11. Briefly vortex and centrifuge to ensure all DNA is in solution. Samples are now ready for quantitation/amplification and/or storage at -15 to -25℃.

12. Wipe down the interior of the Speed-Vac and the lid with a kimwipe pre-moistened with DNA Away or similar, followed by ethanol.

2.3.4 SpeedVac References:

- SpeedVac DNA130 – Thermo Fisher Scientific User Manual.

3. Quantitation Procedure

A. Purpose and Introduction

DNA analysis of forensic samples is dependent upon the quantity and quality of the DNA present in an evidentiary sample. The purpose of quantitation is to determine a reliable estimate of the amount of total human DNA and male DNA present in a sample.

The Quantifiler Trio Kit quantifies human DNA at a small autosomal target (80 bp), a large autosomal target (214 bp) and a Y-chromosome target (75 bp). Each target consists of PCR primers and dye-labeled TaqMan® probes for the amplification of multicopy genomic loci. The reporter dye (VIC, ABY, and FAM, respectively) is linked to the 5' end of the probe and a non- fluorescent quencher at the 3' end of the probe. The 5' nuclease assay process occurs during PCR amplification, during every cycle. During PCR, the TaqMan probe anneals specifically to a complementary sequence between the forward and reverse primer sites. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. AmpliTaq Gold DNA polymerase cleaves probes that are hybridized to the target. Cleavage separates the reporter dye from the quencher dye. This results in increased fluorescence by the reporter. The increase in fluorescence signal occurs only if the target sequence is complementary to the probe and is amplified during PCR.

B. Materials and Instrumentation

- Quantifiler™ Trio Kit (Life Technologies #4482910)
- Desktop computer with Life Technologies HID Software, Version 1.3
- ABI Prism 7500 SDS instrument
- Hoods
- Laptop computer with QIAgility Software, Version 4.17.1.03
- QIAgility instrument
- Consumables:
  - 1.5 mL tube (Promega: # V1231).
  - 200 µl PCR tube (Life Technologies #N8010540).
  - 2.0 mL tube (FisherBrand # 05-408-138).
  - 200 µl PCR tube (Life Technologies 96-well plate #4306737).
  - 50 µl tips (QIAgen #902-013).

C. Safety Considerations

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

D. Minimum Standards and Controls

The Quantifiler Trio kit contains a pooled male DNA standard (100 ng/ul) that is diluted to prepare five DNA standards. These standards represent the following quantities in ng/ul: 50, 5, 0.5, 0.05 and 0.005. This series of standards will be used to create the virtual standard curve during the QC process for a specific lot number of Quantifiler Trio and then will be used to create two calibrators (1/10 and 1/50) to be included in all subsequent runs.

Results are obtained through PCR amplification of samples and calibrators. The HID Real-Time PCR Analysis Software v1.3 enables the virtual standard curve created during QC of the Quantifiler Trio lot to be applied to a set of data to minimize variation between runs and user. The virtual standard curve defines the appropriate standard curve values (y-intercept and slope) for all targets applicable for a specific lot number. Once the applicable virtual standard curve is applied, the threshold cycle values (C_t) for the case samples are compared to the size curve. By comparison to the C_t values of the virtual standard curve, the software can determine the approximate concentration of the case sample DNA.

The calibrators are concurrently run with each Quantifiler Trio setup and are used to monitor the reliability and conformity of a specific data set.

In addition, the Quantifiler Trio kit contains an internal positive control (IPC) of a defined quantity that acts to confirm the efficiency of the PCR amplification. Normal detection of the IPC allows for confirmation that the amplification occurred as expected, while abnormal or no detection is an indicator of an inefficient or failed amplification and suggests inhibition.

The Quantifiler Trio chemistry is highly accurate, sensitive and specific.

- Determines the ratio of human male to female DNA, even with minute male and excess female DNA.
- <1 pg/µl limit of detection.
- Quantifies DNA concentrations from <0.005 ng/ul to >50 ng/ul (standard curve linear rage is 0.005 ng/ul to 50 ng/ul), enabling the applicability to the broad range of concentrations found in forensic samples.
- Detects human and higher primate DNA

Internal validation studies of known serial dilutions detected both male and human signal below 1 pg/ul; however, quantitation values at this level were not always reproducible and did not consistently yield autosomal or Y STR results. The Quantifiler Trio PCR primers are designed to target and amplify specific DNA sequences; however, given these observed limitations, quantitation values below or equal to 1 pg/ul (0.001 ng/ul) are considered inconclusive for the presence of

human or male DNA.

E. Other Considerations

Prior to initial amplification, all samples are required to be quantitated.

When amplifying a sample in a different or additional chemistry, the sample and associated reagent blank should be re-quantitated prior to amplification if the original quantitation was performed with a legacy method. If insufficient volume remains of the reagent blank for both quantitation and amplification, then the reagent blank may be amplified only.

Reagent blanks and corresponding samples previously dried down by a vendor lab for shipment and storage will be reconstituted with nuclease free water in the volume remaining from the original testing documentation. Quantitation should occur prior to amplification of the reconstituted samples.

When the IPC is normal (between 26.7 and 29.8) and the case sample quantity is less than or equal to 1 picogram, then no amplification has to occur on unknown samples. No notation needs to be made. If, however, an analyst continues to amplification a note will be made as to why.

If all case samples are dropped at quantitation, the corresponding reagent blank may also be dropped at quantitation as long as the quantitation value is equal to 0 ng/µl. All reagent blanks that have a quantitation value greater than 0.001 ng/µl must be amplified and all the requisite paperwork included in the case file.

An elevated IPC >29.8 suggests that inhibition has occurred, and the sample should be first diluted then cleaned using the Microcon.

The degradation index (DI) is for use as a general indicator of whether large DNA fragments may perform more poorly relative to small DNA fragments in STR reactions. It is calculated using the following formula:

Small autosomal target DNA [ng/ul] / Large autosomal target DNA [ng/ul]

Observed data compiled from casework samples supports the threshold of a DI $\geq$ 3 as the point at which single source samples begin to demonstrate drop-out resulting in partial or minimal profiles. A DI of >1 indicates that degradation may be impacting the profile; however, the completeness of a profile is not typically impacted until the DI $\geq$3.

Hoods must be cleaned after each use with DNA Away or or similar, di water and ethanol, followed by a 15-minute UV cycle. Pipettes and centrifuges are wiped cleaned with DNA Away, di water and ethanol.

F. Validation

The Quantifiler Trio technology is generally accepted in the forensic community. These procedures have undergone internal validation at the CMPD.

3.1 Manual Quantification of DNA Using Quantifiler Trio

- Storage (protected from light): -15°C to -25°C upon receipt/ 2°C to 8°C after initial use.

Preparation in pre-amplification area:

1. Allow reagents and samples to come to room temperature. If necessary, thaw the primer mix, standards, and case samples. Gently vortex and pulse-spin each before opening. Fill out the “7500 Setup” worksheet throughout the procedure, as appropriate.

2. Fresh sets of Calibrators are prepared weekly. If they have been prepared, skip to Step 3. Prepared Calibrators should be marked with the preparation date and should not be used beyond one week of preparation.

   Prepare the calibrators:
   - Label two 1.5mL tubes (one as 1/10 and one as 1/50).
   - Add 27ul of Dilution Buffer to the 1/10 tube.
   - Add 3ul of the Trio Human DNA standard to the 1/10 tube and mix.
   - Add 20ul of Dilution Buffer to the 1/50 tube.
   - Add 5ul of the 1/10 calibrator to the 1/50 tube and mix.

- *Note: Dilutions may be made in alternate volumes as long they are the same concentrations as described above.*

3. Set up a 96 well optical reaction plate. The template will have the calibrators in wells A1 and B1 (1/10 and 1/50 respectively).

4. Prepare at least the following volume of master mix:

   PCR Reaction mix = 10 µl x (number of samples +1)
   Primer mix = 8 µl x (number of samples +1)

5. Vortex the master mix thoroughly, then briefly centrifuge.

6. Dispense 18 µl of the master mix in the appropriate wells. Make certain to avoid pipetting bubbles into the well.

7. Add 2 µl of the standards or samples to the appropriate wells. Make certain to avoid pipetting bubbles into the well.

8. Seal the plate with an Optical Adhesive Cover. Spin the plate in the amp room.

9. Proceed to 3.3 for Run Preparation on the 7500

3.2 QIAgility Quantification of DNA Using Quantifiler Trio

Workbook:

1. Save the QG_Quant tab created in the Workbook as a text tab delimited (.txt) file (e.g. AGM_QG_MMDDYY).

2. Save current Workbook to flash drive (bring to QIAgility).

Preparation in pre-amplification area:

3. Allow reagents and samples to come to room temperature. If necessary, thaw the primer mix, standards, and case samples. Gently vortex and pulse spin each.

4. Fresh sets of Calibrators are prepared weekly. If they have been prepared, skip to Step 5. Prepared Calibrators should be marked with the preparation date and should not be used beyond one week of preparation.

   Prepare the calibrators:
   - Label two 1.5mL tubes (one as 1/10 and one as 1/50).
   - Add 27ul of Dilution Buffer to the 1/10 tube.
   - Add 3ul of the Trio Human DNA standard to the 1/10 tube and mix.
   - Add 20ul of Dilution Buffer to the 1/50 tube.
   - Add 5ul of the 1/10 calibrator to the 1/50 tube and mix.

   Note: Dilutions may be made in alternate volumes as long they are the same concentrations as described above.

5. Transfer 20 µl of all samples to be quantitated to a 96-well plate. The samples will be loaded in the order in which they will be quantitated (vertically starting at well A1).

6. Seal with Areaseal Sealing Film and centrifuge plate. Remove seal after centrifugation.

7. Insert the flash drive into the QIAgility computer and open current Workbook. Minimize the Workbook to be available for future use.

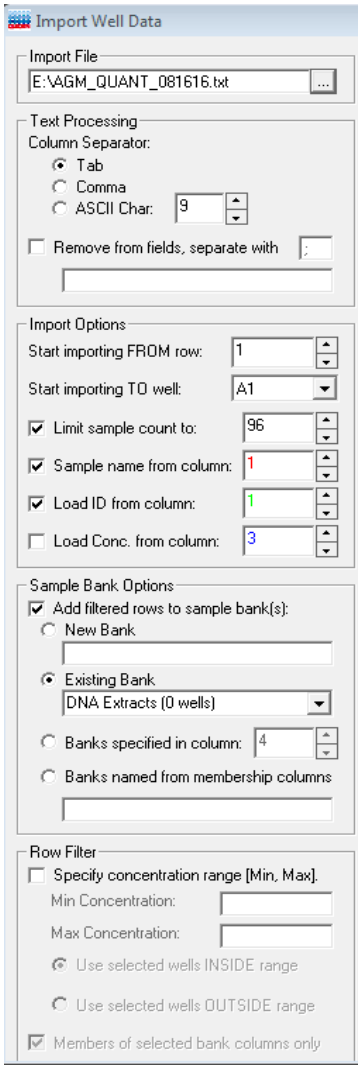
8. Turn on QIAgility.

9. Open the appropriate QT template (Trio VSC) being used by double-clicking on the desktop icon. Confirm the deck lay-out and the available tips are the same layout as on the QIAgility software and adjust as necessary. If adding a new set of tips to the tip holder, first clean the tip holder with purified water and wipe dry.

10. Once more tips (if needed) are physically added to the QIAgility deck, add tips to the software by selecting wells on deck position A1, A2, or B2, right clicking, and selecting “Set selected tips to Available”.

11. From the QIAgility software, click on C2: Sample block (so red box appears – not on individual well). On the right hand window, click “Import.” Navigate to .txt file previously saved from workbook (e.g. AGM_QG_MMDDYY).

12. Do not change the Import fields. The settings below are the default.



13. Click “Import”.

14. Click “Finish”.

15. Click Home button (house icon on tool bar).

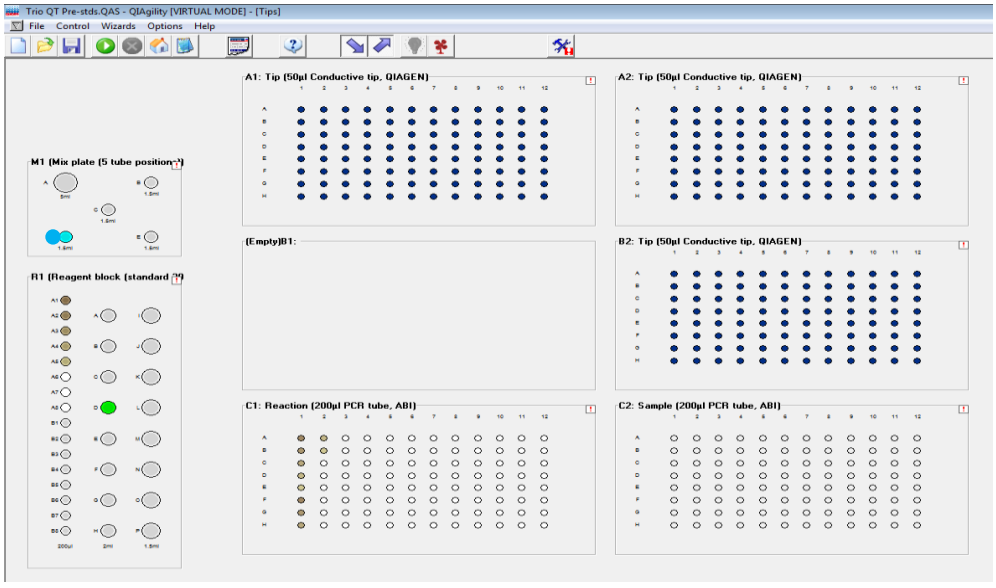
16. Click Run button (green arrow icon on tool bar).
17. Save Run File in appropriate folder (e.g. Runs 2016 Q3) as (e.g. AGM_Trio_MMDDYY).

    (C: Program Files → QIAgility → Data → Run Folder) – use “Recent Places” to navigate

18. Click on Pre-Run Report and note the user supplied volume specified in Position: R1 Well D (Trio Master Mix). Open the Workbook and fill out the “7500 Setup” worksheet with volume in µl.

19. Prepare the Master Mix in a 2.0 mL tube.

20. Set up the DNA extracts, reagents and tubes on the QIAgility deck as specified in the Pre-run report.



- 1.5 mL tube in block **M1 well D = 1/10 calibrator**
- 1.5 mL tube in block **M1 well E = 1/50 calibrator**
- 2.0 mL self-standing screw cap tube in block **R1 well D = Master Mix**
- 200 µl PCR tube in block **C1** (96 well plate)
- 200 µl PCR tube in block **C2** (96 well plate containing sample extracts)

21. Save Pre-Run Report in appropriate folder (e.g. Pre-Run Report 2016 Q3) (C: Program Files → QIAgility → Data → Pre-Run Reports) – use “Recent Places” to navigate.

22. Close the Pre-Run Report. Check the boxes as each line item is verified. Click OK. Run will commence. (Run will stop if an error occurs). If the error is "Insufficient Volume Detected" appears, then hit retry. If retry doesn't work or any other error occurs see the DNA Technical or DNA Team Leaders.

23. Once the run has completed right click on the Post-run report and select all. Right click again on Post-run report and copy.

24. Open the Workbook "QG Post-run" tab. Go to well A1, right click, and paste special as HTML. If there are more than three rows of exceptions, you must stop and wait for the assistance of the Technical Leader or a DNA Team Leader.

25. Save Post-run report in appropriate folder (e.g. Post-run report 2016 Q3) (C: Program Files → QIAgility → Data → Post-run reports) – use "Recent Places" to navigate.

26. Close Post-run report.

27. At prompt, do not save.

28. Open the Workbook "7500 setup" tab and create a 7500 import file. Save as text tab delimited (.txt.) file (e.g. AGM_MMDDYY).

29. Remove the plate and seal with an Optical Adhesive Cover. Spin the plate in the amp room.

30. Proceed to 3.3 for Run Preparation on the 7500

3.3 Run preparation on the 7500

1. Turn on the 7500

2. Press the tray door to open it.

3. Load the plate into the plate holder in the instrument. Ensure that the plate is correctly aligned in the holder.

4. Load standard 96-well plates with the notched A12 position at the top-right of the tray.

5. Close the tray door.

6. Start the ABI Prism 7500 HID v1.3 software (icon on the desktop).

7. Check that the Username is either "CMPD_1" or "CMPD_2".

8. Select the Quantifiler Trio icon from the Home screen.

9. In the Experiment Name field name the plate using the format “initials”_”MMDDYY”. For group processing or combined analysts plates, use the “QIA” prefix instead of individual initials.

10. Click on File → Import → Browse to select the file for import.

11. Click Start Import (there will be a message that the current set-up will be overrode – click OK).

12. Click on Plate Setup → Assign Targets to verify that the correct plate layout has been imported.

13. Click the green Start Run button.

14. When the run is completed proceed to Analysis and Interpretation.

3.4 Analysis and Interpretation

1. The analysis settings for Trio can be located by clicking the **Analysis Settings** button. The settings should be as follows:

   Threshold: 0.2 (IPC only, 0.1)
   Baseline Start (cycle): 3
   Baseline End (cycle): 15

2. At the completion of the Trio run a QC summary will appear. When analyzing an experiment using a virtual standard curve, all “unknown” samples on the plate will generate the IPCCT (Internal PCR Control Ct) flag by default. This is not an indication that the IPC has failed.

3. Go to the Experiment menu and select the Virtual Standard Curve tab. Select the VSC associated with the applicable Trio lot number used. Click on Add Selected VSC.

4. To analyze the samples and apply the VSC, select all the samples on the Trio run and click the green **Analyze** button.

5. Print the Standard Curve to document the slope and y-intercept values associated with the VSC used.

6. Select all the wells associated with the run and use the Export Tool to create the text file (.txt) that will be imported into the DNA Workbook.

7. Quantitation values associated with the Calibrators should be reviewed. Calibrator ranges were established through validation and represent an average observed value +/- 3 std. dev.

Calibrator Dilutions	Human	Y
1/10	7.7 – 13.7	8.4 – 13.3
1/50	1.7 – 2.5	1.7 – 2.3

All quantitation values are estimations. Some variation between runs and users has been observed and is expected. Calibrator values out of range do not necessarily fail a run. Values outside the established ranges will be flagged in the DNA Workbook. If a calibrator is flagged, approval by the DNA Technical Leader or designee to use the quantitation data for amplification is required. Approval will be based on how far out of range a value is and how many of the calibrators were out of range.

8. Interpretation of the results will be based on the following table:

Quantifiler Trio result	**IPC result**	**Interpretation**
Human > 1.0 pg	OK	STR amplification
Human < or = 1.0 pg Male > 1.0 pg	OK	STR amplification
Human and male < or = 1.0 pg	OK	**No** STR amplification
No amplification	No amplification	Invalid QPCR result – possible inhibition
Amplification (low C_t value)	No amplification	Disregard IPC
Amplification (high Ct value)	No amplification	Partial PCR inhibition
Amplification, >5 ng/µl	Amplification appears reduced	High sample concentration may suppress IPC amplification

9. If the sample(s) has a quantitation value of less than or equal to 1.0 pg (0.001 ng) no further amplification is required. Samples that are associated with a Cold Case especially those that exhibit an elevated degradation may be amplified if DNA is detected.

10. If PCR inhibition is suspected, after the sample has been diluted or cleaned using the Microcon procedure it will be re-quantified before amplification.

11. Failed runs will include the 7500 Setup Worksheet, the standard curve, and the 7500 Results Worksheet along with the reason for failing the run. All raw data files (.eds files) must be retained and provided for discovery upon request.

12. Reagent blanks with detected amounts >1.0 pg may be investigated for possible bubbles using the Multicomponent Plot and depending upon the STR results may require re-quantitation or further rework under the guidance of the DNA Technical Leader or designee.

3.5 Male screening – Sexual Assault Evidence

3.5.1 Drop at Quantitation

For evidence items associated with a sexual assault case involving a female and male(s), the M:F ratio value and the total male detected from the Trio quantitation kit will be used to determine if amplification is required. If the M:F ratio is >1:60, then samples will not generally be further processed using STR DNA typing. If the male quantity is less than or equal to 1 picogram, then samples will not be further processed using STR DNA typing.

The ratio is automatically calculated in the quant software and then is populated on the 7500 Results Worksheet if the ratio is 1.0 or greater. The total human uses the small autosomal quantitation value and male uses the Y quantitation value. The calculation in the software uses the equation Male DNA: Female DNA Ratio = Quantity of Male DNA/Quantity of Male DNA : (Quantity of Human DNA - Quantity of Male DNA)/Quantity Male DNA.

3.5.2 Sample selection for amplification

In instances when multiple samples associated with a sexual assault case are extracted and quantitated, not every sample with the minimum male DNA present is required to be amplified. Based on quant value (male to female ratio and total quantitation), case scenario, and sample type some DNA extracts may not be amplified and typed. When processing with a single perpetrator, only one extract is required to be amplified. Cases with multiple perpetrators, consensual partner, low male quantitation amounts, and involving younger juveniles may require all extracts meeting minimum quantitation values for amplification to be amplified.

Analyst discretion as to which samples are amplified and brought forward for autosomal STR DNA typing is allowed. Non-sperm cell fractions will be amplified if the corresponding sperm cell fraction is amplified and collectively counts as one sample.

Due to the condensed turn-around time for priority, expedited, and court cases, all samples meeting minimum quantitation values for amplification will be amplified.

3.5.3 Y-STR recommendation

Samples that are not processed using autosomal STR DNA typing may qualify for Y-STR testing. Samples recommended for Y-STR testing must have more than 1 picogram of male DNA. DNA extracts that were eluted in >40 µl will be concentrated and re-quantitated before amplification if the targeted male value of 1ng cannot be met through maximum amplification volume. With approval from the DNA Technical Leader or designee, samples may be concentrated and re-quantitated prior to making this recommendation.

Not all cases or samples recommended for Y-STR testing will be approved. Refer to QA 18 for case acceptance criteria.

Guidelines for recommending Y-STR testing:

- Sexual assault evidence
- No foreign male profile(s) observed in intimate samples of SAK
- >1pg/µl of male DNA
- > 1:60 male to female ratio
- Multiple perpetrators and/or consensual partner
- Consider case details
- Sufficient extract volume (including corresponding reagent blank(s) or substrate material remains

3.6 References

- Quantifiler User's manual, Life Technologies
- CMPD Quantifiler Trio Validation
- QIAgility Validation
- Automate Differential/male screening validation
- CMPD Material Modification Study – QIAgility Preparation of Quant Standards
- CMPD - Evaluation of SAK Sample Amplification Threshold
- CMPD – HID v1.3 software validation – including VSC

4. Amplification Procedures

4.1 GlobalFiler

A. Purpose and Introduction

Short tandem repeat (STR) genetic markers are polymorphic DNA loci that contain a repeated nucleotide sequence. The STR repeat unit can be from two to seven nucleotides in length. The number of repeat units at an STR locus differs from individual to individual, so alleles of many different lengths are possible. This high power of discrimination makes STRs useful for human identification purposes.

STR loci are amplified using the polymerase chain reaction (PCR). The GlobalFiler kit amplifies all the original core CODIS loci and the current core CODIS loci (TPOX, D3S1358, FGA, D5S818, CSF1PO, D7S820, D8S1179, TH01, vWA, D13S317, D16S539, D18S51, D21S11, D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, and D22S1045) as well as the autosomal loci SE33, the gender marker Amelogenin, 1 Y-STR (DYS391), and 1 insertion/deletion polymorphic marker on the Y chromosome (Y indel). The GlobalFiler allelic ladder contains 589 total alleles (343 alleles and 246 virtual bins) but does not include all known alleles, such as rare microvariants.

B. Materials and Instrumentation

- GlobalFiler Kit
- TE^{-4} Buffer
- Applied Biosystems Veriti thermal cycler
- Hood
- Laptop computer with QIAgility Software, Version 4.17.1.03
- QIAgility instrument
- Consumables:
    - 5.0 mL QIAGEN QIAgility tapered tube (QIAgen # 990552)
    - 2.0 mL tube (Fisherbrand # 05-408138)
    - 96-well plate (Life Technologies #4306737)
    - 8-well strip caps (Life Technologies #4323032)

C. Safety Considerations

Standard Laboratory Practices

Warning: Potential biohazard.

D. Minimum Standards and Control

GlobalFiler Positive Amplification Control: add 5µl Control DNA 007 (supplied in kit) and 10 µl TE^{-4} to the reaction. An amplification positive control must be run with each amplification and will be labeled with the date of the amplification. If the amplification fails or the known type is not obtained, the results from the entire amplification are inconclusive. The Positive Control will be labeled on the electropherogram as “Pos Ctrl” and the corresponding date of the amplification.

GlobalFiler Reagent Blank: Add 15 µl of the reagent blank control to the reaction even if amplifying less volume of the sample. Exceptions may be made with the DNA Technical Leader or designee approval. Refer to DNA QA Manual section 15.2 (Contamination Contingency Plan) if a pattern of peaks is present in the reagent blank.

GlobalFiler Amplification Blank: Add 15 µl of TE^{-4} to the reaction. Refer to DNA QA Manual section 15.2 (Contamination Contingency Plan) if peaks are present in the amplification blank. An amplification blank must be run with each amplification and will be labeled with the date of the amplification. The Negative Control will be labeled on the electropherogram as “Neg Ctrl” and the corresponding date of the amplification.

The optimal template quantity for samples quantified with Quant Trio and amplified with GlobalFiler is approximately 0.75 ng. Validation samples that targeted the range of 0.5 ng – 1.0 ng produced complete profiles. The amplification worksheet indicates the amount of DNA amplified.

If the DI is $\geq$3, the amplification target may be increased for single source samples or known standards. Re-extraction of degraded known standards is not required but may be considered on a case-by-case basis in consultation and approval with the DNA Technical Leader or designee.

An addition of more DNA to the amplification of mixture samples with a DI of $\geq$3 has not shown to improve the interpretability of the data obtained. If the target amount of DNA is increased, it should not exceed 2 ng. If the analyst believes that targeting more than 2 ng is necessary, then they should consult the DNA Technical Leader or designee.

The reagent blank is not required to be amplified concurrently with the samples in the associated extraction batch. The reagent blank must be amplified using the same chemistry on the same model of thermal cycler using the same cycling parameters. The reagent blank must be typed using the same model genetic analyzer and injection parameters as the rest of the extraction batch.

For reagent blanks A and B associated with DCC samples that are not combined:

- If the quantitation values for both reagent blanks is 0.0000 ng/ul, only RB-A must be amplified.
- If one or both reagent blanks show a quantitation value above 0.001 ng/ul, both must be amplified.

For reagent blanks A and B associated with DCC samples that are combined to create reagent blank C, the reagent blank will be amplified if it has a quantitation value greater than 0.001 ng/µl or if any associated extracts are amplified.

All reagent blanks that have a quantitation value greater than 0.001 ng/µl must be amplified and all the requisite paperwork included in all associated case file(s).

Reagent blanks with quantitation values >1.0 pg and no STR profile obtained will be re-quantitated under the guidance of the DNA Technical Leader or designee.

If considering amplification of a sample with a different or additional chemistry, and the associated reagent blank has been depleted, no amplification of the sample may occur if the extraction was performed after July 1, 2009.

Any reamplification of samples for reinterpretation with STRmix must also have the associated reagent blank amplified using the same chemistry on the same model of thermal cycler using the same cycling parameters. The associated reagent blank must also be run on the same model genetic analyzer and injection parameters.

If the extraction pre-dates July 1, 2009:

1) re-quant and re-amp the corresponding RB when sufficient volume exists to perform the testing; however, when necessary,
2) at least re-amp the corresponding RB if there is insufficient volume to perform a re-quant or when there is no other option,
3) neither re-quant or re-amp the corresponding RB.

Typically, the reagent blank is amplified at the maximum volume for the chemistry, however, with prior TL approval, the reagent blank may be amplified at the maximum volume as any associated sample from the extraction batch.

Positive and Negative controls associated with each sample set will be amplified using the same kit lot used for the forensic samples and will be concurrently run in the same thermal cycler, and on the same genetic analyzer.

If a sample is re-amplified for replicate interpretation utilizing STRmix, both amplifications must be run on the same models of thermal cycler and genetic analyzer (3500). There is no requirement for the same thermal cycler or 3500 instrument to be used.

Reamplifications will be designated on the Amplification Worksheet with DNA #-R. The addition of the -R is required when individual samples are selected for reamplification. The failure of an entire amplification does not require the addition of the -R for each sample but will be noted on the Amplification Worksheet that it is a reamplification.

All samples that are amplified together will be run on the same model genetic analyzer (3500). Re-injections will be run on the same model genetic analyzer as the original injection. There is no requirement for the same instrument to be used.

E. Other Considerations

Hoods must be cleaned after each use with DNA Away or similar, di water and ethanol, followed by a 15 minute UV cycle. Pipettes and centrifuges are wiped cleaned with DNA Away, di water and ethanol.

After the completion of amplification plate setup the QIAgility robot is cleaned by wiping down the block surfaces on the deck with di water and Ethanol, followed by a 15 minute UV cycle. The exterior surfaces and door are wiped down with di water only.

F. Validation

This procedure is generally accepted in the forensic community.

This procedure has undergone internal validation at the CMPD.

4.1.1 Manual Procedure GlobalFiler

- Storage (protected from light): -15°C to -25°C upon receipt/ 2°C to 8°C after initial use.

- After the resultant quantitation data has been imported from the 7500, open the “Amp Setup” tab in the Workbook.

- Verify that all the correct samples are present in the Amp Setup worksheet. These are the samples that were selected as “Yes” from the Trio Results worksheet. Samples may be dropped if the quantitation values are insufficient to be amplified (0-0.001 ng/ µl), are being targeted at a higher amount of DNA, or do not meet the criteria for M:F ratio as outlined in Biology SOP 3.5.

1. Label appropriate number of 0.2 mL MicroAmp tubes. Alternately, a 96 well plate can be used for amplification.

2. Allow samples and reagents to come to room temperature. If necessary, thaw the PCR reaction mix, and the primer mix. Gently vortex and pulse-spin each tube

before opening. Add the following reagents to a pre-sterilized microcentrifuge tube in order:

- 7.5 µl GlobalFiler Master (PCR Reaction) Mix X number of samples
- 2.5 µl GlobalFiler Primer Set X number of samples

Vortex gently in order to avoid drops of liquid on the sides and cap of the tube.

3. Add 10 µl of the combined Master Mix and Primer Set to labeled 0.2 mL amplification tubes or to appropriate plate wells.

4. Add the appropriate amount of TE^{-4} and DNA to each tube (or to appropriate plate wells) to a final volume of 25 µl. Close the tubes or seal the plate with caps.

5. Place tubes or 96 well plate in thermal cycler, tap the touch screen and press the power button in the bottom left corner – select the "globalfiler [cmpd]" protocol from the Home Screen – press "Run" in the bottom left hand corner to Start – Press "Start Run Now" to verify Start.

GlobalFiler Amplification Parameters for the Veriti Thermal Cycler (in 100% ramping mode):

- 95° C for 1 minute.
- 28 cycles, each consisting of ramp to 94° C, 10 second hold; ramp to 59° C, 1.5 minute hold.
- 1 cycle at 60° C for 10 minutes.
- Hold at 4° C.

After amplification, store tubes protected from light in an amplified DNA dedicated refrigerator for up to 2 weeks or until completion of a technical review.

4.1.2 QIAgility Procedure – GlobalFiler Only

Workbook:

1. After the resultant quantitation data has been imported from the 7500, open the "Amp Setup" tab in the Workbook.

2. Verify that all the correct samples are present in the Amp Setup worksheet. These are the samples that were selected as "Yes" from the Trio Results worksheet. Samples may be dropped if the quantitation values are insufficient to be amplified (0-0.001 ng/ µl), are being targeted at a higher amount of DNA, or do not meet the criteria for M:F ratio as outlined in Biology SOP 3.4.

3. Using the "QIAgility" buttons on the Amp Setup worksheet – Click (1) "Create QIAgility Amp Setup File" and save as text tab delimited (.txt) file (e.g.

AGM_GF_MMDDYY) – Do **not** "Add the Positive and Negative Controls" at this step. The controls will be added after the Workbook is updated from the QIAgility Post-run report.

Preparation in pre-amplification area:

4. Allow samples and reagents to come to room temperature. If necessary, thaw the PCR reaction mix, and the primer mix. Gently vortex and pulse-spin each tube before opening.

5. The sample plate with the DNA extracts should currently be on the QIAgility deck in Position C2: Sample. Remove the AeraSeal Sealing Film if applicable.

6. Insert the flash drive into the QIAgility computer and open current Workbook. Minimize the Workbook to be available for future use.

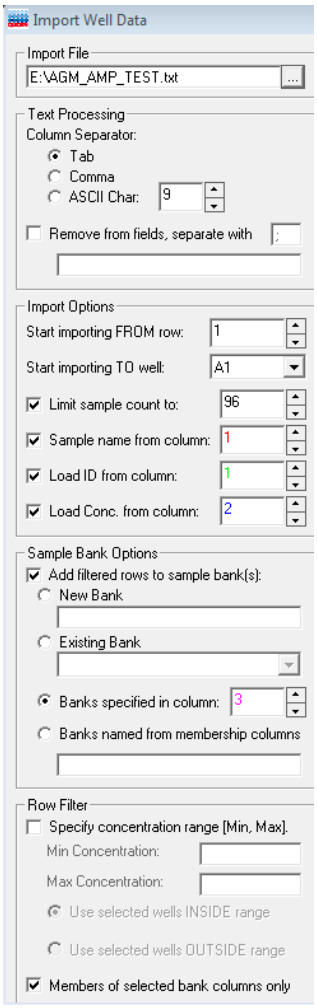
7. Turn on the QIAgility.

8. Open the appropriate QT template (GlobalFiler) being used by double-clicking on the desktop icon. Confirm the deck lay-out and the available tips are the same layout as on the QIAgility software and adjust as necessary. The tips used are QIAgility 50µl Tips. If adding a new set of tips to the tip holder, first clean the tip holder with purified water and wipe dry.

9. Once more tips (if needed) are physically added to the QIAgility deck, add tips to the software by selecting wells on deck position A1, A2, or B2, right clicking, and selecting "Set selected tips to Available".

10. From the QIAgility software, click on C2: Sample block (so red box appears – not on individual well). On the right hand window, click "Import." Navigate to .txt file previously saved from workbook (e.g. AGM_GF_MMDDYY).

11. Do not change the Import fields. The settings below are the default.



12. Click “Import”

13. Click “Finish”

14. From the QIAgility software, click on C1: Reaction block (so red box appears – not on individual well), and go to the Reaction List in the top right hand corner. For all “Normalize…” lines that are active – select and right click. Select “Create Sample Bank from Target Wells”.

15. Click on “Existing Bank” and highlight “Diluted Samples” and then click “Add Selection and Close”.

16. Go to the Reaction List in the top right hand corner. Select and delete all lines that are inactive.

17. Click Home button (house icon on tool bar).

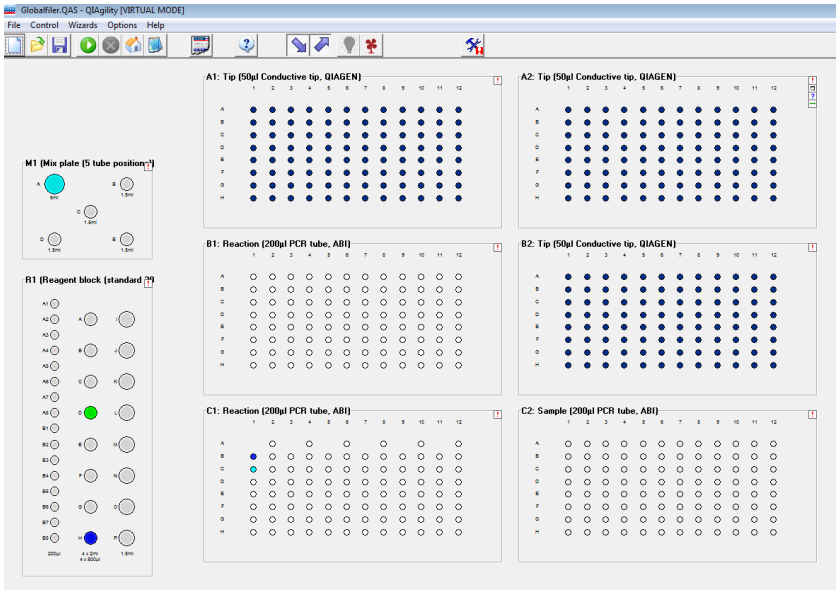
18. Click Run button (green arrow icon on tool bar).

19. Save Run File in appropriate folder (e.g. Runs 2016 Q3) as (e.g. AGM_GF_MMDDYY).
    (C: Program Files → QIAgility → Data → Run Folder) – use “Recent Places” to navigate

20. Click on Pre-Run Report and note the user supplied volume specified in Position: R1 Well D (GlobalFiler Master Mix). Open the Workbook and fill out the “Amp Setup” worksheet with Master Mix volume in µl.

21. Prepare the combined Master Mix and Primer Set solution in a 2.0 mL tube.

22. Set up the DNA extracts, reagents and tubes on the QIAgility deck as specified in the Pre-run report.



- **5.0mL QIAGEN QIAgility tapered tube** in block **M1 (well A)** {TE} (can remain on QIAgility – verify that sufficient volume is contained in tube)
- **2.0mL tube** in block **R1 (well D)** {Master mix}
- **500µl screw cap tube** in block **R1 (well H)** {positive control} (007 tube from GlobalFiler Kit)
- **200µl PCR tube** in block **B1 and C1** (96 well plates)
- **200µl PCR tube** in block **C2** (96 well plate containing sample extracts)

23. Save Pre-Run Report in appropriate folder (e.g. Pre-Run Report 2016 Q3)
(C: Program Files → QIAgility → Data → Pre-Run Reports) – use “Recent Places” to navigate.

24. Close the Pre-Run Report. Check the boxes as each line item is verified. Depending on the concentration of the samples being amplified, the software may indicate a problem in the normalized samples (volume <1.0µl). This will not affect the run, so this box can be "checked". Run will commence. (Run will stop if an error occurs). If the error is "Insufficient Volume Detected" appears, then hit retry. If retry doesn't work or any other error occurs see the DNA Technical or DNA Team Leaders.

25. Once the run has completed right click on the Post-run report and select all. Right click again on Post-run report and copy.

26. Open the Workbook "QG Amp Post-run" tab. Go to well A1, right click, and paste special as HTML. Go to the Amp Setup tab of the Workbook and click QIAgility button (2) – "Update from Post Run Report". If there are more than three rows of exceptions, you must stop and wait for the assistance of the Technical Leader or a DNA Team Leader.

27. Save Post-run report in appropriate folder (e.g. Post-run report 2016 Q3) (C: Program Files → QIAgility → Data → Post-run reports) – use "Recent Places" to navigate.

28. Close Post-run report.

29. Click on C1: Reaction block and go to right hand side of screen and click "Export".

30. Select "Custom, tab separated" from the drop down menu and save (.txt) file to flash drive (e.g. AGM_GF_MMDDYY_data). This file is for import into the "QG 3500 Prep" worksheet of the Workbook.

31. Close Post-run report.

32. Remove the plate and seal with strip caps. Spin the plate in the amp room.

Note: *To clean after use: Using Kimwipes, wipe down the block surfaces on the deck with di water and ethanol and wipe down exterior surfaces and door with di water only. Close the instrument lid. Click on the "light bulb" icon on the toolbar. The "UV Lamp Control" dialog box will appear. Set time for 15 minutes, click the box "Close software when UV finishes", and click "Start". This may be performed after step 33.*

Run preparation in amplification room:

33. Place tubes or 96 well plate in thermal cycler, tap the touch screen and press the power button in the bottom left corner – select the "globalfiler [cmpd]" protocol from the Home Screen – press "Run" in the bottom left hand corner to Start – Press "Start Run Now" to verify Start.

GlobalFiler Amplification Parameters for the Veriti Thermal Cycler (in 100% ramping mode):

- 95° C for 1 minute.
- 28 cycles, each consisting of ramp to 94° C, 10 second hold; ramp to 59° C, 1.5 minute hold.
- 1 cycle at 60° C for 10 minutes.
- Hold at 4° C.

After amplification, store plate protected from light in an amplified DNA dedicated refrigerator for up to 2 weeks or until completion of a technical review.

4.2 Yfiler Plus

A. Purpose and Introduction

A Y-STR is a short tandem repeat (STR) on the Y-chromosome. Y-STRs are used in forensics, paternity, and genealogical DNA testing. The Y chromosome is only found in males. The Y chromosome in any paternal line is practically identical. This causes a significantly smaller amount of distinction between Y-STR samples, and; therefore, a lower power of discrimination between individuals. The inclusion of seven rapidly-mutating (RM) Y-STRs with mutation rates above 1 × 10(-2) helps improve resolution of paternal lineage differentiation as well as helps with discrimination of closely-related males. Y-STR analysis may be performed on samples with trace quantities of male DNA and/ or male DNA in the presence of high level of female DNA.

The Yfiler Plus amplification kit is a 6-dye, short tandem repeat (STR) multiplex assay optimized to allow amplification from multiple male-specific sample types such as male-male and male-female mixtures. The kit amplifies 27 Y-STR loci in a single PCR reaction. The loci amplified are DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439, DYS437, DYS448, DYS456, DYS458, DYS460, DYS481, DYS533, DYS635, YGATAH4, DYF387S1a/b, DYS449, DYS518, DYS570, DYS576, and DYS627. The Yfiler Plus allelic ladder contains 342 total alleles and microvariants (virtual bins) reported in the Y-STR database yhrd.org to improve genotyping accuracy.

B. Materials and Instrumentation

- Yfiler Plus Kit
- TE^{-4} Buffer
- Applied Biosystems Veriti thermal cycler
- Hood

- Consumables:
  - 96-well plate (Life Technologies #4306737)
  - 8-well strip caps (Life Technologies #4323032)
  - 0.2 mL MicroAmp Reaction Tube (Life Technologies #N8010540)

C. Safety Considerations

Standard Laboratory Practices

Warning: Potential biohazard.

D. Minimum Standards and Control

Yfiler Plus Positive Amplification Control: The 007 positive control contains 2 ng/µl of male DNA. Dilute the positive control and add a total amount of 1.0 ng. Once diluted to 0.1 ng/µl, add10 µl Control DNA 007 (supplied in kit). The positive control dilution may be made in any volume that achieves the same concentration as long as there is sufficient volume to add to the amplification reaction. An amplification positive control must be run with each amplification and will be labeled with the date of the amplification. The positive control dilution expires one month from the date of preparation and should be stored at 2-8^{o} C between uses. Positive Control dilutions should be discarded after the one month expiration or once the "in-use" Yfiler Plus amplification kit is finished. If the amplification fails or the known type is not obtained, the results from the entire amplification are invalid and must be repeated. The Positive Control will be labeled on the electropherogram as “Pos Ctrl_[date]-Y” where the date is the corresponding date of the amplification.

Yfiler Plus Reagent Blank: Add 10 µl of the reagent blank control to the reaction. Refer to DNA QA Manual section 15.2 (Contamination Contingency Plan) if a pattern of peaks is present in the reagent blank.

Yfiler Plus Amplification Blank: Add 10 µl of TE^{-4} to the reaction. Refer to DNA QA Manual section 15.2 (Contamination Contingency Plan) if peaks are present in the amplification blank. An amplification blank must be run with each amplification, and will be labeled with the date of the amplification. The Negative Control will be labeled on the electropherogram as “Neg Ctrl_[Date] -Y” where the date is the corresponding date of the amplification.

The optimal template quantity for samples quantified with Quant Trio and amplified with Yfiler Plus is approximately 1 ng. Validation samples that targeted the range of 0.5 ng – 1.0 ng produced complete profiles. The amplification worksheet indicates the amount of DNA amplified.

The reagent blank is not required to be amplified concurrently with the samples in the associated extraction batch. The reagent blank must be amplified using the

same chemistry on the same model of thermal cycler using the same cycling parameters. The reagent blank must be typed using the same model genetic analyzer and injection parameters as the rest of the extraction batch.

If considering amplification of a sample with a different or additional chemistry, and the associated reagent blank has been depleted, no amplification of the sample may occur.

Typically, the reagent blank is amplified at the maximum volume for the chemistry, however, with prior TL approval, the reagent blank may be amplified at the maximum volume as any associated sample from the extraction batch.

Positive and Negative controls associated with each sample set will be amplified using the same kit lot used for the forensic samples and will be concurrently run in the same thermal cycler, and on the same genetic analyzer.

All samples that are amplified together will be run on the same model genetic analyzer (3500). Re-injections will be run on the same model genetic analyzer as the original injection. There is no requirement for the same instrument to be used.

E. Other Considerations

Hoods must be cleaned after each use with DNA Away or or similar, di water and ethanol, followed by a 15 minute UV cycle. Pipettes and centrifuges are wiped cleaned with DNA Away, di water and ethanol.

F. Validation

This procedure is generally accepted in the forensic community.

This procedure has undergone internal validation at the CMPD.

4.2.1 Manual Procedure Yfiler Plus

- Storage (protected from light): -15°C to -25°C upon receipt/ 2°C to 8°C after initial use.
- Yfiler Plus amplifications will be performed manually based on male quantitation values obtained from the Trio Quantitation Kit.
- Open the “Y Amp Setup” tab in the Workbook. Type in the DNA #’s of the samples that you are amplifying. Add a “-Y” to the sample DNA # to distinguish it from the GlobalFiler amplification. Type in the male quant values previously obtained. Samples with less than 0.001 ng/µl of male DNA will not be amplified unless approved by DNA Team Leader or designee.

1. Label appropriate number of 0.2 mL MicroAmp tubes. Alternately, a 96 well plate can be used for amplification.

2. Bring reagents and samples to room temperature. If necessary, thaw the PCR reaction mix and the primer mix. Gently vortex and pulse-spin each tube before opening. Add the following reagents to a pre-sterilized microcentrifuge tube in order:

   - 10 µl Yfiler Plus Master (PCR Reaction) Mix X number of samples
   - 5 µl Yfiler Plus Primer Set X number of samples

   Vortex gently in order to avoid drops of liquid on the sides and cap of the tube.

3. Add 15 µl of the combined Master Mix and Primer Set to labeled 0.2 mL amplification tubes or to appropriate plate wells.

4. Add the appropriate amount of TE^{-4} and DNA to each tube (or to appropriate plate wells) to a final volume of 25 µl. Close the tubes or seal the plate with caps.

5. Place tubes or 96 well plate in thermal cycler, tap the touch screen and press the power button in the bottom left corner – select the "yfiler plus [cmpd]" protocol from the Home Screen – press "Run" in the bottom left hand corner to Start – Press "Start Run Now" to verify Start.

Yfiler Plus Amplification Parameters for the Veriti Thermal Cycler (in 9600 Emulation mode):

- 95° C for 1 minute.
- 30 cycles, each consisting of ramp to 94° C for a 4 second hold and ramp to 61.5° C for a 1-minute hold.
- 1 cycle at 60° C for 22 minutes.
- Hold at 4° C.

After amplification, store tubes protected from light in an amplified DNA dedicated refrigerator for up to 2 weeks or until completion of a technical review.

4.3 References

- JFS Nov. 2004 Vol. 49, No. 6 Collins, Hennessy, Leibert, Roby, Reeder, and Foxall

- Characterization of Human Lymphoid Cell Lines GM9947 and GM9948 as Intra- and Interlaboratory Reference Standards for DNA Typing. C.J. Fregeau, et al. (1995) Genomics 28:184-197

- Applied Biosystems Veriti ™ Thermal Cycler, User Guide.

- Forensic DNA Typing: Biology and Technology behind the STR Markers.

John M. Butler. Academic Press 2001.

- National Institute of Standards & Technology, Certificate of Analysis for Standard Reference Material 2391, PCR-based DNA Profiling Standard (May 26, 1995).
- CMPD Veriti Internal Validation Study and Performance Check
- QIAgility Validation
- GlobalFiler PCR Amplification Manual
- Yfiler Plus PCR Amplification Manual
- GlobalFiler Validation Study on 3130 performed in CMPD laboratory by Life Technologies
- Yfiler Plus Validation Study on 3500 performed in CMPD laboratory by Life Technologies
- CMPD GlobalFiler Internal Validation Study on 3500
- CMPD Internal YFP Positive Control Material Modification Study 2020

5. Sample Preparation and Operation of 3500 Genetic Analyzer

A. Purpose and Introduction:

This procedure provides a method for preparation and typing of amplified samples for STR analysis using capillary electrophoresis on the 3500 Genetic Analyzer. Instructions cover the capillary electrophoresis technique and the data collection software.

Spatial: The 3500 genetic analyzer's Data Collection software uses images collected during the spatial calibration to establish a relationship between the signal emitted by each capillary and the position where that signal falls on and is detected by the CCD camera.

Spectral: A spectral calibration creates a matrix that is used during a run to reduce raw data from the instrument to the data stored in sample files.

B. Materials and Instrumentation

- GeneScan-LIZ Internal Size Standard (GS600)
- Hi-Di Formamide
- Applied Biosystem 3500 Genetic Analyzer
- PC with 3500 Series Data Collection Software v. 4.0 or v. 4.0.1
- 3500 Anode Buffer
- 3500 Cathode Buffer
- 3500 8 Capillary Array, 36cm
- 3500 POP-4 Performance Optimized Polymer (POP-4)
- 3500 Conditioning Reagent
- Hi-Di Formamide
- Multi-Capillary DS-36 (Dye Set J6) Matrix Std
- Nuclease Free Water
- Hood

C. Safety Considerations

- Appropriate safety precautions should be taken and proper personal protective equipment worn when working with hazardous or potentially hazardous materials.
- Warning formamide is a chemical hazard. Formamide is a teratogen and is harmful by inhalation, skin contact, and ingestion. This should be used in a biological safety cabinet or under a chemical hood.
- Electrical Shock Hazard: The ABI Prism 3500 capillary electrophoresis unit contains a high voltage power supply. Handle with caution. Under no

circumstances should any safety system be bypassed. Arcing may result from incomplete drying of instrument components.

- Laser Hazard: The ABI Prism 3500 contains a laser. Operate only with doors closed. This unit should be serviced by ThermoFisher personnel only.

D. Minimum Standards and Controls

Spectral Calibration: Is required once a year unless needed before due to the following:

- a new dye set is used on the instrument
- a capillary array is installed or replaced
- the capillary length or polymer type is changed
- after the laser or CCD camera has been realigned/replaced by a service engineer
- when a decrease in spectral separation (pull-up and or/pull down peaks) is observed in the raw or analyzed data

Spatial Calibration: Is required when:

- a capillary array is installed or replaced
- the capillary array is temporarily removed from the detection block
- the instrument is moved

Autosampler Calibration: done only as needed.
Symptoms of autosampler alignment problems may include: poor injection for a small number of capillaries, low signal strength, or no evidence of sample.

Changing the Capillary Array: as needed.
The following problems may indicate that a new capillary array is required:

- poor sizing precision or allele calling
- poor resolution and/or decreased signal intensity

E. Other Considerations

- Formamide is needed to help the amplified product stay in the denatured state. However, over time formamide will degrade into formate. This will lead to preferential injection of formate ions over DNA resulting in decreased sensitivity. The Internal Lane Standard will also take on a “Golden Gate” appearance.

- Ionized formamide (containing formate ions) does not appear frozen when removed from the freezer and should be discarded.
- Hoods must be cleaned after each use with DNA Away or similar, di water and ethanol, followed by a 15 minute UV cycle. Pipettes and centrifuges are wiped cleaned with DNA Away or similar, di water and ethanol.

- GlobalFiler samples can be re-injected for up to approximately 72 hours from when the plate run was initiated (plates started on a Friday can be re-injected on Monday). Yfiler Plus samples can be re-injected for up to approximately 48 hours from when the plate run was initiated.

- The case analyst will determine if re-injections are necessary and will select the samples for re-injection in the appropriate DNA workbook.

- If the 3500 has been cleaned between the original run and the re-injection run, the lot numbers and expiration dates of the polymer, anode buffer, and cathode buffer will need to be updated on the re-injection worksheet.

- If it has been greater the maximum allowed time (72 hours for GF and 48 hours for YFP) since the initiation of the run, then the samples selected for re-injection, will instead need to be re-setup on a new plate. All reinjections should contain at least two ladders and a positive control. In the case file there will be a 3500-setup worksheet for each GMID-X project made.

- Samples may be re-setup instead of re-amplifying if no visible evaporation has occurred and no more than two weeks have passed since the original amplification was done.

F. Validation

- The use of formamide to denature DNA is generally accepted in the scientific community.
- Capillary electrophoresis for the analysis of DNA fragments is generally accepted in the scientific community.
- The use of the 3500 Genetic Analyzer was validated internally at the CMPD.

5.1 3500 Plate Preparation Procedure:

3500 plates may be Globalfiler only, Yfiler Plus only, or a combination of both Globalfiler and Yfiler Plus. Note that the plate name, assay name, results group, and file name convention should be accurate for the samples being run.

Plate record nomenclature should follow the following format:

Initials_MMDDYY_Chemistry

Initials are the initials of the analyst if they are setting up their own plate and they are the only user on the plate.

“QIA” will be used for the initials for a group processing/operator run and/or a multi-

user run.

The date is the date of the 3500 run starting.

GlobalFiler Chemistry = GF
Yfiler Plus Chemistry = YFP
Combination of GlobalFiler and Yfiler Plus = GY

If the same analyst (or group processing operator) is running two plates of the same chemistry on the same day, differentiate them by adding “A” and “B” after the chemistry abbreviation to coincide with the plate location on the 3500.

(e.g. AGM_091119_GFA and AGM_091119_GFB)

If re-setting up a plate the same day, then add _2, to the end of the plate name.
Prepare the Formamide: Size Standard Mixture

- GeneScan -600 LIZ 0.4 µL X number of samples
- Formamide 9.6 µL X number of samples

1. Add 10 µL of the Formamide: Size Standard Mixture to the wells being used on the 96 well reaction plate. The 3500 injects samples in sets of 8. If the number of samples does not make a complete set of 8, fill the remaining wells with formamide; however, do not name this well on the injection list.

2. Add 1 µL of sample, controls, or applicable allelic ladder to the appropriate well. An allelic ladder will be placed approximately every 16 samples, and each plate will contain at least two ladders for each chemistry run.

3. Cover the reaction plate with a 96 well plate septa.

4. Briefly spin the reaction plate in the centrifuge to ensure that the contents of each well are mixed, collected at the bottom, and all bubbles removed.

5. To denature, heat the reaction plate in the thermal cycler at 95° C for 3 minutes.

6. Place the reaction plate immediately in the freezer or on ice for 3 minutes.

7. Place the sample plate into the plate base.

8. Snap the plate retainer onto the plate and the plate base.

9. Verify that the holes of the plate retainer and the plate septa are aligned. Damage to the array tips will occur if not aligned properly.

10. Place plate on instrument. Load onto instrument in Plate A position first and then Plate B position if running two plates at once.

11. Import sample list using the text file created from the 3500 Setup Worksheet.

5.2 Creating 3500 Setup Sheet and Injection List:

Note: can be combined chemistries – just ensure that all controls are properly labeled and that the correct assay name is applied to each set of samples.

5.2.1 GlobalFiler

1. On the 3500 Prep or QG 3500 Prep tab: Click “Prepare for 3500” button or “Add Samples from QIAgility Amp” (depending on whether the QIAgility has been used at the quantitation and amplification steps).

2. Ensure that the Complaint #, DNA # or ID (Ladder_”WELL”, Pos Ctrl., Neg Ctrl), Item # and description are populated. Remove Ladders from portion of plate not used and add a Ladder to the end of your samples (if less than 16 samples).

3. Click “Send to 3500 Import File”.

4. Save the Injection List as a text tab delimited file (.txt). This injection list will be imported on to the 3500.

5. Print out worksheet on the “3500 Setup or QG 3500 Setup” tab for the case file.

5.2.2 Yfiler Plus

1. On the 3500 Prep or QG 3500 Prep tab: Click “Prepare for 3500” button

2. Ensure that the Complaint #, DNA # or ID (Ladder_”WELL”-Y, Pos Ctrl -Y, Neg Ctrl – Y), Item # and descriptions are populated. Remove Ladders from portion of plate not used, and add a Ladder to the end of your samples (if less than 16 samples).

3. Click “Send to 3500 Import File”.

5. Save the Injection List as a text tab delimited file (.txt). This injection list will be imported on to the 3500.

6. Print out worksheet on the “3500 Setup or QG 3500 Setup” tab for the case file.

5.3 Running Samples on the 3500:

1. Import the plate record (.txt file) by clicking on the Workflow tab along the top tool bar. Click on “New Plate – Create New Plate from Standard Format File”. Navigate to the import file and select. If no error message appears, you may proceed to the next step.

2. Click on “Assign Plate Contents”

3. Verify the correct assays, file name conventions, and results group have been imported.

   GlobalFiler:
   Assay Name: GF_POP4_16s
   Results Group: CMPD
   File Name Convention: CMPD_GF

   Yfiler Plus:
   Assay Name: YFP_POP4_16s
   Results Group: CMPD
   File Name Convention: CMPD_YFP

   The default injection time for the 3500 is 1.2 kilovolts for 16 seconds.

   The injection time will not be changed.

4. Place plate on instrument (if you haven’t already). Load onto instrument in Plate A position first and then Plate B position if running two plates at once. If there is still a plate on the instrument, Unlink from “Workflow →Load Plates for Run” and remove from the instrument.

5. Click Link Plate for Run – ensure that plate is in correct position and click “Link”

6. Click OK

7. Click on Create Injection List which will display the Preview Run screen where you can modify the injection list before starting the run (if needed).

   An Expiration and Usage Limit warning may appear – capillary arrays may exceed the expiration date; however, all other consumables must be compliant. → Click Yes

   A message indicating that wells assigned to Results Group CMPD on the current plate do not contain an allelic ladder. It is OK to proceed.

8. Click Start Run to start your run.

9. If there is an instrument error and no data is generated, the run failure will be documented on the 3500 Setup Worksheet. The reason for the error, if known, should be included in the documentation.

10. If the run completed successfully, click on Review Results. Verify that you are on the Samples View (the View can be changed by using the View dropdown menu at the top). Make sure that View Fragment/HID Results is highlighted on the left side of the screen.

11. Once the data has been reviewed in GMID-X and samples have been selected for re-injection (if necessary), a new import file will be created and used for re-injections. You will have to unlink the current plate and link the newly imported plate. The plate can be unlinked from the Load Plates for Run screen. Follow the steps above to initiate the re-injection run.

12. Once all the data has been collected (original and re-injections), Unlink the plate(s) from "Workflow →Load Plates for Run" and remove from the instrument.

5.4 HID Files (Raw Data files) and R Drive:

The raw data (.hid) files are stored within folders labeled with the run name. These files can be accessed using the desktop shortcut to the 3500 Data folder. Naming conventions for both CMPD_GF and CMPD_YFP raw data files are <well position_sample name>.

All raw data files must be transferred from the 3500 instrument computer to the appropriate location within the DNA Technical Data folder on the R drive. Computer→ (R:)→ Department→ Crimelab→ Sensitive→ DNA Technical Data.

Within DNA Technical Data:
- Single analyst runs will be stored in the appropriate location within the analyst's personal folder.
- Runs that contain samples from multiple analysts or performed with group processing will be stored in the appropriate location within the QIA folder.

Any data that is on the R drive will be automatically backed up by the city and will not require any additional data backup steps by the Biology Section. Once all raw data has been moved to the R drive, the samples can be added to GMID-X to create a project.

The group processing operator is responsible for:
- Saving the raw data files for group processing runs on the R: drive

- Saving the workbook to the appropriate location on the R drive
- Informing analyst(s) their 3500 run data is ready to review
- Distributing the completed worksheets to the appropriate analysts
- If notified in a timely manner, the group processing operator may perform any necessary re-injections.

5.5 References:

- CMPD Internal Validation GlobalFiler
- CMPD Internal Validation Yfiler Plus
- Forensic DNA Typing: Biology and Technology behind the STR Markers. John M. Butler. Academic Press 2001.
- 3500 Genetic Analyzer User Guide. Applied Biosystems
- GlobalFiler PCR Amplification Kit User's Manual. Applied Biosystems.
- Yfiler Plus PCR Amplification Kit User's Manual. Applied Biosystems
- GlobalFiler PCR Amplification Kit – PCR Amplification and CE Quick Reference – Applied Biosystems
- 3500_3500xL User Bulletin Software v4.0 – Applied Biosystems
- CMPD Internal Performance Check 3500-Jesse (Includes Longevity Study and Performance Check of Data Collection Software v4.0.1)

6. GeneMapper® ID-X Analysis

A. Purpose and Introduction

GeneMapper® ID-X (GMID-X) software is used to apply the Analysis Method and Size Standard to raw data generated by the 3500 Genetic Analyzer thus allowing for automatic conversion of the allele sizes (in base pairs) into allele designations. It also generates electropherograms and builds tables containing the genotype information. Genotypes are assigned by comparing the sizes obtained from the unknown sample files with the sizes obtained for the alleles in the allelic ladder(s). Use of this software corrects for spectral overlap within samples, corrects for differences in baseline, and defines the ranges of interest within the data.

Note: *This section describes how to perform a GeneMapper® ID-X analysis starting with a new project.*

B. Materials and Instrumentation

- PC computer running GMID-X approved operating system
- Life Technologies GeneMapper® ID-X Analysis Software, version 1.6

C. Safety Considerations

- Prolonged computer analysis may cause muscle soreness, eye fatigue, or other discomforts. To minimize the occurrence of discomforts, periodic breaks from computer analysis are encouraged.

D. Limitations

- The software is designed to help assign allele calls to true peaks by assessing data that has been collected utilizing the Applied Biosystems Data Collection software. Allele calls are based on base pair size and known allelic ladders. This, however, does not replace the need for analyst interpretation. All data requires analyst review and relevant data will have a second review by the case file technical reviewer to ensure that all true peaks have been accurately labeled and called by the software.

- All allele calls are dependent on an appropriate ladder and internal size standard.

E. Other Considerations

- The GMID-X software version 1.6 may be used as an expert system to assess allelic ladders and amplification positive controls. The software will evaluate, the allele calls, the base pair values, the peak heights, and sizing quality flags

for the allelic ladders and amplification positive controls against the expected values and determine if they are in concordance and, therefore; acceptable to be used for data analysis. An analyst may override the software evaluation for amplification positive controls with DNA Technical Leader or designee approval but must make note of their evaluation on the Data Evaluation Worksheet.

- The GMID-X v1.6 software will not be used as an expert system to assess negative amplification controls or reagent blanks.

- The analyst as well as the technical reviewer will evaluate the following data attributes: the presence of a primer peak, internal lane size standard quality, allele calls of ladder(s), positive control(s), negative control(s), reagent blank(s), pullup/spectral efficiency, signal strength/saturation, resolution, migration rate, baseline, and artifacts.

F. Validation

- This procedure is generally accepted in the forensic community.
- These procedures have undergone internal validation at the CMPD.

Procedure:

Prior to creating a project, case analysts on group processing/multiple analyst runs will do the following:

Within the DNA Technical Data folder:

- Copy the applicable run folder from the QIA folder to the appropriate location within the analyst's personal folder.
- In the personal folder copy, remove the raw data files of samples and controls that belong to other analysts. All allelic ladders on the run will be retained. This copy of the run folder will be used to create the project.

6.1 Creating a GeneMapper® ID-X Project

1. Open GeneMapper® ID-X version 1.6

   All analysis will be performed under analyst's name.

2. Add Samples: select "Edit" → "Add Samples to Project"

Navigate to the run folder containing the raw data sample files. Run folders are stored within the DNA Technical Data folder: Computer→ (R:)→ Department→ Crimelab→ Sensitive→ DNA Technical Data. Select the applicable run folder, select "Add to List" and then "Add".

6.2 Check Settings

6.2.1 Settings - Sample Type

Verify that the correct sample type has been filled in for each sample:

Sample	Sample Type
Allelic Ladder	Allelic Ladder
Positive Control	Positive Control
Negative Control	Negative Control
Reagent Blank	Negative Control
Evidence/reference sample	Sample

6.2.2 Settings: - GlobalFiler (3500)

- Specimen Category: no export
- SFN: No
- Analysis Method: CMPD 3500 GF analysis_2
- Panel: GlobalFiler_Panels2_v1-dup
- Size Standard: GS600_LIZ_(60-460)
- Custom Control: None
- Matrix: None
- Other fields automatically filled in by the software and instrument

6.2.3 Settings: - Yfiler Plus (3500)

- Specimen Category: no export
- SFN: N/A
- Analysis Method: CMPD YFP analysis method_2
- Panel: Yfiler_Plus_Panel_v4
- Size Standard: GS600_LIZ_(60-460)
- Custom Control: None
- Matrix: None
- Other fields automatically filled in by the software and instrument

6.3 Analysis

Once the columns have been verified, the samples can be analyzed by selecting Analysis → Analyze. A message box may appear indicating that the samples were not normalized. If the message box appears, click “OK” and check the “Don’t show again until the next session” box.

The Save Project Dialog box will open and prompt the user to enter a name for the project.

GMID-X projects names should be the same as the plate record name with the following exceptions. Group/multi-user plates will use the QIA prefix and have the case analyst initials added as a suffix to differentiate the data from different analysts.

Examples of GMID-X project nomenclature:

QIA_012119_GF_AGM	individual project created by AGM for AGM's cases from QIAgility Group Processing or from combined plate with other analysts
AGM_012119_GF	individual project created by AGM for AGM's cases / only AGM on the plate

The security group should be set to: GeneMapper ID-X Security Group when saving projects.

Once all samples have been analyzed by the GMID-X software, the Analysis Summary tab will appear. This tab shows an overview of all samples, allelic ladders, and controls for the run. To proceed with the analysis of the project there must be a passing positive control and negative control along with at least one passing allelic ladder. A failed allelic ladder does not require the Sample Type to be changed to "Sample". Allelic ladders with flagged CGQ and/or SQ values are not used by the software to create bin offsets.

The assigned case analyst is ultimately the responsible party and must review and take ownership of their data. The case analyst will evaluate their data and applicable controls to ensure they meet quality control measures and determine any necessary re-injections. The analyst will document this review by completing the Data Evaluation Worksheet. Any failure of controls and additional laboratory work associated with controls, must be communicated to the DNA TL or designee and shall be approved prior to using the associated data for comparison.

The assigned case analyst's review of the data includes ensuring their data meets quality control measures, reviewing the ILS sizing quality (SQ), assessing the Process Quality Value (PQV) flags, and making any edits they deem necessary. Sized evidence and reference data that is generated will be included in the appropriate case file's allele table, regardless of whether the data is used for interpretation or comparison. Prior to creating the allele table, NUFI will be added to the sample name of any evidence/reference not used for interpretation/comparison based on quality control measures.

GeneMapper ID-X Software uses PQV flags as a tool to help the analyst in their

analysis of sample data. These quality flags help identify potential data quality issues, however, the analyst is ultimately responsible for visually inspecting all loci and for making decisions regarding the interpretation of a profile and whether it is suitable for comparison.

The GeneMapper ID-X Software uses color-coded flags to display the PQV results. If the SQ has a green square, the software considers the sizing quality in the passing range. If the sizing quality has a yellow triangle, the SQ value falls between the passing and low-quality range. If the SQ= , the analyst can manually evaluate the SQ and decide if it is suitable for interpretation and/or comparison for single source evidence samples, controls or references only. Amplification Positive Controls may be used with TL or designee approval. All other SQ= samples will be re-injected/re-run. If the ILS sizing quality has a red stop sign, the software considers the SQ low quality. These samples are not suitable for interpretation/comparison.

Flag Indicator	Description
Pass	SQ is in the Pass range (Default = 0.75 – 1.0)
Check	SQ is between the Pass range and the Low Quality range (Default = 0.26 – 0.74)
Low Quality	SQ is in the Low Quality range (Default = 0.0 – 0.25)
Note: The software does not genotype samples with SQ.	

The GQ (Genotype Quality) PQV Flag indicates the individual loci's genotype quality. The CGQ (Composite Genotype Quality) PQV Flag indicates the overall genotype quality.

Flag Indicator	Description
Pass	All individual marker GQ values are .
Manually overridden	Status was originally , , , , , or but was set to by the user.
Check	One or more individual marker GQ values are and no marker GQ values are .
Low Quality	One or more marker GQ values are (affected by marker-level PQVs).
, , or	At least one marker in the sample was edited.

Editing of artifacts that will be omitted from the STRmix import file will use either "Rename Artifact Label" or "Rename Allele Label" depending on the initial software designation of artifact or allele. In the menu, "ART" will be selected followed by a screen that will prompt you to enter the reason for the change.

Use the following edit codes in the “Reason for Change” screen:

Artifact	Code
Pullup	PU
Spike	SP
Minus A	MA
Stutter	ST
N+4	NP
Other Artifacts	ART (e.g. dye blobs, bacterial DNA, SNPs) – add additional notes on worksheet

When viewing the data, the Globalfiler analysis methods/panels will have stutter filters in place. Analysts will only have to edit stutter peaks in autosomal reference samples and YSTR samples. Potential stutter will not be edited in autosomal evidence samples.

Edit codes are assigned based on the analyst’s assessment. If the software designates an artifact as a “spike,” confirm that this is indeed a spike and not a multi-channel pull-up artifact. Such artifacts should be coded as “PU” for pull-up. If this artifact is indeed a spike then it still must be changed and coded as “SP” to document the analyst’s assessment.

Editing of off ladder OL alleles will be done using the “Rename Allele Label” → “Custom Allele Label” → type in allele call (“OL” is not needed and will interfere with STRmix™ import). The allele call will be entered as the reason. (e.g. 16.1).

Multiple allele labels or artifact labels can be edited at one time by using the control button when selecting the relevant labels. The reason for change entered will be applied to all allele or artifact labels selected.

Once an edit is made the locus header changes to gray and will alert the analyst/technical reviewer that a change has been made at that location. It is not necessary to manually override the locus headers or the CGQ of any samples or controls.

The STRmix™ software checks the input files for the presence of invalid peaks (e.g. “OL”) and, if present, will flag them as an issue for the analyst to review. This can be used as a tool to aid analysts in identifying possible artifacts that were not edited in evidence and reference profiles.

After the data has been reviewed and all applicable edits have been made, perform the following steps to update the workbook and the Data Evaluation Worksheet:

1. In GMID-X, select all the samples and controls in the project and go to

"File → Export Table" and save table. (GMID-X Sample Table)

2. Open the current workbook for the batch of samples associated with the run and from the "Data Evaluation Worksheet" tab click the "Import GMIDX Sample Table" button. Select the sample table previously exported.

3. Review the ILS column. If ILS sizing quality is in the range of 1.0 to 0.75 (SQ flag-green square), then it will be noted as "Pass." If the sizing quality is in the range of 0.26-0.74 (SQ flag-yellow triangle), then it will be noted as "Check." SQ values .25 or less (SQ flag-red stop sign), will be noted as "Fail".

4. Any issues that affect the entire run will be recorded in the Notes section at the bottom of the Data Evaluation Worksheet. The reasons why individual sample/control data is not meeting quality control measures will be recorded in the corresponding last column of the Data Evaluation Worksheet. The analyst may type/handwrite the reason or choose the appropriate option from the dropdown menu.

5. If a sample/control needs to be re-injected, select "Yes" from the dropdown menu. The reasons why the data is being re-injected will be recorded in the last column of the Data Evaluation Worksheet. The analyst may type/handwrite the reason or choose the appropriate option from the dropdown menu.

    - For samples that require re-injection, use the RI prep and RI setup sheets to prepare a new plate record and set-up sheet. The subsequent project will be named the same with the addition of "RI." If notified in a timely manner, the group processing operator may perform any necessary re-injections. The case analyst is responsible for reviewing the data and updating the workbook with the information generated from the re-injections performed from the original plate.

6. In the STRmix column, select "Yes" or "No" from the drop-down menu depending on whether the sample is suitable for STRmix analysis. This column does not need to be filled out for controls or references. "Yes" indicates the sample will be analyzed in STRmix. "No" indicates the sample will not be analyzed in STRmix. If the answer is "No", add the reason why the sample is not suitable for STRmix in the corresponding last column of the Data Evaluation Worksheet. The analyst may type/handwrite the reason or choose the appropriate option from the dropdown menu.

    - The analyst can then click on the "Go to STRmix Interpretation"

button on the Data Evaluation worksheet to access the STRmix Interpretation Worksheet.

7. If a sample is being manually interpreted (Y-STR samples or NOC greater than four with visually obvious complete major), the analyst will place “No” in the sample’s STRmix column and choose “Manual interpretation” from the drop down menu in the last column.

    - The analyst can then click on the “Go to Manual Interpretation” button on the Data Evaluation worksheet to access the Manual Interpretation Worksheet.

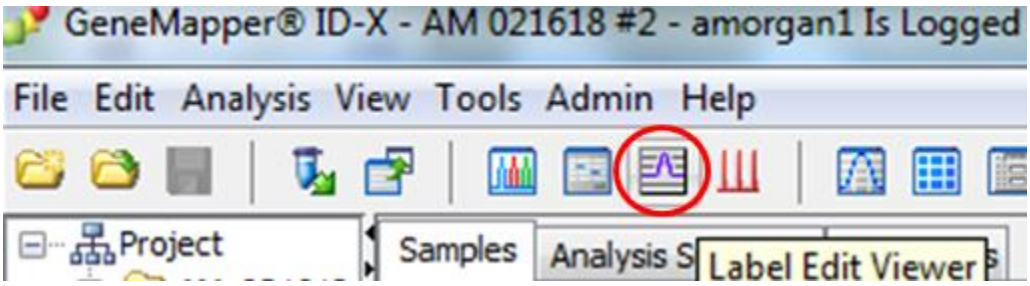
8. The analyst will document the elements of the data they reviewed by placing a check in the data evaluation check box on the top of the Data Evaluation Worksheet. The analyst will initial and date the page.

Any edits that were made in the project will be documented by importing the Artifacts Edits table into the workbook using a button on the Data Evaluation Worksheet. The project should be saved after all edits have been made to ensure all edits are included in the Edits table. The Artifacts Edits Worksheet is only generated/included in the case file when edits are made in the GMID-X project. If there are no edits, a check will be placed in the “No Artifact edits” box on the Data Evaluation Worksheet.

Perform the following steps to import the Artifact Edits table into the workbook:

1. In GMID-X select all samples and controls in “Samples” view.

2. Select the Label Edit Viewer button.



3. Click “Export” button and save table.

4. From the “Data Evaluation” worksheet in the workbook click the “Import Artifact Edits” button. Select the allele edit table previously exported.

5. Confirm all edits were recorded. Double check that the correct date and

time fields are imported. The analyst will date and initial the page.

A copy of the printed Setup Worksheet, Data Evaluation Worksheet, Artifacts Edits Worksheet (if applicable), and any Interpretation Worksheets from each run will be placed in the case file.

If samples within a project need to be changed after being submitted for technical review, a separate project will be created with the ladders, positive control, and applicable samples requiring the noted change. This project will have the original project name, but "TRC" (Technical Review Change) will be added. An Artifacts Edits Worksheet will be created, or the edit will be added to the already generated Artifacts Edits Worksheet in the applicable case files. The reason the TRC was created will be added to the Artifacts Edits Worksheet

6.4 Creating GMID-X Export Tables for Data Interpretation Software

6.4.1 ArmedXpert Table

Table for Import into ArmedXpert (Used for interpreting applicable autosomal legacy data.)

After completing analysis and saving all changes, ensure that the Samples Plot window is closed.

Click the Genotypes tab to bring up the genotypes table – Ensure that the Table Setting is ArmedXpert.

File > Export Table.

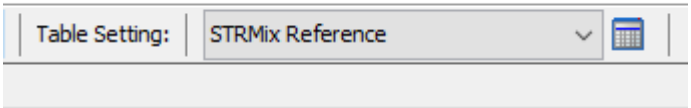
Save .txt file – this file does not have to permanently be retained; however, it will be imported into ArmedXpert.

6.4.2 STRmix Tables

After the analyst has reviewed their data, they determine which evidence profiles are suitable for STRmix™ interpretation. Evidence and reference tables will be created from GMID-X to import STRmix™ suitable profiles and their applicable references into the software.

Reference table for import into STRmix™

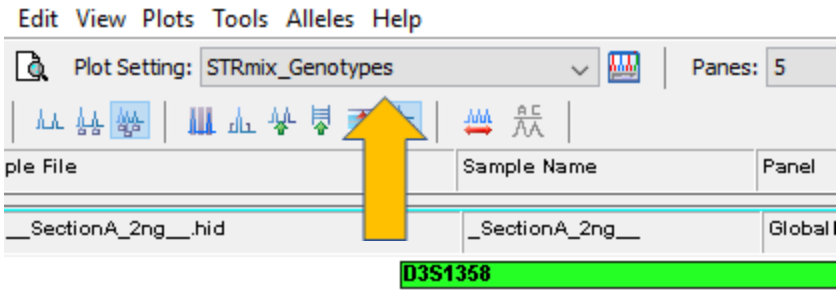
1. In the GeneMapper main screen, change your table setting to STRmix Reference.



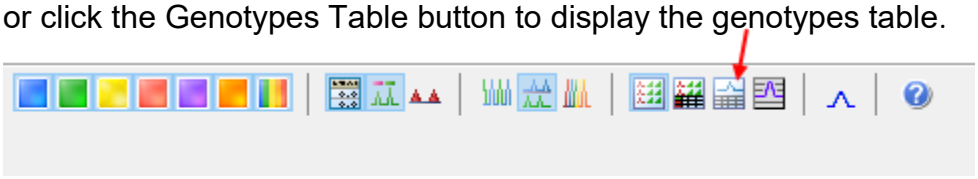
2. Highlight the reference samples that need to be imported into STRmix™ .

3. Click the Display Plots button. 

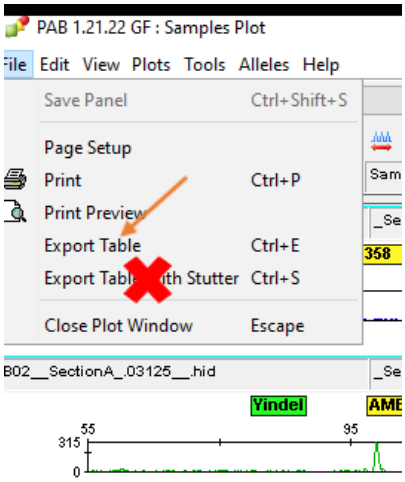
4. Either change your plot setting to STRmix _Genotypes



or click the Genotypes Table button to display the genotypes table.



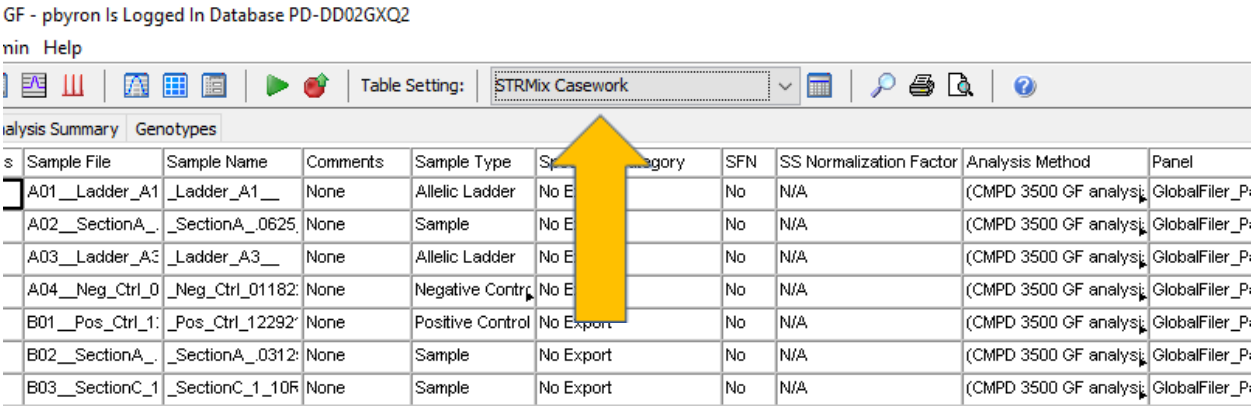
5. Click on File. Choose Export Table.



6. Export the file as a .txt file. This file does not have to be permanently retained; however, it will be imported into STRmix™.

Evidence table for import into STRmix™

1. In the GeneMapper main screen, change your table setting to STRmix Casework.



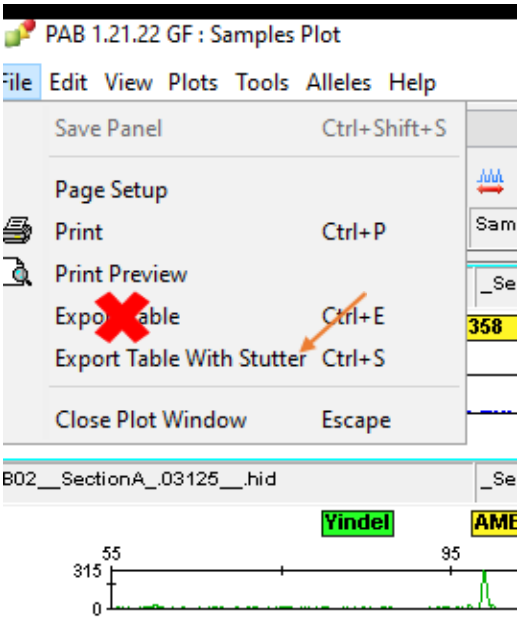
s	Sample File	Sample Name	Comments	Sample Type	Sp…	…gory	SFN	SS Normalization Factor	Analysis Method	Panel
	A01__Ladder_A1	_Ladder_A1__	None	Allelic Ladder	No E		No	N/A	(CMPD 3500 GF analysi	GlobalFiler_P
	A02__SectionA_.	_SectionA_.0625	None	Sample	No E		No	N/A	(CMPD 3500 GF analysi	GlobalFiler_P
	A03__Ladder_A3	_Ladder_A3__	None	Allelic Ladder	No E		No	N/A	(CMPD 3500 GF analysi	GlobalFiler_P
	A04__Neg_Ctrl_0	_Neg_Ctrl_01182	None	Negative Contr	No E		No	N/A	(CMPD 3500 GF analysi	GlobalFiler_P
	B01__Pos_Ctrl_1	_Pos_Ctrl_12292	None	Positive Control	No Export		No	N/A	(CMPD 3500 GF analysi	GlobalFiler_P
	B02__SectionA_.	_SectionA_.0312	None	Sample	No Export		No	N/A	(CMPD 3500 GF analysi	GlobalFiler_P
	B03__SectionC_1	_SectionC_1_10F	None	Sample	No Export		No	N/A	(CMPD 3500 GF analysi	GlobalFiler_P

2. Highlight the evidence samples that are suitable for STRmix™ analysis.

3. Click Display Plots button. 

4. The analyst can either change the plot setting to STRmix _Genotypes or click the Genotypes Table button at the top.

5. Click on File. Choose Export Table with Stutter.



6. Export the file as a .txt file. This file does not have to permanently be retained; however, it will be imported into STRmix™

6.5 Creating an Allele Table using GeneMapper

An allele table containing the DNA results from the relevant evidence and reference samples will be printed and included in each case file. The allele table contains the information shown on the electropherogram, which has stutter filters applied and applicable artifacts edited. Prior to creating the allele table, NUFI will be added to the sample name of any evidence/reference not used for interpretation/comparison based on quality control measures. Sized evidence and reference data that is generated will be included in the appropriate case file's allele table, regardless of whether the data is used for interpretation or comparison. Within each case, the reference samples will appear in separate tables from the evidence samples. In cases with multiple projects, an analyst may handwrite the applicable project names on the tables to assist their technical reviewers.

To create the allele table:
1. In the GeneMapper main screen, the table setting should be STRMix Casework. (This table setting should be used when making both evidence and reference allele tables).

2. Highlight the relevant evidence case samples. Generally, five samples should be selected to fit on one page. This number may be less depending on length of item description/number of alleles at each loci.

3. Click on the Report Manager button at the top.

4. The Report Setting should be set to AlleleTable_CMPD. Verify all selected samples are present.

5. Click Edit. Choose View Table by Marker. (Note: In this view, GMID may not show all selected samples.)

6. Click File. Choose Export. The file will be exported as a .pdf.

7. Navigate to location where file was exported. Print allele table in Landscape orientation to be included in case file. If too many samples were selected, GeneMapper will cut off the allele table for one/multiple samples and display the results on separate pages. If this is observed, repeat the steps to create an allele table but highlight fewer samples.

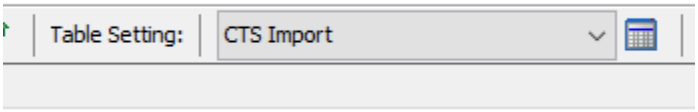
8. Close Report Manager. Report does not have to be saved. Repeat steps until all relevant evidence case samples have been included in allele tables.

9. Once the evidence samples are complete, repeat steps with relevant reference samples. Evidence and reference samples will appear in separate allele tables.

6.6 Uploading DNA Data to CTS Website (Proficiency Tests Only)

Once all edits have been made and interpretation is complete:

1. In the GeneMapper main screen, change your table setting to CTS Import.



2. Highlight your evidence and reference proficiency test samples.

3. Click the Display Plots button.

4. Either change your plot setting to STRmix_Genotypes or click the Genotypes Table button to display the genotypes table.

5. Click File. Choose Export table. This is the text file you will import into the CTS website.

6. Once logged into the CTS website, from the home screen click on the blue arrow icon under “Actions” and the "Upload data" page will open.

7. Choose the sequence type and the amplification kit arrangement option. The sequence type will control the amplification kit options that are displayed under the Amplification Kit Arrangement section. The amplification kit chosen will control the loci order.

8. Click on the “Choose File” button to locate the file to upload. Select the text file that you exported from GeneMapper. Click on the file, then click “Open”.

9. After the file has been chosen, click on the “Load File” button to access sample information.

10. Select the sample for each item number on the top section of the page. Click the arrow to open the drop-down list above each necessary item number.

11. Select the appropriate sample from the drop-down list for that specific item number. You can select a sample for all necessary items in your test.

12. Once chosen, all alleles will be shown for that selected sample. Repeat for each additional item number. If the CTS Locus does not match the File Locus, you can click on the arrow next to each CTS Locus to choose the correct Locus.

13. The middle section of the page shows the substitution options. The left side allows you to substitute a blank space with something else. The right side of the section allows you to choose how to display homozygotes. By default, a homozygous allele will be represented by a single allele i.e. 8. Click on the box next to any character if you want the homozygous allele to be shown as the two identical alleles i.e. 8,8.

14. Once these sections are complete, click on the “Next” button found below this section. The next window shows all changes to the data based on your chosen options for a final review. Click on the “Save” button to successfully transfer this data into the test.

15. The files have now been uploaded to the test. From the “My Data Entry” page, click on the “Open Test” button to review the data that has been uploaded.

6.7 Exporting/ Removing GMID-X Projects:

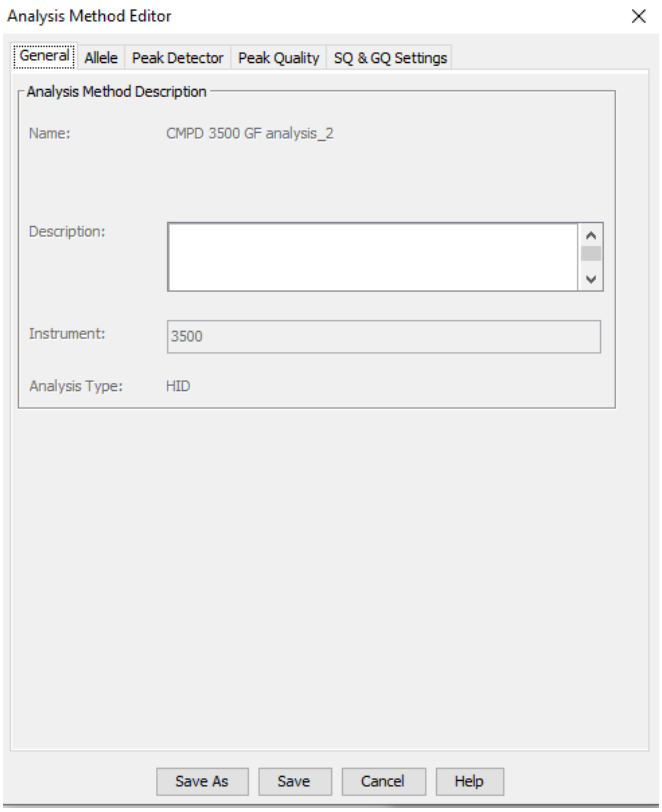
After completing GMID-X analysis, the case analyst will export their project to their personal folder within the DNA Technical Data folder on the R drive. Analysts can export projects using Tools→ GeneMapper ID-X Manager→ Select project →Export and navigate to the location. Once the project has been saved on the R drive, the case analyst will then remove the project from the GMID-X software by using the “Delete” button within GeneMapper ID-X Manager .

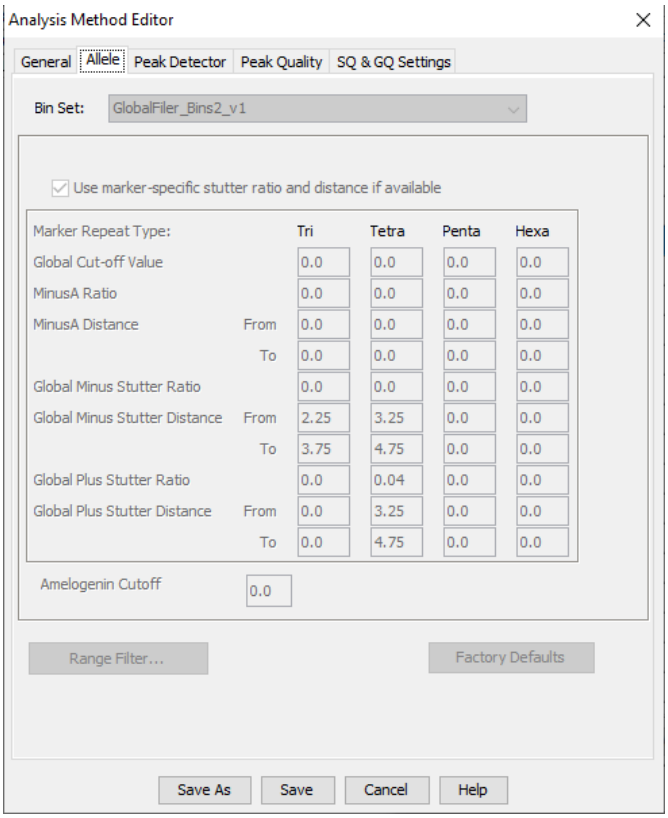
When the file is ready for technical review, the reviewing analyst will import the case work analyst’s individually named project from the R: drive. Once the review is complete, they will remove the imported project from the GMID-X software.

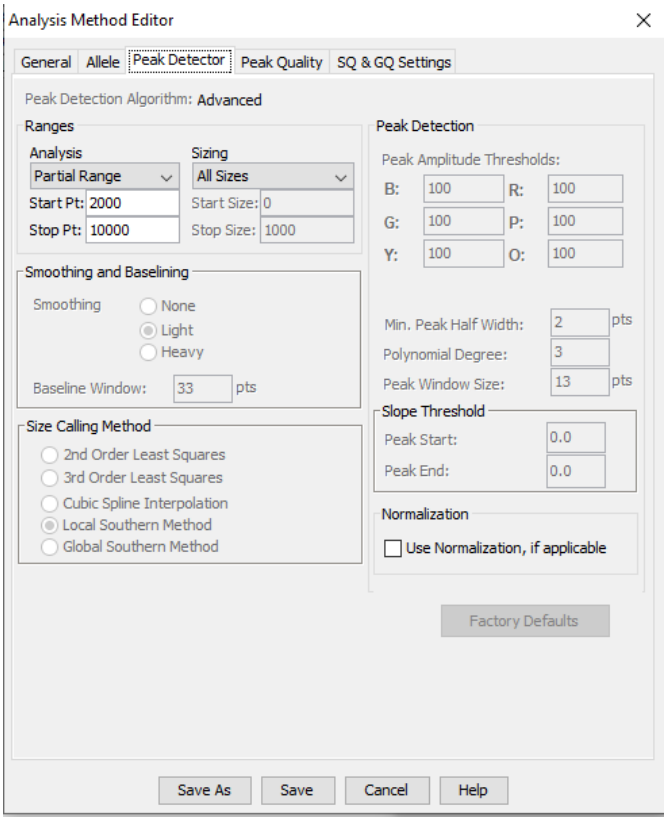
All projects will be permanently saved and backed-up on the R drive. Removing the project from the GMID-X server should be done only after it is confirmed that the project is saved.

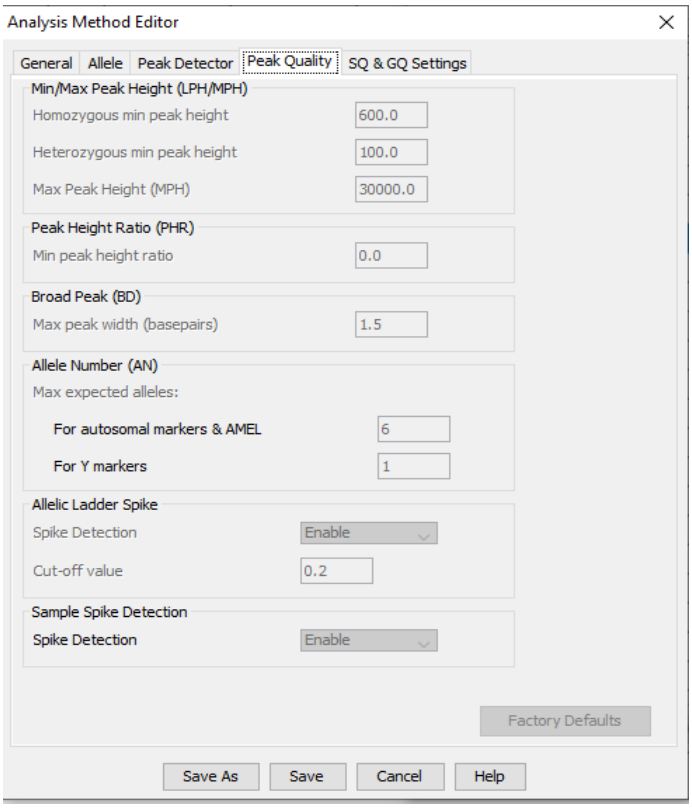
6.8 Analysis Method Editor Parameters for GlobalFiler on 3500:

CMPD 3500 GF analysis_2:


Analysis Method Editor
General
Allele
Peak Detector
Peak Quality
SQ & GQ Settings
Analysis Method Description
Name:
CMPD 3500 GF analysis_2
Description:
Instrument:
3500
Analysis Type:
HID
Save As
Save
Cancel
Help

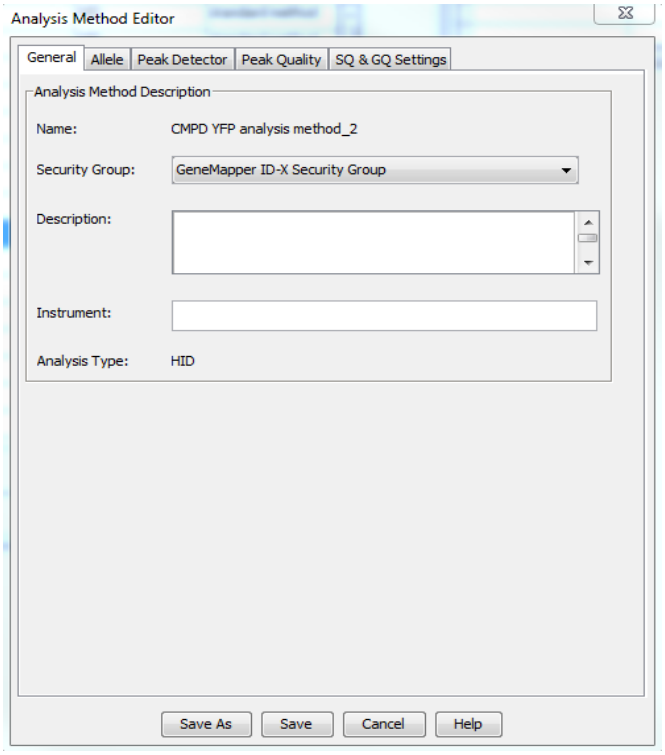

Analysis Method Editor
General
Allele
Peak Detector
Peak Quality
SQ & GQ Settings
Bin Set:
GlobalFiler_Bins2_v1
Use marker-specific stutter ratio and distance if available
Marker Repeat Type:
Tri
Tetra
Penta
Hexa
Global Cut-off Value
MinusA Ratio
MinusA Distance
From
To
Global Minus Stutter Ratio
Global Minus Stutter Distance
Global Plus Stutter Ratio
Global Plus Stutter Distance
Amelogenin Cutoff
Range Filter...
Factory Defaults
Save As
Save
Cancel
Help

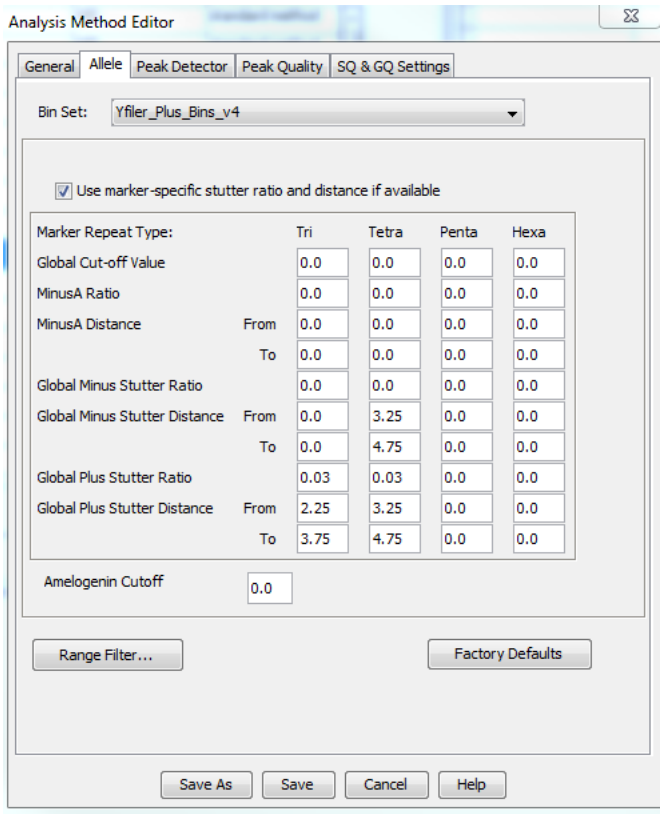

Analysis Method Editor
General
Allele
Peak Detector
Peak Quality
SQ & GQ Settings
Peak Detection Algorithm: Advanced
Ranges
Analysis
Partial Range
Start Pt: 2000
Stop Pt: 10000
Sizing
All Sizes
Start Size: 0
Stop Size: 1000
Smoothing and Baselining
Smoothing
None
Light
Heavy
Baseline Window: 33 pts
Size Calling Method
2nd Order Least Squares
3rd Order Least Squares
Cubic Spline Interpolation
Local Southern Method
Global Southern Method
Peak Detection
Peak Amplitude Thresholds:
B: 100
R: 100
G: 100
P: 100
Y: 100
O: 100
Min. Peak Half Width: 2 pts
Polynomial Degree: 3
Peak Window Size: 13 pts
Slope Threshold
Peak Start: 0.0
Peak End: 0.0
Normalization
Use Normalization, if applicable
Factory Defaults
Save As
Save
Cancel
Help

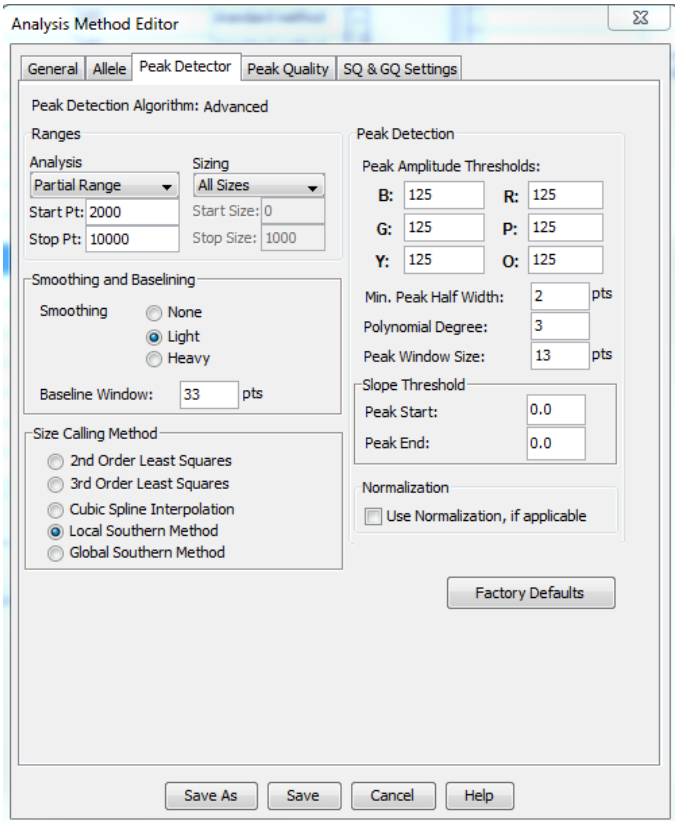

Analysis Method Editor
General
Allele
Peak Detector
Peak Quality
SQ & GQ Settings
Min/Max Peak Height (LPH/MPH)
Homozygous min peak height 600.0
Heterozygous min peak height 100.0
Max Peak Height (MPH) 30000.0
Peak Height Ratio (PHR)
Min peak height ratio 0.0
Broad Peak (BD)
Max peak width (basepairs) 1.5
Allele Number (AN)
Max expected alleles:
For autosomal markers & AMEL 6
For Y markers 1
Allelic Ladder Spike
Spike Detection Enable
Cut-off value 0.2
Sample Spike Detection
Spike Detection Enable
Factory Defaults
Save As
Save
Cancel
Help

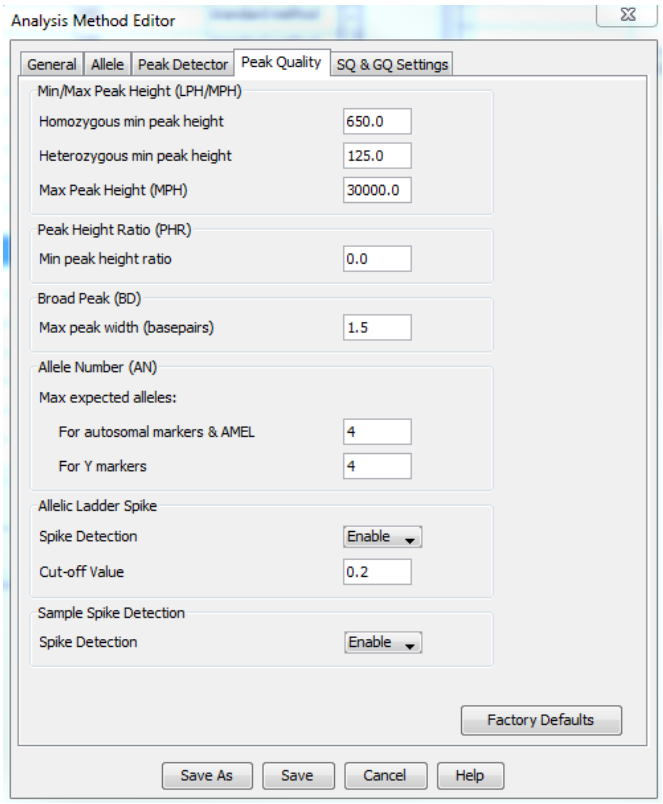
6.9 Analysis Method Editor Parameters for Yfiler Plus on 3500:

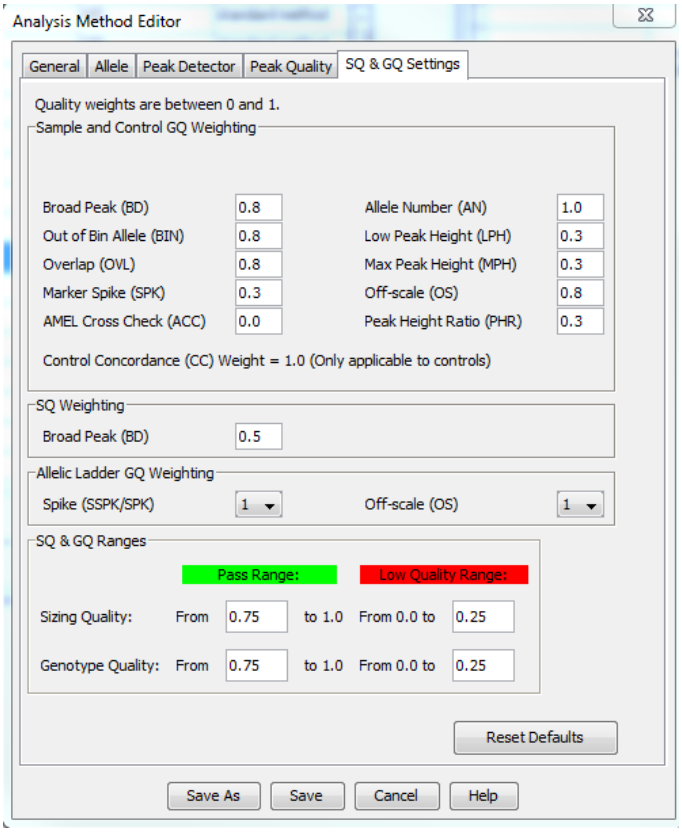
CMPD YFP analysis method_2:




Analysis Method Editor
General
Allele
Peak Detector
Peak Quality
SQ & GQ Settings
Bin Set:
Yfiler_Plus_Bins_v4
Use marker-specific stutter ratio and distance if available
Marker Repeat Type:
Tri
Tetra
Penta
Hexa
Global Cut-off Value
MinusA Ratio
MinusA Distance
From
To
Global Minus Stutter Ratio
Global Minus Stutter Distance
Global Plus Stutter Ratio
Global Plus Stutter Distance
Amelogenin Cutoff
Range Filter...
Factory Defaults
Save As
Save
Cancel
Help


Analysis Method Editor
General
Allele
Peak Detector
Peak Quality
SQ & GQ Settings
Peak Detection Algorithm: Advanced
Ranges
Analysis
Partial Range
Sizing
All Sizes
Start Pt:
Stop Pt:
Start Size:
Stop Size:
Smoothing and Baselining
Smoothing
None
Light
Heavy
Baseline Window:
pts
Size Calling Method
2nd Order Least Squares
3rd Order Least Squares
Cubic Spline Interpolation
Local Southern Method
Global Southern Method
Peak Detection
Peak Amplitude Thresholds:
B:
R:
G:
P:
Y:
O:
Min. Peak Half Width:
Polynomial Degree:
Peak Window Size:
Slope Threshold
Peak Start:
Peak End:
Normalization
Use Normalization, if applicable
Factory Defaults
Save As
Save
Cancel
Help


Analysis Method Editor
General
Allele
Peak Detector
Peak Quality
SQ & GQ Settings
Min/Max Peak Height (LPH/MPH)
Homozygous min peak height
650.0
Heterozygous min peak height
125.0
Max Peak Height (MPH)
30000.0
Peak Height Ratio (PHR)
Min peak height ratio
0.0
Broad Peak (BD)
Max peak width (basepairs)
1.5
Allele Number (AN)
Max expected alleles:
For autosomal markers & AMEL
4
For Y markers
4
Allelic Ladder Spike
Spike Detection
Enable
Cut-off Value
0.2
Sample Spike Detection
Spike Detection
Enable
Factory Defaults
Save As
Save
Cancel
Help


Analysis Method Editor
General
Allele
Peak Detector
Peak Quality
SQ & GQ Settings
Quality weights are between 0 and 1.
Sample and Control GQ Weighting
Broad Peak (BD)
0.8
Out of Bin Allele (BIN)
0.8
Overlap (OVL)
0.8
Marker Spike (SPK)
0.3
AMEL Cross Check (ACC)
0.0
Allele Number (AN)
1.0
Low Peak Height (LPH)
0.3
Max Peak Height (MPH)
0.3
Off-scale (OS)
0.8
Peak Height Ratio (PHR)
0.3
Control Concordance (CC) Weight = 1.0 (Only applicable to controls)
SQ Weighting
Broad Peak (BD)
0.5
Allelic Ladder GQ Weighting
Spike (SSPK/SPK)
1
Off-scale (OS)
1
SQ & GQ Ranges
Pass Range:
Low Quality Range:
Sizing Quality:
From
0.75
to 1.0
From 0.0 to
0.25
Genotype Quality:
From
0.75
to 1.0
From 0.0 to
0.25
Reset Defaults
Save As
Save
Cancel
Help

6.10 References:

- User Guide GMID-X v1.6
- CMPD Internal Validation
- CMPD – Stutter Percentage – Internal Study - June 2018
- CMPD – Stutter Re-evaluation – Internal Study – January 2022
- CMPD – STRmix™ Internal Validation
- GlobalFiler PCR Amplification Kit User's Manual. Life Technologies
- Yfiler Plus PCR Amplification Kit User's Manual. Life Technologies
- Forensic DNA Typing: Biology and Technology behind the STR Markers. John M. Butler. Academic Press 2001.

7. Data Evaluation and Interpretation - GlobalFiler

A. Purpose and Introduction

- The purpose of this procedure is to provide guidance for the data evaluation of autosomal STR data generated using the GlobalFiler amplification kit. This evaluation will assess the data, consider artifacts, determine number of contributors, and determine the suitability for STRmix™.

- This SOP is to serve as reference material and is not intended as a replacement for coursework and training. These guidelines are not an exhaustive list of all scenarios.

- Final discretion regarding interpretation is left to the analyst as experience is a significant factor in interpreting STR profiles.

B. Materials

- Data Evaluation Worksheet

- An allele table will be generated for each Complaint # and all sized profile data associated with the Complaint # will be included regardless of whether the data is used for interpretation or comparison.

- A note referencing the page number(s) of previously run data should be added to the bottom of subsequent submission allele tables if applicable.

C. Safety Considerations

- Prolonged computer analysis may cause muscle soreness, eye fatigue, or other discomforts. To minimize the occurrence of discomforts, periodic breaks from computer analysis are encouraged.

D. Limitations

- Analysis of forensic casework can produce several possible results. This documentation is to standardize the interpretation of profiles obtained.

F. Other Considerations

- All possible casework scenarios cannot be exhaustively described; therefore, extensive training, casework experience, and critical thinking skills are all essential factors in the proper interpretation and reporting of DNA mixture results. To the extent possible, evidence profiles should be interpreted prior to comparison to the known DNA profile(s) of the individual(s) in question.

- Within each case, all submitted reference samples will be compared to all

evidence profiles that are suitable for comparison.

- All questioned profiles will only be compared to reference profiles with the following exception:

    - Complete single source profiles (identical genotypes at all locations with a weight of one) may be compared to one another and reported as being consistent for the purpose of combining the reporting statement and reporting one statistic If a case has multiple, complete single source profiles with identical results (identical genotypes at all locations with a weight of one) and is associated to a reference, then only one of those samples needs to have LR calculations performed.

- There is no requirement to exclude profiles with unknown donors from one another, enumerate separate unknown donors, or to add “consistent with” wording to link unknown donors in the results section of the report.

G. Validation

These procedures are generally accepted in the forensic community and have undergone internal validation at the CMPD.

7.1 Data Evaluation

The initial evaluation of STR data in GMID-X will include looking at the presence of the primer peak in the raw data, the internal lane standard sizing quality, the allele calls for ladders and controls, the spectral efficiency, signal strength, resolution, migration, baseline, and artifacts.

All amplified data (including controls) will be assessed for the presence of preferential amplification. While preferential amplification of an allele, locus, or dye channel does occasionally occur, extreme occurrences can manifest as complete drop out of locus data or a severe reduction in the inter locus balance of peak amplitudes. It has been documented with sporadic GlobalFiler amplifications, that there is a preferential amplification of the NED (yellow) dye channel as compared to the other dye channels. This may be attributed a lack of available MgCl2 within the amp reaction and, therefore; it is important that a full volume of master mix is pipetted into each well and that the master mix is well mixed before pipetting.

Things to remember when evaluating data and determining any additional testing steps.

- Evaluate NED peaks – they can appear 3-15x higher than other peaks or other peaks can be dropped out completely.

- Look for drastic reverse degradation of NED and SID channels (purple) with possible dropout of lower molecular weight loci.

- View all dye channels together for each sample to get an idea of relative peak

height across the sample and review in the context of the quantitation data.

- This phenomenon is more common in samples with higher amounts of DNA (buccal standards and positive controls).

Reamplification is required to remedy the issue. All incidents of preferential amplification should be communicated to the DNA TL or designee to determine the severity of the issue and the suitability of impacted data. Depending on the severity of the issue, some single source data may be suitable for comparison.

7.1.1 Controls:

Any failure of controls and additional laboratory work associated with controls, must be communicated to the DNA TL or designee and shall be approved prior to using the associated data for comparison.

- A yellow triangle sizing quality flag is an indicator that the data may not be suitable for typing. If the sizing quality is starting to fail but the data is not impacted or if the failure occurs outside the analysis window (60 to 460 base pairs), then the data may be suitable for typing. For single source evidence samples, references, and controls only, an analyst may check the data visually and determine that it is suitable for interpretation/comparison and notate it on the Data Evaluation Worksheet. All other data with a yellow triangle sizing quality flag will be re-injected/re-run. Amplification Positive Controls may be used with TL or designee approval. Data with a red stop sign sizing quality flag is not suitable for interpretation/comparison.

- GMID-X (software version 1.6) may be used as an expert system to assess the allelic ladders. The analyst will document any ladders that don't meet quality controls measures on the Data Evaluation worksheet. GeneMapper automatically excludes low-quality ladders from analysis, so the sample type does not have to be changed from Allelic Ladder to Sample. If all ladders fail, the DNA samples on the plate will not be used for interpretation or comparison.

- An amplification negative control must be present in each amplification set and serves as a check for sample contamination introduced during amplification set-up. This blank control consists of the PCR Master Mix to which sterile TE Buffer is added instead of DNA. If a negative control contains a pattern of peaks (two or more peaks above the lower analytical threshold), any DNA specimens co-amplified with the reagent aliquots contained in the negative control may be considered inconclusive for typing. Reference Biology Quality Assurance Manual 15.5 for the Contamination Contingency Plan.

- An amplification positive control is required for each amplification set and is used to assess the proper performance of amplification and typing procedures. The manufacturer supplies DNA Control 007 as a Yfiler Plus kit component. The Control 007 is human genomic DNA. If the positive control exhibits a discordant result, all co-amplified samples may be considered inconclusive for typing. GMID-

X v1.6 may be used as an expert system to assess the amplification positive controls. If the positive control exhibits unexpected results, any co-amplified DNA samples will be evaluated for suitability for interpretation/comparison.

- A reagent blank must be present in each extraction set. The reagent blank serves as a check for possible contamination by adventitious DNA in samples, reagents or supplies during the testing procedure. Adventitious DNA can be non-amplified or amplified. If a reagent blank contains a pattern of peaks (two or more peaks above the lower analytical), any DNA specimens co-extracted with the reagent aliquots contained in the reagent blank may be considered inconclusive for typing. Reference Biology Quality Assurance Manual 15.5 for the Contamination Contingency Plan.

7.1.2 Stutter Peaks:

- STRmix™ models the height of both allelic and stutter peaks. As part of the STRmix™ implementation, expected stutter ratios pertaining to the kit and instrumentation used within the CMPD laboratory were determined. Within STRmix™, stutter in evidence samples is modelled per locus and per allele. When viewing the data, the GeneMapper GlobalFiler analysis methods/panels will have locus specific stutter filters in place. The analyst will use the Export Table with Stutter option to include stutter alleles in their evidence table(s) that will be imported into STRmix™ for analysis.

- The PCR amplification of STR loci typically produces a minor product peak four bases shorter (n-4) (for tetranucleotide STRs) or three bases shorter (n-3) (for trinucleotide STRs) than the corresponding main allele peak. This is a well-known aspect of STR analysis and should not cause concern when detected. On occasion with high level samples, stutter will appear as a peak that is eight bases shorter (n-8) or four bases larger (n+4) than the allele.

- Peaks may be considered as stutter in GlobalFiler and filtered in GeneMapper ID-X if their peak height percentages to the larger peaks are equal to or less than approximately:

Locus	Stutter
D3S1358	15.41%
vWA	13.45%
D16S539	10.68%
CSF1PO	11.59%
TPOX	6.94%
D8S1179	10.48%
D21S11	15.97%
D18S51	14.54%
DYS391	8.84%
D2S441	8.97%
D19S433	11.51%

TH01	4.83%
FGA	13.72%
D22S1045	20.49% (n-3)
D5S818	10.60%
D13S317	10.75%
D7S820	9.84%
SE33	14.49%
D10S1248	12.25%
D1S1656	13.22%
D12S391	16.48%
D2S1338	13.67%

D18S51	5.10% (n+4)
D22S1045	7.50% (n+3)

SE33	4.59% (n-2)
D1S1656	2.45% (n-2)

D18S51	4.10% (n-8)
FGA	2.96% (n-8)
D22S1045	5.65% (n-6)

SE33	4.53% (n-8)
D1S1656	2.69% (n-8)

- For any given GlobalFiler locus, minor peaks that are above the applicable analytical threshold and are located at a position one repeat unit smaller either (n-4) or (n-3) OR at D22S1045 (n+3), SE33 (n-2), D1S1656 (n-2), or one repeat unit larger (n+4) must be evaluated as to whether it represents stutter or a real allele.

- Plus stutter (n+4) percentages are generally below 2% of the main peak height and at times may exceed the 2% when the main peak is at or approaching saturation. A global 4% n+4 stutter filter is applied to all data except at the loci D18S51 and D22S1045.

- A 2% global n-8 stutter filter is applied to all data except at the loci D18S51, FGA, D22S1045, SE33, and D1S1656. For these five loci there were observed n-8 stutter values greater than 2%. TPOX and DYS391 were not evaluated due to one or no instances of n-8 stutter observed in the data set.

- The data used to support the application of the above stutter percentages can be found in the 3500 GlobalFiler stutter reevaluation study. For any given locus, minor peaks that are above the applicable analytical threshold and located in a documented stutter position may be considered as a real allele if their peak heights exceed the stutter percentage established for that locus as indicated above. Occasionally, stutter may exceed these values and an evaluation of the

entire profile is necessary including the allele at which the stutter is occurring. The number of base pair repeats of the main peak directly impacts the stutter percentage. The larger the number of repeat units in the main peak the higher the observed (n-) stutter percentage will be. The above percentages are averaged across the entire locus and may not completely account for stutter observed at the larger alleles at a locus.

7.1.3 Repeat Sequences:

All allele designations will be based on the number of repeat sequences as defined in the GlobalFiler technical manual and in the GeneMapper ID-X software distributed from Life Technologies.

7.1.4 Alleles:

For any given locus a peak above the applicable analytical threshold with no major peaks at a position one repeat unit larger to it will be interpreted as an allele unless it is likely due to a technical reason such as spiking or pull-up or a biological reason such as co-extracted bacterial DNA or a primer binding site mutation artifact (SE33).

7.1.5 Variants (off ladder):

The designation of alleles containing an incomplete repeat motif (i.e., an off ladder allele falling within the range spanned by the ladder alleles) should include the number of complete repeats and, separated by a decimal point, the number of base pairs in the incomplete repeat (e.g., FGA 18.2 allele). If an allele falls above the largest or below the smallest allele of the allelic ladder, the allele should be designated as either greater than (>) or less than (<) the respective ladder allele. STRmix™ will not accept non-numeric values such as > or <, so the locus will have to be ignored if the sample is suitable for STRmix interpretation/comparison.

If the profile is suitable for interpretation/comparison, refer to the table below to determine what if any further processing is needed to confirm it:

STR Result	Action
Two or more single source questioned samples exhibit the same variant allele	No further processing needed. Allele is considered confirmed.
A known sample exhibits a variant allele that is consistent with the variant allele for a single source questioned sample	No further processing needed. Allele is considered confirmed.
One single-source questioned sample exhibits a variant allele, no additional questioned or known sample(s) exhibit the same variant allele	Sample will be re-injected to confirm.
A known sample exhibits a variant allele and no questioned sample(s) exhibit the same variant allele	Sample will be re-injected to confirm.

Note: If STR results obtained do not fit any of the above scenarios, consult with the TL to determine if further processing is necessary.

Once the variant is confirmed, it can be included in the statistical calculations. The analyst will choose one of the injections to interpret in the STRmix™ software. If the analyst is uncertain to which locus the variant allele belongs, the loci in question will be ignored in the STRmix interpretation.

When importing an evidence sample into the STRmix™ software, the software may indicate there are OLs present in the sample that are not visible in the electropherogram. The analyst can compare the height/size information in the import table/evidence issues screen to the electropherogram to determine what is being considered an OL. If the OL is in a stutter position, it is not necessary to re-inject to confirm the allele designation. The analyst will change the OL in the import table to the appropriate allelic designation, save their changes, and re-import the sample into STRmix. This change to the import table should be noted on the appropriate worksheet.

7.1.6 Tri-alleles:

Occasionally a DNA profile will exhibit a three allele (tri-allele) genotype at one or more loci. Tri-allele genotypes are not included in statistical calculations. The STRmix™ software cannot account for tri-allelic loci within its calculations.

Tri-alleles may be easier to visualize in single source samples. If an analyst observes a tri-allele in a single source evidence sample, the locus with the tri-allele will be ignored in the STRmix™ interpretation. Tri-alleles may be less apparent in mixture samples, Reference the STRmix™ Interpretation SOP for further guidance.

If a tri-allele genotype is observed in a sample, refer to the table below to determine if further processing is needed:

STR Result	Action
Two or more single source questioned samples exhibit the same tri-allele genotype	No further processing needed
A known sample exhibits a tri-allele genotype that is consistent with the tri-allele genotype for a single source questioned sample	No further processing needed
One single-source questioned sample exhibits a tri-allele genotype, no additional questioned or known sample(s) exhibit the same tri-allele genotype	Sample will be re-amplified.
A known sample exhibits a tri-allele genotype and no questioned sample(s) exhibit the same genotype	Sample will be re-amplified.

Note: If STR results obtained do not fit any of the above scenarios, consult with the TL to determine if further processing is necessary.

If applicable, once the tri-allele has been confirmed, the analyst will choose one of the amplifications to interpret in the STRmix™ software and clearly note which one is used on the applicable Data Evaluation Worksheet.

7.2 Thresholds:

7.2.1 Analytical Threshold:

The analytical threshold is defined as the minimum RFU at which detectable peaks can be reliably distinguished from non-allelic peaks for evidentiary samples. Non-allelic peaks such as spikes, pull-up, elevated background noise, and PCR artifacts are typically associated with instrument noise or background.

The analytical threshold for 3500 GlobalFiler data has been established from validation studies at 100 RFU.

7.2.2 Stochastic Threshold:

The stochastic threshold is defined as the RFU value where stochastic effects are observed (e.g. allelic dropout and peak height ratio imbalance). Based on validation results, the stochastic threshold is set at 600 RFU for 3500 GlobalFiler data.

Although not utilized in STRmix™, the stochastic threshold can be used as a qualitative assessment of the data and will be used as the guideline for determining a homozygote allele in single source samples.

7.2.3 Upper Analytical Threshold:

The peaks in a DNA profile are measured using fluorescence which is captured by the CCD camera. The amount of fluorescence is proportional to the amount of DNA present. The camera can become saturated when there is too much fluorescence detected. This means it is no longer possible to accurately measure the height of the peaks observed or estimate how much DNA is actually represented by this result. Saturated data will have pink bar(s) above the peaks in the GMID-X software. Numerous artifacts may also be observed in profiles with saturated data. The upper analytical threshold is set at 30,000 RFU for 3500 GlobalFiler data.

Forensic DNA profiles displaying saturation are not suitable for STRmix™ analysis. Saturated data at Amelogenin, Yindel, or DYS391 may be considered acceptable because these locations are not interpreted by STRmix™.

Reference samples with saturated data will be evaluated by the analyst to determine whether the reference is suitable for comparison. STRmix™ does not interpret the reference sample (i.e. height information is not included in the import file), but uses the genotype information for comparison. Care must be taken to ensure accurate genotype information is obtained in a saturated

reference sample and excessive artifacts are not interfering with allele calls.

The Technical Leader should be consulted prior to sample re-amplification. If there is insufficient extract to amplify or other concerns, the interpretation of the sample will be discussed with the Technical Leader and documented.

7.2.4 Lower Analytical Threshold:

The lower analytical threshold is defined as the relative fluorescent unit (RFU) value where allelic peaks can be reliably distinguished from non-allelic peaks for reagent blanks and/or negative amplification controls.

The lower analytical threshold for 3500 GlobalFiler data has been established from validation studies at 50 RFU.

7.3 Extraneous Peaks

- Peaks other than the true alleles may be detected on the electropherograms display. It is important to identify the most likely cause for the appearance of extra peaks.

- Additional artifacts were observed and had reproducible base pair values in multiple samples. The artifact list is not comprehensive; however, the most common are characterized in the GlobalFiler User Guide and subsequent bulletins.

Locus	Dye Channel	Size
D3S1358	Blue	~132 bp
D3S1358	Blue	~126-127 bp
vWA	Blue	~204 bp
D16S539	Blue	~228 bp *
D8S1179	Green	~114-121 bp
D8S1179	Green	~124 bp
D21S11	Green	~207 bp
D2S441	Yellow	N-2 to N-2.5 bp
D2S441	Yellow	~76 bp
D19S433	Yellow	N-6 bp
D19S433	Yellow	N+2 to N+4 bp
N/A	Red	~250 bp
D5S818	Red	~180 bp
D5S818	Red	~142 bp
SE33	Red	N-90 bp **
SE33	Red	~352.5 bp
D2S1338	Purple	~345 bp

* Related to artifacts ~95 bp in green dye channel and ~424 bp in red channel most likely associated with some species of pig or wild boar DNA.

** Sample dependent artifact likely caused by the presence and amplification of human DNA containing a Single Nucleotide Polymorphism (SNP) internal to the SE33 primer binding sites that allows an unlabeled non-SE33 primer to bind. Has typical signal intensity of ~15% to 30% of the parent peak height. Depending on the location of the parent SE33 allele containing the SNP, the artifact could appear in D13S317, D7S820 or SE33.

- These artifacts will have their labels edited on the GeneMapper ID-X electropherograms using the editing function. Artifact code designations will be noted as below in the table. The designation "ART" will be used for all artifacts. Additional notations as to the type of artifact will be made in the subsequent "Reason(s) for Change" comment screen in GeneMapper ID-X and will show up on the Artifacts Edits Worksheet.

Artifact	Code	Comment
Stutter	ART	ST
Pullup	ART	PU
Spike	ART	SP
Minus A	ART	MA
N+4	ART	NP
Other Artifacts	ART	ART

7.3.1 Incomplete 3' (A) Nucleotide Addition:

The DNA polymerase used in STR/PCR amplification catalyzes the addition of a single nucleotide to the 3' ends of double stranded PCR products. This non-template addition results in a PCR product which is one base pair longer than the actual target DNA sequence. STR/PCR amplifications have been optimized to favor the A addition. Incomplete A addition is recognized as "split peaks" (n-1), where a target allele is represented by two peaks one base pair apart. If this is observed within a DNA profile, re-amplification may be necessary. Please consult the Technical Leader for guidance.

7.3.2 Spectral Failure:

A matrix failure is a result of the instrument's inability to separate the colors (spectral overlap) used to fluorescently label STR/PCR products. A matrix failure is observed as a peak beneath a peak or as an elevation of the baselines for any color and can occur as reagents change slightly over the course of time. A peak of another color is "pulled up" as a minor component beneath the true allele peak. If excessive pull-up is observed in a sample/3500 run, re-amplification may be necessary. The possible cause (i.e. mis-amplification of single sample/instrument issues) should be investigated.

7.3.3 Stutter:

- Peaks in known references and/or amplification positive controls that are not filtered by the software but still determined to be stutter will be edited as such by

the analyst.

- Although each applicable peak will be evaluated for the possibility of being stutter, no edits of stutter will be performed on forensic samples. This evaluation may aid in the determination of the number of contributors and the intuitiveness of the STRmix interpretation.

7.3.4 Spike:

A spike is an extra peak that can be caused by a polymer crystal, an electrical impulse, or bumping of the instrument. There may be circumstances when a spike impacts the data (i.e. spike interfering with an allele call) and re-injection will be necessary.

7.4 Primary Evaluation of Samples

Within the STRmix internal validation, studies were conducted to determine the software's reliability over a broad range of DNA typing results. The guidelines that follow are based on information obtained from the internal validation. Forensic DNA profiles undergo multiple assessments following GeneMapper ID-X (GMID-X) analysis to determine if the sample is suitable for STRmix™ interpretation.

Profiles or portions of profiles deemed not suitable for comparison due to the limited completeness or complexity of the data will be used for neither inclusion nor exclusion.

The primary evaluation of forensic samples will include evaluating for general overall quality, including an assessment of the quantitation amount, amount of DNA amplified, degradation, and baseline.

- Forensic DNA profiles displaying saturation are not suitable for STRmix™ analysis. Saturated data, indicated by pink bar(s) above the peaks in the GMID-X software, will likely have inaccurate peak heights which will affect the performance of the models within STRmix™. Saturated data at Amelogenin, Yindel, or DYS391 may be considered acceptable because these locations are not interpreted by STRmix™. The Technical Leader should be consulted prior to sample re-amplification. If there is insufficient extract to amplify or other concerns, the interpretation of the sample will be discussed with the Technical Leader and documented.

- Forensic DNA profiles containing data at less than seven loci (not including Amelogenin or Y markers) are not suitable for STRmix™ analysis.

- STRmix™ cannot effectively/accurately interpret loci with the following: unresolved 1bp microvariants/alleles (i.e. peak $\geq$ 100 RFU), tri-alleles, off ladder allele designations containing > or <, suspected primer binding site mutations, somatic mutations, null alleles, or other anomalous issues (such as vWA 14 allele unintuitive genotypes) that cannot be remedied through additional laboratory work. In these instances, the Ignore box for the locus within the Kit Settings\Loci window should be checked to omit the locus from interpretation. For

unresolved 1bp microvariants that are below the AT, the locus will not be ignored since this data would not be part of the STRmix™ evaluation and will not impact genotypes.

The Ignore Locus function refers specifically to omitting a locus or loci within the STRmix software, analysts are still responsible for visually inspecting and evaluating these locations. If references are submitted for comparison, analysts will compare any ignored locations manually. Ignored loci are not considered uninterpretable loci, instead they are loci where statistics cannot be applied. Ignoring a locus from an evidence/reference profile does not make the profile partial. Additionally, an ignored locus is not considered a location without data for the purposes of determining if there are at least seven loci with data. The reasoning for using the ignore loci function will be documented on the appropriate worksheet and ignored loci wording will be added to the report. The Technical Leader should be consulted prior to ignoring a locus or loci within STRmix™.

7.5 Determination of Number of Contributors (NOC)

After the primary evaluation, the sample will be assessed to determine the number of contributors. The true number of contributors to a forensic sample is unknown. Defining the sample as being attributed to a discrete number of contributors does not mean that it is impossible for additional contributors to be present, only that the data presents as such and that the interpretation was performed under that assumption.

Analysts should use their professional judgment when assessing the number of contributors to a forensic profile. The initial assessment of the number of contributors to a DNA profile is based on a comprehensive review of the profile and occurs prior to comparison to a reference sample(s). The presence of low-level DNA, multiple contributors, apparent degradation, inhibition, or significant stochastic effects may increase the complexity in determining the number of contributors.

The presence of a conditioned contributor within a mixture may be used to assist with the estimation of number of contributors to a sample. Additionally, non-sperm cell and sperm cell fractions from the same sample can be used to assist in the determination of the number of contributors of the alternate fraction.

Overestimating and underestimating the number of contributors within STRmix™ generally has little significant effect on major and well-represented minor contributors but can affect trace contributors. The risk of false inclusion increases when the number of contributors is overestimated, while the risk of false exclusion increases when the number of contributors is underestimated. The CMPD STRmix™ internal validation demonstrated analysts were more likely to underestimate the number of contributors than overestimate.

7.5.1 Method for Assigning the Number of Contributors for a Profile

When determining the number of contributors to a profile, the entire profile will be

reviewed.

To assign the number of contributors to a profile:

- Review the profile in its entirety, assessing the quantitation amount, amount of DNA amplified, level of degradation, presence of low-level peaks, noisy or clean baseline and general quality of the profile. The presence of apparent degradation, inhibition, or significant stochastic effects in a profile shall be considered and may influence the assessments made at each locus. Careful attention should be used when considering a partial profile recovered from a sample with a target amount of less than 250 pg. The stochastic threshold, although not utilized in STRmix™, can be used as a qualitative assessment of the data.

- Find the locus/loci with the greatest number of unambiguous alleles to determine an initial number of contributors. Loci that show the most variation are FGA, SE33, D1S1656, D12S391, and D2S1338. If one or more contributors appear to be trace or more prominent contributors, check that this pattern is represented at other loci. Gender markers can also be used to assist in the determination of number of contributors.

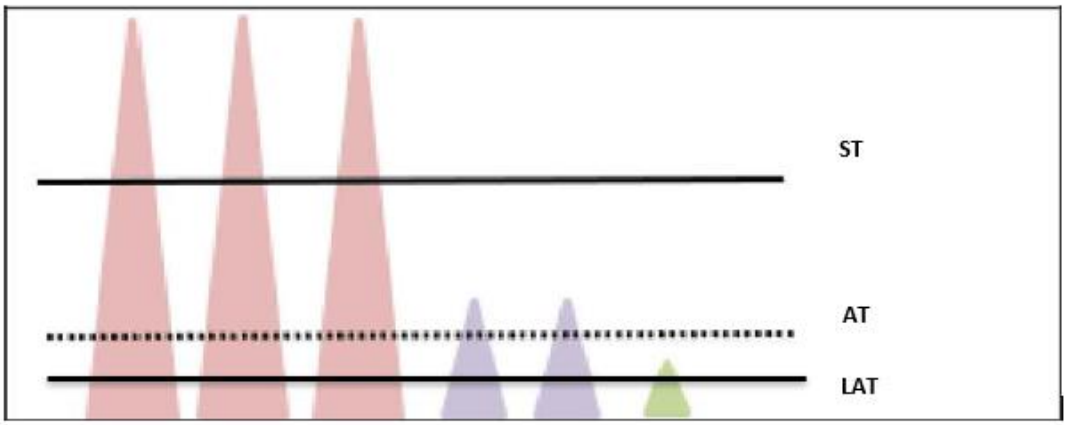
- Through previous validation a minimum 50% peak height ratio (PHR) was observed for heterozygote allele pairs. Generally increased amounts of input DNA result in the higher PHR of heterozygote pairs. It is recognized that low template or degraded samples may deviate from the 50% rule, and a broader combination of genotypes should be considered.

  Review peak height imbalances, particularly at the loci with the greatest number of alleles and consider the possibility of allele sharing or “stacking.” Generally, homozygote alleles will appear with peak heights twice as large as heterozygotes. Visually attempt to pair alleles according to expected peak height ratios. Peak height imbalances may indicate the presence of an additional contributor and may warrant increasing the assigned number of contributors value.

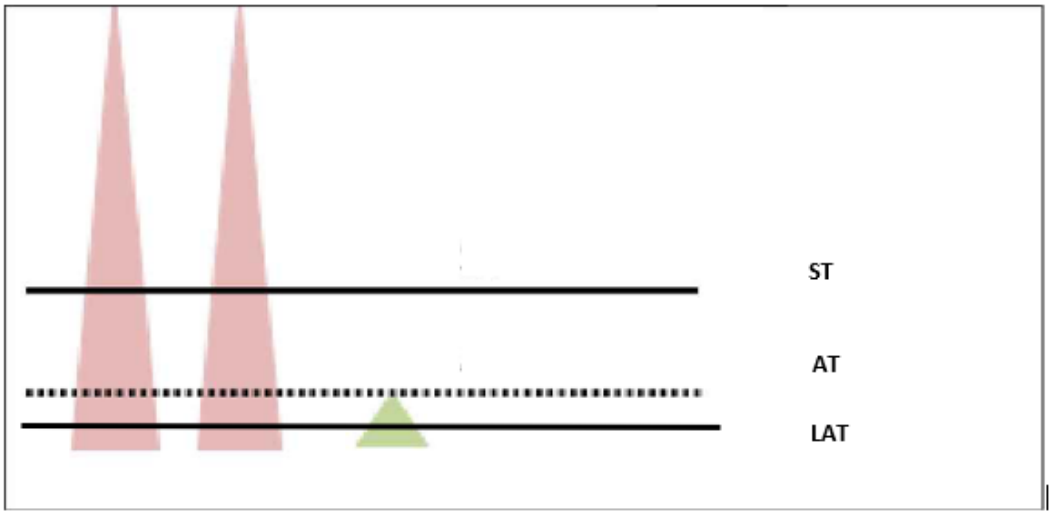
- Data seen between the analytical threshold and the lower analytical threshold can also be considered in determining the number of contributors. Data at this level in stutter positions should be assessed in the context of the profile. This would include evaluating the height of parent alleles and the height of other non-called peaks in stutter positions. Particular care should be used when examining data between the analytical and lower analytical threshold for the presence of microvariants. Data between the analytical and lower analytical threshold can support the addition of a contributor if there are low-level peaks or peak height imbalances in the profile. Increasing the number of contributors is supported when 1.) the analyst cannot clearly differentiate between one or more contributors in a forensic sample and the data between analytical and lower analytical threshold (The stochastic threshold may assist in this assessment) and 2.) the presence of this data, combined with the total number of called alleles at a locus, creates a sum total that supports a higher number of contributors. If there are no

indications the data between the analytical and lower analytical threshold is influencing the alleles in the profile, an additional contributor should not be added to the total number of contributors. Data observed below the lower analytical threshold will not be considered when assigning number of contributors, as this is not sufficiently differentiating from the baseline.

For example, consider increasing the NOC when the peak between LAT and AT “green peak’ is in a non-stutter position and could pair with the alleles called above AT “purple peaks.”



Increasing the NOC is not warranted if the peak between LAT and AT “green peak” cannot pair with the alleles above AT and ST “pink peaks”.



- Alleles in stutter positions can be used to inform the number of contributors. Elevated stutter amounts can indicate an allelic contribution by an additional contributor.

- Apply the general contributor proportions (number and mixture ratio) across the profile to see if the pattern fits across multiple loci. If the pattern generally fits,

assign this number of contributors to the mixture; otherwise consider adjusting the number of contributors.

- There may be circumstances when a profile appears single source, but one additional allele below the drop-in cap of 150 RFU is observed. Drop-in is a rare and sporadic event and should be considered within that context. The analyst will review the entire profile, including data between the analytical threshold and the lower analytical threshold. If the additional allele is not consistent with originating from the single source contributor, it may indicate drop-in has occurred. The profile will be considered single source for STRmix™ interpretation. The suspected drop-in peak is not suitable for comparison.

When evaluating mixture profile data, an analyst should carefully consider if the chosen number of contributors limits the software to consider an allele as non-allelic (i.e. stutter) or drop-in. Drop-in is a rare and sporadic event and should be considered within that context. The analyst should review the entire profile and choose a number of contributors value that is the most likely number required to reasonably explain the observed data.

There is no conservative approach to estimating the number of contributors to a mixed DNA profile. The number of contributors value should be the most likely number required to reasonably explain the observed data. If the number of contributors is ambiguous, the analyst should initially deconvolute the sample in STRmix™ using the lowest number of contributors value that can reasonably explain the data. Generally, mildly elevated stutter (typically within 5% of the applied GMID-X filters), peak height imbalance, and fluctuation of contributor ratio at only a few loci can be considered ambiguous information. These factors individually should not be the sole reason to increase the number of contributors. The profile should be evaluated in its totality.

Replicate amplifications can assist in the interpretation of complex and/or low-level mixtures by adding relevant information. Additionally, replicates can aid in determining the number of contributors and be used as a troubleshooting measure. Replicate amplifications of forensic samples are performed at the discretion of the analyst and require the Technical Leader’s or designee’s approval. When using replicate amplification(s), all qualifying replicates should be examined when determining the assigned number of contributors. In addition to the guidelines listed above, consider the sum total of distinct alleles across replicates. For example, if the first replicate has 5 called alleles at FGA, and the second replicate has 6 called alleles (4 alleles in common with the first replicate and 2 different alleles), the distinct total number of alleles visualized at this locus is 7.

After STRmix™ deconvolution/LR calculations with the initial determined number of contributors, an analyst may review their interpretation/LR results and determine that the results did not meet their expectations of the data and the number of contributors may need to be re-evaluated. Any subsequent analysis adjusting the number of contributors would need approval from the Technical Leader or designee. All STRMix™ runs shall be documented in the case file.

7.6 Assessing for Conditioned Contributors

In certain situations, it may be reasonable to expect an individual is present in a forensic DNA profile. When an individual is an expected donor to a DNA profile, their reference profile may be used to condition the forensic profile during STRmix™ analysis. A forensic profile may be conditioned on more than one reference. Conditioning improves the deconvolution of any additional contributors to the mixture and can better inform LR calculations. Conditioning should be utilized when applicable. A reference profile must have complete results at all locations to be used as a conditioning profile. Exceptions may be made at the discretion of the Technical Leader or designee.

To condition on an individual, it must be reasonable to expect their presence on an item based on case details and item information. Decisions regarding whether a reference can be considered as a reasonably expected contributor will be made on a case-by-case basis and supporting documentation will be added to the relevant case file**.** Particular care should be used when evaluating a suspect reference as a potential conditioned contributor to an item of evidence. In many circumstances, due to case scenario, it will not be appropriate to condition on a suspect reference. Possession cases are one example of when it would not be appropriate to condition on a suspect reference.

Examples when it is reasonable to expect an individual include:

- Intimate samples which originate directly from an individual's body, such as swabs of any skin surface or orifice and fingernail scrapings/clippings. (May expect the victim or consensual partner(s))
- Items documented to have been removed directly from someone's person
- Samples which individuals own/have regularly touched or handled (includes bedding, individual's cell phone, steering wheel of car, items recovered from a location where an individual is documented to reside). These items will generally be accessible to a limited number of individuals.
- On body fluids identified on any of the previously listed samples/items
- Carryover in a differential extraction from one fraction into the other

Once a reference profile meets the guidelines to be considered a reasonably expected contributor, the analyst will manually compare the reference profile to the forensic DNA profile. If the manual review of the data supports the presence of the reference profile, the analyst may proceed to STRmix™ analysis and condition on the reference profile. The number of contributors used in this deconvolution must consider the presence of the conditioned contributor. This may require the analyst to adjust their initially considered number of contributors after evaluating the data.

If after the manual review it is ambiguous as to whether the reasonably expected reference profile is present (contributor is partially represented), a deconvolution of the forensic profile will be performed without conditioning. The number of contributors used in this deconvolution will be based on the data and will not consider the reasonably expected contributor. Once the interpretation report has been reviewed, the reference

sample will then be compared to the deconvolution by performing a Likelihood Ratio calculation. The resulting LR must be greater than 1000 in a contributor position suitable for comparison to support conditioning using this contributor. If the LR is greater than 1000 in a contributor position suitable for comparison, the analyst will then perform a deconvolution of the forensic DNA profile conditioned on this reference profile. The number of contributors will be re-assessed for this subsequent deconvolution to consider the presence of the conditioned contributor. The Technical Leader does not need to approve this subsequent deconvolution. A note will be added to the STRmix Interpretation worksheet documenting the reason it is not being used and the preliminary LR will not be reported. All STRmix deconvolutions will be documented in the case file.

If the LR is 1000 or less or if the reference is placed in a contributor position that is not suitable for comparison, conditioning using this individual is not supported and the forensic DNA profile will not be conditioned on this reference profile. The analyst can proceed with any additional comparisons to the original deconvolution.

A conditioning reference profile will be included in both the H1 hypothesis and H2 hypothesis. The use of conditioning in interpretation will be stated in the report.

A single source non-sperm/sperm cell fraction can only be used as a conditioning profile in the alternate fraction with Technical Leader or designee approval. The analyst will use the STRmix™ Reference table setting to export the sample intended for conditioning into STRmix™.

With CODIS Administrator/backup CODIS Administrator approval, an analyst may use a profile/contributor to condition a forensic profile for CODIS entry purposes only.

7.7 Profile Suitability for STRmix™ Interpretation

Single Source Profiles

Generally, a profile will be determined to be single source if :

- There are no more than two alleles at a locus (except for a tri-allelic individual or if an additional allele has been evaluated as drop-in)
- Any peaks in stutter positions have been evaluated and determined to be non-allelic rather than an additional contributor. This decision will be based on an evaluation of the entire profile, including data between the analytical threshold and the lower analytical threshold. Additional information to assess include the possible stutter percentage, the location of the possible stutter, and any additional possible stutters present
- Heterozygous alleles have >50% PHR at all loci, and if <50% PHR is present, it is reasonable based on the context of the profile.

If an analyst has assessed the profile as single source, STRmix™ may be used to deconvolute and interpret:

- Full single source profiles

- Partial single source profiles that have at least seven loci with data (not including Amelogenin or Y markers) and four complete genotypes. The stochastic threshold, although not utilized in STRmix™, can be used as a qualitative assessment of the data and will be used as the guideline for determining a homozygote allele.

Single source profiles that are suitable for STRmix™ analysis will be interpreted in the software. Once the deconvolution reports have been reviewed and meet expectations, the analyst should perform manual comparisons of the data to any applicable standards. The analyst can then perform LR calculations if necessary. The LR calculations should be reviewed for consistency with the manual assessment. If an analyst has made a manual exclusion of a standard to a single source or partial single source profile, the LR calculation does not have to be performed in STRmix™.

If a case has multiple, complete single source profiles with identical results (for example, identical genotypes at all locations with a weight of one) and is associated with a reference, then only one of those samples needs to have LR calculations performed. Results statements may be combined in the report with a “consistent with” statement.

It is not necessary to use STRmix™ to deconvolute and/or calculate an LR for single source profiles in a non-sperm cell fraction that is consistent with an expected donor or for any single source profile where an individual is an expected donor.

Mixture Profiles

A DNA mixture is generally defined as a DNA profile where the following is observed:0.0

- more than two alleles are detected at one or more loci (except for a tri-allelic individual)
- peaks are present in stutter positions that have been evaluated as an additional contributor rather than non-allelic. This decision will be based on an evaluation of the entire profile, including data between the analytical threshold and the lower analytical threshold. Additional information to assess includes the possible stutter percentage, the location of the possible stutter, and any additional alleles or possible stutters present in the profile.
- the genotype combination for a heterozygous genotype is significantly imbalanced (<50%) and is not attributable to known stochastic effects

If an analyst has assessed the profile as a mixture:

- Two-, three-, and four-person mixture profiles with at least seven loci with data (not including Amelogenin or Y markers) are suitable for STRmix™ interpretation.

- DNA mixtures assigned a number of contributors value greater than four are not suitable for STRmix™ interpretation.

Once the mixture data has met the above guidelines, the RFUs of the data should be examined. If one or more alleles are above the stochastic threshold (not including Amelogenin or Y markers), then the profile may proceed to STRmix™ analysis.

If all alleles in the profile are below the stochastic threshold (not including Amelogenin or Y markers), STRmix™ analysis will proceed if the following parameters are met:

For NOC=2, 4 loci must have at least 2 alleles
For NOC=3, 4 loci must have at least 3 alleles
For NOC=4, 4 loci must have at least 4 alleles

These parameters are designed to ensure at least partial representation of all contributors at a minimum of four loci. Mixtures not meeting these parameters are not suitable for STRmix™ analysis.

Mixture profiles that are suitable for STRmix™ analysis will be interpreted in the software. The analyst should evaluate the STRmix™ interpretation report in the context of the DNA profile. Analysts will review the results for intuitiveness, as well as evaluate the primary and secondary diagnostics. Further suitability assessment of the components of the mixture will be made based on template amount and mixture proportion. Once the analyst determines the deconvolution report meets their expectations, the analyst should perform initial manual comparisons of the data to any applicable standards. The analyst can then perform LR calculations. All applicable standards will have LR calculations performed. The LR calculations should be reviewed for consistency with the manual assessment.

For a differentially extracted sample, based on an evaluation of the DNA profiles obtained from the sperm and non-sperm cell fractions, it may not be necessary to analyze both fractions with STRmix™. As mentioned previously, it is not necessary to use STRmix™ to deconvolute and/or calculate an LR for single source profiles in a non-sperm cell fraction that is consistent with an expected donor.

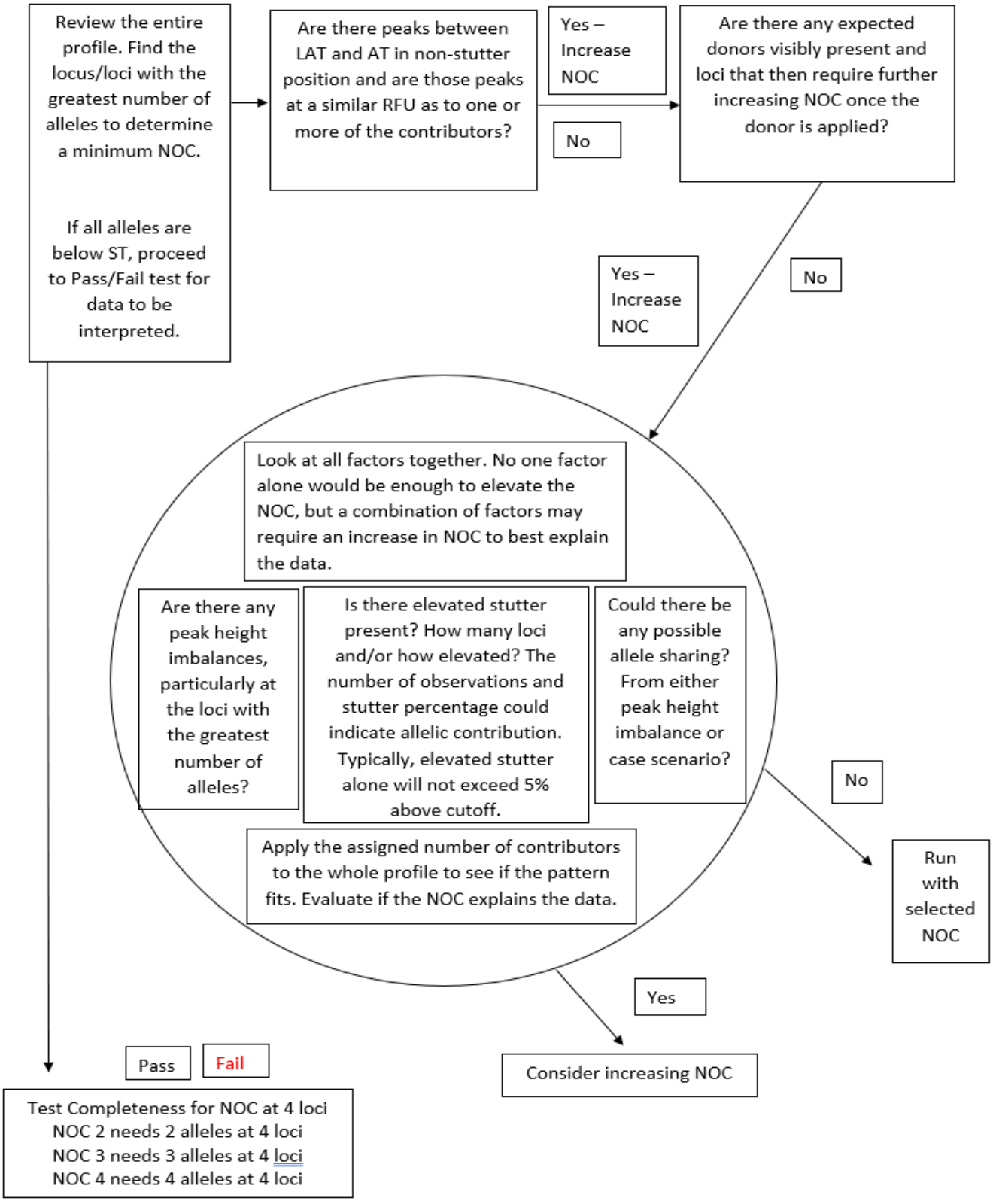
For a mixture profile that is consistent with two expected donors (e.g. victim and consensual partner) it is not necessary to use STRmix™ to deconvolute and/or calculate an LR. This may be reported as consistent with the two expected donors. With DNA Technical Leader or designee approval, comparisons of the mixture profile to another submitted reference do not require the use of STRmix™.

Carryover is a phenomenon observed in differentially extracted samples where epithelial cell/non-sperm cell DNA carries over into the sperm cell fraction or sperm cell DNA carries over into the non-sperm cell fraction resulting in a mixed DNA profile. For differentially extracted samples where a contributor is determined to be carryover, no further interpretation is necessary with STRmix™ to assess the carryover contribution and LR calculations will not be performed. This means that profiles obtained from the non-sperm cell fraction that are a mixture of the expected donor and carryover from the same male obtained from the sperm cell fraction do not need STRmix interpretation or

LRs performed on the entirety of the profile. Profiles obtained from the sperm cell fraction that are a mixture of male donor(s) and carryover from the non-sperm cell fraction do not need the carryover portion interpreted in STRmix or LR's performed. Documentation that carryover was observed will be noted in the case file.

Due to case circumstances or case data, there may be instances when both fractions of a differential extraction need to be analyzed in STRmix™. Some examples include multiple suspects, type of assault, and ambiguous results.

7.8 NOC Determination Flowchart



7.9 References:

- SWGDAM Interpretation Guidelines for Autosomal STR typing by forensic DNA testing laboratories, 2010, Available at www.swgdam.org
- The Federal Bureau of Investigations Quality Assurance Standards Audit Document for Forensic DNA Testing Laboratories
- NRC II, 1996
- Journal of Forensic Science, Volume 44(6):1277-1286.
- Journal of Forensic Science, Volume 46 (3): 453-489.
- Journal of Forensic Science, Erratum Volume 60 (4): 1114-1116
- FSI Genetics, Volume 7, Issue 3, Pages e82-e83.
- Forensic Science International: online August 12, 2017, Corrigendum to 'U.S. Population Data for 29 Autosomal STR Loci' [Forensic Sci. Int. Genet. 7 (2013) e82–e83].
- SWGDAM Interpretation Guidelines for Autosomal STR typing by forensic DNA testing laboratories, 2017, Available at www.swgdam.org

8. DNA Interpretation Software

8.1 STRmix™

A. Purpose and Introduction:

STRmix™ is a fully continuous probabilistic genotyping software. Probabilistic genotyping is the use of biological modelling, statistical theory, computer algorithms, and probability distributions to calculate likelihood ratios and/or infer genotypes for the DNA typing results of forensic samples. The STRmix™ software is a tool to assist the DNA analyst in the interpretation of forensic DNA profiles. Once the analyst assigns the number of contributors, the software deduces possible genotype combinations that could reasonably explain the data and provides a weighting to the different combinations. If a reference is available, STRmix™ provides a statistic by calculating the likelihood ratio (LR). The LR is the probability of observing the DNA typing results given that the hypothesis one proposition is true divided by the probability of observing the DNA typing results given that the hypothesis two proposition is true.

B. Materials and Instrumentation:

- STRmix™ Software, version 2.9.1 running on a workstation or server.

- A computer with 64 gigabytes of RAM can analyze 4 person mixtures without the need for low memory mode.

C. Safety Considerations:

Prolonged computer analysis may cause muscle soreness, eye fatigue, or other discomforts. To minimize the occurrence of discomforts, periodic breaks from computer analysis are encouraged.

D. Limitations:

The software can assist in the interpretation of single source profiles and mixture profiles of two, three, or four contributors, including partial or complete profiles. The STRmix™ software requires the analyst to assess the data and determine the number of contributors.

E. Other Considerations:

Through an evaluation of the electronic data in GeneMapper ID-X and the use of the STRmix™ software, the analyst will interpret the data. Notes conveying the initial assessment of the data will be made on the Data Evaluation Worksheet. Any profile deemed not suitable for comparison and therefore not interpreted using STRmix™ will be noted on the Data Evaluation Worksheet.

Data generated on the 3500 Genetic Analyzer instruments using GlobalFiler chemistry and previously reported as not suitable for comparison in whole or in part may be reinterpreted using STRmix™ on a case-by-case basis with the approval of

the DNA Technical Leader or designee. See QA 7.5 and QA 18.2.8 for additional guidance.

F. Validation:

- These procedures are generally accepted in the forensic community and meet SWGDAM guidelines.

- This procedure has undergone internal validation at the CMPD.

Procedure:

8.1.1 Prior to running STRmix™:

- The analyst must be able to determine the number of contributors.

- The analyst must determine whether the mixture is suitable for interpretation.

- The analyst must determine whether conditioning is appropriate.

- The sample profiles must be analyzed, and data exported in the appropriate text file using GMID-X

  NOTE: If no electronic files are available for the known standard for comparison, then the import file can be created manually.

- The analyst must log into the STRmix™ computer to ensure they are the user that appears in subsequent STRmix™ reports. An analyst must close the software and log out of the computer when they are finished. Switch User should not be used on computers with STRmix™ software due to potential license errors.

- An analyst should make sure the prior analyst's STRmix™ interpretations/comparisons are completed before beginning their analysis.

8.1.2 STRmix Interpretation Worksheet

The decision whether a sample is suitable/not suitable for STRmix interpretation is documented on the Data Evaluation Worksheet. The analyst can then click on the "Go to STRmix Interpretation" button on the Data Evaluation worksheet to access the STRmix Interpretation Worksheet. A copy of the printed STRmix Interpretation Worksheet will be included in the case file. The analyst will fill out/verify the following fields within the STRmix Interpretation Worksheet:

Sample Information:
Any sample that the analyst has marked "Yes" for STRmix on the Data Evaluation worksheet should be listed in the Sample Information column. Within the Sample Information column, the top cell lists the DNA #, Item # and description of the sample. The bottom cell can be used by the analyst to add any additional comments pertaining to that sample. The analyst should verify all appropriate samples are listed within this column.

NOC:
The analyst will click on the drop-down menu in the top cell of the NOC column and choose the analyst determined number of contributors.

Proposition: Hp = / Proposition: Hd = :
In these columns the analyst will document the general propositions they are using for the sample. This information is typically filled in after the deconvolution is reviewed. These cells will be marked N/A if there are no references for comparison or if the analyst has manually excluded all reference profiles. Manual exclusions will be documented in the bottom cell of the appropriate sample's sample information column.

The following abbreviations will be used in these columns:

Con= Conditioned contributor
Ref = Reference
U = Unknown, unrelated individual

For example, a sample with NOC=3 and two references submitted for comparison can appear in either format seen below: (Note: The proposition only has to be written once.)

NOC	Proposition: Hp =	Proposition: Hd =	List any conditioned individual(s) below:	List any reference individual(s) for comparison below:
NOC=3	Ref+2U	3U	N/A	24-00000, 24-00001

Or

NOC	Proposition: Hp =	Proposition: Hd =	List any conditioned individual(s) below:	List any reference individual(s) for comparison below:
NOC=3	Ref + U + U	U + U + U	N/A	24-00000, 24-00001

A sample with NOC=3, a conditioned contributor, and no additional submitted references can appear as the following:

NOC	Proposition: Hp =	Proposition: Hd =	List any conditioned individual(s) below:	List any reference individual(s) for comparison below:
NOC=3	N/A	N/A	24-00000	N/A

If an evidence sample has multiple individuals with individual inclusionary LRs, the analyst will perform a compound LR. The analyst will note the performance of a compound LR in the bottom cell of the applicable sample's sample information column. The analyst only has to note the compound LR was performed, no further details are necessary.

If a compound LR is specifically requested, the analyst will note that a compound LR was performed in the bottom cell of the applicable sample's sample information column. The analyst will also note the propositions and references used in the compound LR comparison.

The STRmix software uses HP and HD to describe the propositions. The lab may substitute H1 for HP and H2 for HD for reporting purposes and within the protocols.

List any conditioned individual(s) below:
In the top cell of this column, the analyst will write/type/list the DNA # (s) of any individuals the interpretation is being conditioned on. This cell will be marked N/A if there are no conditioned contributors.

List any reference individual(s) for comparison below:
In the top cell of this column, the analyst will write/type/list the DNA # (s) of any references the interpretation will be compared to. This cell will be marked N/A if there are no references for comparison.

Diagnostics were reviewed checkbox/ Is additional work needed checkbox:
Once the analyst has reviewed the applicable deconvolution/comparison(s) primary and secondary diagnostic results, the analyst will place a check in the Diagnostics were reviewed checkbox.

If, after review, the analyst has determined that additional STRmix analysis is necessary, the analyst will place a check in the Is additional work needed checkbox.

Sample sent to COSTaR? :

The decision on whether a sample is suitable for COSTaR will be documented by choosing "Yes" or "No" from the drop-down menu in the cell to the left of the Sample sent to COSTaR box.

If the answer is "No", document the reason using the drop-down menu in the cell to the right of the Sample Sent to COSTaR box. The analyst can also type/handwrite a reason into this cell.

8.1.3 Troubleshooting-Table Import into STRmix™

Data checks are performed on the input profiles as they are added to the STRmix™ software. When STRmix™ detects an issue in the evidence input file, for example missing stutter or invalid peaks, the software will prompt the user to resolve the issue.

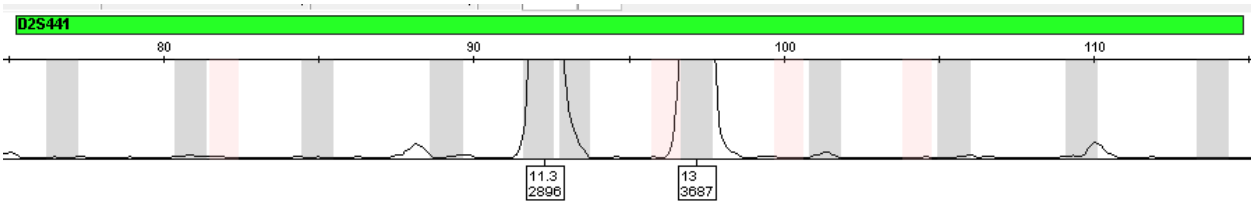
If a sample is flagged for missing stutter, the analyst should first confirm the evidence table was exported correctly. If the table is correct, the analyst should then closely examine the locus electropherogram data. If the stutter is not present or is below analytical threshold, the analyst can proceed with interpretation without ignoring the locus in the software.

Stutter that is missing due to a 1bp resolution issue will be treated differently depending on whether the sample is single source or a mixture.

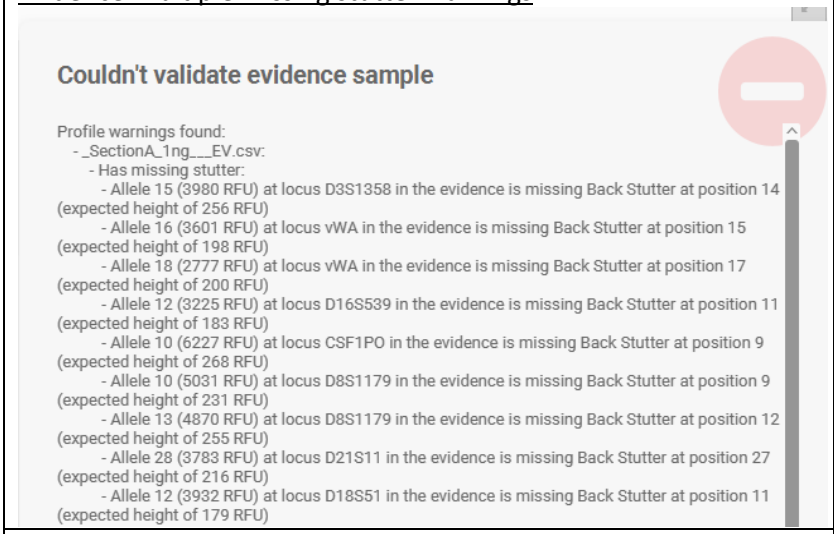
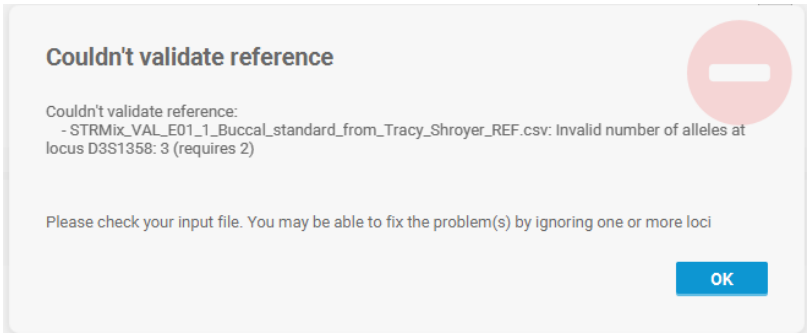
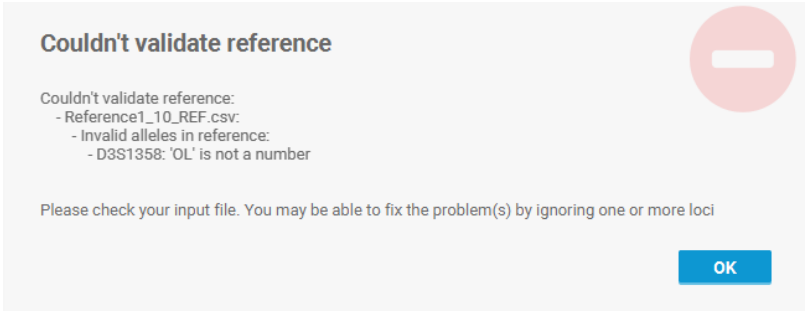
- In a single source sample, the analyst can proceed with interpreting the sample without ignoring the locus in the software. This may cause the stutter variance to be elevated but should not affect the ability of STRmix™ to assign the correct genotype to the locus.

- In mixture samples, if the stutter is 1) distinguishable from the neighboring allele and 2) determined to be below the analytical threshold, the analyst may proceed with interpretation without ignoring the locus in the software. If the missing peak in stutter position is 1) distinguishable from the neighboring allele and 2) above the analytical threshold, the locus should be ignored in the software. This is because there is missing data present that may affect the deconvoluted genotypes and their weights. See the troubleshooting table for a detailed example.

  In some instances, the stutter peak will not be able to be distinguished from the neighboring allele. See the electropherogram below where the 12 is not being resolved/is not distinguishable from the 11.3 allele. Loci with stutters that cannot be distinguished from neighboring alleles in mixture samples will be ignored in the software.

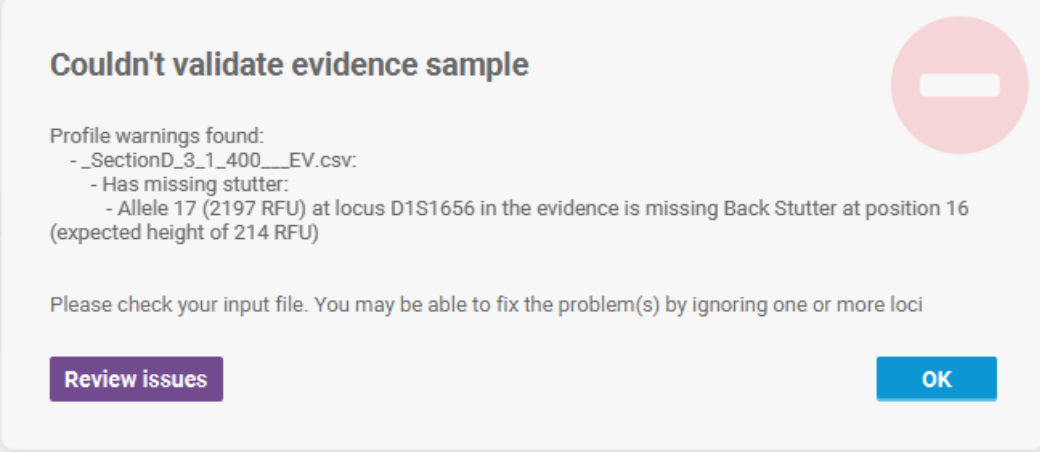


The table below lists additional scenarios that may occur when importing evidence and reference tables into the STRmix™ software. If an issue arises that is not referenced, please consult the Technical Leader.

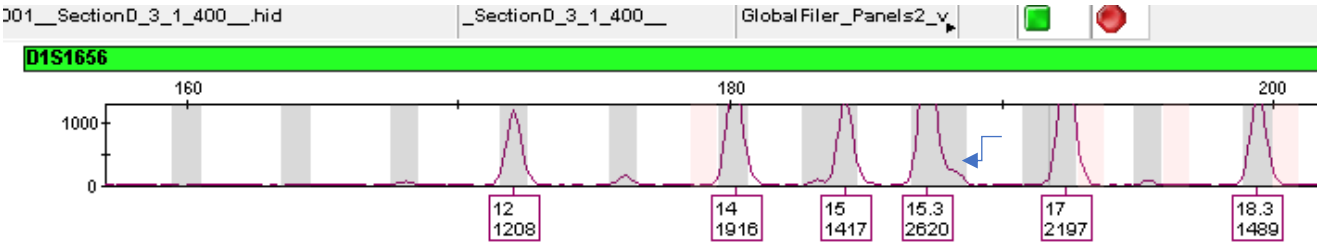
Observation	Action
Evidence-Multiple missing stutter warnings 	• Click purple Review Issues button to investigate further • If Issue “Has missing stutter”: appears at multiple loci, possible evidence profile exported without stutter • Review import table to confirm samples exported without stutter • Export table from GMID-X using Export Table with Stutter option
Reference-Invalid number of alleles 	• There can only be two alleles at each locus for reference profiles. Confirm correct sample exported. • Review import file/electropherogram. Confirm table exported in correct format and not exported with stutter. • Possible artifact not edited in e-gram. Edit artifact in GMID-X and re-export table. • Possible tri-allele- ignore locus in software.
Reference-Invalid alleles 	• Non-numeric values such as OL, <, or > are not accepted. Allele values greater than 70 are not accepted. • Review electropherogram. If sample contains > or < allele, locus will be ignored in software. If OL is an artifact, edit artifact in GMID-X and re-export table. • If off-ladder allele, confirm OL or add appropriate allelic designation in GMID-X. Re-export table.
Evidence-Invalid alleles 	• Non-numeric values such as OL, <, or > are not accepted. Allele values greater than 70 are not accepted. • Press the purple Review Issues button to investigate further. • Review electropherogram. If sample contains > or < allele, locus will be

	ignored in software. If OL is an artifact other than stutter, edit artifact n GMID-X and re-export table. • If OL is in stutter position (this can be confirmed by comparing the height/size information in the import file/evidence issues screen to the electropherogram), change the OL in the import file to the correct allelic designation. Save changes. Note change on appropriate worksheet. OL alleles in stutter positions do not have to be re-injected. • If off-ladder allele, confirm OL or add appropriate allelic designation in GMID-X. Re-export table.
Observation	Action

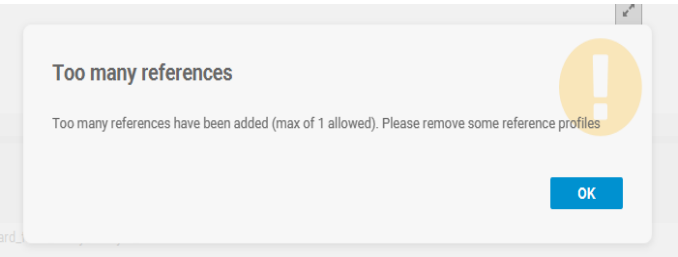
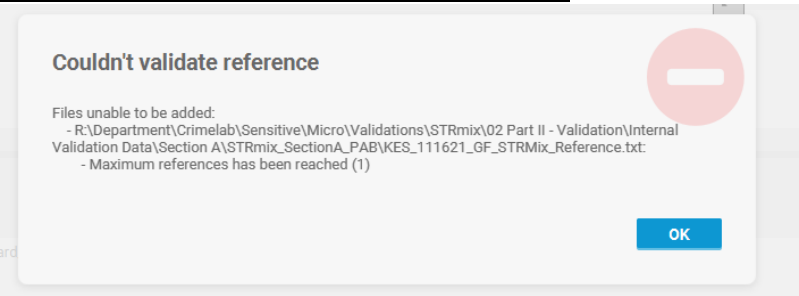
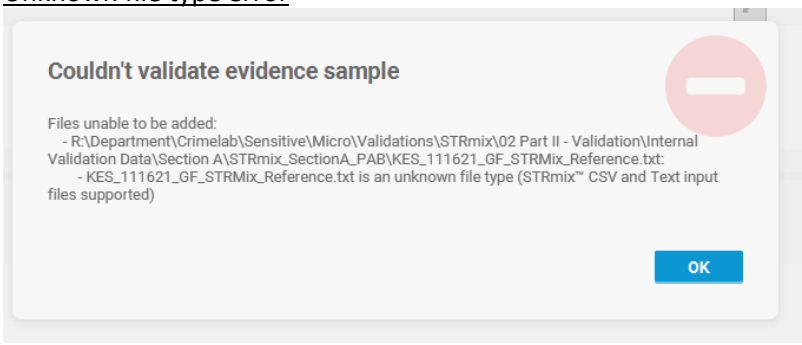
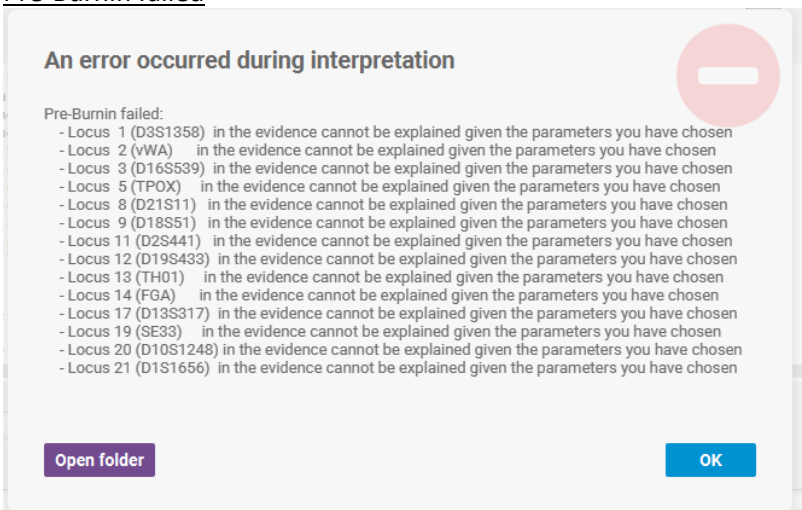
Evidence-Missing Stutter-1 BP Resolution Issue-Mixture

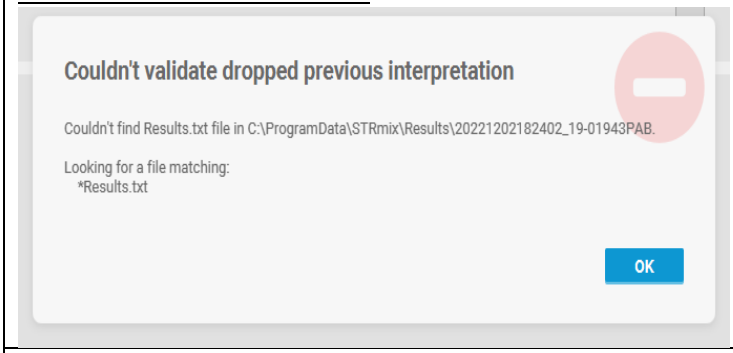


- Click purple Review Issues button to investigate further.
- Check import table to ensure correct table exported/sample was exported with stutter.
- If import file correct, investigate the missing stutter by examining the electropherogram. Use the evidence issues screen to help identify where the issue is located.

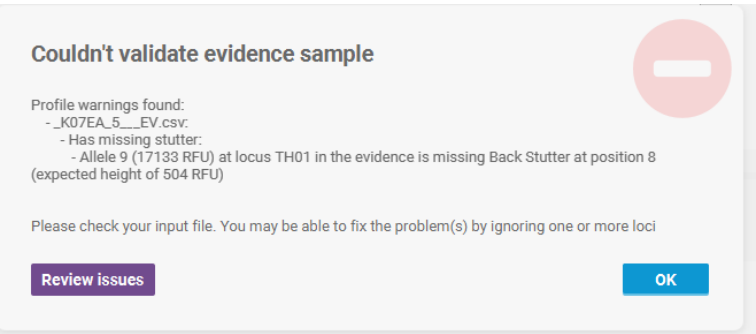


- In this example, there is a 1 bp resolution issue in a mixture sample. The stutter in the 16 position is 1) distinguishable from the neighboring allele and 2) above analytical threshold, but is being hidden by the 15.3 allele. The locus should be ignored in the software.
- See the Troubleshooting-Table Import into STRmix™ section for more guidance on missing stutter due to 1 bp resolution issues in single source samples and mixtures.

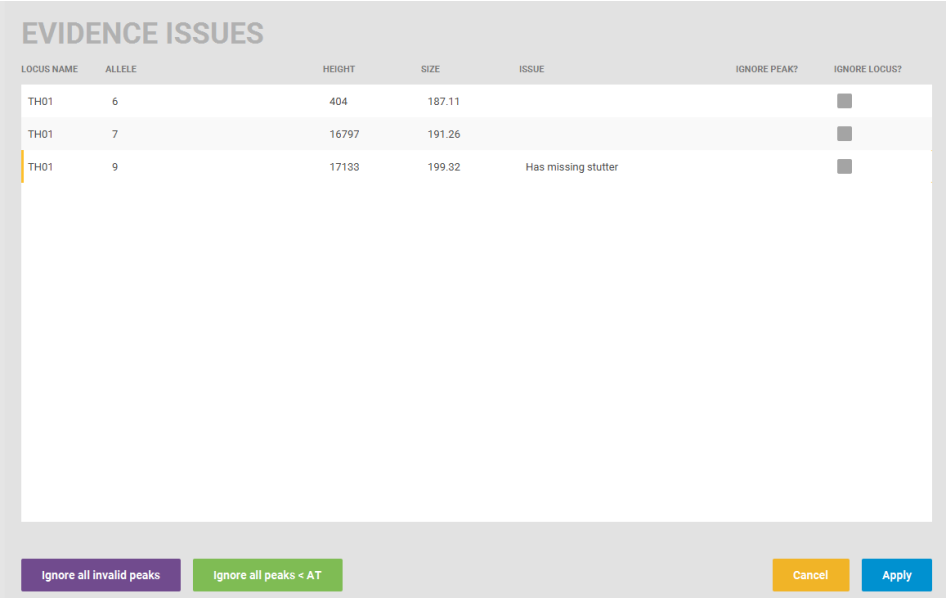
Observation	Action
Interpretation Screen-Too many references 	• In the interpretation screen, the total number of reference profiles added to the Reference Profile Data screen cannot be greater than the number of contributors. References should only be added in this screen when they are used for conditioning. If seeking to compare multiple standards separately to deconvolution, use Investigation Batch tool.
LR from Previous-Maximum references reached 	• This error occurred in the LR from Previous Screen. The total number of reference profiles added to the Reference Profile Data screen cannot be greater than the number of contributors. If seeking to compare multiple standards separately to deconvolution, use Investigation Batch tool.
Unknown file type error 	• Make sure the table setting is correct in the GeneMapper ID-X main screen (STRmix Casework or STRmix Reference.) Re-export the table in the correct format.
Pre-Burnin failed 	• The number of contributors that was entered cannot explain the data. Check the correct number was entered. Re-evaluate the number of contributors. This typically occurs when number of contributors is underestimated.

Observation	Action
Could not find Results .txt file 	• The interpretation the analyst is comparing the reference to was not completed. Choose the correct deconvolution for comparison.

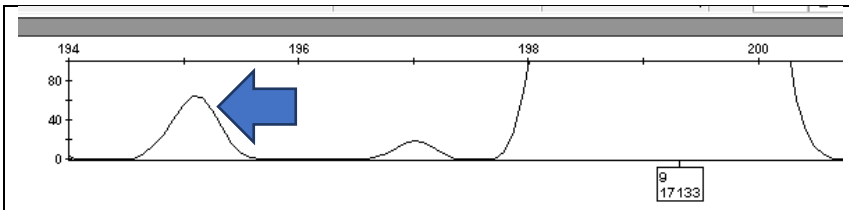
Evidence-Missing Stutter-Not present in electropherogram



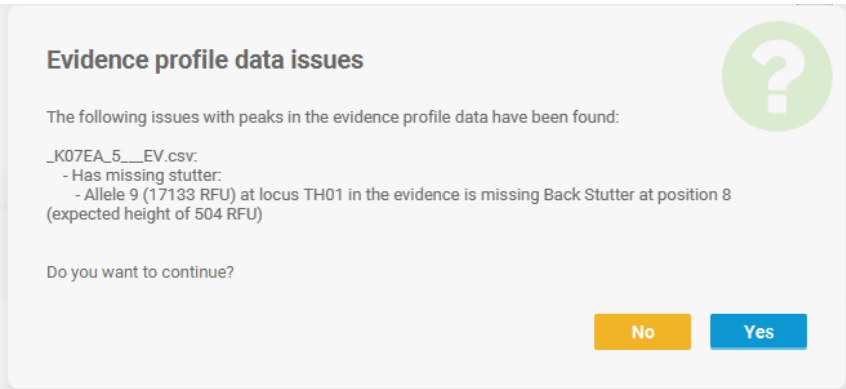
- Click purple Review Issues button to investigate further.



- Check import table to ensure correct table exported/sample was exported with stutter.
- If import file correct, investigate the missing stutter by examining the electropherogram. Use the evidence issues screen to help identify where the issue is located.



- In this instance, looking at the electropherogram has confirmed the information in the import table is correct. The stutter at the 8 allele is below analytical threshold. Press the yellow cancel button to leave the Evidence Issues Screen. Proceed with interpreting the sample.
- An additional warning screen may pop up. Press Yes.



- The missing stutter information will be listed in the Evidence Peak Issues section of the Interpretation Report.

8.1.4 Backup of STRmix™ Data

All STRmix™ interpretation/comparison folders that are created will be retained in the appropriate folder(s) within the DNA Technical Data folder on the R: drive. Batch log text files are generated when batch mode is used and will be retained with all corresponding batch samples in the appropriate folder on the R: drive. STRmix™ interpretation/comparison folders and batch log files will be retained even if created inadvertently or if not used for reporting purposes.

Within the DNA Technical Data folder, analysts will have personal folders organized by year. Within the yearly folders, analysts will store STRmix™ data within a STRmix™ sub-folder. Within this sub-folder, analysts will store STRmix™ interpretation/comparison folder(s) along with applicable batch log(s) in folders named after the GMID-X project where the relevant sample data was analyzed. The suffix _STRmix will be added to the folder name (Example: QIA_080823_GF_AGM_STRmix). If comparison reference(s) are analyzed in separate projects/at later dates, the STRmix™ comparison folder(s), along with applicable batch log(s), will be stored under the project name(s) where the relevant reference sample was analyzed. If replicate analysis is used, the STRmix™ replicate interpretation, along with applicable batch log(s), will be stored under the project name where the re-amplified sample was analyzed. If additional scenarios arise, the analyst can choose which folder to save the STRmix™ information in and will document the location within the case file.

The following lists directions for operating the various functions within the STRmix™ software.

8.1.5 Data Interpretation

1. Click on the STRmix™ icon to start the software.

2. Click on the Interpretation button.

3. Add the Complaint Number (format: 231016-000000) to the Case Number field. Add the DNA # with analyst initials (Example: 23-03396_AGM) to the Sample ID field.

4. The analyst is not required to add any information to the Case Notes field but may add any information they find relevant.

5. Under MCMC settings, add the analyst determined number of contributors to the number of contributors field. Click Next.

6. Ensure CMPD_GlobalFiler_3500 is the Profiling Kit selected.

7. This screen contains an Evidence Profile Data field and a Reference Profile data field. The evidence table can be brought into the Evidence Profile Data field by dragging the table into the field. If multiple evidence profiles are contained in the table, the Add Evidence Profile data window will open and list the samples within the table. Place a check mark in the box to the left of the sample you would like to interpret. (Note: Unless performing replicate interpretation, only one sample is selected.) Press Confirm. If there is only one profile in the table, it will automatically be added to the field.

8. Evidence profile data can also be added by clicking on the green plus sign under Evidence Profile Data, pressing the Select button, and navigating to the location of the evidence table. Press Open. If there is only one profile in the table, it will automatically be added to the input file field. Press Confirm. If multiple evidence profiles are contained in the table, the Add Evidence Profile data window will open and list the samples within the table. Place a check mark in the box to the left of the sample you would like to interpret. (Note: Unless performing replicate interpretation, only one sample is selected.) Press Confirm.

9. The purple Review issues/Evidence Issues button can be used to investigate any evidence data notifications. Additional information can be found in the Troubleshooting-Table Import into STRmix™ section. Any changes made to the import text file and any ignored locus/loci should be noted on the appropriate

worksheet. Issues with the data (i.e. OL pull-up artifact in data that was not edited) should be corrected in the GMID-X project and the table re-exported.

10. To ignore a locus/loci in the interpretation, click on the purple Kit Settings button. Click on LOCI. Place a checkmark in the ignore box of the locus/loci you would like to ignore. {note: The ignored loci are not retained and will return to the default for the next interpretation.}

11. All profile deconvolutions will be performed separately from statistical analysis. References will not be added at this time, unless used for conditioning. Skip to step 15 if not conditioning.

12. To condition on a reference(s), drag the appropriate reference table into the Reference Profile Data field. If the table contains multiple references, the Add Reference Profile data window will open and list the samples within the table. Place a check mark in the square to the left of the reference you would like to add. Press Confirm. If there is only one reference in the table, it will automatically be added to the field. Any reference added will automatically have a check mark in the HP box that cannot be removed. Add a check to the HD box to condition on the reference.

13. The reference table can also be added by clicking the green plus sign under Reference Profile Data, clicking the green plus sign again, clicking Select, and navigating to the location of the table. Press Open. Only one reference can be added at a time when using this method. If there is only one profile in the table, it will automatically be added to the input file field. Press Confirm and then Add. If the table contains multiple references, the Add Reference Profile data window will open and list the samples within the table. Place a check mark in the square to the left of the reference you would like to add. Press Confirm and then Add. Any reference added will automatically have a check mark in the HP box that cannot be removed. Add a check to the HD box to condition on the reference.

14. Any reference data notifications will be investigated. Additional information can be found in the Troubleshooting-Table Import into STRmix™ section. Issues with the data (i.e. OL pull-up artifact in data that was not edited) should be corrected in the GMID-X project and the table re-exported. Any ignored locus/loci should be noted on the appropriate worksheet. See step 10 for directions on how to ignore loci.

15. The red minus sign on the left of the screen will remove profiles from the Evidence Profile Data and Reference Profile Data field, if necessary.

16. Verify that the Perform Database Search box is checked. This is the default setting. All STRmix™ deconvolutions will be searched against the staff database. When the database search box is checked, the software will automatically perform this search once the interpretation is complete.

17. Click the blue Start button in the bottom right corner to start the interpretation. Alternatively select Queue from the dropdown on the Start button to queue the Interpretation into Batch Mode. See the Batch Mode protocol for more information on this option.

18. If not performed as part of a batch, the Interpretation Report and the Database Search Report will automatically open once the interpretation is complete.

19. Click Finish to return to the Main Screen. The interpretation and database search results folders can be accessed by selecting the Open STRmix™ Results Directory Icon  in the upper right portion of the screen. Clicking on this icon opens the location (C:\ProgramData\STRmix\Results) where the software automatically stores the results. The results folders are named using the information that was entered into the Case Number field followed by the Sample ID field. Database Search folders will have -DBSearch attached to the folder name.

20. Copy the applicable analysis results folders from the C drive into the appropriate location on the R drive. Once this information has been moved to the R drive, it should be deleted from the computer's C drive.

8.1.6 LR from Previous

The LR from Previous function is used to run a likelihood ratio calculation for a previous interpretation. This function can be used for running a compound LR, for the comparison of one standard to one interpretation, and for comparing a reference that has a locus/loci ignored. To compare multiple standards individually to a previous interpretation, see directions for Investigation Batch.

1. Click on the STRmix™ icon to start the software.

2. Click on Investigation.

3. The Results folder from a previous interpretation can be dragged into the gray previous interpretation box at the top of the screen.

4. Click LR from Previous.

5. If the Results folder was added in Step 3, the Previous Interpretation, Case Number, Sample ID, and Number of Contributors will be filled in. The Sample ID will have a -LRPrev added to it. Add the first and last initial of the reference you are comparing to -LRPrev. (Example -LRPrevFS) If there are multiple references with the same initials in the case, add additional information like the middle initial or the full name to distinguish them.)

6. If not added previously, drag in the applicable Results folder to the Previous Interpretation field. The Previous Interpretation, Case Number, Sample ID, and Number of Contributors will populate automatically. The Sample ID will have a -LRPrev added to it. Add the first and last initial of the reference you are comparing to -LRPrev. (Example -LRPrevFS) If there are multiple references with the same initials, add additional information like the middle initial or the full name to distinguish them.)

7. Click Next.

8. This screen contains an Evidence Profile Data field and a Reference Profile data field. The Evidence Profile Data field contains the sample file name of the evidence profile that was previously interpreted. This field is locked, no information can be added or deleted from it.

9. Drag the appropriate reference table into the Reference Profile Data field. If the table contains multiple references, the Add Reference Profile data window will open and list the samples within the table. Place a check mark in the square to the left of the reference you would like to add. Press Confirm. If there is only one reference in the table, it will automatically be added to the field. Any reference added will automatically have a check mark in the HP box and no check mark in the HD box. The HP and HD options cannot be changed.

10. The reference table can also be added by clicking the green plus sign under Reference Profile Data, clicking the green plus sign again, clicking Select, and navigating to the location of the table. Press Open. Only one reference can be added at a time when using this method. If there is only one profile in the table, it will automatically be added to the input file field. Press Confirm and then Add. If the table contains multiple references, the Add Reference Profile data window will open and list the samples within the table. Place a check mark in the square to the left of the reference you would like to add. Press Confirm and then Add. Any reference added will automatically have a check mark in the HP box and no check mark in the HD box. The HP and HD options cannot be changed.

11. Multiple references will only be added to the Reference Profile Data field when the analyst is performing a compound LR. To perform a compound LR, add all

applicable references into the Reference Profile Data field. All references added will automatically have a check mark in the HP box and no check mark in the HD box. The HP and HD options cannot be changed.

12. Any reference data notifications will be investigated. Additional information can be found in the Troubleshooting-Table Import into STRmix™ section. Issues with the data (i.e. OL pull-up artifact in data that was not edited) should be corrected in the GMID-X project and the table re-exported.

13. Any ignored locus/loci should be noted on the appropriate worksheet. To ignore a locus/loci in the interpretation, click on the purple Kit Settings button. Click on LOCI. Place a checkmark in the ignore box of the locus/loci you would like to ignore. {note: The ignored loci are not retained and will return to the default for the next interpretation.}

14. Click the blue Start button in the bottom right corner to start the comparison. Alternatively select Queue from the dropdown on the Start button to queue the LR calculation into Batch Mode. See the Batch Mode protocol for more information on this option.

15. If not performed as part of a batch, the LR from Previous Report will automatically open once the comparison is complete.

16. Click Finish to return to the Investigation Screen. The comparison results folder can be accessed by selecting the Open STRmix™ Results Directory Icon in the upper right portion of the screen. Clicking on this icon opens the location (C:\ProgramData\STRmix\Results) where the software automatically stores the results. The comparison results folder is named using the information that was entered into the Case Number field followed by the Sample ID field and -LRPrev(Initials).

17. Copy the applicable comparison results folder from the C drive into the appropriate location on the R drive. Once this information has been moved to the R drive, it should be deleted from the computer's C drive.

8.1.7 Replicate Interpretation

1. Click on the STRmix™ icon to start the software.

2. Click on the Interpretation button.

3. Add Complaint Number (format: 231016-000000) to the Case Number field. Add DNA #, analyst initials and _REP to the Sample ID field. (Example: 23-03396_AGM_REP).

4. The analyst is not required to add any information to the Case Notes field but may add any information they find relevant.

5. Under MCMC settings, add the analyst determined number of contributors value to the number of contributors' field. Click Next.

6. Ensure CMPD_GlobalFiler_3500 is the Profiling Kit selected.

7. This screen contains an Evidence Profile Data field and a Reference Profile data field. All evidence profiles that will be used for replicate interpretation must be added to the Evidence Profile Data field. This will result in the Evidence Profile Data field containing multiple evidence samples.

8. The evidence table can be brought into the Evidence Profile Data field by dragging the table into the field. If multiple evidence profiles are contained in the table, the Add Evidence Profile data window will open and list the samples within the table. Place check marks in the boxes to the left of the replicate samples you would like to interpret. Press Confirm. If there is only one profile in the table, it will automatically be added to the field. Multiple evidence tables can be used to import all applicable evidence profiles.

9. Evidence profile data can also be added by clicking on the green plus sign under Evidence Profile Data, pressing the Select button, and navigating to the location of the evidence table. Press Open. If there is only one profile in the table, it will automatically be added to the input file field. Press Confirm. If multiple evidence profiles are contained in the table, the Add Evidence Profile data window will open and list the samples within the table. Place a check mark in the box to the left of the samples you would like to interpret. Press Confirm. Multiple evidence tables can be used to import all applicable evidence profiles.

10. The purple Review issues/Evidence Issues button can be used to investigate any evidence data notifications. Additional information can be found in the Troubleshooting-Table Import into STRmix™ section. Any changes made to the import text file and any ignored locus/loci should be noted on the appropriate worksheet. Issues with the data (i.e. OL pull-up artifact in data that was not edited) should be corrected in the GMID-X project and the table re-exported.

11. To ignore a locus/loci in the interpretation, click on the purple Kit Settings button. Click on LOCI. Place a checkmark in the ignore box of the locus/loci you would like to ignore. {note: The ignored loci are not retained and will return to the default for the next interpretation.}

12. All profile deconvolutions will be performed separately from statistical analysis. References will not be added at this time, unless used for conditioning. Skip to step 16 if not conditioning.

13. To condition on a reference, drag the appropriate reference table into the Reference Profile Data field. If the table contains multiple references, the Add Reference Profile data window will open and list the samples within the table. Place a check mark in the square to the left of the reference you would like to add. Press Confirm. If there is only one reference in the table, it will automatically be added to the field. Any references added will automatically have a check mark in the HP box that cannot be removed. Add a check to the HD box to condition on the reference.

14. The reference table can also be added by clicking the green plus sign under Reference Profile Data, clicking the green plus sign again, clicking Select, and navigating to the location of the table. Press Open. Only one reference can be added at a time when using this method. If there is only one profile in the table, it will automatically be added to the input file field. Press Confirm and then Add. If the table contains multiple references, the Add Reference Profile data window will open and list the samples within the table. Place a check mark in the square to the left of the reference you would like to add. Press Confirm and then Add. Any reference added will automatically have a check mark in the HP box that cannot be removed. Add a check to the HD box to condition on the reference.

15. Any reference data notifications will be investigated. Additional information can be found in the Troubleshooting-Table Import into STRmix™ section. Issues with the data (i.e. OL pull-up artifact in data that was not edited) should be corrected in the GMID-X project and the table re-exported. Any ignored locus/loci should be noted on the appropriate worksheet. See step 11 for directions on how to ignore loci.

16. The red minus sign on the left of the screen will remove profiles from the Evidence Profile Data and Reference Profile Data field, if necessary.

17. Verify that the Perform Database Search box is checked. This is the default setting. All STRmix™ deconvolutions will be searched against the staff database. When the database search box is checked, the software will automatically perform this search once the interpretation is complete.

18. Click the blue Start button in the bottom right corner to start the interpretation. Alternatively select Queue from the dropdown on the Start button to queue the Interpretation into Batch Mode. See the Batch Mode protocol for more information on this option.

19. If not performed as part of a batch, the Interpretation Report and the Database Search Report will automatically open once the interpretation is complete.

20. Click Finish to return to the Main Screen. The interpretation and database search results folders can be accessed by selecting the Open STRmix™ Results Directory Icon  in the upper right portion of the screen. Clicking on this icon opens the location (C:\ProgramData\STRmix\Results) where the software automatically stores the results. The results folders are named using the information that was entered into the Case Number field followed by the Sample ID field. Database Search folders will have -DBSearch attached to the folder name.

21. Copy the applicable analysis results folders from the C drive into the appropriate location on the R drive. Once this information has been moved to the R drive, it should be deleted from the computer's C drive.

8.1.8 Performing a Manual Database Search

This procedure should be used to perform a staff search if it was not automatically performed after STRmix™ interpretation (i.e. outsourcing data). A staff search will typically be performed automatically and does not require a subsequent manual search.

1. Click on the STRmix™ icon to start the software.

2. Click on Investigation.

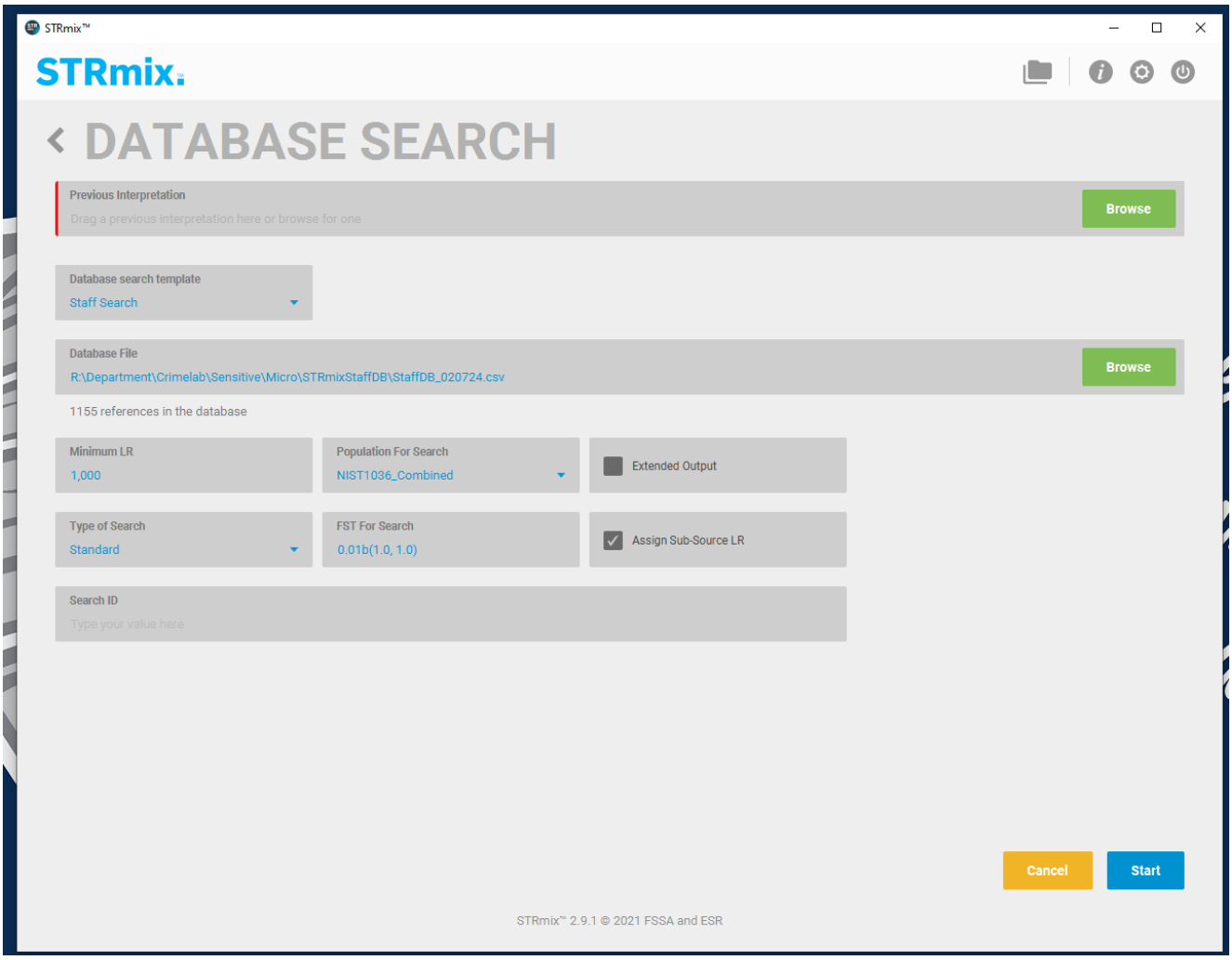
3. The Results folder from a previous interpretation can be dragged into the gray previous interpretation box at the top of the screen.

4. Click on Database Search.

5. If the Results folder was added in Step 3, the Previous Interpretation and Search ID fields will be filled in. The Search ID field will contain the information from the Case Number and Sample ID fields followed by -DBSearch.

6. If not added previously, drag in the applicable Results folder to the Previous Interpretation field. The Previous Interpretation field and the Search ID field will be updated. The Search ID field will contain the information from the Case Number and Sample ID fields followed by -DBSearch.

7. The Database Search template will be set to Staff Search. Verify all template parameters are correct. See below. Note: The staff database file will be updated as new samples are added, so the date at the end of the file name will change.



8. Click Start.

9. The Database Search Report will automatically open. Click Finish to return to the Investigation Screen. The search results folder can be accessed by selecting the Open STRmix™ Results Directory Icon in the upper right portion of the screen. Clicking on this icon opens the location (C:\ProgramData\STRmix\Results) where the software automatically stores the results. The search results folder is named using the information that was entered into the Search ID field. (Case Number_ Sample ID-DBSearch).

10. Copy the applicable search results folder from the C drive into the appropriate location on the R drive. Once this information has been moved to the R drive, it should be deleted from the computer's C drive.

8.1.9 Batching Options

8.1.9.1 Investigative Batch

The Investigation Batch function can be used to compare multiple standards individually to previous interpretations.

1. Click on the STRmix™ icon to start the software.

2. Click on Investigation.

3. Click on Investigation Batch.

4. The screen has an Interpretations Field and an Investigations Field. The STRmix™ results folders from previous interpretations can be dragged into the Interpretations field. Multiple interpretations can be added to this field. Any interpretations added must be intended to be compared to the same set of references.

5. The Investigations field is where the reference samples are added, but the reference table cannot be pulled directly into this field. Click on the Investigations side green plus sign. The LR from Previous Batch Screen should open. If the Database Search Batch screen opens instead, press the Cancel button. There is a drop-down menu on the right side of the Investigations green plus sign. Once you click on the drop-down menu, the two options listed are Add LR From Previous and Add Database Search. Click on Add LR from Previous. The LR from Previous Batch screen should open.

6. Drag the appropriate reference table(s) into the Reference Profiles field. If the table contains one reference, it will automatically be added to the Reference Profiles field. If the table contains multiple references, the Add Reference Profile data window will open and list the samples within the table. Place a check mark in the square to the left of the references(s) you would like to add. Press Confirm. The reference samples should be added to the References Profiles field.

7. The right side of the LR from Previous Batch Screen contains settings information that will not be changed. One of the fields is a required Run ID that STRmix™ automatically generates, generally in the format of LRPrev_ followed

by a number. This information does not have to be changed. The Run ID will become part of the folder name when the comparisons are complete.

8. When all applicable reference samples are added, click Save.

9. The analyst will return to the Investigation Batch screen. The red minus signs can be used to remove interpretations or a set of comparisons (labeled by the Run ID on the Investigations side). If a reference needs to be added or removed from an existing investigations set, highlight the Run ID, and press the pencil icon to edit.

10. When the applicable interpretations and references have been added, click Queue.

11. At this point, STRmix™ will notify the analyst of any reference data issues. The analyst will have to investigate and resolve the issue(s) before the investigation batch can proceed. This may involve removing the reference from the comparison set temporarily and re-exporting it when the table has been adjusted. Additional information can be found in the Troubleshooting-Table Import into STRmix™ section. Issues with the data (i.e. OL pull-up artifact in data that was not edited) should be corrected in the GMID-X project and the table re-exported. Comparisons with references that need a locus/loci ignored cannot be performed in Investigation Batch. Those comparisons will be performed using the LR from Previous function.

12. STRmix™ will open the Batch Mode Screen. Each of the comparisons that were set up in the previous screen should be listed in the Calculations in Batch Field.

13. If no other comparisons need to be added, click the Start Batch button. If additional comparisons need to be added to the batch, click on the dropdown menu next to the Add to Batch Button. Choose the appropriate analysis. See the Batch Mode protocol for more information on this option.

14. Reports will not open automatically when batch mode is used. The comparison results folders can be accessed by selecting the Open STRmix™ Results Directory Icon  in the upper right portion of the screen. Clicking on this icon opens the location (C:\ProgramData\STRmix\Results) where the software automatically stores the results. The comparison results folders are named using the Case Number field, Sample ID field, Run ID, and the sample name of the reference.

15. Whenever batch mode is used, a batch log text file is created and will also appear in the C drive location. The batch log is named with the date and time the batch is started. The analyst is responsible for retaining this text file.

16. Copy the applicable comparison results folders and batch log(s) from the C drive into the appropriate location on the R drive. Once this information has been moved to the R drive, it should be deleted from the computer's C drive.

8.1.9.2 Batch Mode

Batch mode can be used to perform batches of the interpretations/comparison procedures previously mentioned. The directions below detail creating a batch using the Batch Mode Module.

1. Click on the STRmix™ icon to start the software. Click on Batch Mode.

2. Click on the drop-down menu on the right side of the Add to Batch button and choose the analysis you would like to add.

3. Add the appropriate information for the analysis and then press Queue.

4. The Batch mode screen opens and any interpretation/comparison you added should be listed in the Calculations in Batch field. To remove an interpretation/comparison, highlight it and then press the Remove button.

5. To add additional interpretations or comparisons, repeat steps 2 through 4. As you add additional interpretations/calculations, they will be added to the Calculations in Batch field.

6. When all analyses have been added to the batch, press the Start Batch button.

7. Reports will not open automatically when batch mode is used. The analysis results folders can be accessed by selecting the Open STRmix™ Results Directory Icon in the upper right portion of the screen. Clicking on this icon opens the location (C:\ProgramData\STRmix\Results) where the software automatically stores the results.

8. Whenever batch mode is used, a batch log text file is created and will also appear in the C drive location. The batch log is named with the date and time the batch is started. The analyst is responsible for retaining this text file.

9. Copy the applicable analysis results folders and batch log(s) from the C drive into the appropriate location on the R drive. Once this information has been moved to the R drive, it should be deleted from the computer's C drive.

8.1.10 Increasing the Number of Iterations

1. Click on the STRmix™ icon to start the software.

2. Click on the Interpretation button.

3. Add Complaint Number (format: 231016-000000) to the Case Number field. Add DNA #, analyst initials and _ITR to the Sample ID field. (Example: 23-03396_AGM_ITR).

4. The analyst is not required to add any information to the Case Notes field but may add any information they find relevant.

5. Under MCMC settings, add the analyst determined number of contributors to the number of contributors field.

6. Click on the purple Run Settings button.

7. In the Burn-In Accepts (per chain) box, change the value to 100,000. In the Post Burn-In Accepts (per chain) box, change the value to 500,000. {note: These values are not retained and will return to the default for the next interpretation.}

8. Click Apply.

9. Click Next and proceed with the routine interpretation steps.

10. The interpretation and database search results folders can be accessed by selecting the Open STRmix™ Results Directory Icon in the upper right portion of the screen. Clicking on this icon opens the location (C:\ProgramData\STRmix\Results) where the software automatically stores the results.

11. Copy the applicable analysis results folders from the C drive into the appropriate location on the R drive. Once this information has been moved to the R drive, it should be deleted from the computer's C drive.

12. In the MCMC section of the interpretation report, the Burn-In/Post Burn-in accepts per chain value will be bolded.

8.1.11 Additional Analysis

If additional analysis is necessary for a sample that has previously had STRmix™ interpretation performed, the following will be added to the information in the Sample ID field. This is to distinguish the Results folders for the two separate analyses of the same DNA sample.

_NOC – The number of contributors is being changed in the second interpretation.

Original Sample ID: 23-03396_AGM → 23-03396_AGM_NOC

_CON – The sample is now being conditioned on a reference

Original Sample ID: 23-03396_AGM → 23-03396_AGM_CON

_IGN The second analysis now ignores a locus/loci

Original Sample ID: 23-03396_AGM → 23-03396_AGM_IGN

If additional scenarios occur, the analyst will add a three-letter suffix to the subsequent sample ID field(s) so the analyses can be distinguished. The analyst will add to the Comments on the STRmix Additional Analysis Worksheet the three-letter designation and the analysis it pertains to within the case file.

If a compound LR is being performed the analyst will add _CPD after _LRPrev in the Sample ID.

8.1.12 Creating a Customized Report

The STRmix™ interpretation reports have been set to display the first 10 genotype combinations/weights for each locus. The Reports module allows the analyst to generate a customized report from a previously completed STRmix™ interpretation that displays all genotype combinations/weights for each locus. These customized reports will be generated at the discretion of the analyst and will be documented within the case file.

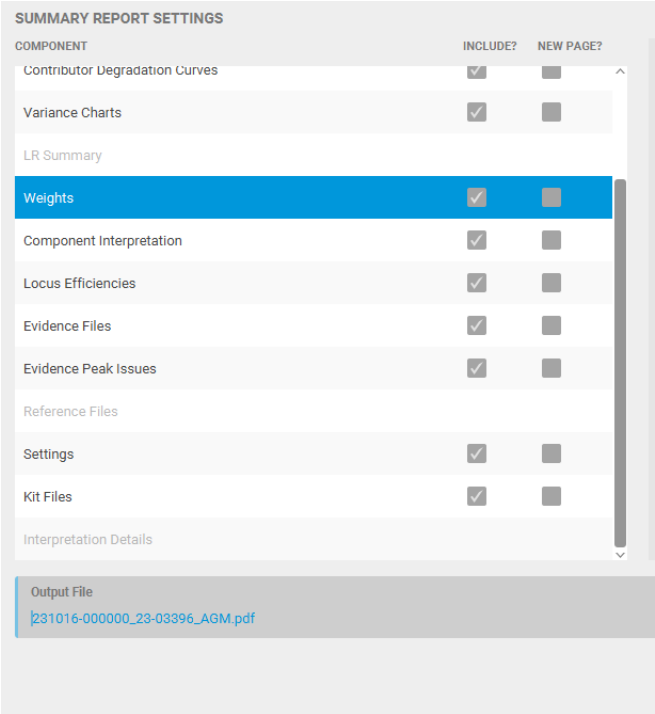
1. Click on the blue-green Reports module in the main screen.

2. Drag the appropriate deconvolution folder into the Previous Calculation field.

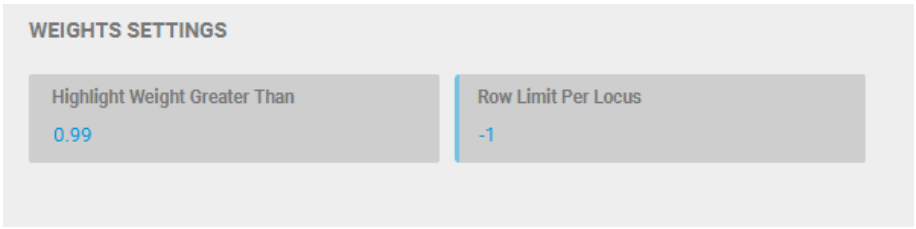
3. The Previous Calculation field will populate with the location/name of the deconvolution folder and the reports window will open.

4. On the left side of the screen, there are two headers. Reports to run should be selected and will have a blue line underneath it. In the Reports to run field, highlight Summary report and put a check mark in the box next to it. The CODIS report box will remain blank.

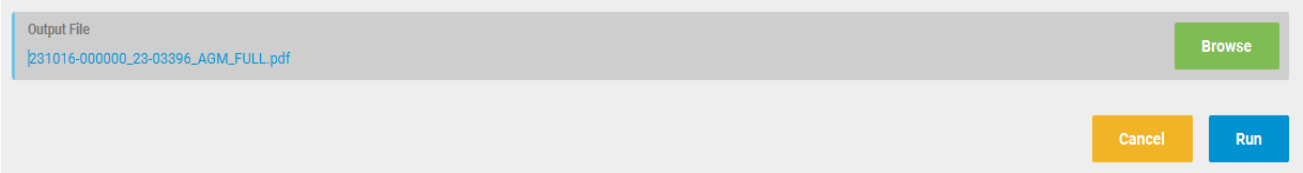
5. The Summary Report Settings window should be in the center of the screen. Click on Weights.



6. The Weights Settings options should appear on the right-hand side. Change the value in the Row Limit Per Locus box from 10 to -1. If the software doesn't allow changes, close STRmix™ completely and re-start the directions from the beginning.

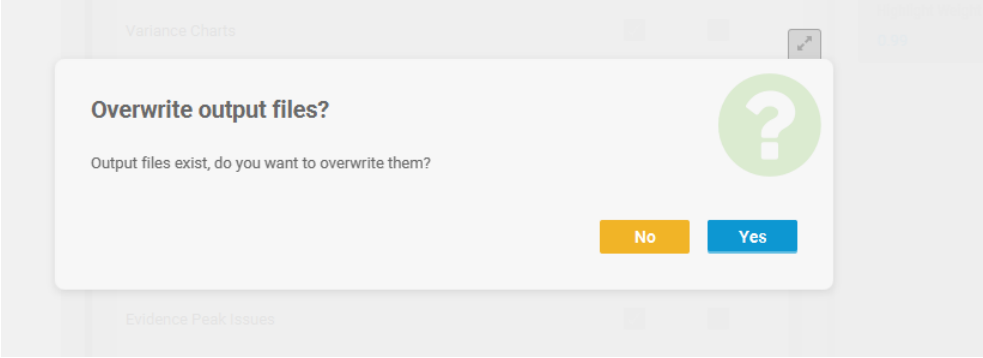


7. The Output File field appears at the bottom of the screen. The field automatically populates with the original analysis report name. Add _FULL to the end of the file name to distinguish the customized report from the original report.



8. Press the blue Run button.

9. If the following message appears, the analyst did not rename their output file. Press No and add _FULL to the end of the file name to distinguish the new customized report from the original report. Pressing yes at the below prompt will overwrite the original report.

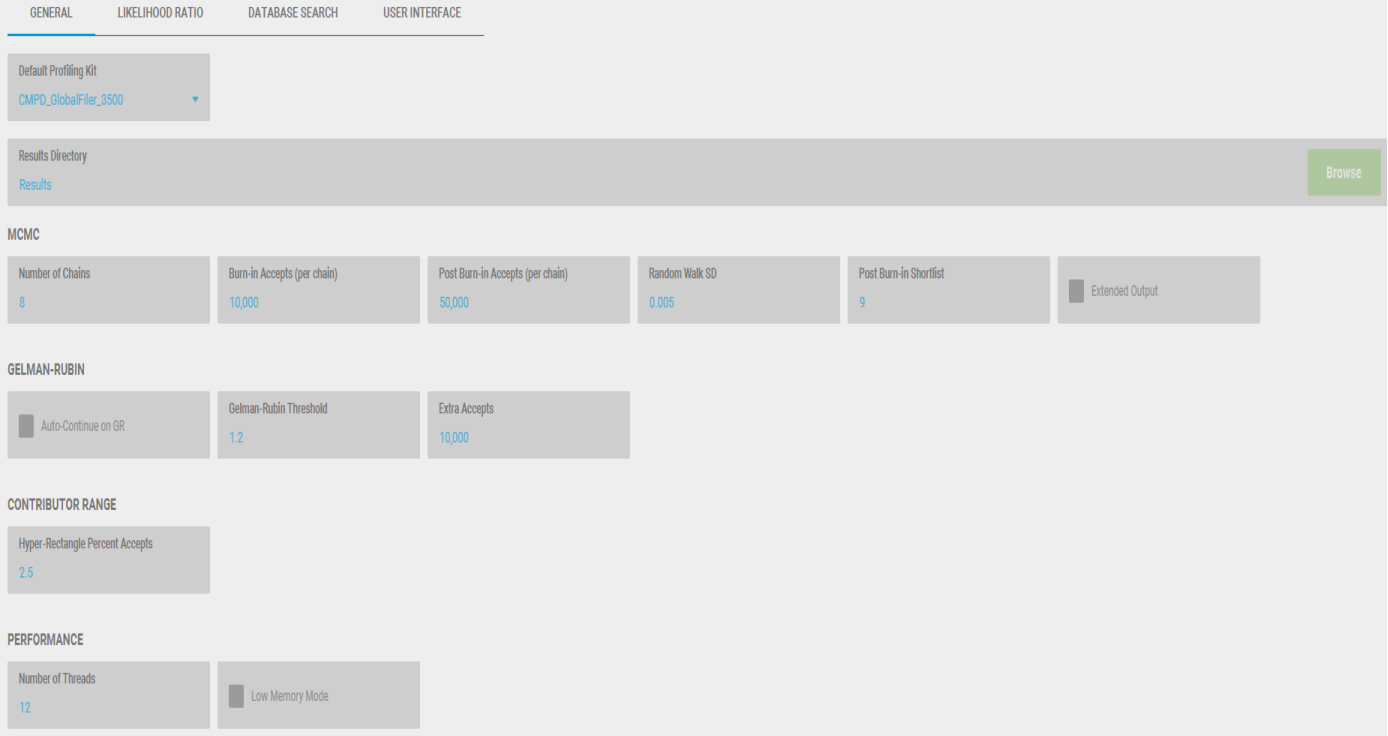


10. The customized report will automatically open. This report will be saved in the Reports folder of the original interpretation.

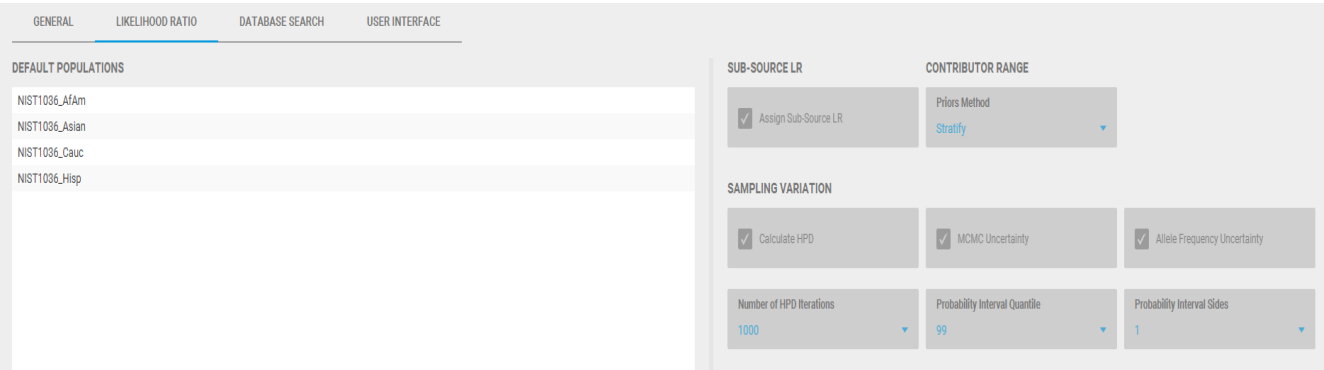
11. Press Cancel and yes to exit reports.

8.1.13 Settings

General



Likelihood Ratio



8.1.14 References

- Scientific Working Group on DNA Analysis Methods (SWGDAM). Guidelines for the Validation of Probabilistic Genotyping Systems. 2015.
- STRmix™ v2.9 Implementation and Validation Guide
- STRmix™ v2.9 User's Manual
- STRmix™ v2.9 Operation Manual
- STRmix™ v2.9.1 Release and Testing Report – February 2022
- CMPD STRmx v2.9.1 Internal Validation – ESR – September 2022
- Estimation of STRmix™ V2.9 parameters for the Charlotte-Mecklenburg Police Department (CMPD) Crime Laboratory (GlobalFiler™ 3500, 28 Cycle)
- Internal Validation of STRmix™ v2.9 with performance check of STRmix™ v2.9 software installation, staff database search, and GeneMapper® ID-X v1.6 export table function – performed by CMPD

8.2 ArmedXpert

A. Purpose and Introduction:
ArmedXpert was first used at CMPD in March of 2016 with the Identifiler kit and then the GlobalFiler kit starting in September of 2016. Capillary electrophoresis instrumentation was changed to the 3500 in October of 2019.

Legacy data is defined as data generated prior to GlobalFiler amplification on the 3500 Genetic Analyzer instruments (lab work completed prior to approximately October 1, 2019). Legacy data will not be reinterpreted; however, analysts may perform statistics on previously interpreted applicable legacy data using the ArmedXpert software. Data generated on the 3500 using GlobalFiler may be reinterpreted for use with STRmix including suitability and NOC assessment.

Data previously interpreted with ArmedXpert should have statistical calculations already performed and contained in the case file. Data that was previously interpreted, but pre-dates the implementation of ArmedXpert may utilize the software for statistical calculations.

Date	Chemistry	Instrument	Interp.
2000/2001	ProfilerPlus/Cofiler (13 Core loci)	310	
June 2003	PowerPlex 16 (15 loci – 13 Core plus Penta E and Penta D)	310	
January 2008	Identifiler (15 loci – 13 Core plus D2S1338 and D19S433)	310	
August 2010	Identifiler (15 loci – 13 Core plus D2S1338 and D19S433)	3130	
March 2016	Identifiler (15 loci – 13 Core plus D2S1338 and D19S433)	3130	AX
September 2016	GlobalFiler (20 Core loci plus SE33)	3130	AX
October 2019	GlobalFiler (20 Core loci plus SE33)	3500	AX

Before ArmedXpert is used by the analyst, the GMID-X raw data and electropherograms must be reviewed to determine that all allele calls are correct, any artifacts are edited, and all samples and controls used for interpretation and comparison pass quality control measures. ArmedXpert is not an expert system.

B. Materials and Instrumentation:
ArmedXpert v.3.0.8.0

C. Safety Considerations:
Prolonged computer analysis may cause muscle soreness, eye fatigue, or other discomforts. To minimize the occurrence of discomforts, periodic breaks from computer analysis are encouraged.

D. Limitations:
ArmedXpert is a software program used to facilitate the interpretation of data using the binary method. Prior to the implementation of probabilistic genotyping, it assisted in the mixture interpretation of two or three contributors by considering all possible combinations at each locus using thresholds determined by validation. ArmedXpert is a tool that does not replace the need for analyst interpretation. All data requires analyst review as well as a second review by the technical reviewer to ensure accuracy.

E. Other Considerations:
ArmedXpert employs Random Match Probability (RMP) for statistical calculations. RMP is the probability that the DNA of a randomly chosen person has the same profile as the DNA of an evidentiary sample. While the RMP is commonly thought of in terms of single-source profiles, this formula also applies to mixture calculations where the number of contributors is determined. At a locus, the RMP calculation (also referred to as a “modified RMP” or “mRMP”) is the sum of the probabilities for all of the genotypes that represent possible contributors to a DNA mixture under the assumption of a defined number of contributors.

The statistics for GlobalFiler will include all allele frequencies from the NIST Database for four population subgroups (Caucasian, African American, Hispanic, and Asian) and theta values where applicable.

F. Validation:

- These procedures are generally accepted in the forensic community.
- This procedure has undergone internal validation at the CMPD.

Procedure:

- Ensure that the correct database is selected for the type of data that is being interpreted by selecting Options → Connection Properties from the ArmedXpert Login screen.

GlobalFiler settings (3500): Connection Name = GlobalFiler DataSource = R:Department\Crimelab\Sensitive\Micro\ArmedXpert\Data\ArmedXpert6.db

Yfiler Plus settings: Connection Name = YFP = R:Department\Crimelab\Sensitive\Micro\ArmedXpert\Data\ArmedXpertYFP.db

Note: ArmedXpert has limited functionality with Y-STRs and is used exclusively to generate allele tables.

8.2.1 Importing Data from GMID-X Table

- Generate a GeneMapper table (.txt file) following instructions in Biology SOP 6.4.1.
- Click the ArmedXpert menu button (double helix) in the top left corner→Import → Other→ browse to location where Genotypes table created from GeneMapper ID-X was saved→ select → Open

8.2.2 Manually Entering Data (only if GMID-X table cannot be created)

- Click on the Data tab → New Sample → Set Sample Source Type = GlobalFiler (or applicable chemistry) → Enter sample name (DNA # - major/foreign/minor) → Click Add Peak (keep clicking depending on the number of alleles – the BP, RFU, Stutter leave empty) → Send To: (select in drop-down from tables already in AX project of Add Table to create new) → Add → Close (x in table corner)

8.2.3 Organizing Data:

Under the Views Tab → Samples then navigate to Data → Sort Samples
Auto-sort by → Sample Name → OK
Click ArmedXpert menu button
Save → prompt to save Genotypes Table → Yes New Project → NAME (same as .ser)
Data→Combine Sources → Expand Genotypes Table under Samples & Sources →
Select (Control and Shift buttons work here) all samples that are being reported together under same case (Generally all the same Complaint #'s)
Using the single carrot button ">" – send selected
Change Title to Complaint # under which the report is being issued → Combine
Repeat →Combine Sources (remembering to use double reverse carrot "<<" to clear previous selections

In the ArmedXpert software to navigate between tables go to Data tab → Organize → expand project → double click table to open.

8.2.4 Printing Allele Table

To Print → Click the ArmedXpert menu button (double helix) in the top left corner→ Export → Plugins→ Vertical report without RFUs (save as an excel workbook .xls on your desktop) → Open and print each tab (if necessary resize the column width to see all the alleles).

8.2.5 Using the "Interpretation" tool

Analysts may use the ArmedXpert software to calculate RMP statistics after comparing a newly submitted buccal standard and applicable legacy data. Additionally, a visually obvious major with greater than four contributors can be interpreted using the ArmedXpert software.

The following lists procedures for performing single source or major profile analysis within the ArmedXpert software. Additional archived ArmedXpert procedures may be used for the calculation of statistics on DNA mixtures with the approval of the DNA Technical Leader or designee.

Single Source (legacy data):

- Single source profile: Interpretation tab →Single Source → navigate to sample → print statistics page

Partial Single Source (legacy data with drop-out noted, but no stats in case file):

- Partial single source (drop-out/allele any designations): Interpretation tab → Begin Mixture Interpretation → Pick via mouse → Popout calls → (change name of Profile 01 to DNA # by selecting Rename Profile" from drop down menu, Profile 02 does not need to be removed) → Select the allele combinations → Save as part of the project (this profile will be added to the Combined table) → View call report →RMP→ select profile → change Contributors to 1 (bottom of page) → adjust any allele combinations to reflect interpretation→ print statistics page

Major (legacy data with no stats in case file):

- Major profile: no conclusion on minor(s) – Interpretation tab→ Begin Mixture Interpretation → Pick via mouse → Popout calls → (change the name of Profile 01 to "Major DNA #" by selecting "Rename Profile" from drop down menu, Profile 02 does not need to be removed)→ Select the major profile by checking the boxes or clicking the allele combinations → Save as part of the project (this profile will be added to the Combined table) → View call report→ RMP → select profile → change Contributors to 1 (bottom of page) → adjust any allele combinations to reflect interpretation → print statistics page

Legacy data comparisons will need to have the ArmedXpert allele table and any resulting statistics printed and placed in the case file. The ArmedXpert allele table will contain the evidence DNA profile(s) being used for comparison(s). A note will be added to this table referencing the page number(s) of the evidence data's original allele table. Applicable reference(s) can be present in either an ArmedXpert or GMID-X allele table. The ArmedXpert allele table and statistics will be printed in portrait orientation with the most conservative statistic highlighted.

There is no requirement to permanently save or retain these (.axp) files, since all supporting documentation will be in the case file.

Complete major profiles or fully deduced profiles previously interpreted prior to ArmedXpert that need a statistic performed, will have the corresponding statistics printed and placed in the case file. An allele table or previous interpretation documentation with genotypes must be clearly represented in the case file to apply the statistic to the designated major/deduced DNA profile. Applicable reference(s) can be present in either a ArmedXpert or GMID-X allele table. The ArmedXpert allele table and statistics will be printed in portrait orientation. There is no requirement to permanently save or retain these (.axp) files, since all supporting documentation will be in the case file.

8.2.6 RMP Conclusions

8.2.6.1 Inclusions

- The patterns are consistent with having come from the same individual and are determined by careful, objective, qualitative and quantitative evaluation of the entire pattern produced by the various loci tested.

- Inclusion reporting terminology:

    - Match – Used to describe a full match that occurs when all loci are completely deduced – no allele / locus dropout

    - Consistent – partial single source, partial major, minor profile, partial foreign, unresolvable two or three person mixtures – genotypes not fully resolved

8.2.6.2 Exclusions

The patterns are not consistent with having come from the same individual and are determined by careful, objective, qualitative and quantitative evaluation of the entire pattern produced by the various loci tested.

8.2.6.3 Inconclusive Results

Comparisons between data and known references that can neither be included or excluded will be interpreted as inconclusive; however, this interpretation will be evaluated on case-by-case basis and will need the approval of the DNA Technical Leader or designee.

8.2.6.4 No Reportable Results

A DNA profile may be considered not reportable due to not meeting quality control measures.

Examples of no reportable results may include but are not limited to:

- a profile having an association with a CMPD elimination profile.
- sample/sample's associated controls having unexpected results/contamination being present.

If a complete or partial single source profile has an association with a CMPD staff profile, it will be verified by a CODIS Admin. This will be reported as "consistent with" and no further comparisons or statistics will be performed. Complete and partial single source evidence profiles that have a CODIS Admin. verified association with a CMPD staff profile will be reported as "consistent with." No further comparisons or statistics will be performed.

The reporting statement for mixture evidence profiles with a CODIS Admin. verified association to a CMPD staff profile will state that the DNA results cannot be reported

due to quality control measures not being achieved. No comparisons or statistics will be performed using the mixture profile unless the associated CMPD staff member submits a buccal standard. See Biology QA 20.5.1.2 for additional guidance on buccal standard submission. Once the buccal standard is received and analyzed, the mixture may be conditioned on the presence of the CMPD staff profile and comparisons/statistics performed at the discretion of the Technical Leader or designee.

8.2.7 Population Statistics

8.2.7.1 Formulas:

- The random match probability (RMP) formula as described in NRCII formula 4.1 b will be used for a heterozygous locus frequency: 2pq.

- The random match probability (RMP) formula as described in NRCII recommendation 4.4a will be used for a homozygous locus frequency: $p^2 + p(1-p)\theta$. (using $\theta = 0.01$).

- Statistical assessment of a locus containing a potential undetected allele will be calculated based on NRCII 4.1 recommendations: $[p^2 + p(1-p)\theta] + [2p(1-p)]$ (using $\theta = 0.01$) where p represents the detected allele.

- A conditional probability using the RMP (random match probability) may be used when appropriate to interpret and report genotype frequencies when resolving mixtures. This type of RMP is the sum of the individual genotypes appropriate for a particular contributor at a given locus. All of the formulas are based in accordance with the 2010 SWGDAM guidelines sections 4-5.

- The ArmedXpert software applies the 5/2N minimum allele frequency where applicable.

8.2.7.2 General Population Statistics Considerations:

- Newly calculated statistics will use the NIST-Revised database allele frequencies based on population data published by the National Institute of Standards and Technology (NIST) as part of the NIST 1036 U.S. Population Dataset. Previously calculated statistics associated with a forensic profile that are being reported subsequent to an inclusionary statement, will not be re-calculated, but will reference the database used at the time of the calculation.

- FBI-Amended Database: Allele frequencies based on population data published by the Federal Bureau of Investigation as part of the U.S. STR Population Database.

  The Caucasian, African American, and SW and SE Hispanic databases used are from the following publications:

Statistics are calculated using the FBI population database which is published in the July 2015 Journal of Forensic Science Erratum referencing the Journal of Forensic Science, Volume 44(6):1277-1286., and Journal of Forensic Science, Volume 46 (3): 453-489 by Budowle, et al. The loci D2S1338 and D19S433 are not included in the statistical calculations.

- NIST Database: Allele frequencies based on population data published by the National Institute of Standards and Technology (NIST) as part of the NIST 1036 U.S. Population Dataset.
  The Caucasian, African American, and Hispanic and Asian databases used are from the following publications:

  Statistics are calculated using the NIST population database which is published in the FSI Genetics, Volume 7, Issue 3, Pages e82-e83.

- NIST-Revised Database - Allele frequencies based on population data published by the National Institute of Standards and Technology (NIST) as part of the NIST 1036 U.S. Population Dataset.

  The Caucasian, African American, Hispanic, and Asian databases used are from the following publications:

  Statistics are calculated using the revised NIST population database published online August 12, 2017 as Forensic Sci. Int. Genet.; Corrigendum to 'U.S. Population Data for 29 Autosomal STR Loci' [Forensic Sci. Int. Genet. 7 (2013) e82–e83].

- For each of the frequency databases, the data set for each locus has been verified independently. The databases have been reported to demonstrate little departure from Hardy-Weinberg equilibrium. Linkage equilibrium was also mostly observed for the alleles across loci. As a result, the statistical genotype frequencies and product rule will be used in accordance with the NRCII recommendations.

8.2.7.3 Statistical Designations:

Quadrillion: 1,000,000,000,000,000 (15 zeros)
Quintillion: 1,000,000,000,000,000,000 (18 zeros)
Sextillion: 1,000,000,000,000,000,000,000 (21 zeros)
Septillion: 1,000,000,000,000,000,000,000,000 (24 zeros)
Octillion: 1,000,000,000,000,000,000,000,000,000 (27 zeros)
Nonillion: 1,000,000,000,000,000,000,000,000,000,000 (30 zeros)
Decillion: 1,000,000,000,000,000,000,000,000,000,000,000 (33 zeros)
Undecillion: 1,000,000,000,000,000,000,000,000,000,000,000,000 (36 zeros)
Duodecillion: 1,000,000,000,000,000,000,000,000,000,000,000,000,000 (39 zeros)

Tredecillion: 1,000,000,000,000,000,000,000,000,000,000,000,000,000,000 (42 zeros)

8.2.8 References:

- SWGDAM Interpretation Guidelines for Autosomal STR typing by forensic DNA testing laboratories, 2010, Available at www.swgdam.org
- Evett, et al, Taking account of peak areas when interpreting mixed DNA profiles, *J Forensic Sci. 1998, 43: 62– 69.*
- Clayton, et al, Analysis and interpretation of mixed forensic stains using DNA STR profiling, *FSI 1998, 91; 55-70.*
- Bill, et al, Pendulum – a guideline based approach to the interpretation of STR mixtures, *FSI 2005, 148: 181-189.*
- Bille, et al, Application of Random Match Probability Calculations to Mixed STR Profiles, *J Forensic Sci 2013, 58 (2): 474-485.*
- Kelly, et al, A comparison of statistical models for the analysis of complex forensic DNA profiles, *Science and Justice 54 (2014): 66-70.*
- Journal of Forensic Science, Volume 44(6):1277-1286.
- Journal of Forensic Science, Volume 46 (3): 453-489.
- Journal of Forensic Science, Erratum Volume 60 (4): 1114-1116.
- FSI Genetics, Volume 7, Issue 3, Pages e82-e83.
- CMPD Internal Validation of Armed Xpert

9. STRmix™ Report Interpretation

A. Purpose and Introduction:

Before STRmix™ analysis, the analyst will manually assess their evidence profile. This assessment will include reviewing the electropherogram data and making conclusions regarding general quality, number of contributors, and mixture proportions. Following STRmix™ analysis the analyst is responsible for reviewing the STRmix™ Interpretation Report and making an assessment of the intuitiveness of the output given the electropherogram data.

B. Materials:

- Electropherograms
- Case file
- STRmix™ Interpretation Report
- The current database used for all statistical interpretation is the NIST Revised 1036 population frequency database published in 2017.

C. Safety Considerations

Prolonged computer analysis may cause muscle soreness, eye fatigue, or other discomforts. To minimize the occurrence of discomforts, periodic breaks from computer analysis are encouraged.

D. Limitations

STRmix™ does not eliminate the need for all data, software output, and diagnostics to be reviewed by the analyst in the context of the profile. All data requires analyst review as well as a second review by the technical reviewer to ensure accuracy.

E. Other Considerations

Once the analyst has reviewed the applicable deconvolution and/or comparison(s), they may determine that additional STRmix™ analysis is necessary. Additional STRmix™ analysis may require consultation and approval by the DNA Technical Leader or designee before proceeding and will be noted on the STRmix™ Interpretation Worksheet.

F. Validations

- This procedure has undergone internal validation at the CMPD.

Procedure:

9.1 Reviewing the STRmix™ Interpretation Report

The interpretation report generated from STRmix™ will be reviewed by the analyst. This review includes the following elements:

- Reviewing the Details and Run Parameters sections of the report. Verify the user, number of contributors, profiling kit (CMPD_GlobalFiler_3500), sample file/sample name, and case number are correct.

- If applicable, check if a conditioning reference profile was applied. The conditioned reference's file name should appear next to "Known Contributors under HP:" and "Known Contributors under HD:" in the Run Parameters Section. The conditioned reference will be placed in the contributor one position, regardless of their contribution to the mixture. No LR will be calculated for the conditioned reference.

- Template amounts, mixture proportion (primary diagnostic), degradation, and locus efficiency should be evaluated for consistency with the analyst's expectations and should support the analyst's manual review of the electropherogram. The contributor degradation curve may be helpful in recognizing extreme degradation and when reviewing unintuitive weights.

- Examining the accepted genotype combinations and their weights (primary diagnostic). The results obtained should be intuitive. Genotype combinations that best explain the data should be given higher weights, while genotype sets that are not good explanations of the data should be assigned low or no weight(s). The weights section should accurately reflect the analyst's manual review of the data. For example, more complex profiles, higher order unresolved mixtures, and low-level contributors are expected to have more genotype combinations accepted with diffused weights, while a mixture with resolvable components is expected to have fewer genotype combinations accepted with a distinct separation in weights. The Component Interpretation section can be helpful for reviewing accepted genotypes and weights per contributor.

- Check the secondary diagnostics, which include the total iterations, effective sample size, Gelman-Rubin (GR) result, variances, acceptance rate, log(likelihood), along with any evidence peak issues noted in the report. The analyst will use this review to ensure the STRmix™ analysis performed as expected and that no additional analysis is needed. The secondary diagnostics need to be interpreted in the context of the profile itself. A single diagnostic value outside of the expected range needs further scrutiny and may target an aspect of the sample/run to troubleshoot but does not necessarily invalidate the STRmix™ interpretation results. Collectively sub-optimal secondary diagnostic results may be an indicator of the quality of the results obtained and a more thorough review of the profile and STRmix™ deconvolution is recommended.

    - Total Iterations and Acceptance Rate- The total iterations value indicates the total number of post burn-in iterations that the MCMC ran during its analysis to reach 400,000 accepts. The acceptance rate is calculated by

dividing the number of accepts at post-burn in MCMC by the total number of iterations. The total iterations value and acceptance rate can inform the analyst as to how often a new proposed set of parameters was accepted. 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.

- Effective Sample Size (ESS)- Effective sample size is the number of independent samples the MCMC has taken from the posterior distribution of the MCMC likelihood. ESS is the measure of how many independent iterations the chains represent. 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 was run again.

- Log (likelihood) –The log (likelihood) describes the fit of the observed electropherogram given the proposed profile and mass parameters. Generally, this value should be above 0. The larger the value, the better STRmix™ has been able to describe the observed data. A low/negative value suggests the software has not been able to describe the observed data very well.

- Gelman-Rubin convergence diagnostic –This value informs the analyst whether the MCMC analysis has likely converged. If each chain is sampling from the same space, then the GR result should be approximately equal to one. A GR value greater than 1.2 may indicate that the chains have not converged and requires further examination. A GR value less than 1.2 does not guarantee all chains have converged and a GR value greater than 1.2 does not indicate results are invalid; however, GR values greater than 1.6 will require further analysis. The GR results need to be considered in the context of the profile and other secondary diagnostics.

- Allele and stutter variances – Both the allele and stutter variance constants are the average value across the entire post burn-in MCMC analysis. The variances of a profile can be thought of as a description of its quality and indicate how well the data is being explained by the biological models. It is expected that as peak height decreases, peak height variability increases. If the values have increased markedly from the respective prior modes, it may indicate poor quality and that the data is not being explained well. The variance chart plots may be helpful in evaluating the variance values. Caution should be exercised when the variance values appear on the right-hand side tail of the plots.

- LSAE Variance- The LSAE variance constant is the average value across the entire post burn-in MCMC analysis. The variances of a profile can be thought of as a description of its quality and indicate how well the data is being explained by the biological models. LSAE values that appear high/excessive when compared to the prior mean can indicate the peak heights are quite variable across the profile. The LSAE variance chart plot may be helpful in evaluating the LSAE variance. Caution should be exercised when the variance value appears on the right hand side tail of the plot. This value can be used in conjunction with the locus efficiency plot to identify unusual amplification. The locus efficiency chart should be reviewed to ensure it is reflective of the profile.

- Review the Evidence Input Files information to ensure the correct sample file(s) are included and the profile information is complete and accurate. If a conditioning profile was used, review the reference file information.

- Review the information contained in the Settings section of the report. Pay particular attention to any information that is bolded, this indicates a change from the default settings.

9.2 Database Search Reports

The STRmix™ software will automatically search the results of each deconvolution against the staff database. Only staff profiles with LRs above the minimum LR value of 1,000 will be returned in the results. The database search results are found in a separate folder named with the information input into the case number and sample ID fields followed by -DBSearch.

The Database Search report generated from the STRmix™ software will be reviewed by the analyst. This review will include the following elements:

- Reviewing the Details and Database Search Setup sections of the report. Verify the user, interpretation chosen, profiling kit, sample file, database searched, settings template, minimum LR, population, type of search, and FST value is correct. The information included in boxes below is default information for the staff search. Note: The staff database file will be updated as new samples are added, so the date at the end of the file name will change.

DATABASE SEARCH SETUP

Interpretation chosen	R:\Department\Crimelab\Sensitive\Micro\Validations\STRmix\02 Part II - Validation\Internal Validation Data\Section A\STRmix_SectionA_PAB\PracticeSet3_PAB_SectionA_1ng_2022-04-26-12-32-04
Profiling Kit	CMPD_GlobalFiler_3500
Sample file	_SectionA_1ng___EV.csv
Database searched	R:\Department\Crimelab\Sensitive\Micro\STRmixStaffDB\StaffDB_020724.csv
Number of individuals in database	1,155
Settings template	Staff Search
Minimum LR	1000
Population	NIST1036_Combined
Type of search	Standard
FST	0.01b(1.0, 1.0)
Assign sub-source LR	Y
Extended output	N
Search ID	PracticeSet3_PAB_SectionA_1ng-DBSearch

- Verify the information contained in the Previous Interpretation Details section of the report is correct

- Check to see if there are any samples listed in the LR Results section of the report. The CODIS Administrator or back-up Administrator will be notified of any associations for resolution. If there are no associations, “No matches found at specified Minimum LR cutoff level” should appear in the LR Results section.

9.3 Troubleshooting

STRmix™ interpretation reports that have non-intuitive primary diagnostics and/or with sub-optimal secondary diagnostic(s) need further investigation before proceeding with reference comparisons. This investigation may include:

- Checking the interpretation setup which includes the input files, profiling kit selected, and any conditioning profiles
- Confirming the MCMC default settings of 10,000 burn-in accepts per chain and 50,000 post burn-in accepts per chain
- Checking whether any of the other diagnostics are not intuitive or have excessive values
- Checking the number of contributors used is appropriate and the best estimate for the profile
- Reviewing the forensic profile electropherogram. Ensure the profile/input file does not contain any artifacts that should have been removed. Verify the input file contains accurate allele information, including stutter and alleles that are separated by one base pair.

- If the STRmix™ degradation output is not consistent with the analyst's assessment of the electropherogram data or if the amplification efficiencies display a steep "ski slope" pattern, the sample may benefit from increasing the degradation max setting. For the majority of forensic profiles, the default degradation max setting of 0.01 is sufficient. There may be profiles that display extreme degradation that require a degradation max setting greater than the default. With Technical Leader approval, the degradation max setting can be increased to 0.1. This change in settings will be bolded on the deconvolution report. The reason for the settings change will be documented within the file.

- Checking whether the interpretation requires an increased number of MCMC accepts to allow the MCMC process to converge. Increasing the number of MCMC accepts requires Technical Leader approval. Typically, both the per chain burn-in and post-burn in accepts are increased by a factor of 10. This change in settings will be bolded on the deconvolution report. The reason for the settings change will be documented within the file.

Ideally the analyst will have identified any evidence profile loci to ignore within STRmix™ prior to the deconvolution. There may be situations where identification of anomalies are not possible prior to deconvolution. Analysts are responsible for visually inspecting and evaluating any ignored locations. Ignoring a locus from an evidence profile does not make the profile partial. Additionally, an ignored locus is not considered a location without data for the purposes of determining if there are at least seven loci with data. The reasoning for using the ignore loci function will be documented on the appropriate worksheet and ignored loci wording will be added to the report. Technical Leader or designee approval should be obtained prior to ignoring a locus or loci within the STRmix™ software.

If any of the settings or assumptions are changed from the previous deconvolution, such as number of contributors, MCMC settings, or conditioning on a reference, then a separate deconvolution, along with a new database search, must be performed. Any subsequent analysis adjusting the number of contributors would need approval from the Technical Leader. All STRmix™ interpretations will be documented within the case file.

The table below lists some commonly encountered casework scenarios. If an issue arises that is not referenced, please consult the Technical Leader or designee.

MCMC Diagnostic Parameter	Range found during Validation	QA Flag	Action
Total Iterations	1102493 to 28003281		• Evaluate other diagnostic parameters if found to be too low
Acceptance Rate	2.7562325 to 70.0082025	1 in 100s to 1 in millions	• Consider another STRmix analysis at a greater number of iterations (typically 100,000 burn in accepts and 500,000 post burn-in accepts). **Technical Leader Approval Required.**
Effective Sample Size	607.1024 to 42144.43107	10s to 100s	• Low ESS value (especially in conjunction with a low log (likelihood) parameter) is expected for low quality profiles – no re-run necessary. • Signifies that there is potential for a large difference in weights if re-run.
Log (likelihood)	-3.348 to 97.77	Low (<1) or negative value	• If the rest of the diagnostics appear intuitive and low/negative average (log) likelihood is due to low level & limited data, can still use STRmix deconvolution. • Re-evaluate data. If low level or little data exists, no need to re-run.

			• If error exists in the editing of the profile, correct and re-run. • Unexpected value may be due to low peak heights, limited allelic information, # of contributors is not supported, real allele or stutter data has been removed, or an artifact is present in the data. • If # of contributors is not supported, re-run. **Technical Leader Approval Required.**
Gelman-Rubin Convergence Statistic	1.00 to 1.44	>1.2	• A GR>1.2 may be acceptable if other diagnostics are within range. • Data has been removed that is truly allelic and/or stutter. Re-insert data and re-import. • A GR>1.6 requires additional analysis and possible reevaluation of NOC. **Technical Leader Approval Required.** • Allele call not fully resolved at a given locus – ignore locus and perform analysis again **Technical Leader Approval Required.**

			• Consider another STRmix analysis at a greater number of iterations (typically 100,000 burn in accepts and 500,000 post burn-in accepts). **Technical Leader Approval Required.**
Allele Variance	3.87 to 9.11 Mode: 6.5	Refer to graphs for better estimation. The mean point resulting on the tail-end of graphs is cause for concern	• DNA profile sub-optimal or # of contributors is not supported. Re-evaluate data. • If low level or little data exists, no need to re-run. • If error exists in the editing of the profile, correct and re-run. • If # of contributors is not supported, re-run. **Technical Leader Approval Required.**
Stutter Variance	3.37 to 17.39 Modes: Back Stutter: 4.787 Forward Stutter: 5.242 2bp: 1.559 Double Back: 6.422		
LSAE Variance	0.013 to 0.030 Mean: 0.022		
Loci Efficiency	Average: D3S1358: 98.2% vWA: 92.9% D16S539: 89.9% CSF1PO: 104.8% TPOX: 90.9%	Below Average Performing Loci: D16S539 D19S433 D12S391	• Check E-gram for issues • Check other Diagnostics

	D8S1179: 116.6% D21S11: 93.1% D18S51: 121.2% D2S441: 97.6% D19S433: 94% TH01: 121.1% FGA: 126.3% D22S1045: 109.9% D5S818: 116.9% D13S317: 134.6% D7S820: 118.4% SE33: 141.4% D10S1248: 111.3% D1S1656:109% D12S391: 90.2% D2S1338: 174.2%	Above Average Performing Loci: D2S1338	

9.4 Determining Suitability for Comparison after STRmix™ Interpretation

Trace and minimally represented contributors may be present in a forensic DNA profile. These contributors may be considered part of the number of contributors evaluation; however, based on their lack of completeness and their reduced ability to differentiate between closely related individuals, these components may be deemed not suitable for comparison. The following guidelines should be used to evaluate each contributor position of a mixture to determine if it is suitable for comparison.

Rules: NOC=2

Contributor 1 can be used for comparison if the template is 100 RFU or greater

Contributor 1 can be used for comparison if the template is between 50-99 RFU and the mixture proportion is 60% or greater

Contributor 2 can be used for comparison if the template is 100 RFU or greater

Contributor 2 can be used for comparison if the template is between 50-99 RFU and the mixture proportion is 5% or greater

Rules: NOC=3

Contributor 1 can be used for comparison if the template is 100 RFU or greater

Contributor 2 can be used for comparison if the template is 100 RFU or greater

Contributor 2 can be used for comparison if the template is between 50-99 RFU and the mixture proportion is 30% or greater

Contributor 3 can be used for comparison if the template is 100 RFU or greater

Contributor 3 can be used for comparison if the template is between 50-99 RFU and the mixture proportion is 7.5% or greater

Rules: NOC=4

Contributor 1 can be used for comparison if the template is 100 RFU or greater

Contributor 2 can be used for comparison if the template is 100 RFU or greater

Contributor 2 can be used for comparison if the template is between 50-99 RFU and the mixture proportion is 15% or greater

Contributor 3 can be used for comparison if the template is 100 RFU or greater

Contributor 3 can be used for comparison if the template is between 50-99 RFU and the mixture proportion is 15% or greater

Contributor 4 can be used for comparison if the template is 100 RFU or greater

Contributor 4 can be used for comparison if the template is between 50-99 RFU and the mixture proportion is 10% or greater

9.5 Comparison of Profiles

Once the analyst has examined both the STRmix™ Interpretation report and Database Search report and the results meet expectations, the analyst can then begin manual comparisons between the forensic profile electropherogram(s) and the reference profile(s) electropherogram(s). Analysts will conduct a manual comparison of the appropriate reference profile(s) to forensic profile data and make general conclusions before LR calculations will be performed in STRmix™.

Ideally an analyst will identify any locus to ignore and not use for statistical calculations prior to comparison to reference samples. There may be situations where anomalies are not identified prior to examination of the reference profiles. The below table lists some examples and courses of action.

Example	Action
Tri-allele in reference sample	• Typically handled by ignoring the affected locus in the reference when performing the Likelihood Ratio calculation
Unresolved microvariant (above analytical threshold) not observed in initial viewing of forensic sample	• Deconvolution will be re-run ignoring the affected locus • Original deconvolution will be documented within the case file

Analysts will still perform manual comparisons on any ignored loci in the forensic and reference samples. Ignoring a locus from a reference profile does not make the reference profile partial. The reasoning for using the ignore loci function will be documented on the STRmix Additional Analysis Worksheet and ignored loci wording will be added to the report. Technical Leader or designee approval should be obtained prior to ignoring a locus/loci within the STRmix™ software.

There may be circumstances when the information obtained from a reference, (e.g. conditioning on a newly obtained reference) will prompt a re-evaluation of the number of contributors used in the original interpretation. Deduced or inferred contributors without a submitted reference should not be used to determine the NOC unless observed in the corresponding fractions (NSF and SF) of the same sample. Any subsequent analysis adjusting the number of contributors would need approval from the Technical Leader or designee. The original deconvolution(s) will be documented within the case file.

The propositions chosen by the analyst will be the most reasonable based on the data from the profile and the case information provided. Multiple scenarios may be calculated for each profile using different propositions. The analyst should also review their service requests to see if any specific propositions were requested. Some common proposition examples are provided below:

Number of Contributors	H1 (Numerator)	H2 (Denominator)	Example
1	Reference	Unknown	Single Source
2	Reference + Unknown	Unknown + Unknown	Mixture
2	Conditioned Reference + Reference	Conditioned Reference + Unknown	Mixture with Conditioning
3	Reference 1 + Reference 2 + Unknown	Unknown + Unknown + Unknown	Compound LR

LR calculations are not performed in the following situations:

- Conditioned reference profiles will not have LRs calculated.
- A complete single source or partial single source profile is consistent with an expected donor on a relevant item of evidence.
- The evidence profile is a complete or partial single source profile, and the reference profile is manually excluded
- On carryover contributors observed in the sperm cell or non-sperm cell fractions
- If a case has multiple, complete single source profiles with identical results (for example, identical genotypes at all locations with a weight of one) and is associated to a reference, then only one of those samples needs to have LR calculations performed.

9.6 LR from Previous Report

The LR from Previous Report generated from STRmix™ will be reviewed by the analyst. Output files are reviewed to ensure the parameters used and results obtained are as expected and are consistent with the conclusions from manual review. This review includes the following elements:

- Reviewing the Details and Run Parameters sections of the report. Verify the user, number of contributors, profiling kit, sample file/sample name, and case number is correct. Verify the proposition information in Known Contributors under HP and Known Contributors under HD (if applicable) is correct.

- If a conditioning profile was applied, their reference file name should appear next to “Known Contributors under HP:” and “Known Contributors under HD:” and they will be placed in the contributor one position.

- Review the Summary of Contributors information and the contributor position the reference(s) is/are being placed in. Pay particular attention to contributor position(s) that were deemed not suitable for comparison based on template or template and mixture proportions.

- Review the Per Locus Likelihood Ratios (primary diagnostic) and the LRs obtained, particularly the 99% 1-sided Lower Highest Posterior Density (HPD) Interval values. The per locus likelihood ratios should agree with the analyst’s manual assessment of the electropherogram. If most loci favor inclusion, but one locus displays a very low LR or a LR =0, this indicates further review of the electropherogram, and the interpretation is necessary.

- Review the Reference Files information to ensure the correct reference file(s) is/are included and the profile information is complete and accurate. Review the information contained in the Settings section of the report. Pay particular attention to any information that is bolded, this indicates a change from the default settings.

- Review the information contained in Previous Interpretation Details.

The LR will be calculated using four sub-populations (NIST1036 Caucasian, NIST1036 African American, NIST1036 Hispanic, and NIST1036 Asian). The most conservative value obtained from the 99% 1-sided lower HPD interval LR will be used to determine the conclusion. If all values following the E (scientific notation) are positive, the lowest integer is considered most conservative. If all values following the E (scientific notation) are negative, the highest integer is considered most conservative. If any sub-population has a zero 99% 1-sided lower HPD LR value, the conclusion will be exclusion. The most conservative unrelated HPD LR value will be bolded by the STRmix™ ™ software in the Summary of LR section of the report.

When the reference is placed in a contributor position that is suitable for comparison:

- If the LR result is equal to zero, the reference conclusion will be reported as an exclusion. The statistical results will be retained electronically as part of the case record but are not required to be printed for the case file.

- If the LR result supports the exclusionary hypothesis by being less than .001 and greater than zero, the reference conclusion will be reported as providing support for exclusion. The LR value will not be reported but will be provided upon request. When requested to provide the LR, the analyst will flip the LR statement

and report 1/LR. The statistical results will be retained electronically as part of the case record but are not required to be printed for the case file.

- If the calculated LR value is equal to/between .001 through 1000, then no conclusion can be rendered to the reference, and it will be reported as an inconclusive result. The LR value will not be reported but will be provided upon request. The statistical results will be retained electronically as part of the case record but are not required to be printed for the case file.

- If the LR value is greater than 1000, the reference conclusion will be reported as supporting inclusion. Statistical results will be printed and included in the case file along with being reported in the report. The statistic will be written out in words on the printed LR from Previous results.

Printing instructions for LR from Previous results

1) File Print → Click "Multiple"
2) In the Pages per Sheet drop-down select "2"
3) Make Sure Page Order = "Vertical"
4) Make Sure Orientation = "Portrait"

To go back to printing a single page per piece of paper Click on "Size"

Likelihood Ratio Value and Conclusion:

Likelihood Ratio Value	Conclusion
0	Exclusion
0 < x < .001	Supports exclusion – LR provided upon request.
.001 - 1000	Inconclusive (observed adventitious non-donor inclusionary LRs in validation)- LR provided upon request.
x > 1000	Supports inclusion. Report LR.

When the reference is placed in a contributor position that was deemed not suitable for comparison based on template or template and mixture proportions:

- The reference will be reported as being excluded from the comparable components of the mixture.

  Although not necessarily excluded (LR=0) as a contributor in the other positions, these contributor positions will have lower sub-sub source LRs. A conservative approach will be applied, and an exclusion conclusion will be reported for the reference.

The reported LR value will be shortened to two significant figures with no rounding. LR values 1 million or greater will be reported using a verbal description. LR values equal to or greater than .001 but less than 1 million will be reported using numbers in decimal form. The below chart contains some LR reporting examples.

LR Result	LR Conclusion	Report LR?	Reporting Example
5.9149E8	Supports Inclusion	Yes	590 million
15,036	Supports Inclusion	Yes	15,000
1.7838E-2	Inconclusive	Only if requested	.017
2.6507E-10	Supports exclusion	Only if requested-flip the LR statement and report 1/(LR Result)	3.7 billion
0	Exclusion	No	

9.7 Compound LRs

If an evidence sample has multiple individuals with individual inclusionary LRs, the analyst will then perform a compound LR that combines all these individuals in Hypothesis 1. This will be performed as a routine part of casework to ensure all contributors can explain the mixture if considered together. The conclusion of this routinely performed compound LR will be reported when the LR result does not support inclusion. If compound LR's are specifically requested, both the individual and compound LRs will be included in the report. Compound LR's will be performed only if each individual LR supports inclusion. All requested compound LR conclusions will be reported. Compound LR from Previous reports, whether routine or specifically requested, will be printed and included within the case file. The LR statistic will be written out in words from scientific notation on the printed LR from Previous results (ex. 3.6 E26→ 360 septillion).

It is expected that the compound LR will be greater than the individual LRs and inclusionary. The analyst will review the deconvolution further if the compound LR is lower than the individual LRs and/or if it does not support an inclusionary result for the individuals together. If the compound LR places an individual in a contributor position that is not suitable for comparison, the compound LR conclusion will be considered inconclusive. If the review confirms the results, appropriate wording will be added to the report.

If a compound LR cannot be performed because there are more individuals with inclusionary LRs than there are number of contributors to the profile, wording stating this will be included in the report.

Analysts will ensure that subsequent submissions have any applicable compound LRs performed.

9.8 Possible Conclusions

Not suitable for comparison- DNA profiles that have no data or less than seven loci with data are not suitable for comparison. Partial single source profiles that have at least seven loci with data, but do not have four complete genotypes are not suitable for comparison. DNA mixtures can be not suitable for comparison due to complexity (five or more contributors) or limited information (data at less than seven loci/mixture with all alleles below stochastic threshold and does not meet number of alleles requirement). No comparisons to reference standard(s) shall be performed between DNA profiles that are not suitable for comparison because the data has insufficient information. Profiles that are not suitable for comparison are considered uninterpretable.

Exclusion- A known individual could not have contributed to or is not a part of the forensic profile from a single source sample or the comparable components of a mixed sample. This may be determined based on a likelihood ratio of 0 or an analyst can manually exclude a reference from a single source profile. Manual exclusions will have no statistical analysis performed.

Components of a mixture may be not suitable for comparison based on template amount or template and mixture proportions***.*** If a reference is placed in a contributor position that was deemed not suitable for comparison based on template or template and mixture proportions, a conservative approach will be applied and an exclusion to the comparable components of the mixture will be reported for the reference.

Supports exclusion- Based on CMPD's STRmix™ validation studies LR values greater than zero and less than .001 support the exclusionary hypothesis (H2).

Inconclusive – A determination that no inclusion or exclusion can be drawn from the comparison of a reference sample to a questioned profile. An inconclusive conclusion results from a comparison to a suitable profile/component of a profile that has a likelihood ratio value that fails to provide sufficient support for an inclusion or exclusion. The inconclusive LR range was established from CMPD's STRmix™ validation studies.

Supports inclusion – A known individual is included in a profile if their genotype is present at all loci at which DNA typing results are deemed interpretable with no unexplained differences. The loss of an allele due to preferential amplification, stochastic effects, mutation, or other factors is considered and does not necessarily indicate an exclusion. Based on CMPD's STRmix™ validation studies LR values greater than 1,000 support the inclusionary hypothesis (H1).

9.9 Statistical Calculations

STRmix™ produces a statistic in the form of a likelihood ratio (LR). The LR assesses the probability of the evidence (E) given two alternate propositions; one that aligns with the inclusion of the comparison sample (H1 or Hp) and one that aligns with the exclusion of that comparison sample (H2 or Hd) and attributes the allelic contribution to a random unknown individual (U). An unknown individual is a random, unrelated person whose DNA has not been tested by the laboratory.

The LR is defined as:

$$LR = \frac{Pr\ (E|H1)}{Pr\ (E|H2)}$$

H1: Inclusionary Hypothesis
H2: Exclusionary Hypothesis
E: Evidence or the DNA profile

Statistical calculations will be performed using the STRmix™ software which uses a fully continuous probabilistic genotyping likelihood ratio (LR) model. LR are calculated using the Balding and Nichols formula (NRC II recommendation 4.2)

$$\frac{2[\theta+(1-\theta)p_i][\theta+(1-\theta)p_j]}{(1+\theta)(1+2\theta)}$$ for heterozygote loci

$$\frac{[3\theta+(1-\theta)p_i][2\theta+(1-\theta)p_j]}{(1+\theta)(1+2\theta)}$$ for homozygote loci

The CMPD laboratory will use a FST value of .01 (1%) in the STRmix™ LR calculations.

The LR will be calculated using four sub-populations (NIST1036 Caucasian, NIST1036 African American, NIST1036 Hispanic, and NIST1036 Asian). The most conservative value obtained from the 99% 1-sided lower HPD interval LR will be used to determine the conclusion and will be reported (if applicable) truncated to two significant figures. If all values following the E (scientific notation) are positive, the lowest integers are considered most conservative. If all values following the E (scientific notation) are negative, the highest integers are considered most conservative. If any sub-populations have a zero 99% 1-sided lower HPD LR value, the conclusion will be exclusion. The most conservative unrelated HPD LR value will be bolded by the STRmix™ software in the Summary of LR section of the report. The HPD LR statistic accounts for uncertainty associated with the MCMC and database sampling. By default, there is no minimum allele frequency or count within STRmix™.

Y Indel, Amelogenin, and DYS391 frequencies are not used for calculations.

9.10 STRmix™ Additional Analysis Worksheet

Once the analyst has reviewed the applicable deconvolution and/or comparison(s), they may determine that additional STRmix™ analysis is necessary. For these samples, the analyst will place a check in the "Is additional work needed" checkbox for the applicable sample on the STRmix™ Interpretation Worksheet. The sample should then be listed on the STRmix™ Additional Analysis worksheet. The analyst should verify that all appropriate samples are listed on this worksheet. The analyst will fill out the following fields for each sample:

Observed:

The analyst should describe what they are observing that is prompting the additional STRmix™ analysis. There is a drop-down menu with options and the analyst may also type/handwrite their own comment.

Action:

The analyst should describe the action they are taking that is different than their initial analysis. There is a drop-down menu with options and the analyst may also type/handwrite their own comment.

Comments: The comments box is for any additional comments the analyst would like to add.

Initials (if applicable): If the actions the analyst is taking require DNA Technical Leader or designee approval, their approval will be documented in this location.

A copy of the printed STRmix™ Additional Analysis worksheet will be included in the case file.

9.11 References

- Scientific Working Group on DNA Analysis Methods (SWGDAM). Guidelines for the Validation of Probabilistic Genotyping Systems. 2015.
- STRmix™ v2.9 Implementation and Validation Guide
- STRmix™ v2.9 User's Manual
- STRmix™ v2.9 Operation Manual
- STRmix™ v2.9.1 Release and Testing Report – February 2022

- CMPD STRmx v2.9.1 Internal Validation – ESR – September 2022

- Estimation of STRmix™ V2.9 parameters for the Charlotte-Mecklenburg Police Department (CMPD) Crime Laboratory (GlobalFiler™ 3500, 28 Cycle)

- Internal Validation of STRmix™ v2.9 with performance check of STRmix™ ™ v2.9 software installation, staff database search, and GeneMapper® ID-X v1.6 export table function – performed by CMPD

10. CODIS Entry

A. Purpose and Introduction

CODIS (COmbined DNA Index System) is a collection of databases from forensic laboratories across the United States and consists of three levels: Local (LDIS), State (SDIS), and National (NDIS). Forensic autosomal profiles and suspect profiles will be entered into CODIS using the COSTaR software program. CODIS entry, COSTaR procedures, and autosomal and Y-STR CODIS entry file requirements are described below, while familial search requirements, forensic investigative genetic genealogy requirements, and general guidance on CODIS eligibility determination, can be found in Biology QA 20.

B. Materials and Instrumentation:

CMPD COSTaR workbook

C. Safety Considerations:

Prolonged computer analysis may cause muscle soreness, eye fatigue, or other discomforts. To minimize the occurrence of discomforts, periodic breaks from computer analysis are encouraged.

D. Limitations:

The COSTaR software can assist in the development of profiles suitable for CODIS entry; however, all data requires analyst review as well as a second review by the technical reviewer to ensure accuracy.

E. Other Considerations:

COSTaR is an Excel workbook that is used to assist in creating profiles for entry into CODIS. It was designed primarily for use in analyzing STRmix deconvolution data and developing profiles for entry into CODIS at the Local, State, and National levels. STRmix mixture deconvolution produces multiple genotype possibilities for a single contributor and these genotypes must then be evaluated for CODIS entry suitability. COSTaR will modify profiles to attempt to meet eligibility requirements for the highest level of CODIS possible. Based on the thresholds that are set at each level, COSTaR can remove (“trim”) data or assign obligate alleles in order to increase the moderate match estimate (MME) values for mixture or partial profiles; thereby, increasing the chance that a profile can be entered into CODIS.

F. Validation:

This procedure has undergone internal validation at the CMPD.

Procedure:

10.1 COSTaR

The macros in COSTaR use a four-step process to attempt to create CODIS searchable profiles:

1. Trimming (removing) of the number of alleles at each locus above the user-defined maximum number allowed.
2. Exclusion of loci with dropout probabilities (Q values) above the trimming threshold.
3. Marking of obligate/required alleles to increase discrimination.
4. Trimming of alleles to increase discrimination based on the risk threshold in use.

If after the first two steps, the MME does not meet the statistical threshold for a particular level of CODIS (starting at NDIS), steps 3 and 4 will occur in an incremental fashion until the MME is met or all possible edits have been made, at which point a higher risk threshold will be used or a lower level CODIS profile will be attempted. The above four steps will occur for each of the CODIS search levels: NDIS, SDIS, and LDIS. The maximum number of alleles and the risk thresholds can vary between the different levels of CODIS. COSTaR will first attempt to build a profile for upload to NDIS. If that is not possible, then it will attempt SDIS and finally LDIS, if necessary. Not all profiles entered into COSTaR will result in a CODIS entry. Additional information on the developmental validation and design of COSTaR can be found with the CMPD validation documentation.

The following chart summarizes the criteria needed for CODIS entry of partial and mixture forensic profiles:

CODIS Level	Maximum Trimming Threshold	Maximum Number of Alleles per Locus	Number of Loci Required	MME Threshold
NDIS	5%	4	8 (original core)	1.00E07
SDIS	5%	4	8 (original core)	1.50E04
LDIS	1%	4	7 (any)	1.00E03

A minimum of thirteen original core loci are required for upload of Forensic, Unknown samples to NDIS.

All profiles that are suitable for interpretation in STRmix will be run through COSTaR if case scenario indicates CODIS entry may be warranted. It is not possible to only select contributors suitable for comparison to be imported into COSTaR. All contributors will be imported regardless of comparison suitability and a potential CODIS entry may be generated for all contributors in a profile. The analyst must be aware that if a STRmix contributor is deemed not suitable for comparison, then it is also not suitable for CODIS entry. Refer to SOP 10.1.2.17 for guidance on documenting profiles that will not be entered into CODIS.

It must be documented in the case file if a profile will be processed in COSTaR or not. The analyst will mark either the “yes” or “no” box on the STRmix Interpretation worksheet. If a profile will not be processed in COSTaR the reason must be noted. Potential reasons for not sending a profile to COSTaR include not being CODIS eligible, STRmix interpretation guidelines, insufficient number of core loci, and multiple single source consistent profiles.

The COSTaR workbook is also used for entry of suspect reference profiles into CODIS. When a suspect profile is compared to forensic profiles in STRmix a .csv file is generated which can then be used to import suspect profiles into COSTaR and subsequently into CODIS. Suspect profiles that have been run but are not compared in STRmix will be manually entered into COSTaR in order to generate a file for CODIS entry.

The Reference File Maker workbook of COSTaR can be used to generate .txt reference files that are compatible with STRmix. This workbook will be used for 1) reference profiles that were not generated using GMID-X or 2) a profile/contributor is used to condition a forensic profile for CODIS entry purposes only. Since the function of this workbook is to format reference profiles into a specific text file, this is not considered re-analysis. The Reference File Maker workbook is a separate workbook from the COSTaR workbook.

COSTaR will be used for profiles with no more than 4 contributors. The one exception is for DNA mixtures that are not suitable for STRmix interpretation due to having > 4 contributors, but a visually obvious complete major profile can be determined. The complete major profile may be CODIS eligible and will require manual entry into COSTaR.

A DNA record entered into CODIS is considered the property and responsibility of the CMPD Forensic Biology section (refer to the NDIS Operational Procedures Manual for guidance). Each record entered into CODIS will be entered into the appropriate index based upon the profile type. Each record will receive a unique identifier. Profiles that have been generated by vendor laboratories and whose property and responsibility becomes that of the CMPD Biology lab will be given either a specimen identification number or a unique identification number created by the CODIS Administrator or the back-up Administrator.

10.1.1 Use of the COSTaR Workbook

Opening the COSTaR workbook and navigating to the "COSTaR" tab is the first step in the COSTaR process. Selections on this tab include beginning the COSTaR process for both suspect and forensic profiles. There is also a "Settings" button which analysts should only use for changing their text file location. All other settings will only be changed by the CODIS Administrator or back-up Administrators.

The forensic portion of the COSTaR workbook will be for analysis of single source and mixture profiles that were obtained from testing conducted either at CMPD or an outsourced lab. Analysis of unidentified human remains profiles will also use this workbook.

The suspect portion of the COSTaR workbook will be for entering suspect profiles into CODIS.

Suspect and forensic profiles can be entered using the same COSTaR workbook; however, the suspect profiles should be imported into COSTaR before any forensic profiles. If forensic profiles are entered first and then it is determined that suspect profiles also need entered, a new COSTaR workbook should be opened before beginning the suspect entry process.

A description of the COSTaR workbook function tabs is as follows:

a) The "Contributors" tab displays the individual contributor genotypes and weights. The column with genotype weights highlighted in yellow indicate which contributor the workbook is associated with.

b) The "Allele Weights Summary" tab lists the cumulative allele weighting derived from the STRmix derived genotype weightings, for a particular contributor. The alleles that are removed (trimmed) from the CODIS entry are also shown.

c) The "MME" tab shows both the MME value and the level of CODIS entry.

d) The "Forensic Entry" and "Suspect Entry" tabs show the CODIS entry worksheets and are the basis for importing a profile into the CODIS software.

10.1.2 COSTaR for Forensic Profiles (single source and mixture profiles and unidentified human remains profiles for in-house and outsourced casework):

1. Open the COSTaR workbook and click on the "I would like to enter a forensic profile" button.

2. Navigate to the DNA Technical Data folder where the STRmix data for the case is saved and open the applicable “Results.txt” file.

3. The COSTaR macros will run automatically and .xls COSTaR results files will be generated for each contributor. Individual .xls files for each contributor will be automatically saved to the DNA Technical Data Folder. The file name will automatically be generated as “COSTaR” followed by the STRmix Case Number, Sample ID, mixture proportion % of the contributor (if applicable), and the CODIS upload level (e.g. COSTaR 220627-117201_23-12345_AGM (71) National).

4. The original COSTaR workbook will close in order to create the file for each contributor (including single source profiles). Open the COSTaR file for each contributor. The individual files will need to be opened separately in order to view the results.

5. Navigate to the “Forensic Entry” tab.

6. Use of the CODIS Forensic Entry Worksheet

    a) Enter the Complaint # and DNA #.

    b) Enter the specimen ID. The overall specimen ID should be in the following format: xxxxxx-xxxxxx_yy-yyyyy_z where “xxxxxx-xxxxxx” is the Complaint number, “_yy-yyyyy” is the DNA number, and _z is the contributor number.

        1) The specimen ID cannot contain more than 24 characters. The specimen ID will need to include a designation to the contributor # being entered or notated to indicate a mixture consisting of multiple contributors is being entered.

        2) If the profile to be entered consists of multiple contributors (example: C1 and C2 have the same CODIS entry), _M should be added to the specimen ID in place of the contributor number

    c) Specimen Category default = Non-reviewed profiles. This is to ensure that no profiles are uploaded prior to technical review.
    d) Select from the drop-down menu either “Yes” or “No” for Source ID. The Source ID will be classified as “Yes” if the source of the sample is known or as “No” if the source of the sample is unknown. If a profile consisting of multiple contributors/components is entered and one or none of the contributors is known, the Source ID should be set to “No”. “Source” is defined as a person having a likelihood ratio favoring association to a profile based on STRmix comparisons.

e) Select from the drop-down menu either “Yes” or “No” for partial profile status. “Yes” will be selected for any profile that has a locus marked as partial by COSTaR analysis. Additionally, if any alleles or Q’s are red on the Allele Weights Summary page then partial profile status = “Yes”.

f) Upload to = automatically input by the COSTaR software based on MME and indicates the level of CODIS a profile is eligible for.

g) Select from the drop-down menu the final specimen category for an uploaded profile (refer to the chart below for guidance).

h) Select from the drop-down menu the grant ID a case has been worked under or “N/A” if a grant ID does not apply.

i) Select from the drop-down menu the case type: ADW (ADW or aggravated assault), Burglary (burglary or larceny), Homicide (homicide or death investigation), Rape (rape, sexual assault, fondling), Robbery (robbery), and Other (the type of crime is not described by one of the others listed).

j) Notes are used to record additional information about the profile. This may include which contributors are part of the CODIS entry (example: CODIS entry = C1/C2) or the reason why a profile is not being entered into CODIS (example: DNE (Do Not Enter) = C2 included in the C1 CODIS entry).

k) The MME for the 13 original core loci and the MME for all loci is shown. These should be used to assist with the determination of specimen category. Initials and date by the technical reviewer verify the specimen category selected by the analyst is correct.

7. The COSTaR software will determine alleles that are marked partial, exact genotypes, and obligate alleles and these will be automatically indicated on the CODIS Forensic Entry Sheet. These determinations may not be overridden by an analyst.

a) Partial alleles
   1) [ ] will be placed around alleles that are marked partial.
   2) Individual loci will only be marked partial for single source profiles.
   3) The partial locus indicator will automatically be set to “Yes” when the profile is imported into CODIS.

b) Targeted profiles

1) < > will be placed around alleles to indicate an exact genotype. This will be marked when alleles are used for the purpose of creating a Targeted Forensic profile.
2) COSTaR will automatically determine if a profile should be entered into the Targeted category; no analyst determination is needed.

b) Obligate alleles

1) + will be placed next to obligate alleles.

8. The following should be used as a guide in determining the correct Specimen Category as well as Partial profile status:

Forensic Unknown	Partial profile = No	All loci in profile or component of mixture deconvoluted to 100% with no complete locus drop-out. No other genotypes or Qs considered during deconvolution.
Forensic Partial 2) Single source 3) Alleles enclosed with [ ]	Partial profile = Yes	At least one locus being entered deconvoluted <100% with Qs OR complete drop-out at any loci. No other genotypes considered during deconvolution aside from Qs at all loci being entered.
Forensic Mixture	Partial profile = No	More than two alleles to be entered at any locus OR multiple genotypes considered in profile/component at any locus entered. Data entered for all loci and no Qs considered during deconvolution.
Forensic Mixture	Partial profile = Yes	More than two alleles to be entered at any locus OR multiple genotypes considered in profile/component deconvoluted at any locus entered. Data not entered

		at all loci or Qs considered during deconvolution for loci being entered.
Forensic Targeted (Partials and Mixtures) Alleles enclosed with < >	Partial profile = Yes	Refer to definitions of Forensic Partial and Forensic Mixture. Meets the NDIS MRE threshold when searched at a specified stringency by locus (high or moderate). All loci with one or two alleles searched at moderate stringency will automatically have partial locus indicators set to “Yes”.
Forensic Targeted (Mixtures only) Alleles enclosed with < >	Partial profile = No	Refer to definitions of Forensic Mixture. Meets the NDIS MRE threshold when searched at a specified stringency by locus (high or moderate).
Forensic Targeted – State (Partials and Mixtures) Alleles enclosed with < >	Partial profile = Yes	Refer to definitions of Forensic Partial and Forensic Mixture. Doesn’t meet the NDIS threshold but meets the SDIS MME threshold when searched at a specified stringency by locus (high or moderate). All loci with one or two alleles searched at moderate stringency will automatically have partial locus indicators set to “Yes”.
Forensic Targeted – State (Mixtures only) Alleles enclosed with < >	Partial profile = No	Refer to definitions of Forensic Mixture. Doesn’t meet the NDIS threshold but meets the SDIS MME threshold when searched at a specified stringency by locus (high or moderate).
Mixture - State	Partial profile = No	More than two alleles to be entered at any locus OR

		multiple genotypes considered in profile/component at any locus entered. Data entered for all loci and no Qs considered during deconvolution. Does not meet the NDIS threshold.
Mixture – State	Partial profile = Yes	More than two alleles to be entered at any locus OR multiple genotypes considered in profile/component deconvoluted at any locus entered. Data not entered at all loci or Qs considered during deconvolution for loci being entered. Does not meet the NDIS MME threshold.
Partial - State	Partial profile = Yes	At least one original core locus being entered deconvoluted <100% with Q's OR complete drop-out at any original core loci. No other genotypes considered during deconvolution aside from Q's at all loci being entered. Does not meet the NDIS MME threshold.
Forensic-LDIS	Partial profile = Yes	Does not meet NDIS or SDIS MME threshold for any specimen category, but does meet the LDIS MME threshold

9. Amelogenin is not automatically entered in COSTaR and must be manually entered by the analyst. Use the following as guidance in selecting an option:

    a) For CODIS profiles being entered as single source or partial single source profiles where the gender is clear, the corresponding gender should be selected. Manually enter X, Y if male and X if female.

b) If there is the possibility of dropout at Amelogenin, no results should be entered.

c) For CODIS profiles being entered as mixtures, no results will be entered.

d) DYS391 and Yindel are not imported into COSTaR and will not be seen in any tab for forensic profiles. These loci will also not be entered into CODIS.

10. Print the CODIS Forensic Entry Worksheet by choosing “File” on the tool bar and then “Print”. Print the worksheet in color. Only worksheets for profiles being entered into CODIS will be printed. The worksheet is the only portion of the workbook that will be printed for the case file.

11. To create the text export file for CODIS entry, choose the “Export” button on the “Forensic Entry” tab. The text file will automatically be saved to a designated folder on each analyst’s desktop computer, which has been defined on the COSTaR Settings tab for each user.

    a) A text file should not be created for profiles that are not being entered into CODIS.

    b) Since the text file is only a tool to assist with importing profiles into CODIS it is not required for the analyst to save these files once they have been imported into CODIS

12. A “Results exported to CODIS” button will appear. Choose “OK”. The Contributor # will not be automatically saved at the end of the file name; therefore, if multiple contributors from a single profile are being entered into CODIS, the analyst should adjust the file name to include the Contributor # before creating the next text file (Example: CODIS user name_Complaint #_DNA #_1) to avoid the text file being written over by subsequent exports.

13. Refer to SOP 10.2 for directions on how to import text files to CODIS.

14. Save the workbook before closing out of the workbook or moving on to other profiles.

15. Open the COSTaR workbooks for the remaining contributors (if applicable) and repeat the previous steps.

16. For profiles that will not be entered into CODIS, the file name should be edited to include the addition of “_DNE” at the end of the file name. “DNE” represents Do Not Enter. For profiles that COSTaR deems not eligible for CODIS entry, “Do Not Enter” is automatically added to the file name; no update is needed to this file name.

a) The reason for the profile not being entered should be documented in the Notes section of the CODIS Forensic Entry Worksheet. This may include which contributors are part of the CODIS entry (example: CODIS entry = C1/C2) or the reason why a profile is not being entered into CODIS (example on the CODIS entry worksheet for C2: DNE = C2 included in the C1 CODIS entry). No note is needed for profiles determined by COSTaR to not be eligible for entry.

17. To begin the COSTaR process for additional profiles, open a new COSTaR workbook and begin the process for the next profile or:

    a) On the "Forensic Entry" tab choose "Clear all data". This will clear the allele weights summary, MME, and CODIS forensic entry pages.

    b) An "Are you sure? Be sure to save contributor file!" button will appear. Choose "Yes" as long as the file has been saved. If the file has not yet been saved, choose "No" and save the file before proceeding.

    c) On the Contributors tab, choose "Clear Contributors Data".

    d) Navigate to the "COSTaR" tab and begin the process for the next profile.

10.1.3 Modification of Mixture Profiles with 99% STRmix Component

There may be instances when the STRmix deconvolution generates a full, single source profile based on the 99% genotype component. Since COSTaR uses all allele weights when creating profiles for CODIS entry those alleles with small weightings may be pulled into the CODIS entry. Using the STRmix 99% component as a guide, if a single source profile can be deconvoluted and is complete at all loci, then the CODIS entry may be modified to produce a Forensic, Unknown profile. Modification of profiles may only be considered by approval of the CODIS Administrator or CODIS back-up Administrator.

Use the following steps to modify a profile:

1. Review the 99% component interpretation summary from STRmix. Use this as your guide for profile modification.

2. Save the workbook as originally generated.

3. On the MME tab, delete the alleles that are not part of the 99% component. Leave all called alleles in place and do not move them to another location on the MME entry box. The MME value will automatically update to reflect the changes.

4. Verify the MME reaches the NDIS threshold.

5. Click the “Update CODIS sheet” button.

6. Select the “Forensic Entry” tab and verify that the profile to be entered into CODIS has transferred correctly onto the CODIS Forensic Entry Worksheet.

7. Manually enter the Specimen ID and DNA #. No more than 24 characters are allowed in the specimen ID. The specimen ID should consist of the last twelve digits of the Complaint number followed by the DNA number in the following format: xxxxxx-xxxxxx_yy-yyyyy where “xxxxxx-xxxxxx” is the Complaint number and “_yy-yyyyy” is the DNA number.

   The specimen ID will need to include a designation to the contributor # being entered. For example, xxxxxx-xxxxxx_yy-yyyyy_1 if contributor 1’s profile is being entered.

8. The following should be verified:

   a) The eligibility level will automatically populate; however, the Specimen Category will be defaulted to “Non-reviewed profiles” in order to ensure no profiles are uploaded prior to technical review.
   b) Select from the drop-down menu either “Yes” or “No” for Source ID. The Sample ID will be classified as “Yes” if the source of the sample is known or as “No” if the source of the sample is unknown.” “Source” is defined as a person having a likelihood ratio favoring association to a profile based on STRmix comparisons.
   c) Select from the drop-down menu “No” for partial profile status.
   d) Select from the drop-down menu the final specimen category = Forensic, Unknown.
   e) Select from the drop-down menu the grant ID a case has been worked under or “N/A” if a grant ID does not apply.
   f) Select from the drop-down menu the case type: ADW (ADW or aggravated assault), Burglary (burglary or larceny), Homicide (homicide or death investigation), Rape (rape, sexual assault, fondling), Robbery (robbery), and Other (the type of crime is not described by one of the others listed).

9. Amelogenin is not automatically entered in COSTaR and must be manually entered by the analyst. Use the following as guidance in selecting an option:

   a) For CODIS profiles being entered as single source profiles where the gender is clear, the corresponding gender should be selected. Manually enter X, Y if male and X if female.

   b) If there is the possibility of dropout at Amelogenin, no results should be entered.

10. Print the CODIS Forensic Entry Worksheet by choosing “File” on the tool bar and then “Print”. Print the worksheet in color. Only worksheets for profiles being entered into CODIS will be printed. The worksheet is the only portion of the workbook that will be printed for the case file.

11. To create the text export file for CODIS entry, choose the “Export” button on the “Forensic Entry” tab. The text file will automatically be saved to a designated folder on each analyst’s desktop computer, which has been defined on the COSTaR Settings tab for each user. Since the text file is only a tool to assist with importing profiles into CODIS it is not required for the analyst to save these files once they have been imported into CODIS

12. A “Results exported to CODIS” button will appear. Choose “OK”. The export file will automatically be saved in the following format: CODIS user name_Complaint #_DNA #. The Contributor # will not be automatically saved; therefore, the file name may be edited to include this additional information before importing into the CODIS software.

13. Save the modified workbook as [file name _MOD_Z] where Z is the contributor number. Refer to SOP 10.1.2.3 for file naming convention. Both the original contributor workbook and the modified workbook must be saved. Any forensic profiles that are run through COSTaR must be saved.

14. Refer to SOP 10.2 for directions on how to import text files to CODIS.

10.1.4 Conditioned Contributors

When a contributor is conditioned during STRmix deconvolution, STRmix will set all conditioned genotype weightings to 100%. COSTaR will detect the number of conditioned contributors from information in the STRmix results file and will skip them when creating .xls files. Files will be created and saved for all other contributors.

A conditioned contributor is deemed Contributor 1 in both STRmix and COSTaR; therefore, it is an important reminder that the saved COSTaR file(s) for profiles with conditioned contributors will begin with Contributor 2.

Depending on the number of contributors, number of alleles at a locus, and peak heights, some of the conditioned contributor’s alleles may be considered for other contributors as well. For example, if a 2-person mixture has two alleles from the conditioned contributor and one weaker additional allele, STRmix may consider one or both of the conditioned contributors as possibly being the sister allele to the additional allele. If this occurs, the conditioned contributor’s allele(s) will be part of the CODIS entry.

The COSTaR process is the same for conditioned contributors as it is for profiles without conditioned contributors; therefore, refer to SOP 10.1.2 for the process.

10.1.5 Manual Entry of Profiles

DNA mixtures that have the number of contributors > 4 are not suitable for STRmix interpretation but will be manually assessed for a visually obvious complete major profile. If a visually obvious complete major profile can be determined it may be used for comparison purposes. The complete major profile may require CODIS entry. This will be accomplished by manually entering the profile into COSTaR. This is the only time a forensic profile will be entered manually into COSTaR.

1. Open the COSTaR workbook and click on the MME tab.

2. Manually enter the allele calls for each locus. Heterozygote alleles should be entered in the format X, X and homozygote alleles should be entered in the format X. Yindel and DYS391 should not be entered.

3. Select “Update CODIS sheet”.

4. Select the “Forensic Entry” tab and verify that the profile to be entered into CODIS has transferred correctly onto the CODIS Forensic Entry Worksheet.

5. Manually enter the Specimen ID and DNA #. No more than 24 characters are allowed in the specimen ID. The specimen ID should consist of the last twelve digits of the Complaint number followed by the DNA number in the following format: xxxxxx-xxxxxx_yy-yyyyy where “xxxxxx-xxxxxx” is the Complaint number and “_yy-yyyyy” is the DNA number.

   The specimen ID will need to include a designation to the contributor # being entered. For example, xxxxxx-xxxxxx_yy-yyyyy_1 if contributor 1’s profile is being entered.

6. The following should be verified:

   a) The eligibility level will automatically populate; however, the Specimen Category will be defaulted to “Non-reviewed profiles” in order to ensure no profiles are uploaded prior to technical review.
   b) Select from the drop-down menu either “Yes” or “No” for Source ID. The Sample ID will be classified as “Yes” if the source of the sample is known or as “No” if the source of the sample is unknown.
   c) Select from the drop-down menu “No” for partial profile status.
   d) Select from the drop-down menu the final specimen category of Forensic, Unknown.

e) Select from the drop-down menu the grant ID a case has been worked under or “N/A” if a grant ID does not apply.
f) Select from the drop-down menu the case type: ADW (ADW or aggravated assault), Burglary (burglary or larceny), Homicide (homicide or death investigation), Rape (rape, sexual assault, fondling), Robbery (robbery), and Other (the type of crime is not described by one of the others listed).

7. Amelogenin is not automatically entered in COSTaR and must be manually entered by the analyst. Use the following as guidance in selecting an option:

   a) For CODIS profiles being entered as single source profiles where the gender is clear, the corresponding gender should be selected. Manually enter X, Y if male and X if female.

   b) If there is the possibility of dropout at Amelogenin, no results should be entered.

8. Print the CODIS Forensic Entry Worksheet by choosing “File” on the tool bar and then “Print”. Print the worksheet in color. Only worksheets for profiles being entered into CODIS will be printed. The worksheet is the only portion of the workbook that will be printed for the case file.

9. To create the text export file for CODIS entry, choose the “Export” button on the “Forensic Entry” tab. The text file will automatically be saved as a text file to a designated folder on each analyst’s desktop computer, which has been defined on the COSTaR Settings tab for each user. Since the text file is only a tool to assist with importing profiles into CODIS it is not required for the analyst to save these files once they have been imported into CODIS

10. A “Results exported to CODIS” button will appear. Choose “OK”. The export file will automatically be saved in the following format: CODIS user name_Complaint #_DNA #. The Contributor # will not be automatically saved; therefore, the file name may be edited to include this additional information before importing into the CODIS software.

11. Save the workbook before closing out of the workbook or moving on to other profiles. Any forensic profiles that are run through COSTaR must be saved.

10.1.6 Choosing Contributors for Entry

All profiles to be considered must first meet CODIS eligibility criteria. The decision to run a profile through COSTaR will generally be made after STRmix deconvolution and prior to COSTaR use. If a case has multiple consistent (manually compared) single source profiles that are CODIS eligible only one will be entered into COSTaR to

generate the CODIS entry. The determination of which partial profiles and mixture profiles will be entered into CODIS may occur after evaluating COSTaR results.

COSTaR automatically attempts to generate a CODIS profile for any contributor deconvoluted in STRmix. There is the possibility that all contributors in a mixture profile could be entered into CODIS.

If mixture profiles are generated for multiple contributors, the profiles should be compared to determine if both contributors are represented in a single mixture that can be uploaded.

For mixture profiles where NOC = 2 and there is a conditioned contributor, similar profiles may result in multiple potential CODIS entries. This will generally be seen with profiles generated from sexual assault samples. The goal of the CODIS entry is to only enter the most comprehensive representation of the second contributor; therefore, an evaluation of the alleles different from the conditioned contributor in each profile should be done to determine the most complete profile for CODIS entry.

With approval of the CODIS Administrator or back-up Administrator, an analyst may use a profile or a contributor to condition a forensic profile for CODIS entry purposes only. The CODIS Administrator or back-up CODIS Administrator must document that the conditioning of a profile or contributor is approved for CODIS entry purposes only.

For CODIS eligible forensic profiles, the following should be used as a guide in determining which profiles to enter into CODIS:

Multiple consistent single source profiles with no suspect for comparison	Enter one based on CODIS eligibility criteria
Multiple single source partial profiles with no suspect for comparison	Enter one based on CODIS eligibility criteria, profile completeness, & MME
Multiple consistent single source profiles with suspect for comparison	Enter one based on CODIS eligibility criteria
Multiple single source partial profiles with suspect for comparison	Enter one based on CODIS eligibility criteria, profile completeness, & MME
2 or more contributors have the same mixture profile	Choose 1 profile for entry and document why the other(s) are not being entered
2 or more contributors have differences at > 2 loci (no conditioned contributor)	Enter all profiles (CODIS searches are based on two mismatches)
2 or more contributors have differences at < 2 loci	Enter the most complete profile and document why the other(s) are not being entered
Forensic profile only meets the statistical threshold for acceptance at SDIS and the profile is consistent with a complete suspect profile from the same case	Enter suspect profile Exception: if suspect profile was previously entered in CODIS under another Complaint # then enter the

	forensic profile

10.1.7 COSTaR for Suspect Profiles

Suspect profiles will be entered into CODIS using the COSTaR workbook. Suspect profiles that have been run through STRmix will be imported into COSTaR. Suspect profiles that were not run through STRmix must be entered manually into COSTaR.

Tri-allelic loci and loci with alleles that are outside the allelic ladder range are not able to be interpreted in STRmix and will be ignored in the STRmix software. Additionally, COSTaR is not compatible with these loci. These loci are not considered uninterpretable loci, but instead are loci where statistics cannot be applied. Since a tri-allelic locus or a locus with alleles outside of the allelic ladder range will be ignored in STRmix, no results will be entered into CODIS for that locus. Complete results must be obtained at all 21 STR loci and Amelogenin in order to enter a suspect profile; however, when a locus is omitted due to having a tri-allele or an allele outside of the allelic ladder range, the partial profile status indicator should be set to “Yes”. Omission of a locus due to being tri-allelic or outside the allelic ladder range for CODIS entry purposes is the only reason a suspect profile may be partial.

10.1.7.1 Suspect Profiles Run through STRmix:

1. Open the COSTaR workbook and click on the “I would like to enter a suspect profile” button.

2. Select “Yes” when the box appears that states “Import a suspect profile now”.

3. Navigate to the folder where the STRmix data for the case is saved and open the .csv file associated with the known standard. Note: All known standard .csv files will be saved in the folder associated with the Complaint # the standard was submitted under.

4. The COSTaR macros will run automatically. The workbook will automatically open on the “Suspect Entry” tab.

5. Review the “Suspect Entry” tab to verify that allele calls are correct. If the profile is from a male individual where DYS391 and/or Yindel results are present, these results will not be imported and these boxes should be left blank on the worksheet.

6. Manually enter the specimen ID. The specimen ID should consist of the last twelve digits of the Complaint number the suspect sample was submitted under followed by the DNA number in the following format: xxxxxx-xxxxxx_yy-yyyyy where “xxxxxx-xxxxxx” is the Complaint number and “_yy-yyyyy” is the DNA number.

7. The specimen category is defaulted to Non-reviewed profiles. This is to ensure that no profiles are uploaded prior to technical review.

8. Select from the drop-down menu either “Yes” or “No” for partial profile status.

9. “Suspect Known” on the Comments line indicates the final specimen category.

10. The source ID is automatically set to “yes” upon creation of the text file (See #12 below). This is not visible on the CODIS Suspect Entry Worksheet.

11. Print the CODIS Suspect Entry Worksheet by choosing “File” on the tool bar and then “Print”. Print the worksheet in color. Only worksheets for profiles being entered into CODIS will be printed. The worksheet will be retained in the case file.

12. To create the text export file for CODIS entry, choose the “Export” button on the “Suspect Entry” tab. The text file will automatically be saved to a designated folder on each analyst’s desktop computer, which has been defined on the COSTaR Settings tab for each user. Since the COSTaR workbook is only a tool to assist with importing suspect profiles into CODIS it is not required for the analyst to save the workbook or the text file once the profile has been imported into CODIS.

13. A “Results exported to CODIS! Well done!” button will appear. Choose “OK”. The export file will automatically be saved in the following format: CODIS user name_Complaint #_DNA #.

14. See SOP 10.2 for directions on how to import text files to CODIS.

15. To begin the COSTaR process for additional suspect profiles that have been run through STRmix:

    a) On the “Suspect Entry” tab, choose “Clear all data”. This will clear the profile on the CODIS Suspect Entry Worksheet.

    b) Delete the Specimen ID.

    c) Select “Import Profile” on the Suspect Entry tab. Navigate to the folder where the STRmix data for the case is saved and begin the COSTaR process. Alternatively, return to the COSTaR tab and begin the COSTaR process by clicking on the “I would like to enter a suspect profile” button.

10.1.7.2 Suspect Profiles Not Run through STRmix:

1. Open the COSTaR workbook and click on the “I would like to enter a suspect profile” button.

2. Select “No” when the box appears that states “Import a suspect profile now”.

3. The “Manual Suspect Entry” tab will open. Manually enter the allele calls for each locus. Heterozygote alleles should be entered in the format X, X and homozygote alleles should be entered in the format X. If the profile is from a male individual where DYS391 and/or Yindel results are present, the results for these loci should not be entered.

4. Manually enter the Specimen ID. The specimen ID should consist of the last twelve digits of the Complaint number the suspect sample was submitted under followed by the DNA number in the following format: xxxxxx-xxxxxx_yy-yyyyy where “xxxxxx-xxxxxx” is the Complaint number and “_yy-yyyyy” is the DNA number.

5. The specimen category is defaulted to Non-reviewed profiles. This is to ensure that no profiles are uploaded prior to technical review.

6. Select from the drop-down menu either “Yes” or “No” for partial profile status.

7. “Suspect Known” on the Comments line indicates the final specimen category.

8. The source ID is automatically set to “yes” upon creation of the text file (See #10 below). This is not visible on the CODIS Suspect Entry Worksheet.

9. Print the CODIS Suspect Entry Worksheet by choosing “File” on the tool bar and then “Print”. Print the worksheet in color. Only worksheets for profiles being entered into CODIS will be printed. The worksheet will be retained in the case file.

10. To create the text export file for CODIS entry, choose the “Export” button on the “Suspect Entry” tab. The text file will automatically be saved to a designated folder on each analyst’s desktop computer, which has been defined on the COSTaR Settings tab for each user. Since the COSTaR workbook is only a tool to assist with importing suspect profiles into CODIS it is not required for the analyst to save the workbook or the text file once the profile has been imported into CODIS.

11. A “Results exported to CODIS! Well done!” button will appear. Choose “OK”. The export file will automatically be saved in the following format: CODIS user name_Complaint #_DNA #.

12. See SOP 10.2 for directions on how to import text files to CODIS.

13. To begin the COSTaR process for additional manually entered suspect profiles:

    a) On the “Suspect Entry” tab, choose “Clear all data”. This will clear the profile on the CODIS Suspect Entry Worksheet.

    b) Delete the Specimen ID.

    c) The next profile can now be manually entered. Alternatively, return to the COSTaR tab and begin the COSTaR process by clicking on the “I would like to enter a suspect profile” button.

10.2 Importing Text Files into CODIS

Autosomal DNA profiles will be entered into CODIS by the case analyst prior to turning a case in for technical review. All autosomal DNA CODIS entries will be unmarked for upload by the default CODIS setting. Upon completion of technical review, any profiles being uploaded to LDIS, SDIS or NDIS will have their specimen categories updated to the final designation. SDIS and NDIS profiles will then be marked for upload.

1. Copy text file(s) to a thumb drive.

2. At a CODIS workstation, open Analyst Workbench.

3. Open Specimen Manager and either select the Import icon or choose “Import Specimens” under Specimen Manager on the toolbar.

4. Select “All files (*.*)” to view all text files. Select the text files from the thumb drive to be imported. Multiple files can be imported at once.

5. When prompted with “Select import type”, choose “Data Import”. Verify the “assign to user” is the case analyst. Select “OK”.

6. If the files have been imported correctly, an “Import Confirmation” box will appear and an import received successfully message will appear. Select “OK”.

7. After ~ 1 minute, the file(s) will be automatically executed and the profiles(s) will be able to be viewed in Specimen Manager.

8. Open each profile and verify the information is correct for the following:
   a) Specimen ID
   b) Specimen category = Non-reviewed profiles
   c) Source ID = yes or no
   d) Partial profile = yes or no

e) Comments box = case type (if applicable), grant ID (if applicable), and final specimen category

9. If any changes or additions need made, select “Edit Data for the selected specimen” on the tool bar.

10. Print two copies of the Specimen Detail Report for the case file review process.

10.3 Reference File Maker

The reference file maker will be used for manual creation of reference profiles that can be imported into STRmix for comparison or conditioning purposes. This applies to reference profiles that were not generated using GMID-X or profiles/contributors used to condition a forensic profile for CODIS entry purposes only.

1. Open the “Reference File Maker” workbook of COSTaR.

2. In the Sample File column cell A2, enter the identifier of the sample the STRMix compatible reference profile is being generated for and hit Enter. Hitting “Enter” will auto-fill the sample name in remaining rows. If the sample being entered is from a person, the identifier will consist of the last twelve digits of the Complaint number the sample was submitted under followed by the DNA number and the initials of the person the known standard was collected from in the following format: xxxxxx-xxxxxx_yy-yyyyy_AB where “xxxxxx-xxxxxx” is the Complaint number, “yy-yyyyy” is the DNA number, and AB is the person’s initials.

3. If the sample being entered is a profile that has been conditioned from an evidence sample, the Contributor number (if applicable) will follow the DNA number. For example: xxxxxx-xxxxxx_yy-yyyyy_1.

4. Manually enter the DNA profile information for the reference sample appropriately into allele columns 1 and 2.

   a) Do not enter any partial loci.

   b) Homozygous loci should be entered as a single allele.

   c) If a locus is tri-allelic or has an allele that is outside the allelic ladder range, it will not be entered into the Reference File Maker.

5. The allele size will automatically populate for most alleles.

6. Save as a .txt file that will serve as a reference file that can be used for any STRmix comparisons. This file will ultimately be incorporated into a STRmix file; therefore, it is not required to save the .txt file once imported into STRmix.

10.4 Autosomal CODIS Entry Case File Requirements

1. Prior to turning a case file in for technical review, all forensic profiles considered for CODIS entry must have the eligibility verified by the CODIS Administrator or back-up Administrator (or designee, if necessary). The CODIS Administrator or back-up Administrator will document in the case file if a sample is eligible for entry. There may be instances where a communication log is included in a case file where CODIS eligibility has been addressed. This is to be used as general guidance; however, all potential CODIS entries will still be verified and documented by the CODIS Administrator or back-up Administrator.

2. Two copies of the STR specimen detail report will be printed for the case file. One copy of the specimen detail report will remain in the case file and will include the initials of the analyst, the technical reviewer and the administrative reviewer. The technical reviewer will also initial and date the CODIS Forensic Entry Worksheet contained in the case file to indicate that the MME correlates to the CODIS level being uploaded, and the final specimen category is correct. Once technical review has been completed and signed off on the DNA TR worksheet, the technical reviewer will initial and date the second copy of the STR specimen detail report and place it in the designated CODIS upload box located at the CODIS Administrator's desk. The initials and date of the technical reviewer are to signify that a profile is ready to be marked for upload. The following lists the items on the Specimen Details Report that should be checked for correctness by the case analyst and the technical reviewer: specimen ID, specimen category, source ID, partial profile status, case type, grant ID (if applicable), and correct allele calls. The case type will only be noted for forensic profiles.

3. The CODIS Administrator or back-up Administrator will remove the Specimen Details Reports from the CODIS upload box, update the specimen category and mark the profile for upload. Once the specimen category has been updated, the CODIS Administrator or back-up Administrator will print the specimen detail report reflecting the final specimen category and add their initials. The specimen detail report will then be scanned into PLIMS and attached to the associated case file under the Case Info tab. The paper copy of the Specimen Details Report will then be placed into the corresponding case file within a reasonable timeframe of the profile being marked for upload and the associated case report being issued. Once the specimen category has been updated and the profile has been marked for upload, the second copy of the Non-reviewed specimen category STR specimen detail report may be discarded; this is considered an administrative document and is not required to be maintained since a copy remains in the case file. The print date on the updated specimen details report correlates to the date the profile was marked for upload.

4. All profiles will automatically have the MME calculated when entered into COSTaR and the value will be shown on the MME tab and CODIS Forensic

Entry Worksheet. It is possible the MME value on the specimen detail report may have slight differences due to rounding; however, the differences are not enough to change the overall specimen category.

5. If it is determined prior to or during technical review that an entry needs changes made to it (for example: specimen category, partial profile indicator, etc) the analyst may make those changes as long as the profile(s) has not been marked for upload or uploaded. Two copies of the STR specimen detail report should be re-printed. Since the original entry is an Administrative document it may be discarded. If changes are needed to a CODIS entry after the profile has been marked for upload and uploaded, the CODIS Administrator or back-up CODIS Administrator will need to make the corrections and the original entry paperwork will be maintained in the case file. Any associated paperwork will be added to the case file, as well as being attached in PLIMS.

6. If it is determined during technical or administrative review that a profile needs to be deleted either the CODIS Administrator or back-up CODIS Administrator must delete it. The Specimen Delete report will be maintained in the case file along with the original STR specimen detail report. Due to the CODIS entry worksheet being a technical case file document any supporting documentation of profile deletion must be maintained in the case file.

10.5 Y-STR CODIS Entry Case File Requirements

COSTaR is not compatible with Y-STR entry; therefore, Y-STR profiles must be manually entered in the CODIS software.

1. If both autosomal and Y-STR profiles are being entered at the same time, the autosomal profile will be imported using the COSTaR procedure. The Y-STR profile will be entered manually after the autosomal profile has been imported into CODIS. Two copies of the Specimen Detail Report will be printed for the case file review process.

2. If a Y-STR profile is being added to a previously entered autosomal DNA record, it will be manually entered by the analyst conducting the Y-STR testing. A Y-STR profile must be entered as part of an autosomal DNA record and will not be entered as an individual record.

3. If a Y-STR profile is developed from a buccal standard under a Complaint # that is different than the Complaint # the autosomal profile was developed under, the Y-STR CODIS entry will be added to the Complaint # with the autosomal profile entry. A comment will be entered into the Comment box on the specimen detail report that references the Complaint # the Y-STR testing was done under. Once

technical review is complete, in addition to being saved with the Y-STR case file, a copy of the updated DNA record will be added to the original autosomal case file as well as scanned into the case info tab in PLIMS.

4. If a buccal standard submitted for Y-STR testing has not been previously run, both autosomal and Y-STR typing will be done. The DNA profile will be entered into CODIS under the Complaint # the buccal was submitted under. The autosomal profile will be imported using the COSTaR procedure. The Y-STR profile will be entered manually after the autosomal profile has been imported into CODIS. Two copies of the Specimen Detail Report will be printed for the case file review process.

5. Generally, Y-STR testing is not conducted on forensic samples with autosomal results. However, there may be instances where both an autosomal forensic profile matching a submitted suspect and the suspect profile with Y-STR typing results from the same Complaint # are entered into CODIS (i.e., the suspect is being compared in a different Complaint #). If a CODIS match occurs at LDIS, both the forensic sample information and the buccal standard information will be reported. Both the forensic sample information and the suspect buccal standard information will be relayed to an SDIS lab if a CODIS match occurs. Since a match to a suspect buccal standard cannot occur at NDIS, only the forensic sample information will be relayed to a NDIS lab.

6. In cases where a newly generated Y-STR profile is being added to an existing autosomal profile, the analyst will complete the Y-STR CODIS entry worksheet prior to submitting the case file for technical review. Once the entry worksheet has been verified (initialed and dated) by the technical reviewer, the analyst will enter the Y-STR profile into CODIS and print one copy of the updated Specimen Detail Report (which includes both autosomal and Y-STR profiles) prior to the completion of the technical review. This copy will remain in the case file and will include the initials of the analyst, the technical reviewer and the administrative reviewer.

7. If an autosomal and Y-STR profile are being entered concurrently and it is determined that an entry needs changes made to it, the analyst may make those changes and re-print the STR specimen detail report as long as the profile(s) has not been marked for upload or uploaded. Since the original entry is an Administrative document it may be discarded. If changes are needed to a CODIS entry after the profile has been marked for upload or uploaded, the CODIS Administrator or back-up CODIS Administrator will need to make the corrections and the original entry paperwork will be maintained in the case file. Any associated paperwork will be added to the case file, as well as being attached in PLIMS.

8. If it is determined during technical or administrative review that a record needs to be deleted either the CODIS Administrator or back-up Administrator must delete it. The Specimen Delete Report will be maintained in the case file along with the original STR specimen detail report. Due to the Y-STR CODIS entry worksheet being a technical case file document, any supporting documentation of profile deletion must be maintained in the case file.

9. Partial profile status will be based solely on the autosomal DNA record. If the Y-STR profile is a partial profile, the overall partial profile status for the entire DNA record will not change.

10. There may be instances when Y-STR typing is conducted on a sample that yielded an autosomal profile that was previously entered into CODIS (for example: investigative purposes, testing conducted without a suspect standard, making additional case associations, completeness of a record, etc). The Y-STR profile will be added to the previously entered autosomal CODIS profile for purposes of a more complete record should Y-STR databases be used for searching in the future.

11. Y-STR testing is required before familial searching can be performed. Refer to Biology QA 20.6.3 for guidance on the familial search and Y-STR procedure.

10.6 Unidentified Human Remains

On a case-by-case basis the CMPD lab may accept an unidentified human remains (UHR) case for entry into CODIS. The remains must be “newer” as the lab does not have testing procedures compatible with DNA extraction from older skeletal remains. The preferred samples for testing include blood, buccal, teeth, and/or femur. Coordination with a detective may be necessary if entry into NCIC or NamUs is needed.

Generally, only male remains will be accepted. This is due to the requirement to conduct testing in multiple DNA technologies for CODIS entry. Since the CMPD lab does not have the capability to conduct testing in additional technologies for females (i.e. mtDNA), testing for female UHR’s will typically be done in coordination with an FBI-approved external laboratory. Exceptions may occur on a case-by-case basis for UHR’s believed to be female in nature.

In order to upload to CODIS, UHR profiles must have complete typing results at 8 original core loci. If allelic dropout is suspected at a locus, the entire locus shall be omitted from CODIS entry. Additionally, due to the high mutation rates in the following Y-STR loci, these loci should not be entered into CODIS even if they are fully represented: DYS449, DYS518, DYS570, DYS576, DYS627, and DYF387S1.

Profiles obtained from unidentified human remains (UHR) cases will be run through STRmix whether there is an alternate standard for comparison or for the sole purpose of

CODIS entry. If a UHR profile is being entered into CODIS, follow the COSTaR protocol under SOP 10.1.2.

The following should be used as a guide in determining UHR CODIS entry and removal:

Only the UHR sample is submitted for testing & the profile is entered into CODIS	Hit to convicted offender or arrestee	Hit to solved case or suspect standard	Hit to unsolved case	Hit to missing person sample or deduced missing person sample (alternate standard)
UHR (full body present)	Remove UHR profile from CODIS after confirmation	Remove UHR profile from CODIS	UHR profile remains in CODIS with source ID = no	Remove UHR profile from CODIS
UHR (incomplete body/body parts)	UHR profile stays in CODIS to ID additional body parts if found; source ID = yes	UHR profile stays in CODIS to ID additional body parts if found; source ID = yes	UHR profile remains in CODIS with source ID = no	UHR profile stays in CODIS to ID additional body parts if found; source ID = yes

If UHR's match a submitted alternate standard:

a) Full body present = profile is not entered into CODIS

b) Incomplete body/body parts present = UHR profile is entered into CODIS in order to identify additional found body parts and the source ID is set to "Yes"

10.7 References

- NDIS Operational Procedures Manual, FBI
- Developmental Validation – San Diego Police Dept.
- North Carolina State Crime Lab COSTaR validation summary
- CMPD Internal Validation

11. Data Evaluation and Interpretation Guidelines – Yfiler Plus

A. Purpose and Introduction

The purpose of this procedure is to provide interpretation guidelines for DNA analysis and to accurately reflect the scientific weight given to the various situations encountered when interpreting and calculating statistics.

B. Materials

- Case file

- Allele Table (or Combined Table, when applicable) - will list all profiles obtained or deduced from the case. The allele table will be generated in the ArmedXpert or GMID-X software. An allele table will be generated for each Complaint # and all profiles associated with the Complaint # will be included regardless of whether the data is used for interpretation or comparison. Data from subsequent submissions that will be compared to previously obtained data may be incorporated into one new combined table or the page number(s) referencing the previously run data may be noted at the bottom of the subsequent submission allele table.

C. Safety Considerations

Not Applicable

D. Limitations

- All Y-STR loci are physically linked on the Y-chromosome. Due to the lack of genetic recombination, the entire Y-chromosome haplotype is similarly inherited to a single autosomal locus. It is noted that two specimens that exhibit the same Y-STR haplotype may have originated from either a common individual source, from males within the same paternal lineage, or from unrelated individuals. A paternal lineage consists of those male relatives to whom the same Y-chromosome has been transmitted from a common ancestor. Barring mutation, all male relatives within the same paternal lineage have the same Y-STR profile. Attribution of the YSTR typing results to a single individual, to the exclusion of relatives in the paternal lineage, is generally not possible based on Y-chromosome loci. However, loci with higher mutation rates may enhance the ability to distinguish relatives in the same paternal lineage.

- Analysis of forensic casework can produce several possible results. This documentation is to standardize the interpretation of profiles obtained.

- Before interpretation can be done, the following controls must be checked: Allelic ladders, Y Positive Control, Y Negative Control, Reagent Blanks and internal size standards. If these controls do not meet quality control measures, then the samples may need to be re-analyzed. The check of the controls is done

manually by the analyst, automatically within the GeneMapper ID-X software.

- A Y-STR profile is a haplotype and can be defined as the Combination of alleles occurring on the same chromosome; results for a combination of Y-STRs tested.

E. Other Considerations

- The interpretation of results in casework is a matter of professional judgment and expertise. Not every situation can or should be covered by a predetermined rule. These criteria will be based on validation studies, literature references, and casework experience and will be developed with input from the analysts. These guidelines ensure that conclusions in casework reports are scientifically supported by the analytical data, including appropriate standards and controls. In addition, interpretations are made as objectively as possible and consistently from analyst to analyst.

- Known standards of males that are amplified using the Yfiler Plus amplification kit in a case will be compared to all evidence also amplified using the Yfiler Plus amplification kit in that case. Yfiler Plus amplifications will not be performed in the absence of a suspect standard with rare exceptions for elimination purposes that must be approved by the DNA Technical Leader or designee.

- Due to the palindromic sequences often found on the Y chromosome, Y-STR loci can sometimes exhibit as duplications or even triplications. These multi-allelic patterns may be inherited or passed on across generations as has been observed when studying father-son sample pairs. Y chromosome duplications, which occur in single-source samples, can confuse or complicate mixture interpretation if analysts are not aware of this phenomenon. Sequence regions flanking STR regions may also be duplicated elsewhere in the Y-chromosome and may create artifacts when PCR primers are moved during multiplex assay design. Duplications or triplications of several Y-STRs have been reported for DYS19, DYS390, and DYS391.

- A rapidly mutating locus is a locus where the frequency of mutation is 10-100 fold higher per generation. Because of the higher mutation rate and therefore better discrimination capacity, it is more possible to differentiate between brother pairs or in other cases, father-son pairs. The Yfiler Plus PCR Amplification kit has seven rapidly mutating and highly informative Y-STR loci (iDYF387S1a/b, DYS449, DYS518, DYS570, DYS576, and DYS627).

F. Validation

These procedures are generally accepted in the forensic community and have undergone internal validation at the CMPD.

11.1 Data Evaluation

11.1.1 Controls:

- A yellow triangle sizing quality flag is an indicator that the data may not be suitable for typing. If the sizing quality is starting to fail but the data is not impacted or if the failure occurs outside the analysis window (60 to 460 base pairs), then the data may be suitable for typing. For single source evidence samples, references, and controls only, an analyst may check the data visually and determine that it is suitable for interpretation/comparison and notate it on the Data Evaluation Worksheet. All other data with a yellow triangle sizing quality flag will be re-injected/re-run. Amplification Positive Controls may be used with TL or designee approval. Data with a red stop sign sizing quality flag is not suitable for interpretation/comparison.

- GMID-X (software version 1.6) may be used as an expert system to assess the allelic ladders. The analyst will document any ladders that don't meet quality controls measures on the Data Evaluation worksheet. GeneMapper automatically excludes low-quality ladders from analysis, so the sample type does not have to be changed from Allelic Ladder to Sample. If all ladders fail, the DNA samples on the plate will not be used for interpretation or comparison.

- An amplification negative control must be present in each amplification set and serves as a check for sample contamination introduced during amplification set-up. This blank control consists of the PCR Master Mix to which sterile TE Buffer is added instead of DNA. If a negative control contains a pattern of peaks (two or more peaks above the lower analytical threshold), any DNA specimens co-amplified with the reagent aliquots contained in the negative control may be considered inconclusive for typing. Reference Biology Quality Assurance Manual 15.2 for the Contamination Contingency Plan.

- An amplification positive control is required for each amplification set and is used to assess the proper performance of amplification and typing procedures. The manufacturer supplies DNA Control 007 as a Yfiler Plus kit component. The Control 007 is human genomic DNA. If the positive control exhibits a discordant result, all co-amplified samples may be considered inconclusive for typing. GMID-X v1.6 may be used as an expert system to assess the amplification positive controls. If the positive control exhibits unexpected results, any co-amplified DNA samples will be evaluated for suitability for interpretation/comparison.

- A reagent blank must be present in each extraction set. The reagent blank serves as a check for possible contamination by adventitious DNA in samples, reagents or supplies during the testing procedure. Adventitious DNA can be non-amplified or amplified. If a reagent blank contains a pattern of peaks (two or more peaks above the lower analytical), any DNA specimens co-extracted with the reagent aliquots contained in the reagent blank may be considered inconclusive for typing. Reference Biology Quality Assurance Manual 15.2 for the Contamination Contingency Plan.

11.1.2 Stutter Peaks:

- The PCR amplification of Y STR loci can produce a minor product peak one repeat unit smaller than the corresponding main allele peak (minus stutter) or a minor peak one repeat larger than the corresponding main allele peak (plus stutter). This is a well-known aspect of STR analysis and should not cause concern when detected.

  A stutter peak should be edited as an artifact, even if the peak is being labeled as an allele in the GMID-X software. To determine if the called peak is elevated stutter, evaluate the entire profile, take into consideration the locus and allele, and account for possible shared stutter (n- with n+).

- DYS392 and DYS481 are trinucleotide repeats, DYS438 is a pentanucleotide repeat and DYS448 is a hexanucleotide repeat. Their allele differences result from differences in the number of repeat units 3-bp, 5-bp, and 6-bp respectively. The remaining Yfiler Plus PCR Amplification Kit loci are tetranucleotide short tandem repeat (STR) loci. The length differences among alleles of these particular loci result from differences in the number of 4-bp repeat units.

- Peaks may be considered as stutter in Yfiler Plus and filtered in GMID-X if their peak height percentages to the larger peaks are equal to or less than approximately:

Locus	Minus Stutter %	Plus Stutter %
DYS576	15.57	3.85
DYS389I	9.16	3.45
DYS635	13.38	3.3
DYS389II	19.73	3.73
DYS627	21.07	4.54
DYS460	11.65	4.27
DYS458	15.31	2.52
DYS19	12.68	3.72
YGATAH4	13.44	2.27
DYS448	4.68	2.56
DYS391	10.56	3.41
DYS456	18.30	3.74
DYS390	16.22	3.51
DYS438	6.17	2.76
DYS392	18.80	12.78
DYS518	25.50	4.85
DYS570	15.65	3.38
DYS437	8.13	1.65
DYS385	18.32	3.7
DYS449	24.14	4.77
DYS393	14.07	4.95
DYS439	10.29	3.39

DYS481	34.62	6.04
DYF387S1	15.71	3.00 *
DYS533	12.10	4.60

* locus not assessed for plus stutter in validation – global filter of 3% applied

Locus	Alternate Stutter	%
DYS627	(-2ST)	2.71
DYS19	(-2ST)	10.10
DYS481	(-2ST)	10.03
DYS533	(-2ST)	1.88
DYS19	(+2 bp)	3.73

- The data used to support the application of the above stutter percentages can be found in the Yfiler Plus validation. For any given locus, minor peaks that are above 125 RFU and located in a documented stutter position may be considered as a real allele if their peak heights exceed the stutter percentage established for that locus as indicated above. Occasionally, stutter may exceed these values and an evaluation of the entire profile is necessary including the allele at which the stutter is occurring. The number of base pair repeats of the main peak directly impacts the stutter percentage. The larger the number of repeat units in the main peak the higher the observed (n-) stutter percentage will be. The above percentages are averaged across the entire locus and may not completely account for stutter observed at the larger alleles at a locus.

- Peaks that are not filtered by the software, but still determined to be stutter will be edited as such by the analyst.

11.1.3 Repeat Sequences:

All allele designations will be based on the number of repeat sequences as defined in the Yfiler Plus technical manual and in the GMID-X software distributed from ThermoFisher Scientific.

11.1.4 Alleles:

For any given locus a peak above 125 RFU with no major peaks at a position one repeat unit larger to it will be interpreted as an allele unless it is likely due to a technical reason such as spiking, pull-up, bacterial DNA, or rendered inconclusive due to lack of reproducibility.

11.1.5 Variants:

The designation of alleles containing an incomplete repeat motif (i.e., an off-ladder allele falling within the range spanned by the ladder alleles) should include the number of complete repeats and, separated by a decimal point, the number of base pairs in the incomplete repeat (e.g. DYS390 20.1 allele). If an allele falls above the largest or below the smallest allele of the allelic ladder, the allele

should be designated as either greater than (>) or less than (<) the respective ladder allele. If the profile is suitable for comparison, the variants need to be re-analyzed for confirmation by re-injection of the sample. Once the variant is confirmed it may be used for comparison and included in the statistical calculations.

11.1.6 Null Alleles:

Null alleles can occur with any PCR-based STR typing system. They generally are due to deletions within the target region or primer binding sites or by primer binding site mutations that destabilize hybridization of at least one of the primers flanking the target region. Depending on the size of the deletion, multiple closely located primer binding sites can be impacted causing deletions to several Y-chromosome loci. Profiles suspected of presenting with null alleles will be interpreted in consult with the DNA Technical Leader or designee. It is important that a profile with a suspected deletion not be erroneously interpreted as drop-out.

11.1.7 Peak Height Ratios:

There are two multi-copy loci that are part of the Yfiler Plus amplification kit (DYS385 and DYF387S1) so the ability to assess a stochastic threshold and an appropriate peak height ratio is impacted by a limited amount of observable drop-out. Peak height ratios were above 70% with input amounts of a least 0.5 ng of DNA. For input amounts of 125 pg of DNA and above the peak height ratios ranged from 59% to 99%. It is therefore, expected that peaks at multi-copy loci will generally adhere to a peak height balance of at least 50%. Stochastic effects may cause a departure from the 50% peak height ratio rule due to limited template of one allele compared to another. As a part of this analysis, it is recognized that low template or degraded samples may deviate from the 50% rule. Caution should be taken with low template contributors since a low PHR could also be signaling an additional contributor may be present.

11.1.8 Duplication:

Duplication has been reported in the literature for up to four loci due to a single mutation. The close proximity of certain Y loci permits simultaneous duplications at multiple loci. While single repeat unit duplications are most common, 2-, 3-, 4- and partial repeat unit differences have also been observed. If a single suspected duplication event for a locus is observed at a single repeat unit, the profile may still be deemed single source. Profiles suspected of presenting with multiple duplications or those beyond a single repeat unit will be interpreted in consult with the DNA Technical Leader or designee. It is important that a profile with a suspected duplication not be erroneously reported as a mixture.

A duplication is expected to have a peak height ratio of > 50% and will be reported as both alleles (e.g.13,14) on the allele table.

11.1.9 Analytical Threshold:

All peaks must be above 125 RFU to be used for comparison and statistical purposes. The analytical threshold is defined as the minimum RFU at which detectable peaks can be reliably distinguished from non-allelic peaks for evidentiary samples. Non-allelic peaks such as spikes, pull-up, elevated background noise, and PCR artifacts are typically associated with instrument noise or background. Allelic peaks above the analytical threshold may be used for reporting purposes to include comparison and statistical calculations. Allelic peaks may also be observed below the analytical threshold, and although not reportable should be cautiously considered as part of the overall interpretation when appropriate. The analytical threshold has been established from validation studies at 125 RFU.

11.1.10 Stochastic Threshold:

The stochastic threshold is defined as the RFU value where stochastic effects are observed (e.g. allelic dropout and peak height ratio imbalance) making quantitative interpretation more difficult, especially with mixtures. Based on validation results, this threshold is set at 650 RFU for Yfiler Plus data.

DYS385 and DYF387S1 are the two multi-copy loci that are part of the Yfiler Plus amplification kit. If a single allele is observed at either of these loci, then that peak must be a minimum peak height of 650 RFU to be reported as a single allele. Alleles below this threshold will be noted as *allele, any* (“^”) and may be included in statistics by selecting "yes" in the statistics tool to allow for drop-out at duplicated loci.

11.1.11 Upper Analytical Threshold:

The upper analytical threshold is defined as the RFU value where allelic peaks are known to produce artifacts in other loci which could impact reliable interpretation for the minor component. The upper analytical threshold is set at 30000 RFU for Yfiler Plus data.

A saturated signal above the linear dynamic range for the instrument can also result in unreliable data in the minor profile from increased artifacts (e.g. pull-up/elevated baseline). When the GMID-X software flags a peak for saturation, the RFU is not accurate and, therefore; this data should not be used for quantitative analysis in mixture profiles.

The minor component from a DNA mixture containing major peaks greater than the upper analytical threshold or flagged for saturation will not be interpreted at that locus without approval from the Technical Leader or designee and should instead be re-amplified targeting a lesser template concentration.

11.1.12 Lower Analytical Threshold:

The lower analytical threshold is defined as the relative fluorescent unit (RFU) value where allelic peaks can be reliably distinguished from non-allelic peaks for reagent blanks and/or negative amplification controls. These samples should not contain any detectable DNA, and as such have less PCR artifacts and instrument artifacts (e.g. pull-up) than observed with actual DNA samples. As a result, the lower analytical threshold has been established from validation studies at 50 RFU.

11.1.13 Extraneous Peaks

- Peaks other than the true alleles may be detected on the electropherograms display. It is important to identify the most likely cause for the appearance of extra peaks.

- These peaks will be labeled on the electropherograms in GeneMapper IDX with the editing function. Artifact code designations will be noted as below in the table. The designations for all of these will be “ART” for artifact. Additional notations as to the type of artifact will be made in the subsequent “Reason(s) for Change” comment screen in GeneMapper IDX and will show up on the Artifacts Edits Worksheet.

Artifact	Code	Comment
Stutter	ART	ST
Pullup	ART	PU
Spike	ART	SP
Minus A	ART	MA
N+4	ART	NP
Other Artifacts	ART	ART

- Low levels of female cross-reactivity were observed during the Yfiler Plus developmental validation and in the internal validation in the presence of high amounts of female DNA. Most peaks attributed to cross reactivity did not show well-defined peak morphology and were at an amplitude (below 50 RFU) or base pair location (red channel 412-413 bp) that did not impact the data interpretation.

- Additional artifacts were observed and had reproducible base pair values in multiple samples. Some of these artifacts are characterized in the Yfiler Plus User Guide and others were observed during the internal validation.

Locus	Dye Channel	Size
DYS627	Blue	348-349 bp
DYS391	Green	N-10 bp
DYS437	Red	N-5 bp
DYS437	Red	N-12 bp

DYS576	Blue	~65-90 bp
DYS438	Yellow	N-2 bp
DYS456	Yellow	~90-105 bp
DYS481	Purple	N-5 to 6 bp
YGATAH4	Green	N-14 to 15 bp

Incomplete 3' (A) nucleotide addition:

The DNA polymerase used in Y-STR/PCR amplification catalyzes the addition of a single nucleotide to the 3' ends of double stranded PCR products. This non-template addition results in a PCR product which is one base pair longer than the actual target DNA sequence. Y-STR/PCR amplifications have been optimized to favor the A addition. Incomplete A addition is recognized as "split peaks" (n-1), where a target allele is represented by two peaks one base pair apart.

Spectral failure:

A matrix failure is a result of the instrument's inability to separate the colors (spectral overlap) used to fluorescently label STR/PCR products. A matrix failure is observed as a peak beneath a peak or as an elevation of the baselines for any color and can occur as reagents change slightly over the course of time. A peak of another color is "pulled up" as a minor component beneath the true allele peak.

Spike:

A spike is an extra peak that can be caused by a polymer crystal, an electrical impulse, or bumping of the instrument.

11.2 Possible Results

The results of a Yfiler Plus amplification may result in no DNA profile, a minimal DNA profile, a single source DNA profile, or a mixture of DNA profiles. Profiles obtained may be complete or partial. Complete profiles are profiles where data is obtained at all loci tested and partial profiles are profiles where some data is observed to be missing at the loci tested. When a single allele is observed at either DYS385 and DYF387S1 then it must exceed the peak height of 650 RFU to be reported as a single allele and be considered a complete profile.

11.2.1 No DNA

No data is above the analytical threshold at all loci, the result obtained is no DNA profile.

11.2.2 Single Source Profiles

Single source profiles typically present with a single allele per locus and up to two alleles at DYS385 and DYF387S1. All loci must be evaluated in determining if a sample is single source in origin. A profile demonstrating a called haplotype at fewer than ten loci will not be interpreted and will be characterized as minimal

DNA results and not suitable for comparison.

Reference standards are expected to yield single source profiles. If a reference standard yields extra, unexplained allelic peaks, the sample should be re-injected, re-amplified, and/or re-extracted. If the sample still contains unexplained peaks, it will not be used for comparison and a new standard must be requested.

A single source profile may be compared with known profiles and reported as a match, exclusion, or deemed to be foreign to an expected donor. A single source profile may be complete (exhibiting alleles at all loci tested) or partial (exhibiting alleles at seven or more loci).

If more than one unknown single source profile is observed in a case, the profiles may be characterized as Male 1, Male 2, etc. for reporting purposes.

11.2.3 Mixture Profiles:

Mixture profiles typically present with more than one peak at two or more loci, other than DYS385 and DYF387S1.

A mixture is suspected based on any of the following observations:

- Two peaked pattern observed at one or more locus other than DYS385 or DYF387S1 while considering possible duplications
- Three or four peak pattern if observed in DYS385 or DYF387S1.

If the mixture can be based on discrete number of contributors, it will be interpreted as being 'consistent with' that number.

If the number of contributors cannot be reliably determined, then the mixture will be interpreted as 'at least' a minimum number.

11.2.3.1 Determining the Number of Contributors to the Mixture:

Generally, the number of contributors to a mixed sample may be inferred based on a locus (other than DYS385 or DYF387S1) that exhibits the greatest number of allelic peaks.

Examination of peak height ratios of the detected alleles may indicate the presence of more individuals than suggested by the number of allelic peaks.

Counting the greatest number of allelic peaks and examining peak height ratios may provide guidance in determining the number of contributors the mixture is consistent with or determining the minimum number of contributors.

When using allele count to report that a mixture is consistent with a finite number of contributors, the counting of the alleles assumes that the mixture profile has no

alleles that are below the analytical threshold and therefore undetected.

If there is reason to believe that there may be undetected alleles, the counting of the alleles might only be useful in determining the minimum number of contributors and will be reported as at least that number.

Determining the number of contributors will be used for major profile deconvolution (where appropriate), interpretation, and reporting purposes.

11.2.3.2 Proportions:

An individual's contribution to a mixed biological sample is generally proportional to their quantitative representation within the DNA typing results. Depending on the relative contribution of the various contributors to a mixture, the DNA typing results may potentially be further refined. If a sample is a mixture and there is a distinct contrast in signal intensities (i.e. peak heights) among the different contributors' alleles, a major and possibly a minor contributor may be determined.

11.2.3.3 Mixture of At Least Two Contributors – Major Contributor:

A mixture consisting of at least two contributors may be used for comparison if the major donor demonstrates a ratio of at least 4:1 to the next most prevalent donor. This ratio should be observable throughout the mixture. The major profile must be determined at a minimum of ten loci in order to be deemed a profile suitable for comparison purposes.

When a mixture consists of only a few low-level alleles (less than ¼ peak height of the major allele) in addition to a robust profile, the requirement of demonstrating a ratio of 4:1 for calling a major profile is suspended, due to the inability to mathematically demonstrate the ratio. This allows for calling a profile, with a possible additional contributor to which no comparisons can be made.

Major DNA profiles may be determined from mixtures of at least three individuals with approval of the DNA Technical Leader or designee.

11.2.3.4 Mixture Consistent with Two Contributors – Resolvable:

A mixture consistent with two donors may be resolved to yield major and minor profiles suitable for comparison. The major contributor should demonstrate a ratio of at least 4:1 to the minor contributor in order to be reliably resolved. Both the major and minor profiles must be uniquely determined at a minimum of ten loci in order to be deemed single source profiles for comparison purposes. Due to the limited polymorphic nature of the Y loci, the presentation of a called minor profile is expected to be rare. If the called minor does not meet the minimum requirements for uniquely attributable alleles at ten loci, then the mixture will be interpreted as a mixture of at least two individuals, and the minor contributor will not be suitable for comparison.

By conditioning on the presence of a known individual (elimination standard or male victim standard), a mixture consistent with two contributors may be distinguished into known or expected and foreign. The foreign profile must be uniquely determined at a minimum of ten loci in order to be deemed a profile suitable for comparison purposes.

11.2.3.5 Mixture Consistent with Two Contributors - Unresolvable

A mixture consistent with two donors where one donor cannot be assumed may not be resolvable into major and minor components because of similarity in signal intensities of the two contributors; however, the mixture may still be used for comparison. A minimum of ten loci must demonstrate the unambiguous presence of both contributors to be suitable for comparison.

11.2.4 Not Suitable for Comparison

Data is deemed to be not suitable for comparison when the profile obtained is too minimal or complex for interpretation or if it fails to meet quality assurance requirements as defined by the laboratory.

A sample is deemed to be uninterpretable based on the determination that the DNA results at one or more loci cannot be interpreted due to the poor or limited data quality. If a sample is deemed uninterpretable, a notation will be made in the file that clearly states why the sample is uninterpretable (e.g. minimal profiles or mixture profiles where the number of contributors cannot be determined).

If a partial profile is obtained from a known standard, it will not be used for comparison. The partial profile will be considered incomplete and therefore not suitable for comparison. Exceptions may be approved on a case-by-case basis by the DNA Technical Leader or designee.

11.2.4.1 Minimal

A minimal profile is a DNA result that is deemed uninterpretable due to less than ten loci with data.

Minimal profiles may also be single source profiles or mixture profiles that exhibit excessive drop-out and numerous alleles below stochastic threshold. The following factors should be considered when determining if the profile should be reported as minimal:

- Amount of template DNA in the amplification.
- Stochastic peak imbalance or allele dropout.
- Degradation due to environmental or chemical processes.
- Preferential amplification due to the presence of inhibitors or other factors.

Minimal profiles are not suitable for comparison.

11.2.4.2 Complex

If the profile is a mixture of at least two individuals without a major contributor, this profile is a complex mixture and therefore not suitable for comparison. Due to the indeterminate number of contributors conditioning on the presence of an expected donor should not be performed.

If the profile is a mixture of at least three individuals this profile is a complex mixture and therefore not suitable for comparison. Major DNA profiles may be determined from mixtures of at least three individuals with approval of the DNA Technical Leader or designee.

11.3 Possible Conclusions

Once the Y-STR results have been evaluated to determine if the profile obtained is suitable for comparison, it will be compared to any submitted reference. Two conclusions of the comparison are generally possible: 1) there is an inclusion or 2) there is an exclusion. Results suitable for comparison will be used for both inclusion and exclusion. A profile will not be used for exclusion only. A consistency statement will be based on only those loci that yield conclusive results; therefore, any inclusions or exclusions will be made using interpretable loci.

Rarely and with approval, an inconclusive conclusion may be possible. It is scientifically acceptable for an inclusion or exclusion to be determined for a case when one or more of the loci yield uninterpretable results.

11.3.1 Inclusions

- The pattern of alleles is consistent between the questioned profile and the known reference profile. This is determined by careful, objective, qualitative and quantitative evaluation of the entire pattern produced by the various loci tested.

- A profile suitable for comparison must have at least ten loci that meet the interpretation requirements. Multi-copy loci should have two alleles present or one allele above the stochastic threshold to count towards the ten.

- Inclusion reporting terminology for complete or partial single source profiles, major profiles, and foreign profiles will use the term consistent and include statistics for probative associations.

11.3.2 Exclusions

The patterns are not consistent between the questioned profile and the known reference profile. This is determined by careful, objective, qualitative and quantitative evaluation of the entire pattern produced by the various loci tested.

11.3.3 Inconclusive Results

Comparisons of a known reference to a questioned sample profile suitable for comparison will typically yield either an exclusionary or inclusionary statement. Comparisons to known references that can neither be included or excluded, will be interpreted as inconclusive; however, this interpretation will be evaluated on case-by-case basis, and will need the approval of the DNA Technical Leader or designee.

11.4 Statistical Calculations

11.4.1 General Considerations

Statistical calculations will be reported for inclusionary associations to an individual other than the conditioned, expected, or individual submitted for elimination.

Uninterpretable or inconclusive profiles are not suitable for comparison and will not be used for statistical calculations.

Profiles that are otherwise complete and have ample DNA present but exhibit the absence of an allele at a locus or loci, may be the product of a primer-binding site mutation that inhibits amplification for that allele. This result is a null allele.

A confidence interval is an interval estimate of a population parameter, applied to account for database size and sampling variation and describe the amount of uncertainty associated with the sample estimate.

The counting method is a means of providing a weight to an association to express the rarity of the haplotype in the population of males; achieved by searching the haplotype against a reference database, counting the number of times it is observed against the total number of profiles searched, and applying a form of confidence interval.

The counting method will be employed to determine the total observances in the applicable version of the Y-STR Y-Mix Cal-DOJ tool. The statistic will be calculated with a 95% upper confidence interval, using the Clopper-Pearson method. The exact count statistic and value with the confidence interval applied are reported as two significant figures without rounding.

Newly calculated statistics will use the https://yhrd.org/ R63 (US with subpopulations) allele frequencies. Previously calculated statistics associated with a forensic profile that are being reported subsequent to an inclusionary statement, will not be re-calculated, but will reference the version of the database used at the time of the calculation.

As per the SWGDAM Y-Chromosome STR Interpretation Guidelines, it is recognized that population substructure exists for Y-STR haplotypes. Studies with current population databases have shown that theta values are very small for most populations. The use of the counting method that incorporates the upper-bound estimate of the count proportion offers an appropriate and conservative

approach to evaluating the probative value of a match. The match probability will not be reported.

For single source searches if a multi-copy locus has an allele call with a "^" designation, the single allele will be put into the statistical tool. Select whether or not you wish to allow for drop-out at duplicated loci (use ST as guidance).

A confirmed null allele may be searched as "0" as the allele designation. Alleles that are missing due to the low-level nature of the profile will be left blank.

For profiles that are suitable for comparison, the loci not suitable for statistics will be recorded in the case file on the Interpretation Worksheet, Allele Table, or the statistical calculations pages during evaluation before comparison to standards.

Loci with duplication will not be used for statistical analysis.

Off ladder alleles may be entered. Off-ladder alleles above or below the ladder or virtual bin will be entered as either <# or ># depending on whether the variant occurs at the larger or smaller end of the ladder alleles (e.g. <15 or >17).

For mixtures, if multiple alleles are to be entered, enter them from smallest to largest. For an inclusion involving an unresolved mixture of two donors, the counting method will be used to determine the number of occurrences of each haplotype in the database that could be included in the mixture. Due to inter-locus imbalance and significant overlap of Y haplotypes, only the loci where there is the unambiguous presence of both contributors will be used in the statistical calculation. A locus where one contributor is lost should not be used for inclusion.

Autosomal and Y-STR haplotype frequencies will not be combined.

11.4.2 Equations

For no observed occurrences of a questioned haplotype, the equation for the upper confidence interval is:
$\leq 1-(0.05(1/N))$

Where N = number of profiles searched

If the haplotype has been observed in the database, the formula used for calculating the 95% confidence limit is that of the Clopper-Pearson exact confidence interval (x=number of matches; n=database size; p=sample proportion):

Clopper-Pearson (exact method)

$$\left\{ \theta \,\middle|\, P\left[\mathrm{Bin}\left(n;\theta\right) \le X\right] \ge \alpha/2 \right\} \bigcap \left\{ \theta \,\middle|\, P\left[\mathrm{Bin}\left(n;\theta\right) \ge X\right] \ge \alpha/2 \right\}$$

$$\sum_{k=0}^{x} \binom{n}{k} p_0^k (1-p_0)^{n-k} = 0.05$$

11.4.3 Using the Y-Mix Cal-DOJ Tool

1. Interpret your evidence to determine the alleles of possible contributors.

2. Using the drop-down menus, enter the interpreted alleles into the table on the "Profile" worksheet. To include haplotypes with "0" alleles, you must manually enter "0" as an allele. A confirmed null allele may be searched as “0” as the allele designation.

For mixtures, if the list of alleles for a locus is interpreted as possibly incomplete (i.e., not representative of all possible contributors), the locus should be left blank.

If an allele in the evidence is not present in the list below the table, enter it as a "New Variant" prior to entering it into the table. Alleles must be present in the Observed Alleles list in order to be searched. Variants may be entered in the New Variant row if necessary prior to comparing the profile to the database. Alleles that are greater than or less than the ladder may be entered in the New Variant row as “>#” or “<#” with no space between the sign and the allele designation.

3. Select to search all profiles that have any of the loci in your evidence Limit database to samples with all the loci entered above = No.

Select for a mixture or single source search.

For single source searches only: Select whether or not you wish to allow for drop-out at duplicated loci (use ST as guidance). The setting for the option to allow for dropped alleles at multicopy loci depends upon the interpretation of the result at DYS385 and DYF387S1. If a single allele is called and dropout of the second allele is possible based on the stochastic threshold, set the option to “Yes.” If the interpretation allows for multiple options of the genotype with a single allele common (obligate) between all options, the single allele that is common to all possible options may be entered and the option set to “Yes.” The resulting matches can then be screened to allow for haplotypes that only contain one of the possible options. For instance, the major haplotype could be interpreted as either an 11,11 or an 11,12 at DYS385. An 11 with dropout may be searched. All matching haplotypes other than 11,11 and 11,12 would be excluded as matches from the database search.

Set the Upper Confidence Interval to 95.0%.

Select to report the observed counts from the databases (x/N). Use (x+1)/(N+1)? = No

4. Click on the button-macro "Compare the profile to the database." This will filter the database, leaving only those haplotypes that would be included as possible contributors to your evidence.

5. Counts of non-excluded haplotypes (x), database sizes (N), sample frequencies (x/N), and 95% UCIs are summarized in the table.

6. If you wish to view the filtered list of non-excluded haplotypes, click on the button-macro "View the filtered list."

7. Yfiler Plus statistics will be reported using the profile probability with 95% UCI and the combined probability across all sub-populations.

11.5 References:

- SWGDAM Interpretation Guidelines for Y-STR typing by forensic DNA testing laboratories, 2014, 2017, 2022 Available at www.swgdam.org

- The Federal Bureau of Investigations Quality Assurance Standards Audit Document for Forensic DNA Testing Laboratories

- Willuweit S., Roewer L. (2015), 'The new Y Chromosome Haplotype Reference Database.', Forensic Sci Int Genet 15, 43-8 [Pubmed] [DOI]

- Ballantyne, K.N., Keerl, V., Wollstein, et. al, (2012) A new future of forensic Y-chromosome analysis: Rapidly mutating Y-STRs for differentiating male relatives and paternal lineages. FSI Genetics 6:208–218.

- CMPD Yfiler Plus Internal Validation

- CMPD Y-mix Tool Performance Checks

12. Report Writing

A. Purpose and Introduction

The purpose of this procedure is to standardize the report formatting for the Biology section by creating a standard format for reporting segments.

B. Materials

- Report template
- Case File

C. Safety Considerations

- Not Applicable

D. Limitations

- This protocol contains the appropriate reporting language for many of the scenarios that will be encountered in casework. There may be circumstances where reporting language not contained in this document is warranted. This document is designed to provide general guidelines for proper reporting considering the strengths and limitations of the analysis; modifications may be required.

E. Other Considerations

- The following report wording guidelines are designed to establish consistency in reporting results. Guidelines will provide uniformity in reporting and assist readers in understanding the reports while still providing the necessary scientifically valid information.

  The report has separate sections including Items Received and/or Items Previously Reported, Swabbing and Serology Results and Conclusions, Extraction, Quantitation Results and Conclusions, Methodology, DNA STR Results and Conclusions, Comments, and Appendix. Not all sections are necessary and will be based on the testing performed.

General Guidance:

- “Items Received” –refers to items which the analyst has newly received into their custody for this report. This includes evidence items received by the analyst even if no testing was performed. This could also include DNA extracts (use the parent Item #).

- “Items Previously Reported” –refers to evidence items previously reported with profiles being used for comparison (Qs and/or Ks) and not taken into the

analyst's custody for this report. This could include questioned samples that were previously developed that are suitable for comparison, knowns that were used for conditioning, knowns previously run and are now being compared to newly developed questioned sample profiles, etc. This will follow "Items Received".

- When the previously reported profile was generated by a vendor laboratory, it must be clear in the report that the results were originally generated by another laboratory by adding "Items Previously Reported by [Vendor Lab Name]"

- It is possible that you could have the same item number listed in both "Items Received" and "Items Previously Reported" sections if you are using previously obtained data and also doing new testing on the same item.

- Listed descriptions of the items received will be primarily derived from the PLIMS Item Description. Modifications and alterations may be made to truncate the length of the description or to adhere to reporting guidelines but should not obscure the identity of the item of evidence. Additional descriptors such as "A" and "B" added by the analyst during testing will be addressed in the relevant "Results" section of the report and will not be reflected in the "Items Received" section of the report.

- In the Results section of the report, the initial description of an item of evidence will be a written description of the item and not just an Item number. If the item of evidence is uniquely identifiable and distinguishable, the initial item descriptions can be simplified from the PLIMS descriptions used in the Items Received list. The Item number may be used in the statistical calculation portion of the report instead of the description.

- DNA extract containers are not listed under the items received. If DNA extract(s) are taken into the custody of the analyst for additional testing, only the item description of the DNA extract on which the testing is performed, is listed.

- A report does not have to be generated if the assigned analyst takes the only assigned Item into their personal custody and does not perform any testing for that assignment due to the assignment being cancelled prior to work beginning.

- Items of evidence received but not opened or examined will have notes made regarding packaging and labeling, will be listed under the Items Received, and reported in the Swabbing and Serology Results and Conclusions or the Extraction section of the report as "The following Item(s) were not examined:"

Swabbing/Screening/Serological testing:

- Collected indicates it was removed from the item or the original envelope and placed in a coin envelope for preservation.

- Possible trace material (appearance of glass, fiber, paint or soil) that is specifically noted in an analyst's notes will be included in the report.

- Observation of additional possible serological stains will be included in the report.

- Locations on an evidence item that are swabbed together as one collection may be reported as each location separated by a slash "/" and do not have to be explicitly stated as being swabbed together.

- Stains that test presumptively positive will be individually described.

- If you test one stain on an item and the result is negative, then the location of the stain tested should be reported.

- If you test multiple stains on an item that are all negative, then it is not necessary to report the specific locations of the stains, and the item will be reported collectively as negative. Exceptions to this would be if the service request for a particular evidence Item specifies a location for testing.

- If you test multiple stains on an item and there are both positive and negative results, the locations of the positive stains will be individually described; however, the negative results may be collectively reported as negative.

- Presumptive serological tests of swabs that are packaged together as a single item and collected from the same location will be individually tested and reported separately if the tests of each swab are different.

- The order of serology reporting will be positive serology, swabbing collections, and then negative serology.

Hairs:

- Possible Hair: A term assigned to a trace item collected by an analyst that appears to have the macroscopic characteristics of a hair.

- Apparent Hair: A term assigned to a possible hair that has been microscopically determined to be a hair.

- When a hair extraction is performed, both root and shaft will be reported. If the length of the hair does not allow for the extraction of a shaft, then the results will be reported as "apparent hair".

- Hair standards used as the known standard for comparison that have the same result for both the root and shaft portions of the hair will be reported solely as the hair standard.

- For reporting of root and shaft portions of the hair, if the results are identical, the results may be combined into one sentence.

Reporting-Quantitation results:

- All questioned samples quantitated will have total human and male quantitation results and conclusions conveyed in the report.

- Reagent blank(s) and known standard(s) quantitation results and conclusions are not reported. Documentation of any possible contamination or non-conformance will be in the case file.

Reporting-Observation of male:

- When an unknown single source or partial single source profile contains male marker results at Amelogenin, DYS391, or Yindel, the profile will be reported as male.

- When an unknown single source or partial single source profile contains no male marker results at Amelogenin, DYS391, or Yindel, the profile will be reported as female. The profile will be carefully evaluated to ensure the absence of male results is not due to dropout.

- When a mixture DNA profile contains male marker results at Amelogenin, DYS391, or Yindel, the mixture profile will be reported as containing at least one male contributor unless conditioned on a male individual.

- If male marker results are present at Amelogenin, DYS391, or Y indel in a profile that is not being used for comparison, the profile will be reported as male/having a least one male contributor.

Reporting-Differentials

- Carryover- The differential extraction is a series of steps to separate sperm cell DNA from DNA from other sources, like epithelial cells. The separation may not be completely efficient and may result in carryover. Carryover is a phenomenon observed in differentially extracted samples where epithelial cell/non-sperm cell DNA carries over into the sperm cell fraction or sperm cell DNA carries over into the non-sperm cell fraction resulting in a mixed DNA profile.

- The non-sperm/sperm cell fractions obtained from a differential extraction may be manually assessed to determine if carryover is present. Carryover may be observed in differentially extracted samples when non-sperm cell DNA carries over into the sperm cell fraction or sperm cell DNA carries over into the non-sperm cell fraction. The fractions do not have to be single source to assess carryover. The analyst can evaluate whether the primary contributor(s) is/are carrying over into the alternate fraction. With careful examination of the profile, carryover can be determined regardless of the number of alleles that are observed. This will occur most frequently in samples where two contributors are present, but there may be instances where carryover can be determined in samples with more than two contributors. Documentation that carryover was observed will be noted in the case file.

- Profiles obtained from differential extractions may be manually assessed to determine if the profile is non-probative (e.g. non-sperm cell fraction from expected donor's vaginal swabs matches expected donor) or if components of the profiles are non-probative due to carryover (e.g. sperm cell fraction is primarily male with carryover from non-sperm cell fraction). Non-probative profiles and non-probative components of profiles indicating carryover do not need to be interpreted using STRmix and LR calculations will not be performed. This means that profiles obtained from the non-sperm cell fraction that are a mixture of the expected donor and carryover from the same male obtained from the sperm cell fraction do not need STRmix interpretation or LRs performed on the entirety of the profile. Profiles obtained from the sperm cell fraction that are a mixture of male and carryover from the non-sperm cell fraction do not need the carryover portion interpreted in STRmix or LR's performed.

- If each fraction has ambiguous results that demonstrate the same contributors (e.g. the only additional alleles in either fraction are attributable to carryover from the alternate fraction), the fraction where the probative contributor is better represented may be used for interpretation. The analyst will also consider case information and context when determining which fraction to interpret.

- Due to case circumstances or case data, there may be instances when both fractions of a differential extraction need to be analyzed in STRMix™. Some examples include multiple suspects, type of assault, and ambiguous results. The analyst may reference the report wording in the differential extraction section and replace sperm cell fraction with non-sperm cell fraction where appropriate.

Reporting – DNA Results – General

- All buccal swabs, regardless of type, will be reported as "buccal standard".

- Buccal swabs collected from individuals whose identity needs to remain anonymous (example: employee from a staff match or undercover officer) will be reported as being collected from a CMPD employee. The individual's name will not be reported.

- When reporting the results for an item of evidence there will be a written description of the item and not just an Item number in the body of the DNA results. These descriptions can be truncated from the lengthier descriptions from PLIMS.

- If a portion(s) of the quant results table is not used in reporting, then delete it from the document.

- If there are no swabbing collections or serology tests performed, that portion of the report is deleted.

- If an Item is not examined, list it under the Extraction header.

- If a partial profile is obtained from a reference, it will not be used for comparison. Exceptions may be approved on a case-by-case basis by the DNA Technical Leader or designee.

- Name spellings for reporting purposes will be obtained from the PLIMS item description or from the medical form sticker associated with a SAK. Minor spelling differences found elsewhere in the case documentation do not need to be resolved.

- All known references submitted in a case will be compared to every questioned profile (or portion of) that is suitable for comparison. This is required across subsequent submissions.

- Items submitted and/or run as alternate standards will be used for comparison, conditioning, and/or statistics as applicable if a suitable profile is generated. Subsequent submission of a known reference from the same individual will require a comparison and consistency statement to the alternate standard profile; however, the comparison to forensic samples may not require a regeneration of statistics and will be evaluated on a case-by-case basis in conjunction with the DNA TL or designee.

- An ignored locus does have to be listed in the report. Partial single source profiles with full dropout at a locus/loci, do not have to have loci listed, just reported as partial.

- For comparison reports between a previously run buccal standard and previously generated forensic profiles, there is no disposition statement required since no laboratory work was performed.

Reporting – STRmix Interpretation Results – General

- Conclusion statements should include the following components when applicable:
    - A statement with number of contributors and biological gender statement
    - Any assumptions used in interpretation
    - Summary of components suitable and not suitable for comparison
    - Comparison statement(s) to all applicable reference(s)
    - Propositions and statistics for inclusionary statements

- Profiles being interpreted as single source that do not have reference profiles to compare against will be analyzed with the STRmix software.

- The report wording will state when a sample is conditioned on the presence of the conditioned contributor. The conditioned contributor does not have to be listed in the segment of the report that lists the contributors that are suitable and not suitable for comparison.

- A conditioned contributor is a contributor whose presence is expected on an item and is applied in the STRmix software in both the H1 hypothesis and H2 hypothesis. Once the analyst determines the reference can be considered as a reasonably expected contributor, the analyst will then manually compare the reference to the forensic profile. If their presence is established and the sample is a mixture, the analyst will perform the interpretation conditioned on the reference. If it is ambiguous, the analyst will perform an investigative LR. No LR will be reported for conditioned contributors. The use of conditioning will be stated in the report.

- If a case has multiple, complete single source profiles with identical results (for example, identical genotypes at all locations with a weight of one) associated to a reference, then the results statements may be combined in the report with a “consistent with” statement. Some small variability is inherent to the HPD calculation used by STRmix but run-to-run variation for single source profiles with

a weight of one at all loci, is minimal. A single HPD calculation will be performed and reported for applicable samples.

- Likelihood ratio values that support an inclusionary conclusion will be stated in the report. The most conservative value obtained from the 99% 1-sided lower HPD interval LR will be used. The reported LR value will be shortened to two significant figures with no rounding.

- An LR is not required to report an inclusion determined to be non-probative based on the context of the case, or where the presence of an individual's DNA is reasonably expected. Additionally, likelihood ratios are not reported for individuals used as conditioned contributors.

- Once a conclusion has been determined and reported between a reference and an item of evidence, it does not need to be restated in subsequent reports. Additionally, if a profile has been established as not suitable for comparison/containing no DNA, this does not need to be restated in subsequent reports. Exceptions to this may be permitted based on case circumstance.

- Ignoring a locus from an evidence/reference profile during STRmix interpretation/comparison does not make the profile partial.

Reporting – Previously run data

- Under the Extraction portion of the report list "The following Item(s) were previously extracted:" – listing here the item numbers of the DNA extracts brought into custody to do additional lab work – this could include YSTR, re-amp, re-quant. If you are just using previously reported data, do not list your evidence here, this is for newly received DNA extracts into your custody for lab work.

- For reports that are evaluating previously obtained data for re-interpretation with STRmix and no lab work is performed use:
  - Items Previously Reported
  - DNA STR Results and Conclusion with normal STRmix statement
  - Followed by "The following Item(s) were previously analyzed and reported on [date] and re-interpreted using STRmix if applicable: [list Item #s]
  - Under Item result: "The DNA profile previously obtained from [X] was interpreted as a mixture of…"

- For reports where lab work is performed (re-amplifcation, re-extraction, etc.), depending on the scenario, and STRmix interpretation completed on previously reported samples:
    - Items Received
    - Include Extraction, Quantitation, Methodology as applicable
    - Item results as routine

- For reports that have both lab work and profile evaluation for STRmix, apply the above where applicable.

Reporting - Legacy Data:

- If the mixture can be based on an assumed number of contributors, it will be reported as being 'consistent with' that number. When used with a mixture, the wording “consistent with” conveys a discrete number.

- If the number of contributors cannot be reliably determined, then the mixture will be reported as 'at least' a minimum number based on the assessment. Typically, the ‘at least’ number will be based on the greatest number of called alleles at any locus, but other factors may be used. Major profiles may be determined from these mixtures; however, no interpretation/ comparison will be performed on the minor profile(s). The term “at least’ conveys an indeterminate number of contributors.

- If a discrete number of contributors cannot be determined, but the data supports more than four individuals being present, then the mixture will be reported as “greater than four contributors.”

- The most common frequency from the population sub-groups used in the calculations will be reported.

- In a DNA analysis evaluation, three conclusions are generally possible: 1) there is an inclusion, 2) there is an exclusion, or 3) the results are unsuitable for comparison.

- Interpreted results will be used for both inclusion and exclusion. A profile will not be used for exclusion only.

- Source attribution will not be reported.

- The most conservative RMP for the profile will be reported as two significant figures with no rounding.

- The reported statistics are an estimation for which a deviation of approximately +/- 10-fold may exist.

Y-STR testing

- For items linked to a sexual assault case investigation, Y-STR testing will be recommended by the reporting analyst on qualifying samples.

- Y-STR testing may be recommended and performed in other case types but require pre-approval from the DNA Technical Leader or designee.

- Refer to Biology SOP 3.5.3 and Biology QA 18.2 for further guidance

- Submission of additional references
    - If there is a listed suspect on the service request and a buccal standard has not been collected from that individual, the buccal standard will be requested for comparison in the report.

12.1 Report Wording – Swabbing

Report Wording – Swabbing	
Scenario	**Wording**
No serology performed -one area swabbed	The [area of the item] was swabbed for DNA analysis. The swabs were collected as Item [X].
No serology performed -multiple areas swabbed	Areas of the [Item description] were swabbed separately for DNA analysis. Swabs of [location 1] (Item X.1), [location 2] (Item X.2), [location 3] (Item X.3), [location 4] (Item X.4) and the [location 5] (Item X.5) were collected.
No serology performed- Multiple items listed as the same Item number -Multiple areas swabbed	The [Item description with all items a, b, c etc.] were swabbed separately for DNA analysis. Swabs of [location 1] (Item X.1), [location 2] (Item X.2), [location 3] (Item X.3), [location 4] (Item X.4) of [Item a] and the [location 5] (Item X.5) of [Item b description] were collected.
No serology performed -inventory/items not examined	Areas of the [Item description] were swabbed separately for DNA analysis. Swabs of [location 1] (Item X.1), [location 2] (Item X.2), [location 3] (Item X.3), [location 4] (Item X.4) and the [location 5] (Item X.5) were collected. The interior contents were not examined.
No Serology performed -apparent hairs (micro performed)	An apparent hair that may be suitable for DNA analysis was observed on the [Item description].
No serology performed -item is not suitable	This item was not suitable for DNA analysis; therefore, no examination was performed.

No serology performed -no further analysis per XX	No (further) analysis was needed per instruction from XX.
No serology performed -M-Vac	Utilizing the M-Vac, sample collection for DNA analysis was performed on the [Item description]. The filter was collected as [X.1].
Apparent hair(s) (micro performed) – nuclear DNA suitable	An apparent hair(s) that may be suitable for nuclear DNA analysis was observed on the [Item description] and collected as Item X.1. [No further examination was performed at this time.]
Apparent hair(s)/fragment (micro performed) – not nuclear DNA suitable	An apparent hair(s) [or hair fragment] that may not be suitable for nuclear DNA analysis was observed on the [Item description] and collected as Item X.1. [No further examination was performed at this time.]
Possible hair(s) – no micro performed	A possible hair(s) was observed on the [Item description] and returned to the original container or sealed manila envelope without examination.
No possible or apparent hairs observed – use when specifically required to exam evidence for the presence of possible or apparent hairs	No [possible] [apparent] hairs were observed on the [item].
Possible trace material	Loose possible trace material was observed on [*item*] and was returned to the original container or sealed manila envelope without examination.

12.2 Report Wording - Serology

Report Wording – Serology	
Scenario	**Wording**
Testing stopped after serology	No further testing was conducted on this item.
No staining observed	No stains of serological value were observed on the [item].
RBS observed not tested	An apparent red brown stain was observed but not tested at this time.
Additional RBS observed/not tested	Additional red brown stains were observed but not tested at this time.
Additional areas of possible serological stains observed but not tested (ALS)	Additional possible serological staining was observed, but not tested.
Presumptive positive tests (KM, LMG, AP, Phadebas $\geq$1+)	Presumptive testing for the presence of [blood, semen or saliva] was positive on [item].
Presumptive negative tests (KM, LMG, AP, Phadebas)	Presumptive testing for the presence of [blood, semen or saliva] was negative on [item].

Presumptive test Phadebas <1+	Due to the nature of the test, no conclusion can be made concerning the presumptive testing for saliva on [item].
Presumptive positive for blood (KM or LMG) and confirmatory positive for blood (Hematrace)	Blood of probable human origin was detected on [item].
Presumptive positive for blood (KM or LMG) and confirmatory negative for blood (Hematrace)	Blood of non-human origin was presumptively detected on [item].
Semen presumptive testing positive (AP) with confirmatory positive (micro +)	Semen was detected on [item].
Semen presumptive testing positive (AP) with confirmatory positive (micro - and p30 +)	Semen was indicated on [item].
Semen presumptive testing positive (AP) with confirmatory negative (micro – and p30 –)	Presumptive testing for the presence of [semen] was positive on [item]; however, semen was not detected.
Semen presumptive testing negative (AP) with confirmatory negative (micro -)	Semen was not detected on [item].
Microscopic examination of slide prepared during differential extraction positive (micro +)	Semen was detected on the [Item description] slides prepared during differential extraction.
Microscopic examination of slide prepared during differential extraction negative (micro –)	Semen was not detected on the [Item description] slides prepared during differential extraction.
Serology performed/POSITIVE -Stain collection and multiple no serology areas swabbed	Presumptive testing for the presence of [blood, semen or saliva] was positive on the stain on the [location] of the [Item description]. [Cutting or Swabs] of [a portion of or the entire] stain (Item X.1) were collected. Additional areas of the [Item description] were swabbed separately for DNA analysis. Swabs of [location 1] (Item X.1), [location 2] (Item X.2), [location 3] (Item X.3), [location 4] (Item X.4) and the [location 5] (Item X.5) were collected.

Serology performed/POSITIVE - Additional staining observed but not tested at this time. -Stain collection and multiple no serology areas swabbed	Presumptive testing for the presence of [blood, semen or saliva] was positive on the stain on the [location] of the [Item description]. [Cutting or Swabs] of [a portion of or the entire] stain (Item X.1) were collected. Additional areas of the [Item description] were swabbed separately for DNA analysis. Swabs of [location 1] (Item X.1), [location 2] (Item X.2), [location 3] (Item X.3), [location 4] (Item X.4) and the [location 5] (Item X.5) were collected. Additional red brown stains were observed but not tested at this time.
Serology performed/POSITIVE & NEGATIVE -Stain collection and multiple no serology areas swabbed	Presumptive testing for the presence of blood was positive on [number] stains [labeled A, B and C – add if applicable] on the [location] of the [Item description]. [Cuttings or Swabs] of [a portion of or the entire stain] [labeled A] (Item X.1), [a portion of or the entire stain] [labeled B] (Item X.2) and [a portion of or the entire stain] [labeled C] (Item X.3) were collected. Additional areas of the [Item description] were swabbed separately for DNA analysis. Swabs of [location 1] (Item X.1), [location 2] (Item X.2), [location 3] (Item X.3), [location 4] (Item X.4) and the [location 5] (Item X.5) were collected. Presumptive testing for the presence of blood was negative on [number] stains [labeled D and E – add if applicable] on the [location] of the [Item description].
Serology performed -stain collection with swabs on non-visibly stained area and swabs have observed RBS after collection	No stains of serological value were observed on [Item description]. Areas of the [Item description] were swabbed separately for DNA analysis. Swabs of [location 1] (Item X.1), [location 2] (Item X.2), [location 3] (Item X.3), [location 4] (Item X.4) and the [location 5] (Item X.5) were collected. Presumptive testing for the presence of blood was positive on the swabs of [location] (Item X.X). [reported when press-out or swab-to-swab test after collection – no KM testing of collected swabs]
Blood card (standard) is prepared from a blood alcohol collection tube [FTA CARD] Item received: Blood collection kit from [Name]	This item contained [number] test tubes with apparent blood. A blood stain card (blood standard) from [Name] was prepared and collected as Item [X.1].
Collecting a blood standard from liquid blood (tubes)	Swabs were collected from the blood standard from [Name] and collected as Item [X.1].
Presumptive positive blood testing prior to sending for outsourcing	Presumptive testing for the presence of blood was positive on Item XX. The swabs were repackaged as Item X.1 and retained in the laboratory.

Report Wording – Serology – Comments and Dispositions	
Scenario	**Wording**
Laboratory Activity	Laboratory activity was performed between [DATE] and the report date.
DNA request	A DNA service request has been entered.

No DNA - for possession cases	Please contact an ADA for DNA testing.
Requesting a standard	Please submit a buccal standard from [Name] for comparison purposes.
Photos in Evidence.com [Packaging for selected items listed]	Items X, Y and the packaging for Items X, Y, and Z were photographed. All photographs are contained in the attached case file in PLIMS. Copies of all photographs were saved to Evidence.com.
Photos in Evidence.com [Packaging for all items listed]	Items X, Y and the packaging for these items were photographed. All photographs are contained in the attached case file in PLIMS. Copies of all photographs were saved to Evidence.com.
Collected items retained to GBS	The collected items will be maintained in the laboratory and transferred to General Biology Storage.
Collected items (and/or submitted items) returned to PC	The submitted items (and/or the collected items) were transferred to the General Biology Cage to be returned to Property Control following examination.
Submitted items transferred to another section/unit	The submitted items were transferred to the [Latent Print Section, Firearm Section, Cyber Crime Unit] following examination.
Submitted items transferred to Crime Lab Evidence Locker #1 or #2	The submitted items were transferred to the Crime Lab Evidence Locker [1 or 2] following examination.
Listed items retained in lab for outsourcing	Items listed as retained in the laboratory were transferred to General Biology Storage to be sent to an outsourcing laboratory for DNA analysis which will be the subject of another report. All other items were transferred to the General Biology Cage to be returned to Property Control.

12.3 Report Wording – DNA General

Report Wording – DNA - General	
	Wording
DNA Methodology	DNA was amplified by the STR polymerase chain reaction (PCR) using the GlobalFiler Kit. [or the AmpFlSTR Identifiler Kit, the Power Plex 16 Kit, the AmpFlSTR Profiler Plus and Cofiler Kits].
STRmix methodology	STRmix software was used to aid in the interpretation of DNA profiles, and when appropriate, to perform statistical calculations.
Previously obtained data – reinterpreted with STRmix	The DNA profile previously obtained from X was re-interpreted as….
Extraction	The following Item(s) were [previously] [differentially] extracted [and consumed]: The following Item(s) were not examined:

	The following Item(s) were inventoried and cuttings were taken; however, no DNA testing was performed:

Results Section- Report Wording- Reference Specific	
Scenario	**Wording**
Buccal Standard	A DNA profile was obtained from the buccal standard from [*Reference*].
Blood Standard	A DNA profile was obtained from the blood standard from [*Reference*].
Blood standard (swabs created from liquid blood tube)	A DNA profile was obtained from a sample taken from the blood standard from [*Reference*]. – check to see if this contradicts with serology wording
Partial profile	A partial DNA profile was obtained from the [*blood*] [*buccal*] standard from [*Reference*] that is not suitable for comparison.
Standard submitted under different Complaint # than reported	A DNA profile was obtained from the [*blood*] [*buccal*] standard from [*Reference*], which was submitted under Complaint # [XX]
Previously run standard	A DNA profile was previously obtained from the [*blood*] [*buccal*] standard from [*Reference*], [*which was analyzed and reported under Complaint* # [XX]].
Previously run standard from an outsourcing lab	A DNA profile was previously obtained from the [*blood*] [*buccal*] standard from [*Reference*] which was analyzed and reported by [Lab Name].

Results Section- Report Wording- Alternate Standard Specific	
Scenario	**Wording**
Alternate Reference Samples	A DNA profile was obtained from [*Item*] that will be used as the alternate known standard for comparison purposes; the use of this DNA profile in making the following comparisons assumes that [*Reference*] is the source of the DNA profile obtained from [*Item*].
If not using as an alternate standard due to testing results (Quant)	Due to *insufficient/no human* DNA detected at quantitation for PCR analysis, the [*Item*] was not suitable to be used as an alternate known standard from [*Reference*].
If not using as an alternate standard due to testing results (Mixture)	Due to a mixture of DNA profiles being obtained, the [*Item*] was not suitable to be used as an alternate known standard from [*Reference*].

Results Section – Report Wording - Discharged Cartridge Casing Specific	
Scenario	**Wording**
DCC swabbing, no serology	*The/each* cartridge case was swabbed separately for DNA.

DNA extracts	NOTE: Report template includes consumption, combining, new Item # for DCCs under Extraction.

Report Wording - Direct to DNA Specific	
Scenario	**Wording**
Items Received for SAK	Item X: Sexual assault evidence collection kit from [NAME] including:
Slide(s) made – Direct to DNA (Combine for all slides and report once under methodology statement)	A slide (Item [X]) was prepared for [*samples, ex. the vaginal swabs, the anal swabs, etc*.], placed into a sealed manila envelope, and returned to the original container without examination.

Report Wording - M-Vac Specific	
Scenario	**Wording**
Collection with the M-Vac	Utilizing the M-Vac, sample collection for DNA analysis was performed on the *[area]* of the [*Item*]. The filter was labeled Item [X.1].

Results Section- Report Wording	
Item not processed	
Scenario	**Wording**
If item received, but not processed	This item was not examined.
If work is stopped per ADA or Detective	No (further) analysis was needed per instruction from [*name*].
Item determined to be not suitable for DNA analysis	This item was not suitable for analysis; therefore, no examination was performed.
Item cut by screener, but no DNA testing by DNA analyst	This item was inventoried, and cuttings were taken; however, no DNA testing was performed.

12.4 Report Wording – DNA Quantitation (table in report)

Results Section- Report Wording	
Quantitation Results	
Scenario	**Wording**
Human >1 – Male >1 *This does not include NSF with >1:60 ratio that are amp'd because the SF is amp'd*	Human and male DNA was detected.

Human >1 – Male ≤1	Human DNA was detected; however, the results are inconclusive for the presence of male DNA.
Human >1 – Male = 0	Human DNA was detected; no male DNA was detected.
Human ≤1 – Male >1	Human male DNA was detected.
Human ≤1 – Male ≤1	Human and male quantitation signals were obtained; however, the results are inconclusive for the presence of DNA.
Human ≤1 – Male = 0	Human quantitation signal was obtained; however, the results are inconclusive for the presence of DNA.
Human = 0 – Male >1	Human male DNA was detected.
Human = 0 – Male ≤1	Male quantitation signal was obtained; however, the results are inconclusive for the presence of DNA.
Human = 0 – Male = 0	No human or male DNA was detected.
Human and male DNA detected but male DNA at a ratio >1:60 *This includes non-sperm cell fractions even if amplified.*	Human and male DNA was detected; however, the ratio of male to female DNA is insufficient
>0.001pg of male signal and >1:60 ratio observed in a Female standard	Female reference- Low level male signal detected
>0.001pg of male signal and <1:60 ratio observed in a Female standard	Human and male DNA detected.
Listing all samples dropped at quantitation based on either total human/male or male/total human ratio	The following samples were stopped at quantitation: [list Item #s].
Human and male DNA detected in Direct to DNA samples but samples not amplified due to other sample(s) chosen for amp. This refers only to samples that would not have been dropped based on other criteria.	Human and male DNA detected; however, no further processing at this time.
Listing buccal standard dropped at quantitation due to all Q's dropped	All associated questioned samples were stopped at quantitation; therefore, the following buccal standard(s) were not amplified: [list Item #s]

Recommending YSTR testing Refer to Biology SOP 3.5.3 and QA 18	The following samples may be suitable for YSTR testing: [list Item #s].

12.5 Report Wording – STRmix – Not Suitable for Comparison

STRmix Report Wording – Not suitable for Comparison	
Scenario	**Wording**
No DNA profile obtained	No DNA profile was obtained from [*Item*].
Data at less than seven loci Single Source	The [male, female] DNA profile obtained from [Item] is not suitable for comparison due to the quality of the profile. [This profile contains at least one male contributor. – *use when single source is very low-level and ambiguous – report as at least one male contributor*]
Data at less than seven loci-Mixture	The DNA mixture obtained from [Item] is not suitable for comparison due to the quality of the profile. [This mixture contains at least one male contributor.]
Partial single source profile with at least seven loci with data, but less than four complete genotypes	The partial [male, female] DNA profile obtained from [Item] is not suitable for comparison due to the quality of the profile.
Number of contributors is greater than four	The DNA profile obtained from [Item] is a mixture greater than four contributors and is not suitable for comparison due to the quality of the profile. [This mixture contains at least one male contributor.]
Mixture profile, all alleles below stochastic threshold and does not meet number of alleles requirement	The DNA mixture obtained from [Item] is not suitable for comparison due to the quality of the profile. [This mixture contains at least one male contributor.]
Related individuals	The total number of contributors cannot be determined from the mixture obtained from [*Item*] due to the high level of relatedness between multiple potential contributors. [This mixture contains at least one male contributor.]
Mixture may contain related individuals-not all references submitted	The total number of contributors cannot be determined from the mixture obtained from [Item]. [This mixture contains at least one male contributor.] Please submit buccal standard(s) from [Reference(s)]. Additional interpretation of this mixture may be possible once the requested standards are received and analyzed.
STRmix deconvolution performed, interpretation results not suitable for reporting	The DNA profile obtained from [Item] was not suitable for STRmix analysis. [This mixture contains at least one male contributor.]

12.6 Report Wording – STRmix – Single Source

STRmix Report Wording – Single Source Profiles	
Scenario	**Wording**
Single source profile, no reference(s)	The [male, female] DNA profile obtained from [Item] was interpreted as a single source profile.

Partial single source profile, no reference(s)	The partial [male, female] DNA profile obtained from [Item] was interpreted as a single source profile.
Single source profile/Partial single source profile with one additional allele below drop-in, no reference(s)	The [partial], [male, female] DNA profile obtained from [Item] was interpreted as a single source profile; however, one additional allele was detected from which no conclusions can be made.
Single source profile/Partial single source profile with one additional allele at male marker – run in STRmix as NOC=1	The [partial], [male, female] DNA profile obtained from [Item] was interpreted as a single source profile; however, one additional allele at a male marker was detected from which no conclusions can be made. [Expected Donor Reference] is consistent with the DNA profile obtained from [Item]; however, one additional allele at a male marker was detected from which no conclusions can be made.
Single source profile/Partial single source profile consistent with expected donor with indication of mixture below AT (above LAT)	[Expected Donor Reference] is consistent with the DNA profile obtained from [Item]; however, there is an indication of an additional contributor(s) that is not suitable for comparison.
Single source or partial single source, consistent with expected donor (no STRmix interpretation/LRs)	[*Expected Donor Reference*] is consistent with the DNA profile obtained [*Item*]. [*Expected Donor Reference*] is consistent with the partial DNA profile obtained from [*Item*].
Single source profile, reference(s) submitted	The [male, female] DNA profile obtained from [Item] was interpreted as a single source profile. [This statement can be omitted if the single source profile is consistent with the Expected Donor – use the Expected Donor statement] **<u>Expected Donor</u>** • [*Expected Donor Reference*] is consistent with this DNA profile. [No STRmix interpretation or LRs performed]. **<u>Support for Inclusion</u>** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] Hypothesis 2 (H2): The evidence originated from an unknown, unrelated individual The [*male, female*] DNA profile obtained from [*Item*] is approximately [*LR value*] times more likely if it originated from [*Reference*] than if it originated from an unknown, unrelated individual. • There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **<u>Support for Exclusion</u>** • Given the evidence, the following propositions were considered for the statistical calculation.

	Hypothesis 1 (H1): The evidence originated from [*Reference*] Hypothesis 2 (H2): The evidence originated from an unknown, unrelated individual The LR result provides support for an exclusion. [This statement may be applicable if a manual exclusion cannot be made.] **Exclusion** (LR= 0 or manual exclusion) • [*Reference(s)*] *is/are* excluded from this DNA profile. **Inconclusive** • No conclusions can be made regarding [*Reference*] as a contributor to this DNA profile due to a likelihood ratio result in the inconclusive range. [This statement may be applicable if a manual exclusion cannot be made.]
Partial single source profile, reference(s) submitted	The partial [*male, female*] DNA profile obtained from [*Item*] was interpreted as a single source profile. [This statement can be omitted if the single source profile is consistent with the Expected Donor – use the Expected Donor statement] **Expected Donor** • [*Expected Donor Reference*] is consistent with this partial DNA profile. [No STRmix interpretation or LRs performed]. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] Hypothesis 2 (H2): The evidence originated from an unknown, unrelated individual The partial [*male, female*] DNA profile obtained from [*Item*] is approximately [*LR value*] times more likely if it originated from [*Reference*] than if it originated from an unknown, unrelated individual. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] Hypothesis 2 (H2): The evidence originated from an unknown, unrelated individual The LR result provides support for an exclusion. [This statement may be applicable if a manual exclusion cannot be made.] **Exclusion** (LR= 0 or manual exclusion) • [*Reference(s)*] *is/are* excluded from this partial DNA profile.

	Inconclusive • No conclusions can be made regarding [*Reference*] as a contributor to this partial DNA profile due to a likelihood ratio result in the inconclusive range. [This statement may be applicable if a manual exclusion cannot be made.]
Single source profile/Partial single source profile with one additional allele below drop-in, reference(s) submitted	The [*partial*], [*male, female*] DNA profile obtained from [*Item*] was interpreted as a single source profile; however, one additional allele was detected from which no conclusions can be made. [This statement can be omitted if the single source profile is consistent with the Expected Donor – use the Expected Donor statement] **Expected Donor** • [*Expected Donor Reference*] is consistent with this [*partial*] DNA profile. One additional allele was detected from which no conclusions can be made. [No STRmix interpretation or LRs performed]. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] Hypothesis 2 (H2): The evidence originated from an unknown, unrelated individual The [*partial*], [*male, female*] DNA profile obtained from [*Item*] is approximately[*LR value*] times more likely if it originated from [*Reference*] than if it originated from an unknown, unrelated individual. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] Hypothesis 2 (H2): The evidence originated from an unknown, unrelated individual The LR result provides support for an exclusion. [This statement may be applicable if a manual exclusion cannot be made.] **Exclusion** (LR= 0 or manual exclusion) • [*Reference(s)*] *is/are* excluded from this [*partial*] DNA profile. **Inconclusive** • No conclusions can be made regarding [*Reference*] as a contributor to this [*partia*l] DNA profile due to a likelihood ratio result in the inconclusive range. [This statement may be applicable if a manual exclusion cannot be made.]

12.7 Report Wording – STRmix – Mixture Profiles

STRmix Report Wording – Mixture Profiles	
Scenario	**Wording**
Mixture deconvolution performed, no reference(s), all contributors suitable for comparison	The DNA profile obtained from [*Item*] was interpreted as a mixture of [*analyst noc*] contributors. [*This mixture contains at least one male contributor*.] • All contributors to this mixture are suitable for comparison.
Mixture deconvolution performed, no reference(s), one or more contributors not suitable for comparison	The DNA profile obtained from [*Item*] was interpreted as a mixture of [*analyst noc*] contributors. [*This mixture contains at least one male contributor*.] • Contributor(s) *[list contributor number(s)*] is/are suitable for comparison. Due to limited information, contributor(s) [*list contributor number(s)*] is/are not suitable for comparison.
Mixture deconvolution performed, reference(s) submitted, all contributors suitable for comparison	The DNA profile obtained from [*Item*] was interpreted as a mixture of [*analyst noc*] contributors. [*This mixture contains at least one male contributor*.] • All contributors to this mixture are suitable for comparison. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] and *[Subtract one from analyst noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*analyst noc*] unknown, unrelated individuals The DNA profile obtained from [*Item*] is approximately [*LR value*] times more likely if it originated from [*Reference*] and *[Subtract one from analyst noc]* unknown, unrelated individuals than if it originated from [*analyst noc*] unknown, unrelated individuals. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] and *[Subtract one from analyst noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*analyst noc*] unknown, unrelated individuals The LR result provides support for an exclusion. **Exclusion** (LR= 0) • [*Reference(s)*] *is/are* excluded from this mixture.

	Inconclusive • No conclusions can be made regarding [*Reference*] as a contributor to this mixture due to a likelihood ratio result in the inconclusive range.
Mixture deconvolution performed, reference(s) submitted, one or more contributors not suitable for comparison	The DNA profile obtained from [*Item*] was interpreted as a mixture of [*analyst noc*] contributors. [*This mixture contains at least one male contributor*.] • Contributor(s) *[list contributor number(s)*] is/are suitable for comparison. Due to limited information, contributor(s) [*list contributor number(s)*] are not suitable for comparison. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] and *[Subtract one from analyst noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*analyst noc*] unknown, unrelated individuals The DNA profile obtained from [*Item*] is approximately [*LR value*] times more likely if it originated from [*Reference*] and *[Subtract one from analyst noc]* unknown, unrelated individuals than if it originated from [*analyst noc*] unknown, unrelated individuals. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] and *[Subtract one from analyst noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*analyst noc*] unknown, unrelated individuals The LR result provides support for an exclusion. **Exclusion** (LR= 0 to comparable component) • [*Reference(s)*] *is/are* excluded from the comparable components of this mixture. **Inconclusive** • No conclusions can be made regarding [*Reference(s)*] as a contributor to the comparable components of this mixture due to a likelihood ratio result in the inconclusive range. [Use when LR in inconclusive range obtained to comparable component of mixture]. **Reference placed in contributor position that is not suitable for comparison**

	• [*Reference(s)*] *is/are* excluded from the comparable components of this mixture. [Use when LR in inconclusive or excluded range obtained to not suitable component of mixture]

12.8 Report Wording – STRmix – Mixtures with Conditioned Contributors

STRmix Report Wording – Mixtures with Conditioned Contributors	
Scenario	**Wording**
Mixture deconvolution performed with conditioned contributor(s), no additional reference(s), all contributors suitable for comparison	The DNA profile obtained from [*Item*] was interpreted as a mixture of [*analyst noc*] contributors and conditioned on the presence of [*Conditioned Reference(s)*]. [*This mixture contains at least one male contributor*.] • The additional contributor(s) to this mixture is/are suitable for comparison.
Mixture deconvolution performed with conditioned contributor(s), no additional reference(s), one or more contributors not suitable for comparison	The DNA profile obtained from [*Item*] was interpreted as a mixture of [*analyst noc*] contributors and conditioned on the presence of [*Conditioned Reference(s)*]. [*This mixture contains at least one male contributor*.] • Contributor(s) *[list contributor number(s)*] is/are suitable for comparison. Due to limited information, contributor(s) [*list contributor number(s)*] are not suitable for comparison.
Mixture deconvolution performed with conditioned contributor(s), remaining contributors not suitable for comparison	The DNA profile obtained from [*Item*] was interpreted as a mixture of [*analyst noc*] contributors and conditioned on the presence of [*Conditioned Reference(s)*]. [*This mixture contains at least one male contributor*.] • The additional contributor(s) to this mixture is/are not suitable for comparison due to limited information.
Mixture deconvolution performed with conditioned contributor(s), additional reference(s) submitted, all contributors suitable for comparison	The DNA profile obtained from [*Item*] was interpreted as a mixture of [*analyst noc*] contributors and conditioned on the presence of [*Conditioned Reference(s)*]. [*This mixture contains at least one male contributor*.] • The additional contributor(s) to this mixture are suitable for comparison. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Conditioned Reference*], [*Reference(s)*], and [*remaining noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*Conditioned Reference*] and [*remaining noc*] unknown, unrelated individuals The DNA profile obtained from [*Item*] is approximately [*LR value*] times more likely if it originated from [*Conditioned Reference*], [*Reference(s)*], and *[remaining noc*] unknown, unrelated individuals than if it originated from[*Conditioned Reference*] and [*remaining noc*] unknown, unrelated

	individuals. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Conditioned Reference*], [*Reference(s)*], and *[remaining noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*Conditioned Reference*] and [*remaining noc*] unknown, unrelated individuals The LR result provides support for an exclusion. **Exclusion** (LR= 0) • [*Reference(s)*] *is/are* excluded from this mixture. **Inconclusive** • No conclusions can be made regarding [*Reference*] as a contributor to this mixture due to a likelihood ratio result in the inconclusive range.
Mixture deconvolution performed with conditioned contributor(s), additional reference(s) submitted, one or more contributors not suitable for comparison	The DNA profile obtained from [*Item*] was interpreted as a mixture of [*analyst noc*] contributors and conditioned on the presence of [*Conditioned Reference(s)*]. [*This mixture contains at least one male contributor*.] • Contributor(s) *[list contributor number(s)*] is/are suitable for comparison. Due to limited information, contributor(s) [*list contributor number(s)*] are not suitable for comparison. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Conditioned Reference*], [*Reference(s)*], and *[remaining noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*Conditioned Reference*] and [*remaining noc*] unknown, unrelated individuals The DNA profile obtained from [*Item*] is approximately [*LR value*] times more likely if it originated from [*Conditioned Reference*], [*Reference(s)*], and *[remaining noc*] unknown, unrelated individuals than if it originated from [*Conditioned Reference*] and [*remaining noc*] unknown, unrelated individuals. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation.

	Hypothesis 1 (H1): The evidence originated from [*Conditioned Reference*], [*Reference(s)*], and *[remaining noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*Conditioned Reference*] and [*remaining noc*] unknown, unrelated individuals The LR result provides support for an exclusion. **Exclusion** (LR= 0 to comparable component) • [*Reference(s)*] *is/are* excluded from the comparable components of this mixture. **Inconclusive** • No conclusions can be made regarding [*Reference(s)*] as a contributor to the comparable components of this mixture due to a likelihood ratio result in the inconclusive range. [Use when LR in inconclusive range obtained to comparable component of mixture.] **Reference placed in contributor position that is not suitable for comparison** • *[Reference(s)*] *is/are* excluded from the comparable components of this mixture. [Use when LR in inconclusive or excluded range obtained to not suitable component of mixture]

12.9 Report Wording – STRmix – Differential Extractions

STRmix Report Wording -Differential extractions	
Scenario	**Wording**
Non-Sperm Cell Fraction	
Non-sperm cell fraction, single source or partial single source, consistent with expected donor (no STRmix interpretation/LRs)	[*Expected Donor Reference*] is consistent with the DNA profile obtained from the non-sperm cell fraction of [*Item*]. [*Expected Donor Reference*] is consistent with the partial DNA profile obtained from the non-sperm cell fraction of [*Item*].
Non-sperm cell fraction, mixture consistent with two expected donors (no STRmix interpretation/LRs)	[*Expected Donor References*] is consistent with the DNA mixture obtained from the non-sperm cell fraction of [*Item*].
Non-sperm cell fraction, mixture of expected donor and carryover (no STRmix interpretation/LRs)	The DNA profile obtained from the non-sperm cell fraction of [*Item*] is consistent with a mixture of two individuals. Assuming [*Expected Donor Reference*] is one of the contributors, the remaining allele(s) are consistent with carryover from the sperm cell fraction.

STRmix Report Wording -Differential extractions	
Scenario	**Wording**
Sperm Cell Fraction	
Single source male, no reference(s)	The male DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a single source profile.
Partial single source male profile, no reference(s)	The partial male DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a single source profile.
Mixture deconvolution performed, NOC=2, no reference(s), suitable contributor + carryover	The DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a mixture of two contributors. [*This mixture contains at least one male contributor*.] • Contributor one is suitable for comparison. • The additional contributor is consistent with having **originated** from carryover from the non-sperm cell fraction. [NOC=2, No interpretation/LR for carryover.]
Mixture deconvolution performed, no reference(s), all contributors suitable for comparison	The DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a mixture of [*analyst noc*] contributors. [*This mixture contains at least one male contributor*.] • All contributors to this mixture are suitable for comparison.
Mixture deconvolution performed, no reference(s), one or more contributors not suitable for comparison	The DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a mixture of [*analyst noc*] contributors. [*This mixture contains at least one male contributor*.] • Contributor(s) *[list contributor number(s)*] is/are suitable for comparison. Due to limited information, contributor(s) [*list contributor number(s)*] are not suitable for comparison.
Mixture deconvolution performed with conditioned contributor(s), no additional reference(s), all contributors suitable for comparison	The DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a mixture of [*analyst noc*] contributors and conditioned on the presence of [*Conditioned Reference(s)*]. [*This mixture contains at least one male contributor*.] • The additional contributor(s) to this mixture is/are suitable for comparison.
Mixture deconvolution performed with conditioned contributor(s), no additional reference(s), one or more contributors not suitable for comparison	The DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a mixture of [*analyst noc*] contributors and conditioned on the presence of [*Conditioned Reference(s)*]. [*This mixture contains at least one male contributor*.] • Contributor(s) *[list contributor number(s)*] is/are suitable for comparison. Due to limited information, contributor(s) [*list contributor number(s)*] are not suitable for comparison.
Mixture deconvolution	The DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a mixture of [*analyst noc*] contributors and conditioned on the presence of [*Conditioned Reference(s)*]. [*This mixture contains at least one male contributor*.]

performed with conditioned contributor(s), remaining contributors not suitable for comparison	• The additional contributor(s) to this mixture is/are not suitable for comparison due to limited information.
Single source male, reference(s) submitted	The male DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a single source profile. **Expected Donor** • [*Expected Donor Reference*] is consistent with this DNA profile. [No STRmix interpretation or LRs performed]. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] Hypothesis 2 (H2): The evidence originated from an unknown, unrelated individual The male DNA profile obtained from the sperm cell fraction is approximately [*LR value*] times more likely if it originated from [*Reference*] than if it originated from an unknown, unrelated individual. • There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] Hypothesis 2 (H2): The evidence originated from an unknown, unrelated individual The LR result provides support for an exclusion. [This statement may be applicable if a manual exclusion cannot be made.] **Exclusion** (LR= 0 or manual exclusion) • [*Reference(s)*] *is/are* excluded from this DNA profile. **Inconclusive** • No conclusions can be made regarding [*Reference*] as a contributor to this DNA profile due to a likelihood ratio result in the inconclusive range. [This statement may be applicable if a manual exclusion cannot be made.]
Partial single source male, reference(s) submitted	The partial male DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a single source profile. **Expected Donor** • [*Expected Donor Reference*] is consistent with this partial DNA profile. [No STRmix interpretation or LRs performed.]

	Support for Inclusion • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] Hypothesis 2 (H2): The evidence originated from an unknown, unrelated individual The partial male DNA profile obtained from the sperm cell fraction of the [*Item*] is approximately [*LR value*] times more likely if it originated from [*Reference*] than if it originated from an unknown, unrelated individual. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] Hypothesis 2 (H2): The evidence originated from an unknown, unrelated individual The LR result provides support for an exclusion. [This statement may be applicable if a manual exclusion cannot be made.] **Exclusion** (LR= 0 or manual exclusion) • [*Reference(s)*] *is/are* excluded from this partial DNA profile. **Inconclusive** • No conclusions can be made regarding [*Reference*] as a contributor to this partial DNA profile due to a likelihood ratio result in the inconclusive range. [This statement may be applicable if a manual exclusion cannot be made.]
Mixture deconvolution performed, reference(s) submitted, NOC=2, suitable contributor + carryover	The DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a mixture of two contributors. [*This mixture contains at least one male contributor*.] • Contributor one is suitable for comparison. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] and one unknown, unrelated individual Hypothesis 2 (H2): The evidence originated from two unknown, unrelated individuals The DNA profile obtained from the sperm cell fraction of [*Item*] is approximately [*LR value*] times more likely if it originated from [*Reference*] and one unknown, unrelated individual than if it originated from two

	unknown, unrelated individuals. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] and one unknown, unrelated individual Hypothesis 2 (H2): The evidence originated from two unknown, unrelated individuals The LR result provides support for an exclusion. **Exclusion** (LR= 0) • [*Reference(s)*] *is/are* excluded from this mixture. **Inconclusive** • No conclusions can be made regarding [*Reference*] as a contributor to this mixture due to a likelihood ratio result in the inconclusive range. • The additional contributor is consistent with having **originated** from carryover from the non-sperm cell fraction. [NOC=2, No interpretation/LR for carryover.]
Mixture deconvolution performed, reference(s) submitted, all contributors suitable for comparison	The DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a mixture of [*analyst noc*] contributors. [*This mixture contains at least one male contributor*.] • All contributors to this mixture are suitable for comparison. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] and *[Subtract one from analyst noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*analyst noc*] unknown, unrelated individuals The DNA profile obtained from the sperm cell fraction of [*Item*] is approximately [*LR value*] times more likely if it originated from [*Reference*] and [*Subtract one from analyst noc*] unknown, unrelated individuals than if it originated from [*analyst noc*] unknown, unrelated individuals. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion**

	• Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] and [*Subtract one from analyst noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*analyst noc*] unknown, unrelated individuals The LR result provides support for an exclusion. **Exclusion** (LR= 0) • [*Reference(s)*] *is/are* excluded from this mixture. **Inconclusive** • No conclusions can be made regarding [*Reference*] as a contributor to this mixture due to a likelihood ratio result in the inconclusive range.
Mixture deconvolution performed, reference(s) submitted, one or more contributors not suitable for comparison	The DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a mixture of [*analyst noc*] contributors. [*This mixture contains at least one male contributor*.] • Contributor(s) *[list contributor number(s)*] is/are suitable for comparison. Due to limited information, contributor(s) [*list contributor number(s)*] are not suitable for comparison. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] and *[Subtract one from analyst noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*analyst noc*] unknown, unrelated individuals The DNA profile obtained from the sperm cell fraction of [*Item*] is approximately [*LR value*] times more likely if it originated from [*Reference*] and [*Subtract one from analyst noc*] unknown, unrelated individuals than if it originated from [*analyst noc*] unknown, unrelated individuals. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Reference*] and [*Subtract one from analyst noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*analyst noc*] unknown, unrelated individuals

	The LR result provides support for an exclusion. **Exclusion** (LR= 0 to comparable component) • [*Reference(s)*] *is/are* excluded from the comparable components of this mixture. **Inconclusive** • No conclusions can be made regarding [*Reference*] as a contributor to the comparable components of this mixture due to a likelihood ratio result in the inconclusive range. [Use when LR in inconclusive range obtained to comparable component of mixture] **Reference placed in contributor position that is not suitable for comparison** • *[Reference(s)*] *is/are* excluded from the comparable components of this mixture. [Use when LR in inconclusive or excluded range obtained to not suitable component of mixture.]
Mixture deconvolution performed with conditioned contributor(s), additional reference(s) submitted, all contributors suitable for comparison	The DNA profile obtained from the sperm cell fraction of the [*Item*] was interpreted as a mixture of [*analyst noc*] contributors and conditioned on the presence of [*Conditioned Reference(s)*]. [*This mixture contains at least one male contributor*.] • The additional contributor(s) to this mixture is/are suitable for comparison. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Conditioned Reference*], [*Reference(s)*], and *[remaining noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*Conditioned Reference*] and [*remaining noc*] unknown, unrelated individuals The DNA profile obtained from the sperm cell fraction of the [*Item*] is approximately [*LR value*] times more likely if it originated from [*Conditioned Reference*], [*Reference(s)*], and *[remaining noc*] unknown, unrelated individuals than if it originated from[*Conditioned Reference*] and [*remaining noc*] unknown, unrelated individuals. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Conditioned Reference*], [*Reference(s)*], and *[remaining noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*Conditioned Reference*] and [*remaining noc*] unknown, unrelated individuals

	The LR result provides support for an exclusion. **Exclusion** (LR= 0) • [Reference(s)] is/are excluded from this mixture. **Inconclusive** • No conclusions can be made regarding [Reference] as a contributor to this mixture due to a likelihood ratio result in the inconclusive range.
Mixture deconvolution performed with conditioned contributor(s), additional reference(s) submitted, one or more contributors not suitable for comparison	The DNA profile obtained from the sperm cell fraction of the [Item] was interpreted as a mixture of [analyst noc] contributors and conditioned on the presence of [Conditioned Reference(s)]. [This mixture contains at least one male contributor.] • Contributor(s) [*list contributor number(s)*] is/are suitable for comparison. Due to limited information, contributor(s) [*list contributor number(s)*] are not suitable for comparison. **Support for Inclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Conditioned Reference*], [*Reference(s)*], and *[remaining noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*Conditioned Reference*] and [*remaining noc*] unknown, unrelated individuals The DNA profile obtained from the sperm cell fraction of the [*Item*] is approximately [*LR value*] times more likely if it originated from [*Conditioned Reference*], [*Reference(s)*], and *[remaining noc*] unknown, unrelated individuals than if it originated from [*Conditioned Reference*] and [*remaining noc*] unknown, unrelated individuals. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **Support for Exclusion** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Conditioned Reference*], [*Reference(s)*], and *[remaining noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*Conditioned Reference*] and [*remaining noc*] unknown, unrelated individuals The LR result provides support for an exclusion. **Exclusion** (LR= 0 to comparable component) • [*Reference(s)*] *is/are* excluded from the comparable components of this DNA mixture. **Inconclusive** • No conclusions can be made regarding [*Reference(s)*] as a contributor to the comparable components of this DNA mixture due to a likelihood ratio result

	in the inconclusive range. [Use when LR in inconclusive range obtained to comparable component of mixture.] **Reference placed in contributor position that is not suitable for comparison** • *[Reference(s)] is/are* excluded from the comparable components of this DNA mixture. [Use when LR in inconclusive or excluded range obtained to not suitable component of mixture]

12.10 Report Wording – STRmix – Likelihood Ratios

STRmix Report Wording - LRs	
Scenario	**Wording**
Ignored loci wording (interpretation of forensic profile with ignored locus/loci)	The following [*locus/loci*] [*was/were*] not included in the STRmix interpretation: *D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338*]. [This locus/These loci] is/are suitable for comparison; however, will not be included in statistical calculations.
Ignored loci wording (buccal standard) – report with just that portion of LR from previous results	Due to the observation of [reason – tri-allele or < > OL etc.] [*D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338*] [*was/were*] not included in the STRmix statistical calculations.]
Multiple, complete single source profiles-associated to same reference	The [*male, female*] DNA profile obtained from [*Item*] was interpreted as a single source profile. The DNA profile obtained from this item is consistent with the DNA profile(s) obtained from [*List Item(s)*]. Refer to results for Item [X] for statistical calculations.
Reporting LR value- LR wording for support for exclusion	**Single Source** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 2 (H2): The evidence originated from an unknown, unrelated individual Hypothesis 1 (H1): The evidence originated from [*Reference*] The [*partial*], [*male, female*] DNA profile obtained from [*Item*] is approximately [*LR value*] times more likely if it originated from an unknown, unrelated individual than if it originated from [*Reference*]. This provides support for an exclusion. **Mixture** • Given the evidence, the following propositions were considered for the statistical calculation.

	Hypothesis 2 (H2): The evidence originated from [*analyst noc*] unknown, unrelated individuals Hypothesis 1 (H1): The evidence originated from [*Reference*] and [*Subtract one from analyst noc*] unknown, unrelated individuals The DNA profile obtained from [*Item*] is approximately [*LR value*] times more likely if it originated from [*analyst noc*] unknown, unrelated individuals than if it originated from [*Reference*] and [*Subtract one from analyst noc*] unknown, unrelated individuals. This provides support for an exclusion.
Compound LR Routine	Although there is support for the individual inclusions of [*list Reference(s)*], additional analysis does not provide inclusionary support for the proposition that these individuals are present in the mixture together.
Compound LR- NOC is less than number of references included	The DNA profile obtained from this item was interpreted as a mixture of [analyst noc] contributors. There are [*list number*] of individuals who have a likelihood ratio result that supports inclusion. Although there is support for the individual inclusions of [*List References*] to the mixture, under the stated number of contributors, these individuals cannot be present in the mixture together
Compound LR-Reported	The DNA profile obtained from [*Item*] was interpreted as a mixture of [*analyst noc*] contributors. [Only restate if in subsequent report.] **<u>Support for Inclusion</u>** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 1 (H1): The evidence originated from [*Applicable References*] and [*Subtract references from analyst noc*] unknown, unrelated individuals Hypothesis 2 (H2): The evidence originated from [*analyst noc*] unknown, unrelated individuals The DNA profile obtained from [*Item*] is approximately [*LR value*] times more likely if it originated from [*Applicable References*] and [*Subtract references from analyst noc*] unknown, unrelated individuals than if it originated from [*analyst noc*] unknown, unrelated individuals. There is evidentiary support for the inclusion of NAME as a possible contributor to the DNA profile obtained as compared with the alternate explanation of the evidence. **<u>Support for Exclusion</u>** • Given the evidence, the following propositions were considered for the statistical calculation. Hypothesis 2 (H2): The evidence originated from [*analyst noc*] unknown, unrelated individuals

	Hypothesis 1 (H1): The evidence originated from [*Applicable References*] and [*Subtract references from analyst noc*] unknown, unrelated individuals The LR result provides support for an exclusion. **Exclusion** (LR=0/ LR=0 to comparable component) • [*List Reference(s)*] are excluded from being contributors to the [*mixture/comparable components of this mixture*] when considered together. **Inconclusive** • No conclusions can be made regarding [*list Reference(s)*] as contributors to the [*mixture/ comparable components of this mixture]* when considered together due to a likelihood ratio result in the inconclusive range. **Reference placed in contributor position that is not suitable for comparison** • No conclusions can be made regarding [*list Reference(s)*] as contributors to the comparable components of this mixture when considered together.

12.11 Report Wording – STRmix – Comments and Dispositions

STRmix Report Wording – Template Wording	
Scenario	**Wording**
STRmix methodology	STRmix software was used to aid in the interpretation of DNA profiles, and when appropriate, to perform statistical calculations.
Include only when statistic falls into either inconclusive or supports exclusion	The LR statistics for "inconclusive" and "supports exclusion" conclusions can be provided upon request. Please contact the Biology Section.
Include in all reports with STRmix interpretation and/or LR	The propositions used, the evaluation performed, and any conclusions made are based on the received case information and the assumptions made. If this information changes, it may be necessary to re-evaluate the findings and revise conclusions. Should any additional information become available, please contact the Biology Section.
Additional standard request	Please submit a buccal standard from [Reference]. Additional interpretation of items in this case may be possible once the requested standard is received and analyzed.

Comments and Disposition Statements	
Laboratory Activity	Laboratory activity was performed between [DATE] and the report date.
Laboratory Activity - SAK	Inventory and cutting of Item # [X] was performed on [DATE]. Additional laboratory activity was performed between [DATE] and the report date.

SAK returned to Property	Item # [X] (entire kit, including all listed contents) was transferred to the General Biology Cage to be returned to Property Control.
Submitted items returned to Property	The submitted items were transferred to the General Biology Cage to be returned to Property Control following examination.
Submitted items/Collected Items returned to Property *(a slide prepared during diff ext. is considered a collected item)*	The submitted items and the collected Item(s) were transferred to the General Biology Cage to be returned to Property Control following examination.
Collected Items to remain in lab	The collected item(s) will be maintained in the laboratory and transferred to General Biology Storage.
Item to remain in lab	Item [X] will be maintained in the laboratory and transferred to General Biology Storage.
Submitted items transferred to another section/unit	The submitted items were transferred to the [Latent Print Section, Firearm Section, Cyber Crime Unit] following examination.
Photos in Evidence.com [Packaging for selected items listed]	Items X, Y and the packaging for Items X, Y, and Z were photographed. All photographs are contained in the attached case file in PLIMS. Copies of all photographs were saved to Evidence.com.
Photos in Evidence.com [Packaging for all items listed]	Items X, Y and the packaging for these items were photographed. All photographs are contained in the attached case file in PLIMS. Copies of all photographs were saved to Evidence.com.
Submitted items transferred to Crime Lab Evidence Locker #1 or #2	The submitted items were transferred to the Crime Lab Evidence Locker [1 or 2] following examination.
DNA extracts disposition	All DNA extracts and any remaining extraction material associated with this case will be maintained in the laboratory and transferred to General Biology Storage.
CODIS entry	The following DNA profile(s) have been entered into the Combined DNA Index System (CODIS) and will be searched on a periodic basis. Any associations will be the subject of a separate report. (this statement precedes the table in the report)
CODIS deletion	The DNA profile previously obtained from the [item] (DNA #) has been removed from the Combined DNA Index System (CODIS).

Contamination	
Scenario	**Wording**
Contamination/Other QC issues	No reportable DNA results were obtained from [*Item*] due to quality control measures not being achieved.
Verified by CODIS Admin. - Staff Match, single source	A [*partial*], [*male, female*] DNA profile was obtained from [*Item*]. This DNA profile is consistent with a CMPD staff profile. The DNA profile is not suitable for further interpretation.

Verified by CODIS Admin. - Staff Match, Mixture	No DNA results can be reported from the mixture obtained from [*item*] due to quality control measures not being achieved. Additional interpretation of this mixture may be possible if the buccal standard from the associated CMPD staff member is received and analyzed.

12.12 Report Wording – Y-STR

Y-STR statements	
Y-STR Methodology	DNA was amplified by the STR polymerase chain reaction (PCR) using the Yfiler Plus amplification kit.
Y-STR results for Reference Standard	A Y-STR DNA profile was obtained from the buccal standard from [NAME].
Y-STR single source inclusion	A Y-STR DNA profile was obtained from Item __ (optional description) that is consistent with the Y-STR DNA profile obtained from [NAME]. Therefore, [NAME] or his paternal relative is included as a possible contributor of this Y-STR DNA profile. According to the Cal-DOJ Y-Mix tool Version ___, X of _____ total male individuals could be a potential contributor of this Y-STR DNA profile. By applying a 95% upper confidence interval, it is equivalent to approximately 1 in every # male individuals.
Y-STR partial single source inclusion	A partial Y-STR DNA profile was obtained from Item __ (optional description) (if partial list loci or loci except) that is consistent with the Y-STR DNA profile obtained from [NAME]. Therefore, [NAME] or his paternal relative is included as a possible contributor of this partial Y-STR DNA profile. According to the Cal-DOJ Y-Mix tool Version ___, X of _____ total male individuals could be a potential contributors to this partial Y-STR DNA profile. By applying a 95% upper confidence interval, it is equivalent to approximately 1 in every # male individuals.
Y-STR single source/single source partial exclusion	A [partial] Y-STR DNA profile was obtained from Item __ (optional description) (if partial list loci or loci except). NAME or his paternal relative is excluded as being a possible contributor of this [partial] Y-STR DNA profile.
Y-STR single source profiles with results at fewer than 10 Y-STR loci	Due to the limited nature of the Y-STR DNA results obtained from Item __ (optional description) (if partial list loci or loci except), this data is not suitable for comparison.
Y-STR no foreign DNA detected	No Y-STR DNA results foreign to NAME were obtained from Item __ (optional description).
Y-STR mixed profile consistent with 2 donors- no references and data at 10 or more loci	The Y-STR DNA results obtained from Item __ (optional description) demonstrated the presence of a mixture consistent with two donors.
Y-STR mixed profile consistent with 2 donors- at least 1 reference and data at 10 or more loci, unresolvable	The Y-STR DNA results obtained from Item __ (optional description) demonstrated the presence of a mixture consistent with two donors. [NAME] is included as a possible contributor to this mixture. Therefore, [NAME] or his paternal relative is included as a possible contributor of this mixed Y-STR DNA profile. According to

	the Cal-DOJ Y-Mix tool Version ___, X of _____ total male individuals could be a potential contributors to this mixed Y-STR DNA profile. By applying a 95% upper confidence interval, it is equivalent to approximately 1 in every # male individuals.
Y-STR mixed profile consistent with 2 donors, conditioned, foreign/partial foreign determined, inclusion	The Y-STR DNA results obtained from Item __ (optional description) demonstrated the presence of a mixture consistent with two donors. Conditioned on the presence of [NAME], a [partial] foreign Y-STR DNA profile was determined that is consistent with the DNA profile obtained from [NAME]. (if partial list loci or loci except) Therefore, [NAME] or his paternal relative is included as a possible contributor of this [partial] foreign Y-STR DNA profile. According to the Cal-DOJ Y-Mix tool Version ___, X of _____ total male individuals could be a potential contributors to this [partial] foreign Y-STR DNA profile. By applying a 95% upper confidence interval, it is equivalent to approximately 1 in every # male individuals.
Y-STR mixed profile consistent with 2 donors, conditioned, foreign/partial foreign determined, exclusion	The Y-STR DNA results obtained from Item __ (optional description) demonstrated the presence of a mixture consistent with two donors. Conditioned on the presence of [NAME], a [partial] foreign Y-STR DNA profile was determined. (if partial list loci or loci except) NAME or his paternal relative is excluded as being a possible contributor of this [partial] foreign Y-STR DNA profile.
Y-STR mixed profile consistent with 2 donors, major/partial major and minor/partial determined	The Y-STR DNA results obtained from Item__ (optional description) demonstrated the presence of a mixture consistent with two contributors. A [partial] major DNA profile was determined at all loci (if partial list loci or loci except). A [partial] minor DNA profile was determined at all loci (if partial list loci or loci except). [that matches / is consistent with the DNA profile obtained from [NAME].] **For inclusions:** A [partial] major DNA profile was determined at all loci (if partial list loci or loci except) that is consistent with the Y-STR DNA profile obtained from [NAME]. Therefore, [NAME] or his paternal relative is included as a possible contributor of this [partial] major Y-STR DNA profile. According to the Cal-DOJ Y-Mix tool Version ___, X of _____ total male individuals could be a potential contributors to this [partial] major Y-STR DNA profile. By applying a 95% upper confidence interval, it is equivalent to approximately 1 in every # male individuals. A [partial] minor DNA profile was determined at all loci (if partial list loci or loci except) that is consistent with the Y-STR DNA profile obtained from [NAME]. Therefore, [NAME] or his paternal relative is included as a possible contributor of this [partial] minor Y-STR DNA profile. According to the Cal-DOJ Y-Mix tool Version ___, X of _____ total male individuals could be a potential contributor to this [partial] major Y-STR DNA profile. By applying a 95% upper confidence interval, it is equivalent to approximately 1 in every # male individuals. **For exclusions:** NAME or his paternal relative is excluded as being a possible contributor of this [partial] [major][minor] Y-STR DNA profile.
Y-STR mixed profile, at least 2, conditioned, foreign not suitable	The Y-STR DNA results obtained from Item__ (optional description) demonstrated the presence of a mixture of at least X contributors. Conditioned on the presence of [NAME], the minor profile(s) are not suitable for comparison.
Y-STR mixed profile, major/ partial major, inclusion minors not suitable	The Y-STR DNA results obtained from Item__ (optional description) demonstrated the presence of a mixture of at least X contributors. A [partial] major Y-STR DNA profile was determined (at all loci or list all loci except) is consistent with the Y-

	STR DNA profile obtained from [NAME]. Therefore, [NAME] or his paternal relative is included as a possible contributor of this [partial] major Y-STR DNA profile. According to the Cal-DOJ Y-Mix tool Version ___, X of _____ total male individuals could be a potential contributor to this [partial] major Y-STR DNA profile. By applying a 95% upper confidence interval, it is equivalent to approximately 1 in every # male individuals. The minor profile(s) are not suitable for comparison.
Y-STR mixed profile, major/ partial major, exclusion minors not suitable	The Y-STR DNA results obtained from Item__ (optional description) demonstrated the presence of a mixture of at least X contributors. A [partial] major Y-STR DNA profile was determined (at all loci or list all loci except). NAME or his paternal relative is excluded as being a possible contributor of this [partial] major Y-STR DNA profile. The minor profile(s) are not suitable for comparison.
Y-STR mixed profile with results >2 with >10 loci, unresolvable/uninterpretable	The Y-STR DNA results obtained from Item __ (optional description) demonstrated the presence of a mixture of at least X contributors. Due to the complexity of the Y-STR DNA results obtained, this data is not suitable for comparison
Y-STR mixed profile with results at fewer than 10 Y-STR loci, unable to discern NOC	The Y-STR DNA results obtained from Item __ (optional description) demonstrated the presence of a mixture. Due to the limited nature of the Y-STR DNA results obtained, this data is not suitable for comparison.
Y-STR no results at any loci	No Y-STR DNA results were obtained from Item __ (optional description).
Y-STR possible mutation, not suitable for comparison	Due to the presence of more than one potential mutation event observed in the resulting Y-STR DNA profiles, the profile is not suitable for comparison.
Y-STR profiles not consistent with the same paternal lineage	The (partial) Y-STR DNA profile from Item # and the (partial) Y-STR DNA profile from Item # are not consistent with having originated from the same paternal lineage.
Y-STR Statistics – included in Appendix	The DNA statistics calculated herein used the counting method and were generated from all profiles listed within the National Database with Subpopulations – United States subset of the YHRD total database version R63. The reported statistics are an estimation and represent the profile probability with a 95% upper confidence interval and the combined probability across all sub-populations.
Y-STR CODIS entry for samples with autosomal entries	The Y-STR DNA profile obtained from [item] (DNA #) has been added to the previously entered Combined DNA Index System (CODIS) DNA record.

Combined autosomal and Y-STR statements	
Methodology	DNA was amplified by the STR polymerase chain reaction (PCR) using the Globalfiler and Yfiler Plus amplification kits.
CODIS entry	The following DNA profile(s) have been entered into the Combined DNA Index System (CODIS) and will be searched on a periodic basis. Any associations will be the subject of a separate report. (this statement precedes the table in the report) (After table)- Additionally, the Y-STR DNA profile obtained from [Item] (DNA #) was entered into CODIS.

12.13 Report Wording – Legacy

Results Section- Report Wording	
Legacy Report Wording	
Complete Single Source	
Scenario	**Wording**
Complete single source Match	The [*male*] [*female*] DNA profile previously obtained from [*Item*] matches the DNA profile obtained from [*Reference*]. The probability of selecting an unrelated person at random who could be a contributor of this DNA profile is approximately 1 in _____.
Complete single source Non match	A [*male*] [*female*] DNA profile was previously obtained from [*Item*]. [*Reference*] is excluded as a possible contributor of this profile.
Partial Single Source	
Scenario	**Wording**
Partial single source Consistent with Reference	A partial [*male / female*] DNA profile was previously obtained from [*Item*] at [*loci listed or all loci except*] that is consistent with the DNA profile obtained from [*Reference*]. The probability of selecting an unrelated person at random who could be a contributor of this partial DNA profile is approximately 1 in _____.
Partial single source Reference Excluded	A partial [*male / female*] DNA profile was previously obtained from [*Item*]. [*Reference*] is excluded as a possible contributor of this partial profile.
Complete Major Profiles	
Scenario	**Wording**
Mixture with major profile and minor (s) inconclusive (NOC>4, manual assessment of major, major at all loci)	The DNA profile obtained from (*Item*) is a mixture of greater than four individuals. A [*male / female*] major DNA profile can be determined. No conclusions can be made regarding the minor profile(s); [T*his profile contains at least one male contributor*.]
Complete Major Match	The [*male / female*] major DNA profile [*previously*] obtained from [*Item*] matches the DNA profile obtained from [*Reference*]. The probability of selecting an unrelated person at random who could be a contributor of this major DNA profile is approximately 1 in _____.
Complete Major Non-Match	[*Reference*] is excluded as a possible contributor of the [*male / female*] major DNA profile [*previously*] obtained from [*Item*].
Partial Major Profiles	
Scenario	**Wording**
Partial Major Consistent with Reference	The [*male / female*] partial major DNA profile previously obtained from [*Item*] at [*loci listed or all loci except*] is consistent with the DNA profile obtained from [*Reference*]. The probability of selecting an unrelated person at random who could be a contributor of this partial major DNA profile is approximately 1 in _____.

Partial Major Reference Excluded	[*Reference*] is excluded as a possible contributor of the [*male / female*] partial major DNA profile previously obtained from [*Item*].
Complete Minor Profile	
Scenario	**Wording**
Complete Minor Match	The [*male / female*] minor DNA profile previously obtained from [*Item*] matches the DNA profile obtained from [*Reference*]. The probability of selecting an unrelated person at random who could be a contributor of this minor DNA profile is approximately 1 in _____.
Complete Minor Non-Match	[*Reference*] is excluded as a possible contributor of the [*male / female*] minor DNA profile previously obtained from [*Item*].
Partial Minor Profile	
Scenario	**Wording**
Partial Minor Consistent with Reference	The [*male / female*] partial minor DNA profile previously obtained from [*Item*] at [*loci listed or all loci except*] is consistent with the DNA profile obtained from [*Reference*]. The probability of selecting an unrelated person at random who could be a contributor of this partial minor DNA profile is approximately 1 in _____.
Partial Minor Reference Excluded	[*Reference*] is excluded as a possible contributor of the [*male / female*] partial minor DNA profile previously obtained from [*Item*].
Major Group	
Scenario	**Wording**
Major Group Included	[*Reference* (s)] *is/are* included [*as a major contributor/as major contributors*] to the mixture previously obtained from [*Item*]. The probability of selecting an unrelated person at random who could be included as a major contributor is approximately 1 in _____.
Major Group Excluded	[*Reference* (s)] *is/are* excluded [*as a major contributor/as major contributors*] to the mixture previously obtained from [*Item*].
Partial Major Group	
Scenario	**Wording**
Partial Major Group Included	[*Reference* (s)] *is/are* included [*as a major contributor/as major contributors*] to the mixture previously obtained from [*Item*]. The probability of selecting an unrelated person at random who could be included as a major contributor is approximately 1 in _____. The loci (*list loci*) are not suitable for comparison or statistical calculations.
Partial Major Group Excluded	[*Reference* (s)] *is/are* excluded [*as a major contributor/as major contributors*] to the mixture previously obtained from [*Item*].
Mixture of two or three-no Major	
Scenario	**Wording**

Included	[*Reference* (s)] *is/are* included [*as a contributor/as contributors*] to the mixture previously obtained from [*Item*]. The probability of selecting an unrelated person at random who could be included as a possible contributor is approximately 1 in _____. The loci (*list loci*) are not suitable for comparison or statistical calculations.
Excluded	[*Reference* (s)] *is/are* excluded [*as a contributor/ as contributors*] to the mixture previously obtained from [*Item*].

12.14 Report Wording – Legacy – Intimate Samples

Results Section- Report Wording	
Legacy Report Wording	
Intimate Samples	
Foreign Single Source	
Scenario	**Wording**
Complete foreign single source Match	The foreign [*male / female*] DNA profile previously obtained from [*Item*] matches the DNA profile obtained from [*Reference*]. The probability of selecting an unrelated person at random who could be a contributor of this foreign DNA profile is approximately 1 in _____.
Complete foreign single source Non match	A foreign [*male / female*] DNA profile was previously obtained from [*Item*]. [*Reference*] is excluded as a possible contributor of this foreign DNA profile.
Partial Foreign Single Source	
Partial foreign single source Consistent	The partial foreign [*male / female*] DNA profile previously obtained from [*Item*] at [*loci listed or all loci except*] is consistent with the DNA profile obtained from [*Reference*]. The probability of selecting an unrelated person at random who could be a contributor of this partial foreign DNA profile is approximately 1 in _____.
Partial foreign single source Excluded	A partial foreign DNA profile was previously obtained from [*Item*]. [*Reference*] is excluded as a possible contributor of this partial foreign DNA profile.
Foreign Complete Major	
Scenario	**Wording**
Foreign Complete Major Match	The [*male / female*] foreign major DNA profile previously obtained from [*Item*] matches the DNA profile obtained from [*Reference*]. The probability of selecting an unrelated person at random who could be a contributor of this foreign major DNA profile is approximately 1 in _____.
Foreign Complete Major Exclusion	[*Reference*] is excluded as a possible contributor of the [*male / female*] foreign major DNA profile previously obtained from [*Item*].
Foreign Partial Major	
Scenario	**Wording**

Foreign Partial Major Match	The [*male / female*] partial foreign major DNA profile previously obtained from [*Item*] at [*loci listed or all loci except*] is consistent with the DNA profile obtained from [*Reference*]. The probability of selecting an unrelated person at random who could be a contributor of this partial foreign major DNA profile is approximately 1 in _____.
Foreign Partial Major Exclusion	[*Reference*] is excluded as a possible contributor of the [*male / female*] partial foreign major DNA profile previously obtained from [*Item*].

12.15 Report Wording – Legacy – Comments and Dispositions

Comments Section- Report Wording	
Activity Dates	
SAK and DNA processed	Inventory and cutting of Item # [X] was performed on [*Date*]. Additional laboratory activity was performed between [*Date*] and the report date.
DNA processed	Laboratory activity was performed between [*Date*] and the report date.
Statistics	
FBI and/or FBI-Amended	The DNA statistics calculated herein used the population allele frequencies provided by the Federal Bureau of Investigation (FBI). The reported statistics are an estimation for which a deviation of approximately +/- 10-fold may exist. The most common frequency from the Caucasian, African American, Hispanic, or Asian populations is reported.
NIST and/or NIST-Revised	The DNA statistics calculated herein used the population allele frequencies provided by the National Institute of Standards and Technology (NIST). The reported statistics are an estimation for which a deviation of approximately +/- 10-fold may exist. The most common frequency from the Caucasian, African American, Hispanic, or Asian populations is reported.
Y-STR Statistics: - Cal-DOJ Y-Mix Tool	The DNA statistics calculated herein used the counting method and were generated from all profiles listed within the National Database with Subpopulations – United States subset of the YHRD total database version R63. The reported statistics are an estimation and represent the profile probability with a 95% upper confidence interval and the combined probability across all sub-populations.
Y-STR statement	
Y-STR Request	Based on sample type and quantitation results, a reported evidence item may be suitable for Y-STR testing. Please contact the Biology Section to submit a Y-STR DNA service request.
STRmix Recommendation statement	

STRmix recommendation – when going back to do comparisons of data – data originally not suitable to interpret	Based on the original STR results, a previously reported evidence item may be suitable for reinterpretation and/or retesting utilizing STRmix. Please contact the Biology Section to submit a service request.
Comments and Disposition Statements	
Submitted items returned to Property	The submitted items were transferred to the General Biology Cage to be returned to Property Control following examination.
SAK returned to Property	Item # [X] (entire kit, including all listed contents) was transferred to the General Biology Cage to be returned to Property Control.
Submitted items/Collected Items returned to Property	The submitted items and the collected Item(s) were transferred to the General Biology Cage to be returned to Property Control following examination.
Collected Items to remain in lab	The collected item(s) will be maintained in the laboratory and transferred to General Biology Storage.
Item to remain in lab	Item [X] will be maintained in the laboratory and transferred to General Biology Storage.
Submitted items transferred to another section/unit	The submitted items were transferred to the [Latent Print Section, Firearm Section, Cyber Crime Unit] following examination.
Submitted items transferred to Crime Lab Evidence Locker #1 or #2	The submitted items were transferred to the Crime Lab Evidence Locker [1 or 2] following examination.
DNA extracts disposition	All DNA extracts and any remaining extraction material associated with this case will be maintained in the laboratory and transferred to General Biology Storage.

12.16 Report Wording – Outsourcing

STRmix Report Wording – Outsourcing	
Scenario	**Wording**
DNA Results from [Vendor Lab Name]	Item X: [List the items that have a DNA profile that is 1) being evaluated for CODIS entry 2) a reference used for conditioning or comparison]
DNA Results and Conclusions	The DNA profile(s) from [list out samples with descriptions] generated by [Lab Name] have been reviewed by the CMPD Biology section for CODIS entry.
Laboratory Activity - In the comments section and is the date of assignment	Laboratory activity was performed between [DATE] and the report date.
Not suitable for CODIS entry determined by COSTaR, may be comparable	No DNA profiles were suitable for CODIS entry; however, the profiles may be suitable for comparison.

CODIS entry	The following DNA profile(s) have been entered into the Combined DNA Index System (CODIS) and will be searched on a periodic basis. Any associations will be the subject of a separate report. (this statement precedes the table in the report – table has CMPD Item #, Description, Contributor, and CODIS level)
Disposition – include all in the report comment section	The DNA results and conclusions from the vendor lab testing are contained in the laboratory report from [Vendor Lab Name] that are attached in PLIMS. Case file documentation from the vendor laboratory relied upon by CMPD for the data review is retained by CMPD and may be provided upon request. Additional records outside of the case file are retained and provided by the vendor laboratory. Upon receipt from [Vendor Lab Name], the DNA extracts and any remaining extraction material associated with this case will be maintained in the laboratory and transferred to General Biology Storage.

12.17 Report Appendix

The appendix is part of the template document in PLIMS and is required for all serology and DNA reports (except CODIS, outsourcing review, and legacy).

For DNA reports, the Serology Appendix needs to be deleted. The same information is also contained in the DNA and Serology Appendix.

For a swabbing/serology only report, then the DNA and Serology Appendix needs to be deleted.

Appendix - Serology

Serological Methods

Serological testing results will be included in the report. If not noted, no serological examinations were performed.

Presumptive test- A test used for detecting the possible presence of biological fluids. The results of a presumptive test will not conclusively prove or disprove the presence of a biological fluid.

Confirmatory test- A confirmatory test conclusively identifies a specific substance.

Appendix – Serology and DNA

Serological Methods

Serological testing results will be included in the report. If not noted, no serological examinations were performed.

A presumptive test is a test used for detecting the possible presence of biological fluids. The results of a presumptive test will not conclusively prove or disprove the presence of a biological fluid.

A confirmatory test conclusively identifies a specific substance.

DNA Methods

A differential extraction is a technique that attempts to separate non-sperm cell DNA (non-sperm cell fraction) from potential sperm cell DNA (sperm cell fraction) but does not imply the presence or absence of spermatozoa.

The DNA # is for laboratory reference only.

STRmix is a fully continuous probabilistic genotyping forensic software which combines biological modeling with mathematical processes to 1.) Interpret and attempt to deconvolute DNA profiles in the presence or absence of conditioned contributors 2.) Compare references to evidence samples and provide statistical weight in the form of a likelihood ratio.

DNA Statistics

A likelihood ratio (LR) is a statistic calculated for the comparison of the probability of the evidence given two competing hypotheses.

A reference can be used for conditioning when there is a reasonable expectation of an individual on his/her person, personal items, or other items based on information provided at the time of this report. This may include known consensual partners. A potential conditioned contributor may be informed by calculating a likelihood ratio statistic. If a likelihood ratio was calculated for a potential conditioned contributor, this documentation will be included in the case file. Likelihood ratios are not generated for every conditioned contributor.

The propositions used within the report address the source of the DNA. How or when the DNA was transferred are activity level issues that are not addressed by the reported likelihood ratio.

The DNA statistics calculated herein used the population allele frequencies provided by the National Institute of Standards and Technology (NIST).

The 99% 1-sided lower HPD interval LR statistic provided represents the most conservative value from one of the four population groups calculated (Caucasian, Asian, African-American, Hispanic) per laboratory policy. The data from these calculations can be found in the case file. The HPD LR statistic accounts for uncertainty associated with the MCMC and database sampling.

Number name	Number	# of Zeros
Million	1,000,000	6
Billion	1,000,000,000	9
Trillion	1,000,000,000,000	12
Quadrillion	1,000,000,000,000,000	15
Quintillion	1,000,000,000,000,000,000	18
Sextillion	1,000,000,000,000,000,000,000	21
Septillion	1,000,000,000,000,000,000,000,000	24
Octillion	1,000,000,000,000,000,000,000,000,000	27
Nonillion	1,000,000,000,000,000,000,000,000,000,000	30
Decillion	1,000,000,000,000,000,000,000,000,000,000,000	33
Undecillion	1,000,000,000,000,000,000,000,000,000,000,000,000	36
Duodecillion	1,000,000,000,000,000,000,000,000,000,000,000,000,000	39
Tredecillion	1,000,000,000,000,000,000,000,000,000,000,000,000,000,000	42

DNA Conclusions

Likelihood Ratio Value	Conclusion
0	Exclusion
0 < X < 0.001	Supports exclusion – LR provided upon request.
0.001 - 1000	Inconclusive (observed adventitious non-donor inclusionary LRs in validation)- LR provided upon request.
X > 1000	Supports inclusion. Report LR.

Exclusion- A known individual could not have contributed to or is not a part of the forensic profile from a single source sample or the comparable components of a mixed sample. This may be determined based on a likelihood ratio of 0 or an analyst can manually exclude a reference from a single source profile. Manual exclusions will have no statistical analysis performed.

If a reference sample is placed in a contributor position that was deemed not suitable for comparison based on template or template and mixture proportions, a conservative approach will be applied and an exclusion to the comparable components of the mixture will be reported for the reference sample.

Supports Exclusion-Based on CMPD's STRmix Validation studies LR values greater than zero and less than 0.001 support the exclusionary hypothesis (H2).

Inconclusive- A determination that no inclusion or exclusion can be drawn from the comparison of a reference sample to a questioned profile. An inconclusive conclusion results from a comparison to a suitable profile/component of a profile that has a likelihood ratio value that fails to provide sufficient support for an inclusion or exclusion. The inconclusive LR range was established from CMPD's STRmix Validation studies.

Supports Inclusion- A known individual is included in a profile if their genotype is present at all loci at which DNA typing results are deemed interpretable with no unexplained differences. The loss of an allele due to preferential amplification, stochastic effects, mutation, or other factors is considered and does not necessarily indicate an exclusion. Based on CMPD's STRmix Validation studies LR values greater than 1,000 support the inclusionary hypothesis (H1).

The report and evaluation do not provide any assessment of how likely either of the propositions are to be true but is limited to an evaluation of the results obtained given the propositions. To assign which of the two propositions is the most likely, the DNA results should be combined with other information in the case. This is not considered to be the domain of the expert.

Y-STR

Y-STRs have paternal lineage, passed on from father to son(s). Samples that exhibit the same Y-STR haplotype may originate from either a common individual source, from males within the same paternal lineage, or from unrelated individuals.

If indicated, based on sample type and quantitation results, a reported evidence item may be suitable for Y-STR testing. Please contact the Biology Section to submit a Y-STR DNA service request.

The DNA statistics calculated herein used the counting method and were generated from all profiles listed within the National Database with Subpopulations – United States subset of the YHRD total database version R63. The reported statistics are an estimation and represent the profile probability with a 95% upper confidence interval and the combined probability across all sub-populations.

Provisions for Modification and Update of This Manual

Any updates, modifications, additions, or deletions to this manual prepared after the issue date on the cover sheet will have the following information and an updated issue date located at the bottom of each page.

Summary of Revisions

Date Issued	Summary of Changes Made
3/23/00	Intro pg 7, Sec 1 pg 4-6, Sec 5 all
5/31/00	Sec 5 pg 5, 9-11
6/6/00	Sec 5 all
10/19/00	TOC, Sec 1 pg 4-5,16,22, Sec 3 pg 3-4, Sec 5 all
12/5/00	Sec 3 pg 3,5
7/26/01	Sec 1 pg 4,17,23,25
4/29/02	Sec 1 pg 2,16,18,20,22,24,26, Sec 2 pg 3, Sec 3 pg 2-4, Sec 4 pg 5,6, Sec 5 all
8/26/02	Sec 2 pg 3, Sec 4, pg 6
11/21/02	Sec3 pg 1-3, Sec 5
7/10/03	TOC, Secs 1, 2, 3, 4, 5 (all)
2/26/04	Sec 2 all
5/17/04	Sec 3 pg 1 and 8
1/13/06	TOC, Secs 1, 2, 3, 4, 5 (all)
5/12/06	Sec 1 pg. 15, 16
10/25/06	Sec 3 all
1/30/07	Sec 4 pg. 2, 6, 7
6/18/07	Sec 2 pg. 7, 8
1/30/08	Sec 3 all and Sec 4 pg. 2, 4-7
8/26/08	Sec 1 added 1.5
4/9/09	Sec 2 remove 2.1; Sec 4 change dye colors
11/30/09	Sec 1, add LEV, changes to Reagent Blank treatment
4/13/10	Sec 1, change out Mini kit for Investigator kit; Sec 2, change out 7000 for 7500
7/30/10	Sec 1, add suggested amounts and rpms; Sec 3, additional information on 3130 samples; Sec 4, addition of 3130;
10/12/10	Sec 5, added/changed report wording
1/1/11	Sec. 1, references added; Sec 2 clarification added; Sec 3 option added; Sec 4 310 removed;
4/1/11	Sec 5. 310 reference removed, wordings added
7/18/11	Changes made for ASCLD International and combination of both the Serology SOP's and the DNA SOP's, all but SOP 8
7/20/11	SOP 8- changes made and SOP split into 8 and 9
10/12/11	Changes made for the 7/1/2011 QAS document, 3, 4 & 8
2/7/12	Change to SOP 1 Abacard procedure; SOP 2 remove 2.5.3 and 2.5.4 LEV protocols

2/22/12	Change to statistics calculations and references, creation of Appendix A
6/4/12	Change interpretation of SOP 1, change wording in SOP 8 and 9
8/28/12	Added PrepFiler/Automate procedure to SOP 2 following validation, removed Qiagen for blood and saliva
11/14/13	Added profiles for EE, KMA, MA & DS to Appendix A
2/2014	Added QIAgility additions to SOP 3 and 4
4/4/14	Changes made to all SOP's due to PLIMS additions and review
10/5/14	SOP 1 added IR and Hematrace, remove Tak and Ouch. SOP 2 added sample size for tip. SOP 3 added Trio, removed Quantifiler Human and Male. SOP 4 new target amount. SOP 6 clarify re-injections. SOP 7 clarifications. SOP 8 clarifications. SOP 9 added wording
4/7/15	SOP 4, added 96 well plate usage, SOP 5 added 3130 worksheet usage; SOP 6 added worksheet usage and new software instructions, SOP 7 created for GMID-X
6/22/15	SOP 1- no sperm verification, controls and dates, wireless and light; SOP 2- no notation needed for consuming other that *, handling of penile swabs, notations for samples incubated longer; SOP 3- notations on amplifying samples; SOP 7- injection list added, changes made to accommodate using copies of e-grams; SOP 8- changes in stats; SOP 9- changes in report wording
8/4/15	Volume in 2.6.2.9 changed from 300 µl to 150 µl to correspond with the manufacturer's protocols.
2/24/16	Removed "n-21" from SOP 4. Updated SOP 6 and 7 to reflect workbook. Created a new SOP 8 for Armed Xpert, changed mixture interpretation for SOP 9 and added report wordings to SOP 10
5/2/16	SOP 2- remove separate reagent blanks from the Automate, only one is needed; SOP 5 – remove optional LIZ set up; SOP 6 – add when spectral is needed; SOP 7 – clarify what to print when; SOP 8 – clarify when statistics is needed; SOP 9 –add combined allele tables and remove match and comparison; SOP 10- add CODIS and DEMS report wording
9/1/16	Highlighted material removed from all SOP chapters per change made to lab QM 4.3.3.2 on 8/16/16. SOP 1: update what controls consist of and how to record controls for luminol, hematrace, p30 ABAcard; clarify p30 is to be used in conjunction with a positive AP test; clarify p30 will not be done on AP=0 or negative SOP 2: Update cleaning procedures for magnetic stands, hoods and instruments; add storage conditions for MinElute columns;

	add QIAamp hair extraction only to be used as a backup extraction method; Change minimum elution volume for manually extracted samples to 40 uL; added AP testing of penile swabs; add reference for handling of bone and teeth for PrepFiler extraction; add extraction procedure for differential samples using PrepFiler Express; add storage conditions for kits or components that are not at room temperature SOP 3: add Qiagility consumables; clarify IPC interpretation range(s); add DI interpretation; add cleaning procedures for hood; add Qiagility and male screening instructions and interpretations; add storage conditions of Trio kit before and after opening SOP 4: add list of GlobalFiler loci; add Qiagility consumables; add manual and Qiagility GlobalFiler amplification instructions and parameters; add cleaning procedures for hoods and Qiagility; add storage conditions of amplification kits before and after opening SOP 5: add name of LIZ used with GlobalFiler; add cleaning procedure for hoods; add 3130 set up for GlobalFiler; clarify do not name a blank well on the 3130 injection list SOP 6: add name of GlobalFiler matrix standard kit; update data backup; add that two ladders will be on each re-injection plate; add creation of 3130 set up sheet and injection list for GlobalFiler; clarify parameters for runs using Identifiler and GlobalFiler SOP 7: clarify second review of data is done by the technical reviewer; update creation of Genemapper ID-X project; add GlobalFiler analysis settings; add clarification of notations to be made on the 3130 Injection List worksheet; add information required to be in the Sample Name column and Genotypes Table; electropherograms will no longer be printed SOP 8: clarify second review of data is done by the technical reviewer; update recording of interpretations; update use of statistics; clarify statistical databases to be used and how to select them in Armed Xpert; update data organization; update how to print allele tables SOP 9: add GlobalFiler interpretation guidelines; update profiles to be included in an allele table; add CODIS specimen category will be entered on CODIS entry form; add LIZ base pair range

	for Identifiler and GlobalFiler; add name of GlobalFiler positive control; add stutter definitions; clarify variant documentation; add peak height ratio exceptions; update reagent blank and negative control definitions; update analytical threshold; add stochastic, upper analytical, and lower analytical thresholds for GlobalFiler; add population database designations; add GlobalFiler reference for STR repeat sequences; clarify use of autosomal loci for interpretations; update proportions interpretations; add GlobalFiler population database reference; add clarification of notations to be made on 2130 Injection List worksheet; added numerical statistical designations SOP 10: update definitions for Identifiler, GlobalFiler, full match, partial match, and minimal profile; clarify reporting of a male marker; update statistical population subgroup to be reported; clarify definition of a mixture profile; add exceptions to exclusions or inclusions; update Y-STR recommendation; add wording for GlobalFiler; update wording for partial single source or major profiles; add wording to assume the presence of an individual; clarify wording for presumptive semen testing results; clarify when a sample can be dropped at quantitation; update wording when the actual numerical value from a statistical calculation will be reported; updated the loci not being used in statistical calculations statement
11/21/17	SOP 9- removal of Identifiler and clarification of intimate samples and mixture interpretations SOP 10 – removal of Identifiler and addition of wording being used based upon email changes. Clarified when to use mixture statements, how to report intimate samples, and addition of new statistics statement.
10/15/18	1: changed name; added information about evidence handling, photographs and how to add to DEMS and what to do with nurse photos, added Direct to DNA information, remove luminol, Clarification on reading Hematrace controls. Clarification on when to make slides for spermatozoa search. Press out AP instructions added. Changes to interpretation AP, clarifications to Christmas tree stain, added Phadebas chart. 2: Clarification on alternate standards and consumption and added quantities for the hair extraction, added Automate variable elution, added that 350 uL saved for non sperm fraction. 3: Added when samples can be dropped at quant.

	4: Remove all Identifiler and 9700, add Veriti 5: Remove Identifiler 6: Remove Identifiler, and added pop fill and blank formamide injection, added 2nd reads." 7: Add group processing and 2nd reads. 8: Remove Identifiler reference, added worksheet reference and added TR information. 9: Added 1st and 2nd read information and information on obligate and allele, any, clarified unsuitable/uninterpretable samples, changed stutter percentages to match material modification, clarified upper analytical threshold, added new worksheet reference and clarified partial minor/foreign profiles. 10: Clarification of report wording, added swabbing wording.
11/28/18	SOP 1: Added collection of samples using the M-Vac. SOP 2: Half of sample used for DNA and the remaining retained; added sampling from M-Vac filer for extraction. SOP 7: Removal of requirement to change allelic ladder to sample if failed. SOP 9: Not required to exclude one profile from another. SOP 10: Clarification of mixture statements and addition of M-Vac wording.

5/13/19	SOP 1: p.1 clarified when to request a standard in sexual assault cases. p. 9 added Y-STR DNA testing SOP 2: p. 3 Change final elution volume to 40 µL to accommodate Y STR amplification if necessary p. 17 Added that alternate standards cannot be run on Maxwell p. 25 Changed ratios of lysis buffer/DTT mix for differential extractions and clarifying that slides need to be made for all SAK cases p. 27 added that 200 µL elution volume only used on body swabs SOP 3: p. 1 change of 2.0 mL tube brands p. 2 change of considerations using the degradation index p. 9 3b change to the interpretation of Y-intercept p. 10 added guidance for Y STR samples in male screening SOP 7: Overall changes from 1.4 to 1.6 Overall changes to 1st read 2nd read p. 6 and 7 clarification on naming of projects SOP 8: Added 8.6 on how to upload DNA data to CTS proficiency SOP 10: p. 6 added wording for related individuals and kit sampled by technician 10.2.1 added CODIS wording
10/1/19	**SOP 1**: p.9 1.4.B. Point 1: Removed. Point 9: added that serology testing will be performed after DNA testing; p. 22 1.6.2 C. 1. A. changed SOP reference. **SOP 2**: 2.2.2.15 Removed or if the case is assigned as a Direct to DNA case. 2.6.3.15h. Removed “For Direct to DNA cases” **SOP 3**: E. and 3.3.6 table Changed 2 picogram to 1 picogram. 3.4 changed M:F ratio, changed quantitiy, added YSTR testing, added clarification on the minimal amount of elution. **SOP 4**: 4.1B changed type of tubes; D: changed 3130 to genetic analyzer; 4.1.1 changed quantity of samples that do not need amplification: 1, added that well positions of samples of ladders are reflected on the 3500 set up sheet;

	4.1.2 #2 changed to reflect new workbook. #5 corrected spelling #30 changed 3130 to 3500. Added all of 4.2 for YSTR's **SOP 5**: overall changed 3130 to 3500 and added Yfiler Plus **SOP 6**: overall change 3130 to 3500, removed the use of Nuclease water **SOP 7**: A. added 3500 and Yfiler plus settings, 7.3 removed QIA naming; 7.4.1: added operator responsibility; 7.4.2: removed group processing 1st and 2nd read, added edit code clarification and added clarifications on 1st and 2nd reads; 7.8: added 3500 analysis parameters; NEW 7.9 Yfiler Plus parameters **SOP 8**: Procedure: added 3500 and Yfiler Plus settings; 8.2: removed need to add control check tables for runs that failed as long as the documentation exits in the file. **SOP 9**: Changed to GlobalFiler Interpretation.9.2.1, 9.2.3, & 9.3 changed thresholds to reflect the 3500. 9.3.1 to 9.3.4 added appropriate 3500 thresholds; 9.6.1 clarify when to use "at least"; 9.6.2 #3 added AT=analytical threshold; 9.7.2 point 10: clarification on what is used to evaluate completeness. **SOP 10**: (NEW) Yfiler Plus **SOP 11**: added to definitions: Yfiler Plus, CODIS eligible and suitable profiles, Possible and apparent hair. General guidance: added observed stains that have not been tested to reports. Added report wording Hair vs hairs. Clarifications on when to add Y STR testing in report, 11.1.1 added clarifications on report wording and Y STR wording, added wording for making a blood standard; NEW 11.1.4 YSTR reporting 11.2 Added statements to remove profile from CODIS. Guidance on Y-STR only reports
3/3/20	**SOP 1**: Addition of Cold Case exception and pubic hair combs; changes made to reflect when serology is done on SAK's; remove AP tests and add AP Spot test; SOP 11: Changed report wording to reflect presumptive testing of the semen

1/20/2021	Clarifications, changes due to audit and validations. New references added. **SOP1**: added how to upload to DEMS, remove Bardole-Method, added swabbing SOP, added clarifications on SAK testing and secondary evidence. **SOP2:** Remove Maxwell procedures, add EZ-1 and QIAcube procedures, added information for combining extracts (DCC), added SpeedVac concentration. **SOP3**: added procedure for making standards using the QIAgility, added procedure for setting up the 7500. **SOP4**: modified Y filer positive control dilutions. **SOP6:** added longevity guidelines for re injections. **SOP7:** removed procedures for the 3130, added low analytical threshold procedure. **SOP9**: added information on Y indel, added known artifacts, clarified when to do stats on items reasonably expected to have individual's DNA. **SOP10:** changed comparisons from 7 to 11 loci, added guidance for mixture interpretation. **SOP11**: added guidance on when to report low level mixture in standard, added wording for alternate standards, combining extracts, changed to new Y STR version, removed some mixture wordings, added new male mixture information.
3/21/2022	The following changes are in response to the 2021 FBI QAS, and new instrumentation/performance checks. **SOP 1:** 1.1 B-added note about DEMS quantity; 1.3.3 A-removed requirement for supervisor to approve "Swab and freeze"; 1.4 B-added information about returning kits to PC; added 1.4 C-STIMS workflow; 1.5.3 A-added guidance about using to confirm blood of probable human origin **SOP 2:** B-added materials; D-added notes about Lysep column lot; E-updated minimum elution volumes remove that combining needs to be approved by supervisor: 2.2.1-2-changed ProK concentration; 2.2.2- changed to DNA IQ Digest Buffer through out; 2.2.2 15 added when to make a slide; 2.3.3 5-changed program for speedvac; 12-added cleaning information; 2.4.1-added a balance tube when have 11 samples; changed to DNA IQ Digest Buffer through out; 13- changed to QIAgen screw-cap; 23A-added when to make a slide; Trace protocol: 11d.-changed 1.5 ml QIAgen screw cap; 2.4.2 added stopping points for process; added Low elution Volume Card procedure; 2.5.3 15h.-added when to make a slide **SOP 3-** E. when to requant older samples; 1 2a. alternate method for making standards; added 3.5.1 SAK samples;

	SOP 4- 4.1 and 4.2 D-added notes about reagent blank requirements; 4.1.1-modified how to use the workbook **SOP 6**- 6.1.2 and 6.2.2 10-note to check sample order **SOP 7-** 7.4.2 5- paragraph 5- removed after the instrument number; added analysis methods **SOP 8-** removed old GF settings and requirement to initial pages; added reference **SOP 9-** E.-added "contamination" definition; F.-added when to use "^" for any in a partial single source profile; 9.2.1 added new stutter %; 9.3.4- added note about stutter; 9.5.1 added tri-allele clarification; 9.7.2-added notes for uninterpretable or inconclusive profiles; removed initial requirements **SOP 10-** E.-added "contamination"; 10.7.1-paragraph 2 added notes for uninterpretable or inconclusive profiles; paragraph 4 new calculated stats use R63. Paragraph 8-loci not suitable for stats will be recorded **SOP 11-** 11.1.1-added DCC swabbing statement; added CODIS lever to CODIS input statement; clarified statement "Mixture of at least two or three – Major determined and minor alleles consistent with expected contributor:" 11.1.2 changed report wording for outsourcing
9/16/2022	**SOP 1:** 1.1 A- added Decontamination; 1.4 added survivor act information and guidance on Direct to DNA **SOP 2:** 2B. Removed QIAamp DNA Investigator Kit, Promega 1.5ml spin baskets and the oven. 2D: clarification on amping reagent blank; added that DNA extracts of live rounds cannot be combined with DCC. Removed 2.1 QIAamp Manual Extraction (all sample types); 2.2.1 #19 added that need to allow UV bulb to cool off; 2.2.2 added notes of red brown staining on DCC; removed "Swab and freeze" note.
8/2/23	**SOP 2:** B.- Remove DNA IQ kit, add DNA-free swabs and 15mL disposable beaker; E.-add samples from DCCs and remove Manual DNA IQ extraction method; 2.1- remove DNA IQ Manual extraction; **New** 2.1-add in collection from sexual assault slides; 2.1.2-add requirement to UV beakers; 2.2.4-added bone and tooth extraction **SOP 3:** E.-added "If insufficient volume remains of the reagent blank for both quantitation and amplification, then the reagent blank may be amplified only."**;** 3.4 #7-Removed requirement to bring all DCC extracts forward

4/22/24	**SOP 1:** 1.1.2-added check packaging and for initials, date, & code of individual who packaged evidence; added information on safety of firearms and what to do; 1.1.3- added how to upload photos to Axon (Evidence.com); 1.2-removed Foster and Freeman IR; 1.3.2 D- added information on how to swab a "switch" on a firearm and why; 1.3.3- noting that can swab DCC's before going to Latent Prints; 1.3.4 References- added DCC swab study.1.4.1-All SAK's will be completely inventoried; 1.4.2-explains processing "routine" and "prioritized" kits; 1.4.6- added what to do if DNA analyst does not take all cut samples forward; 1.5-added that may not need to test sero negative stains depending on case scenario; 1.6- semen stains for DNA will be confirmed before sending to DNA; micro slides of penile may be examined depending on results;1.6.2 A.- removed estimation of time frames; B-removed phase contrast and sonicator; C1-named Kernectrot Stain A and Picroindigocarmine Stain B 1 A. removed sonication and added when a p30 test has to be conducted; D.-remove hazardous reagents statement; 1.6.3 A.- if AP pos, micro negative a p30 will be performed; 1.6.4-added Christmas tree stain internal validation; 1.7 D 12-remove DNA present cannot be attributed to saliva if result is <1. **SOP 2:** A.- removed first sentence about DNA providing information of source and DNA is isolated from nucleated cells; D.-removed "the manipulation of the sample", added that Lysep columns must be QC'ed prior to use; E.- Added alternate standards elution volumes will be 40ul; added that designee can approve consumption and removed that, removed need to note why a sample is reextracted; 2.1.2-removed after speed vac that samples must be reconstituted. **SOP 3:** A.- added quant is a reliable estimate for total DNA and male DNA; B.- version 1.3; D. updated standards and controls for the virtual standard curve; E.-added vendor lab samples and controls will be reconstituted in nuclease free water and re-quanted prior to amplification; added information about the degradation index; 3.1 #2 & 3- NEW changed to add new virtual quantitation procedures; 3.2- added QC-Quant tab; added #4 & 5; #9- added Trio VSC; #20- changed to add calibrators; 3.3 #6- removed "depending on what instrument was used"; #8 add name group processing plates, "QIA"; 3.4- #2-4, #7 & #9- updated for VSC; 3.5.1 & 3.5.2 updated to clarify when can drop at quant and when you can amplify or not all samples from kit; 3.6-added software validation for VSC. **SOP 4:** D.-added optimal template and information about the degradation index and DCC reagent blanks when to amp, information on samples amped before July 1, 2009 and

	STRmix; all samples amped will be run on the same 3500 at the same time; 4.1.1 #1-removed well positions statement; #2 removed tube size and added additional master mix; #4 remove plate sample dilution; 4.2 D.-added optimal template information; added all samples amped will be run on the same 3500 at the same time; removed well positions statement; #2 removed tube size and added additional master mix; #4 remove plate sample dilution. **SOP 5:** B.- added PC with 3500 Series Data Collection Software v. 4.0 or v. 4.0.1; C.- added Warning formamide is a chemical hazard and laser needs to be serviced by ThermoFisher; E.-added case analyst will determine if re-injections are needed; 5.1- can combine GF and YF and added how to name and what information to add; #2- add 2 ladders/ run; #11 import using text file from workbook; REMOVE 5.2 YFiler; NEW 5.2 3500 set up sheets; 5.3 #9.-added how to document instrument errors; REMOVED 5.3.2; 5.4- added where to save files. SOP 6: E.- can override software evaluation for amp positive controls with DNA TL approval or designee; added a procedure statement; 6.2.1-updated; 6.3-QIA- added from combined plate with other analysts, and added information of what to do with controls and to notify DNA TL, #4-added any issues for entire run needs to be documented in the notes area; #5- what to add when need to re-inject or re-set up; #6- can handwrite reason for using STRMix or not; #7 & 8 added for STRMix;6.4- can use Armed Xpert for a complete major with 4 contributors; added 6.4.2 for STRMix; 6.5- add NUFI is not using samples before creating allele table in GMID-X; 6.6-upload to CTS just new number;6.7-added to remove or export GMID-X projects;6.10-added STRMix validation. **SOP 7:** 7.1.1-added that any failed controls need to be seen by the DNA TL before using the data; 7.1.2- added that GMID has locus specific stutter filters; 7.1.5-Table added for what to do with profiles suitable for comparison; 7.4 to 7.8 NEW-added for STRMix. **SOP 8, 9**: NEW added for STRMix. **SOP 10:** NEW added for COSTar and STRMix. **SOP 11:** D. Y STR can only be used for the male lineage; 11.1.1- added controls;11.1.2- added analysts can edit stutter;11.1.6- NEW null alleles;11.1.7-added" It is therefore, expected that peaks at multi-copy loci will generally adhere to a peak height balance of at least 50%."; 11.2- types of DNA profules;11.2-added no DNA; 11.2.3-if mixture can be interpreted as discrete NOC then can use consistent with, if not then at least;11.2.4 called not suitable for comparison; 11.2.4.1-

	minimal; 11.2.4.2 complex; 11.3- possible conclusions redefined; 11.5 added reference. **SOP 12:** NEW format and STRMix report wording added.
7/8/24	**SOP 1:** 1.5.3 C.3 change of amount of extract added to the ABAcard; 1.5.4 added the CMPD internal material modification reference. **SOP 3:** 3D added clarification to inconclusive quant values. **SOP 7:** 7.1 added what to look for in evaluating amplified data and what should be communicated with the DNA TL; 7.1.6 Note: added what to do about tri-alleles in STRMix. **SOP 12:** General Guidance (swabbing/screening/serological testing) added that observation of additional possible serological stains will be included in the report; YSTR testing-added quant results must be greater than 1 pg; 12.1 added wording for apparent and possible hairs; 12.2 added additional areas or staining; 12.4 added further results for quantitation; 12.5 added clarification to low level single source; added lockers 1 and 2 to LP or FA transfers.
7/27/2026	**SOP 1:** 1.1.2-added that can mark stains to be tested; 1.2-added wavelengths, distances and angles to ALS; 1.3.2 A-added swabbing or cutting to collection of biological material; 1.3.2 C-added what should be included in an evidence seal; 1.3.3 A- added loci tubes and what to refer to when combining DCC's; 1.4.2 added guidance on what to sample; 1.4.3-added clarification to guidance on how to treat underwear in the kit; 1.4.4-added clarification on what to do with non-swabs in the SAK; 1.4.7-DNA analyst responsible for returning the inventoried items from the kit; 1.4.8-updated workflow; 1.6 intro bullet point 5 changed p30 from confirmatory test to test indicating semen with AP positive; changed table in 1.6 to indicated for p30 positive; 1.6.1 added time consideration to AP pressout; 1.6.3 A,C, E-updated procedure according to material modification study and removed confirmatory;1.7.1C-added some clarification to testing size and blood positive controls; Added 1.8 Hair Collection and Examination **SOP 2:** 2B and title 2.1-added EZ2 kits, 2D-clarification of recording of BTA kit lot number, 2E-Added Suggested Sampling Amounts, 2F-added "or similar" to DNA away, 2.1-step 10. add secure the lid 2.1.2-step 12. add rinsing instructions, step 20. combining of reagent blanks changed to two and added clarification to nomenclature, 2.2.1-added Extraction Procedure

	before steps, 2.3.1-step 3 retain the original extraction tube, 2.3.3-step 10 nuclease water and step 12 “or similar” added. **SOP 3:** 2E-paragrapgh five changed to 0.001 ng/ul for reagent blanks to be consistent with quantitation QC, paragraph 11 added “or similar”, 3.1,3.2-step 1 and 3 allow reagents and samples to come to room temperature., 3.4-step 12 added reagent blank investigation and possible bubbles, 3.5.2-clarified first paragraph last sentence of which cases may require all extracts with qualifying quantitation values to be amped., second paragraph added non-sperm cell fraction amplification. 3.5.3-more info about recommending Y-STR testing. **SOP 4:** 4D-second paragraph added TL exception approval to RB other than max volume, fifth paragraph added no re-extraction for degraded buccal but should be assessed on a case-by-case basis. RB passing requirements updated to 0.001 ng/ul. Reamplification parameters for STRmix added and clarified replicate amplification instrument requirements. 4E, 4.2E-added “or similar”, 4.1.1 step 2, 4.1.2 step 4, 4.2.1 step 2 all added bring reagents and samples to room temperature 4.2D-specified male DNA, one month expiration, and nomenclature of positive and negative controls for Y-STR amp. **SOP 5:** 5E-added “or similar”, 5.2-apply correct assay name, 5.2.2-positive and negative controls nomenclature. **SOP 6:** 6.3-clarified when to use “Rename Artifact Label” or “Rename Allele Label”, 6.4.1-removed mixture with more than four contributors. **SOP 7:** 7.5.1-added TL approval for replicate amp of forensic samples, 7.7-a forensic sample’s deduced profile with no submitted reference can’t be used to assume the presence of that contributor in another forensic sample. **SOP 8:** 8.1.8-clarified to be done with outsourcing data, throughout 8.2-removed reference to interpreting mixtures with greater than four contributors with a major profile. 8.2.6.4-complete or single source no reportable results if associated to a CMPD staff profile, and instructions for reporting and buccal collection if mixture profile associated to CMPD staff profile. **SOP 9:** 9.3-approval prior to performing Ignore Locus, 9.5-determination of NOC limitations on using deduced contributor without submitted reference. 9.6-updated printing requirements for LR and printing instructions. 9.7-printing compound LRs. **SOP 10:** 10.5 step 6-added more instructions for newly generated Y-STR profile being added to existing autosomal profile. **SOP 11:** 11B-“or GMID-X” added. **SOP 12:** 12E-added “Items Previously Reported”, General Guidance-added information on how to report previously

	reported data, item description in the “Results” section, items not examined, stains presumptively positive individually described, if conditioned on a male do not state “at least one male contributor”, no individual names of employee in report, details on re-interpretation reporting, Y-STR testing recommendation 12.1-no hairs obs. 12.2- p30 (+) is now indicated not detected, remove Sgt.name., 12.3-previsouly obtained data, extraction, DCC, 12.4-clarified drop at quant due to ratio, samples not amped at this time, buccal not amped, 12.6-simplified single source reporting for expected donors, added one additional allele reporting, 12.9-mixture consistent with two expected donors only, 12.10-changed ignore locus wording, 12.11-clarified when to include STRmix Comments, slide is collected item, CODIS entry and CODIS delete, 12.12-mixture consistent with two with data at 10 or more loci from both contributors and submitted reference, combined statement for autosomal and Y-STR CODIS entry, 12.16-Outsourcing updated reporting for DNA Results from Vendor Lab Name, DNA Results and Conclusions, CODIS entry, and no CODIS but suitable for comparison, and disposition updates. 12.17-added which appendix and when to include

Approved by: _____ (DNA Technical Leader) Date: 7.27.26

_____ (Section Administrator) Date: 7-27-26

_____ (Laboratory Director) Date: 7/27/2026

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