

Forensic Biology Standard Operating Procedures

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SECTION 1 - INTRODUCTION**1.1 Description**

STR (short tandem repeat) loci consist of short, repetitive sequence elements or repeat units which are 3 to 7 base pairs (bp) in length. These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using PCR (polymerase chain reaction). PCR amplifies the target regions of DNA. Alleles of STR loci are differentiated by the number of copies of the repeat units contained within the amplified region and are distinguished from one another using fluorescence detection following capillary electrophoretic separation.

The Promega PowerPlex® 16 System allows the co-amplification and three-color detection of fifteen STR loci and the Amelogenin locus for gender identification. The system contains the Penta E and Penta D loci in addition to the thirteen core CODIS loci D18S51, D21S11, THO1, D3S1358, FGA, TPOX, D8S1179, vWA, CSF1PO, D16S539, D7S820, D13S317 and D5S818. One primer specific for each of the following – Penta E, D18S51, D21S11, THO1 and D3S1358 is labeled with fluorescein (FL); one primer specific for each of the following loci – FGA, TPOX, D8S1179, vWA, and Amelogenin is labeled with carboxy-tetramethylrhodamine (TMR); and one primer specific for each of the following loci – Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818 is labeled with 6-carboxy-4',5'-dimethyl-7'-dimethoxy-fluorescein (JOE). All sixteen loci are amplified simultaneously in a single reaction tube and analyzed in a single injection by capillary electrophoresis on the Applied Biosystems 3130xl and/or 3500xl Genetic Analyzer.

1.2 STR (Short Tandem Repeat) Typing**1.2.1 Advantages of STR Typing**

STR typing is more tolerant of the use of degraded DNA templates than other typing methods because the amplification products are less than 500bp long. STR typing is also amenable to a variety of rapid DNA purification techniques, which are compatible with PCR but do not provide enough DNA of appropriate quality for Southern blot-based analyses. Visual or instrument-based comparison between the allelic ladder and amplified samples of the same locus allows rapid and precise assignment of alleles.

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1.2.2 Advantages of Using the Loci in the PowerPlex® 16 System

The loci included in the PowerPlex® 16 System satisfy the needs of several major standardization bodies throughout the world. The United States Federal Bureau of Investigation (FBI) has selected 13 STR core loci to be typed prior to searching or entering samples in CODIS (Combined DNA Index System), the U.S. national database of convicted offender and forensic sample profiles. The PowerPlex® 16 System amplifies all CODIS core loci in a single reaction. The PowerPlex® 16 System also contains two low-stutter, highly polymorphic pentanucleotide repeats, Penta E and Penta D. These loci add significantly to the discriminating power of the system, making the PowerPlex® 16 System a single amplification system with a power of exclusion sufficient to satisfactorily resolve paternity disputes. The low stutter (Promega developmental validation and AK SCDL internal validation associated with Penta E and Penta D makes these ideal loci for evaluation of DNA mixtures often encountered in forensic casework. The Amelogenin locus allows gender identification of the DNA source(s).

Reference: PowerPlex® 16 System Technical Manual, Promega (Part #TMD012)

1.3 Alaska DNA Legislation

House Bill 27 (Alaska Statute Sec. 44.41.035) directs the Department of Public Safety (DPS) to establish and maintain a DNA identification registration system and requires DNA registration by persons convicted of a felony crime against a person and of minors 16 years of age or older who are adjudicated as a delinquent for an act that would be a felony crime against a person if committed by an adult. Implementation of this statute began January 1, 2006. Updates to the legislation in 2001, 2003, 2004 and 2005 significantly expanded the scope of the registration system to include burglaries and felony attempts to commit burglary, all crimes against a person, registered sex offenders and child kidnappers, and certain municipal ordinances. As of July 2007, persons arrested for any qualifying offense are also included in the database.

Samples are collected by Department of Corrections, patrol officers, and Department of Health and Social Services / Division of Family and Youth Services and submitted to the crime lab for storage and DNA analysis. The DNA profiles are entered into CODIS.

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1.4 CODIS (Combined DNA Index System)

The purpose of the Combined DNA Index System (CODIS) is to create a national information repository where CODIS sponsored laboratories can share DNA information. The comparison of DNA profiles between different laboratories has the potential for generating investigative leads by locating DNA matches between cases previously thought to be unrelated, and to identify putative perpetrators in unknown suspect cases. CODIS is comprised of three levels: National DNA Index System (NDIS); State DNA Index System (SDIS); and Local DNA Index System (LDIS). The State of Alaska does not maintain separate state and local databases. The national database is maintained by the FBI.

A set of 13 core STR loci for use in NDIS was established by the FBI sponsored Short Tandem Repeat Standardization Project. Participants in the project were from 21 public laboratories throughout the U.S. and Canada. In November 1997, the participants of the project agreed that the set of core loci required for NDIS participation were to be as follows: D3S1358, FGA, vWA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, CSF1PO, TH01 and TPO.

Reference:

AK SCDL DNA Identification Registration System and CODIS Procedures Manual.

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SECTION 2 – REAGENTS AND CHEMICALS

2.1 Reagents: Critical and Non-critical

By definition, “**critical reagents are determined by empirical studies or routine practice to require testing on established samples before use on evidential or casework reference samples**” (FBI QAS, 2009). Reagents which are used in pre-amplification procedures directly involved in DNA extraction from forensic casework or database samples, have been deemed critical reagents to prevent unnecessary loss of sample.

The following pre-amplification DNA/Screening reagents are excluded from the critical reagent list: Phenolphthalein and 3% hydrogen peroxide (for presumptive blood test), BCIP solution, Nuclear Fast Red stain, Picro-indigo carmine stain and Ethanol, anhydrous reagent grade (for sperm slide preparation).

All post-amplification DNA reagents are hereby listed as non-critical reagents. Non-critical DNA reagents need not be verified prior to use in casework.

When a reagent fails to meet the criteria for verification, the DNA Technical Manager shall be notified and an appropriate course of action will be determined. The reagent shall not be used in casework until the issue has been resolved and the approval or an alternate course of action suggested by the DNA Technical Manager has been documented.

2.2 General Instructions for the Preparation, Storage and Verification of Reagents and Chemicals

- Gloves and mask shall be worn while preparing reagents.
 - Use of graduated cylinders or pipettes closest in capacity to the volume of the fluids being measured is recommended.
- Chemical and reagent quantities may be adjusted to prepare more or less than the specified amount.
All critical reagents shall be stored in sterile/autoclaved containers.

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- Reagent containers are to be labeled with the following:
 - Name of reagent
 - Lot number (the date of preparation and preparer's 2 or 3 letter initials are used as the lot # for reagents prepared in-house and reagents where a lot # is not provided by the commercial vendor; i.e. 06-0101MLC would be the lot # for a reagent prepared on Jan. 1, 2006 by MLC)
 - Any necessary safety information
- If chemicals or reagents are transferred to another container, the second container shall also contain the reagent name, lot # (or preparation date) and any necessary safety information.
- One member of the DNA discipline shall be designated for purchasing of supplies and reagents.
- Chemicals/Reagents received without a manufacturer's expiration date will be assigned an expiration date as follows:
 - Solid chemicals will expire 15 years from the date received
 - Liquid chemicals will expire 10 years from the date received
- All chemicals and reagents prepared or purchased shall be logged in the laboratory's information management system (LIMS) or in the Reagent Log binder maintained in the DNA discipline.
- All newly received/prepared critical reagents and chemicals shall be verified prior to use on casework/database samples. Chemicals/reagents requiring verification should be clearly marked as such.
- Verification of a reagent that is only used as a component of another reagent is achieved by verifying the final preparation and does not need to be documented separately.
- Reagents used in the same procedure may be verified simultaneously. If the verification fails, the components will need to be verified separately.
- Verification paperwork is filed electronically in the appropriate folder in the LIMS. The electronic verifications for the positive control/reference sample(s) and negative control/blank(s) are included in the verification paperwork. A reagent is deemed verified if the expected genetic profile is obtained for the known sample and no contamination is noted in the reagent blank/negative control.
- Additional control log paperwork may be referenced by noting the batch in which the verification was performed. The Reagent Log and reagent location (e.g. shelf, storage box, etc.) shall be updated with the verification date and analyst. Reagents and chemicals may be re-verified and labeled with the re-verification date to indicate that the item is suitable for use through the new expiration date. Re-verification must be approved in advance by the DNA Technical Manager. This approval will be documented in the form of an email or memorandum.

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2.3 Chemicals and Reagents not Requiring In-House Preparation and/or Verification

Chemicals/Reagents purchased from a commercial vendor and requiring no preparation or verification prior to use in procedures or preparation of other reagents are listed below. They shall be stored as prescribed by the manufacturer and shall expire on the date provided by the manufacturer. Expiration dates are assigned as previously described if not provided by the manufacturer.

- Aluminum Sulfate [solid]
- BCIP (5-bromo-4-chloro-3-indolyl phosphate) [solid]
- Citric Acid, anhydrous [solid]
- Dithiothreitol [solid]
- Ethanol, anhydrous reagent grade [liquid]
- 10X Genetic Analyzer Buffer (GAB) from Applied Biosystems [liquid]
- Concentrated Hydrochloric acid (HCl) [liquid]
- 3% Hydrogen Peroxide [liquid]
- Indigo Carmine dye [solid]
- Nuclear Fast Red [solid]
- Phenolphthalein [solid]
- PowerPlex[®] 16 Matrix Standards from Promega [liquid]
- Saturated Picric Acid [liquid]
- POP⁴ Polymer from Applied Biosystems [liquid]
- Potassium Hydroxide [solid]
- Sodium Acetate, anhydrous [solid]
- Sodium acetate buffer solution (3M, pH 5.2) [liquid]
- Sodium Carbonate (Na₂CO₃) [solid]
- Sodium Hydroxide pellets [solid]
- 1N or 2N Sodium Hydroxide (NaOH) [liquid]
- Sodium Phosphthalein Monophosphate, disodium salt
- Xylenol [liquid]
- Xylene Substitute [liquid]
- Zinc [solid]

Points and procedures regarding selection of a suitable vendor are prescribed in the Laboratory Quality Assurance Manual.

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2.4 Preparation and Verification of Reagents and Chemicals

AmpliAq Gold® DNA Polymerase (critical reagent)

Purchased from Applied Biosystems and stored at -20°C; expires on date provided by manufacturer.

Verification

Amplify the 9947A control and a negative amplification control using the new Taq Polymerase lot.

BCIP Solution (5-bromo-4-chloro-3-indolyl phosphate)

Dissolve 0.025g BCIP in 50mL sodium acetate buffer (0.05M, pH 5.5). Store at 2-8°C; solution expires 4 weeks from date of preparation.

Verification

Test the reagent with a positive semen control and a negative dH₂O control prior to first use, and on each day used in casework

Buffer G2 (critical reagent)

(when purchased outside of kit)

Purchased from Qiagen and stored at room temperature; expires 10 years from date received.

Verification

Extract and amplify reference sample and a corresponding reagent blank with the new kit lot.

Buffer G1 (critical reagent)

Purchased from Qiagen and stored at room temperature; expires 10 years from date received.

Verification

Extract and amplify a reference sample and a corresponding reagent blank with the new kit lot.

Citric Acid Buffer (0.14M, pH 4.9)

(for STMP solution #1 preparation) Add 1.35g Citric Acid, anhydrous and 0.5g sodium hydroxide to 50mL de-ionized water. Adjust to pH 4.9 with NaOH /1:1 HCL.

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DTT (1M) (critical reagent)

Working solution Dissolve 0.77g dithiothreitol in 5mL sterile de-ionized water in a sterile conical tube. Add 50µL of 3M Sodium Acetate buffer solution, pH 5.2. Do not autoclave. Aliquot (0.1mL recommended) and store at -20°C. Aliquots expire one year from date of first thaw.

Verification

Extract and amplify a reference semen sample and a corresponding reagent blank with the new DTT lot.

Ethanol (Absolute, 200 proof) (critical reagent)

Verification

Extract and amplify a reference sample and a corresponding reagent blank with the new Ethanol lot.

EZ1 DNA Investigator Kit (critical reagent)

Components: Reagent Cartridges, Buffer G2, Proteinase K solution, carrier RNA

Purchased from Qiagen and stored at room temperature. Reagent cartridges may be stored at 2-8°C for long-term storage. All components, except carrier RNA, expire on date provided by manufacturer.

Carrier RNA solution is prepared by reconstituting the carrier RNA in 310µL of sterile, de-ionized water. Vortex and spin briefly. Prepare 20µL, single use aliquots in 0.5mL tubes and store at -20°C. Reconstituted carrier RNA expires one year from date of preparation.

Verification

Extract and amplify reference sample and a corresponding reagent blank with the new kit lot.

H₂O₂ (30% Formamide)

Purchased from Applied Biosystems. Aliquot (0.4mL and 1.0mL recommended) and store at -20°C. Aliquots are intended for one-time use and should not be re-frozen. Expires 10 years from date received.

1X Genetic Analyzer Buffer (GAB)

In a sterile conical tube, add 5mL 10X Genetic Analyzer Buffer to 45mL sterile de-ionized water. Mix by inversion. This solution is stored at room temperature and should be prepared fresh and as needed (approximately weekly).

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MagAttract DNA Mini M48 Kit (critical reagent)

Components: MagAttract Suspension B, Buffer MTL, Buffer MW1, Buffer MW2, RNase-free water, Buffer G2, Proteinase K solution

Purchased from Qiagen and stored at room temperature; expires one year from date of verification. Buffer MW2 and RNase-free water are not used by the laboratory and are discarded upon receipt.

Buffer MW1 is prepared by adding 26mL of Ethanol (Absolute, 200 proof) to the MW1 bottle.

Verification

Extract and amplify a reference sample and a corresponding reagent blank with the new kit lot.

Nuclear Fast Red stain

Dissolve 5.0g of aluminum sulfate in 100ml of deionized water. Add 0.1g of Nuclear Fast Red. Stir and let cool. Filter the solution through filter paper and store at 40°C. Store at 2-8°C; expires one year from date of preparation.

One-step PSA ABacards

Purchased from Abacus Diagnostics. Stored according to manufacturer's instructions; expires on date provided by manufacturer.

One-step Herpes Traces ABacards

Purchased from Abacus Diagnostics. Stored according to manufacturer's instructions; expires on date provided by manufacturer.

Permout

Purchased from a commercial vendor and stored at room temperature.

Working solution: Permout diluted with Xylene if necessary.

Use until the reagents adequately satisfy the purpose they are used for.

No expiration date although manufacturer provides one. (Please refer to the DNA QA manual)

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Phenolphthalein (for Kastle-Meyer Test)

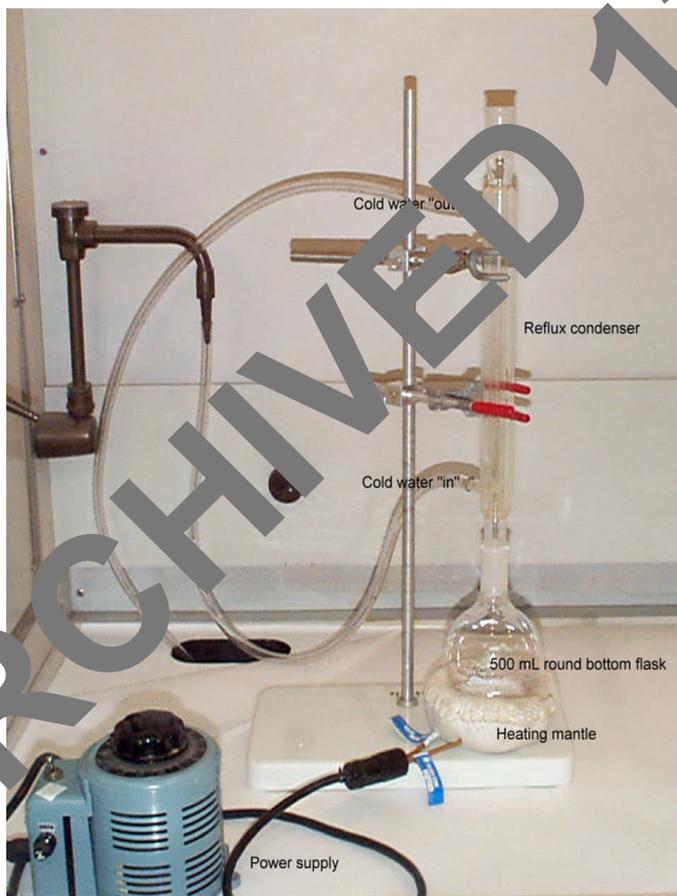
Stock Solution

Reflux 2g phenolphthalein, 20g potassium hydroxide, and 100mL deionized water with 20g of zinc until the solution becomes colorless (approximately 30 minutes to 1 hour after boiling begins - **See Figure 1**). Store the solution at 2-8°C in a dark bottle to which some zinc has been added to keep it in the reduced form.

Working solution

Combine 20mL phenolphthalein stock solution (obtained from the biological screening discipline) with 80mL Ethanol (anhydrous reagent grade). The solution is stored at 2-8°C in a dark bottle. This reagent has no expiration date and may be used as long as the appropriate reactions are observed with the positive and negative blood controls, prior to use on evidentiary items.

Figure 1. Phenolphthalein stock solution preparation



- Assemble the reflux apparatus as shown.
- Turn on cold water at source. Allow the system to fill and cool. Adjust flow so that no bubbles are formed in the condenser.
- Add the chemicals, deionized water and zinc to the 500mL round bottom flask.
- Reassemble the apparatus. Place the flask on the heating mantle.
- Turn on the power supply. Heat the flask to a gentle boil (100°C for approximately 15 minutes) Adjust temperature setting to 75°C and allow the solution to reflux until colorless (approximately 2-3 hours). Store the solution with the zinc from the flask at 2-8°C in a dark bottle.
- Clean glassware with EDTA and water.

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Picro-indigo-carmin stain

Add 0.33g of Indigo Carmine dye to 100mL of saturated picric acid. Filter the solution through filter paper. Store at 2-8°C; expires one year from date of preparation.

PowerPlex® 16 Amplification and Typing Kit (critical reagent)

Components: 9947A DNA, 10X Primer Pair Mix, Gold ST*R 10X Buffer, Allelic Ladder, Internal Lane Standard (ILS 600)

Purchased from Promega Corporation. 9947A is diluted, aliquotted and stored at -20°C. (This procedure applies to DNA casework and database sections). Other components are stored according to manufacturer's instructions. 9947A expires one year from date of dilution. Other components expire on kit expiration date provided by manufacturer.

Verification

- Use 975µL sterile de-ionized H₂O to bring the 9947A to an appropriate concentration for setting up amplification reactions.
- Amplify the 9947A positive control, negative control and a known sample twice using (1) the primers and buffer from the kit currently in use and the (2) the primers and buffer from the kit being verified to compare amplification sensitivity. Refer to the [Section 11](#) (Data Interpretation) for evaluating the kit components
- The relative fluorescence units (RFU) for the known sample amplified with the new kit are compared to the results obtained with the kit currently in use to estimate the sensitivity of the new kit. This is important for adjusting the target value with the new kit of units.

Proteinase K solution (critical reagent)

(when purchased outside of a kit)

Purchased from Megazyme or another suitable vendor and stored at room temperature; expires on date provided by manufacturer or 10 years from date received if no expiration date is given.

Verification

Extract and amplify a reference sample and a corresponding reagent blank with the new proteinase K lot.

Sodium Acetate buffer (0.01M, pH 5.5)

(for BCIP preparation)

Dissolve 0.34g Sodium Acetate (anhydrous) in 200mL deionized water. Adjust the pH to 5.5. Bring to a volume of 250mL with deionized water. Store solution at room temperature; expires one year from date of preparation.

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Sodium Thymolphthalein Monophosphate

Solution #1

Dissolve 0.01g Sodium Thymolphthalein Monophosphate in 50mL Citric Acid Buffer.

Solution #2

Add 0.53g Sodium Carbonate (Na_2CO_3) and 0.20g Sodium Hydroxide (NaOH) to 50mL deionized water.

Store at 4° C; solutions are stable for 4 months.

Verification

Test the reagent with a positive semen control and a negative DNA control prior to first use, and on each day used in casework

Sterile De-ionized Water (H_2O)

(critical reagent)

Fill glass bottles with nanopure de-ionized H_2O . Autoclave and store at room temperature. Expires 1 year from date prepared.

- When autoclaving, include a Sterikon™ plus Bio-Indicator, or equivalent. The DNA Technical Manager shall approve use of reagents autoclaved without a Sterikon™ (or equivalent). This approval will be documented in the Reagent Log. After autoclaving, place the autoclaved ampoule, and an ampoule that was not autoclaved, in the 56°C incubator for 48 hours. Refer to the manufacturer's instructions to evaluate the results of the ampoules.

Verification

Amplify the 9947A control and a negative amplification control using the new water lot.

Quantifiler QF kit

(critical reagent)

Components: DNA Standard A, Human Primer Mix and Reaction Mix

Purchased from Applied Biosystems. DNA Standard A and Primer Mix are stored at -20°C. Reaction Mix is stored at 2-8°C. Components expire on date provided by manufacturer. Standard curve prepared fresh approximately weekly.

Verification

- Test new QF lot with a standard curve and NTCs.
Print out standard curve data and confirm that data falls within specified guidelines (refer to [Section 7](#)).
- Print out Q-PCR set-up worksheet and Q-PCR result summary page.

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SECTION 3 – SCREENING OF BIOLOGICAL EVIDENCE

The laboratory does not routinely examine every piece of evidence submitted for analysis. Generally, the only items screened in a sexual assault case will be the samples present in the assault kit(s). Underwear, clothing or other items, such as condoms and bedding, may also require screening under special circumstances.

The amount of time between the alleged assault and the collection of the evidence should be considered when deciding if the analysis of these samples/items is appropriate. Samples present in the assault kit(s) may not be examined due to the length of time between the offense and collection. Information pertaining to the date of offense and date of examination may be found on the Forensic History Form, the Victim Information/Medical History Form or on the outside of the kit box/envelope.

3.1 Hair and Fiber Evidence

The following sexual assault kit components may need to have trace evidence evaluated/isolated before biological screening/testing can be conducted:

- Foreign Material Sheet
- Debris Collection
- Pubic Hair Combing (combing will not be examined if the victim/suspect has bathed/showered or when the sample was collected 2 days after the offense)
- Miscellaneous Evidence
- Underwear/Clothing

Hair and fiber evidence may be recovered from evidentiary items by the following methods:

Tape Lifting

Clear plastic latent fingerprint tape (or other suitable adhesive tape) can be applied adhesive side down to the surface of the evidence item. Press the tape down, and then pull away. Hairs and fibers will adhere to the adhesive on the tape. Place the tape adhesive side down on the shiny-side of freezer paper (or on another appropriate surface).

Scraping

The item to be examined is suspended above the examination surface and gently scraped with a clean metal spatula. Scraping in a downward direction allows surface debris to fall onto the examination surface for collection. The debris is transferred to an appropriate storage container.

Hand-picking

Clean forceps are used to collect trace evidence from evidence items. The isolated material is then placed in an appropriate container. Isolated trace evidence should be sealed in a suitable container and clearly marked with identifying information. Isolated trace evidence may be placed within the originating item or it may be repackaged as an entirely new item of evidence.

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The laboratory does not currently provide microscopic comparisons for hair and fiber evidence submitted or recovered in case work. The laboratory will screen the evidence submitted in these cases for probative value. When needed, evidence may be sent "outside" for analysis. Examination of trace evidence need not occur in cases where the trace is not likely to have probative value. In these cases, trace collections are re-packaged with the original evidence and the bench notes will reflect that no examination of the trace evidence was conducted.

3.1.1 Screening Hairs

References

Linch, Charles A., et al, "Evaluation of the Human Hair Root for Hair Typing Subsequent to Microscopic Comparison", *J Forensic Sci* 1998; 43(2): 305-314.

Petraco, Nicholas, et al, "The Morphology and Evidential Significance of Human Hair Roots", *J Forensic Sci* 1988; 33(1): 68-76.

An intact hair will have a root portion and a shaft portion. It is important to start with visual examination followed by macroscopic examination of the morphology of individual hairs. A full examination at magnifications of 10X, 40X and up to 60X -100X with incident illumination will enable the analyst to record the overall shape of the hair, whether or not a root is present and, where present, its shape and appearance, the basic features of the shaft, and medullary structure.

Hairs recovered in case work will be examined visually and macroscopically, using a stereoscope, to determine the following:

- Is the hair Animal or Human in origin
- If Human, serologic region (head hair, pubic hair or inconclusive)
- If Human, is the hair suitable for nuclear DNA analysis

DNA analyses are destructive techniques and consume portions of the hair. If the analyst concludes that the hair(s) examined may be suitable for nuclear DNA analysis, the hair's root will be documented (photographed) and included in the analyst's screening notes.

Documentation will also assist in the technical review process.

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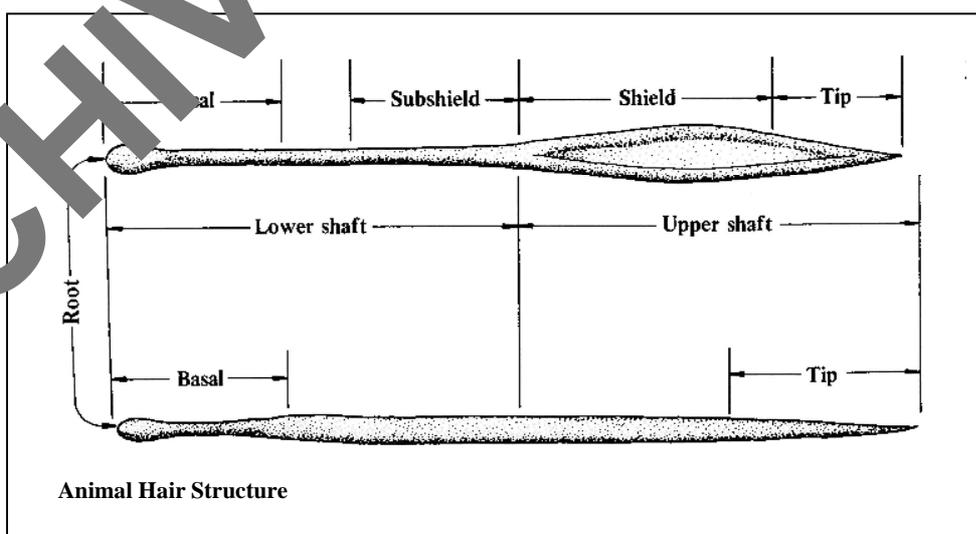
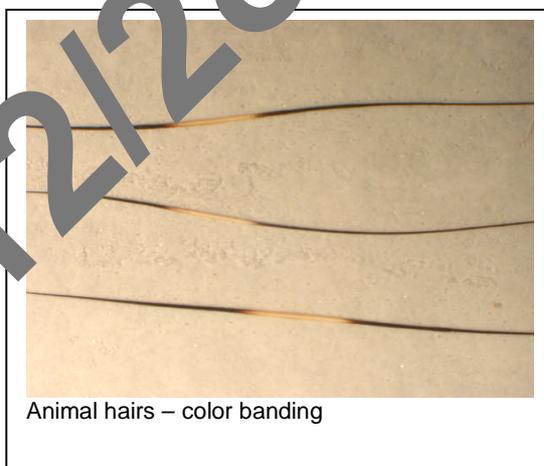
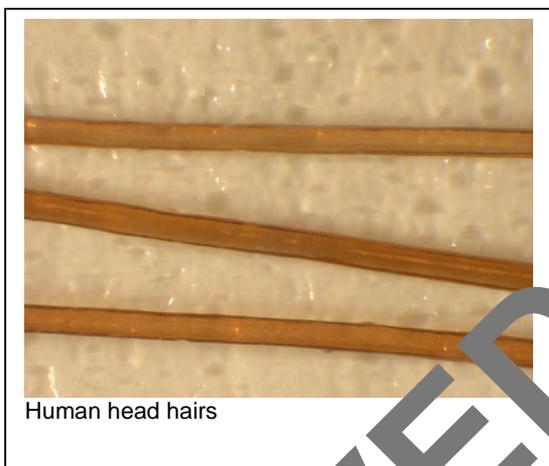
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3.1.2 Animal v. Human Hair Identification

There are distinct differences between human hair and animal hair. The differences between human and animal hair characteristics show up most often in the color(s) of the hair, medullary structure, root shape and hair shape or form.

Human hairs are generally consistent in color and pigmentation throughout the length of the hair shaft, whereas animal hairs may exhibit radical color changes in a short distance, called color banding.



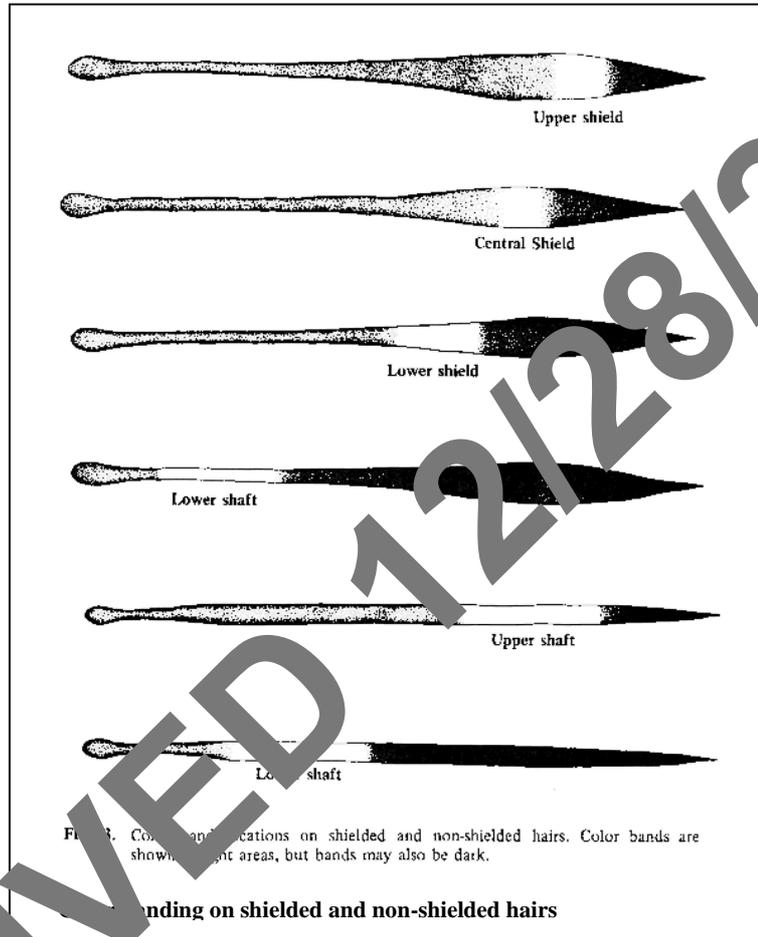
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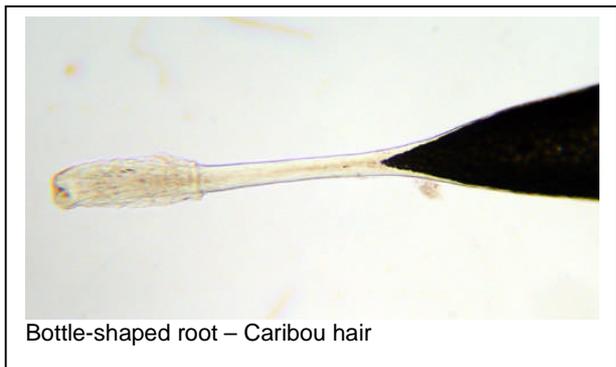
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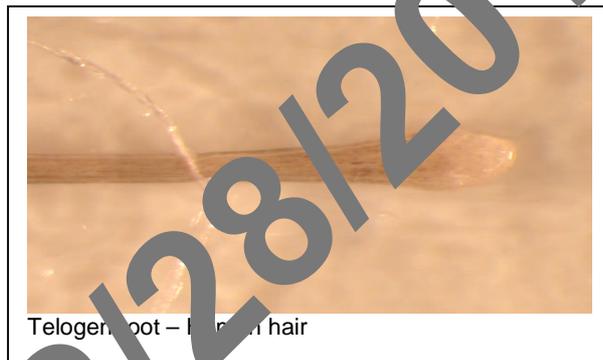
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Root shape is a strong demarcator for animal hairs. Roots are usually the first indication that the hair is not human. The root of human hairs is commonly club-shaped, whereas the roots of animal hairs are highly variable.



Bottle-shaped root – Caribou hair



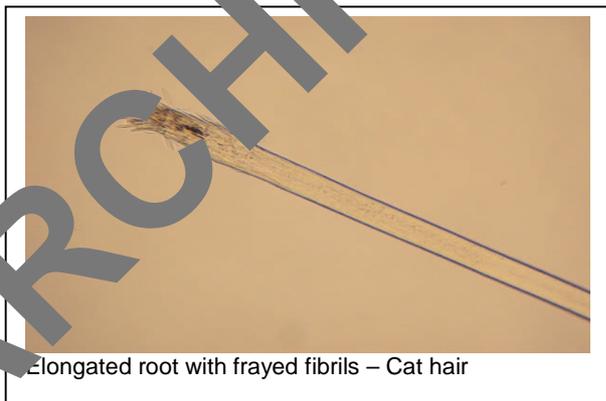
Telogen root – Human hair



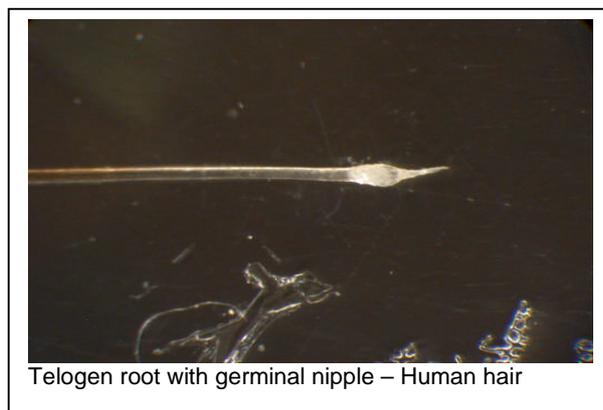
Spade-shaped root - Dog hair



Anagen root – Human hair



Elongated root with frayed fibrils – Cat hair



Telogen root with germinal nipple – Human hair

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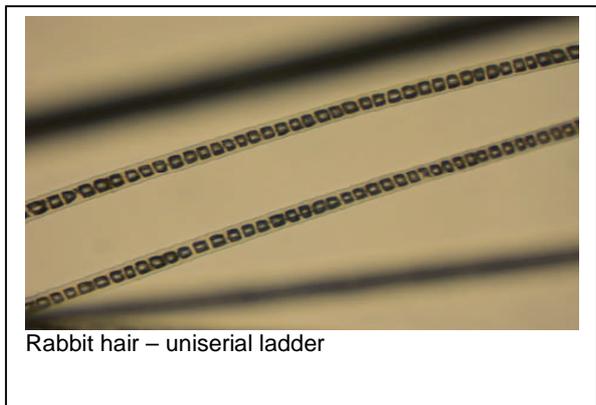
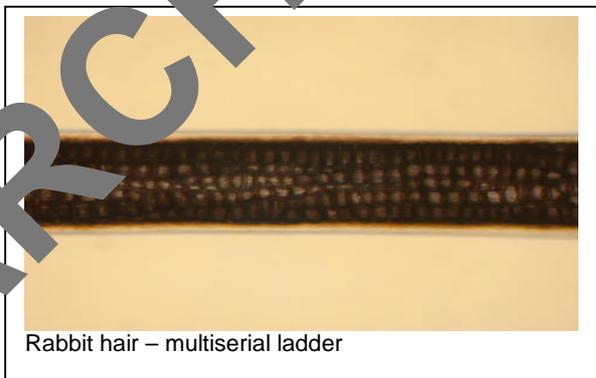
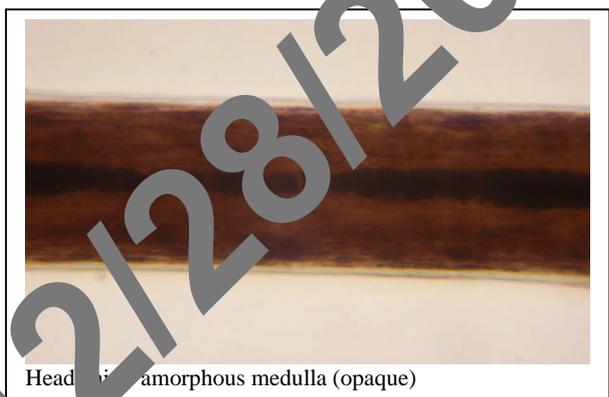
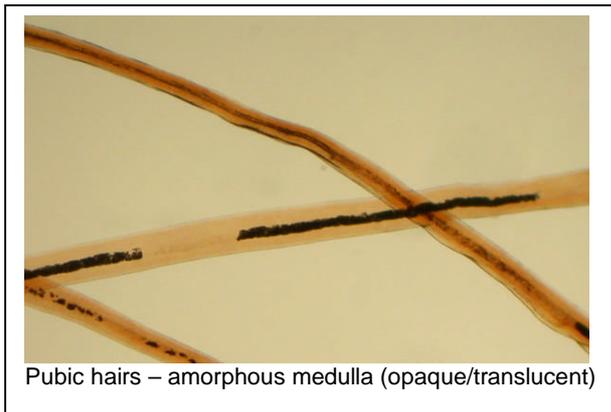
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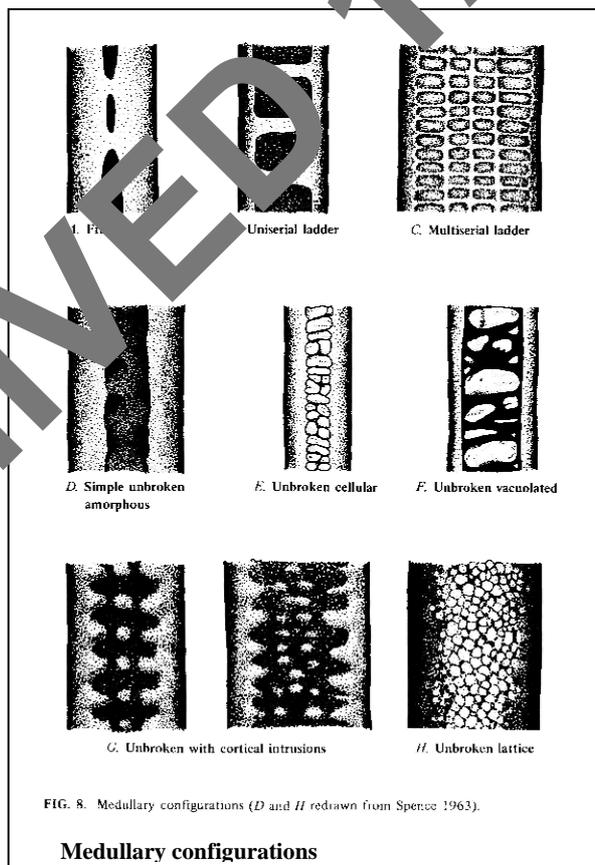
The medulla, when present in human hairs, is amorphous in appearance, may be transparent or opaque, and the width is generally less than one-third the overall diameter of the hair shaft. The medulla in animal hairs is normally continuous and structured and generally occupies an area greater than one-third the overall diameter of the hair shaft.



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3.1.3 Determining Body Area or Somatic Region

Human hairs to be examined typically come from the head or pubic regions of the body. However, hairs originating from other body areas such as the face and limbs may also be encountered and analysts should become familiar with the morphologies of all types of human hairs. Hairs from other body areas may be of limited comparative value.

If the analyst is unable to identify the somatic region of the hair the analyst should indicate so in their notes. The analyst may consult with a qualified hair analyst, if one is available, or they may indicate that the hair examined exhibits head-like or pubic-like characteristics in their notes.

General Body/Somatic Characteristics:

Head Hairs

1. Long with moderate shaft diameter and diameter variation.
2. Medulla absent to continuous and relatively narrow when present compared with its structure in hairs from other body areas.
3. Often with cut or split tips.
4. May show artificial treatment (solar tanning, dyeing, or mechanical damage).
5. Soft texture (pliable).
6. Little or no taper.

Pubic Hairs

1. Shaft diameter coarse with wide variations and buckling.
2. Medulla relatively broad and usually continuous when present.
3. Follicular tag often present on the root.
4. Tips usually rounded or abraded.
5. Stiff texture (wooly).

Limb Hairs (arm/leg)

1. Diameter fine with little variation.
2. Gross appearance of hair is arc-like in shape.
3. Medulla is broad, discontinuous and with a granular appearance.
4. Stiff texture.

Facial Hair (beard/mustache)

1. Diameter very coarse with irregular or triangular cross-sectional shape.
2. Medulla very broad and continuous may be doubled.
3. Blunt or razor (angular) cut tips.

Chest Hairs

1. Shaft diameter moderate and variable.
2. Tips are often darker in color, long to fine, arc-like.
3. Stiff texture
4. Medulla may be granular.

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Axillary/Underarm Hairs

1. Resemble pubic hairs in general appearance, but are less wiry.
2. Moderate diameter variation with less buckling than pubic hairs.
3. Medullary appearance similar to limb hairs
4. Tips long and fine may appear yellowed or bleached.

Eyebrow/Eyelash hairs

1. Very short, stubby, coarse, pointed tips.
2. Saber-like appearance.

3.1.4 Determining if a Hair is Suitable for Autosomal STR Analysis

Human hairs are amenable to nuclear DNA and mitochondrial DNA analyses. DNA analysis should always be considered in those cases when the source of a hair is crucial to an investigation. The condition and macroscopic assessment of the hair will determine which type of DNA analysis should be employed.

Hair roots that are in the active growing phase (anagen) contain an abundance of nucleated cells in the root and in the surrounding sheath material. Shed hairs from telogen follicles are the most commonly encountered in case work. Telogen hairs without follicular tissue may not be amenable to nuclear DNA analysis because of the lack of nucleated cells. These hairs may contain sufficient mitochondrial DNA in their roots and hair shafts for analysis.

While the appearance of anagen roots is a little variable, most often they present as a flattened, ribbon-like structure. Often with pigmentation present all the way down to the end of the root. Anagen hairs sometimes have cellular sheath material attached, but this is not always the case. The sheath can appear clear to cloudy. Anagen hairs do not fall out in the normal course of events and require some force to detach them from the dermal papilla. The hair may be twisted, stretched, or broken because of the forces used to remove it from the follicle.

The catagen phase is transitional (about 2 weeks) and is difficult to identify. You will not likely see hairs in this stage. The root sheath will be a bit dry and may appear to be club-shaped; there will be some diminished pigmentation of the root; medullation, when present, will be farther out in the hair shaft. Note hairs collected at autopsy will most often resemble a hair in the 'catagen' phase. Analysts should consult a qualified hair analyst, if one is available.

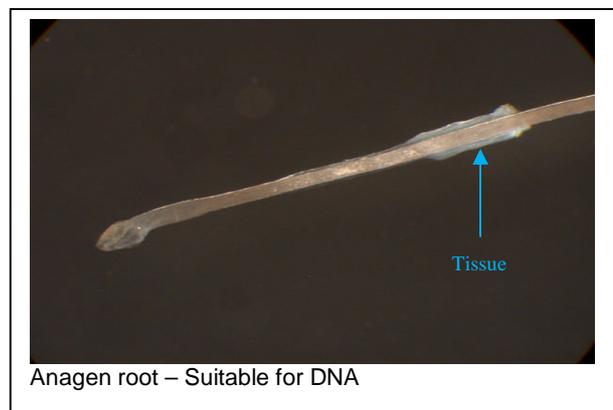
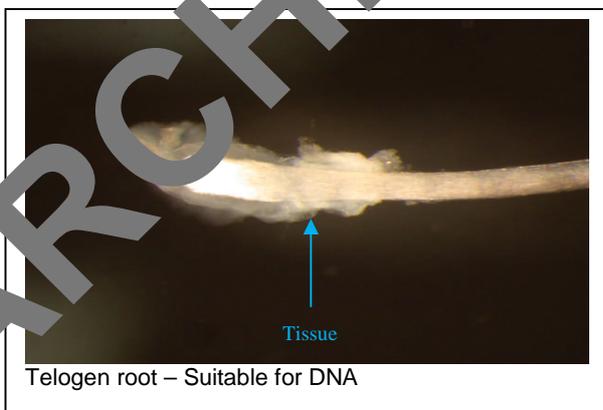
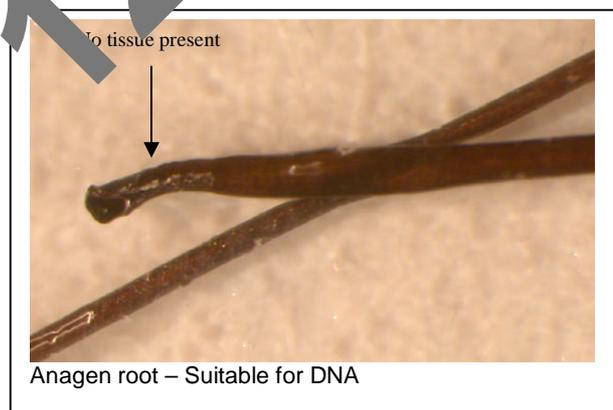
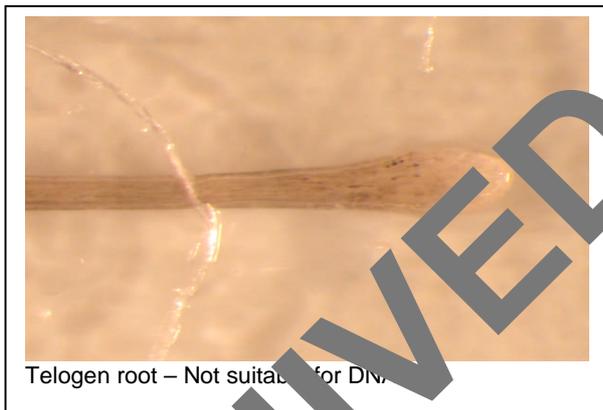
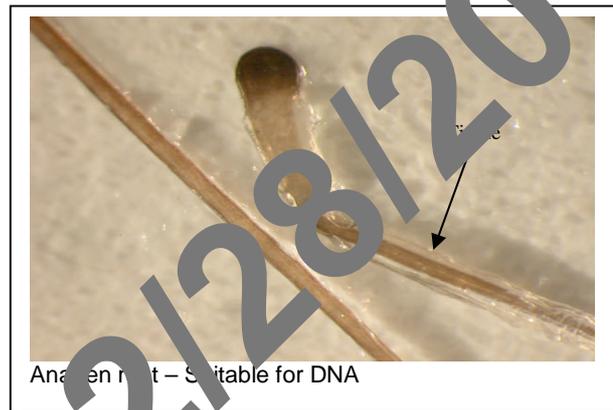
A telogen root will have no sheath material around the root area, there will be no pigmentation close to the root area, and it will be clear. There should be a lot of cortical fusi between the pigmentation and the root bulb. Some telogen hairs may contain a follicular tag (more common in pubic hairs than head hairs).

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When using nuclear DNA, there needs to be some amount of tissue/sheath material present. The best hair would be an anagen hair or early catagen. About 2 centimeters of good root area is needed to get sufficient material for DNA analysis. While only one centimeter is needed for mtDNA analysis.



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3.2 Vaginal/Cervical, Rectal and Oral Swabs and Smears

Samples collected within the following time frame should be examined for the presence of spermatozoa:

- Vaginal/Cervical - 7 days
- Rectal - 2 days
- Oral - 1 day

These guidelines, based on published literature, apply only to living adult female victims. Different considerations may apply to samples collected from children, adult males, and homicide victims.

Procedure

- Stain and grade the smear(s) ([Section 4.4](#)). Document your observations
- If spermatozoa (1+ - 4+) are detected on the smear(s), place the envelope containing the swabs/smear(s) from the assault in an envelope and create a separate item of evidence to be retained in the laboratory freezer. Note: if swabs are not available, the smear(s) should be retained for possible DNA analysis.
- If no spermatozoa or fewer than 1+ are observed on the smear(s), sample/cut a small portion of the swab(s) and follow the Extraction Protocol (see [Section 4](#)), beginning with Step 1.
- Note: If the sample consists of less than 5 sperm heads, the identification must be verified by another analyst. Two or more spermatozoa must be identified to report the presence of spermatozoa.

3.3 Miscellaneous Evidence Swabs

Miscellaneous evidence samples such as blood, semen, saliva or DNA (contact/touch) swabs may also be collected and submitted for analysis.

Samples present in the Miscellaneous Swabs - Semen envelope or any other envelope labeled as a possible semen or a wood's + sample may be examined for the presence of spermatozoa ([Section 4](#)). Swabs collected from the external genital area may be retained for saliva/foreign DNA in cases of alleged or possible oral assault.

Samples present in the Miscellaneous Swabs - Blood envelope or any other envelope labeled as a possible blood sample may be examined for the presence of blood ([Section 4](#)).

Samples present in the Miscellaneous Swabs - Saliva envelope or any other envelope labeled as a possible saliva, breast swab, bite mark, or licked/sucked area will be retained as a separate item of evidence for further analysis.

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Samples present in the Miscellaneous Swabs – DNA envelope or any other envelope labeled as a possible contact/touch or skin cell sample may be retained as a separate item of evidence for further analysis.

The laboratory does not conduct presumptive testing for the presence of saliva, urine or feces. Currently there are no confirmatory tests for the presence of these body fluids.

3.4 External Genitalia Swabs

Swabs collected from the external genitalia area may be examined for the presence of semen/spermatozoa ([Section 4](#)).

Samples from the external genitalia area include the Mons Pubis, Labia Majora, Labia Minora, Introitus, Perineum, Anus, Penis and Scrotum.

Samples may be retained for saliva in cases of alleged or possible oral assault (as needed). No presumptive testing will be conducted.

Penile swabs obtained from the victim/suspect within 24 hours of the offense may be examined for the presence of blood, epithelial cells or spermatozoa (useful in cases involving multiple suspects or to help establish recent contact) prior to isolation. The analyst will add any relevant case information to the packaging and/or to the notes section in JusticeTrax ([Section 3.11](#)).

3.5 Fingernail Scrapings, Finger and Hand Swabs

Fingernail scrapings and finger and hand swabs may contain biological evidence such as blood, tissue, epithelial cells and spermatozoa as well as trace evidence (fibers). Victim fingernail scrapings are examined in instances where it is believed/or indicated that the victim has scratched the assailant or in cases involving homicide. Suspect fingernail scrapings and finger and hand swabs may be useful in cases involving digital penetration. Due to the limited quantity of material recovered in most cases, the presence of potential biological evidence suitable for DNA analysis will be given priority over biological and trace evidence examinations. If the case scenario suggests the presence of spermatozoa is likely, the analyst will make a note on the retained items packaging and in the notes section in JusticeTrax ([Section 3.12](#)).

3.6 Condoms

Condoms may contain evidence that can be isolated and compared to the DNA profiles of suspects or victims in sexual assault cases.

- Document the condition of the condom. Imaging may be used as needed.
- Label one pair of sterile cotton swabs “outside”. Moisten the swabs using sterile deionized water. Swab the “outside” surface of the condom, as received.

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- Prepare a smear from the swabs collected.
- Repeat for the “inside” surface of the condom.
- Stain and grade the prepared smears ([Section 4.4](#)).
- If spermatozoa and/or nucleated epithelial cells are present, create a new item of evidence that includes the swabs and prepared slides. If spermatozoa and/or nucleated epithelial cells are not present, the swabs and slides/smears may be packaged with the original item.

Note: Acid phosphatase/BCIP testing should not be conducted when screening condoms. The laboratory has encountered condoms containing seminal fluid/spermatozoa that tested negative using BCIP.

3.7 Contact/Wearer Sources of DNA

Items not examined for the presence of biological evidence may be sampled by swabbing the item for contact/touch sources of DNA. Standard screening protocols apply.

- Document the item by imaging.
- Moisten a sterile cotton-tipped swab using sterile water.
- Swab the area(s) of interest. If sampling clothing, swab the entire interior surface of the garment.
- Package and retain the isolated sample(s) for DNA analysis.

3.8 Known DNA Samples

Case related known DNA samples (also referred to as reference samples) are required for DNA analysis. Known blood samples present in the assault kit(s) will be dried, packaged and retained for further analysis as needed. Do not process more than one case at a time.

- Wear appropriate protective gear when handling liquid blood samples (i.e., lab coat, gloves, eye protection, and mask).
 - Clean the hood by using a diluted bleach solution (10 – 20%) or a suitable cleaner.
 - Place a poly towel in the hood.
- Prepare and label a FTA® Classic Card with the Lab Number, Item number, Name and additional identifier (DOB, Social Security Number, APSIN, etc).
- Mark the exterior packaging of the item with the case number, item number, your initials, and the date opened (if needed).
- Open the vial of blood. Using a sterile plastic pipette, carefully fill each of the four circles on the drying card.
- With evidence tape, seal the vial of blood around the cap area. Label with your initials and date.

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- Reseal the exterior packaging with evidence tape. Label with your initials and date.
- Allow the card to completely air dry in the hood.
- Prepare and label a foil envelope with the case number, item number, and description of the contents ([Section 3.12](#)). Place the card into the foil envelope, add a desiccant packet and seal with evidence tape. Label with your initials and date.
- Retain per laboratory protocols.

3.9 Semen Stain Analysis

Semen is important physical evidence in rape cases. The detection and identification of semen is initiated by presumptive testing for the presence of acid phosphatase and confirmed by microscopically identifying spermatozoa, and occasionally by testing for human seminal protein p30. Semen stains with sufficient spermatozoa present are normally subjected to DNA profiling to provide information concerning the semen donor.

Suspected semen stains can be located by a number of methods, including careful visual examination, the use of tactile senses, fluorescence under alternate light sources, and chemical presumptive testing.

Visual and Tactile Examination

- Most dried semen stains on cloth are detectable visually because their color is different from that of the material on which the semen has been deposited. Semen stains that have been exposed to warm, moist conditions may assume a yellow coloration due to the growth of bacteria.
- Seminal stains frequently have a stiffening effect on fabric. Semen may form a crusty stain.
- Examine the item using an alternate light source such as an Omniprint™ 1000B, or UV lamp ([Section 3.9.1](#)). Semen stains frequently fluoresce. They can appear as a bright fluorescence on a dark background or as a dark area when the background itself fluoresces.
- Mark the suspect stains with a pen and proceed with chemical presumptive tests and microscopic confirmatory tests.

Chemical Examination

Semen stains can be located by detecting the presence of acid phosphatase activity. Suspected stains can be lightly swabbed with a water-moistened cotton swab and tested with BCIP or STMP solutions ([Section 4](#)).

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3.9.1 Omniprint™ 1000A/1000B Operating Instructions

General Safety Guidelines

- It is essential that proper eye protection be provided for and worn by anyone operating an intense light source such as the Omniprint™ 1000B. Permanent eye damage can occur from specular (direct illumination to the eye) or reflected refractive light hitting the eye. Label goggles for the specific wavelength. Do not allow use of inappropriate or incorrect goggles. Some goggles offer only a narrow band of protection.
- Remove all unnecessary reflective surfaces from the area (exam room). Avoid looking at reflections in shiny spherical objects such as doorknobs, watch crystals, tools, jewelry, window panes, mirrors or any other surface that may reflect light.
- Exposing the skin to the beam of light (directly from the unit) can cause burns and other skin damage. There is no hazard with skin exposures to the beam emitting from the liquid light guide or fiber optic cables as temperatures are decreased, but the direct emission of the light from the discrete setting is very warm.

Proper Operation of the System

- Check to see that both switches are in the “off” position.
- Plug the unit into a three-prong grounded outlet. If an extension cord is used, it must be a heavy duty grounded cord.
- Turn on the power rocker switch (marked “Line”). The switch will light, and the fan will begin to operate. You may now turn the lamp switch on (marked “Lamp”). The lamp should light within a few seconds.
- You may hear a ticking noise and see brief flashes of light while the lamp is attempting to ignite. This is normal.
- If the lamp fails to ignite within two minutes, and you hear a ticking noise, turn the unit off. You may have a bad lamp. Lamps are guaranteed for 500 hours of use, provided it has been used in the prescribed manner (periods of at least 15 minutes). Replace the lamp (see Lamp Changing Instructions provided with the manual). The lamp should be left running for periods of at least 15 minutes. The lamp must cool after it has been turned off and should not be restarted until it has fully cooled. Although the minimum suggested operating time is 15 minutes, it is important to note it is better to operate the lamp for continuous periods, rather than turning the lamp on and off. This procedure will increase lamp life.
- If you do not hear a ticking noise and the lamp does not light, this is an indication that the power supply is not functioning properly. Please contact Omnicrome for further instructions.

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- Operation in high ambient temperatures – the Omniprint™ 1000A/1000B is equipped with over temperature protection. If the instrument is being operated at ambient temperatures exceeding 100° F, the over temperature protector may shut off the lamp. When the unit cools sufficiently, the lamp will come back on automatically.

Filter Selections

The wavelengths are selected by simply turning the knob marked "Wavelength Selector" in either direction. A green LED light will appear next to the selected wavelength.

- **UV** (Omniprint™ 1000B) ultraviolet filter (long wave UV) is perfect for locating evidence treated with sensitive powders or dyes, serology and bite mark evidence. Use only the liquid light guide or direct output optical piece with this filter selection. The fiber optic cable will not transmit UV light below 340 nm.
- **450nm** band when used with yellow goggles or filters provides near UV excitation. Generally the optimum setting for the detection of physiological stains such as semen, saliva, urine and blood. Forensic podonomologists and pathologists prefer this setting for bite mark and bruising detection and photographic documentation.
- **485nm** band has a narrow bandwidth of 24nm. This wavelength, when used with the orange goggles or filters, provides great contrast and is most commonly used on surfaces treated with chemical dyes such as Rhodamine 6G. Also useful for the detection of trace evidence such as fibers and paint flecks.
- **525nm** band when used in conjunction with the orange goggles is useful for trace evidence and fingerprints treated with fluorescent powders and dyes.
- **530nm** broad band filter covers all wavelengths below 530nm. Use with the orange viewing goggles or filters. This wavelength is perfect for locating and viewing all types of evidence as it has the highest output power. Due to the broad bandwidths, it is recommended that other settings be used if background fluorescence occurs.
- **570nm** band has a 35nm bandwidth for excitation of ink fluorescence. In addition, this setting will give optimum results on DFO treated prints on paper items that exhibit strong background fluorescence. Red goggles and camera filters are recommended.
- **IR** (Omniprint™ 1000B) infrared filter is used in question document examinations, gunshot residue detection and possible bloodstains. Use only the fiber optic cable or direct output optical piece with this filter selection. The liquid light guide will be damaged if used with this filter due to the heat of infrared light. Open "white" light covers all wavelengths from 400 to 700 nm. This is useful for visible search procedures and lighting for evidence photography.

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Shutdown Procedure

- Push the “Lamp” rocker switch off. You must now wait for the unit to cool down. The system includes dual fans for cooling.
- After feeling that the body of the unit and the exhaust are cool, the “Power” rocker switch may be turned off. The cool-down period is approximately 5 minutes.
- Never turn the lamp back on until the unit has completely cooled.
- If the unit is to be moved, remove the light guide(s) by gently pulling the cable out of the aperture. Gently wind the cable into a loose coil and place cable in safe place or back into the case.
- The liquid light guide must not be wrapped or coiled too tightly as this can permanently damage the cable. The liquid light guide is very fragile and will become damaged if it is kinked, stepped on, bent, or frozen. Damage may not be evident, but there will be a decrease in output power (brightness).

3.10 Blood Stain Analysis

A common form of physical evidence found at a scene of a crime involving physical violence is that of blood. Blood may be present in the form of pools, spatters, or stains. The detection and characterization of blood is initiated by presumptive testing for peroxidase-like activity of hemoglobin. Stains testing positive to a presumptive test for blood may be further characterized by determining if the stain is animal or human in origin or by DNA profiling to provide information as to the blood’s source.

Suspected bloodstains can be located by a number of methods, including careful visual and stereoscopic examination, the use of alternate light sources and chemical presumptive testing.

Visual Examination

- Most dried bloodstains on cloth can be detected visually because their color is different from that of the material on which the blood has been deposited. Blood stains range in color from a reddish-brown to black.
- Blood deposited on dark colored items may be difficult to locate visually. The use of an infrared hand held scope, video-imaging system using an infrared filter, or Oniprint™ 1000B may assist in the location of possible bloodstains. Use of these sources will provide the contrast needed to search for stains that are not visible otherwise.
Bloodstains on clothing tend to be wicked into the substrate unlike dirt and debris, which will be found on and around the fibers. This can easily be seen microscopically.
- Bloodstains deposited on a hard surface such as glass, a knife, and so on, will dry on the surface as a film or a crust.

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Chemical Examination

- Visible stains can be lightly swabbed with a water-moistened cotton swab and tested for peroxidase-like activity with Phenolphthalein reagent and hydrogen peroxide ([Section 5.1](#)).
- Invisible stains can be located using alternate light sources ([Section 3.9.1](#)) followed by detection of peroxidase-like activity ([Section 5.1](#)).
- Stains testing positive to a presumptive test for blood may be further characterized as to their origin using Human Hemoglobin analysis by immunoassay to determine species ([Section 5.2](#)).

3.11 Processing, Documentation and Isolation of Biological Stains

- Receive evidence.
- Wear appropriate protective gear when handling biological evidence (i.e., lab coat, gloves, eye protection, mask).
- Mark the exterior packaging of each item of evidence with the case number, item number, the analyst's initials, and the date (as they are processed). If possible, the item itself should be labeled away from stained areas with the case number, item number, and the analyst's initials. (Exercise caution in labeling articles that will be submitted for latent print examinations so that any latent print evidence is not obscured or obliterated).
- Clean the lab bench by using a 10% bleach solution or other disinfectant cleaner. Ensure that any bleach residue is thoroughly removed by wiping it down with another clean paper towel. Place evidence on clean laboratory paper. Open and examine one item at a time, gaining access to the evidence without totally destroying the packaging or previous seals where possible. Mark each item individually away from stained areas.
- If any preliminary testing was done prior to submitting the item to the laboratory, record the nature of the test and the results obtained. Identify field-tested stains as "possible" blood or semen stains.
- Take detailed notes describing each item and its container. Include the condition of the item, nature and location of the staining and any damage observed. Use sketching, imaging, and/or photography. For items requiring latent fingerprint examinations, the analyst should consult with a latent fingerprint analyst prior to biological testing.
- Collect other trace evidence that may be present and of possible forensic significance ([Section 3.1](#)).

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- Isolate those stains that have tested positive to a presumptive test for blood, the presence of spermatozoa, or PSA activity for further analysis. If the sample is "limited", do not attempt any further tests. If the stain is large, only a generous portion needs to be isolated. Document in your case notes that a portion of the stain remains on the item. Package each isolated stain to be retained in an individually sealed envelope. Label each envelope with the lab number, a unique identifier (item/stain #), your initials and date. Place all sealed/isolated stain envelopes into a larger manila envelope. Label this envelope with the agency number, lab number, unique identifier (item#), your initials, date and list of the envelope's contents.
- Control samples will be isolated where appropriate by a DNA analyst only. Control samples submitted, as evidence will be retained, as needed.
- Report findings. The report will indicate the items that have been tested, the unique identification number (item #) given to those items, and the test results for each item.

Other Considerations

- To avoid contamination, do not allow one evidence stain to come into contact with other biological samples. Do not collect or package two separate stains together. Do not allow evidence samples to come into contact with any surface that may contain residue from another biological sample (i.e., dirty tweezers, blood stained glove, contaminated work surface). Tools can be cleaned by thoroughly rinsing with a stream of distilled water and drying thoroughly with paper tissue or by rinsing with ethanol and flaming. Repeat this process twice before using the tool to manipulate another sample.
- Small biological stains (i.e., 2mm size bloodstain) are most susceptible to contamination. Perform hand hygiene before collecting small stains. If stains have to be manipulated by tool, consideration should be given to using new, disposable instruments.
- Change gloves between items or between pertinent groups of items (i.e., suspect and victim items from different crime scenes).
- It is important to save as much sample as possible to permit reanalysis at a later date if needed. The analyst should not perform any presumptive and/or confirmatory testing methods on any stains or samples where the stains or samples would:
 - Be consumed by the testing conducted (or)
 - Would prevent re-analysis by the crime lab or any outside laboratory/agency.

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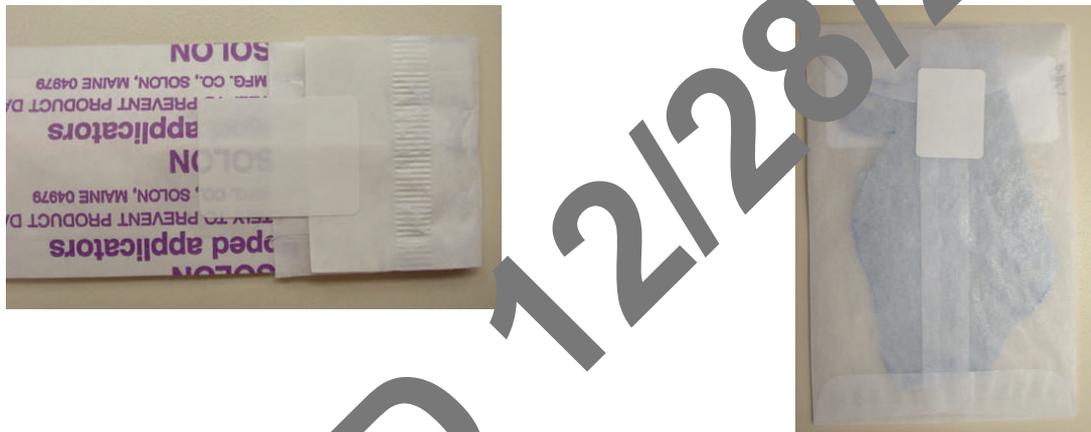
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3.12 Packaging of Isolated Stains

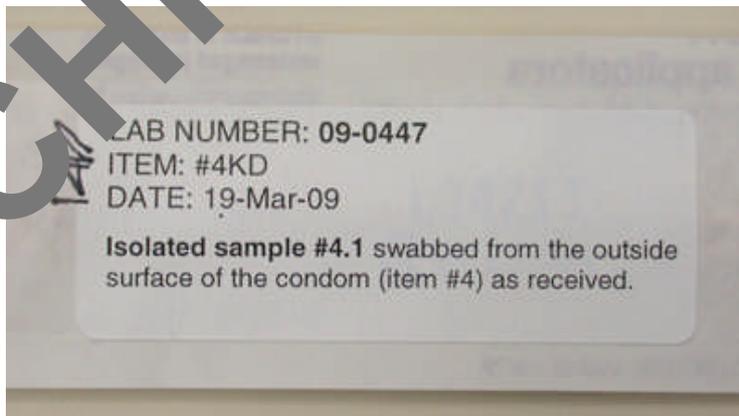
- Place the dried swabs back in their original package(s) or a swab carton. Place any isolated cuttings into a glassine envelope. Secure the package(s). Scotch tape, a MACO® label (3/4" X 1"), or any other suitable label or tape may be used (Figure 1). Please do not use evidence tape.

Figure 1



- Label each swab package, swab carton, or glassine envelope with the lab number, unique identifier (item #/your initials), a brief description/isolated stain number, and the analyst initials along the edge of the label (Figure 2).

Figure 2



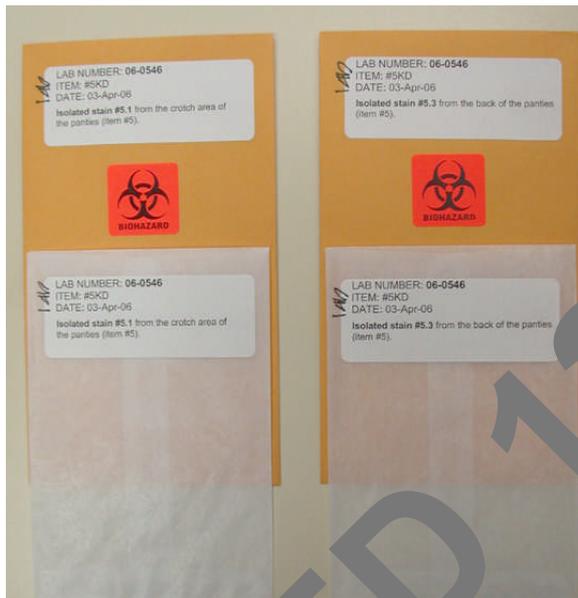
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- Place each isolated stain/sample to be retained into another envelope and seal with evidence tape. Label each envelope with the lab number, unique identifier (item #), a brief description, your initials, and a biohazard label (Figure 3).

Figure 3



Note: An envelope does not need to be used when only one isolated stain or multiple swab packages represent a single isolated stain/sample are collected.

- Place all sealed isolated stain envelopes into one larger envelope. Label this envelope with the lab number, unique identifier (item #), your initials, and a brief description of the contents. Note any relevant case information if applicable (Figure 4). Place a biohazard label, and the LIMS bar-coded label on the envelope (Figure 4).

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Figure 4



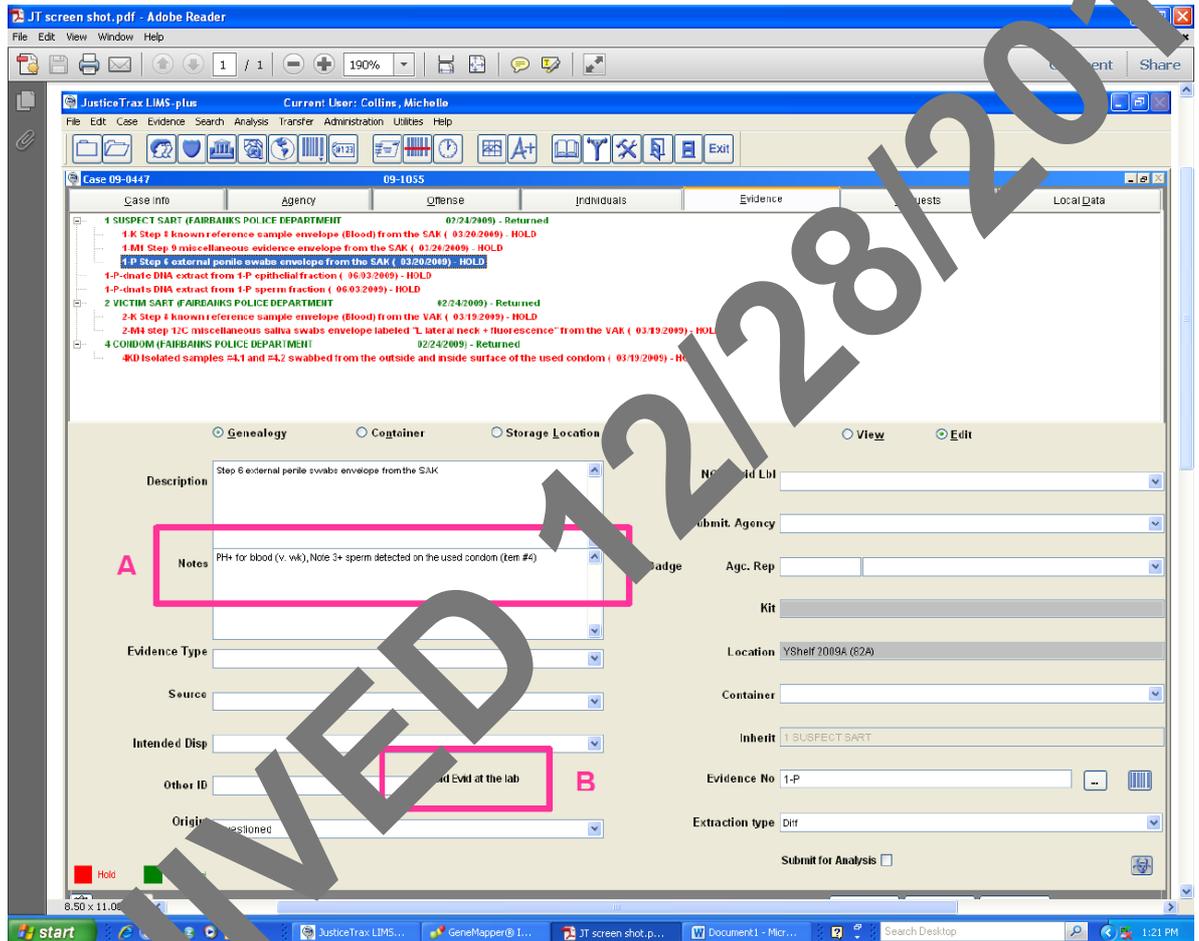
- In the “Notes” section in JusticeTrax (A) document any relevant case/sample information as needed (Figure 5). Example: sperm 2+ detected on the vaginal smear, victim had consensual intercourse with boyfriend the day before the offense, etc.

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Figure 5



Mark items to be retained for DNA analysis in JusticeTrax by checking the box labeled "Hold Evid at the lab" (B).
 Transfer the evidence to the tote (if applicable) and return it to the evidence section of the laboratory.

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- Saferstein, R., "Chapter 12 Forensic Serology" in: Criministics: An Introduction to Forensic Science, Fifth ed. 1995: 346-382.
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SECTION 4 – PRESUMPTIVE AND CONFIRMATORY TESTING FOR SEMEN/SEMINAL FLUID

4.1 BCIP (5-bromo-4-chloro-3-indolyl phosphate)

BCIP solution is used for rapid screening of suspected semen stains to detect acid phosphatase.

Reference

F.S. Baechtel, J. Brown and L.D. Terrell, "Presumptive Screening of Suspected Semen Stain In Situ Using Cotton Swabs and Bromochloroindolyl Phosphate to Detect Prostatic Acid Phosphatase Activity," J. For. Sci., 32, pp. 880-887, (1987).

Procedure

- Place 200µL of BCIP substrate solution in labeled glass test tubes; one test tube for each sample. (Prepare a test tube for a positive control, a negative control, and for each Q sample).
- **Negative Control:** Moisten a sterile swab with a minimal amount of sterile deionized water. Place the swab in labeled test tube with BCIP solution. (A Negative Control is required for each run and should be the first sample prepared).
- **Q Samples:** Moisten sterile swab with a minimal amount of sterile deionized water. Lightly swab the questioned stain with the swab. Place the swab in a labeled test tube with BCIP solution.
- **Positive Control:** Moisten a sterile swab with a minimal amount of sterile deionized water. Swab a human semen sample with the swab for a positive control. Place the swab in labeled test tube with BCIP solution. (A Positive Control is required for each run and should be the last sample prepared).
- Put the test tubes in a rack. Place the rack in a 37°C water bath and incubate for 15 minutes. Document the water bath temperature in the log book. Record the following in case notes: lot # and expiration dates for the BCIP solution, lot # for the sterile water, and the sample # of the human semen stain used.

Interpretation of Results

A positive test result is an aqua (blue-green) color. The appearance of an aqua color indicates the presence of acid phosphatase activity.

Additional Notes

- The BCIP procedure is not specific for semen.
- The BCIP procedure is 99% accurate in predicting a true negative stain, i.e. one out of 100 negative BCIP tests may be false. (reference Baechtel paper)

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- The BCIP procedure has not been found to be useful in screening latex condoms. The laboratory has encountered condoms containing numerous spermatozoa that yielded negative BCIP results.
- This test should not be performed on body cavity swabs since spermatozoa may be found on vaginal swabs which do not test positive to the BCIP reagent.

4.2 Thymolphthalein Monophosphate

Thymolphthalein Monophosphate is used for screening of suspected semen stains to detect acid phosphatase activity.

References

Roy, A. V., Brower, M. E. and Hyden J. E., "Sodium Thymolphthalein Monophosphate: A new Acid Phosphatase Substrate with Greater Specificity for the Enzymatic Enzyme in Serum", Clinical Chemistry Vol. 17, No. 11, 1971.

Seiden, H. and Duncan, G., "Presumptive Screening Test for Seminal Acid Phosphatase Using Sodium Thymolphthalein Monophosphate", Proceedings from a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, FBI Academy 1983.

Procedure

- Positive & Negative Control: A known human semen standard and a sterile water blank are required for each day that the presumptive test reagents are used to demonstrate that they are working properly.
- Moisten a sterile swab with a minimal amount of sterile deionized water. Lightly stroke the questioned stain with moistened swab.
- Add one drop of Solution #1. Wait one minute.
- Add 2-3 drops of Solution #2.
- **Note:** this method may also be performed by pressing a piece of filter paper moistened with deionized water over a suspected semen stain for a few seconds.

Interpretation of results

The rapid development (within 15-30 seconds) of a blue color indicates the presence of a high concentration of acid phosphatase. Lower levels of acid phosphatase result in a green color.

Acid phosphatase catalyzes the hydrolysis of the ester-linked phosphate group from thymolphthalein monophosphate producing thymolphthalein and phosphate.

Thymolphthalein is blue in the presence of base. Acid phosphatase is present in high concentration in seminal fluid, however the enzyme is present in other body fluids including vaginal fluid and feces. Other sources include plants, fungi and bacteria. Sources other than semen generally produce slow, weak reactions.

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4.3 Extraction of Suspected Semen Stains

After determining the possible presence of acid phosphatase in a stain with BCIP, the following procedure will provide an extract of the soluble substances and a pellet of the particulate material for analysis. Body cavity swabs are not subjected to BCIP testing prior to extraction.

Procedure

- Label the appropriated number of tubes needed (2.0mL polypropylene Eppendorf™ tube or equivalent). Using clean utensils, place a small cutting of the stain/swab(s) into each tube. Swabs collected from the same area may be grouped.
- Add 200µL of sterile deionized water to each cutting/sample in the tube and soak for at least 30 minutes at 37°C. The extraction may also be accomplished overnight at 37°C or in the refrigerator at 4°C.
- Label a microscope slide for each sample. Transfer the material in each tube with a clean toothpick or a sterile pipette tip. Pipette 3µL of the extract onto a microscope slide. Follow the staining protocol ([Section 4.4](#)) and examine the slides for spermatozoa.

Optional: To improve recovery of the spermatozoa, the following procedure may be substituted:

- Agitate the cutting/sample using a clean toothpick for approximately one minute. Use a clean toothpick to transfer the cutting into a spin basket and insert into the 2.0mL tube containing the extract. Centrifuge for 1 minute (to maximize the recovery of extract and to allow cells to form a pellet). Discard the basket with the cutting. (The supernatant may be used for PSA/P30 analysis.)
- Label a microscope slide for each sample. Re-suspend the pellet and pipette 3µL of the extract onto the microscope slide and allow the sample to dry. (The 3µL may be withdrawn from the pellet, but this should be clearly stated in case notes for interpretation by DNA analysts). Follow the staining protocol ([Section 4.4](#)) and examine the slides for spermatozoa.
- If no spermatozoa are observed, a P30 analysis on the aqueous portion of the extract (supernatant) may be performed for cases involving, for example, homicide, sexual abuse of a minor, or with suspects reported to have had a vasectomy (see [Section 4.5](#) – PSA/P30 Protocol).

Microscope slides are packaged with the item after examination. All case related staining extracts are discarded after analyses are completed.

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4.4 Staining Protocol

Human sperm have a distinctive size and morphology and, with differential staining, can be conclusively identified.

References

Stone, I.C., "Staining of Spermatozoa with Kernechtrot and Picroindigocarmine for Microscopical Identification", SWIFS, Criminal Investigation Laboratory, Sept. 1992

Gaensslen, R., Sourcebook in Forensic Serology, Immunology, and Biochemistry, U.S. Government Printing Office, 1983.

Procedure

- Heat fix cells to a microscope slide by gently flaming or by placing the slide in a 37°C oven for 15 minutes.
- Slides may be examined microscopically prior to staining. Intact spermatozoa can often be detected. If intact spermatozoa are observed, staining is not required.
- Cover the slide/debris with Nuclear Fast Red stain and let stand for 10-15 minutes covered.
- Gently wash away the Nuclear Fast Red with deionized water.
- Without drying the slide, cover the slide/debris with Picroindigocarmine stain (PICS) and let stand for 15-20 seconds. Gently wash the slide with reagent grade ethanol. Allow to air dry.
- Add Permunt and cover slip. Examine the slide microscopically (200-400x).
- Document your observations. Record the presence of nucleated epithelial cells and if the spermatozoa present are intact.

Interpretation of Results

Nuclear material is stained red by the Nuclear Fast Red dye. Sperm heads are usually well differentiated with the acrosome staining significantly less densely than the distal region of the head. Picroindigocarmine stains the epithelial membranes green. Nuclei inside the epithelial cells appear purple. Yeast cells also stain red; however, the stain is uniform throughout the cells and extends into polyp-like structures, which are occasionally observed with yeast cells.

- If spermatozoa are detected, score the number observed on a scale of 1+ to 4+.
 - 1 + Hard to find
 - 2 + Some in fields, easy to find
 - 3 + Many or some in most fields
 - 4 + Many in every field
 - <10 Few

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- Record the presence of nucleated epithelial cells (NECs), non-nucleated cellular debris and presence of tails. NECs may also be graded using the scale given above.
- At least two spermatozoa must be observed to report the presence of spermatozoa. An observation of less than five spermatozoa must be verified by another qualified analyst.

4.5 PSA (P-30 or Prostate-specific Antigen)

PSA (P-30) is a glycoprotein produced by the prostate gland and secreted into seminal plasma. PSA is a reliable forensic marker for the identification of seminal fluid. The use of the ABACard® by this method is a rapid and sensitive detection method for PSA. The sensitivity of this system is 4 nanograms of PSA per milliliter of body fluid, which is comparable to the ELISA technique.

This test is normally performed after obtaining a positive presumptive test for seminal fluid (acid phosphatase) and sperm search results are negative for the presence of spermatozoa.

References

Manfred N. Hochmeister, et al, "Evaluation of Prostate-specific Antigen (PSA) Membrane Test Assays for the Forensic Identification of Seminal Fluid", J Forensic Sci 1999;44:1057-1060.

J. Kearsey, et al, "Validation Study of the 'Onestep ABACard® PSA Test' Kit for RCMP Casework", Can. Soc. Forensic Sci. J. Vol. 34. No 2 (2001) pp. 63-72.

Theresa F. Spear and Neelam Khoskebari, "The Evaluation of ABACard® p30 Test for the Identification of Seminal Fluid", CACNews 1st Quarter 2001, pp. 22-24.

Procedure

- Label the sample tubes. Using clean utensils, place a portion of the suspected semen stain or swab into a sterile 2.0mL microcentrifuge tube.
- Add 200µl of sterile deionized water to each sample tube. Allow the sample(s) to extract at 37°C for a minimum of 30 minutes. The sample may be extracted at 2-8 °C overnight ([Section 4.3](#)).
- Known human PSA standard and sample blank are to be run as an assay control. Dilute 2 - 10µl PSA standard L-F500 in 200µl of sterile deionized water. Record all lot numbers and expiration dates.
- Place the substrate into a spin basket and centrifuge the sample for 3-5 minutes.
- Remove the device and dropper from the sealed pouch.
- Label the PSA card with the case number, item number, your initials and the date. The sample(s) must be brought to room temperature prior to testing.

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- Remove 150µl (or 4 drops) of the supernatant and add it to the sample region ("S" well) on the PSA card. This aliquot may be stored between 2-8°C if not used immediately.
- Allow the sample to stand for 10 minutes. Positive results can be seen as early as 1 minute depending on the P-30 concentration.
- The results of the test should be documented using digital imaging, 35 mm photography, or by photocopying the card(s).

Interpretation of Results

Positive: The formation of two pink lines, one in the test area (T) and one in the control area (C) indicates a positive test result and that the P-30 level is at or above 4 ng/ml. Another analyst should confirm weak results (if needed, based on intensity of the digital image).

Negative: The formation of only one pink line in the control area (C) indicates a negative test result. This may indicate that (a) No PSA is present above 4 ng/ml or (b) presence of "high dose hook effect". Presence of "high dose hook effect" may give a false negative result due to the presence of high concentrations of PSA in the sample. In such cases the sample may be retested using a 1:10 to a 1:1000 dilution of the sample in question using the remaining 100µl of sample.

Inconclusive: There is no formation of a pink line in either the test area (T) or the control area (C) of the card. Repeat the test and reexamine the test procedure carefully.

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SECTION 5 – PRESUMPTIVE AND CONFIRMATORY TESTING FOR BLOOD

5.1 Phenolphthalein (Kastle-Meyer Test)

This is an oxidative test for the presumptive identification of blood based on the catalytic activity of the heme group of hemoglobin. If the sample is of a limited nature and the presumptive test is likely to consume sufficient sample to prevent successful DNA typing, then the presumptive test is not performed. The limited nature of the sample will be documented on the DNA extraction worksheet.

References

Culliford, B., *The Examination and Typing of Bloodstains in the Crime Laboratory*. U.S. Government Printing Office, 1971.

Saferstein, R., *Forensic Science Handbook Volume 1*. Prentice Hall, Inc., 1983.

Gaensslen, R., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. U.S. Government Printing Office, 1983.

Procedure

- Record the lot number for the phenolphthalein reagent and sterile deionized water in your lab notes. Record the lot number and expiration date of the 3% Hydrogen Peroxide.
- Positive/Negative Controls: A known human blood standard and sterile water blank are required for each day that the presumptive test reagents are used to demonstrate that they are working properly.
- Questioned samples: Moisten a sterile cotton-tipped swab with sterile water. Rub the swab over the suspected bloodstain.
- Add 1-2 drops of the phenolphthalein working solution to the swab. Wait a few seconds and observe any color changes.
- Add 1-2 drops of the 3% hydrogen peroxide solution.

Interpretation of Results

The appearance of a rapidly developing pink color after the addition of 3% hydrogen peroxide (H₂O₂) is a presumptive positive test for the presence of blood. A pink color forming after one minute should not be considered as a positive result, as auto-oxidation can occur in air and light.

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Catalytic blood detection tests depend upon an oxidation reaction in which the oxidant, H_2O_2 , oxidizes a colorless substance, such as phenolphthalein, leucomalachite green or ortho-tolidine, to a colored one. The heme group of hemoglobin exhibits a peroxidase-like activity which catalyzes the breakdown of H_2O_2 .

Color catalytic tests are very sensitive, but not specific. The positive color test alone should not be interpreted as positive proof of blood. However, a negative result is proof of the absence of detectable quantities of heme or its derivatives.

The major sources of "false positive" reactions are chemical oxidants and vegetable peroxidases. Color development before the addition of H_2O_2 may be due to the presence of chemical oxidant. While copper and nickel salts are the most frequent chemicals responsible for false positive reactions, others include rust, some bleaches, iodine, insecticides, wood preservatives, and pigments.

Fruit and vegetable peroxidases react similar to blood but slower and more weakly. In addition, stains created by fruits and vegetables can generally be visually distinguished from blood and their peroxidases do not react with strongly basic solutions such as phenolphthalein (pH 14).

It should be noted that the presumptive test for blood is not species specific. Blood and other tissues from animals besides humans will also give a positive reaction with this test.

A negative result is indicative of the absence of detectable quantities of heme or its derivatives. In addition, if a field testing reagent such as Bluestar has already oxidized a sample, it may not react in subsequent testing.

5.2 One Step ABACard HemaTrace

The One Step ABACard HemaTrace test is designed to be used as a confirmatory test for human (primarily) blood. The test is a simple, rapid and a sensitive detection method for human hemoglobin (hHb). The sensitivity of this system is 0.05µg of hHb per milliliter.

Reference

Manfred J. Hochmeister, et al, "Validation Studies of an Immunochromatographic 1-Step Test for the Forensic Identification of Human Blood," J Forensic Sci 1999;44:1057-1060.

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Procedure

1. Label the sample tubes. Place a portion of the suspected bloodstain or swab into an entire tube of the extraction buffer provided (deionized water or other buffers suitable for DNA extraction may be used as well). This test is pH dependent; the pH must be in the range of 1-9. A known human blood standard (positive control) and a negative control (extraction buffer or deionized water) are to be run as an assay control. Record all lot numbers and expiration dates.

Optional: Extraction Method for old stains:

Extract sample in 2-3 drops of 5% ammonia (NH₃) for 2-5 minutes. Allow the ammonia to evaporate. Add 2-3 drops of extraction buffer. Confirm that the sample pH is in the range of 1-9 by using pH paper.

2. Allow the sample(s) to extract for 1 – 5 minutes.
3. Allow the sample(s) to warm to room temperature if refrigerated.
4. Remove the device/card and dropper from the sealed pouch.
5. Label the HemaTrace card with the lab number, item number, your initials and the date.
6. Using the dropper provided, add 4 drops or pipette 150 µl of the extract into the sample region (S) on the card.
7. Allow the sample to stand for 10 minutes. Positive results can be seen as early as 2 minutes depending on the heme concentration.
8. Document the test results using digital imaging, 35 mm photography, or by photocopying the card(s).

Interpretation of Results

The control line in the control area (C) can be considered an internal procedural control. A distinct pinkish line will always appear if the test has been performed correctly. If the control line does not appear, the test is invalid and a new test must be performed.

Positive: The formation of two pink lines, one in the test area (T) and in the control area (C) indicates a positive test result and that the human hemoglobin level is at or above 0.05 µg/mL. A second analyst should confirm weak results.

Negative: The formation of only one pink line in the control area (C) indicates a negative test result. This may indicate that:

- No human hemoglobin is present above 0.05 µg/mL or
- Presence of “high dose hook effect”. Presence of “high dose hook effect” may give a false negative result due to the presence of high concentrations of human hemoglobin in the sample. In such cases the sample may be retested using a 1:100 or 1:1,000 fold dilution of the sample in question using the remaining sample.

Inconclusive: There is no formation of a pink line in either the test area (T) or the control area (C) of the card. Repeat the test and re-examine the test procedure carefully.

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SECTION 6 – DNA EXTRACTION

6.1 General instructions pertinent to DNA extraction

- Appropriate personal protective equipment must be worn while handling evidence and during DNA extraction.
- DNA extraction must be performed in a designated area in the laboratory, with equipment dedicated to the DNA extraction area. Work surfaces should be cleaned with a 10% bleach solution before and after use.
- Sterile techniques appropriate to forensic procedures must be used when handling evidence items for DNA extraction.
- Analysts will use a clean cutting surface and sterile consumables for sampling items of evidence. Non-disposable items used for evidence handling, such as hole punchers and metal forceps, will be cleaned before and after use.
- Questioned samples and reference samples should be extracted separately in time and/or location.
- Extraction of samples with potentially high levels of DNA (for example, whole blood and/or buccal swabs) should be performed separately, or after, extraction of samples with potentially lower levels of DNA (for example, single hairs and/or small bloodstains).
- Tubes must be centrifuged before opening them. A de-capping device may be used, if desired, to open tubes. Only one sample tube must be open at a time.
- DNA extraction information should be documented on the appropriate worksheet. This worksheet must include the batch name, a description of the item, extraction procedure used (e.g. EZ1 Trace or Large Volume protocol for casework samples), extraction instrument, sampling and elution volume (for casework extractions) and the date extraction is started (e.g. the date the evidence is collected/sampled). If the questioned samples and reference samples are extracted on the same day, the time when each set of extractions was begun should be documented in the worksheet notes.
- Each batch of extractions must include an appropriate reagent blank(s) for each type of extraction performed. All reagents (lot# and expiration date) and reagent blanks used for a batch of samples must be documented in an appropriate worksheet documenting the standards and controls used in that batch.
- The volume amounts of buffers and other reagents in the extraction protocols are suitable for most forensic stains and reference samples. The volume of these reagents may be adjusted as noted in the protocols according to the size and nature of the sample (for example, when using the Large Volume Protocol).

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- Digital imaging of evidence
 - It may be appropriate for a DNA analyst to image an item of evidence that has not previously been examined and documented by another discipline in the laboratory (for example, a cigarette butt).
 - Digital images will be retained in the case file as part of the bench notes.
- Guidelines for the workflow between Biological Screening analysts and DNA analysts for processing evidence in sex assault cases:
 - Case reports issued by the DNA discipline do not need to include the results of presumptive tests and microscopic examinations when the tests were first performed and reported in a biological screening report.
 - DNA analysts do not need to perform presumptive blood testing when the testing was previously performed and reported in a biological screening report.
 - DNA analysts will continue to prepare sperm search slides of previously screened items and isolated stains and document their observations in the DNA extraction worksheet. These slides do not need routine technical review since the purpose of this action is to facilitate proper sampling of the item for DNA extraction and to monitor efficiency of the differential extraction process.
 - For items that may contain limited amounts of biological evidence (e.g. penile swabs, and/or fingernail scrapings/body swabs), the Biological Screening analyst will note any relevant case information on the item(s) packaging and/or in the notes section in the laboratory's information management system (LIMS). This action will alert the DNA analyst to the possible presence or absence of spermatozoa in the samples not previously screened.
 - For items not previously screened, and when appropriate, the DNA analyst qualified in sperm search and microscopy techniques will proceed with processing the sample and prepare a sperm search slide for microscopic inspection. A second qualified analyst may be requested, on a case-by-case basis, to preview this slide to verify these results. If this verification is performed, it is documented on the DNA extraction worksheet and by the second analyst's initials on the slide. Slide(s) will be retained and packaged with the evidence. The DNA case report, in such instances, will include a statement stating the presence or absence of spermatozoa in the sample(s).
 - Workflow between the Biological Screening analysts and DNA analysts will be consistent with the above stated guidelines while processing forensic casework as well as proficiency test samples.

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6.2 Sampling of Forensic Items of Evidence

Note: Whenever feasible, a portion of the original evidence will be retained to provide an opportunity for re-testing. If the entire original sample is consumed in extraction, the total elution volume must be at least 100µL. For cases with a suspect, if an analysis determines that the entire original sample and DNA extract must be consumed for the analysis, the analyst shall obtain written permission from the District Attorney's Office prior to consuming the DNA extract. Samples and extracts for unknown suspect cases do not require written permission prior to being consumed in analysis.

6.2.1 Bloodstains

- Typical bloodstains are sampled by cutting approximately 1.5 cm² of the stained material using a sterile, disposable scalpel. The size of the cutting may vary depending on the size and condition of the stain. It may be appropriate to sample the entire stain.
- Depending on the substrate, other sampling methods may be appropriate. For example, bloodstains on thick fabric or filter paper may be sampled using a 3mm hole punch. Bloodstains may also be sampled by swabbing with a damp, sterile swab if the substrate is difficult to cut or potentially contains PCR inhibitors (e.g. cigars or denim).
- Bloodstained swabs are usually sampled by cutting a portion of the stained area of the swab.

6.2.2 Swabs

- Swabs are generally sampled by cutting approximately half of the swab lengthwise.
- Any swab(s) that is sampled should be labeled with the case number, item number, date and analyst's initials using a cryo-baby sticker.
- For contact DNA swabs (not thought to contain biological fluids) sample the entire swab to maximize the chance of obtaining an interpretable DNA profile (elution volume must be at least 100µL). However, in such a circumstance, at least fifty percent of the extract will be retained for future use.
- Typical less swab material is cut from known buccal swabs (< 1/3 of one swab or a portion of two swabs).

6.2.3 Fingernail Scrapings

- Fingernail scrapings are received either as swabs from underneath the nails or debris from under the nails that is scraped into a paper bindle.
- Swabs from under the nails are generally sampled by cutting the swab tips into the sample tube. However, as in the case of contact swabs, the entire swab may be used in extraction and at least fifty percent of the extract retained for future use.

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- When sampling debris in a paper bundle, a moistened swab is used to swab the scraper, visible debris and the inside of the paper bundle. Typically one swab is used per hand. The entire swab is then cut into the sample tube.

6.2.4 Fingernails or Fingernail Clippings

- Fingernails may be submitted as evidence collected from a potential suspect at the scene. Alternatively, fingernail clippings may be submitted in lieu of or in addition to fingernail scrapings. Clippings should be imaged prior to sampling.
- Swabs from the nail(s) may be collected for profiling of tissue and cellular debris external to the nail itself. The entire swab tips are cut into the sample tube.
- If surface material, i.e. blood or dirt, is observed on the nail, the nail may be washed by immersing in 200 μ L of dilute G2 buffer (1:1000 ratio of H₂O:G2 buffer) in a microcentrifuge tube with gentle agitation. This may be appropriate when attempting to obtain a DNA profile of the surface material separately from the nail itself.
- The washing is then transferred to a new microcentrifuge tube and may be extracted by adding 10 μ L Proteinase K.
- Any clippings that remain after digestion are re-packaged with the evidence.

6.2.5 Hairs

Note: Prior to DNA analysis, hair evidence shall be examined by a qualified Biological Screening analyst to determine suitability for DNA analysis.

For mounted hairs, score the edge around the cover slip with a sterile scalpel. Remove the cover slip by prying it off or by soaking the slide in xylene. Use a pipette to wash the mounting medium away with xylene. Pick up the hair with clean forceps and wash in a beaker, on a watch glass, or on a microscope slide with absolute ethanol, then wash the hair with sterile, deionized water in a clean container.

Note: Process reference (known) hair sample alongside the questioned hair sample(s) as an internal control.

- Examine the hair, microscopically if necessary, for the presence of surface materials (i.e. blood or dirt) and document the findings. The hair may be placed on a clean piece of white paper or on a microscope slide for examination.
- If surface material is observed on the hair, the hair should be washed by immersing in 200 μ L of dilute G2 buffer in a microcentrifuge tube with gentle agitation. This may be appropriate when attempting to obtain a DNA profile of the surface material separately from the hair itself.
- The washing is then transferred to a new microcentrifuge tube and may be extracted by adding 10 μ L Proteinase K.
- Cut approximately 1cm of the proximal (root) end of the hair for digestion. Using clean forceps, place the hair root into a sterile 1.5mL tube.

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- Cut approximately 1cm of the shaft adjacent to the root for separate analysis as a substrate/shaft control. Add the shaft portion of the hair to a sterile 1.5mL tube.
- The remaining portion of the hair is re-packaged with the evidence.

6.2.6 Other tissue samples

Refer to the EZ1 DNA Investigator handbook for instructions on the appropriate sample size based on tissue type. The extraction procedure is as for other sample types.

6.3 Qiagen BioRobot M48 DNA Extraction – Known Database Samples

Note:

- All required reagents, except ethanol and sterile de-ionized water, are provided as listed in MagAttract DNA Mini M48 Handbook.
- Prepare Buffer MW1 according to the instructions given in the 'Important Notes' section of MagAttract Mini M48 Handbook (add 26mL of Absolute Ethanol to the MW1 concentrate).
- Ensure that Buffer MTL does not contain a white precipitate by shaking the bottle before use. Check again when transferring Buffer MTL into the Reagent Container. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve the precipitate.
- Prepare a master mix of diluted Buffer G2 and Proteinase K solution as follows:
(Number of samples + 3) x 255µL Buffer G2
(Number of samples + 3) x 255µL sterile de-ionized water
(Number of samples + 3) x 10µL Proteinase K solution

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6.3.1 Sample Digestion

- Aliquot 500µl of the Buffer G2/Proteinase K master mix into labeled 1.5mL sample tubes.
- For serrated buccal swabs, carefully eject the tip of the sample swab into the appropriate sample tube. For other cotton swabs, cut a portion of the swab tip into the appropriate sample tube or snap the entire swab end into the tube. Alternatively, if two swabs were collected, the entire cotton tip of one swab may be used. Dried stain cards are sampled with a 3mm hole punch (1-3 punches). The hole punch is cleaned by punching a clean piece of filter paper a few times.
- Vortex the samples for 10 seconds.
- Incubate in a 56°C incubator for at least 60 minutes, up to overnight.
- Centrifuge the tube briefly to remove any condensation from the lid.
- Transfer 200µl of the liquid from the sample tube into a new 1.5mL capless tube and place on the BioRobot M48 for DNA extraction. If absorption by the swab is unusually great, transfer as much of the digest as possible, up to 200µl.

6.3.2 DNA Extraction

- Ensure that power for the BioRobot M48 has been switched on. The power switch is located on the left side of the instrument.
- Turn on the power for the computer and monitor.
- Upon startup, the computer controlling BioRobot M48 is set to launch the 'Qiasoft M' software startup window by default.

Note: If this setting has been changed, the 'Qiasoft M' Operating System can also be launched from the 'Qiasoft M' icon on the computer desktop or from the Microsoft Windows "Start" menu, where it is located in Qiasoft M Operating System → Qiasoft M V2.0 for BioRobot M48.

- Select the protocol group from the drop-down menu, by selecting **Forensics→gDNA→Normalization 1.1**.
- Choose the elution tube type (use either 1.5mL or 2.0mL). Enter the number of samples (in multiples of 6), set sample volume to 200µl and final elution volume to 100µl.
- The 'Qiasoft M' software will guide you through the remaining steps required to set up BioRobot M48 for the Normalization protocol.

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Note:

- Wear gloves, a lab coat and a mask when loading the required items on the worktable.
- Remove the caps from the elution tubes as they are being loaded on the worktable.
- Close the workstation door and start the purification procedure. All steps are automated, and a software message on the screen will indicate the estimated time to completion of the run.
- Retrieve and cap the elution tubes containing the purified DNA from the cooling block.
- Extracted DNA is now ready for amplification (quantification of known samples is optional). DNA extracts can be stored for several weeks at 2°C to 8°C. Extracts should be transferred to -20°C for long-term storage.
- Clean the BioRobot M48 by wiping down with ethanol, followed by distilled water, after each use.

CAUTION:

Do not use bleach to clean the M48, since it can react with the extraction reagents.

6.3.3 Troubleshooting

Please consult the Troubleshooting Guide in the MagAttract DNA Mini M48 Forensic Handbook or the BioRobot M48 User's Guide.

6.3.4 Maintenance

Refer to Qiagen M48 Maintenance Log (AK SCDL document).

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6.4 Qiagen BioRobot EZ1 Advanced-XL DNA Extraction

Note:

- Use the Trace or Large Volume protocols for all questioned and known reference casework samples.
- The Large Volume protocol requires an additional reagent blank because of the addition of Buffer MTL after digestion. When using the Large Volume protocol, be sure to aliquot enough dilute G2 to your reagent blank tube (≥700µl of dilute G2 buffer) before digestion to have enough post-digestion solution to extract two separate reagent blanks to accompany the Trace and Large Volume protocols, respectively. Alternatively, a second reagent blank tube can be set up prior to digestion and used exclusively as the reagent blank for Large Volume protocol sample(s).
- Questioned samples shall be eluted in TE buffer.
- Known samples may be eluted in water or TE buffer.

Elution volumes are selected based on sample type and/or quantity:

- Larger elution volumes (200µl) may be selected for large blood stains, 4+ sperm samples, 4+ epithelial fractions and reference samples
- Smaller elution volumes (50µl) are recommended for contact DNA samples and samples with few sperm or epithelial cells.
- Larger elution volumes (100µl) are recommended for known reference samples.
- If the entire sample was consumed, the total elution volume shall be no less than 100µl, unless given written permission to elute into 50µl (see [Section 6.2](#) for guidance on consuming samples).
- If more than one elution volume is used in a set of extractions, the corresponding reagent blank(s) should use the most stringent elution volume used in that set.

6.4.1 Casework Samples – Direct (non-differential) Extraction

1. Prepare the pre-digest solution:
 - a. (Number of samples + 3) x 230 µl G2 buffer
 - b. (Number of samples + 3) x 230 µl sterile de-ionized water
 - c. (Number of samples + 3) x 10 µl Proteinase K
2. Add enough pre-digest solution to each sample cutting to allow for approximately 200µl of free liquid in the tube after absorption by the substrate. A typical sample cutting, such as half each of two swabs, requires about 450µl of pre-digest buffer.

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Note: for hair samples and nail clippings*, also add 10 μ l DTT.
(* when the source or owner of the nail needs to be determined)

3. Incubate in a 56°C incubator. Known samples shall be incubated for at least one hour. Question samples will typically be incubated overnight. Exceptions will be noted in the bench notes.
4. Following incubation, transfer 200 μ l of the digest buffer into a sterile 1.5 mL screw-cap tube. If absorption by the swab is unusually great, transfer as much of the digest as possible, up to 200 μ l. For all casework questioned samples, add 1 μ l carrier RNA solution to the transferred digest solution. It is not necessary to add carrier RNA to reference sample extracts.

Note: If necessary, prepare the carrier RNA solution by reconstituting a previously verified lot of lyophilized carrier RNA in 310 μ l of sterile, deionized water. Vortex, and spin briefly. Prepare 20 μ l, single use aliquots in 0.5ml tubes and store at -20°C.

Option 1 – Large-Volume Protocol:

- For samples that typically yield less DNA or if the sample required additional digest buffer for thorough cell lysis, the analyst may transfer up to 500 μ l of the digest solution to an EZ1 sample tube, add 1 μ l carrier RNA solution, and add 400 μ l of Buffer MTL.
- Load the sample(s) onto the EZ1, select the Large-Volume Protocol, and elute in 50 μ l TE buffer. An additional reagent blank with Buffer MTL added must be extracted with all Large-Volume Protocol samples.

Option 2 – Large-Volume Protocol for Consumed Samples:

- If the entire evidence sample has been consumed and digested, the analyst shall transfer the entire volume of digest solution to an EZ1 sample tube (or tubes - with no more than 500 μ l in each tube), add 1 μ l of carrier RNA solution and add 400 μ l of Buffer MTL.
- Run the large volume protocol and elute in 100 μ l TE buffer.
- Occasionally use of more than one tube may be required to digest and extract the entire sample efficiently. In such situations, the EZ1 extracts obtained from all such digests may be combined in one tube and proceed to the quantitation step.
- **NOTE:** If any sample is extracted using the Large-Volume protocol and eluted in 50 μ l, then the reagent blank accompanying that sample will suffice as the reagent blank for a consumed sample extracted using the Large-Volume protocol and eluted 100 μ l. If no other Large-Volume protocol samples are extracted, ensure that a reagent blank is extracted using the Large-Volume protocol and eluted in at least 50 μ l.

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5. Reagent cartridges may be stored at room temperature (short term) or between 2°C and 8°C (long term). If the reagent cartridges have been stored between 2°C and 8°C, warm them up by leaving them at room temperature for several hours or placing them in a 37°C incubator for approximately 1 hour.
6. Turn on the power switch on the back of the instrument.
7. Directions are displayed on the screen on the front of the instrument. Press the START button to select a protocol (Trace or Large-Volume), elution buffer, and elution volume.

Note: If selecting the Large-Volume protocol, ensure that 400 µl of mL buffer has been added to the sample(s) prior to loading on the instrument.

8. Follow the instructions displayed on the screen to set up the reagent cartridges, digest tubes, tip holders, and elution tubes.

Note: Ensure that the reagent cartridges snap into place in the cartridge rack and that the caps are removed from the sample tubes and elution tubes prior to loading on the deck. The caps from the sample tubes may be discarded. The caps from the elution tubes are retained and replaced upon completion of the protocol.

9. If fewer than 14 samples are being extracted, reagent cartridges and consumables need to be used only for the occupied channels.
10. Upon completion of the protocol, remove the elution tubes containing the purified DNA and cap the tubes.
11. The extracted DNA is now ready for quantification (optional for known samples) and/or amplification. DNA extracts can be stored for several weeks at 2°C to 8°C. Extracts should be transferred to -20°C for long-term storage.
12. Clean the instrument by wiping down with ethanol, followed by distilled water, after each use.
13. Clean the piercing unit after each use by selecting option 2 ("Man") on the main menu, then option 3 ("Clean"). Then wipe each piercing unit down with ethanol.
14. Refer to EZ1 Advanced XL Maintenance Log (AK SCDL document) for UV decontamination procedures.

CAUTION:

Do not use bleach to clean the EZ1, since it can react with the extraction reagents.

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6.4.2 Casework Samples - Differential Extraction

1. Prepare the 1:1 diluted G2 buffer:
 - i. (Number of samples + 3) x 1040µl G2 buffer
 - ii. (Number of samples + 3) x 1040µl distilled water
2. Cut a portion of the sample and place in a 2.0ml tube.
3. Add 600µl of sterile de-ionized water to each of the tubes.
4. Vortex briefly. Incubate overnight in a 37°C incubator. Pre-soaks/water soaks with less incubation time shall be performed only with documented pre-approval of the Forensic Biology Supervisor or DNA Technical Manager after evaluation of the sample.
5. Agitate the samples for 1-2 minutes by vortexing or using a sterile pipette tip or a sterile toothpick to remove the cells from the substrate.
6. For each sample, transfer the substrate to a spin basket and place the basket back in the sample tube. Centrifuge the sample tubes for 5 minutes at 10,000 to 14,500 RPM.
7. Transfer approximately 550µl of the supernatant to a new, labeled 2.0mL tube. Also transfer the spin basket containing the substrate. Retain until the case has been completed.
8. Re-suspend the cell pellet and spot 3µl on a microscope slide. Stain and grade the slide ([Section 4.4](#)).

Note: Two or more spermatozoa must be identified and verified by a second qualified analyst to report the presence of spermatozoa.

9. If sperm and epithelial cells are observed proceed to step 10.

OPTIONS

If few or no sperm/epithelial cells are observed, a second extraction may be performed by adding more of the original evidence item to the sample tube and repeating steps 2-8. Alternatively, the substrate may be added back to the sample tube for digestion or it may be digested separately. If adding the substrate back to the sample tube, place substrate back in the tube and proceed to step 10. If digesting separately, place substrate into a new 1.5mL tube and label it as "Q#_{sub}"; analysis of the substrate resumes at step 17.

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When no sperm are observed, it is not necessary to divide the sample into separate epithelial and sperm fractions (document on the DNA worksheet). If not separating the sample, add up to 390µl of 1:1 diluted G2 buffer + 10µl of Proteinase K solution and proceed to step 18.

When no NECs are observed, it is not necessary to divide the sample into separate fractions (document on the DNA worksheet). Add 190µl of 1:1 dilute G2 buffer + 10µl Proteinase K solution + 10µl DTT and proceed to step 18.

Note: Package sperm slides created from questioned stains with the evidence if a Biological Screening analyst has not reported the results of microscopic examination. It is not necessary to retain reagent blank sperm slides.

10. To the re-suspended cell pellet (may include substrate if added back), add up to 390µl of 1:1 diluted G2 buffer + 10µl of Proteinase K solution.
11. Vortex and spin briefly to force the material into the extraction fluid.
12. Incubate for at least two hours in a 56°C oven. During the incubation step label a new sterile tube for each sample, including the reagent blank. Label these as the epithelial fractions.
13. Centrifuge the sample tubes for 5 minutes at 10,000 to 14,500 RPM. If the substrate was added back to the tube (step 9 option), using a sterile toothpick to place the substrate into a spin bucket and centrifuge for 5 minutes at 10,000 to 14,500 RPM. Remove all but 50µl of the supernatant and transfer to the epithelial fraction tube. Analysis of the epithelial fraction resumes at step 19.

Optional: At this stage the analyst may perform a second epithelial cell digest (repeat steps 10-13) on samples with a large number of nucleated epithelial cells. This is called a “double digest” and should be documented on the DNA worksheet and on the Standards and Control worksheet for the accompanying reagent blank. This may be based on an initial microscopic examination or on a second examination.

14. Wash the sperm pellet by adding approximately 500µl of dilute G2 buffer. Vortex briefly and centrifuge at approximately 10,500 to 14,000 rpm for 5 minutes.
15. Remove and discard the supernatant.
16. Repeat the wash two more times for a total of three washes. If few sperm were observed, the number of washes for the sperm fraction may be decreased.
17. Add 190µl of 1:1 diluted G2 buffer + 10µl Proteinase K solution + 10µl DTT.

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Note: If the substrate was digested separately, the volume of diluted G2 buffer may be increased to account for absorption by the substrate.

18. Vortex and spin briefly to force the material into the extraction fluid.
19. Incubate overnight in a 56°C oven.
Reagent cartridges may be stored at room temperature (short term) or between 2°C and 8°C (long term). If the reagent cartridges have been stored between 2°C and 8°C, warm them up by leaving them at room temperature for several hours or placing them in a 37°C incubator for approximately 1 hour.
20. Insert the DNA Investigator card into the slot located on the front of the BioRobot EZ1 Advanced-XL.
21. Turn on the EZ1 power switch on the back of the instrument.
22. Directions are displayed on the screen on the front of the instrument. Press the START button to select a protocol (Trace or Large-Volume), elution buffer and elution volume.

Note: If selecting the Large-Volume protocol, ensure that 400µl of MTL buffer has been added to the sample(s) prior to loading on the instrument.

24. Follow the instructions displayed on the screen to set up the reagent cartridges, digest tubes, tip holders, and elution tubes.

Note: Ensure that the reagent cartridges snap into place in the cartridge rack and that the caps are removed from the sample tubes and elution tubes prior to loading on the deck. The caps from the sample tubes may be discarded. The caps from the elution tubes are retained and reused upon completion of the protocol.

Note: If fewer than 14 samples are being extracted, reagent cartridges and consumables need to be used only for the occupied channels.

25. For sperm fractions:
Transfer 200µl of the digest to a 1.5mL screw cap tube, add 1µl carrier RNA solution, run Trace protocol and elute in 50µl to 200µl TE buffer.

For epithelial fractions:
Transfer 200µl of the digest to a 1.5mL screw cap tube, add 1µl carrier RNA solution, run Trace protocol and elute in 50µl to 200µl TE buffer.

Note: If necessary, prepare the carrier RNA solution by reconstituting the carrier RNA in 310µl of sterile, de-ionized water. Vortex and spin briefly. Prepare 20µl, single use, aliquots in 0.5mL tubes and store at -20°C.

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Options for fractions with none/few/1+ cells and samples not separated:

Option 1 – Large-Volume Protocol:

- For samples that typically yield less DNA or if the sample required additional digest buffer for thorough cell lysis, the analyst may transfer up to 500 μ l of the digest solution to an EZ1 sample tube, add 1 μ l carrier RNA solution, and add 400 μ l of Buffer MTL to each tube(s).
- Load the sample(s) onto the EZ1, select the Large-Volume Protocol, and elute in 50 μ l TE buffer. An additional reagent blank with Buffer MTL added must be extracted with all Large-Volume Protocol samples.

Option 2 – Large-Volume Protocol for Consumed Samples:

- If the entire evidence sample has been consumed and digested, the analyst shall transfer the entire volume of digest solution to an EZ1 sample tube (or tubes - with no more than 500 μ l in each tube), add 1 μ l carrier RNA solution and add 400 μ l of Buffer MTL to the tube(s).
- Run the Large-Volume protocol and elute in 100 μ l TE buffer.
- NOTE: If any sample is extracted using the Large-Volume protocol and eluted in 50 μ l, then the reagent blank accompanying that sample will suffice as the reagent blank for a consumed sample extracted using the Large-Volume protocol and eluted in 100 μ l. If no other Large-Volume protocol samples are extracted, ensure that a reagent blank is extracted using the Large-Volume protocol and eluted in at least 50 μ l.

26. Upon completion of the EZ1 protocol, remove and cap the elution tubes containing the purified DNA.
27. The extracted DNA is now ready for quantification and amplification. DNA extracts can be stored for several weeks at 2°C to 8°C. Extracts should be transferred to -20°C for long-term storage.
28. Clean the instrument by wiping down with ethanol, followed by distilled water, after each use.
29. Clean the piercing unit after each use by selecting option 2 ("Man") on the main menu, then option 3 ("Clean"). Then wipe each piercing unit down with ethanol.
30. Refer to EZ1 Advanced XL Maintenance Log (AK SCDL document) for UV decontamination procedures.

CAUTION:

Do not use bleach to clean the EZ1, since it can react with the extraction reagents.

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6.4.3 Troubleshooting

Please consult the Troubleshooting Guide in the EZ1 DNA Investigator Handbook or the BioRobot EZ1 Advanced-XL User's Guide.

6.4.4 Maintenance

Refer to Qiagen EZ1 Advanced-XL Maintenance Log (AK SCDL document)

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SECTION 7 – DNA QUANTIFICATION USING APPLIED BIOSYSTEMS QUANTIFILER™ KIT ON STRATAGENE® Mx3000P™

As mandated by the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories, all forensic questioned samples must be quantified prior to amplification. Quantification is not mandatory for database samples or forensic known samples.

References: Quantifiler® Kits User's Manual and Mx3000P Online User's Manual (<http://www.stratagene.com/manuals/>)

7.1 Preparing the Mx3000P™ for a Run

- Open the MxPro-Mx3000P™ Software
- In the New Options pop up select Quantitative PCR(Multiple Standards) and ensure the box for Turn lamp on for warm-up? is checked. Select OK
- Click File>Open and navigate to QPCR-template.mxp which is located in DNA_Share in the Mx3000P Data Storage file.
- Save the file with the run date and analyst's initials (i.e. QPCR-YY-MMDDKAH) in the appropriate analyst folder.
- Select the appropriate well types using the scroll down bar labeled Well type on the right hand side of the screen. Choose Unknown for all samples irrespective of type (questioned or known) and choose NTC (no template control) for the wells with no template control. Two wells of each plate are to be run as NTCs. These wells contain only master mix. The first two columns always contain the standard curve.

Note: The lamp takes twenty minutes to warm-up therefore open the Mx3000P software before sample preparation, to allow time for lamp to warm-up. On the bottom right of the screen a box will indicate when the lamp is ready.

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7.2 Preparation of DNA Quantitation Standards

On the bench top prepare a three-fold serial dilution of the Quantifiler™ Human DNA Standard (provided in kit) in sterile H₂O as follows:

- Label eight sterile 0.5mL microcentrifuge tubes A through H.
- Add 30µL sterile H₂O to tube A.
- Add 20µL sterile H₂O to tubes B through H.
- Thaw the Quantifiler™ Human DNA Standard completely. Vortex for 3 to 5 seconds and spin briefly.
- Transfer 10µL of DNA Standard (200ng/µL stock) into tube A. Vortex and spin briefly.
- Transfer 10µL of prepared Standard A into tube B. Vortex and spin briefly.
- Continue the serial dilution through tube H.

The approximate quantities of DNA in prepared Standards A through H are as follows:

- Standard A ≈ 50ng/µL
- Standard B ≈ 16.7ng/µL
- Standard C ≈ 5.56ng/µL
- Standard D ≈ 1.85ng/µL
- Standard E ≈ 0.62ng/µL
- Standard F ≈ 0.21ng/µL
- Standard G ≈ 0.068ng/µL
- Standard H ≈ 0.023ng/µL

Note: In-house experiments have demonstrated that the standard curve is stable for at least one week and should be stored in the freezer with documentation of the date made, expiration date and the H₂O lot number.

7.3 Preparing the Reactions

Calculate the volume of each component needed to prepare the reactions in duplicate. Calculate the volume of each component needed to prepare the reactions in duplicate.

- Quantifiler™ Human Primer Mix at 10.5µL per reaction.
- Quantifiler™ PCR Reaction Mix at 12.5µL per reaction.

Include three additional reactions in your calculations for every sixteen samples prepared, to provide excess volume for loss that occurs during reagent transfers. A 96-well plate QPCR worksheet is to be used for well mapping and can be found in DNA Share. Questioned samples will be run in duplicate. Known samples, if quantified, need not be run in duplicate.

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- Thaw the primer mix completely, then vortex 3 to 5 seconds and centrifuge briefly before opening the tube.
- Swirl the PCR Reaction Mix gently before using. **DO NOT** vortex.
- Pipette the required volumes of components into an appropriately sized tube.
- Vortex the master mix for 3 to 5 seconds.
- Place a new Stratagene®, or equivalent, 96 well plate into well plate working rack.

Note: Stratagene® 96-well plates are different than the plates used for amplification.

- Dispense 23µL of the PCR master mix into each reaction well of the 96-well plate.
- Add 2µL of sample or standard (prepared as stated in [Section 7.2](#)) to the appropriate wells. No sample is added to the NTC wells.
- Use powder-free gloves and a Kimwipe to cover wells with Stratagene®, or equivalent, optical strip caps.
- Apply downward force to each cap then view from side to ensure all caps are seated evenly.
- Centrifuge samples in a plate centrifuge to make sure there are no bubbles in the wells.

7.4 Sample Loading

- Use care when loading and unloading the 96 well plate; the thermal block can be hot.
- Verify that the status LED (upper LED on the instrument) is solidly lit, indicating that the instrument is ready for use.
- Open the door of the Mx3000P™, located at the front of the instrument.
- To gain access to the thermal block, unlatch the hot-top assembly by pulling forward on the handle and then lifting the hot-top up and away from the thermal block.
- Insert the 96 well plate into the thermal block. (Plate goes in with the 'A' well in the upper left)
- Close hot-top and door on the instrument.

7.5 Starting the Run

- Confirm that the worksheet has been saved as described above in Section 7.1.
- Click on the Run icon in the upper right hand corner of the screen. The run status box will appear.
- Check the Turn lamp off at end of run box if this will be the last run of the day.
- Click Start.

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7.6 Analyzing the Data

- When the run is complete click the Analysis icon in the upper right hand corner of the screen.
- You are now in the Analysis Selection/Setup screen. Make sure that all wells on the 96-well plate map are highlighted. Clicking All in the upper left hand corner of the 96-well plate map will select/de-select all 96 wells at once. If you have fewer than 96 samples, individual well or columns can be individually selected by holding down the ctrl key while selecting the wells.
- On the right hand side, under Algorithm enhancements, click off the Moving average and Amplification based threshold boxes leaving only the Adaptive baseline box checked.
- Click on the Results tab at the top of the 96-well plate map.

7.6.1 Checking the Standard Curve

- Under Well types shown in the lower right hand corner of the window ensure Standard is the only option selected.
- In the Area to analyze box select Amplification plus and in the Assays shown box in the lower left hand corner of the window ensure FAM is the only one selected by deselecting ROX, HEX, and CY5.
- Check the Ct values of the standards in the box at the lower right hand corner of the screen. If a standard fails to amplify (no Ct value) or if there is a large variation in the Ct value of the duplicate standards, you can remove the data point for that well. This is done by returning to the Analysis Selection/Setup screen and de-selecting the well. When returning to Results, the curve will automatically be re-calculated. **Note:** No more than 3 points should be taken out and the first and last points (A and H) should always be retained.
- Next, in the Area to analyze box in the upper right of the screen select the Standard curve option. Under Assays shown (lower left) make sure FAM is the only one selected. **Print this view.**

$R^2 \geq 0.99$. If < 0.98 see Quantifiler™ Kits User's Manual (p. 5-4).

- Slope Range -2.9 to -3.3 ± 0.3
- Efficiency Range 85% to 115%

- Generally, the Ct value of prepared Standard A should lie between 22 and 23 and the Ct value of prepared Standard H should lie between 32 and 33. When the standard curve shifts to the right (higher Ct values) a higher amount of template DNA should be targeted for STR analysis. When the standard curve shifts to the left (lower Ct values) a lower amount of template DNA should be targeted for STR analysis. How much of an adjustment to be made will depend on the degree of shift of the standard curve.

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Note: If the standard curve plot values are marginally outside the given ranges, the data may be acceptable at the discretion of the Technical Manager.

7.6.2 Checking the Internal PCR Control (IPC)

- In the Area to analyze box select Amplification plots. Under Assays shown (lower left) select HEX only to view the Internal PCR Control (IPC). Select the Standard, Unknown and NTC boxes in the Well types shown box in the lower right hand corner of the window.
- Check all IPC Ct values (found in lower right hand box). The values should be between 20 and 30.
 - A high Ct value or no Ct for the IPC can indicate inhibition or competition between extremely high concentrations of human genomic DNA.
 - If a sample has a Ct value >30, make a note of this so you can account for possible inhibition. This will need to be considered when setting up for STR amplification.
 - If a sample has a low IPC Ct value or no IPC Ct, but a high initial template quantity, it is unlikely that PCR inhibitors are present.
 - If a sample has no IPC Ct and a high initial template quantity, it is not possible to distinguish between the absence of DNA and PCR inhibition.
- Document the average IPC Ct value for all unknown samples (this will be recorded on the bottom of the Initial Template Quantity sheet discussed in [Section 7.6.5](#))

7.6.3 Checking the Passive Reference

Check the passive reference for background noise by only selecting the ROX in the Assays shown box in the lower left corner of the screen. If the ROX baseline appears elevated or jagged, refer to the Quantifiler® Kits User's Manual.

7.6.4 Checking the No Template Controls (NTC)

- Check the NTCs by only selecting the NTC box in the Well types shown on the bottom right of the window and select both FAM and HEX on the bottom left in the Assays shown box.
- Make sure the HEX Ct value is between 20 and 30 and the FAM has no Ct value.
- HEX and FAM Ct values other than these could indicate contamination of the master mix. If the master mix was contaminated, the quantification may need to be repeated (consult with the Technical Manager).

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7.6.5 Checking the Initial Template Quantity

- On the right hand side of the screen under Area to analyze select Initial template quantity and in Assays shown select FAM only. **Print this screen** to show quantity of DNA in ng/ μ L.
- From this view you can determine the amount of sample to amplify for STR analysis.
 - Reagent blanks should give no value for initial template quantity.
 - Very low initial template quantity values could be the result of dust or debris interfering with the optical path. All blanks will be amplified regardless of the quantification result. Therefore, the quantification result alone should not be used to assess contamination of a blank.
- At the bottom of this page, document the average IP C_t value for all unknown samples (refer to [Section 7.6.2](#)).

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SECTION 8 – DNA SHORT TANDEM REPEAT (STR) AMPLIFICATION

8.1 General instructions pertinent to DNA amplification

- Analysts must wear appropriate personal protective equipment (PPE) during PCR set-up.
- PCR set-up is performed in a designated area in the laboratory, with equipment dedicated to the work area. Work surfaces must be cleaned with a 10% bleach solution before and after use.
- Sterile techniques appropriate to forensic procedures will be used when handling samples during PCR set-up.
- Centrifuge all tubes before opening them. Only one sample tube should be open at a time.
- If reference samples and questioned samples are being set-up for amplification on the same day, always set-up questioned samples before reference samples and on a separate 96-well plate; the time when the amplifications were begun should be documented in the worksheet notes.
- DNA amplification work is documented on the appropriate worksheet. This worksheet will include the sample code, sample concentration (if applicable), volume of sample amplified, and all amplification reagent lot numbers.
- Each amplification plate must include a positive and negative amplification control.
- Always add template DNA to the sample well/tube after addition of the PCR master mix.

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8.2 PowerPlex 16 Amplification on the Applied Biosystems GeneAmp® PCR System 9700 thermal cycler

8.2.1 Cycling Parameters for Forensic Casework Samples and Offender Database Samples to be Analyzed on the Applied Biosystems 3130xl

The thermal cycler(s) should be turned on prior to setting up samples to allow for time to warm up. Select user (alaska) and program (pp16-new):

95°C for 11 minutes, then:

96°C for 1 minute, then:

ramp 100% to 94°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 10 cycles, then:

ramp 100% to 90°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 22 cycles, then:

60°C for 30 minutes
4°C hold

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8.2.2 Cycling Parameters for Offender Database Samples to be Analyzed on the Applied Biosystems 3500xl

The thermal cycler(s) should be turned on prior to setting up samples to allow for time to warm up. Select user (alaska) and program (pplex16-28 cycles):

95°C for 11 minutes, then:

96°C for 1 minute, then:

ramp 100% to 94°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 10 cycles, then:

ramp 100% to 90°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 18 cycles, then:

60°C for 30 minutes
4°C hold

8.3 Amplification Setup of Forensic Casework Samples

- Allow the samples to warm to room temperature, then vortex and spin briefly in a microcentrifuge. Samples that have been stored at 2-8°C or frozen for a week or more may be warmed in an incubator to bring them to room temperature. These samples should be vortexed longer to optimize recovery.
- Transfer the amplification reagents to the designated PCR set-up area. Place the amplification reagent tubes in a rack that is dedicated to PCR set-up.

Note: Do not expose reagents to light for extended periods of time.

- Obtain a 96-well amplification plate.
- Ensure that all kit components have thawed completely before use. Reagents should be vortexed and centrifuged briefly to ensure uniform mixing and collection of tube contents.

Note: Centrifugation of the primer mix should be minimal to avoid primer collecting at the bottom of the tube.

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- Prepare a PCR master mix by adding the following volumes of reagents to an appropriately sized tube:

of samples x 2.5µL Gold STR 10X Buffer
of samples x 2.5µL PowerPlex® 16 10X Primer Pair Mix
of samples x 0.8µL AmpliTaq Gold™ DNA Polymerase (5U/µL)

Prepare enough for a few extra reactions to allow for loss during pipetting. This calculated automatically on the DNA Amplification/electrophoresis worksheet.

- Vortex the master mix and spin briefly. Transfer 5.8µL master mix to each sample well. Cover the entire well plate with Glad® Press'n Seal (or equivalent).
- Prepare the samples to be amplified as follows (in individual 0.5mL tubes):

Forensic Case Samples

Add approximately 0.8ng to 2.0ng template DNA. The amount of template DNA may be higher or lower depending on the sample (i.e. low-level samples that are likely to be mixtures may be targeted higher, while bloodstains likely to be single-source may be targeted lower). Add sterile deionized water to bring the sample to a final volume of 19.2µL.

Quantification Value <0.05ng/µl

Samples with a quantification value <0.05ng/µl (or PCR target template estimate of less than 0.95ng) shall be routinely amplified in duplicate to ascertain reproducibility of alleles above and below 100RFU. Data interpretation and reporting of conclusions will depend on whether the profile indicates a single source or mixed DNA sample.

Quantification Value >0.05ng/µl

Samples with a quantification value >0.05ng/µl will NOT be routinely amplified in duplicate if the profile generated includes artifacts (e.g. stutter/true allele) and/or ambiguous allele peaks, the sample will be re-amplified to verify reproducibility of the data.

Negative Quantification Value

Samples with no detectable DNA, including reagent blanks and negative controls, may be amplified one time (i.e. duplicate amplification not required).

Note: When amplifying greater than 10µL of template DNA, be aware that inhibitors that were not detected during the quantification step may interfere with amplification.

Reagent Blank Sample(s)

Add 19.2µL of the extraction reagent blank.

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Positive Control Sample

Vortex and spin the tube briefly and add 5µL of the diluted 9947A Control DNA (or other positive control DNA supplied by the PCR kit vendor) to a 0.5mL tube containing 14.2µL of sterile, de-ionized water.

Note: Over extended periods of storage of the positive control, it may be necessary to increase the volume of 9947A and decrease the volume of water to achieve the required concentration of the sample.

Negative Control Sample

Add 19.2µL of sterile de-ionized water.

- Transfer the entire 19.2µL of the above prepared samples to the appropriate sample well, already containing the PCR master mix. During sample addition, the pipette tip is inserted through the Press 'n Seal.
- Once all samples have been added, remove the Press 'n Seal and cover the plate with a sheet of amplification tape.
- Transfer the plate to the PCR room and place directly into the thermal cycler. Cover well plate with a silicone spacer prior to closing the thermal cycler cover. Start the run.
- Store amplified products at -8°C.
- All amplified products will be disposed of upon completion of the technical and administrative reviews of the case.

8.4 Amplification Set-up of Offender Database Samples

- Allow the samples to warm to room temperature, then vortex and spin briefly in a microcentrifuge. Samples that have been stored at 2-8°C or frozen for a week or more may be warmed in an incubator to bring them to room temperature. These samples should be vortexed longer to optimize recovery.
- Transfer the amplification reagents to the designated PCR set-up area. Place the amplification reagent tubes in a rack that is dedicated to PCR set-up.

Note: Do not expose reagents to light for extended periods of time.

- Obtain a 96-well amplification plate.
- Ensure that all kit components have thawed completely before use. Reagents should be vortexed and centrifuged briefly to ensure uniform mixing and collection of tube contents.

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Note: Centrifugation of the primer mix should be minimal to avoid primer collecting at the bottom of the tube.

- Prepare a PCR master mix by adding the following volumes of reagents to an appropriately sized tube:

(# of samples + 6) x 2.5µL Gold STR 10X Buffer
(# of samples + 6) x 2.5µL PowerPlex® 16 10X Primer Pair Mix
(# of samples + 6) x 0.8µL AmpliTaq Gold™ DNA Polymerase (5U/µL)

Note: The volumes are calculated automatically on the Database Batch Worksheet.

- Vortex the master mix and spin briefly, when possible. Transfer 18µL of master mix to the sample wells for the amplification positive and negative controls and the extraction reagent blank(s).
- Add the following volume of sterile, de-ionized water to the master mix:

(# of samples + 2) x 17.2µL

Note: This volume is calculated automatically on the Database Batch Worksheet.

- Vortex the master mix and spin briefly. Transfer 23µL of master mix to the remaining sample well(s).
- Cover the well plate with Rad® Press 'n Seal film. During sample addition, the pipette tip is inserted, puncturing through the Press 'n Seal film. This will aid in tracking the sample well(s) which have sample already transferred to them and minimizing the chance of well to well contamination.
- Prepare the positive and negative amplification controls and reagent blank as described below:

Reagent Blank Sample(s)

Add 19.2µL of the extraction reagent blank(s) to the appropriate well(s).

Positive Control Sample

Vortex and spin the tube briefly and add 5µL of the diluted 9947A Control DNA to a 0.5mL tube containing 14.2µL of sterile, de-ionized water. Transfer the entire 19.2µL to the appropriate well.

Note: Over extended periods of storage of the positive control, it may be necessary to increase the volume of 9947A and decrease the volume of water to achieve the required concentration of the sample.

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Negative Control Sample

Add 19.2µL of sterile, de-ionized water to the appropriate well.

Database Sample(s)

- Transfer 2µL of each offender extract to the appropriate well or tube.
- Once all samples have been added, remove the Press 'n Seal and cover the plate with a sheet of amplification tape.
- Transfer the plate to the PCR room and place directly into the thermal cycler. Cover well plate with a silicone spacer prior to closing the thermal cycler cover. Start the run.
- Store amplified products at 2-8°C.
- All amplified product will be disposed of upon completion of the reviews and upload of the batch.

Re-amplification Samples

It may be necessary to adjust the volume of water (and of offender DNA extract) for re-amplifications or when processing older samples. The total volume, per sample, should always equal 19.2µL.

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SECTION 9 – CAPILLARY ELECTROPHORESIS OF AMPLIFIED DNA

9.1 Applied Biosystems 3130xl Genetic Analyzer

References: *Applied Biosystems 3130/3130xl Genetic Analyzer Maintenance, Troubleshooting, and Reference Guide*

Promega Protocol GE163, "PowerPlex® Matrix Standards, v100 Custom"

9.1.1 Start the Software and Instrument

- Turn the computer on and log into the computer.
- Turn on the 3130xl Genetic Analyzer. Wait for the green status light to appear.
- Launch the Data Collection Software.
Start > All Programs > Applied Biosystems > Data Collection > Run 3130xl Data Collection v3.0
- The service console displays all of the applications running. When all squares are green, all the applications are running. This could take several minutes.

Note: When shutting down the Data Collection Software use the service console by clicking the **stop all** button. For detailed start up and shut down procedures see the Applied Biosystems 3130/3130xl Genetic Analyzers Quick Reference Card.

9.1.2 Preparing the Instrument

9.1.2.1 Replacing Buffer (1x solution prepared from Applied Biosystems 10x stock solution)

Both the anode and cathode buffer reservoirs should be changed about once a week or more often if the instrument has been running frequently. Using old buffer will result in an increase in spikes and baseline noise.

- Press the tray button on the outside of the instrument to bring the autosampler to the forward position and place fresh 1X genetic analyzer buffer in the cathode reservoir in position 1 (front left) as necessary.
- Place fresh de-ionized water into the reservoirs in positions 2 and 4 (back left and back right) whenever the anode and cathode buffers are replaced.

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- Replace the 1X genetic analyzer buffer in the anode buffer jar on the instrument whenever the cathode buffer (position 1) is replaced.
- Document in the 3130xl-1 (or 3130xl-2) binder(s) under the buffer tab.

9.1.2.2 Replenishing Polymer

Polymer is replenished on an as needed basis when the bottle is almost empty or has expired. Only 3130 POP-4™ is to be used.

- Allow the polymer to equilibrate to room temperature prior to placing on the instrument.
- Launch the Replenish Polymer Wizard in the Data Collection Software: **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Wizards (top of screen) > Replenish Polymer Wizard**
- Follow prompts given in the wizard to load fresh polymer on the instrument.
- Document in the 3130xl-1 (or 3130xl-2) binder(s) under the polymer tab.
- Polymer may be replenished as part of the Water Wash Wizard ([Section 9.1.4.1](#)).

Note: If changing the polymer or performing other maintenance, replace the 1X genetic analyzer cathode and anode buffer reservoirs after other maintenance is complete. All buffer and water reservoirs should be filled to the fill lines.

9.1.3 Replacing the Capillary Array

- The following indications may suggest that a new capillary array is required:
 - Poor sizing precision or allele calling
 - Poor resolution and/or decreased signal intensity
- Launch the Install Array Wizard in the Data Collection Software: **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Wizards (top of screen) > Install Array Wizard**
- Follow the prompts given in the wizard to install or replace an array.
- Document in the 3130xl-1 (or 3130xl-2) binder(s) under the array tab.

Note: Space and Spectral Calibrations must be performed anytime an array is replaced. A water wash, water trap flush, and TH01 9.3-10 Resolution must also be performed to verify performance of the array. Documentation of the water wash, water trap flush, and TH01 9.3-10 Resolution is recorded in the 3130-1(or 3130-2) binder(s) under the water wash and 9.3-10 Tabs respectively.

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9.1.4 Water Wash, Water Trap Flushing, and TH01 9.3-10 alleles Resolution/Performance Check

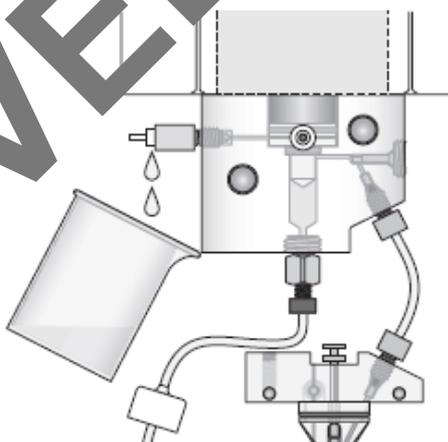
The water wash, water trap flush, and TH01 9.3-10 Resolution are performed as part of monthly maintenance and/or anytime an array is replaced. A water wash may also be performed when there is a noticeable decrease in the data quality that was not resolved by replacing the cathode and anode buffer vessels.

9.1.4.1 Performing the Water Wash

- Launch the Water Wash Wizard
GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Wizard (top of screen) > Water Wash Wizard
- Follow the wizard instructions to completion.

Exception: A 15mL conical (filled to about 10mL) is used in place of the plastic bottle to hold the water for the wash. The plastic tube is inserted to the bottom of the conical to prevent aspiration of air into the pump block.

9.1.4.2 Flushing and Filling the Water Trap



- Flush the water trap each time you perform the Water Wash Wizard.
- Fill a 20ml syringe with **WARM** distilled or deionized H₂O. Expel any bubbles from the syringe.
- Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting clockwise with the other hand.

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- Open the Luer fitting by grasping the body of the fitting and turning it and the attached syringe approximately one-half turn counterclockwise.
- Open the exit fitting at the top left side of the pump block by turning it approximately one-half turn counterclockwise.
- Hold empty beaker under the exit fitting. Flush the trap by steadily pushing approximately 5ml of H₂O through the syringe plunger (**DO NOT USE EXCESSIVE FORCE**).
- Close the Luer fitting by turning one-half turn clockwise.
- Close the exit fitting by turning one-half clockwise
- Remove the syringe from the Luer fitting while holding the fitting with one hand while turning the syringe counterclockwise with the other hand.
- Document in the 3130xl-1 (or 3130xl-2) binder(s) under the water wash/flush trap tab.

9.1.4.3 TH01 9.3-10 alleles Resolution/Performance Check

A performance check allows you to periodically self-check the instrument system's resolution and its ability to correctly size peaks within the base pair using Promega PowerPlex® 16 Classic Allelic Ladder.

- Prepare a loading master mix by adding the following volumes of reagents to an appropriately sized tube:
 - 20µl ILS 600
 - 20µl allelic ladder
 - 180µl Hi-Di™ Formamide
- Vortex the master mix and spin briefly. Transfer 11µl of the master mix to the appropriate well (i.e. 1-H2).
- Place septa onto the 96-well plate.
- Briefly centrifuge the well plate to remove any bubbles. Denature samples at 95°C for 1 minute and then snap chill for 3 minutes.
- Place the 96-well plate into the plate base provided with the instrument and snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Press the tray button to bring the autosampler to the forward position and place the plate in the autosampler (position A or B) with placement of the notched corner in the lower right.
- Close the instrument doors.
- Refer to [Section 9.1.8](#), Creating a Plate Record. (Note: All sample names can be designated by AL)
- Refer to [Section 9.1.9](#), Linking a Plate and Starting a Run.
- Document in the 3130xl-1 (or 3130xl-2) binder(s) under the maintenance tab. Electropherograms zoomed into the 9.3-10 TH01 locus are printed and filed under the 9.3-10 tab in the 3130xl-1 (or 3130xl-2) binder(s).

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9.1.5 Spatial Calibration

A spatial calibration maps the pixel positions of the signal from each capillary in the spatial dimension of the CCD camera. A spatial calibration must be run when the array has been removed for cleaning or replacement.

9.1.5.1 Performing a Spatial Calibration

- Launch the Spatial Calibration
GA instruments > ga3130xl > 12-F0196 (or 12-F0197) > Spatial Run Scheduler
- In the Spatial Protocols section, select one of the following:
 - If the capillaries contain fresh polymer, select protocol 3130SpatialNoFill_1
 - Otherwise, select Protocol > 3130SpatialFill_1
- Click **Start**. The calibration run lasts approximately:
 - 2 minutes without filling the capillaries
 - 6 minutes with filling the capillaries

9.1.5.2 Evaluating a Spatial Calibration

- All peaks should have similar heights
- One orange cross should mark the top of each peak.
- The shape should be a single sharp peak for each capillary. Small shoulders are acceptable.
- Position values are always higher than the previous one for every capillary. Theoretical spacing between capillaries is 15.
- If the calibration passed click **Accept** to write the calibration data to the database.
- If the calibration failed click **Reject** then refer to the Applied Biosystems 3130/3130xl Genetic Analyzer's Maintenance, Troubleshooting, and Reference Guide.

9.1.6 Spectral Calibration

A spectral calibration creates a matrix. A spectral calibration should be performed whenever the array has been changed, the CCD camera or laser have been realigned or replaced or if you see a decrease in spectral separation.

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9.1.6.1 Performing a Spectral Calibration

- Thaw the PowerPlex® Matrix Standards, 3100/3130. Vortex and spin briefly.
- Before mixing the dye fragments (as described below), a 1:5 dilution of each dye fragment must first be prepared by mixing 2µL of the dye fragment in 8µL of sterile de-ionized distilled water.
- A matrix standard master mix is prepared by combining the diluted dye fragments in a tube as follows:
 - Hi-Di™ Formamide 80µL
 - diluted FL Matrix Standard 5µL
 - diluted JOE Matrix Standard 5µL
 - diluted TMR Matrix Standard 5µL
 - diluted CXR Matrix Standard 5µL
- Vortex and spin briefly.
- On the 3130xl Genetic Analyzer, 16 wells of a 96-well plate are used for creating a matrix for the 16 capillaries. Load 25µL of the fragment mix into each of the 16 wells (i.e. A1-H2 of a 96-well plate) and cover with a plate septa.
- Briefly centrifuge the well plate to remove any bubbles.
- Denature samples at 95°C for 3 minutes then snap chill for 3 minutes.
- Place the 96-well plate into the plate base provided with the instrument.
- Align the septa with the plate retainer and snap the retainer onto the reaction plate and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Press the tray button on the front of the instrument and wait for the autosampler to stop moving.
- Open the doors and place the plate assembly on the autosampler in position A or B. There is only one proper orientation for the plate, with the notched end of the plate base in the lower right corner.
- Ensure the plate assembly fits flat in the autosampler. Failure to do so may allow the capillary tips to lift the plate assembly off the autosampler.
- Once the plate is in place the position of the plate turns to yellow in the Run Scheduler view on the monitor.
- Close the instrument doors.
- Create a Plate Record
 - **GA Instruments > ga3130xl > Plate Manager**
 - Click **New** (bottom of screen) and name the spectral with the date (i.e. SpectralYY-MMDD_KAH).
 - In the dialog box that appears, select **Spectral Calibration** in the Application drop-down list and select the **96-well plate type**. Add initials in the owner and operator windows and click **OK**.

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- In the Spectral Calibration Plate Editor dialog box, enter Spectral as the sample name for each of the appropriate cells. Under the Instrument Protocol column, select the protocol **PP16_spectral**. Ensure that this information is present for each row that contains a sample name. Click **OK**.
- Start the Run
GA instruments > ga3130xl > 12-F0196 (or 12-F0197) > Run Scheduler > Plate View
- Click **Find All**, scroll (if necessary) and select the plate you just created. Now click on the yellow area of the plate you have just placed on the autosampler. The area should turn green once a plate is linked.
- On the top left of the screen the run button will turn green. Click on the run button. A Prompt appears stating "You are about to start processing plates" click **OK**.
- The status of the run can be monitored in the "Instrument Status" window and also in the "Capillaries Viewer" or "Cap/Array Viewer."

Note: Do not leave the computer on these two windows for extended periods of time due to large amounts of memory needed to view the data.

9.1.6.2 Evaluating a Spectral Calibration

- Upon Completion of the run check the status of the spectral run
GA instruments > ga3130xl > 12-F0196 (or 12-F0197) > Instrument Status
- A minimum of 12 of the 16 capillaries should pass calibration.
- View the spectral array raw data for each capillary and verify the following:
GA instruments > ga3130xl > 12-F0196 (or 12-F0197) > Spectral Viewer
 - Each capillary should have a Q-value > 0.9 and a condition number range between 3 and 12.
 - Order of peaks in the spectral profile from left to right; blue-green-yellow-red.
 - Order of the peaks in the raw data profile from left to right; red-yellow-green-blue.
- If less than the recommended number of capillaries pass, the spectral calibration should be repeated. First, re-inject the standards to see if the minimum number of capillaries pass. If re-injecting does not work, then create a matrix standard master mix prepared by combining the 1:5 diluted dye fragments (as prepared in 9.1.6.1) in a tube as follows and repeat the spectral calibration:

○ Hi-Di™ Formamide	460µL
○ diluted FL Matrix Standard	10µL
○ diluted JOE Matrix Standard	10µL
○ diluted TMR Matrix Standard	10µL
○ diluted CXR Matrix Standard	10µL

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- For additional troubleshooting see *PowerPlex® Matrix Standards, 3100/3130 Part #TBD022* or Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide.
- If the results are acceptable, the spectral calibration is automatically active for that dye set and saved as day,date,and time of run.

9.1.7 Sample Preparation for the 3130xl

Internal Lane Standard 600 (ILS 600) is included with the PowerPlex® 16 System as the internal lane standard for four-color detection and analysis of amplified samples.

- Prepare a loading master mix by adding the following volumes of reagents to an appropriately sized tube:

of samples x 1µL ILS 600
of samples x 9µL Hi-Di™ Formamide

Prepare enough for a few extra reactions to allow for loss during pipetting. This is calculated automatically on the Database Batch Extraction Reagent Worksheet (database) or the PP16Amp-3130xl Worksheet (casework) found on DNA_Share.

- Vortex the master mix and spin briefly. Transfer 10µL of master mix to the appropriate sample wells of a 96 well plate.
- Add 10µL of Hi-Di™ Formamide to unused wells of a set of 16. (i.e. A1...H2).
- Add 1µL of allelic ladder and up to 3µL of each amplified sample (reagent and amplification block volumes must correspond to the largest volume of amplified sample added to the plate) to the appropriate wells. At least one ladder must be contained within each injection of 16 samples. When all samples have been added, cover with plate septa.

Note: Instrument detection limits vary. Therefore, injection time or the amount of ladder/amplified product may need to be increased or decreased. The interpretation of data is discussed in greater detail in [Section 11](#) (Data Interpretation) of this manual.

- Brief centrifugation of the 96 well plates will remove bubbles that may affect analysis.
- Denature samples at 95°C for 3 minutes and then snap chill for 3 minutes. Place the 96-well plate into the plate base provided with the instrument.
- Align the septa with the plate retainer and snap the retainer onto the reaction plate and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.

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- To place the plate assembly into the instrument:
 - Press the tray button on the front of the instrument and wait for the autosampler to stop moving.
 - Open the front doors.
 - Place the plate assembly on the autosampler in position A or B.
 - There is only one proper orientation for the plate, with the notched end of the plate base in the lower right corner.
 - Ensure the plate assembly fits flat in the autosampler. Failure to do so may allow the capillary tips to lift the plate assembly off the autosampler. Once the plate is in place the position of the plate turns to yellow in the Run Scheduler view on the monitor.
 - Close the instrument doors.
- Refer to [Section 9.1.8](#), Creating a Plate Record
- Refer to [Section 9.1.9](#), Linking a Plate and Starting a Run

9.1.8 Creating a Plate Record

9.1.8.1 Create a Plate Record from the Data Collection Software

- Navigate to the following:
GA instruments > ga3130xl > Plate Manager
- Click New and complete the New Plate Dialog Box
 - Enter a name for the plate.
Casework samples
Batch Number initials_CK_date plate set-up (i.e. 11-0103CM_Q_0130)
 - Database sample
DE Batch number initials (i.e. DB11-0314AB_KAL) this may vary depending on the analyst.
 - A description for the plate record is optional
 - In the Application drop-down list, select **GeneMapper-Generic**
 - In the Plate type drop-down list, select **96-well**
 - Enter your own initials for the owner and the operator.
 - Click **OK** and the GeneMapper Plate Editor opens.
- In the Sample Name column of a row, enter sample name and/or sample code (invalid characters for naming are / \ : * ? < > space).
- In the Comment column, enter any additional comments or notations for the sample.
- Leave the following columns blank: Sample Type, Size Standard, Panel, Analysis Method and Snp Set.
- Text can be entered for User-Defined columns 1 to 3 or they can be left blank.
- In the Results Group 1 column, select the analyst's initials from the drop-down list.

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- In the Instrument Protocol 1 column, select a protocol from the drop-down list. The protocols for PowerPlex 16 start with PP16_3kV_ and have options of 3sec, 5sec, 8sec, and 10sec injections.
- If samples are to be injected more than once:
 - Select Edit > Add Sample Run. Additional Results Group and Instrument Protocol columns are added to the right end of the plate record. This can be done before, during, or after the run.
 - Complete the columns for the additional run(s)
 - Click **OK** to save and close the plate record.

Note: After clicking OK within the Plate Editor, the completed plate records stored in the Plate Manager database. Once in the Plate Manager database, the plate record can be searched for, edited, exported, or deleted.

9.1.8.2 Create a Plate Record from an Export File

- Open a plate record template export file (i.e. .txt exported from instrument).
- Copy and paste sample name and/or sample code info into the export file. Ensure all spaces after sample name have been deleted. (Rows not in use may be deleted but do not delete any columns.) Save the record.
- Using the 3130xl Data Collection Software navigate to the following:
GA instruments > ga3130xl > Plate Manager
- Click **Import** and navigate to the saved plate record.
- Click **OK** to save and close the plate record.

9.1.9 Linking a Plate and Starting a Run

- In the Tree pane of the Data Collection Software
GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Run Scheduler
 - Click **Find All**. All plates in the database will display in plate record section. (You may also type the name of the plate in the box **Scan or Type Plate ID** to pull up plate.)
 - Select the plate record of the plate to be run, then click the plate position indicator that corresponds to the plate you are linking. The plate position indicator will be yellow if there is a plate in that position of the autosampler. The indicator will turn green once you click to link the plate.
- Confirm that your data has a valid export path by performing the following:
- Navigate to **GA instruments > Results Group**
 - Double click results group to be used.
 - Click **destination** tab.
 - Click **Test**. Verify whether or not the test succeeded and the file path.

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- To review the run schedule before beginning the run navigate to the following: **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Run scheduler > Run View**
- Once a plate is linked, the green run arrow will turn green. Click the green arrow in the toolbar.
- A dialog box stating "You are about to start processing plates" click **OK**
- The software automatically performs a run validation:
 - If the validation passes, the run starts
 - If any of the validation tests fails, the run will not start. Refer to the Applied Biosystems 3130/3130xl Genetic Analyzer's **Interference, Troubleshooting, and Reference Guide**.

9.1.10 Viewing Data During a Run

- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Run scheduler > Plate View**
 - This view shows a list of plates in the database and which plate is linked.
- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Run scheduler > Run View**
 - This view shows a list of all the runs scheduled, if they are validated, collecting data, or completed. It also shows where each run is located on the 96-well plate.
- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Instrument Status**
 - This view tells you the system status, EP voltage, EP current, Laser Power, Laser Current, Oven Temperature, array and polymer information.
- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Instrument Status > Event Log**
 - This view shows the event log and error log.
- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Capillaries Viewer**
 - Select the check boxes of the capillaries for which you want electropherograms displayed. As more capillaries are selected, the refresh rate becomes slower. The view can be zoomed in and out using the magnifying glass icons in the toolbar.
- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Cap/Array Viewer**
 - This window is used during a run to examine the quality of the data, which is displayed as color data for the entire capillary array. All the capillaries are viewed similar to a gel view.

Note: Do not leave the computer on these last two windows for extended periods of time due to large amounts of memory needed to view the data.

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9.1.11 Maintenance

9.1.11.1 Monthly Maintenance

- Run the Water Wash Wizard, flush water trap, and perform TH01 9.3-10 resolution ([Section 9.1.4](#)).
- Defragment the hard drive
Start > All Programs > Accessories > System Tools > Disk Defragmentation
- Restart the computer and the Instrument
- Wipe down 3130xl (inside and out)

9.1.11.2 As Needed Maintenance

- Ensure adequate levels of buffer and water in reservoirs
- Purge old plate records
 - Navigate to **GA instruments > Database Management**
 - Click on the **Cleanup Processed Plate** in the Database Status box. This will purge the Oracle database of all of the processed plates, except spectral and spatial plate files.

9.2 Applied Biosystems 3500xl Genetic Analyzer

*References: Applied Biosystems 3500xl Genetic Analyzers Reference Guide
PowerPlex®16 System part #TBD022 revised 8/10
PowerPlex® Matrix Standards, 3500/3500 Part #TBD022 Revised 12/10*

9.2.1 Start the Software and Instrument

- Turn the computer on and log into the computer
- Turn on the 3500xl Genetic Analyzer. Wait for the green status light to turn on.
- Ensure both the Daemon and Service Monitor has started by observing a green checkmark icon in the lower right hand corner. This indicates that all 3500 services have loaded. This may take several minutes.
- Launch the Data Collection Software:
Start > Programs > Applied Biosystems > 3500
Login to the 3500 Series Data Collection Software

9.2.2 Preparing the Instrument

- Navigate to the Dashboard of the software
- Check consumables by clicking **Refresh** to update consumable status.

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9.2.2.1 Replacing Anode Buffer Container (ABC)

The Anode Buffer Container (ABC) must be replaced after 7 days or 50 injections.

- Allow buffer container to equilibrate to room temperature prior to placing on the instrument.
- Ensure that most of the 1X buffer is in the larger side of the ABC container prior to removing the seal by tilting the container slightly.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Place the ABC into the Anode end of the instrument, below the pump. (RFID tag will face the instrument).
- Document in the 3500xl binder under the ABC buffer tab.

9.2.2.2 Replacing Cathode Buffer Container (CBC)

The Cathode Buffer Container (CBC) must be replaced after 7 days or 50 injections.

- Allow buffer container to equilibrate to room temperature prior to placing on the instrument.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Wipe away any condensation on the exterior of the CBC using lint free lab cloth.
- Tilt the CBC back and forth gently to ensure the buffer is evenly distributed and remove the seal.
- Ensure the top of the CBC is dry (failure to do this may result in arcing) and place the appropriate spacers on both sides of the CBC.
- Install the CBC on the autosampler.
- Document in the 3500xl binder under the CBC buffer tab.

9.2.2.3 Replenishing Polymer

Applied Biosystems recommends replacing the polymer (POP4) after 7 days however; the software allows you to continue using past 7 days. The polymer must be replaced after 960 samples or 120 injections.

- Click **Maintenance** (top right of the screen). In the Maintenance Wizards screen, click **Replenish Polymer** (this will take 10 to 20 minutes to complete) and follow the prompts.
- Polymer may be replenished as part of the water wash wizard ([Section 9.2.4.1](#)).
- Document in the 3500xl binder under the polymer tab.

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9.2.3 Replacing the Capillary Array

Applied Biosystems recommends replacing the capillary array after 160 injections however; the software allows you to continue using past 160 injections.

- The following indications may suggest that a new capillary array is required.
 - Poor sizing precision or allele calling
 - Poor resolution and/or decreased signal intensity
- In the Maintenance Wizards screen click **Install Capillary Array** (this will take 15-45 minutes to complete) and follow the prompts.
- Document in the 3500xl binder under the capillary array tab

Note: Spatial and Spectral Calibrations must be performed anytime an array is replaced. A water wash, water trap flush and TH01 9.3-10 Resolution must also be performed to verify performance of the array. Documentation of the water wash, water trap flush, and 9.3-10 resolution is recorded in the 3500xl binder.

9.2.4 Water Wash, Water Trap Flush and TH01 9.3-10 alleles Resolution/Performance Check

The water wash, water trap flush, and TH01 9.3-10 resolution are performed as part of monthly maintenance and/or anytime an array is replaced.

9.2.4.1 Performing the Water Wash

Can take over 40 minutes to complete

- Click **Maintenance** (top left of screen) on the dashboard.
- Select Wash Pump and Channels to run the wizard. Follow the prompts to completion

Note: An empty ABC reservoir may be used instead of emptying the reservoir currently on the instrument. Simply remove from the instrument, cover, and set aside. At the completion of the Water Wash Wizard replace the ABC with the reservoir previously removed from the instrument or a new reservoir.

9.2.4.2 Flush the Water Trap

Benefits to prolong the life of the pump and to clean any diluted polymer.

- Fill the supplied 20ml Luer lock syringe with warm deionized water. Expel any bubbles from the syringe.
- Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe clockwise.
- Open the Luer fitting by grasping the body of the fitting and turning it counterclockwise approximately one-half turn to loosen.

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- Flush 5ml of deionized water through the trap taking extra care not to use excessive force.
- Remove the syringe from the Luer fitting by holding the fitting with one hand while turning the syringe counterclockwise.
- Close the Luer fitting by lightly turning clockwise until the fitting seals against the block.
- Empty the water trap waste container.

9.2.4.3 TH01 9.3-10 alleles Resolution/Performance Check

A performance check allows you to periodically self-check the instrument system's resolution and its ability to correctly size peaks within one base pair using Promega PowerPlex® 16 Classic Allelic Ladder. A 9.3-10 Resolution should be performed whenever the capillary array has been changed and as part of the monthly maintenance of the instrument.

- Prepare a loading master mix by adding the following volumes of reagents to an appropriately sized tube:
 - 15µl ILS 600
 - 30ul allelic ladder
 - 285µl Hi-Di Formamide
- Vortex the master mix and spin briefly. Transfer 11µl of the master mix to the appropriate wells (i.e. A1-H3).
- Briefly centrifuge the well plate to remove any bubbles. Denature samples at 95°C for 3 minutes and then snap chill for 3 minutes.
- Place the 96-well plate into the plate base provided with the instrument and snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler.
- Close the instrument doors.
 - Refer to [Section 9.2.8](#), Creating a Plate Record (Note: Samples names can be designated with AL)
 - Refer to [Section 9.2.9](#), Linking a Plate and Starting a Run

9.2.5 Spatial Calibration

A spatial calibration establishes a relationship between the signal emitted by each capillary and the position where that signal falls on and is detected by the CCD camera. A spatial calibration must be performed when the capillary array has been replaced, the detector door has been opened, or the instrument has been moved.

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9.2.5.1 Performing a Spatial Calibration

- Access the Spatial Calibration screen:
 - Click **Maintenance** and then select **Spatial Calibration** in the navigation pane.
- Under Options, select **NO-Fill** or select **Fill** to fill the array with polymer before starting the calibration.
- Select **Perform QC Checks** to enable the system to check each capillary against the specified range for spacing and intensity.
- Click **Start Calibration**.

9.2.5.2 Evaluating a Spatial Calibration

- Evaluate the spatial calibration profile to ensure that you see:
 - One sharp peak for each capillary. Small shoulders are acceptable
 - One marker (+) at the top of every peak.
 - Peaks are about the same height.
- If the results meet the above criteria, click **Accept Results**. If the results do not meet the above criteria, click **Reject Results** and refer the Applied Biosystems 3500/3500xl Genetic Analyzer User guide, "Spatial calibration troubleshooting" page 300.
- If the results are acceptable, click **View Spatial Calibration Report**. Click **Print**, select **CutePDF Writer**, specify a name for the report (i.e. Spatial Report 3500xl 03-09-2011 SEJ) and save the file under DNA_Share in the 3500xl equipment maintenance folder.

9.2.6 Spectral Calibration

A spectral calibration creates a de-convolution matrix that compensates for dye overlap. A spectral calibration should be performed whenever the capillary array is changed, the CCD camera or laser are realigned or replaced, or if you see a decrease in spectral separation.

9.2.6.1 Performing a Spectral Calibration

- In the Dashboard, Click **Start Pre-heat** at least 30 minutes prior to the start of the run.
- Ensure the consumables are not expired and adequate injections remain.
- Ensure the pump assembly is free of bubbles, run the Remove bubble wizard if needed.
- Thaw the PowerPlex® Matrix Standards. Vortex and spin briefly.
- Make a 1:10 dilution of each dye fragment by mixing 2µl of the dye fragment in 18µl of sterile de-ionized distilled water.

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- A matrix standard master mix is prepared by combining the diluted dye fragments in a tube as follows:

○ Hi-Di™ Formamide	668µl
○ diluted FL Matrix Standard	8µl
○ diluted JOE Matrix Standard	8µl
○ diluted TMR Matrix Standard	8µl
○ diluted CXR Matrix Standard	8µl

- Vortex and spin briefly.
- On the 3500xl Genetic Analyzer, 24 wells of a 96 well plate are used for creating a matrix for the 24 capillaries. Load 25 µl of the matrix standard master mix into each of the 24 wells and cover with a plate septa.

Note: the software uses predetermined positions for the calibration. You cannot specify standard location on the plate. The standards must be loaded in wells A1-H3.

- Briefly centrifuge the plate containing the standards and verify that each sample does not contain bubbles and is positioned correctly in the bottom of the well.
- Denature samples at 95°C for 3 minutes then snap chill for 3 minutes.
- Place the sample plate into the plate base provided with the instrument.
- Snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler. Close the instrument doors.
- Access the Spectral Calibration screen:
 - Select **Maintenance**, then click **Spectral Calibration** in the navigation pane.
- Select **96** for the number of wells in the spectral calibration plate and specify the plate location (A or B) in the instrument.
- Select **Matrix Standard** as the chemistry standard and **Promega4dye** as the dye set.
- Select **Allow Borrowing**.
- Click **Start Run**.

2.2 Evaluating a Spectral Calibration

- Passing and failing capillaries are shown in green and red respectively. Borrowed capillaries are shown in yellow with an arrow indicating the adjacent capillary from which results were borrowed. Up to three adjacent-capillary borrowing events are allowed.
- If fewer than the recommended number of capillaries pass, the spectral calibration run will be repeated automatically up to three times.

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- View the spectral and raw data for each capillary. Ensure that the data meet the following criteria:
 - Order of the peaks in the spectral profile from left to right blue-green-yellow-red.
 - Order of the peaks in the raw data profile from left to right red-yellow-green-blue
 - The Quality Value is ≥ 0.95 and the Condition Number is ≤ 80
- If the data for all capillaries meet the above criteria, click **Accept Results**
- If any capillary data does not meet the criteria click **Reject Results** and refer to the Applied Biosystems 3500/3500xl Genetic Analyzer User Guide "Spectral calibration troubleshooting" page 301.
- If the results are acceptable, click **Export Spectral Calibration Results**. Click **View Spectral Calibration Report**, click **Print**, select **CuePDF Writer**, specify a name (i.e. Spectral Report 3500xl 03-03-2011 SE) for the report and save the file under DNA_Share in the 3500xl equipment maintenance folder.

9.2.7 Sample Preparation for the AB 3500xl

- Prepare the instrument by checking the consumables (click **Refresh** to update consumable status) to ensure consumables are not expired and adequate injections remain.
- Click **Start Pre-heat** to warm the oven.
- Check the pump assembly for bubbles and run the Remove Bubble wizard if necessary.
- Prepare a loading master mix by adding the following volumes of reagents to an appropriately sized tube:
 - # of samples x 0.5 mL of ILS 600
 - # of samples x 9.5 μ l of Hi-Di Formamide

Note: Prepare enough for a few extra reactions to allow for loss during pipetting. This is calculated automatically on the DB-BlankWkst3500xl Reagent Worksheet found on DNA_Share.

- Vortex the master mix and spin briefly. Transfer 10 μ l of master mix to appropriate sample wells of a 96 well plate.
- Add 10 μ l of Hi-Di Formamide to unused wells of a set of 24 (i.e. A1-H3).
- Add 1 μ l of allelic ladder and up to 2 μ l of each amplified sample to the appropriate wells. Reagent and amplification blank volumes must correspond to the largest volume of amplified sample added to the plate. When all samples have been added, cover with plate septa.
- Briefly centrifuge the 96 well plate to remove any bubbles. Denature samples at 95°C for 3 minutes and then snap chill for 3 minutes.
- Place the 96-well plate into the plate base provided with the instrument and snap the plate cover onto the plate, septa, and plate base.

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- Verify that the holes of the plate retainer and the septa are aligned.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler. Close the instrument doors.
- Refer to [Section 9.2.8](#), Creating a Plate Record
- Refer to [Section 9.2.9](#), Linking a Plate and Starting a Run

9.2.8 Creating a Plate Record

9.2.8.1 Create a Plate Record from the Data Collection Software

- In the dashboard click **Create New Plate**
- In the Define Plate Properties screen:
 - Enter a plate name (i.e. DB10-1222_3_PL), this may vary depending on analyst
 - Select 96 for the number of wells
 - Select HID for plate type
 - Select 36cm for capillary length
 - Choose POP4 for polymer
 - Owner, barcode and description are optional fields.
- Click **Save Plate**
- Click **Assign Plate Contents**
- Using either plate view or table view enter sample names in the corresponding wells.
- Under the heading Assays, File Name Conventions, and Results Groups click **Add from Library** select one of the following Assays:
 - Flex16_10sec
 - RPI6_16_20sec
 - Flex16_24sec
- Select a naming convention, and a results group of your choice. Click **add to plate** and then click **close**.
- Select the wells for which to specify the assay file name conventions and results groups and enable the checkbox next to the name to assign it to the selected wells.
- Click **Save Plate** and save.

9.2.8.2 Create a Plate Record from an Export File

- Open a plate record template export file (i.e. .xls exported from instrument).
- Copy and paste sample name info into the export file. (Rows not in use may be deleted but do not delete any columns) Save the record.

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- Using the 3500xl Data Collection Software, click on **Create New Plate** on the dashboard.
- In the Define Plate Properties screen:
 - Enter a plate name (i.e. DB10-1222AB_KAL)
 - Select 96 for the number of wells
 - Select HID for plate type
 - Select 36cm for capillary length
 - Choose POP4 for polymer
 - Owner, barcode and description are optional fields.
- Click **Save Plate**.
- Click **Assign Plate Contents**.
- Click **Import** at the top of the screen and navigate to the saved plate record (make sure .xls files are searched).
- Click **Save Plate** and save.

9.2.9 Linking a Plate and Starting a Run

- Click **Link Plate for Run**. A message will appear saying Plate loaded successfully. Click **OK**.
- Ensure plate is linked to proper position on 3500xl.
- Select **Create Injection List**.
- Click **Start Run**.

Note: Ensure all prompts have appeared and the run begins before walking away.

9.2.10 Viewing Data During a Run

Refer to Applied Biosystems 3500/3500xl Genetic Analyzer User Guide

9.2.11 Maintenance

9.2.11.1 Monthly Maintenance

Run the Water Wash Wizard, flush water trap, and perform TH01 9.3-10 Resolution ([Section 9.2.4](#)).

Defragment the hard drive

Start > Programs > Accessories > System Tools > Disk Defragmenter

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- Check for computer updates
Start > type in **Windows Update** in the search bar. Click on **Windows Update**
- Restart the computer and Instrument
- Wipe down the 3500xl (inside and out)

9.2.11.2 Maintenance to be performed as needed

- Ensure adequate levels of buffer in reservoirs
- Purge old plate records
 - Click **Library** and select **Plates** in the navigation pane. All plates stored within the library will appear on the screen.
 - Select the plates to be deleted (more than one may be selected at a time).
 - Right click the mouse and select **delete**.

Note: Do not use the purge feature to delete items in the library. Doing so will delete all items with the exception of factory stored items. Thus, all Promega assays and protocols will be deleted.

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SECTION 10 – DATA ANALYSIS USING AB GENEMAPPER SOFTWARE

References: *GeneMapper™ ID-X Software User Guide*
GeneMapper™ ID-X Software Tutorial
GeneMapper™ ID-X Software User Bulletin

10.1 Analysis of Casework Data with GeneMapper™ ID-X

10.1.1 Logging in to GeneMapper™ ID-X

- Open GeneMapper™ ID-X
- Select the User Name and Database Host from the drop-down list and enter the appropriate password.
- Click OK.
- The main project window will open.

10.1.2 Creating a Project

- To add samples from the collection software at a workstation go to the edit menu and select Add Samples to project > My Computer > DNA Share on the laboratory "I Drive" for per current designation(s).

Note: Location is dependent on network mapping and may vary slightly.

- Select the raw data folder to be imported or select individual samples and click Add to list. Then click Add.
- Select the appropriate Sample Type, Analysis Method, Panel and Size Standard for each sample and click the green arrow on the tool bar to analyze the samples. NOTE: Ensure that casework (not database) analysis parameters are selected. The user will be prompted to name and save the project to the appropriate Security Group (GeneMapper ID-X Security Group). Name the project with the Batch name, Q or K, the run date (YYMMDD) and the injection time. Click OK.
 - Example: 05-0204JMS_Q_050204_5sec
- Analysis is complete when the green arrows in the Status column on the left are gone.

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10.1.3 Viewing the Data

- Check your ILS by highlighting all samples and clicking on the Size Match Editor icon with the red peaks on the toolbar. Make sure all samples have ILS peaks sized correctly. Database analysis will include notes on the Sizing Quality (SQ<0.9)) of the ILS. Casework analysis will include similar notes if and when deemed necessary. See STR Data Interpretation ([Section 11](#)) for details.
- To view each sample, highlight the sample and click on the icon with the colored peaks to display plots. (This can also be done by View > display plots or ctrl + L).
- The electropherogram can be printed from the sample's plot window. Various plots have been created for different sample types. Commonly used plot displays include: Traditional Genotype Plot, Casework Blank, Casework artifacts and Casework zoom view.
- To view information on a sample's injection time and other run information, highlight the sample of interest and click View on the toolbar Sample Info (Ctrl + F1).
- To view raw data for a sample, click View > Raw Data.
- To view allele calls by sample and locus click the Genotypes tab from the main project window.

10.1.4 GeneMapper™ ID-X Manager – Analysis Methods Settings

The settings for the Analysis Methods are viewed by selecting GeneMapper ID-X Manager under the Tools drop-down menu, then clicking on the Analysis Methods tab then double clicking to select a particular Analysis Method. A range of Analysis Methods are available to analyze casework data. Options for Analysis Methods vary by RFU and analysis run. These methods shall not be modified. New methods shall only be created or modified with permission from the DNA Technical Manager.

10.1.4.1 Available Casework Analysis Methods

25RFU Casework-2400
25RFU Casework-2800
25RFU Casework-3200
50RFU Casework-2400
50RFU Casework-2800
50RFU Casework-3200
100RFU Casework-2400
100RFU Casework-2800
100RFU Casework-3200
50RFU red Casework-2800
Blank Casework

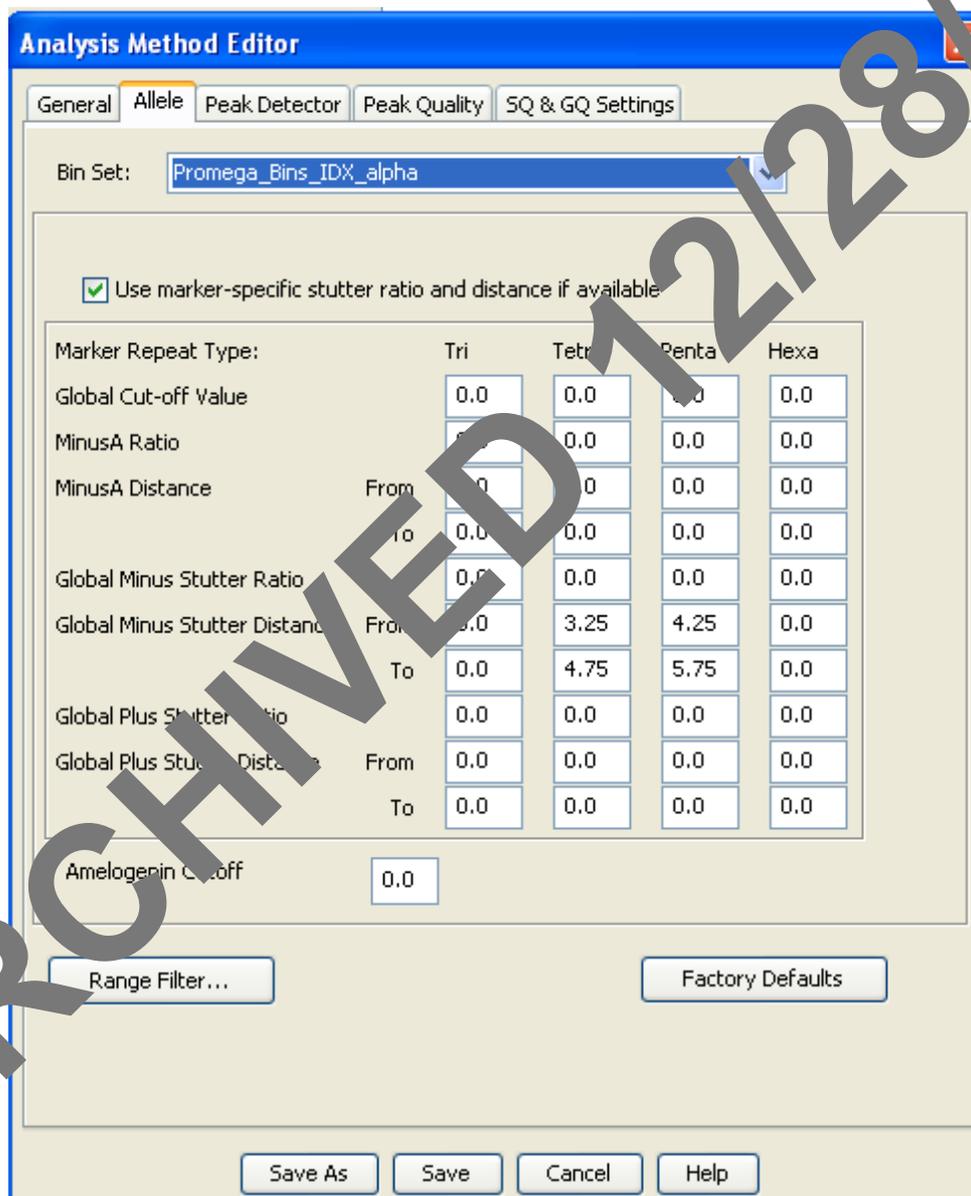
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10.1.4.2 Allele Tab Settings

The Allele Tab Settings that follow are consistent within all Analysis Methods



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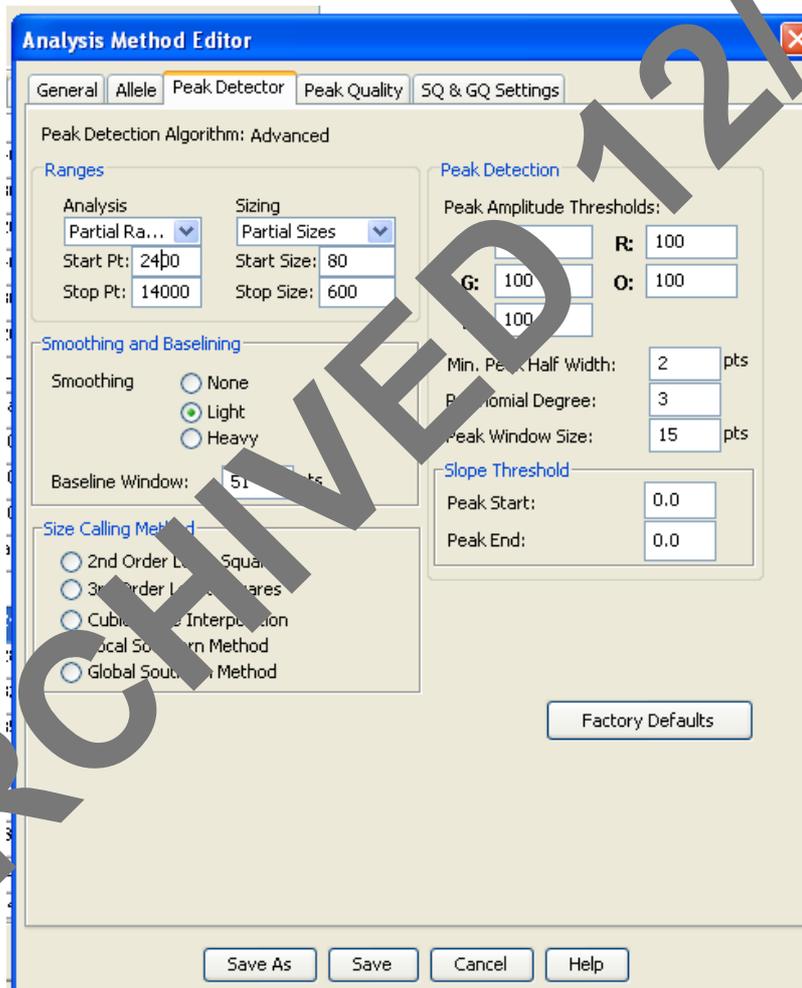
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10.1.4.3 Peak Detector Tab Settings

The Analysis Range and/or the Peak Amplitude Thresholds are the only settings that vary between the alternative Analysis Methods. The Analysis Range is selected to ensure capture of the 80bp ILS peak for all allelic ladders, positive controls, and questioned and known samples in the project. The Analysis Range is selected to capture the primer peaks (start point equal to 0) for reagent blanks, negative amplification controls, and samples that do not yield at least a partial profile. Typically the Peak Amplitude Threshold for all analyses is 100RFU. The use of a threshold other than 100RFU will be documented on the electropherogram(s) and must be approved by the DNA Technical Manager or CODIS Administrator.

Typical 100RFU Analysis Method



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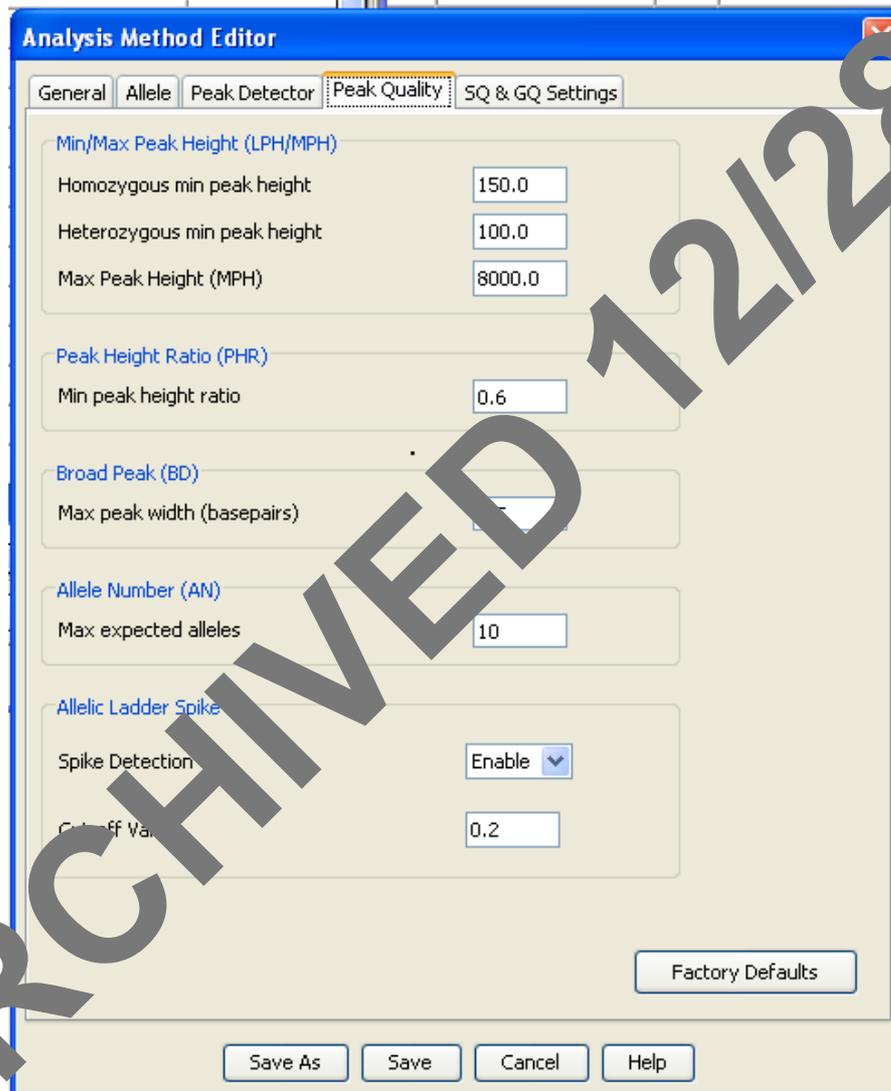
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10.1.4.4 Peak Quality Tab Settings

These settings do not vary among the 100RFU Analysis Methods, and they are not used in analysis of any samples where the sample type is set to Negative Control.

100RFU Analysis Methods



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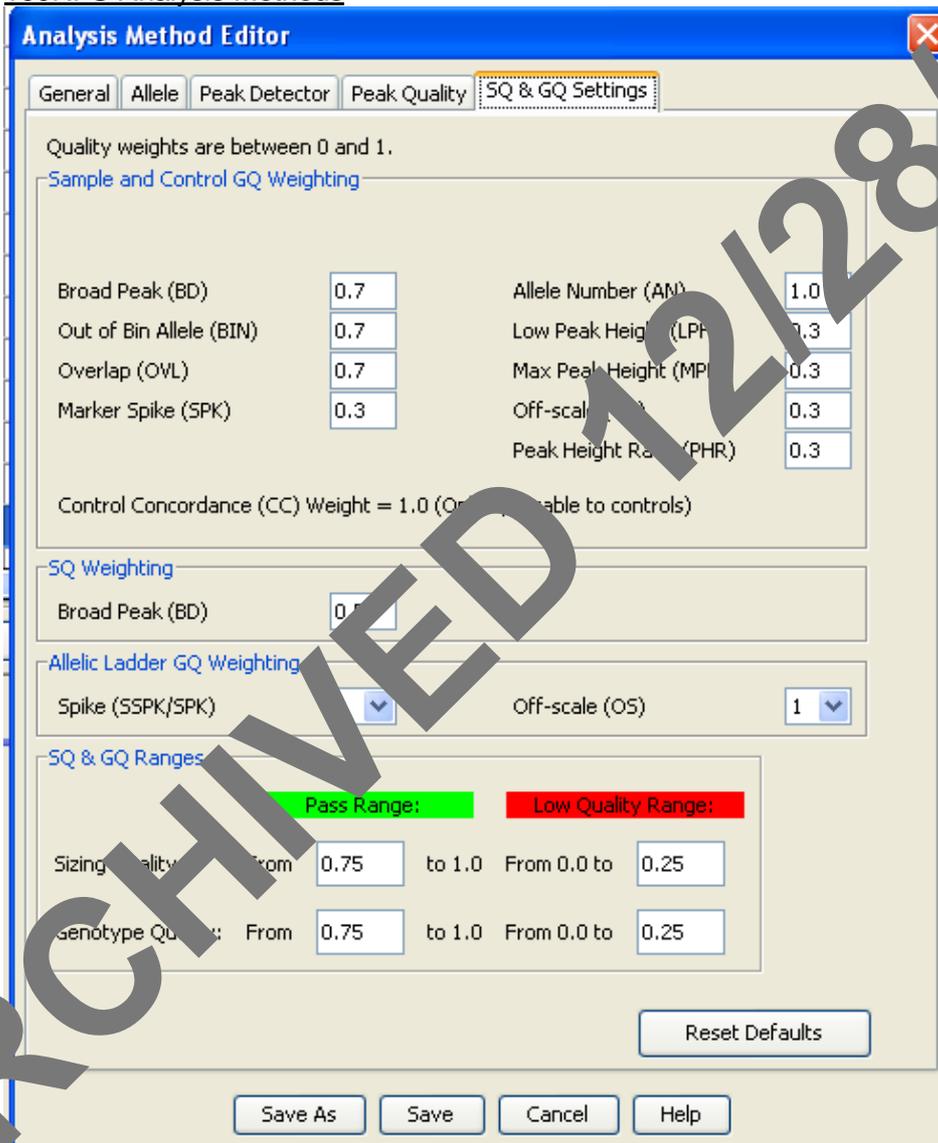
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10.1.4.5 SQ and GQ Tab Settings

These settings do not vary among the 100RFU Analysis Methods, and they are not used in analysis of any samples where the sample type is set to Negative Control.

100RFU Analysis Methods



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10.2 Analysis of Database Samples with GeneMapper™ ID-X

The analysis of database samples using GeneMapper ID-X is the very similar whether the data will be reviewed manually or using approved Expert System procedures. The procedures for analysis can be found in [Section 12.3](#).

10.3 Data Retention

Raw data files will be retained at the laboratory.

GeneMapper ID-X project files will be deleted after report distribution.

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SECTION 11 – DATA INTERPRETATION

References: GeneMapper ID-X User Guide, Tutorial and User Bulletins as appropriate.

The guidelines below are used for the interpretation of STR data. Guidelines for interpretation herein are for use at the discretion of the qualified analyst, based on their training and experience, experimental results, and case specifics. In all cases, interpretations must be independently reviewed and verified based on established peer review procedures. When the DNA Technical Manager is consulted for a sample that does not meet the criteria below and he/she approves its use, this must be documented by the DNA Technical Manager's signature and date on the appropriate interpretation electropherogram(s). The following definitions apply to case work STR interpretations:

"Reportable" alleles are those alleles whose peak heights are equal to or above the 100 RFU reporting (analytical) threshold **and are generally reproducible when re-amplified**. Such alleles will be routinely used in making comparisons between known and questioned sample genetic profiles under the interpretation guidelines stated in this manual.

"Observed" genetic data include data (peaks) below the reporting (analytical) threshold (100 RFU) and/or alleles whose peak heights are greater than 100 RFU, but that **may not be reproducible in duplicate or multiple amplifications** of the same sample (DNA extract). Such alleles will not be routinely included in the STR results table. "Observed" alleles may be considered for use when reporting the presence of multiple sources of DNA in a sample and/or when reporting the presence of male DNA. On occasion, depending on the quality of the data/genetic profile obtained, such alleles may be used in compositing data after careful evaluation of the data.

11.1 Interpretation of the Internal Lane Standard and Allelic Ladders

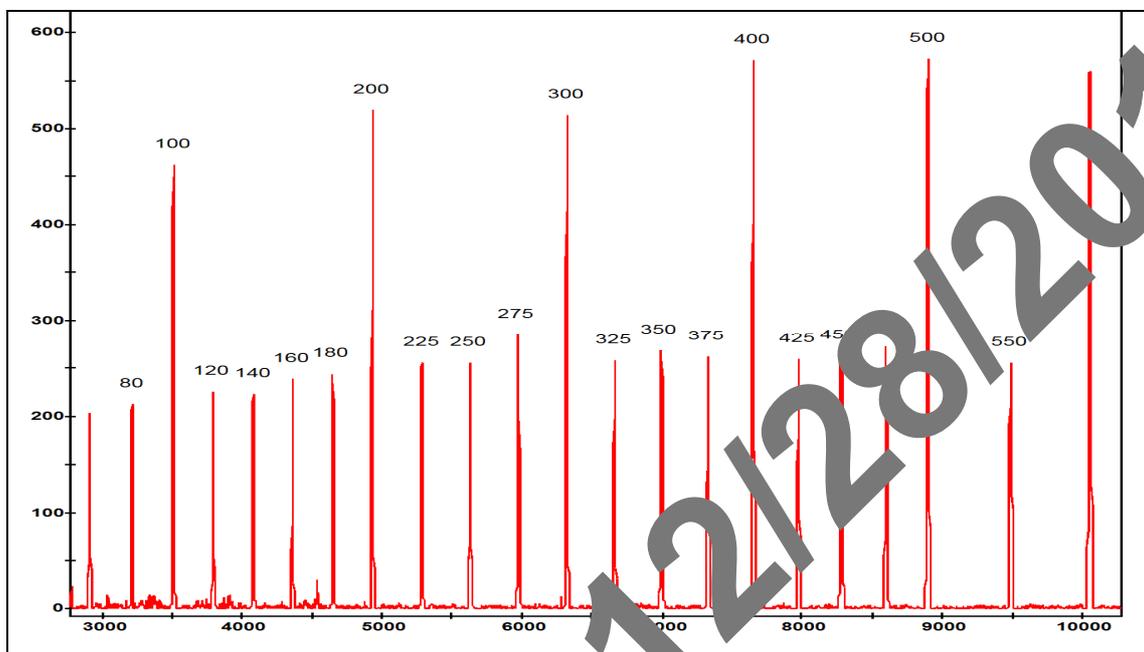
11.1.1 Internal Lane Standard (ILS)

The first step in interpreting data from a run is to assess the ILS for each sample. The Promega PowerPlex16® System uses ILS 600. The analyst should verify that all peaks from 80-550 base pairs are present and labeled as shown.

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The peak heights for the ILS peaks should be at least 100 RFU (relative fluorescence units) to be identified and labeled by the GeneMapper™ ID-X software.

When the peaks are not labeled as shown above, the analyst must determine the cause for the incorrect labeling. If the peaks at either the low or high ends are not visible, the data may be re-analyzed after changing the analysis range in the Analysis Method.

If a peak is not labeled because it falls below the 100 RFU reporting threshold, the analyst may, with the documented approval of the DNA Technical Manager, lower the acceptable threshold for the ILS peak(s). Notes reflecting this action shall be made on relevant electropherogram(s) / bench notes.

Use caution when allowing for lower ILS peak heights since sample peaks may also fall below 100 RFU when ILS peaks fall below 100 RFU. Therefore, re-injection of a sample(s) when the ILS peaks are below 100RFU is advisable.

Refer to the GeneMapper™ ID-X Software User Guide for details on manually labeling a size standard.

The Technical Manager shall approve using samples when some of the ILS peaks between 80 and 550 base pairs are not labeled or fall below 100RFU. Such approval will also be documented on the relevant electropherograms / bench notes as applicable.

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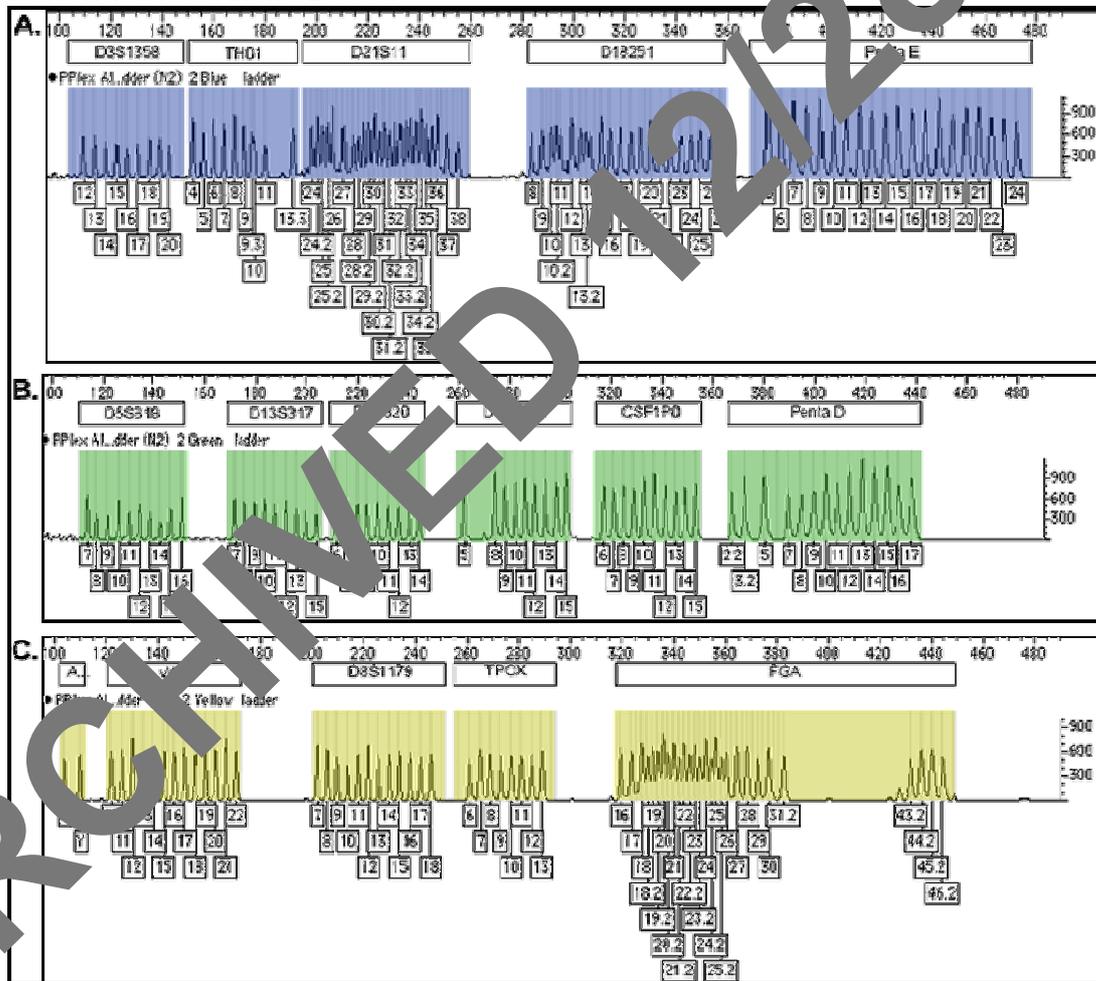
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When correct labeling of the ILS cannot be achieved for a sample(s) and the Technical Manager does not approve its use, the sample(s) shall be re-injected.

11.1.2 Allelic Ladder

When the analyst has verified the correct labeling of the ILS for the samples in a run, the next step is to verify that the peaks in the allelic ladder(s) are labeled correctly. The GeneMapper™ ID-X software uses the allele calls of the ladder to assign allele calls to all the other samples in the project. The allele calls for the Promega PowerPlex System allelic ladder are shown below:

PowerPlex16® Allelic Ladder



The allelic ladder contains the most common alleles determined for each of the 13 FBI STR core loci, the Penta D and Penta E loci, and Amelogenin.

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In addition, alleles not labeled in the allelic ladder (virtual alleles), may be detected and labeled in some of the samples analyzed.

All the peaks in the allelic ladder must be labeled correctly for the software to assign the correct allele calls to samples in the run.

At least one allelic ladder must be included in a GeneMapper™ ID-X project with the sample type designated as “allelic ladder”.

When more than one ladder in a project has the sample type designated as “allelic ladder”, the software will average the allelic ladders to make the allele calls for the samples in the project.

Artifacts such as spikes or “pull-up” peaks may be present that are labeled as off-ladder (OL) peaks in the allelic ladder. If all the true allele peaks are labeled correctly, such OL peaks will not affect the sample allele calls. If a spurious peak (not a true allelic peak) is labeled as a true allele (i.e. it falls within an allele size range and is designated and labeled as an allele) at any particular locus, the remaining allele designations / labels at that locus will shift and will impact the correct allele call / labeling of the samples in the project. Allelic ladders with such artifacts shall not be used to analyze data in the project.

Additional information about the allelic ladder can be found in the Promega PowerPlex16® System Technical Manual.

11.2 Interpretation of Amplification Controls and Reagent Blank Samples

The validity of the STR results obtained for any amplification and run is dependent on obtaining acceptable results for the reagent blanks and amplification controls associated with the forensic samples. Failure to obtain clean blanks and the correct type for the positive amplification control requires careful assessment of the data in that batch of samples. A portion of an extraction, amplification and run may need to be repeated depending on the results of the controls/blanks.

Note: Although appropriate quality assurance practices are stringently applied and enforced, it is not unexpected that low-levels of adventitious DNA (i.e., levels that result in peak heights for allelic data that are below the Laboratory’s reporting threshold) attributable to staff scientists and/or consumable products directly involved in the analytical steps of such a highly sensitive technique may be detected.

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11.2.1 Positive Amplification Control

The standard DNA template 9947A is used as the positive amplification control for the Promega PowerPlex16® System. The positive control is used to evaluate the performance of the amplification and typing procedures.

If the incorrect STR profile is obtained for the positive control, the analyst should verify that the ILS and allelic ladder peaks are labeled properly. If the ILS and allelic ladder are labeled correctly, but one or more of the alleles in the positive amplification control are labeled incorrectly or labeled as "OL", then the analyst when appropriate may remove a ladder(s) from the project or create different projects for different infections in an order to obtain acceptable allele calls for the positive amplification control. If the allele peak heights of the positive control fall below 100 RFU, but the other samples in the batch have acceptable allele peak heights, then the positive control sample data may be analyzed at 50 RFU to verify allele calls with the technical manager's approval. If only a partial profile is obtained from the positive control but another sample(s), eg. ICS (internal control specimen/sample) is/are available in the same batch, then, this will be noted.

If the analyst is unable to achieve correct labeling of the positive amplification control after attempting the actions described above, the Technical Manager should be consulted for determining the next course of action. It may be permissible for an Internal Control Specimen (ICS) ([Section 1.1.3](#)) to substitute as the positive amplification control.

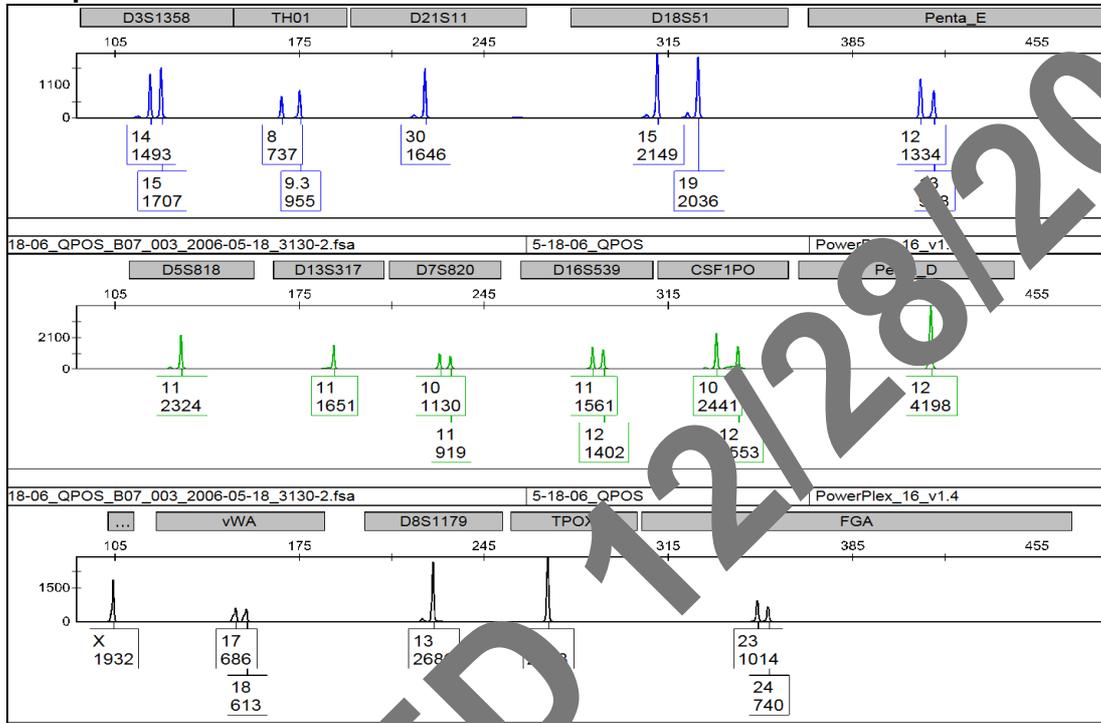
If no interpretable profile is obtained with any of the positive amplification control(s), then a positive amplification control and 10% of the samples in the run shall be re-amplified and re-typed.

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STR profile of 9947A



If all interpretable results agree for the samples between the first and second amplification/run, and the positive amplification control yields a correct STR profile for the second amplification/run, then the STR data obtained in the initial amplification/run may be used.

11.2.2 Negative Controls

11.2.2.1 Reagent Blank

A reagent blank is carried through the entire analytical process as part of each extraction batch. It contains all the reagents - except DNA template - used during extraction, amplification and typing for each type of extraction method in every batch of samples.

The purpose of the reagent blank is to detect DNA contamination that might occur from the reagents, the extraction environment or between the samples being processed and/or due to improper handling of the samples by the analyst.

The reagent blank must be run at the most stringent set of conditions for the batch (e.g., 100RFU threshold, longest injection time, largest capillary electrophoresis preparation volume). This applies to database samples as well as casework samples.

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The reagent blank should not yield any true STR allelic peaks above the 100 RFU reporting threshold. When peaks greater than or equal to 100RFU are present in the range between 80-550 base pairs, the analyst will determine if the peaks are artifacts (e.g. spike, pull-up) or true allele peaks.

The presence of peaks above 100 RFU will not invalidate the reagent blank as long as the peaks can be shown to be artifacts (see [Artifacts Section](#)).

When probable true allele peaks are detected at several or all STR loci above the reporting threshold (100RFU) and the analyst and the Technical Reviewer cannot come to a consensus on how to document the observation, the Technical Manager will be consulted to determine the appropriate course of action.

Since the failure of a reagent blank may indicate a problem at the extraction level, the cause of the failure will be fully investigated and documented in the anomaly log book. Steps will be taken (e.g. procedural modifications, corrective action, analyst retraining) to minimize recurrence. Formal corrective action reports may be completed based on the nature of the discrepancy. The Laboratory Quality Assurance Manual and DNA Quality Assurance Manual contain additional information on this subject.

11.2.2.2 Negative Amplification Control

The negative amplification control contains only the PCR master mix (reagents used to prepare the PCR amplification mixture) for each batch of samples and the water used to dilute the DNA samples.

The purpose of this control is to detect contamination that might occur from the PCR reagents, the PCR set-up environment or between the samples being prepared and/or due to improper handling of the reagents by the analyst.

The negative control must be run at the most stringent set of conditions for the batch (e.g. 100RFU threshold, longest injection time, largest capillary electrophoresis preparation volume).

The negative amplification control should not yield any true STR allelic peaks above the 100 RFU detection/reporting threshold. If peaks greater than 100RFU are present in the range between 80-550 base pairs, the analyst will determine if the peaks are artifacts (e.g. spike, pull-up) or true alleles.

The presence of peaks above 100 RFU will not invalidate the negative amplification control as long as the peaks can be shown to be artifacts.

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When probable true allele peaks are detected at several or all STR loci above the reporting threshold (100RFU) and the analyst and the Technical Reviewer cannot come to a consensus on how to document the observation, the Technical Manager will be consulted to determine the appropriate course of action.

Since the failure of a negative amplification control may indicate a problem at the amplification level, the cause of the failure will be fully investigated and documented in the anomaly log book. Steps will be taken (e.g. procedural modifications, corrective action, analyst retraining) to minimize recurrence. Formal corrective action reports may be completed based on the nature of the discrepancy. The Laboratory Quality Assurance Manual and DNA Quality Assurance Manual contain additional information on this subject.

11.3 Internal Control Specimen (ICS)

An internal control specimen (ICS) is a quality control sample processed with each batch of forensic casework samples and treated as any other casework sample. The purpose of this control is to demonstrate that all analytical processes are working at optimal levels as verified by using a sample that was previously processed and typed to demonstrate that it worked properly. ICS samples may include any known sample, including those obtained from laboratory staff, where the sample has been previously typed to generate a genetic profile.

An electropherogram of the ICS shall be included in the central log folder so the technical reviewer can verify the STR results and determine that the analytical process was appropriate.

The Technical Manager, or a qualified DNA analyst, shall determine whether or not to approve the ICS when only a partial profile or no profile is obtained. The data quality of other positive control samples in the batch and/or the quality of known /reference samples will be reviewed to determine the approval process.

If an incomplete STR profile is obtained and reported for the ICS, the Technical Manager will decide on the appropriate course of action. Generally, case reports from the analyst and the batch of samples will be suspended until the issue is resolved. The discrepancy will be documented and corrective and future preventive actions may be taken as deemed necessary by the Technical Manager.

The Technical Manager, or a designated individual, may approve the issue of specific reports on a case-by-case basis. This approval will be documented and maintained with the central log folder pertaining to the batch of cases affected by the discrepancy.

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11.4 Interpretation of Casework Samples

For all casework questioned and reference samples, the minimum reporting (analytical) threshold is 100 RFU. Allelic peaks greater than or equal to 100 RFU that are reproducible if re-amplified, may be included in the STR results table and used in statistical analyses. Only alleles equal to or greater than 100 RFU will be considered in data interpretation.

A genetic profile will not be considered suitable for comparison when alleles are detected in less than 4 of 13 core STR loci with peaks (homozygous, heterozygous / partial) equal to or greater than 100 RFU. This is applicable to both single source samples and DNA mixtures. Exceptions for single source samples may be appropriate. The Technical Manager will be consulted for determining the appropriate course of action for any exceptions to routine guidelines.

If one or both Amelogenin peaks fall below 100RFU for a sample, the sample may be analyzed at 50RFU in order to determine the data at the Amelogenin locus. This allowance shall be applied only to the Amelogenin locus. The analyzed data and the relevant electropherogram shall document the lowered threshold for the Amelogenin locus, the peak height(s) and the base pair size(s) of the X and Y peaks. Amelogenin data determined and documented in this manner is suitable for inclusion in the STR results table. (This is applicable to database samples as well).

When peak(s) below 100RFU exhibit optimal peak morphology and fall in an allele bin, the electropherogram and the STR results table will be marked by an asterisk (*) indicating data below reporting threshold was observed in this sample.

When peaks are observed below the reporting threshold of 100RFU, an additional electropherogram showing a close-up view of the affected loci should be printed for review and inspection. The electropherogram(s) shall also include a note documenting that data below reporting threshold (dbdt or dbrt) was/were observed.

Ideally, allelic peak heights should fall between 500 and 2000 RFU. Peak heights outside this range may be acceptable; however, it is possible that these samples will require careful interpretation. Alleles with peak heights less than 500 RFU may occasionally exhibit peak height imbalance due to stochastic effects caused by low template copy number.

An initial assessment of a questioned sample STR profile should be done without considering the STR profiles of the known/reference samples for the case. Additionally, the analyst should determine whether a sample is a single source sample or a DNA mixture and determine which alleles may be reported, prior to comparing the questioned sample to known/reference samples analyzed.

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The probative value of samples will be considered when determining if statistical analysis is appropriate. Probative value of evidence is assessed on a case-by-case basis. Evidence that is sufficiently useful to answer an investigative question and /or prove or disprove one of the legal elements of the case would be considered probative in nature (i.e. sperm fraction from vaginal swabs, intimate sample with DNA foreign to owner, various contact DNA samples, etc).

11.4.1 Low-Level DNA Samples

Forensic DNA samples often contain low levels of DNA. Low-level DNA samples are samples that exhibit peaks below the reporting threshold. When only a few DNA molecules are used to initiate PCR, unequal sampling of the alleles present from a heterozygous individual may occur leading to stochastic effects (i.e. peak height imbalance, elevated stutter, non-reproducible alleles, etc).

Peak height imbalance owing to low template copy number stochastic effects can often be mitigated by re-amplifying the sample with more template DNA. Alternatively, it may be appropriate to perform a duplicate amplification of the samples to assess reproducibility of the data. All components of a DNA sample mixture may not exhibit reliable reproducibility (of allelic peaks) when there are stochastic effects during PCR. This phenomenon will impact which alleles may be reported and used in data interpretation. Only alleles equal to or greater than 100 RFU shall be reported on the STR results table and considered suitable for comparison.

Questioned samples with a quantification value of $<0.05\text{ng}/\mu\text{l}$ and greater than zero (or PCR target template estimate of less than 0.95ng) shall be routinely amplified in duplicate to ascertain reproducibility of alleles above and below 100RFU. Samples with a quantitative result of zero do not require duplicate amplification. Data interpretation and reporting of conclusion(s) will depend on whether the profile indicates a single source or mixed DNA sample.

Questioned samples with a quantification value $>0.05\text{ng}/\mu\text{l}$ will not be routinely amplified in duplicate. If the profile generated includes artifacts (e.g. stutter/true allele) and/or ambiguous allele peaks, the sample will be re-amplified to verify reproducibility of the data.

Note: If a sample is amplified in triplicate – the reproducible alleles in at least two amplifications shall be reported on the STR results table and considered suitable for comparison.

Samples with PCR inhibitors may require re-extraction or additional methods of sample clean-up. The DNA Technical Manager must be consulted prior to using methods not defined in this manual.

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11.4.2 True Alleles

True alleles are peaks that are detected and labeled by the GeneMapper™ ID-X software with a number and should lie within the range of the alleles in the ladder.

Peaks that are labeled as OL (off ladder) at a particular locus have a base size that is different from any of the alleles in the ladder and any of the virtual alleles. These peaks may be true alleles but additional analysis is required to verify this.

11.4.2.1 Artifacts

Artifacts are not true alleles. Artifacts can occur in data and much of their nature and origin have been determined and documented.

When an artifact is present within the size range of the alleles at a locus and the presence of the artifact interferes with interpretation of the data at that locus, the STR results may be suitable for reporting as long as the affected locus is excluded. Re-analysis of the sample may not be necessary, but this will be determined by reviewer and/or Technical Manager input.

The affected locus may be reported as 'NR' (not reported) on the STR results table with a similar corresponding notation on the electropherogram.

Alternatively, the alleles at the affected locus may be reported if the locus is omitted from statistical analysis. This is indicated by 'NS' (locus/data not used in statistical analysis) on the STR results table and on the electropherogram.

When it is not possible to distinguish between an artifact and a true allele, this is indicated on the STR results table and the electropherogram as Artifact/True allele (A/TA).

11.4.2.2 Off-Ladder (OL) Alleles

The allelic ladder contains the most commonly observed alleles for the STR loci. Alleles which are not assigned a numeric designation in the standard ladder will be designated as off-ladder (OL) alleles.

Before determining whether an OL is a true allele or an artifact, the analyst must consider all other possible causes of OL occurrence.

OL alleles considered to be true alleles may require re-amplification to confirm. If the OL allele is observed in multiple samples derived from multiple tissue types or sources with the same STR profile, only one of the samples needs to be re-amplified for verification of the OL allele(s). The DNA Technical Manager must approve reporting an OL allele without re-amplification (e.g. limited amount of sample available for analysis).

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When the OL allele lies within the range of alleles for a particular locus, the allele will be reported as a variant of the integer (i.e. X.1, X.2, etc.). The appropriate allele call is determined by simultaneously examining the base sizes for the sample allele peak and the associated allelic ladder peaks in the relevant locus. An electropherogram will be printed for the sample and the associated allelic ladder for the locus, showing the OL allele(s) in the locus, with the bins and base pair sizes.

When the OL allele lies either above or below the largest or smallest allele in the ladder, the OL allele will be designated as either greater than (>) or less than (<) the alleles from the ladder that is closest in proximity to the OL allele.

11.4.2.3 Incomplete 'A' nucleotide addition (-A or split peaks)

AmpliTaq Gold™, like many other DNA polymerases, catalyzes the addition of a single nucleotide (predominantly adenosine) to the 3' ends of double stranded PCR products. This non-template addition results in a PCR product that is one base longer than the actual template, and the PCR product with the extra nucleotide is referred to as the "+A" (base peak) form.

The "-A" form is the peak that represents the correct template length. The "+A" peak will normally be the predominant form, and the "-A" should usually not be detected.

The final step of the STR amplification process is a terminal extension step (60°C for 30 minutes) to promote complete non-template 'A' nucleotide addition.

Failure to attain complete terminal nucleotide addition results in "split peaks", two peaks that are one base apart. Typically, split peaks are the result of an excess of template DNA in the PCR reaction.

If the labeled "A" peaks are not pervasive throughout the sample and the sample appears to otherwise be a single source sample, an electropherogram may be printed to document the allele call, base size and peak heights at the affected loci and the OL peaks labeled as such.

When the sample exhibits artifacts due to an excess of template DNA in several loci, it may be more appropriate to re-amplify the sample with less template DNA.

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11.4.2.4 Pull-up

Multi-component analysis is the process that separates the four different fluorescent dye colors into distinct spectral components. The four dyes (Fluorescein, JOE, TMR and CXR) emit their maximum fluorescence at different wavelengths, but there is some overlap of the emission spectra. A spectral calibration is performed for a specific dye set to create a matrix that corrects for the spectral overlap.

Pull-up is the result of incomplete separation of the emission spectra and is typically observed as a non-allelic peak at the same base size as a peak in another dye. Pull-up typically occurs at high RFU values, when an excess of template DNA has been amplified.

Pull-up that gets labeled with an allele or OL designation should be documented by an electropherogram showing the base size for the true allele and the pull-up peak. If the pull-up is prevalent enough to interfere with data interpretation, the sample may need to be re-injected or re-amplified with less DNA template.

Pull-up may also occur when the matrix file is not current. A new spectral calibration is performed whenever a new capillary array is placed on the instrument or whenever any part of the optics have been realigned and/ or replaced. A new spectral calibration may also need to be performed when an overall decrease in spectral separation is observed.

11.4.2.5 Stutter

The PCR amplification of tetranucleotide STR loci typically produces a minor product peak four bases shorter or five bases shorter (infrequently observed), than the corresponding base peak. This is the result of slippage of the Taq polymerase and is referred to as the stutter peak or stutter product.

In the pentanucleotide loci, the stutter peak is five bases shorter than the corresponding base peak.

The proportion of stutter product relative to the main allele peak is calculated by dividing the height of the stutter peak by the height of the main allele peak. The stutter percentage is fairly reproducible for a particular locus. The following table lists the values of the expected percentage of stutter for the loci in the PowerPlex® 16 System when run on the Applied Biosystems 3100/3130xl. The stutter values are based on data obtained by internal validation studies as well as other published data (Krenke, B., et al: Validation of a 16-locus Fluorescent Multiplex System. July 2002, J Forensic Sci.,47(4)).

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Estimated Stutter Percentage for PowerPlex® 16 loci on the 3130xl

D3S1358 13%	TH01 5%	D21S11 15%	D18S51 13%	Penta E 7%	
D5S818 13%	D13S317 10%	D7S820 10%	D16S539 10%	CSF1PO 10%	Penta D 5%
Amelogenin NA	vWA 13%	D8S1179 10%	TPOX 5%	F1A 13%	

Peaks in the stutter position greater than the listed values may indicate the presence of DNA from more than one individual.

When there is no indication of a mixture, other than the listed stutter, the analyst will document the stutter as such on the electropherogram.

When the sample is a DNA mixture, it may not be possible to distinguish between a stutter peak(s) and a minor component allele(s). In such situations, the peak may be labeled as an Artifact/True allele (A/TA). This shall be documented on the electropherogram (and on the STR result table if appropriate).

As with other artifacts, if the presence of the stutter peaks interferes with data interpretation, the sample may need to be re-injected or re-amplified with less template DNA.

11.4.2.6 Spikes

Spikes are non-allele peaks that may arise due to air bubbles, urea crystals or voltage spikes. Spikes are typically quite sharp and easy to distinguish from a true allele.

Spikes usually appear in more than one color at the same base size and are not reproducible on re-injection.

If the presence of a spike(s) interferes with the interpretation of the sample, the sample will be re-injected.

If the sample is not to be re-injected, the spike(s) shall be documented as such on the electropherogram.

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11.4.2.7 Dye Blobs

Dye blobs, like spikes, are non-allele peaks that are easily distinguished from true alleles. Dye blobs may be the result from residual dye molecules left over from the synthesis of the primers or they may be dye molecules that fell off the primer during the amplification. Samples with dye blobs that interfere with data interpretation will be re-injected. If the sample is not to be re-injected, the dye blob(s) shall be documented on the electropherogram as artifacts.

11.4.2.8 Persistent Kit Artifacts

Occasionally, one or more lots of PCR kits may exhibit persistent artifacts that may not be observed in the verification process. These artifacts may or may not appear as true alleles. Sometimes, these artifacts are so common that they are observed in a high number of routine samples.

If the presence of the kit artifact does not interfere with data interpretation, the Technical Manager shall determine if these artifacts can be documented as such on the electropherograms, without requiring re-analysis of the sample. This may not always be possible in mixed samples, where it can be difficult to distinguish an artifact from a true allele.

When the Technical Manager has acknowledged a particular kit artifact based on documented verification of the kit, his/her documented approval is not required on subsequent electropherograms containing that artifact.

11.4.3 Single Source Samples

Typically, each locus is characterized by one or two labeled peaks or alleles. If two peaks/alleles (heterozygous locus) are detected at a locus, they should ideally be of equal intensity – that is, the peak height ratio (PHR) is approximately 1:1.

Peak height ratios are calculated by dividing the peak height of the allele with the lower RFU value of the heterozygous pair by the peak height of the allele with the higher RFU value, and then multiplying this dividend by 100 to express the PHR as a percentage. The minimum expected PHR for single-source samples, where there is no indication of a mixture, is flagged in GeneMapper™ ID-X at 60%. This ratio, however, may be lower with lower amounts of DNA. Peak-height-ratio imbalance may also result from degraded DNA or the presence of PCR inhibitors. Allelic dropout may occur in degraded/inhibited samples and the possibility of a second allele should be considered.

Samples with more than two peaks at a locus or with alleles of significantly different peak heights may indicate the presence of mixtures, tri-alleles, primer binding site mutations or other biological or procedural artifacts. Such observations require careful consideration.

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The frequency of a single source profile (including single source major/minor components from a DNA mixture) will typically be calculated when STR results cannot exclude an individual as the source of DNA in the sample.

The probative nature of single source matches will be considered when determining if statistical analysis is appropriate. It is not necessary to calculate a profile frequency when the questioned sample is an intimate sample (i.e., body swabs) and there is no detectable DNA foreign to the "owner" of the sample (i.e. the person from whom the sample was collected) or if the questioned sample is consistent with a consensus and/or elimination known/reference sample(s).

Loci where the reported genotypes are identical between the questioned and the known sample shall be used in the statistical analysis and the frequencies of such profiles reported.

11.4.3.1 Tri-alleles

Occasionally, a single source sample may be observed to have three alleles at one or more loci. Samples exhibiting potential tri-alleles may be re-extracted and re-amplified to confirm the genotype observed at the locus (or loci). Statistical calculations, however, will not be performed at these loci.

If the potential tri-allele containing sample is re-extracted and re-amplified and found to be reproducible, the analyst may include the locus with the tri-allele as in the STR results table. If the potential tri-allele is not reproducible, it will be reported as an Artifact/True allele (A/TA).

If the same tri-allele pattern is also observed in multiple samples (from the same case) but from different tissue sources – for example, blood / semen / saliva / hair, with the same STR profile, the potential tri-allele may be considered a reproducible observation and may be included on the STR results table without the A/TA designation. No re-extraction of samples would be required in such instances.

Note: Tri-allele patterns have been observed in single source samples and there are documented instances where different tissues from the same individual may or may not exhibit the tri-allelic pattern.

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11.4.3.2 Comparison of DNA Results

The interpretation process generally results in one or more of the following conclusions:

- Exclusion
- Cannot exclude
- Inconclusive
- Insufficient DNA/Data
- No genetic profile

Exclusion (Non-match):

An exclusion is declared when the known sample has alleles that are not observed in the questioned sample and there is no scientific explanation for the non-match.

Scientific explanations may include, but are not limited to, factors such as inhibition, degradation, low template DNA and/or primer binding site variations or mutations.

Parent-offspring exclusion (also Parent-offspring inconclusive) is declared when, upon comparison of the DNA results from an offspring sample to that from an alleged biological parent(s), the profiles are found to not share at least one allele at two or more corresponding loci.

Cannot exclude (Match):

The donor of the known sample cannot be excluded as a source of the questioned sample when there are no significant differences between the allele designations obtained from these samples. The Random Match Probability (RMP) will be calculated for the forensic sample profile.

Partial Profile: If one allele is identified and a second potential allele is below the reporting threshold (100%), the identified allele will be used for comparison purposes first and if concordance is established the peak below threshold will be assessed to support the inclusion and/or exclusion of an individual(s). Any locus/loci that is utilized for an inclusion based on the individual's allele(s) falling in a peak below threshold position will not be used in the statistical calculation. The significance of a match will decrease for partial profiles because there are fewer loci to compare.

Parent-offspring inclusion is declared when upon comparison of the DNA results from an alleged offspring sample to that from an alleged biological parent(s), the profiles are found to share at least one allele at all corresponding loci for which interpretable DNA results were obtained.

Inconclusive:

When the nature of a forensic profile is such that a meaningful comparison cannot be made due to varying reasons, then the results are deemed inconclusive.

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Insufficient DNA/Data:

If insufficient DNA is observed in a sample (e.g. data below reporting threshold, drop-out, low-level DNA) the data may not be suitable for comparison. This data will be considered insufficient and cannot be utilized for meaningful comparative analysis. As such, this data will be omitted from the STR results table.

No genetic profile:

No genetic profile is declared when there is no data observed. This also may include low-level data observed at less than 4 of 13 core STR loci.

11.4.4 DNA Mixtures

DNA mixtures derived from more than one individual are frequently identified in biological evidence recovered in forensic investigations. DNA mixtures may consist of blood, semen, saliva, epithelial cells or a combination of any of these sources. Such evidence may be derived from:

- intimate swabs/samples collected from an individual
- blood/contact DNA found on weapons, seen in assaults or homicides
- wear areas on clothing
- biological evidence recovered from high traffic areas (i.e., floors, carpets, door handles, steering wheels, etc.)
- other biological samples collected from crime scenes

An evidence item taken directly from an identified anatomical location (i.e., vaginal swab, penile swab, fingernail scrapings, etc.) and / or a piece of intimate apparel (i.e., undershirts, panties, bra, etc.) typically will yield DNA from the individual from which the evidence item was taken. In such circumstances, it is common to recover a DNA mixture which includes DNA consistent with that individual.

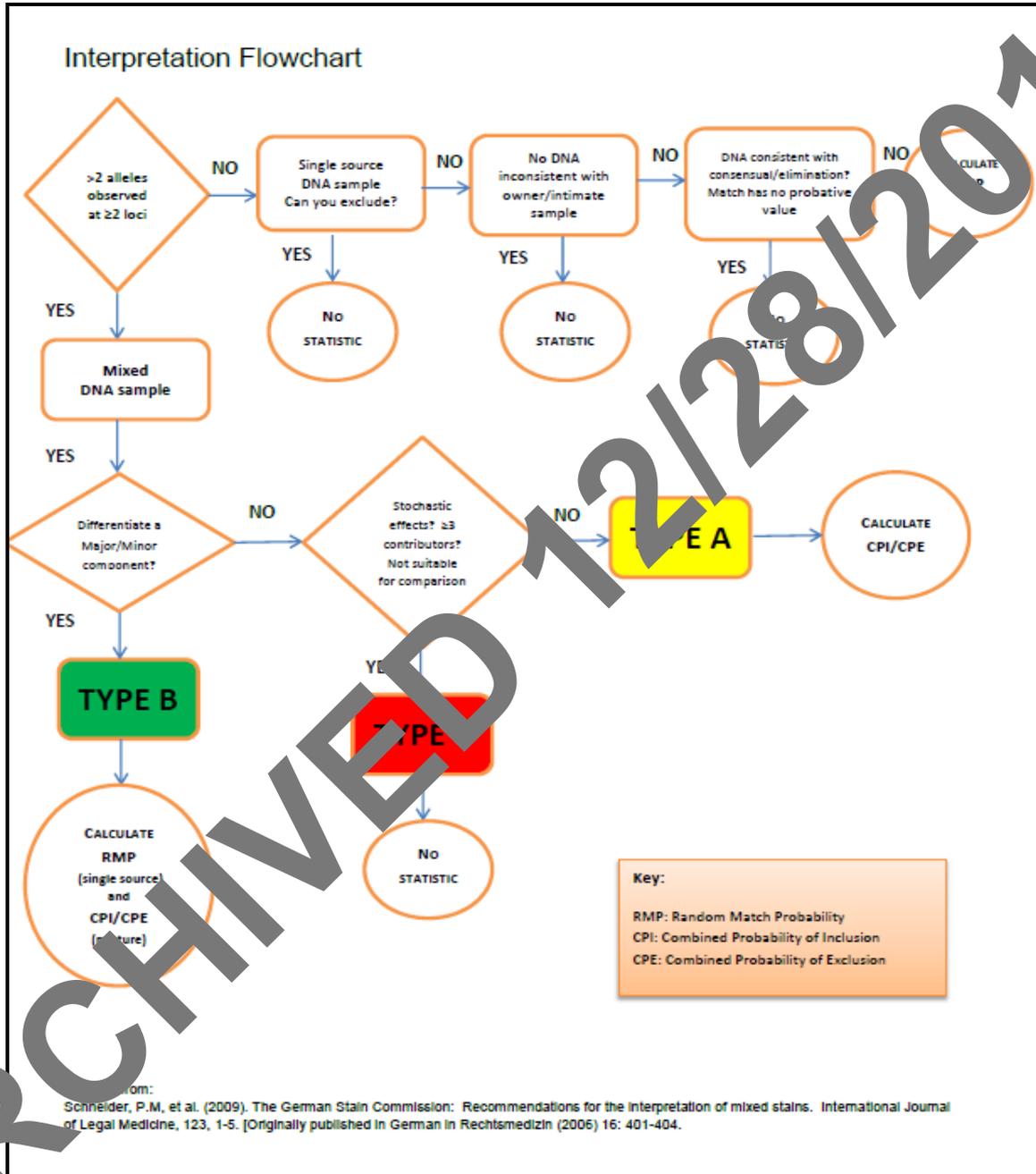
All loci for which DNA results were obtained must be considered when evaluating potential mixture samples. A sample may be considered to have originated from more than one individual when more than two alleles are observed at more than two loci. The minimum number of contributors may be defined by evaluating the locus that exhibits the most allelic peaks (i.e., if at most five alleles are detected at a locus, the DNA results are consistent with having arisen from at least three individuals).

Identification of DNA mixtures can be straightforward or difficult, depending on the number of loci typed, the number of contributors and the relative proportion of DNA contributed by each individual to the mixture. Therefore, interpretation of DNA mixtures and statistical analyses requires careful consideration of factors affecting allele designation, heterozygote detection and peak height variations within and between loci.

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Indications of a DNA mixture sample can include:

- (1) more than two alleles at two or more loci,
- (2) a peak in a stutter position that is unusually high
- (3) significant peak height ratio imbalance for a heterozygous genotype
- (4) Observation of low-level DNA/data below reporting threshold

Occasionally, factors other than DNA mixtures may influence such observations. Before determining that a sample is a mixture, the analyst shall eliminate the possibility of split peaks, pull-up, stutter, kit artifacts, and low template stochastic effects.

When a sample is determined to be a mixture, the analyst must consider whether it can be dissected into major and minor components. The number of potential contributors of DNA and intralocus peak height ratios should be considered when making this determination.

An analyst may not dissect a mixture profile into major and minor components if a mixed sample profile indicates the possibility of several or more (potentially) shared alleles between the contributors. Dissecting the profile into major and minor components may prevent inclusion of the minor alleles in statistical analysis.

As the number of potential contributing sources in a mixture increases or, if the number of shared alleles between the contributing sources increases, dissecting a mixture profile into major and minor components becomes increasingly difficult. Caution should be exercised if attempting such action and the conclusions reported should remain conservative. Technical reviewer and DNA Technical Manager approval and documentation may be required to resolve analysis and interpretation of such data.

The probative nature of DNA mixtures will be considered when determining if statistical analysis is appropriate. It is not necessary to calculate a profile frequency when the questioned sample is an in-state sample (i.e., body swabs) and there is no detectable DNA foreign to the "owner" of the sample (i.e. the person from whom the sample was collected) and a consensual and/or elimination known/reference sample(s).

11.4.4.1 DNA Mixture Type Categorization

It is not possible to anticipate the nature of all potential DNA results, or the nature of the evidentiary samples from which they may be obtained. These categories do not exhaust the possible list of the results that may be encountered by the forensic scientist nor the conclusions that a forensic scientist may render based on his/her interpretation of those results.

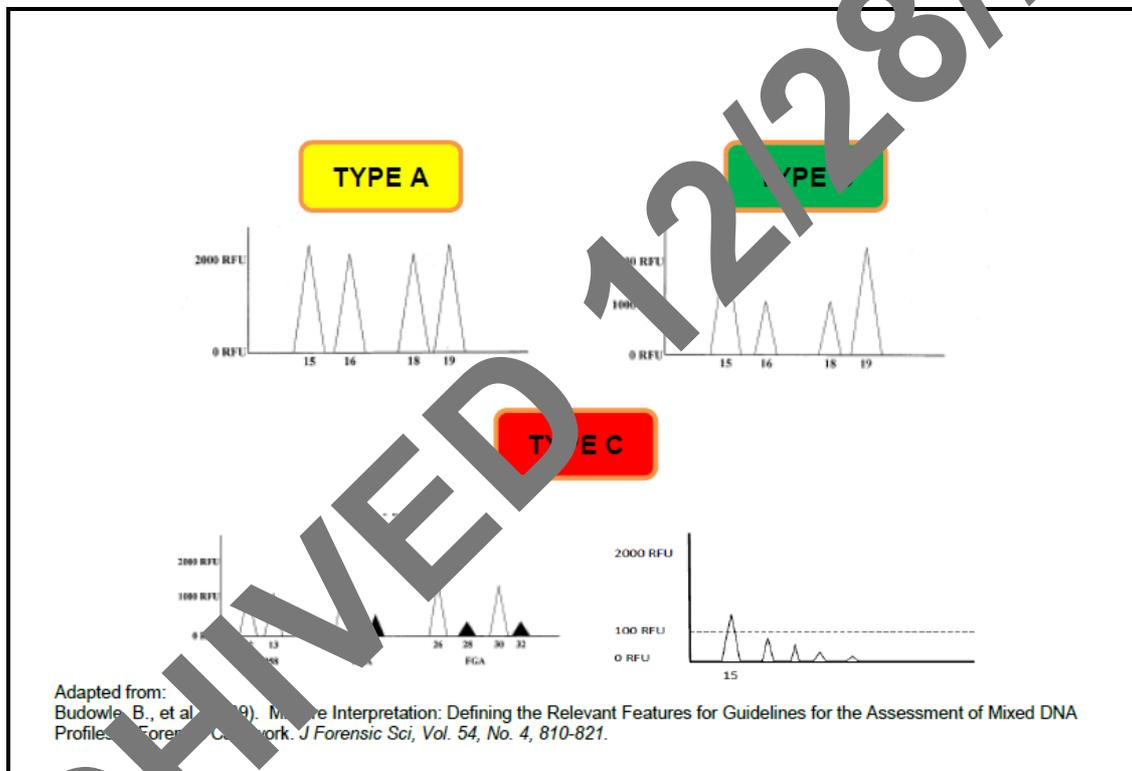
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Three categories of mixed samples have been designated to guide general decision making as illustrated in the Interpretation Flowchart. Mixtures with a known contributor (i.e. intimate samples) are possible in each category:

- **TYPE A:** mixtures with indistinguishable contributors, including multiple major contributors
- **TYPE B:** mixtures with major/minor contributors
- **TYPE C:** mixtures containing DNA from at least three sources and/or mixtures that exhibit potential stochastic effects (e.g. 1 major plus 2 or more minor contributors, 2 major plus 1 or minor contributor, indistinguishable)



TYPE A:
Description: If the amounts of biological material from multiple contributors are similar, it may not be possible to further refine the mixture profile. When major or minor contributors cannot be distinguished because of similarity in signal intensities (i.e. peak heights), the sample is considered to be an indistinguishable mixture.

The classification as indistinguishable does not imply that the profile is uninterpretable. Individuals may still be included or excluded as possible contributors to an indistinguishable mixture. When appropriate, statistical analysis may be performed using the mixture formula.

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TYPE B:

Description: If the amounts of biological material from multiple contributors are dissimilar, it may be possible to further refine the mixture profile. When major or minor contributors can be distinguished because of differences in signal intensities (i.e. peak heights), the sample is considered to be a distinguishable mixture. The differences are evaluated on a case-by-case basis.

Separating a two-source mixture into major and minor components is easier when the number of loci exhibiting four alleles increases and the ratio of the major contributor to the minor contributor increases above a 2:1 ratio.

If a mixture can be successfully separated into major and minor components, the minor component alleles are reported within parentheses. Due to the possibility that the minor contributor's alleles may be shared by the major contributor and that such alleles may be below reporting threshold, determination of the minor contributor profile may be possible at only some loci.

The minor component may be suitable for comparison if alleles equal to or greater than 100 RFU occur at least 4 of 13 core loci. When evaluating if a minor component is suitable for comparison the number of potential contributors and whether the peak falls into stutter position should be considered.

An analyst may partially dissect a DNA mixture profile into major and minor components and report the loci on the STR results table in brackets (e.g. [10,11,12]) where an unambiguous major or minor component is not distinguishable. Peak height ratios may be noted on the electropherogram to document possible genotypes, if appropriate.

Distinguishable mixtures may be used for the purposes of comparison. A Random Match Probability Statistic may be calculated and reported for single source major and/or minor component(s). The mixture formula may be used when an individual cannot be excluded from the sample when considering all reportable (equal to or greater than 100 RFU) alleles.

TYPE C:

Description: In general, type C mixtures contain DNA from at least three (3) individuals and may also exhibit stochastic effects.

If the sample demonstrates a clear reproducible major component at a minimum of 10 of 13 core STR loci a conclusion as to the source of the major contributor DNA may be reported and will be included in statistical analysis. This may also encompass mixture profiles that exhibit more than one major contributor.

If no clear major component is observed this type of genetic profile may be deemed unsuitable for comparison. As such, this profile will not be included on the STR results table and no statistical analysis will be performed.

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A sample may be deemed not suitable for comparison if the majority of alleles in a sample are not reproducible and/or insufficient DNA is detected (i.e. partial profile). Additionally, if a mixture contains DNA from known close relatives a comparison may not be appropriate.

11.4.4.2 Comparison of DNA Results

The interpretation process generally results in one or more of the following conclusions:

- Exclusion
- Cannot exclude
- Inconclusive
- Insufficient DNA/Data
- Complex mixture

Exclusion:

An exclusion is declared when the known sample has alleles that are not observed in the questioned sample and there is no scientific explanation for the absence.

Cannot Exclude:

When alleles detected in a known/reference sample are also detected in at least 10 of the 13 core STR loci in a DNA mixture, and when there is a scientific explanation for any inconsistencies, an individual may be considered a potential contributor to the sample.

If alleles that could not have been contributed by the individuals for whom known/reference sample profiles were submitted for comparison with the questioned samples, the conclusions for this sample may include a statement indicating that DNA that could not have been contributed by X/Y/Z was detected in the sample.

Partial Profile: If reportable alleles (100 RFU or greater) are detected in a mixture at less than 10 core STR loci the sample may still be suitable for comparison. The Technical Manager will be consulted to determine the appropriate course of action.

Note: If a genetic profile obtained from a sample does not satisfy the laboratory's inclusion/exclusion reporting criteria it is possible it may be utilized for exclusionary purposes only. The Technical Manager will be consulted to determine the appropriate course of action.

Inconclusive:

If the STR results neither support an inclusion (i.e. DNA consistent in at least 10 of 13 core STR loci) nor an exclusion, the comparison will be deemed inconclusive and reported as such.

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Insufficient DNA/Data:

If insufficient DNA is observed in a sample (i.e. data below reporting threshold, drop-out, low-level DNA) the data may not be suitable for comparison. This data will be considered insufficient and will not be utilized for comparison. As such, this data will be not be included on the STR results table.

Complex Mixture:

A complex mixture may be declared when DNA from at least four (4) sources is detected in a sample, there is evidence of stochastic effects (i.e. drop out) at several or all loci, and/or a majority of the alleles are not reproducible when amplified in duplicate. Accordingly, the sample will be deemed not suitable for comparison.

11.4.5 Consensus Data Reporting

When a sample is amplified in duplicate, in accordance with [Section 11.4.1](#), only the reproducible alleles will be included on the STR results table.

On occasion, compositing data from multiple injections/amplifications (e.g. data from more than 2 amplifications) from the same extract may be deemed appropriate after inspection of the quality of data. Such data will require technical manager approval for compositing.

Compositing data from multiple injections/amplifications from the same extract may be appropriate when entering or searching profiles in CODIS. The electropherograms should indicate that reproducible alleles were included on the STR results table, but that data was composited for SLD entry.

Profiles obtained by compositing data are currently not being submitted to NDIS.

11.5 Manual Interpretation of Offender Database Samples

The minimum peak height acceptable for database sample STR loci alleles is 75 RFU. The threshold may be raised to a maximum of 250RFU for samples with an elevated baseline. Reportable alleles are those whose peak heights are equal to or above the 75 RFU detection threshold. Lower threshold limits for hit confirmations or ILS (Internal Locus Standard) may be authorized by the DNA Technical Manager after assessing overall data quality and sample availability.

The interpretation and review of database sample data may be conducted by viewing the data electronically. Printed copies of all sample and control data electropherograms are not required. The analyst and reviewer will make appropriate comments and document review on the database batch worksheet.

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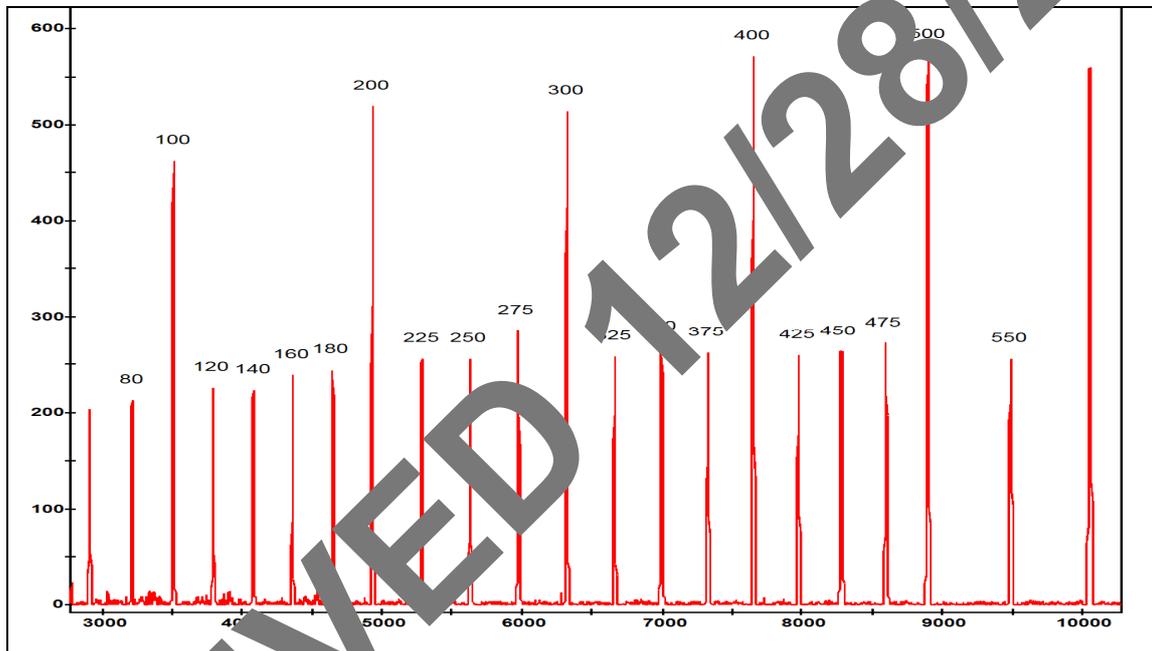
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11.5.1 Interpretation of the Internal Lane Standard and Allelic Ladders

11.5.1.1 Internal Lane Standard (ILS)

The first step in interpreting data from a run is to assess the ILS for each sample (including all controls and allelic ladders). The Promega PowerPlex16® System uses ILS 600. The analyst should verify that all peaks from 80-550 base pairs are present and labeled as shown below.



The peak heights for the ILS peaks should be at least 75 RFU (relative fluorescence units) to be identified and labeled by the GeneMapper™ ID-X software.

When the peaks are not labeled as shown above, the analyst must determine the cause for the incorrect labeling. If the peaks at either the low or high ends are not visible, the data may be re-analyzed by selecting an alternate analysis method with a higher or lower analysis range. The quality of the ILS (SQ<0.9) should be noted where deemed necessary (please see Database Sample Analysis section). The SQ value should be noted especially when using the AB 3500xl for both, database sample as well as forensic casework analysis.

If a peak is not labeled because it falls below the 75 RFU detection threshold, the analyst may, re-inject the sample and/or set up an additional plate with the same amplified product in order to obtain proper ILS sizing. On occasion, adding more ILS to the sample well and re-injecting may be sufficient to achieve this.

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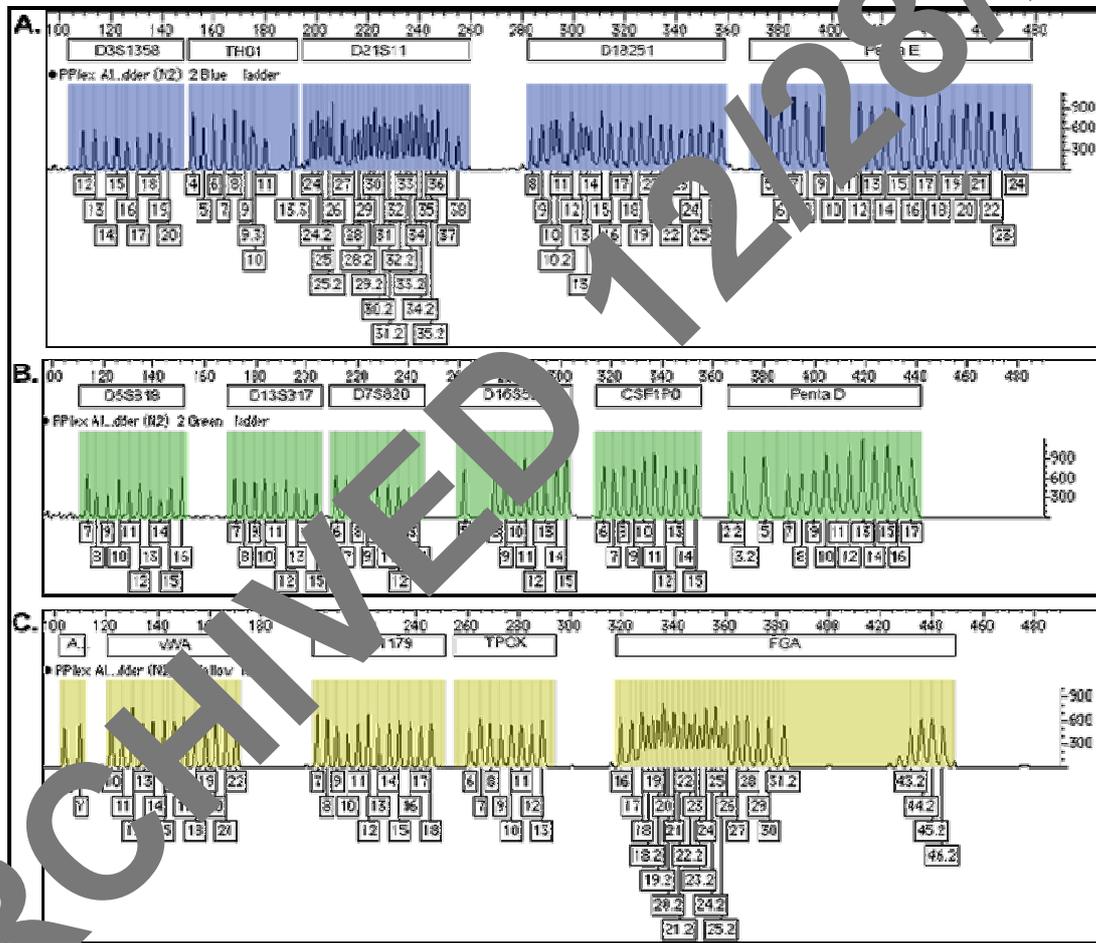
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11.5.1.2 Allelic Ladder

Verify that the peaks in the allelic ladder(s) are labeled correctly. The GeneMapper™ ID-X software uses the allele calls of the ladder to assign allele calls to all the other samples in the project. The allele calls for the Promega PowerPlex16® System allelic ladder are shown on the following page:

PowerPlex16® Allelic Ladder



The allelic ladder contains the most common alleles determined for each of the 13 FBI STR core loci, the Penta D and Penta E loci, and Amelogenin.

In addition, alleles not labeled in the allelic ladder (virtual alleles), may be detected and labeled according to base pair sizing in some of the samples analyzed.

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All the peaks in the allelic ladder must be labeled correctly for the software to assign the correct allele calls to samples in the run.

At least one allelic ladder must be included in a GeneMapper™ ID-X project per folder, with the sample type designated as “allelic ladder”.

When more than one ladder in a project has the sample type designated as “allelic ladder”, the software will average the allelic ladders to make the allele calls for the samples in the project.

Artifacts such as spikes or “pull-up” peaks may be observed that are labeled as off-ladder (OL) peaks in the allelic ladder. If all the true allele peaks are labeled correctly, such OL peaks will not affect the sample allele calls. If a spurious peak (not a true allelic peak) is labeled as a true allele (i.e. it falls within an allele size range and is designated and labeled as an allele) at any particular locus, the remaining allele designations / labels at that locus will shift and will impact the correct allele call / labeling of the samples in the project. Allelic ladders with such artifacts shall not be used to analyze data in the project.

Additional information about the allelic ladder can be found in the Promega PowerPlex16® System Technical Manual.

11.5.2 Interpretation of Control Samples

11.5.2.1 Positive Amplification Control

The standard DNA template 9947A is used as the positive amplification control for the Promega PowerPlex16® system. In database batches, a predetermined duplicate offender/staff sample may also serve as the positive amplification control.

If no interpretable type is obtained with either 9947A and/or one of the duplicate offender/staff samples, at least one positive amplification control and 5% of the samples in the run should be re-amplified and re-typed. If all interpretable results agree between the first and second amplification/run and the correct STR profile is obtained for the second amplification/run of one of the positive controls, then the STR data obtained in the initial amplification/run may be used.

The Technical Manager may approve the use of data from a run where only partial profiles were obtained from the positive control samples after appropriate review of the data set. Approval of such positive control samples should have documented Technical Manager approval on the database batch worksheet or relevant electropherograms.

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11.5.2.2 Reagent Blank

A reagent blank is carried through the entire analytical process as part of each extraction batch. It contains all the reagents - except DNA template - used during extraction, amplification and typing for each batch of samples.

The purpose of the reagent blank is to detect DNA contamination that might occur from the reagents, the extraction environment or between the samples being processed and/or due to improper handling of the samples by the analyst.

Verification of the presence of amplified product in the reagent blank shall be verified by viewing the presence of unincorporated primer peaks.

The reagent blank must be run at the most stringent set of conditions for the batch (e.g., 75RFU threshold, longest injection time, largest capillary electrophoresis preparation volume).

The reagent blank should not yield any true STR alleles above the 75 RFU threshold. If peaks greater than 75RFU are detected in the range between 80 base pairs and 550 base pairs, the analyst will determine if the peaks are artifacts (e.g. spike, pull-up) or true alleles.

The presence of peaks above 75 RFU will not invalidate the reagent blank as long as the peaks can be shown to be artifacts. Documentation of any artifacts detected in the reagent blank will be noted on the database batch worksheet.

When peaks of any height detected in the reagent blank cannot be shown to be artifacts and/or are detected at several or all STR loci the Technical Manager will be consulted to determine the appropriate course of action.

Since the failure of a reagent blank may indicate a problem at the extraction level, the cause of the failure will be fully investigated and documented in the anomaly log book. Steps will be taken (e.g. procedural modifications, corrective action, analyst retraining) to minimize recurrence. Formal corrective action reports may be completed based on the nature of the discrepancy. The Laboratory Quality Assurance Manual and DNA Quality Assurance Manual contain additional information on this subject.

11.5.2.3 Negative Amplification Control

The negative amplification control contains only the PCR master mix (reagents used to prepare the PCR amplification mixture) for each batch of samples and the water used to dilute the DNA samples.

The purpose of this control is to detect contamination that might occur from the PCR reagents, the PCR set-up environment or between the samples being prepared and/or due to improper handling of the reagents by the analyst.

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Verification of the presence of amplified product in the negative amplification control shall be verified by viewing the presence of unincorporated primer peaks.

The negative control must be run at the most stringent set of conditions for the batch (e.g., 75 RFU threshold, longest injection time, largest capillary electrophoresis preparation volume).

The negative amplification control should not yield any true STR allelic peaks above the 75 RFU threshold. If peaks greater than 75RFU are observed in the range between 50-550 base pairs, the analyst will determine if the peaks are artifacts (e.g. spike, pull-up) or true alleles.

The presence of peaks above 75 RFU will not invalidate the negative amplification control as long as the peaks can be shown to be artifacts. Documentation of any artifacts present in the negative control will be noted on the database batch worksheet.

When peaks of any height detected in the negative amplification control cannot be shown to be artifacts and/or are detected at several or all STR loci, the Technical Manager will be consulted to determine the appropriate course of action.

Since the failure of a negative amplification control may indicate a problem at the amplification level, the cause of the failure will be fully investigated and documented in the anomaly log book. Steps will be taken (e.g. procedural modifications, corrective action, analyst retraining) to minimize recurrence. Formal corrective action reports may be completed based on the nature of the discrepancy. The Laboratory Quality Assurance Manual and DNA Quality Assurance Manual contain additional information on this subject.

11.5.2.4 Internal Control Specimen (ICS)

Approximately 5% of the samples extracted in a database batch will consist of internal quality control samples. These samples, duplicate offender/staff samples, have been previously or are being simultaneously typed and are placed throughout the batch to be processed along with the other samples.

The technical reviewer or CODIS Administrator will verify that the correct STR results are obtained for all internal control samples.

An ICS with a partial profile or no interpretable profile does not invalidate the batch provided other positive / ICS controls in the batch yield the correct results.

If an incorrect STR profile is obtained for one or more of the control samples, the Technical Manager will be consulted to determine an appropriate course of action. Generally, other profiles obtained in the associated batch will not be entered into CODIS until the issue is resolved. The CODIS Administrator, or a designated individual, may approve the entry of select profiles on an individual basis in such situations.

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11.5.3 Interpretation of Offender Database Samples

Batches of database samples are uploaded into SDIS by creating an Export Table for CODIS. Prior to creating this table, the analyst must confirm that only eligible profiles are marked for export. This is done by selecting the appropriate Specimen Category within GeneMapper ID-X. Samples not exported to CODIS are marked "no export".

Samples uploaded into CODIS must not contain any labeled artifacts or OL alleles. Artifact or OL peaks are either deleted or relabeled by right clicking on the peak and choosing an appropriate option. The analyst should note any changes made to the allele calls in the appropriate column on the database batch worksheet.

11.5.4 Tri-alleles / dropout / partial profiles

Occasionally, a single-source database sample may be observed to have three alleles at one (rarely more) locus/loci. Samples exhibiting tri-alleles will be re-amplified and may have to be re-extracted to confirm the observed genotypes.

Peaks may be observed that are below the reporting threshold of 75 RFU. When these peaks exhibit peak morphology similar to allelic peaks and fall within an allele bin, the analyst should consider the possibility of allelic dropout. If allelic dropout is suspected in one or more core STR loci, the analyst may re-amplify and/or re-inject the sample to obtain a complete profile. The sample may have to be re-extracted if these methods fail.

If a partial genetic profile is obtained from a sample the analyst will make the "best attempt" to re-process the sample in order to generate a complete genetic profile. The repeat analysis may include one or more of the following: re-extraction using more sample, maximum template quantity in PCR, maximum template volume, maximum allowable PCR cycling conditions, maximum "prep" or amplified sample loading volume for capillary electrophoresis, maximum injection time. **NOTE:** Applicable reagent blank and negative PCR control will be processed under the most stringent conditions).

If the above "best attempt" also fails to generate a complete genetic profile, a maximum of three core STR loci with elevated stutter and/or stochastic issues (e.g., partial dropout, heterozygous peak height ratio less than 50%) may be permissible in a sample. Samples that do not contain at least partial data at each of the 13 core STR loci are not eligible for upload to NDIS. The allele 10 mutation at the D5 locus, which can cause issues with allele heights, is an accepted phenomenon and is not counted towards the maximum three core STR loci with stochastic issues. The presence of the observed mutation/variant should be documented on the database batch worksheet. The CODIS administrator will determine the eligibility of such profiles for upload.

When only partial data is obtained at one or more PowerPlex® 16 STR loci, it is indicated by selecting "Yes" in the "Partial Profile" field in CODIS. The comments field in CODIS may be used to indicate in which loci the dropout is occurring.

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11.5.5 Composite Data Reporting

Data will not be composited from multiple amplifications/injections of a database sample to obtain a more complete profile.

11.5.6 Off-Ladder (OL) Alleles

For interpretation of database samples, the laboratory maintains a record of known (previously observed) OL alleles. When an OL allele has been previously observed within the laboratory and the possibility of an artifact has been eliminated, the allele may be entered into CODIS without re-amplification. If an OL allele is being observed by the laboratory for the first time, the sample will be re-amplified, prior to CODIS entry, to confirm the genotype. On occasion, re-extraction of the sample may be needed for verification. Once confirmed the OL will be added to the record of previously observed OL alleles.

Database samples in which virtual alleles that are automatically assigned allele designation(s) by GeneMapper ID-X need not be re-analyzed for verification. Such alleles, including those "greater" or "smaller" than the largest or smallest alleles in the allelic ladder will be appropriately labeled during CODIS upload.

11.5.7 Artifacts

When an artifact is present within the size range of the alleles in a core locus and the presence of the artifact interferes with interpretation of the data in that locus, re-analysis of the sample may be required to obtain a profile eligible for NDIS. If the interfering artifact is located in one of the pentaploci, the analyst may choose to enter the profile, omitting all data from the affected pentaplocus. It is recognized that artifacts which do not interfere with data interpretation do not require re-analysis.

When any of the artifacts (previously defined) are not pervasive throughout the sample and do not interfere with data interpretation, the sample does not require re-analysis. Labeled artifacts will prevent a sample from being uploaded into CODIS. Therefore, the analyst may remove the artifact label. The presence of artifacts is documented on the DNA worksheet.

11.5.7.1 Stutter

Peaks in the stutter position greater than 20% may indicate the presence of DNA from more than one individual. When there is no indication of a mixture, and the data is determined to be elevated stutter, the analyst will document the stutter on the database batch worksheet. It is unlikely that the presence of stutter peaks would interfere with the interpretation of database samples and therefore, re-amplification and/or re-injection of the sample may not be required.

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11.5.7.2 Spikes

Peaks labeled as such by the GeneMapper™ ID software cannot be manually removed. However, they do not interfere with upload to CODIS and no re-analysis is required if the spike does not interfere with interpretation of the sample.

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SECTION 12 – ANALYSIS AND INTERPRETATION OF SINGLE SOURCE DATABASE SAMPLES UTILIZING GENEMAPPER® ID-X EXPERT SYSTEM SOFTWARE

References: Alaska Scientific Crime Detection Laboratory GeneMapper® ID-X version 1.0.1 Developmental Validation; GeneMapper® ID-X User Guide, Tutorial and User Bulletins as appropriate; National DNA Index System (NDIS) Expert Systems Operational Procedures, FBI Laboratory September 2009.

These guidelines are for the interpretation of STR data for single source database samples analyzed with Expert System (ES) software. The platform consists of GeneMapperID-X® v1.0.1, ABI 3130xl (data collection v3.0) and Promega PowerPlex® 16 utilizing an amplification of 32 PCR cycles and global stutter filter of 20%. The Federal Bureau of Investigation has reviewed and approved the developmental validation of this platform for upload of single source samples to the National DNA Index System (NDIS).

12.1 Logging on to GeneMapper® ID-X Version 1.0.1

- Open GeneMapper® ID-X
- Select user name from the drop down list and enter the appropriate password.
- Click OK.
- The main project window will open.
-

12.2 Creating a Project

- To add samples from the collection software at a workstation go to the edit menu and select **Add Samples to Project**. Navigate to **My Computer > Uncontrolled Documents > Section Shares > DNA Share > Data** on the laboratory "I Drive" as per current designation(s). Note: Location is dependent on network mapping and may vary slightly.
- Select the run to be imported or select individual samples and click **Add to List**.
- When all samples have been selected, click **Add**.
- Select the appropriate sample type for allelic ladder(s), positive control(s), or negative control(s). The default is set to sample.

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- Select the appropriate analysis parameters (including sample type and analysis method) for each sample. Reagent and amplification blanks shall be analyzed with the analysis method **Blank_Method_Global**. All other samples and control shall be analyzed with the analysis method **75RFU ES Method**. Click the  on the tool bar to analyze the samples in the project. The user will be prompted to name and save the project.
- Analysis is complete when the green arrows on the left of each sample name are gone.

12.3 Analysis Settings

The settings for the Analysis Methods are viewed by selecting GeneMapper® ID-X Manager under the Tools pull-down menu. The settings shown in this section are for the default analysis methods for blanks and samples analyzed with the ES validated method at 75RFU. There are several additional methods available for analysis when manual review will be performed. Software security settings have been established allowing only the CODIS State Administrator permission to edit sample analysis settings. However, any samples which do not pass expert system review may be manually reviewed and reanalyzed with alternate methods.

12.3.1 Available Database Analysis Methods for Manual Review

75RFU Database-2400
75RFU Database-2800
75RFU Database-3200
100RFU Database-2400
100RFU Database-2800
100RFU Database-3200
150RFU Database-2400
150RFU Database-2800
150RFU Database-3200
250RFU Database-2400
250RFU Database-2800
250RFU Database-3200
Blank Database Global

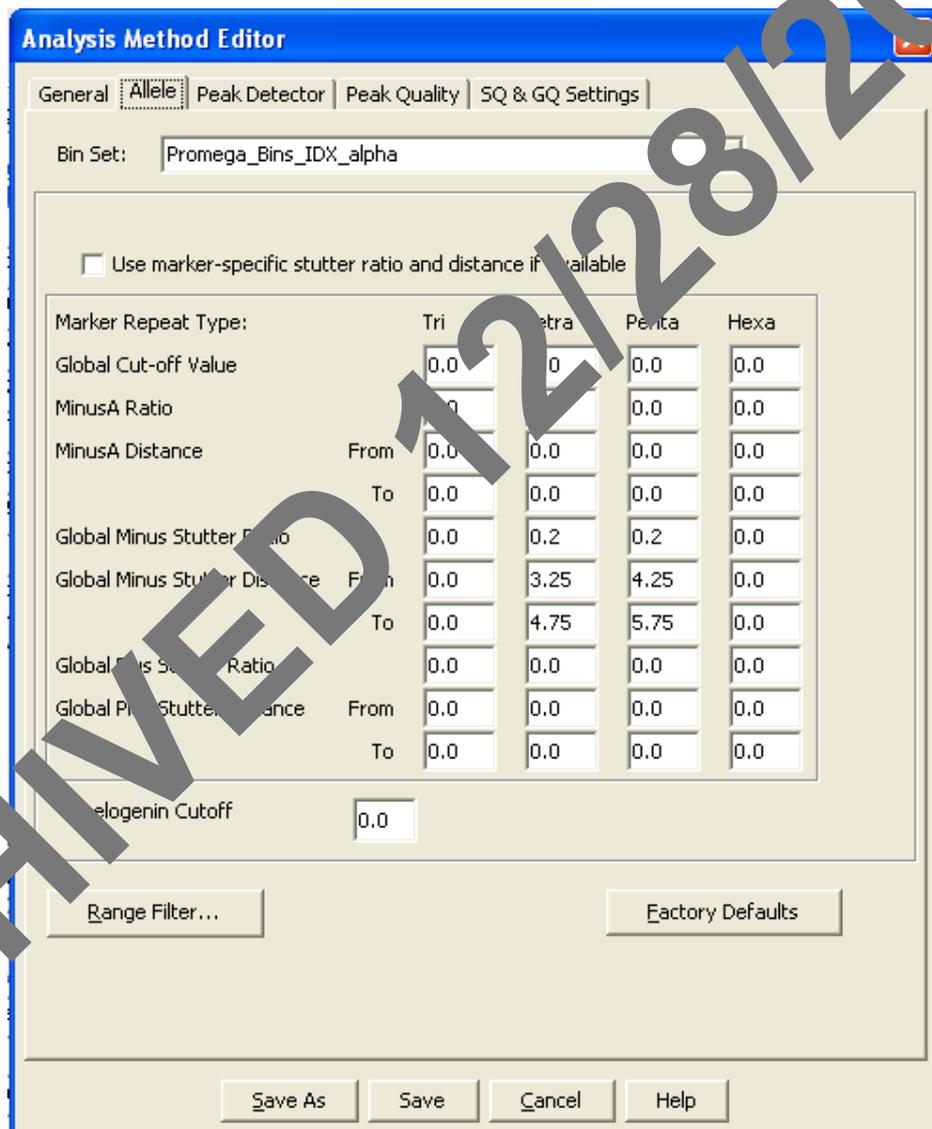
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12.3.2 Allele Tab Setting

The Allele Tab Settings are viewed by opening a selected analysis method from the GeneMapper® ID-X Manager screen. The Allele Tab Settings that follow are applicable for all database analysis methods.



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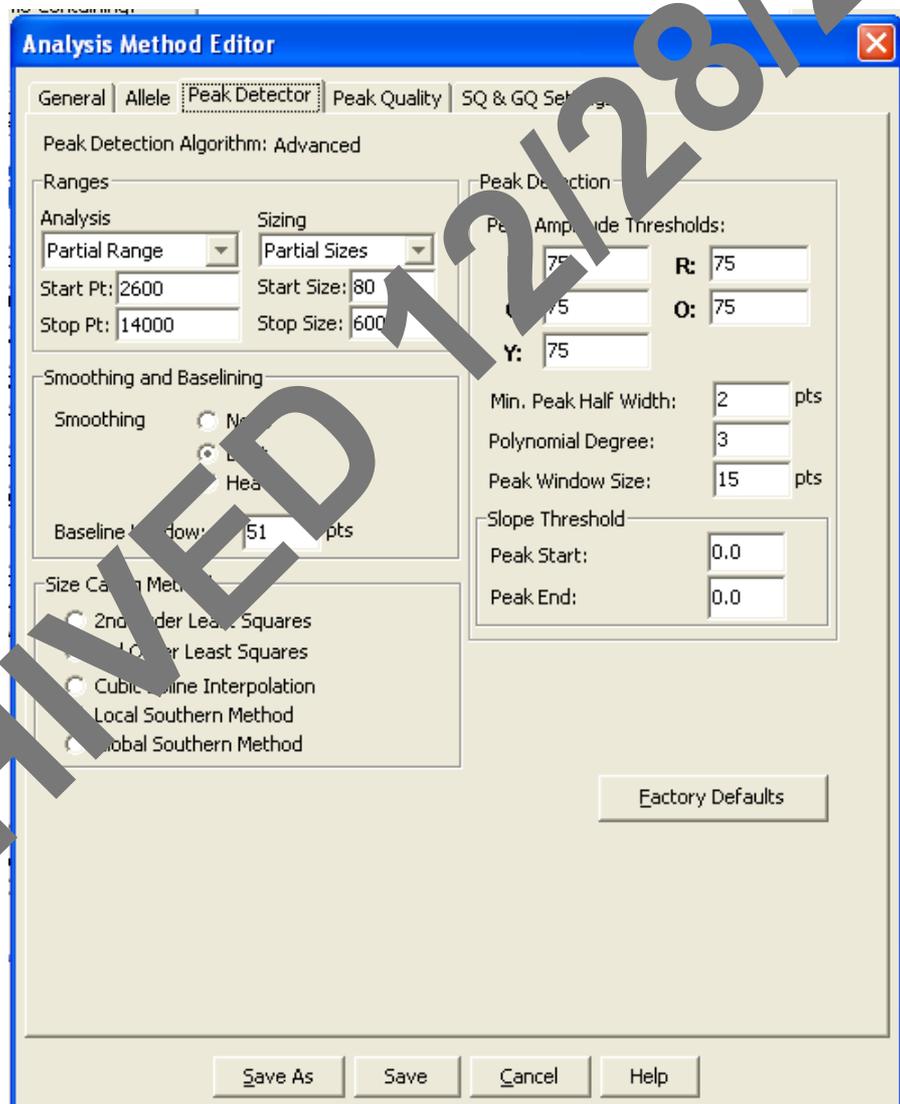
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12.3.3 Peak Detector Tab Settings

The Analysis Range and/or the Peak Amplitude Thresholds may be adjusted only for samples that are manually reviewed. All other settings are the same across all database analysis methods. Samples that are submitted for and pass expert system review without manual review prior to upload are analyzed as followed:

75RFU ES Method

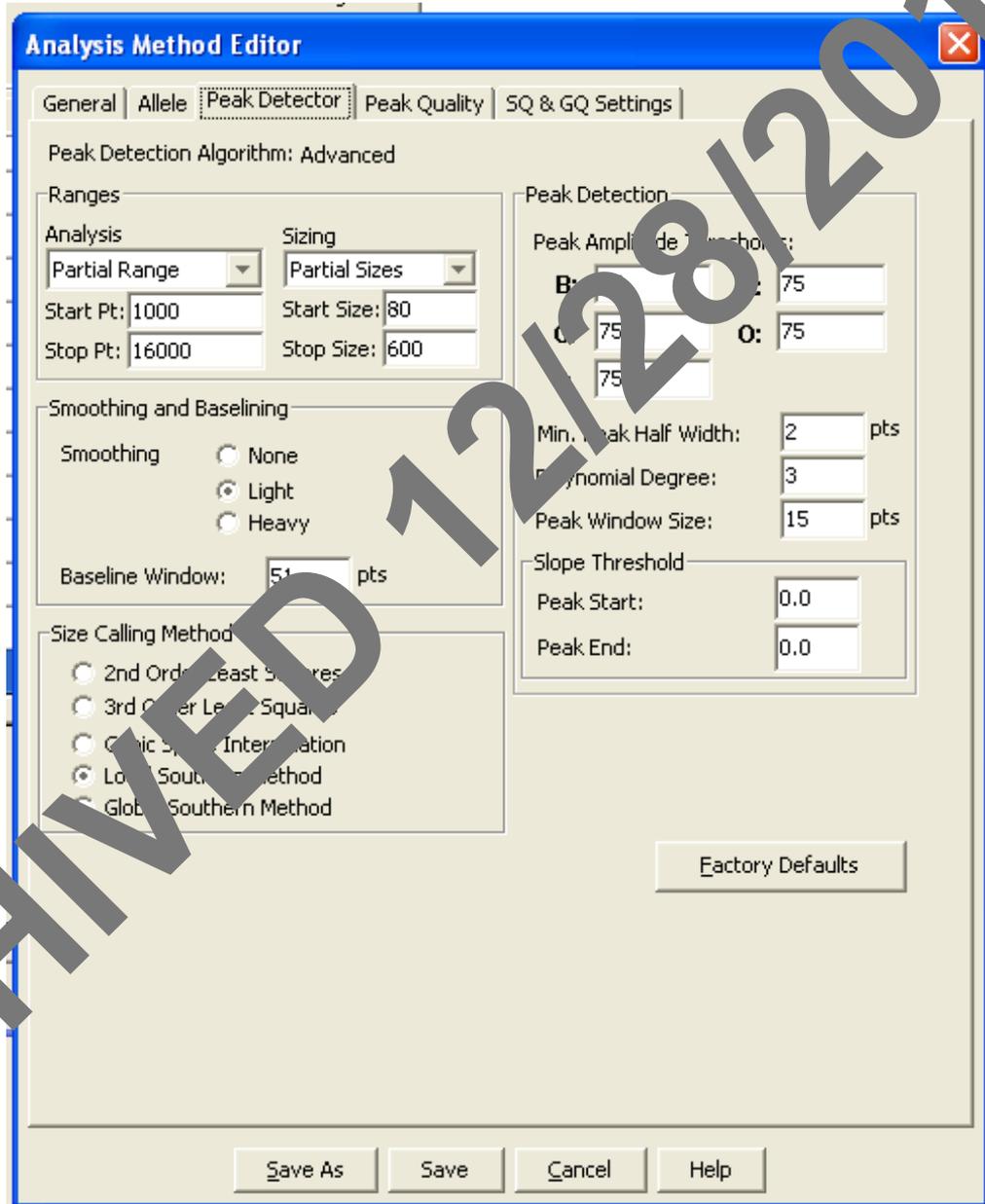


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Blank Method Global



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12.3.4 Peak Quality and SQ/GQ Tab Settings

The reason for the Blank Analysis Method is to facilitate viewing the primer peaks. The Control Concordance (CC) PQV for the negative control is set when Negative Control is selected as the Sample Type. The weight of this flag is not able to be edited and is set to 1.0. No peaks are allowed.

For samples that will be manually reviewed, Peak Quality and SQ & GQ Settings tab settings are not relevant, as all of these samples will be manually reviewed and interpreted, regardless of flagging.

The peak quality and SQ/GQ settings as approved by the Federal Bureau of Investigation after review of the developmental validation for the analytical platform used is as follows:

75RFU ES Method – Peak Quality

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Quality' tab selected. The settings are as follows:

Setting	Value
Min/Max Peak Height (LPH/MPH)	
Homozygous min peak height	150.0
Heterozygous min peak height	100.0
Max Peak Height (MPH)	8000.0
Peak Height Ratio (MPH)	
Min peak height ratio	0.5
Peak Width (basepairs)	1.5
Allelic Ladder Spike	
Cut-off Value	0.2

Buttons at the bottom: Save As, Save, Cancel, Help, Factory Defaults.

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75RFU ES Method – SQ & GQ Settings

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)	0.7	Allele Number (AN)	1.0
Out of Bin Allele (BIN)	0.7	Low Peak Height (LPH)	0.3
Overlap (OVL)	0.7	Max Peak Height (MPH)	0.3
Marker Spike (SPK)	0.3	Off-scale (OS)	0.3
		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

High Quality Range: 0.75 to 1.0 Low Quality Range: 0.0 to 0.25

Sizing Quality:	From 0.75	to 1.0	From 0.0 to	0.25
Genotype Quality:	From	to 1.0	From 0.0 to	0.25

Reset Defaults

Save As | Save | Cancel | Help

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12.4 Data Interpretation using the Expert System

Data interpretation of samples which pass ES Process Quality Values (PQV) do not require manual review. Samples which do not pass ES PQV may be reanalyzed at alternate analytical thresholds and/or ranges, however all samples reanalyzed in such a manner require manual edit/review. A maximum of three core STR loci with technical issues (e.g. partial dropout and/or peak height ratio less than 50%), may be permissible in a sample before an analyst is required to re-extract/re-amplify/re-inject the sample, as appropriate, in an attempt to obtain a more complete genetic profile. Samples that do not contain at least partial data at each of the 13 core STR loci are not eligible for upload to NDIS.

In all cases, samples which require manual edit/review shall be independently reviewed and verified by another qualified analyst according to technical/peer review procedures. When the technical manager is consulted on a sample that does not meet the laboratory interpretation guidelines and he/she approves its use, this approval will be documented by the technical manager's signature on the Database Batch Worksheet and/or printed electropherogram.

12.4.1 Examine Allelic Ladder Quality

Allelic Ladder Quality per run folder (based on SQ and CGQ only)					
Run Folder	Total # of Analyzed Ladders				
2_DB10-07VLD_AB_110-07-3136	5	5	0	0	

In the Analysis Summary Tab look at the **Allelic Ladder Quality per run folder**. If all allelic ladders pass (designated by ) then the analyst is not required to conduct a visual inspection of the allelic ladders and may proceed directly to evaluation of controls in the project.

Failed allelic ladders require manual review and can be viewed through the hyperlink under the failed category (designated by  and ). At least one passing allelic ladder is required per run folder. If an analyst has multiple allelic ladders in a run folder, the allelic ladder sample type of a low quality ladder may be changed to "sample", thus removing it from bin offsetting calculations. If the run folder does not contain a passing ladder, then the analysis plate should be reinjected.

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12.4.2 Examine Control Quality

Control Quality per project (based on sample PQVs: SOS, SSPK, MIX, OMR, SQ, CGQ)

Control Type	Total # of Samples	All thresholds met	One or more thresholds not met
Positive Control	1	1	0
Custom Control	0	0	0
Negative Control	3	3	0
Total	4	4	0

In the Analysis Summary Tab look at the **Control Quality per project**. If all positive and negative controls pass (designated by ) then the analyst is not required to conduct a visual inspection of the controls and may proceed directly to validation of samples in the project.

Failed controls require manual review and can be viewed through the hyperlink under the category one or more thresholds not met (designated by ). Depending on the circumstance for the failed control, reanalysis of the project and manual review may be required. If the reason for a failed positive control can be resolved by manual review, (e.g., pull-up artifact, low sizing quality of the ILS), then the analyst may proceed. Authorization from the technical manager is required for failed reagent blanks or negative amplification controls that cannot be resolved by manual review. Authorization from the technical manager is also required for any ICS sample being used in place of a positive control. Peer review is required for any control that is manually reviewed. The Composite Genotype Quality (CGQ) shall not be overridden for the failed controls.

12.4.3 Examine Sample Quality

12.4.3.1 Samples that Pass ES Review

In the Analysis Summary Tab look at the **Sample Quality per project**

Sample Quality per project (based on sample PQVs: SOS, SSPK, MIX, OMR, SQ, CGQ)

	Total # of Samples	All thresholds met	One or more thresholds not met
Samples	86	42	44

Click the hyperlink for the **All thresholds met** category (). These samples have met all quality thresholds and may be uploaded without manual review. Under **Specimen Category** select from the dropdown menu the appropriate category (e.g., Arrestee, Convicted Offender, Staff Duplicate) and save the project.

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12.4.3.2 Samples that Require Manual Review - Reviewing Low Quality Samples

- Select the Analysis Summary tab in the project window. Click the hyperlink for **One or more thresholds not met** (🔴).
- Note the (🟡) and (🔴) sample-level Process Quality Values (PQVs) for the low-quality samples.
- Select all samples in the filtered table. Scan the sizing qualities of the ILS by clicking  (Size Match Editor). Make notations on the Database Batch Worksheet where the sizing quality is <0.5. Close SQ window.
- Select all samples in the filtered table then click  (Display Plots)
- Verify that **Traditional Genotype Plot** is selected from the Plot settings dropdown list. You can click-drag the individual view panes to accommodate review.
- Investigate markers with a yellow or red marker level flag;

Sample(s) requiring re-analysis

- Analysis range and analytical threshold of ES failed samples may be adjusted. The analytical threshold may be set to a maximum of 250 RFU, but no lower than 75 RFU. Samples may be reanalyzed by selecting the appropriate analysis method from the Analysis Method dropdown menu, then click the green arrow on the toolbar to analyze the samples in the project.
- After reanalysis, select **ES Review Modified Pass** from the table settings. This will filter the table to show only those samples that now pass ES PQC settings. Under **Specimen Category** select from the dropdown menu the appropriate category and save the project.

Sample(s) not requiring re-analysis (e.g. did not pass ES Review at 75RFU using the same Method and did not need an alternate analysis range or analytical threshold)

- If, upon review, it is determined that the sample meets laboratory interpretation guidelines **without editing** (e.g., maximum of three of the core STR loci have stochastic issues), then right-click on the (🟡) or (🔴) Composite Genotype Quality PQC in the sample header. A prompt will appear asking whether to **override** the composite genotype quality for the sample. Select Yes. Overriding the CGQ allows the analyst to manually accept the genotype for a particular sample and also provides evidence that the sample was visually inspected by the analyst. The CGQ should appear as a green stop sign.

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If upon review it is determined that the sample meets laboratory interpretation guidelines **with editing** (e.g., pull-up/elevated baseline or an off ladder allele occurs). Edit the samples first by left-clicking to select the extraneous peak then right-clicking to select the appropriate action (e.g., delete or rename). The analyst will be prompted to enter the reason for the edit. Once editing for the sample is complete, and if the sample meets laboratory interpretation guidelines, then the Composite Genotype Quality PQC for the sample can be overridden by right clicking the (▲) or (●) CGQ in the sample header. A prompt will appear asking whether to override the composite genotype quality for the sample. Click yes only if the rest of the flags have been reviewed and the genotype is confirmed. This will change the CGQ to a green stop sign.

- Select **ES Edited Pass** from the table settings. This will filter to the table to show only those samples that have CGQ that have been overridden. Under **Specimen Category** select from the dropdown menu the appropriate category and save the project.

Sample(s) which do not pass

- If upon review it is determined that a sample does not meet laboratory interpretation guidelines (e.g., possible tri-allele, OL not previous seen, 4 or more loci with stochastic issues, complete dropout at 1 or more core loci), then the sample CGQ is left as is and the Specimen Category left as No Export.
- To view failed samples select **No Export** from the table settings. This will filter the table to show only those samples that have been designated as no export.

12. Conduct Profile Comparison

Additional quality checks may be performed by the analyst within GeneMapper® ID-X by navigating to tools and selecting Profile Comparison. These include:

- Sample Concordance – Determines if any single source profiles within the project are concordant. If none are concordant then all profiles in the project are unique.
- Sample Comparison – Determines if any of the sample profiles in the project are potential contributors to another sample profile in the project.
- Lab Reference Comparison – Determines if any laboratory staff reference profiles are potential contributors to sample profiles in the project.

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- Control/QC Comparison - Determines if any custom control profiles are potential contributors to sample profiles in the project.

Details on using these comparison tools can be found in the vendor supplied GeneMapper® ID-X user manual.

12.5 Performing Peer Technical Review of ES Projects

A technical review by a qualified DNA analyst is required of all database projects. The level of review dictated for an individual sample is dependent on whether the samples passed ES PQVs and/or if manual edit/review was required and passed.

12.5.1 Logging on to Genemapper®ID-X Version 1.0.1

- Open GeneMapper®ID-X
- Select user name from the drop-down list and enter the appropriate password.
- Click OK.
- The main project window will open.
- Select **Open Project** from the File drop down list on the toolbar. Select the name of the project to be reviewed.

12.5.2 View Allelic Ladder and Quality Control Samples

- In the Analysis Summary Tab look at the **Allelic Ladder Quality per run folder** and **Control Quality per project**. Verify that the allelic ladders and controls have passed ES review. Initial appropriate line(s) on the Database Batch Worksheet. By initialing the worksheet the reviewer is verifying that the controls passed ES review. Manual review of ES passed ladders and controls is only required if a threshold is not met - (▲) or (●).
- Any controls that do not pass ES review shall be manually reviewed to determine if they meet laboratory guidelines. When the technical manager is consulted on a control that does not meet initial ES laboratory guidelines, the technical manager's signature should be documented on the Database Batch Worksheet and/or printed electropherogram.

12.5.3 View Samples which Pass ES PQV Flags

- Select **ES Passed** from the table settings. Verify that each sample that has passed ES PQV flags is designated as such on the Database Batch Worksheet and that the Specimen Category is correct.

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- Initial appropriate line(s) on the Database Batch Worksheet. By initialing the worksheet the reviewer is verifying that the sample passed ES review, manual review is not required.

12.5.4 View Samples which Pass ES PQV Flags with modified analysis settings

- Select **ES RFU Modified Pass** from the table settings. All samples shall be manually verified to ensure that the samples that pass ES modified settings meet laboratory interpretation guidelines. Verify Specimen Category for each sample.
- Initial appropriate line(s) on the Database Batch Worksheet. By initialing the worksheet, the reviewer is verifying that the sample has been manually reviewed and that it meets laboratory interpretation guideline.

12.5.5 View Samples which Pass Laboratory Interpretation Guidelines with Manual Review/Edit

- Select **ES Edited Pass** from the table setting. All samples shall be manually verified to ensure that the samples that did not pass ES PQV flags meet laboratory interpretation guidelines through edit/review. Verify Specimen Category for each sample.
- Initial appropriate line(s) on the Database Batch Worksheet. Confirm that the edits/comments are appropriate. By initialing the worksheet, the reviewer is verifying that the sample has been manually reviewed and that it meets laboratory interpretation guidelines.

12.5.6 View Samples that Do Not Meet Laboratory Interpretation Guidelines

- Select **Do Export** from the table settings. All samples shall be manually verified that they do not pass laboratory interpretation guidelines and are not exported for upload to CODIS.
- Initial appropriate line(s) on the Database Batch Worksheet. Confirm that the edits/comments are appropriate. By initialing the worksheet, the reviewer is verifying that the sample has been manually reviewed and that it does not meet laboratory interpretation guidelines.

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12.6 Recertification of an NDIS Approved Expert System for the Review of Offender/Arrestee DNA Profiles

Section 10 of the NDIS Expert Systems Procedural Manual requires that laboratories utilizing NDIS approved Expert Systems (ES) verify the integrity of the ES through recertification using a defined data set of no less than 200 samples. In order to be recertified for continued use, the ES shall demonstrate complete concordance of the defined data set with the known DNA typing results. This quality control challenge is run through the NDIS approved ES on a quarterly basis (regular intervals of three months) such that four challenges are made each calendar year after commencement of the ES. The recertification is also to be conducted after repair, service, or calibration of the ES in accordance with Standard 10.4.1.5 of the FBI's *Quality Assurance Standards for DNA Databasing Laboratories*.

12.6.1 Recertification Dataset

The dataset used for the recertification consists of the 200 samples used for the original calibration of the developmental validation. Each sample's genotype resulting from the analysis of one DNA specimen from one person and includes a variety of challenges included in developmental validation.

12.6.1.1 Opening GeneMapper® ID-X version 1.0.1

- Open GeneMapper® ID-X
- Select user name from the drop-down list and enter the appropriate password.
- Click OK.
- The main project window will open.

12.6.1.2 Open and Analyze the Calibration Project

- Select GeneMapper® ID-X Manager from the Tools drop down list. In the Projects Tab select **Import**. Navigate to **My Computer > Uncontrolled Documents > Section Shares > DNA_Share > VALIDATION AND VERIFICATIONS > ID-X ES Validation > ID-X Quarterly QC Calibration > Original Validation Data** on the laboratory "I Drive" as per current designation(s). Note: Location is dependent on network mapping and may vary slightly.
- Select **ES Calibration Samples.ser** then click **Import**. Choose Security Group: Databasing Security Group. Click Ok. Wait for project to import. Close GeneMapper® ID-X Manager.
- Click on File > Open Project from the file menu on the toolbar. The project name will be ES Calibration Samples YY-MMDD (from last saved .ser file). Click OK.

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- Select the **75RFU ES Method** as the analysis method for the project and click the (▶) on the tool bar to analyze the samples.
- Analysis is complete when the green arrows on the left of each sample name are gone.
- Review Analysis Summary
 - Select the **Analysis Summary Tab** in the open GeneMapper® ID-X project.
 - Verify that a total of 200 samples were analyzed with 93 samples in the All thresholds met category, and 107 samples in the One or more thresholds **not met category**.

Sample Quality per project (based on sample PQVs: SOS, SSPK, MIX, OMR, SQ, CGQ)			
	Total # of Samples	All thresholds met	One or more thresholds not met
Samples	200	93	107

12.6.1.3 Creating Tables for Calibration Comparison

12.6.1.3.1 Create ES Calibration Report

The report table fields are established to electronically document that electronic drift of analysis settings has not occurred. The report fields include:

Sample File	Marker	Allele 1-6	Height 1-6	GQ
SQ	SOS	SSPK	MIX	OMR
CGQ	PHN	PHR	LPH	MPH
SPK	APK	BD	OS	

- Select all samples in the Samples Tab.
- Click on the Report Manager in the Tools drop down menu.
- Select **ES Calibration** from the Report Setting dropdown in the report header.
- Select **Export** from the File drop down. Navigate to **My Documents > Uncontrolled Documents > Section Shares > DNA_Share > VALIDATION AND VERIFICATIONS > ID-X ES Validation > ID-X Quarterly QC Calibration**
- Create a new folder with the month and year by right-clicking. Name the file in the newly created folder as **ID-X_Calibration_YY-MMDD_Validation_Table**.
- Select **Export**.
- Close Report Manager. When prompted to save report, say 'no'.

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12.6.1.3.2 Create Failed Sample List

An additional table is generated showing the sample file names for those samples which do not pass based on ES interpretation values.

- Return to the Analysis Summary tab
- Click on the hyperlink to the 107 samples where **one or more thresholds were not met.**
- Highlight all the samples in the **Sample File** column. Copy the file names by pressing the Ctrl + C.
- Open Word Pad. Paste the sample file names by pressing Ctrl + V. Select **Save As** in the File dropdown and navigate to **My Computer > Uncontrolled Documents > Section Shares > DNA_Share > VALIDATION AND VERIFICATIONS > ID-X ES Validation > ID-X Quarterly QC Calibration.** Save as a text document in the recently created folder as **ID-X_Calibration_YY-MMDD_Failed_Samples.** Save as a text document not in rich text format (RTF) as defaulted. A prompt will warn that saving a text-only file will remove all formatting. Confirm by selecting 'Yes'.
- Close Word Pad.

12.6.2 Recertification Interpretation

Verification of Flagging Values

- Open NotePad ++
- Click **Open** in the File drop down on the toolbar.
- Navigate to **My Computer > Uncontrolled Documents > Section Shares > DNA_Share > VALIDATION AND VERIFICATIONS > ID-X ES Validation > ID-X Quarterly QC Calibration > Original Validation Data > ID-X_Original_Validation_Table_75RFU.**
- Select **Open.**
- Click **Open** in the File drop down on the toolbar.
Navigate to **My Computer > Uncontrolled Documents > Section Shares > DNA_Share > VALIDATION AND VERIFICATIONS > ID-X ES Validation > ID-X Quarterly QC Calibration** and select the recently saved validation table from [section 12.6.1.3.1.](#)
- Select **Open.**
- On the Toolbar select **Plugins > Compare > Compare.**

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- If all of the values between the two tables are concurrent a prompt will appear stating Files Match. If there are differences between any of the values between the two tables, then those differences will be highlighted. Satisfactory recertification is obtained only if the files match. If there are any discrepancies then they shall be investigated and the Technical Manager notified immediately. ES analysis should be halted until the discrepancy has been resolved and deemed satisfactory by the Technical Manager.
- Select **Close All** in the File drop down on the tool bar.

Verification of Flagging Interpretation

- Click **Open** in the File drop down on the toolbar.
- Navigate to **My Computer > Uncontrolled Documents > Section Shares > DNA_Share > VALIDATION AND VERIFICATIONS > ID-X ES Validation > ID-X Quarterly QC Calibration > Original Validation Data** and select **Failed Samples from Original Project**.
- Select **Open**.
- Click **Open** in the File drop down on the toolbar.
- Navigate to **My Computer > Uncontrolled Documents > Section Shares > DNA_Share > VALIDATION AND VERIFICATIONS > ID-X ES Validation > ID-X Quarterly QC Calibration** and select the recently saved **Wordpad file showing the failed samples created in [section 12.6.1.3.2](#)**.
- On the Toolbar select **Plugins>Compare>Compare**.
- If all of the values between the two tables are concurrent a prompt will appear stating Files Match. If there are differences between any of the values between the two tables then those differences will be highlighted. Satisfactory recertification is obtained only if the files match. If there are any discrepancies then they shall be investigated and the Technical Manager notified immediately. ES analysis should be halted until the discrepancy has been resolved and deemed satisfactory by the Technical Manager.
- Select **Close All** in the File drop down on the tool bar.

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12.7 Terms and Abbreviations

Parameter	Description
Allele Number (AN)	Indicates if the software detects within a marker size range: <ul style="list-style-type: none"> No alleles, or More than the Max Expected Alleles , or No X alleles in Amelogenin
Broad Peak (BD)	Indicates if the width of any peak within a marker size range exceeds the max peak width (in basepairs) set in the analysis method. Broad peaks may be a result of dye artifacts or poor resolution. Peaks with poor resolution: <ul style="list-style-type: none"> May be caused by electrophoresis issues, or May not be true DNA peaks
Composite Genotype Quality (CGQ)	Indicates overall genotype quality (GQ) for the sample as determined based on the presence of labeled peaks detected (after filtering) and the GQ weighting.
Genotype Quality (GQ)	Marker genotype quality indicator. Determined differently for samples and allelic ladders. The GQ is used to determine the CGQ . See ID-1 user manual or ID-X developmental validation for more information on how the GQ is calculated.
Low Peak Height (LPH)	Indicates if any peak heights (RFU) within a marker size range are below the following thresholds: Homozygous min peak height, Heterozygous min peak height
Marker	Designates the STR locus.
Max Peak Height (MPH)	Indicates if any peak heights (RFU) within a marker size range exceed the Max Peak Height (RFU) value set in the analysis method.
Mixture (MIX)	Indicates a potential mixed-source sample. A sample is considered a potential mixture if it meets either of the following conditions: <p>Condition 1: Two or more markers contain three or more called alleles.</p> <p>Condition 2: One or more markers contain three or more called alleles <i>and</i> another two or more markers have PHR flagged.</p>
Out of Bin Allele (BIN)	Indicates if labeled peaks do not fall inside bins. These peaks are labeled Off-Ladder (OL).
Outside Marker Range (OMR)	Indicates if labeled peaks are detected between two marker size ranges defined in the panel. <u>Does not indicate if labeled peaks are detected before the first marker or after the last marker.</u>

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Peak Height Ratio (PHR)	Indicates if the peak height ratio between the lowest and highest peaks within a marker size range is less than the Min Peak Height Ratio.
Process Quality Values (PQV)	Process Quality Value (PQV) flags indicate the quality of data the sample and marker levels as defined by user established thresholds.
Sample Off-scale (SOS)	Indicates if any fluorescence signal exceeds the detection threshold of the instrument.
Sample Spike (SSPK)	Indicates if spikes are detected within or between two defined marker size ranges. Does not indicate if spikes are detected before the first marker or after the last marker in each dye. The software uses a proprietary algorithm that detects spikes based on the peak morphology.
Sizing Quality (SQ)	Evaluates the similarity between the fragment pattern for the size standard dye specified in the size standard definition and the actual distribution of size standard peaks in the sample.
Spike (SPK)	Indicates if spikes are detected within a marker size range. The software uses a proprietary algorithm that detects spikes based on the peak morphology.

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SECTION 13 – DOCUMENTATION: CASE RECORDS AND REPORT WRITING

Refer to the Laboratory Quality Assurance Manual for general laboratory guidelines governing casework documentation and report writing. Refer to the Laboratory Quality Assurance Manual information regarding discovery requests and the release of information from the laboratory.

Analysts may use the following abbreviations in their bench notes and on documents generated during analysis.

Biological Screening Abbreviations and Definitions	
Phenolphthalein/Kastle-Meyer	PH, Pheno, or KM
Fluorescence/Alternate Light Source	F or ALS
Acid Phosphatase activity	AP, BCIP, STMP
Nucleated Epithelial cells	NECs
Prostate-Specific Antigen	PSA or p30
Human Hemoglobin	hHb, Species
Positive test result	Pos or (+)
Negative test result	Neg or (=)
CIDI	Case,item,date & initials

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DNA Abbreviations and Definitions	
BL	Noisy baseline
SH	-A / +A Shoulders
PHR	Peak Height Ratio <60%
SPK	Spike
PU	Pull-Up
DO	Complete Drop-Out at a locus
PDO	Partial Drop Out at a locus
D5Mut	D5 Mutation
OL	Off Ladder
OMR	Outside Marker Range
OS	Off Scale Saturation
NR	Data not reported
NS	Data not used for statistics
A/TA	Artifact (ie stutter) or true allele
IF	Instrument failure
ILS	ILS failure
DBDT/DBRT	Data below reporting threshold of 100RFU(*)
ART	Artifact
TRI	Tri-allele
()	Minor component allele
[]	Major/Minor components not separated
RQ	Questioned reagent blank, direct extraction
RBE/RBS	Epithelial and sperm reagent blanks
RBK	Known reagent blank
CIDI	Case, item, date & initials
LV	Large Volume (EZ1 protocol)

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13.1 Documentation Generated During Screening and DNA Analysis

All paperwork generated during the course of analysis will be maintained either in the case record, or in a central log record in the LIMS or on the laboratory network shared drive. All pages in the case record and central log record contain the case number, analyst's initials, item # (if appropriate, i.e. digital images) and page number (page X of Y) indicating the total number of pages in the record. When one analyst is performing a specific procedure on behalf of another analyst, the resulting pages should contain the handwritten initials (or secure electronic equivalent) of both analysts.

13.1.1 Biological Screening Bench Notes

Biological screening bench notes consist of any documentation generated during the analysis of a case and are specific to that case. Upon completion of the case, the biological screening bench notes and the laboratory report are maintained in LIMS. The bench notes for each case may contain the following worksheets:

- Victim Assault Kit Worksheet
- Suspect Assault Kit Worksheet
- Evidence Examination Worksheet
- Microscopic Worksheet
- PSA/Semen Identification Worksheet
- HemaTrace®/Species Identification Worksheet
- Clothing Worksheet
- Case Images Worksheet

The worksheets contain details of all of the items processed and include the items packaging, contents and description, images of the evidence processed (when applicable), documentation of all presumptive tests performed and the test results, the location of all testing, the location of all isolated stains/samples, trace evidence collected, reagents used, and the date the evidence was opened and sealed (where applicable) in accordance with laboratory policies and procedures outlined in the Laboratory QA Manual.

13.1.2 DNA Central Log Records

Each batch of cases assigned to a DNA analyst will be named with the batch date (typically, this is the day you take custody of the evidence) and the analyst's initials (i.e. YY-MMDDinitials 06-0102MLC). This batch name is recorded for each case in the LIMS. Upon completion of the technical review of a batch, the central log documentation is retained in LIMS, in both the case record and the annual DNA record.

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The central log for each batch of cases will contain at least the following information in an appropriate format:

- A cover sheet containing:
 - batch name
 - the name of the analyst(s)
 - a list of cases worked in the batch
 - the raw data folders and GeneMapper™ (current version) project folders for all data used to generate results/conclusions for cases in the batch.
- A Casework Standards, Controls and Reagents worksheet detailing:
 - the extraction methods used
 - specific instrument used
 - elution volumes
 - the extraction controls
 - date of reagent blank (should be on or after the date of extraction)
 - extraction reagent lot numbers and reagent expiration dates. (reagents not used can be deleted from worksheet)
- Q-PCR worksheet(s) containing the lot numbers and expiration dates of reagents/standards used in quantitation, as well as a map of the 96 well plate.
- The standard curve for the quantification.
- The quantitation results sheet (Initial template quantity) showing the estimated concentration of all samples quantified (handwritten, avg of the 2 values obtained for Q's).
- The amplification/electrophoresis worksheet(s) detailing:
 - the samples/controls amplified
 - the well plate locations
 - the amount of sample amplified
 - the amount of sample (amplified product) injected for electrophoresis,
 - the electrophoresis injection modules,
 - the lot numbers and expiration dates of all reagents used in amplification and electrophoresis.
 - If electrophoresis results indicate that a sample should be re-injected, the reason for reinjection should be documented in the comments/notes field of the amp/3130 worksheet (See table above for list of abbreviations)
 - Instrument failure
 - ILS failure
 - Noisy baseline
 - Data below detection (*)
 - Drop-out
 - Saturation/Off Ladders alleles
 - Artifacts (ie pull-up, dye blobs, spike, bubbles, shoulder)

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Note: Multiple injections of the same sample at more than one injection time (for efficiency purposes) in a CE run does not require documentation of a reason. The analyst will select the injection best suited for interpretation, based on the interpretation guidelines

- Reasons for a re-amplification and/or re-extraction (examples)
 - Potential Tri-allele
 - Off-ladder allele
- Electropherograms for all control samples generated during analysis of the batch.
 - ILS - The printouts must show the correct labeling of the 10-550 peaks of the internal lane standard and the tops of every peak.
 - Negative/blank controls - primer peaks must be visible by selecting Blank Casework as the Analysis Method. The negative/blank controls must be injected for at least as long as the longest injection time of a casework sample. [Plot setting: Casework Blank]
 - ICS – print and label “ICS” in upper right corner and add to central log as a control. The analyst should include the necessary information for the reviewer to verify that the obtained profile matches the expected profile. This additional documentation is not retained once the technical review is completed.
 - Identify clearly the Positive Control sample provided by the PCR kit vendor.
 - Exceptions must be approved, in writing, by the Technical Manager or another designated individual.
- Electropherograms of all genetic ladders used for genotyping (Print in landscape view (horizontal) with ILS).
- Page numbering of the central log does not need to be completed until all laboratory work is completed for all cases in the batch.

13.1 DNA Bench Notes

DNA bench notes consist of any documentation generated during the analysis of a case that is specific to that case. Upon completion of a case, the DNA bench notes and the DNA Laboratory report are maintained in the LIMS. The bench notes for each case will contain at least the following:

- The STR Results table (an administrative document) comprises the first page(s) of the bench notes and contains genetic profiles from questioned and known samples suitable for comparison.
 - It is not necessary to include in the STR Results table the profiles obtained from samples that serve as internal controls (i.e. the epithelial fraction of a vaginal swab) as long as the profile is consistent with expected results

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- If results are not suitable for comparison, do not include in the STR Results Table the Questioned sample profile (ie. sample with no called alleles, majority of alleles are not reproducible, minor profile not suitable for comparison, etc).
- If interpretable genetic profiles are not obtained from any of the questioned samples, the known sample profiles need not be included in the STR Results Table.
- Add a * after the item description if there is data – i.e. alleles below reporting threshold in a sample.
- Add “epithelial” or “sperm fraction” to sample description when necessary.
- Minor alleles go in () below the major alleles.
- If an allele is determined to be A/TA, this notation goes after the allele call in superscript (ie. 10^{A/TA}).
- Loci with no reportable alleles are left blank and the boxes grayed out.
- All notes at the bottom of the STR Results Table are retained.

Note: For Proficiency Tests, it is not necessary to create a separate a STR Results table. The DNA results and conclusions pages of the paperwork from the test provider are completed and become the first pages of the DNA bench notes.

- The DNA extraction worksheet(s) contains:
 - All of the questioned and known items processed,
 - Sample code (with the bag)
 - Documentation of item packaging
 - Presumptive testing performed and the results if applicable
 - Amount of sample used (sample size)
 - Date sampled (this is the date that you cut your evidence)
 - Extraction method and specific instrument used
 - Elution volumes
 - Quantitative results
 - Disposition of the DNA extract
- Digital image printouts of questioned samples, when appropriate (ie cigarette butt, fingernail clippings, hairs).
 - Electropherograms for all of the items amplified, showing the correct labeling of the 80-50 peaks of the internal lane standard [Plot setting: Traditional Genotype Plot]. All electropherograms must contain the lab # and item # in the Sample Name column.
 - If the sample has no labeled peaks, the electropherogram must also include the primer peaks [Analysis method: Blank casework; Plot setting: Casework Blank]. Exceptions must be approved, in writing, by the Technical Manager or another designated individual.
 - Zoomed in views (electropherograms) for samples may be appropriate when there are artifacts [Plot setting: Casework artifact] . Zoom in on locus with artifact, there is no need to print entire profile. For data below the reporting threshold [Plot setting: Casework zoom]- Print entire profile.

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- If analyzing at 50rfu for Amelogenin, print a second electropherogram zoomed [Plot setting: Casework artifact] for Amel locus only. Add note "Analyzed at 50rfu for Amel only" to main electropherogram and hand write in a "Y" and circle it.
- Special case: When an OL is a true allele or a potential tri-allele exists, print using Traditional Genotype Plot. Also print a casework artifact view that includes the allelic ladder and the locus on one sheet.
- The following handwritten notations may be included on the electropherogram:
 - " * *DBDT* " / **DBRT** in the upper right hand corner
 - Loci with peak height imbalance (highlighted in orange in GND-X) must be initialed
 - Artifacts (ie pull-up, dye blobs etc) are noted as "artifacts" on both the traditional genotype view and the zoomed in electropherogram
 - "No genetic profile" added to bottom left when no reproducible alleles are above reporting threshold
 - For samples that have been double amplified and yielded data suitable for comparison, add "Reproducible alleles on STR Results Table" to bottom left of the first electropherogram. All non-reproducible alleles are crossed out and initialed on all electropherograms.
 - For samples that were double amplified and did not yield data suitable for comparison, add "Reproducible alleles noted" and the reason that the data is not suitable for comparison to the bottom of electropherogram
 - For peaks in stutter position (that appear close to the expected stutter percentages) indicate the % of the peak relative to the main peak and the expected stutter percentage (for example, 12%>5% or 5.2%~5%). When the peak can be reasonably interpreted as elevated stutter, add a notation to this effect. When it is not possible to discern whether the peak is a stutter peak or a true allele, add "A/TA".
 - If data is not suitable for comparison, add a note at bottom of electropherogram i.e. "data not included in STR Results table due to insufficient DNA", "minor component not suitable for comparison therefore not included in STR Results table", "minor component consistent with lower and therefore not included in STR Results table" etc.
 - If able to determine a major vs minor profile add () around the minor alleles.

Printouts for all samples for which a statistical analysis was performed will include:

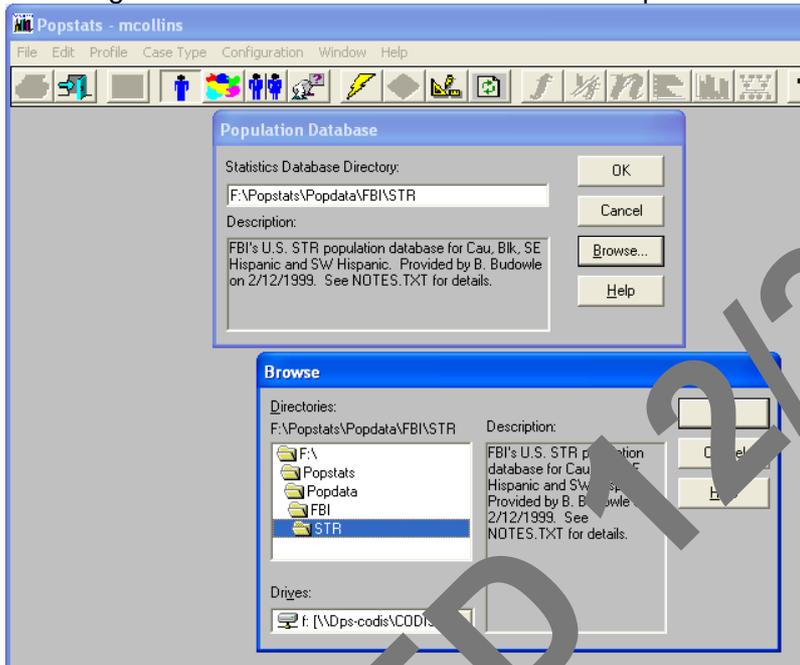
- Specimen ID: lab #_Item#
- Comments section: add any additional info (ie sperm fraction, major profile)
- Print for all populations
 - The population database is selected under the Configuration pull-down menu

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Selecting the Caucasian and African-American Population Databases

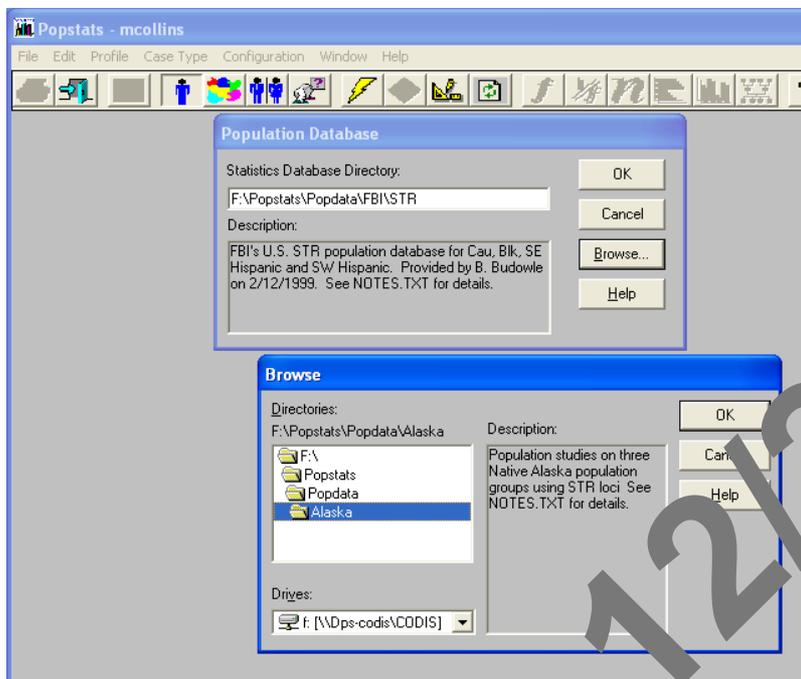


Selecting the Alaska Native Population Databases
(next page)

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- The casework review checklist is completed and signed by the analyst and reviewers.
 - Analyst will complete the first column of boxes before handing in for technical review.

All pages in file must be page numbered before handing in for technical review

13.2 Report Writing

Proficiency test reports will be written in a manner similar to casework reports. Proficiency tests for DNA reports will not require the STR typing form used for casework reports. Instead, the completed STR table from the test provider's form will be placed in the bench notes instead of the STR typing results table.

13.2.1 Guidelines for Biological Screening Examinations / Reports

All biological screening reports will contain the following:

- Date report was issued
- Laboratory and agency case numbers (automatically populates)
- Name of submitting agency and case officer (automatically populates)
- List of all items analyzed
 - include the agency item # in () if the item was re-numbered at the laboratory
 - this is not necessary if the laboratory number contains the original agency item #

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- Results of presumptive/confirmatory tests (in body of report)
- Results, conclusions and opinions for all tested items (guidelines for reporting are provided in the tables below and may be modified, as necessary, on a case by case basis)
- Explanation of why testing was stopped, if appropriate
- The disposition of all retained items
- Any known samples that are required for DNA analysis, when applicable
- Statement regarding Y-STR testing, when applicable

Sperm Findings	Report
Spermatozoa observed	Spermatozoa were observed (microscopically) on the...
Few spermatozoa observed	Few spermatozoa were observed (microscopically) on the...
No spermatozoa observed	No spermatozoa were observed (microscopically) on the...

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Semen Findings	Report
BCIP/STMP negative	No semen was detected chemically (BCIP/STMP) on (item)
BCIP/STMP positive	Positive results are not reported
P-30/ABA card positive (no spermatozoa observed)	The presence of PSA/semen was detected on (item) by immunoassay. This test detects the presence of the human prostate specific antigen (p30) found in seminal fluid. Item (#) has been retained in the laboratory and may be suitable for Y-STR analysis. For more information please contact the laboratory's DNA Technical Manager, Alan Chidambaram (269-5621) or (alan.chidambaram@alaska.gov).
P-30/ABA card positive (no spermatozoa observed)	No PSA/semen was detected on (item) by immunoassay.
Blood Findings	Report
Phenolphthalein positive	A stain(s) testing positive to a presumptive test for blood was/were located/detected on (item)
Phenolphthalein negative	A presumptive test for blood gave negative results on (item)
Speciation	Report
ABA card positive	Stain(s) present on (item) tested positive using an immunoassay test for the presence of human hemoglobin. This test is specific to human, higher primate and ferret blood.
ABA card negative	No human hemoglobin was detected by immunoassay.

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Hair/Fiber Evidence	Report
Hairs/Fibers recovered (positive biological findings)	Hairs/fibers were observed/recovered from (item). No examinations were conducted due to positive biological/sperm findings.
No Hairs/Fibers recovered	No human hairs were observed/recovered in from
Human hairs (suitable for nuclear DNA)	A human head hair(s) was/were found on/in (item). Tissue or tissue-like debris was observed stereoscopically on the root(s). This/These hair(s) may be suitable for nuclear DNA.
Human hairs (not suitable for nuclear DNA)	A human head hair(s) was/were found on/in (item). No tissue or tissue-like debris was observed stereoscopically on the root(s). This/These hair(s) are not suitable for nuclear DNA. No further examinations were conducted.
Human hair fragments (not suitable for nuclear DNA)	One hair fragment, not suitable for nuclear DNA analysis was found in/on (item/envelope). No further examinations were conducted.

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13.2.2 Reporting Guidelines for DNA

All DNA discipline reports will contain the following:

- Date report was issued
 - Laboratory and agency case numbers (automatically populates)
 - Name of submitting agency and case officer (automatically populates)
 - List of all items analyzed
 - include the agency item # in () if the item was re-numbered at the laboratory
 - this is not necessary if the laboratory number contains the original agency item #
 - When DNA analysis is reported for samples retained by another discipline in the laboratory, or comparisons are made to profiles reported by another laboratory, the report should reference the previously issued report
 - i.e. "Reference **biological screening** report dated **Month Day, Year** by **Analyst.**"
 - include the issuing laboratory, if other than the AK SCDL.
 - Results of presumptive/confirmatory tests, not previously reported in a biological screening report (in body of report)
 - DNA analysts should report the results of microscopic examinations of swabs when only the smears (submitted in the kit) were examined and reported by a biological screening analyst
 - Results, conclusions and opinions for all tested items, based on the DNA typing results
 - Results of any statistical analyses performed
 - Explanation of why testing was stopped, if appropriate
 - Description of analyses performed, including a list of STR loci amplified (this is contained in the Methods section of the report templates for DNA Major Crimes and DNA Property Crimes)
 - Description of statistical analyses performed including the methods and loci used in the calculations (this is contained in the Methods section of the report templates for DNA Major Crimes and DNA Property Crimes)
 - The disposition of the evidence ((this is contained in the Methods section of the report templates for DNA Major Crimes and DNA Property Crimes)
- Signatures of the reporting analyst and the technical reviewer (the analyst should electronically sign the report by setting the milestone to Draft Complete prior to submitting for technical review; scanning barcode is only required for analyst's signature to appear on the report)

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Blood Findings	Report
Phenolphthalein positive	A stain(s) testing positive to a presumptive test for blood was/were located/detected on (item)
Phenolphthalein negative	A presumptive test for blood gave negative results on (item)
Sperm Findings	Report
Spermatozoa observed	Spermatozoa were observed (microscopically) in this sample.
Few spermatozoa observed	Few spermatozoa were observed (microscopically) in this sample.
No spermatozoa observed	No spermatozoa were observed (microscopically) in this sample.

For single source samples	Report
Single Source: exclusion	XXX was excluded as the source of DNA detected in this sample.
Single Source: fail to exclude	XXX cannot be excluded as the source of DNA detected in this sample.
Same profile (for 2+ items)	The genetic profiles obtained from these samples were the same.
Single source statistic	The estimated frequency (13 of 13 core loci) of the genetic profile from the above sample(s) is approximately 1 in XXX quadrillion (Caucasian population), 1 in XXX quadrillion (African-American population), 1 in XXX quadrillion (Athabaskan population), 1 in XXX quadrillion (Inupiat population) and 1 in XXX quadrillion (Yupik population).

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For single source and/or mixtures	Report
Unknown Male DNA	A genetic profile from an unknown male individual was obtained from this sample.
Female present	The genetic profile obtained from the sample was consistent with being from an unknown female individual.
Male DNA present	DNA from (a OR at least one) male individual was observed in this sample.
No DNA inconsistent	No DNA inconsistent with XXX was detected in this sample.
DNA consistent	DNA consistent with XXX was detected in this sample.
When cannot exclude but don't have all 13 core loci	DNA consistent with XXX was detected at XX of 13 core loci in this sample. Therefore, XXX cannot be excluded as a source of DNA detected in this sample. (change 13 if not all loci yielded data above reporting threshold)

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For mixtures	Report
More than 1 individual	DNA from more than one individual was observed in this sample.
To indicate # of individuals	DNA from at least XX individuals was observed in this sample.
Mixture: exclusion	XXX was excluded as a source of DNA detected in this sample.
Mixture: fail to exclude	XXX cannot be excluded as a source of DNA detected in this sample.
Foreign DNA present	DNA inconsistent with XXX was also detected in this sample.
Complex Mixture	Due to the complexity of the genetic profile obtained from this sample, no meaningful comparisons can be made to known reference samples.
Mixture statistic	The estimated probability (13 of 13 core loci) of an individual from each of the following population groups contributing to the DNA detected in the above sample(s) is as follows: approximately 1 in XXX quadrillion (Caucasian population), 1 in XXX quadrillion (African-American population), 1 in XXX quadrillion (Athabaskan population), 1 in XXX quadrillion (Inupiat population) and 1 in XXX quadrillion (Yupik population).

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Paternity	Report
Cannot exclude	XXX cannot be excluded as the biological father/mother of XXX. For additional information please contact the DNA Technical Manager Abirami Chidambaram at (907)-269-5621.
Excluded	XXX was excluded as the biological father/mother of XXX.
Not Suitable for comparison	Report
Not suitable	The data obtained from this sample was not suitable for comparison (due to XXXXXX).
No profile (No reproducible alleles above 100RFU in at least four core STR loci)	No genetic profile was obtained from this sample.
Non-reproducible	The majority of data in this sample was not reproducible. Therefore, this sample was not suitable for comparison.
No conclusions	Report
Inconclusive	No conclusions are reported as to whether XXX contributed DNA to this sample.
Insufficient	No (other) conclusions are reported for this sample due to insufficient DNA.

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SECTION 14 – STATISTICAL ANALYSIS OF STR DATA

Statistical analyses will be performed when the genetic typing results fail to exclude an individual(s) as a source of the DNA in a questioned sample and the result is probative. A statistical analysis may not be necessary when an individual is identified as a possible source of DNA in a sample that is intimate to that individual or the result is not germane to the case (i.e. DNA consistent with consent partner detected).

To interpret the significance of a DNA “match” between samples, it is necessary to know the population distribution of alleles at the loci in question. This requires a database containing the frequency of the alleles (observed $\#/2N$) at each of the loci in question. If an allele has been observed five times or less, a value of $5/2N$ is used, where N is equal to the number of individuals tested for the database. The allele frequencies are used to calculate the genotype frequency for each locus.

The allele frequencies for the Athabaskan, Yupik, and Inupiat Alaskan Native populations were calculated using data generated by the Alaska Scientific Crime Detection Laboratory from 101 Athabaskan, 100 Yupik, and 109 Inupiat blood/saliva samples (B. Budowle et al., Population studies on three Native Alaska population groups using STR loci, Forensic Science International 129 (2002) 51-57).

The allele frequencies for the Caucasian and African-American populations are from the U.S. Department of Justice, FBI Popstats database. These allele frequencies can be found at the end of this section. The Alaska Scientific Crime Detection Laboratory routinely reports the frequency probability for all five populations in the DNA Laboratory Report.

Statistical analyses are calculated with the U.S. Department of Justice, FBI Popstats program, using either the Random Match Probability Formula for single source samples or the Mixture Formula for mixed samples. The resultant values may be truncated for reporting but should never be rounded up (i.e. 34,675,000 may be reported as 34 million or 34.6 million, but not 34.7 million).

14.1 Random Match Probability Formula

To calculate the frequency of a locus genotype, the following formulae are used:

$$\begin{aligned} \text{Heterozygotes} &= 2pq \\ \text{Homozygotes} &= p^2 + p(1-p)\theta \end{aligned}$$

The p and q represent the frequencies of two different alleles. θ (theta) is an empirical measure of population subdivision/substructure or “relatedness”.

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For the Caucasian and African-American populations, $\theta=0.01$
For the Alaskan Native populations, $\theta=0.03$

The multi-locus genotype frequency is estimated by multiplying together the genotype frequencies from the different loci. The frequency of a DNA profile in a population is the inverse of the multiple locus genotype frequency.

14.2 Combined Probability of Inclusion (CPI; Mixture Formula)

To calculate the probability of a random individual in the population being a contributor to a mixture for a locus, the following formula is used:

$$(p_1 + p_2 + \dots + p_n)^2 = P_{\text{LOCUS}}$$

The p_1 , p_2 , and p_n are the frequencies of occurrence of the alleles at the locus.

The combined mixture profile probability is calculated by taking the product of the individual locus probabilities. To estimate the number of individuals in a population that could have contributed to the mixture, the inverse of the combined mixture profile frequency is calculated.

14.3 Calculating Frequencies/Probabilities Using Popstats

- Open Popstats and click the appropriate icon to enter either a Single Sample Target Profile or a Forensic Mixture Target Profile. The selection may also be made under the Case Type pull-down menu.

Note: Do not maximize the window

- In the Reference field, enter the lab case # and item #.
- In the Comments field, enter the agency name and type of case.
- Enter the alleles deemed appropriate for statistical analysis (see the section on [Data Interpretation Guidelines](#) in this manual).

Note: It is not necessary to enter alleles for the Penta loci as population data for these loci are not contained in the database.

- Click the calculator icon or select Calculate Statistics from the Profile pull-down menu.
- Click the 1/f icon or select Inverse Summary of Probability Statistics in the Window pull-down menu.
- Click the printer icon or select Print Report from the File pull-down menu. Select Forensic\BrowardRpt.exe and print. Close the print window.

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- The above steps must be performed for both the Alaska Native database and the FBI STR database. To select a database, choose Population database from the Configuration pull-down menu.
- Select Browse in the Population Database window. Navigate to FBI/STR folder or the Alaska STR, double click on either folder the folder. Choose OK twice.
Note: Be sure that the correct sample type (single sample or forensic mixture) selected after changing the population database.
- Repeat the above steps for the second population. The profile does not need to be re-entered when the database is changed but the case and item number information needs to be re-entered.

14.4 STR Loci and Allele Frequencies used in LOPSTATS calculations

D3S1358

Allele	African-American (N=210)	Caucasian (N=200)	Athabaskan (N=101)	Inupiat (N=109)	Yupik (N=100)
<12	0.0119	0.0123	0.0248	0.0229	0.0250
12	0.0119	0.0123	0.0248	0.0229	0.0250
13	0.0119	0.0123	0.0248	0.0229	0.0250
14	0.1214	0.1404	0.1337	0.0275	0.0250
15	0.2905	0.2465	0.4109	0.4541	0.3550
15.2	0.0119	0.0123	0.0248	0.0229	0.0250
16	0.3619	0.2315	0.2574	0.4404	0.5250
17	0.1000	0.2118	0.1436	0.0551	0.0900
18	0.0571	0.1626	0.0545	0.0229	0.0250
19	0.0119	0.0123	0.0248	0.0229	0.0250
>19	0.0119	0.0123	0.0248	0.0229	0.0250

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<i>Allele</i>	<i>African-American (N=180)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<11	0.0139	0.0128	0.0248	0.0229	0.0250
11	0.0139	0.0128	0.0248	0.0229	0.0250
12	0.0139	0.0128	0.0248	0.0229	0.0250
13	0.0139	0.0128	0.0248	0.0229	0.0250
14	0.0667	0.1020	0.1733	0.1743	0.0800
15	0.2361	0.1122	0.0297	0.1055	0.1300
16	0.2694	0.2015	0.3960	0.1881	0.2000
17	0.1833	0.2628	0.2327	0.2226	0.1350
18	0.1361	0.2219	0.1139	0.1835	0.1200
19	0.0722	0.0842	0.0545	0.0367	0.1250
20	0.0278	0.0128	0.0248	0.0229	0.0250
21	0.0139	0.0128	0.0248	0.0229	0.0250
>21	0.0139	0.0128	0.0248	0.0229	0.0250

D8S1179

<i>Allele</i>	<i>African-American (N=180)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<8	0.0139	0.0128	0.0248	0.0229	0.0250
8	0.0139	0.0128	0.0248	0.0229	0.0250
9	0.0139	0.0128	0.0248	0.0229	0.0250
10	0.0250	0.1020	0.0297	0.0642	0.0250
11	0.0361	0.0587	0.0248	0.0229	0.0250
12	0.1020	0.1454	0.2079	0.1881	0.0950
13	0.0722	0.3393	0.4555	0.3578	0.4500
14	0.3333	0.2015	0.1980	0.2477	0.3350
15	0.2139	0.1097	0.0941	0.1239	0.0950
16	0.0444	0.0128	0.0248	0.0229	0.0250
17	0.0139	0.0128	0.0248	0.0229	0.0250
>17	0.0139	0.0128	0.0248	0.0229	0.0250

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<i>Allele</i>	<i>African-American (N=180)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<18	0.0139	0.0128	0.0248	0.0229	0.0250
18	0.0139	0.0306	0.0248	0.0229	0.0250
18.2	0.0139	0.0128	0.0248	0.0229	0.0250
19	0.0528	0.0561	0.0248	0.1009	0.2000
19.2	0.0139	0.0128	0.0248	0.0229	0.0250
20	0.0722	0.1454	0.0693	0.0459	0.0350
20.2	0.0139	0.0128	0.0248	0.0229	0.0250
21	0.1250	0.1735	0.1634	0.0229	0.0350
21.2	0.0139	0.0128	0.0248	0.0229	0.0250
22	0.2250	0.1888	0.0941	0.1650	0.2150
22.2	0.0139	0.0128	0.0248	0.0229	0.0250
22.3	0.0139	0.0128	0.0248	0.0229	0.0250
23	0.1250	0.1582	0.1287	0.1247	0.1050
23.2	0.0139	0.0128	0.0248	0.0229	0.0250
24	0.1861	0.1378	0.1178	0.2248	0.1200
24.2	0.0139	0.0128	0.0248	0.0229	0.0250
24.3	0.0139	0.0128	0.0248	0.0229	0.0250
25	0.1000	0.0688	0.1881	0.1514	0.2100
26	0.0361	0.0128	0.1089	0.1101	0.0700
26.2	0.0139	0.0128	0.0248	0.0229	0.0250
27	0.0222	0.0128	0.0248	0.0275	0.0250
28	0.0167	0.0128	0.0248	0.0229	0.0250
29	0.0139	0.0128	0.0248	0.0229	0.0250
30	0.0139	0.0128	0.0248	0.0229	0.0250
>30	0.0139	0.0128	0.0248	0.0229	0.0250

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D21S11

<i>Allele</i>	<i>African-American (N=179)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=101)</i>
<24.2	0.0140	0.0128	0.0248	0.0229	0.0250
24.2	0.0140	0.0128	0.0248	0.0229	0.0250
24.3	0.0140	0.0128	0.0248	0.0229	0.0250
26	0.0140	0.0128	0.0248	0.0229	0.0250
27	0.0615	0.0459	0.0248	0.0229	0.0250
28	0.2151	0.1658	0.0297	0.0229	0.0250
29	0.1899	0.1811	0.1683	0.2082	0.3150
29.2	0.0140	0.0128	0.0248	0.0229	0.0250
30	0.1788	0.2321	0.4257	0.1977	0.2000
30.2	0.0140	0.0383	0.0248	0.0229	0.0250
30.3	0.0140	0.0128	0.0248	0.0229	0.0250
31	0.0922	0.0714	0.0545	0.1055	0.0650
31.2	0.0754	0.0995	0.0238	0.1881	0.2000
32	0.0140	0.0153	0.0248	0.0229	0.0250
32.1	0.0140	0.0128	0.0248	0.0229	0.0250
32.2	0.0698	0.1122	0.1238	0.1284	0.1100
33	0.0140	0.0128	0.0248	0.0229	0.0250
33.2	0.0335	0.0356	0.0594	0.0413	0.0750
34	0.0140	0.0128	0.0248	0.0229	0.0250
34.2	0.0140	0.0128	0.0248	0.0229	0.0250
35	0.0279	0.0128	0.0248	0.0229	0.0250
35.2	0.0140	0.0128	0.0248	0.0229	0.0250
36	0.0140	0.0128	0.0248	0.0229	0.0250
>36	0.0140	0.0128	0.0248	0.0229	0.0250

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D18S51

<i>Allele</i>	<i>African-American (N=180)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<11	0.0139	0.0128	0.0248	0.0229	0.0250
11	0.0139	0.0128	0.0248	0.0229	0.0250
12	0.0583	0.1276	0.1535	0.0688	0.0250
13	0.0556	0.1225	0.2970	0.1514	0.1850
13.2	0.0139	0.0128	0.0248	0.0229	0.0250
14	0.0639	0.1735	0.1287	0.1193	0.1350
14.2	0.0139	0.0128	0.0248	0.0229	0.0250
15	0.1667	0.1276	0.0941	0.2008	0.3300
15.2	0.0139	0.0128	0.0248	0.0229	0.0250
16	0.1889	0.1071	0.1584	0.0367	0.0400
17	0.1639	0.1556	0.0693	0.2477	0.2250
18	0.1306	0.0918	0.0545	0.0275	0.0300
19	0.0778	0.0357	0.0297	0.0159	0.0250
20	0.0556	0.0255	0.0248	0.0229	0.0250
21	0.0139	0.0128	0.0248	0.0229	0.0250
21.2	0.0139	0.0128	0.0248	0.0229	0.0250
22	0.0139	0.0128	0.0248	0.0229	0.0250
>22	0.0139	0.0128	0.0248	0.0229	0.0250

D5S818

<i>Allele</i>	<i>African-American (N=180)</i>	<i>Caucasian (N=195)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<7	0.0139	0.0128	0.0248	0.0229	0.0250
7	0.0139	0.0128	0.1832	0.0826	0.0650
8	0.0556	0.0128	0.0248	0.0229	0.0250
9	0.0139	0.0308	0.0248	0.0229	0.0250
10	0.0639	0.0487	0.0743	0.0688	0.1150
11	0.2611	0.4103	0.5050	0.3578	0.4800
12	0.3556	0.3539	0.1436	0.2936	0.2350
13	0.2444	0.1462	0.0743	0.1514	0.0850
14	0.0139	0.0128	0.0248	0.0229	0.0250
15	0.0139	0.0128	0.0248	0.0229	0.0250
>15	0.0139	0.0128	0.0248	0.0229	0.0250

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D13S317

<i>Allele</i>	<i>African-American (N=179)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<8	0.0140	0.0128	0.0248	0.0229	0.0250
8	0.0363	0.0995	0.0693	0.1055	0.1100
9	0.0279	0.0765	0.1881	0.1330	0.1030
10	0.0503	0.0510	0.2277	0.2661	0.4250
11	0.2374	0.3189	0.2772	0.3853	0.2700
12	0.4832	0.3087	0.1881	0.0826	0.0900
13	0.1257	0.1097	0.0396	0.0229	0.0250
14	0.0363	0.0357	0.0248	0.0229	0.0250
15	0.0140	0.0128	0.0248	0.0229	0.0250
>15	0.0140	0.0128	0.0248	0.0229	0.0250

D7S820

<i>Allele</i>	<i>African-American (N=210)</i>	<i>Caucasian (N=203)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<6	0.0119	0.0123	0.0248	0.0229	0.0250
6	0.0119	0.0123	0.0248	0.0229	0.0250
7	0.0119	0.0172	0.0248	0.0229	0.0250
8	0.1738	0.1026	0.1485	0.2615	0.3650
9	0.1571	0.0478	0.1089	0.1239	0.0650
10	0.3238	0.0990	0.2673	0.0780	0.1200
10.1	0.0119	0.0123	0.0248	0.0229	0.0250
11	0.2238	0.2020	0.2426	0.3395	0.2750
11.3	0.0119	0.0123	0.0248	0.0229	0.0250
12	0.0905	0.1404	0.2178	0.1743	0.1400
13	0.0119	0.0296	0.0248	0.0229	0.0350
14	0.0119	0.0123	0.0248	0.0229	0.0250
>14	0.0119	0.0123	0.0248	0.0229	0.0250

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D16S539

<i>Allele</i>	<i>African-American (N=209)</i>	<i>Caucasian (N=202)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<8	0.0120	0.0124	0.0248	0.0229	0.0250
8	0.0359	0.0198	0.0248	0.0229	0.0250
9	0.1986	0.1040	0.1337	0.0688	0.0780
10	0.1101	0.0668	0.2921	0.0826	0.0800
11	0.2943	0.2723	0.2772	0.6147	0.3550
12	0.1866	0.3391	0.2426	0.2202	0.3400
13	0.1651	0.1634	0.0446	0.0229	0.0400
14	0.0120	0.0322	0.0248	0.0229	0.0250
15	0.0120	0.0124	0.0248	0.0229	0.0250
>15	0.0120	0.0124	0.0248	0.0229	0.0250

TH01

<i>Allele</i>	<i>African-American (N=210)</i>	<i>Caucasian (N=203)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<5	0.0119	0.0123	0.0248	0.0229	0.0250
5	0.0119	0.0123	0.0248	0.0229	0.0250
6	0.1095	0.2266	0.1535	0.0229	0.0850
7	0.4405	0.1724	0.6089	0.8762	0.7700
8	0.1857	0.0256	0.0594	0.0229	0.0550
8.3	0.0119	0.0123	0.0248	0.0229	0.0250
9	0.1452	0.1130	0.0644	0.0275	0.0250
9.3	0.1043	0.3054	0.1139	0.0596	0.0700
10	0.0143	0.0123	0.0248	0.0229	0.0250
>10	0.0119	0.0123	0.0248	0.0229	0.0250

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TPOX

<i>Allele</i>	<i>African - American (N=209)</i>	<i>Caucasian (N=203)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<6	0.0120	0.0123	0.0248	0.0229	0.0250
6	0.0861	0.0123	0.0248	0.0229	0.0250
7	0.0215	0.0123	0.0248	0.0229	0.0250
8	0.3684	0.5443	0.2178	0.2477	0.2100
9	0.1818	0.1232	0.0248	0.0413	0.1200
10	0.0933	0.0370	0.0248	0.0229	0.0250
11	0.2249	0.2537	0.4010	0.5184	0.3250
12	0.0239	0.0394	0.3515	0.1789	0.1200
13	0.0120	0.0123	0.0248	0.0229	0.0250
>13	0.0120	0.0123	0.0248	0.0229	0.0250

CSF1PO

<i>Allele</i>	<i>African - American (N=210)</i>	<i>Caucasian (N=203)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<6	0.0119	0.0123	0.0248	0.0229	0.0250
6	0.0119	0.0123	0.0248	0.0229	0.0250
7	0.0429	0.0123	0.0248	0.0229	0.0250
8	0.0857	0.0123	0.0248	0.0229	0.0250
9	0.0333	0.0197	0.1040	0.0642	0.0350
10	0.2714	0.2537	0.1683	0.2936	0.3550
10.3	0.0119	0.0123	0.0248	0.0229	0.0250
11	0.2048	0.3005	0.2129	0.1973	0.1400
12	0.3200	0.3251	0.4257	0.4312	0.3600
12.1	0.0119	0.0123	0.0248	0.0229	0.0250
13	0.0548	0.0714	0.0792	0.0229	0.0300
14	0.0119	0.0148	0.0248	0.0229	0.0300
15	0.0119	0.0123	0.0248	0.0229	0.0450
>15	0.0119	0.0123	0.0248	0.0229	0.0250

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SECTION 15 – REVIEW OF STR DATA

This section supplements information contained in the Laboratory Quality Assurance Manual and the DNA Quality Assurance Manual.

15.1 Review of Casework Analyzed In-House

All case reports issued by a DNA analyst and all supporting documentation that is part of the case record or the central log, will be subjected to a technical review and an administrative review. When a case has been through technical and administrative review, the report will be issued in accordance with laboratory policies and procedures. Genetic profiles suitable for CODIS will be uploaded in accordance with CODIS policies and procedures.

15.1.1 Technical and Administrative Review

Technical review of DNA casework will be conducted by a second qualified DNA analyst, in accordance with the FBI QAS Guidelines and the Laboratory and DNA Quality Assurance Manuals. The analyst and the reviewer may consult a third qualified DNA analyst, if necessary, when there is a disagreement on how to report a result. If the analysts are unable to come to an agreement, the DNA Technical Manager will be consulted to make the final decision.

Discipline checklists are used to document completion of the individual components of the technical and administrative reviews. The completion of the final laboratory report, technical review and administrative review are tracked electronically in LIMS.

15.2 Review of Offender Database Samples Analyzed In-House

Genetic profiles and all supporting documentation generated in the course of analysis (for entry into CODIS or for hit confirmation) will be subjected to a technical review. Technical review of offender samples will be conducted by a second qualified DNA analyst, in accordance with the FBI QAS Guidelines and the Laboratory and DNA Quality Assurance Manuals. The analyst and the reviewer may consult a third qualified DNA analyst, if necessary, when there is a disagreement on how to report a result. If the analysts are unable to come to an agreement, the DNA Technical Manager will be consulted to make the final decision.

A review checklist is used to document completion of the individual components of the technical and administrative review. When the technical review is complete, offender samples may be uploaded to CODIS. Detailed procedures for upload and resolution of any resulting database matches are contained in the laboratory's CODIS manual.

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15.3 Review of Outsourced Offender Database Samples

The laboratory does not currently use a vendor laboratory for DNA analysis.

The laboratory may perform a technical review of data generated by a vendor laboratory under contract with an Alaska law enforcement agency.

Please refer to the DNA Quality Assurance Manual for additional information.

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Appendix I

R1 Page	R0 page	Location	Revision made
5	5	2.1	The reagent shall not be used in casework until the issue has been resolved and the approval or an alternate course of action suggested by the DNA Technical Manager has been documented.
11	11	2.4	Phenolphthalein (for Kastle-Meyer Test)
35	35	3.12	Updated Image
36	36	3.12	Updated Image
47	47	6.1	Updated: If the questioned samples and reference samples are extracted on the same day, the time when each set of extractions was begun should be documented in the worksheet notes.
48	48	6.1	Updated: For items not previously screened, and when appropriate, the DNA analyst qualified in sperm search and microscopy techniques will proceed with processing the sample and prepare a sperm search slide for microscopic inspection. A second qualified analyst may be requested, on occasion, review this slide to verify these results. If this verification is performed, it is documented on the DNA extraction worksheet and by the second analyst's initials on the slide. Slide(s) will be retained and packaged with the evidence. The DNA case report, in such instances, will include a statement stating the presence or absence of spermatozoa in the sample(s).
49	49	6.2.2	Revised: "• For contact DNA swabs (not thought to contain biological fluids) sample the entire swab to maximize the chance of obtaining an interpretable DNA profile (elution volume must be at least 100µL). However, in such a circumstance, at least fifty percent of the extract will be retained for future use. "

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49	49	6.2.3	Revised: "• Swabs from under the nails are generally sampled by cutting the swab tips into the sample tube. However, as in the case of contact swabs, the entire swab may be used in extraction and at least fifty percent of the swab retained for future use."
55	55	6.4.1: Option 2	Updated: "• Run the Large Volume Protocol and elute in 10 ml TE buffer. • On occasion, use more than one tube may be required to get and extract the entire sample efficiently. In such situations, the EZ1 extracts obtained from all tubes digests may be combined in one tube and proceed to the quantitation step."
57	57	6.4.2: Step 4	Revised: "4. Vortex briefly. Incubate overnight in a 37°C incubator. Pre-soaks/water soaks with less incubation time shall be performed only with documented pre-approval of the Forensic Biology Supervisor or DNA Technical Manager after evaluation of the sample."
60	60	6.4.2: Option 2	Updated: "Option 2 – Large-Volume Protocol for Consumed Samples: • If the entire evidence sample has been consumed and digested, the analyst shall transfer the entire volume of digest solution to an EZ1 sample tube (or tubes - with no more than 500ml in each tube), add 1ml of carrier RNA solution and add 400ml of Buffer MTL to the tube(s)."
72	72	8.3	Updated: "Vortex and spin the tube briefly and add 5µL of the diluted 9947A Control DNA (or other positive control DNA supplied by the PCR kit vendor) to a 0.5mL tube containing 14.2µL of sterile, de-ionized water. "

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R1 Page	R0 page	Location	Revision made
77	77	9.1.4	Updated: Added the term "alleles"
78	78	9.1.4.3	Updated: Added the term "alleles"
88	88	9.2.4	Updated: Added the term "alleles"
89	89	9.2.4.3	Updated: Added the term "alleles"
103	103	11	<p>"Reportable" alleles are those alleles whose peak heights are equal to or above the 100 RFU reporting (analytical) threshold and are generally reproducible when re-amplified. Such alleles will be routinely used in forensic comparisons between known and questioned sample genetic profiles under the interpretation guidelines stated in this manual.</p> <p>"Observed" genetic data includes data (peaks) below the reporting (analytical) threshold (100 RFU) and/or alleles whose peak heights are greater than 100 RFU, but that may not be reproducible in duplicate or multiple amplifications of the same sample (DNA extract). Such alleles will not be routinely included in the STR results table.</p> <p>"Observed" alleles may be considered for use when reporting the presence of multiple sources of DNA in a sample and/or when reporting the presence of male DNA. On occasion, depending on the quality of the data/ genetic profile obtained, such alleles may be used in compositing data after careful evaluation of the data.</p>

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104	104	11.1.1	If a peak is not labeled because it falls below the 100 RFU reporting threshold, the analyst may, with the documented approval of the DNA Technical Manager, lower the acceptable threshold for the ILS peak(s). Note reflecting this action shall be made on relevant electropherogram(s) / bench notes.
104	104	11.1.1	Use caution when looking for lower ILS peak heights since some peaks may also fall below 100 RFU when ILS peaks fall below 100 RFU. Therefore, re-identification of a sample(s) when the ILS peaks are below 100RFU is inadvisable.
104	104	11.1.1	The Technical Manager shall approve using samples when some of the ILS peaks between 80 and 60 base pairs are not labeled or fall below 100RFU. Such approval will also be documented on the relevant electropherograms / bench notes as applicable.
106	106	11.2	The reagent blank should not yield any true STR allelic peaks above the 100 RFU reporting threshold. When peaks greater than or equal to 100RFU are present in the range between 80-550 base pairs, the analyst will determine if the peaks are artifacts (e.g. spike, pull-up) or true allele peaks.
109	109	11.2.2.1	The reagent blank should not yield any true STR allelic peaks above the 100 RFU reporting threshold. When peaks greater than or equal to 100RFU are present in the range between 80-550 base pairs, the analyst will determine if the peaks are artifacts (e.g. spike, pull-up) or true allele peaks.

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109	109	11.2.2.1	When probable true allele peaks are detected at several or all STR loci above the reporting threshold (100RFU) and the analyst and the Technical Reviewer cannot come to a consensus on how to document the observation, the Technical Manager will be consulted to determine the appropriate course of action.
110	110	11.2.2.2	When probable true allele peaks are detected at several or all STR loci above the reporting threshold (100 RFU) and the analyst and the Technical Reviewer cannot come to a consensus on how to document the observation, the Technical Manager will be consulted to determine the appropriate course of action.
110	110	11.3	<p>Revised: ICS samples may include any known sample, including those obtained from laboratory staff, where the sample has been previously been typed to generate a genetic profile..</p> <p>An electropherogram of the ICS shall be included in the central log folder so the technical reviewer can verify the STR results and determine that the analytical process was appropriate.</p>

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110	110	11.3	Revised: The Technical Manager, or a designated individual, may approve the issue of specific reports on a case-by-case basis. This approval will be documented and maintained within the central log folder pertaining to the batch of cases affected by the discrepancy.
		11.4	Revised: For all casework, questioned and reference samples, the minimum detection/reporting threshold is 100 RFU. Allelotype(s) greater than or equal to 100 RFU that are not reproducible if re-amplified, may be included in the STR results table and used in statistical analyses. Only detected alleles equal to or greater than 100 RFU will be considered in data interpretation.
		11.4	Revised: Exceptions for single source samples may be appropriate. The Technical Manager will be consulted for determining the appropriate course of action for any exceptions to routine guidelines..
111	111	11.4	Updated: Amelogenin data determined and documented in this manner is suitable for inclusion in the STR results table. (This is applicable to database samples as well).
111	111	11.4	Revised: When peak(s) below 100RFU exhibit optimal peak morphology and fall in an allele bin, the electropherogram and the STR results table will be marked by an asterisk (*) indicating data below reporting threshold was observed in this sample.

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111	111	11.4	Revised: When peaks are observed below the reporting threshold of 100RFU, an additional electropherogram showing a close-up view of the affected loci should be printed for review and inspection. The electropherogram(s) shall also include a note documenting that data below reporting threshold (db1 or db2) was/were observed.
		11.4.1	Revised: Low-level DNA samples are samples that exhibit peaks below reporting threshold.
		11.4.1	Revised: Peak height imbalance owing to low template copy number stochastic effects can often be mitigated by re-amplifying the sample with more template DNA. Alternatively, it may be appropriate to perform a duplicate amplification of these samples to assess reproducibility of the data. All components of a DNA sample mixture may not exhibit reliable reproducibility (of allelic peaks) when there are stochastic effects during PCR. This phenomenon will impact which alleles may be reported and used in data interpretation. Only alleles equal to or greater than 100 RFU shall be reported on the STR results table and considered suitable for comparison.
113		11.4.2.1	11.4.2.1 Artifacts
			Artifacts are not true alleles. Artifacts can occur in data and much of their nature and origin have been determined and documented.

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R1 Page	R0 page	Location	Revision made
113		11.4.2.2	Revised: OL alleles considered to be true alleles may require re-amplification to confirm. If the OL allele is observed in multiple samples derived from multiple tissue types or sources with the same STR profile, only one of the samples needs to be re-amplified for verification of the OL allele(s). The DNA Technical Manager must approve reporting an OL allele without re-amplification (e.g. limited amount of sample available for analysis).
118		11.4.3.1	Revised: If the potential tri-allele containing sample is re-extracted and re-amplified and found to be reproducible, the analyst may include the locus with the tri-allele as in the STR results table. If the potential tri-allele is not reproducible,, it will be reported as an Artifact/True allele (A/TA).
119		11.4.3.2	Revised: Scientific explanations may include, but are not limited to, factors such as inhibition, degradation, low template DNA and/or primer binding site variations or mutations. Parent-offspring exclusion (also Parent-offspring inconclusive) is declared when, upon comparison of the DNA results from an offspring sample to that from an alleged biological parent(s), the profiles are found to not share at least one allele at two or more corresponding loci.
		11.4.3.2	Partial Profile: If one allele is identified and a second potential allele is below the reporting threshold (100 RFU), the identified allele will be used for comparison purposes first and if concordance is established the peak below threshold will be assessed to support the inclusion and/or exclusion of an individual(s).

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120	120		Deleted: Parent-offspring inconclusive
			Revised: . In such circumstances, it is common to recover a DNA mixture which includes DNA consistent with that individual.
			Revised: Identification of DNA mixtures may be straightforward or difficult, depending on the number of loci typed, the number of contributors and the relative proportion of DNA contributed by each individual in the mixture. Therefore, interpretation of DNA mixtures and statistical analyses requires careful consideration of factors affecting allele designation, heterozygote detection and peak height variations within and between loci.
122	122	11.4.4	14) Observation of low-level DNA/data below reporting threshold
124	124	11.4.4.1	Due to the possibility that the minor contributor's alleles may be shared by the major contributor and that such alleles may be below reporting threshold, determination of the minor contributor profile may be possible at only some loci.
			The minor component may be suitable for comparison if alleles equal to or greater than 100 RFU occur at least 4 of 13 core loci.
124	124		The mixture formula may be used when an individual cannot be excluded from the sample when considering all reportable (equal to or greater than 100 RFU) alleles.
125	125	11.4.4.2	Partial Profile: If reportable alleles (100 RFU or greater) are detected in a mixture at less than 10 core loci the sample may still be suitable for comparison. The Technical Manager will be consulted to determine the appropriate course of action.

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126	126		<u>Insufficient DNA/Data:</u> If insufficient DNA is observed in a sample (e.g. data below reporting threshold, dropout, low-level DNA) the data may not be suitable for comparison. This data will be considered insufficient and will not be utilized for comparison. As such, this data will be not be included on the STR results table.
126	126		<u>Complete mixture:</u> A complete mixture may be declared when DNA from at least four (4) sources is detected in a sample, there is evidence of stochastic effects (e.g. dropout) at several or all loci, and/or a majority of the alleles are not reproducible when amplified in duplicate.
			Revised: 11.4.5 Consensus Data Reporting When a sample is amplified in duplicate, in accordance with Section 11.4.1, only the reproducible alleles will be included on the STR results table. On occasion, compositing data from multiple injections/amplifications (e.g. data from more than 2 amplifications) from the same extract may be deemed appropriate after inspection of the quality of data. Such data will require technical manager approval for compositing.
126	126	11.5	Revised: Reportable alleles are those whose peak heights are equal to or above the 75 RFU detection threshold. Lower threshold limits for hit confirmations or ILS (Internal Lane Standard) may be authorized by the DNA Technical Manager after assessing overall data quality and sample availability.

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127	127	11.5.1.1	The quality of the ILS (SQ<0.9) should be noted where deemed necessary (please see Database Sample Analysis section). The SQ value should be noted especially when using the AB 3500xl for both, database samples as well as forensic case or analysis.
129	129	11.5.2.1	Revised: Approval of such positive control samples should have documented Technical Manager approval on the initial batch worksheet or relevant electropherograms.
132	132	11.5.4	Revised: Occasionally, a single-source database sample may be observed to have three alleles at one (rare) or more) locus/loci. Samples exhibiting tri-alleles will be re-amplified and may have to be re-extracted to confirm the observed genotypes.
			Revised: Peaks may be observed that are below the reporting threshold of 75 RFU. When these peaks exhibit peak morphology similar to allelic peaks and fall within an allele bin, the analyst should consider the possibility of allelic dropout. If allelic dropout is suspected in one or more core STR loci, the analyst may re-amplify and/or re-inject the sample to obtain a complete profile. The sample may have to be re-extracted if these methods fail.

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132	132	11.5.4	<p>Update: If a partial genetic profile is obtained from a sample the analyst will make the "best attempt" to re-process the sample in order to generate a complete genetic profile. The repeat analysis may include any or all of the following: re-extraction using more sample, maximum template quantity in PCR, maximum template volume, maximum allowable PCR cycling conditions, maximum "prep" or amplified sample volume for capillary electrophoresis, and maximum injection time. NOTE: Applicable reagent blank and negative PCR control will be processed under the most stringent conditions).</p>
			<p>If the above "best attempt" also fails to generate a complete genetic profile, a maximum of three core STR loci with elevated stutter and/or stochastic issues (e.g., partial dropout, heterozygous peak height ratio less than 50%) may be permissible in a sample. Samples that do not contain at least partial data at each of the 13 core STR loci are not eligible for upload to NDIS. The allele 10 mutation at the D5 locus, which can cause issues with allele heights, is an accepted phenomenon and is not counted towards the maximum three core STR loci with stochastic issues. The presence of the observed mutation/variant should be documented on the database batch worksheet. The CODIS administrator will determine the eligibility of such profiles for upload.</p>

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133	133	11.5.6	Revised: For interpretation of database samples, the laboratory maintains a record of known (previously observed) OL alleles. When an OL allele has been previously observed within the laboratory and the possibility of an artifact has been eliminated, the allele may be entered into CODIS without re-amplification. If an OL allele is being observed by the laboratory for the first time, the sample will be re-amplified, prior to CODIS entry, to confirm the genotype. On occasion, re-extraction of the sample may be needed for verification. Once confirmed the OL will be added to the record of previously observed OL alleles.
154		13	Deleted: DNA QAM from Refer to the Laboratory Quality Assurance Manual information regarding discovery requests and the release of information from the laboratory.
154		Biological Screening Abbreviations and Definitions	Deleted : Apostrophe from NEC's to read NECs for Nucleated Epithelial cells.
155		DNA Abbreviations and Definitions	Edited: DNA Abbreviations and Definitions - DBDT or DBRT = Data below reporting threshold of 100RFU(*).
155		DNA Abbreviations and Definitions	Added: LV= Large Volume (EZ1 protocol)

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158		13.1.2	<p>Revised: Note: Multiple injections of the same sample at more than one injection time (for efficiency purposes) in a CE run do not require documentation of a reason. The analyst will select the injection best suited for interpretation, based on the interpretative guidelines.</p> <p>§ Reasons for a re-amplification and/or re-extraction (examples)</p> <ul style="list-style-type: none"> • stutter allele • off-ladder allele
158	158	13.1.2	<p>Additional: Identify clearly the Positive Control sample provided by the PCR kit vendor.</p>

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158	158	13.1.3	<p>Revised : <i>The term "chart" will no longer refer to the STR Results Table</i> -</p> <ul style="list-style-type: none"> o It is not necessary to include in the STR Results table the profiles obtained from samples that serve as internal controls (i.e. the epithelial fraction from a vaginal swab) as long as the profile is consistent with expected results . o If results are not suitable for comparison, do not include in the STR Results Table the Questioned sample profile (i.e.. sample with no called alleles, majority of alleles are not reproducible, minor alleles not suitable for comparison, etc.). o If interpretable genetic profiles are not obtained from any of the questioned samples, the known sample profiles need not be included in the STR Results Table. o Add a <i> </i> after the item description if there is data (i.e. alleles - below reporting threshold in a sample. o Add "epithelial" or "sperm fraction" to sample description when necessary. o Minor alleles go in () below the major alleles. o If an allele is determined to be A/TA, this notation goes after the allele call in superscript (i.e.. 10A^{TA}). o Loci with no reportable alleles are left blank and the boxes grayed out. o All notes at the bottom of the STR Results Table are retained

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160	160		<p>Revised and edited terminology and content. The following handwritten notations may be included on the electropherogram</p> <ul style="list-style-type: none"> o “ * DBDT” / DBRT in the upper right hand corner o Loci with peak height imbalance (highlighted in orange in GMID-X) must be initialed o Artifacts (i.e. pull-up, dye blobs etc.) be noted as “artifacts” on both the traditional prototype view and the zoomed in electropherogram o “No genetic profile added to bottom left when no reproducible alleles above reporting threshold o For samples that have been double amplified and yielded data suitable for comparison, add “reproducible alleles on STR Results Table” to bottom left of the first electropherogram. All non-reproducible alleles are crossed out and initialed on all electropherograms. o For samples that were double amplified and did not yield data suitable for comparison, add “Reproducible alleles noted” and the reason that the data is not suitable for comparison to the bottom of electropherogram o For peaks in stutter positions (that appear close to the expected stutter percentages) indicate the % of the peak relative to the main peak and the expected stutter percentage (for example, 12%>5% or 5.2%~5%). When the peak can be reasonably interpreted as elevated stutter, add a notation to this effect. When it is not possible to discern whether the peak is a stutter peak or a true allele, add “A/TA”. o If data is not suitable for comparison, add a note at bottom of electropherogram i.e. “data not included in STR Results table due to insufficient DNA”, “minor component not suitable for comparison therefore not included in STR Results table”, “minor component consistent with owner and therefore not included in STR Results table” etc.

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162	162	13.2.1	Revised: 13.2.1 Guidelines for Biological Screening Examinations / Reports
163	163	13.2.1	Sperm Findings report table - Revised Detected to read as Observed
164	164	Table	No semen was detected chemically (BCIP/STMP) on (item)
164	164	Blood Findings	A presumptive test for blood gave negative results on (item)
167	167	DNA Results Table	Edited to match Biological Screening Table terminology
		DNA Results Table terminology	Edited to read - +
			Unknown Male DNA: A genetic profile from an unknown male individual was obtained from this sample.
			Female present: The genetic profile obtained from this sample was consistent with being from an unknown female individual.
			When cannot exclude but don't have all 13 core loci:
			Therefore, XXX cannot be excluded as a source of DNA detected in this sample. (change 13 if not all loci yielded data above reporting threshold)
169	169	Table	Edited -Complex Mixture : Due to the complexity of the genetic profile obtained from this sample, no meaningful comparisons can be made to known reference samples.