

Forensic Biology Procedure Manual

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## Section 1: An Overview of Forensic Biology Workflow

Casework in the Forensic Biology discipline is routinely processed in batches. Typical batch size is 5-10 cases but may be larger or smaller as needed. Each batch of casework has a corresponding central log, where documentation of batch QA controls and lab processing is recorded. The Forensic Biology Casework Procedures Manual describes the protocols used for lab processes, interpretation, report writing, and technical and administrative reviews.

Batched casework moves through this general laboratory workflow:

1. Biological screening: presumptive tests for blood and/or semen
2. Prioritizing analysis, sampling, and extraction: analyst uses the available documentation, such as Request for Laboratory Services and sexual assault kit paperwork, along with biological screening results (if applicable) to make decisions about which evidence is best suited for DNA analysis. Evidence is prepared for the DNA extraction process, and a protocol is selected based on type of evidence.
3. Quantification and quant-based decisions: the quantification process determines the amount of DNA present in a sample, as well as the amount of male DNA and the quality of the DNA. Based on the quantification results, analysis of samples may stop or proceed to amplification
4. Amplification and genetic analysis: Samples are amplified by short tandem repeat (STR) polymerase chain reaction (PCR) analysis, or by Y-STR analysis, which is PCR specific to the markers on the Y-chromosome. Capillary electrophoresis is used to analyze the amplified DNA.
5. Interpretation and report writing analyst assess the profiles, makes comparisons, and calculates statistics, as appropriate
6. Review: All reports undergo technical and administrative reviews prior to release

If an analyst completes only part of the workflow and analysis and/or interpretation of the evidence is completed by another analyst, the first analyst must document and self-review the full extent of their work. This documentation may include bench notes, central log documentation, and / or a Biological Screening / DNA Screening report, as appropriate. The same principle applies to technical reviews, where specific elements of a given case may be technically reviewed by different analysts. The review documentation, including the bench notes and/or the review checklist, must clearly indicate if technical review has been performed by more than one reviewer.

This manual is designed to address typical situations that arise during forensic casework. Analysts must seek prior approval of protocol changes that impact the use of evidence. The DNA Technical Manager may authorize adaptations of these protocols to specific case situations and will document this approval with a Protocol Exception Form.

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### ***Forensic Biology Abbreviations and Definitions***

The following abbreviations are commonly used in bench notes and on other documents generated during biological screening.

<b>Biological Screening Abbreviations and Definitions</b>	
Phenolphthalein/Kastle-Meyer	PH, Pheno, or KM
Fluorescence/Alternate Light Source	F or ALS
Acid Phosphatase activity	AP, FBB
Nucleated Epithelial Cells	NECs
Prostate-Specific Antigen	PSA or p30
Human Hemoglobin	hHb, Species
Positive test result	Pos or (+)
Negative test result	Neg or (=)
Reddish-brown color observed	R-B
Case, item, date & initials	CIDI
Scientific Crime Detection Laboratory	SCDL
Victim Forensic History	VFH
Suspect Forensic History	SFH

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The following abbreviations are commonly used in bench notes and on other documents generated during DNA analysis, particularly extraction and quantification:

DNA Analysis Abbreviations and Definitions	
ICS	Internal Control Specimen
SAK	Sexual assault kit
E or Epi	Epithelial fraction
S or Sp	Sperm fraction
SS	Sperm/substrate fraction
Sub	Substrate fraction
RBS	Reagent blank sperm / Reagent blank sperm & substrate
RBE	Reagent blank epithelial
RBsub	Reagent blank substrate
RBQ	Reagent blank questioned (direct)
RBD	Reagent Blank Direct with DTT
RBK	Reagent Blank Known
Quant/QT	Quantification / Quantifiler Trio
No Male	No male DNA detected
Low Male	Female: male ratio is 4:1 or higher. Y-STRs recommended.
Low DNA	Quantitation indicates that consumption of sample is recommended.
NFA	No further analysis
PTC	Permission to consume
PCR	Polymerase Chain Reaction
STR / Y-STR	Short Tandem Repeat / STR analysis specific to Y chromosome markers. When noted on a Quantifiler Trio report, indicates that the extract is suitable to proceed to STR or Y-STR analysis

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The following abbreviations are commonly used on electropherograms, mixture interpretation worksheets, and other documents generated during or referring to DNA analysis.

DNA Interpretations Abbreviations and Definitions	
e-gram	Electropherogram
BL	Noisy baseline
PHR	Peak Height Ratio
PH or PkHt	Peak Height
RFU	Relative Fluorescent Units
AT	Analytical threshold
ST	Stochastic threshold
IT	Interpretational threshold
PU	Pull-Up
DO	Complete Drop-Out at a Locus
PDO	Partial Drop Out at a Locus
OL	Off Ladder
OMR	Outside Marker Range
OS	Off Scale/Saturation
NP	No genetic profile obtained
NR	Data not reported
NS	Data not used for statistics
NA	Locus not amplified (when comparing between kits)
A/TA	Artifact (i.e. stutter) or true allele
IF	Instrument failure
ILS	ILS failure
ART	Artifact
TRI	Tri-allele
OBL / 2 OBL	Obligate allele / 2 obligate alleles
UND	Undetermined
NS <sub>mix</sub>	Data not used for mixture statistic

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DNA Abbreviations and Definitions (continued)	
ND	Not deduced
CR	Contributor ratio
SP	Shared peak (used in Y-STR deduction)
E	Elimination allele (used in Y-STR deduction)
P	Deduced probative allele (used in Y-STR deduction)
( )	Minor component allele
[ ]	Major/Minor components not separated

***\*Note: Notations such as strikes or comments that are made electronically within the GeneMapper ID-X software prior to the date in the footer of each page are attributable to the logged-in analyst, as noted on the bottom of the printed electropherogram; those notations do not require any additional documentation. Notations added later require date and initials of the analyst, either individually or collectively (e.g. Minor allele notation added 6-26-20 cmd)***

In addition to the previous, these abbreviations are commonly used in bench notes generated during database DNA analysis.

DNA Abbreviations and Definitions	
D5Mut	D5 Mutation
HT	Allele below homozygote threshold, w/o a detected sister

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## **Section 2 Biological Screening**

### **2.1 Hair and Fiber Evidence**

Depending on individual case circumstances, it may be appropriate to collect/evaluate trace evidence. Collection and examination of trace evidence need not occur in cases where the trace is not likely to have probative value. 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.

The collection and any subsequent examination of trace evidence is documented in the laboratory bench notes.

The laboratory does not currently provide microscopic comparisons for hair and fiber evidence submitted or recovered in casework. If required, trace evidence may be sent to another laboratory for such analysis.

#### **2.1.1 Screening Hairs**

Screening of hairs is no longer performed. Possible hairs recovered in casework will be examined visually. Observations such as length and color should be included in bench note documentation.

### **2.2 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 digital imaging.
- Moisten a sterile cotton-tipped swab using sterile water.
- Swab the area(s) of interest (those likely to have the most contact with bare skin).

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- If the sample is not proceeding immediately to DNA Screening and/or DNA analysis, the isolated sample(s) are packaged and retained as a separate item of evidence.
- If the sample is proceeding immediately to DNA Screening and/or DNA analysis, any remaining extract is packaged and retained as a separate item of evidence after DNA analysis is complete.

### **2.3 Blood Stain Analysis**

Blood may be present in the form of pools, spatters, or stains. The detection and characterization of blood is achieved by visual examination and subsequent 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 several methods, including visual and stereoscopic examination, the use of alternate light sources and chemical presumptive testing.

#### **2.3.1 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. Bloodstains range in color from a reddish-brown to black.

Blood deposited on dark colored items may be difficult to locate visually. The use of a stereomicroscope (6.3 – 40 x) may assist in the location of possible bloodstains. Bloodstains on clothing and other porous substrates 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 stereoscopically.

Bloodstains deposited on non-porous substrates will dry on the surface as a film or a crust.

#### **2.3.2 Chemical Examination**

Suspected blood stains can be tested for peroxidase-like activity with Phenolphthalein reagent and hydrogen peroxide. Stains can be sampled with a dry or moistened swab or piece of filter paper, or by teasing a few stained fibers from the substrate.

Stains testing positive to this presumptive test for blood may be further characterized as to their origin (human or higher primate) using Human Hemoglobin analysis by immunoassay.

##### **2.3.2.1 Detection of Heme with 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 enough sample to prevent successful DNA typing, then the presumptive test should not be performed. The limited nature of the sample will be documented in the analyst's bench notes.

Color catalytic tests are very sensitive, but not specific. The positive color test alone should not be interpreted as positive proof of blood. The major sources of "false positive" reactions are chemical oxidants and vegetable peroxidases. Color development before the addition of H<sub>2</sub>O<sub>2</sub>



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may be due to the presence of chemical oxidant. Fruit and vegetable peroxidases react like blood but slower and weaker. Additionally, 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.

#### Procedure

- **Positive & Negative Controls:** A human blood standard control is swabbed and tested each day that the presumptive test reagents are used. A swab moistened with the same lot of sterile water used for the questioned samples is also tested to function as a reagent control.
- **Questioned samples:** Moisten a sterile cotton-tipped swab with sterile water. Rub the swab over the suspected bloodstain. Stains may also be sampled with a dry swab or a folded piece of filter paper, or by teasing a few stained fibers from the substrate.
- Add 1-2 drops of phenolphthalein working solution to the swab. Wait a few seconds and observe any color changes.
- Add 1-2 drops of a 3% hydrogen peroxide solution.
- Record all lot numbers, expiration dates and control results in the central log.

#### Interpretation of Results

The positive control must yield a positive result (i.e. rapid pink color change) and the negative control give no color change, for the test results on evidentiary samples to be valid.

**Positive:** The appearance of a rapidly developing pink color after the addition of 3% hydrogen peroxide ( $H_2O_2$ ) is a presumptive positive test for the presence of blood. A pink color forming after ten seconds should not be considered as a positive result, as auto-oxidation can occur in air and light.

**Negative:** No color change immediately (within a few seconds) after addition of 3% hydrogen peroxide ( $H_2O_2$ ). A negative result is indicative of the absence of detectable quantities of heme or its derivatives.

**Inconclusive:** In some instances, a particular substrate makes it very difficult or impossible to see a color change from clear to pink. Typically, this occurs when the red or pink color of the substrate (e.g. red fabric dyes or some food products) is transferred to the testing swab. However, any time that the color change or lack thereof is not readily apparent may be appropriately deemed inconclusive.

#### **2.3.2.2 Detection of Human Hemoglobin (hHb) using OneStep ABACard HemaTrace**

This test is a simple, rapid and a sensitive confirmatory test for human (and other higher primates) blood.

#### Procedure

- Place a portion of the suspected bloodstain or swab into an entire labeled tube of the extraction buffer provided.
- Allow the sample(s) to extract for 1 – 5 minutes.
- If refrigerated, allow the sample(s) to warm to room temperature.

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- Remove the device/card and dropper from the sealed pouch and label the HemaTrace card with CIDI.
- Add 4-5 drops of the extract, using the dropper provided, into the sample region (S) on the card.
- Allow the sample to stand for 10 minutes. Positive results can be seen as early as 2 minutes depending on the hHb concentration.
- Document the lot number of the ABACard and expiration date and the test results in either the bench notes or the central log.

#### Interpretation of Results

The control line in the control area (C) is an internal procedural control. A distinct pinkish line will always appear if the test has been performed correctly and the reagents are working properly. 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) is a positive result, indicating that the human hemoglobin concentration is at least 0.05µg/ml. Another analyst should confirm weak results. This verification will be documented in the bench notes.

**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 0.05µg/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. If suspected, based on visual inspection of the stain, the sample should be retested using a 1:100 or 1:1,000-fold dilution of the 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.

## **2.4 Semen Stain Analysis**

The detection and identification of semen is achieved by visual examination, presumptive chemical testing for the presence of acid phosphatase and by testing for human seminal protein p30.

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

### **2.4.1 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 fluid frequently has a stiffening effect on fabric, forming a crusty stain.

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#### **2.4.1.1 Alternate Light Source Examination using the Omniprint™ 1000B**

*Reference: Omniprint™ 1000B-110 Operating Instructions*

Semen stains frequently fluoresce when examined with alternative wavelengths of light. 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.

##### 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 or refractive light hitting the eye. Choose goggles based on 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 or exam room. Avoid looking at reflections in shiny and/or spherical objects such as door knobs, 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. The lamp may have failed. 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 Omnichrome for further instructions.
- Operation in high ambient temperatures – the Omniprint™ 1000B is equipped with over temperature protection. If the instrument is being operated at ambient temperatures

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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.

- 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 odontologists and pathologists prefer this setting for bite mark and bruising detection and photographic documentation.
- Prior to the examination of casework evidence, a known semen stain control is examined with the wavelength set at 450nm. This ensures that the system is functioning properly. The stain must exhibit the expected fluorescence for the unit to be used in casework. This check is performed each day that the unit is in use and is documented in the central log or in the bench notes if there is no central log, as for a Biological Screening case.

#### 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 a 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).

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#### **2.4.2 Chemical Examination**

Semen stains can be located by detecting the presence of acid phosphatase activity. Visible stains can be lightly swabbed with a water-moistened cotton swab and tested by Fast Blue B.

##### **2.4.2.1 Detection of Acid Phosphatase using Fast Blue B**

This is a presumptive test for the detection of seminal fluid. Samples with positive results are further analyzed by performing a PSA test. Negative results typically require no further analysis.

##### Procedure

- Positive & Negative Controls: A human semen standard control and sterile water blank must be tested each day that the reagents are used in casework.
- Moisten a sterile swab with a minimal amount of sterile deionized water. Rub the questioned stain with moistened swab.
- Add 1-2 drops of Solution #1 ( $\alpha$ -Naphthyl Phosphate).
- Add 1-2 drops of Solution #2 (Fast Blue B).
- The lot numbers, expiration dates of the reagents and the results of the test are documented in the central log.

Note: this test may also be used to 'map' the location of semen stains by pressing a large section of moistened filter paper on the test item. After the paper is removed from the item, add the reagents to the paper.

##### Interpretation of Results

The development of a purple-ish color within one minute is a preliminary indication of the presence of acid phosphatase, a component of semen. The positive control should portray a purple-ish color and the negative control should not exhibit a color change. The controls must function as expected for the test results on questioned stains to be valid.

##### Additional Notes

- The FBB procedure is not specific for semen.
- Possible sources of acid phosphatase other than semen, i.e. vaginal fluids, or feces, generally produce slow, weak reactions.

##### **2.4.2.2 Detection of PSA by the Abacus ABACard® (with option to make microscopic slide)**

This is a rapid and sensitive detection method for PSA (P-30 or Prostate-specific antigen), a component of seminal fluid.

This test is normally performed on stains which test FBB positive and on body swabs collected within 48 hours where semen may be present.

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### Procedure

- A. Place the sample in a 1.5 mL QIAcube microcentrifuge tube.
- B. Add enough sterile water to the sample to just cover the material (300-500 µL works well for one full swab tip), 150 µL at a minimum.
- C. Incubate at 37° C for at least 30 minutes but no more than two hours.
- D. Centrifuge briefly to remove liquid from inside cap. Move substrate to a spin basket and spin for 5 minutes at ~14500 rpm.
  - Remove the device and dropper from the sealed pouch.
  - Using the dropper provided, add enough extract to wet membrane (usually 4-8 drops) into the sample region (S) on the card.
  - Allow the sample to stand for 10 minutes. Positive results can be seen as early as 1 minute depending on the P30 concentration.
  - The lot number and expiration date of the PSA cards and the results of the test are documented in the analyst's bench notes.

### E. Interpretation of Results

Positive: The formation of two pink lines, one in the test area (T) and in the control area (C) is a positive result, indicating the concentration of P30 is at least 4ng/ml. Another analyst should confirm weak results. This verification will be documented in the bench notes.

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 4ng/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. If this is suspected, based on preliminary acid phosphatase test results, the sample should be retested using a 1:10 to a 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 reexamine the test procedure carefully.

### F. After the PSA test has been completed:

- If the PSA test is positive, return the substrate to the tube to proceed to differential extraction. Store at approximately 4 °C until ready to start extraction.
- If the PSA test for a consumed sample is negative and not proceeding to further analysis, the substrate must be air-dried and repackaged.
- If the PSA test for a non-consumed sample is negative and not proceeding to further analysis, the substrate may be discarded.

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G. Slides are not routinely made, but can be made at this stage upon case-specific request:

Re-suspend cell pellet by vortexing, then spin briefly to remove liquid from cap. Remove 3  $\mu$ L to a microscope slide and proceed to stain.

#### Staining of Slides

This is a differential staining process to aide in the conclusive identification of spermatozoa.

- Heat fix cells to a microscope slide by placing the slide in a 37°C oven for 15 minutes.
- Cover the slide/debris with Nuclear Fast Red stain and let stand for at least 10 minutes.
- Gently wash away the Nuclear Fast Red with deionized water.
- Immediately cover the slide/debris with Picroindigocarmine stain (PICS) and let stand for 15-30 seconds.
- Gently wash the slide with reagent grade ethanol. Allow to air dry.
- Add Permount and a cover slip.
- Examine the slide microscopically (200-400x) and record the results based on the interpretations given below.

#### 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 may 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 no spermatozoa are observed on a slide, a second analyst should examine the slide for a microscopic verification. If the same conclusion is reached on the same slide, the verification by the second analyst is documented.



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### **Section 3     *Prioritizing analysis, sampling, and extraction***

Protocols for DNA extraction are found in the Appendices of the manual. Special triage guidelines specific to cases with sexual assault kits (SAKs) are described in the next section.

Analysts rely upon available information to make assessments about which items are most appropriate for analysis on a case-by-case basis. The extraction process for a single sample generally accommodates up to two full swabs worth of material (such as two full swabs, four half-swabs, and so on) per tube.

Typically, swabs within a single packet are sampled equally. Documentation on the packaging must be clear if swabs are not sampled equally.

- If all swabs within a sealed envelope are equally sampled, it is sufficient to write identifying information on the sealed envelope without tagging the swabs as well.
- If a package contains 2 separate swab packets, each with one swab, and only one packet is opened for sampling, then the packet itself is labeled to differentiate.
- For any instance where swabs are sampled unequally (e.g. a portion of only one of two swabs in the same packet), the sampled swab should be tagged with a label containing the case number and item number.

Elution volumes of 40 µL are routinely used for all questioned extracts, as sample size permits.

Bench notes must document the amount of sample used to create the extract.

Any questioned extract remaining after analysis is DNA Stabled and retained, either packaged in a moisture-resistant envelope inside the original package (such as a sex assault kit), or as its own created item if the original piece of evidence is returned (such as a soda can).

When a sample is used in its entirety to create an extract:

- Both the bench notes and the sample must clearly indicate that the sample has been cut in its entirety.
- For non-suspect cases, the entire extract may be consumed without written permission from Department of Law.
- For cases with a named suspect, half the extract must be retained unless written permission from Department of Law for consumption of the sample has been obtained and documented in LIMS.

DNA extracts can be stored for several weeks at 2°C to 8°C. Extracts should be dried down with DNASTable LD for long-term storage. Substrates (in their spin baskets) and reference sample extracts should be retained at 2°C to 8°C until completion of technical review. Substrates and reference sample extracts are routinely discarded after successful completion of technical review.



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### 3.1 Overview of QA samples in a casework batch

An extraction set is a set of samples taken through **the same extraction protocol** (e.g. sperm/substrate, epithelial and/or direct) **at the same time by the same analyst**. An extraction set may include more than one QIAcube or EZ1 run, if they are run concurrently. A casework batch usually involves multiple extraction sets. Each extraction set requires its own controls, described in the table below

<b>Protocol</b>	<b>Type</b>	<b>Consists of:</b>	<b>Assessed by:</b>	<b>Retain/Discard</b>
Questioned sample extraction	Internal Control Specimen (ICS)	Prepared sample previously typed (staff buccal or reference material)	Quant for all ICSs, amplify and verify type for at least one ICS per casework batch	Discard after technical review
	Reagent blank	Two blanks, each created under the most stringent conditions of any sample in the set	Blank with higher quant: DNA Stable and amplify by STR. Second blank: if batch includes sex assault casework, DNA Stable and amp by Y-STR (If no sex assault casework, DNA Stable and retain the second blank)	Retain any questioned blanks not consumed
Known sample extraction	ICS	Casework reference previously typed or staff buccal	Quant for all ICSs, amplify and verify type for at least one ICS per casework batch	Discard after technical review
	Reagent blank	One blank, created under the most stringent conditions of any sample in the set	Amplify 15 µL STR and 17.5 µL by Y-STR if any knowns were run by Y-STR	Discard after technical review (may retain if future Y-STR analysis is likely or if any K's retained)

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### 3.1.1 Reagent blanks (QAS 9.5.1.1)

- Duplicate reagent blanks are created for each extraction set of questioned samples taken through the same extraction protocol (e.g. sperm/substrate, epithelial and/or direct) on the same day by the same analyst at the same time.
- Exception: proficiency tests which are worked alone and not part of a batch of casework, only require one questioned reagent blank. For proficiency tests run as standalone batches, it is not necessary to DNA Stable and consume the reagent blanks; and the same reagent blank may therefore be used for one full-volume amplification using STR analysis and one full-volume amplification using Y-STR analysis.
- Retained reagent blanks are typically named to include extraction type, batch date, analyst, and replicate 1 or 2 (RBS 14-1025CD-1 and RBS 14-1025CD-2). Different naming conventions may be used in central log documentation if the name effectively and clearly distinguishes among the reagent blanks in the batch.
- Both reagent blanks are quantified and DNA Stable'd. Exception: proficiency tests which are worked alone and not part of a batch of casework, only require DNA Stable if a corresponding proficiency test extract is also DNA Stable'd.
- Typically, it is only necessary to create a single reagent blank for reference samples. Because reference samples are rarely consumed and reference extracts are routinely discarded, it is not necessary to create two reagent blanks for reference samples.
- Reagent blanks must be made to the most stringent conditions of any sample in the extraction set. If you are in doubt of how to set up a reagent blank, please confer with the Technical Manager before proceeding.
- Documentation for the reagent blank is in the central log

### 3.1.2 Internal Control Specimen

- Each extraction set must contain an ICS. An ICS may have more than one fraction, such as a sperm/epithelial fraction for a differential extraction.
- Note: Hair extractions should be considered as their own extraction set, with their own hair ICS and reagent blanks.
- All ICSs are extracted and quantified.
- At least one ICS per casework batch is amplified and verified. Additional ICSs may need to be amplified and verified for quality assurance purposes or troubleshooting. See [ICS interpretation](#) section for more details.
- This control is used to check for the efficacy of the extraction process and to reveal any large-scale issues with mislabeling / sample switching. As such, it should not be placed in the exact middle of an extraction set.
- Documentation of each ICS, including its source, is included on the Standards & Controls worksheet in the Central Log.

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### **3.2 Types of evidence – sampling and extraction**

Extraction protocols are listed in the appendices.

*Reference: EZ1 DNA Investigator Handbook*

#### **3.2.1 Triage Practices for Sexual Assault cases with Sexual Assault Kit (SAK) evidence**

*A condensed summary of these guidelines can be found in [Appendix B Sexual Assault Kit Routine Guidelines for Initial Processing](#)*

For the purposes of these triage guidelines, any collection of questioned swabs from the body of a victim or suspect, collected in association with an alleged sexual assault, is treated as a SAK, regardless of whether the packaging includes the specific phrase “Sexual Assault Kit”.

The goal in these cases is to deliver prompt results on a limited number of samples to assist each investigation at an early stage. In cases where the SAK(s) are likely to provide the most probative evidence, the relevant swabs in the SAK(s) will be the only evidence examined in the initial testing process. Additional testing of more questioned samples from the SAKs or additional items (such as clothing or bedding) will only be performed after consultation with Department of Law and/or the submitting officer. In high-priority cases, that consultation may happen before any testing begins, and therefore may be considered with more samples worked in the first round of testing.

Because the length of time between alleged events and kit collection can have a significant impact on triage decision, cases with conflicting information about timelines may benefit from outreach to law enforcement for more information.

For SAKs collected within 48 hours of events, PSA testing will be used on swabs where semen is suspected. Swabs with PSA positive results will proceed to differential extraction; and swabs with negative PSA results will proceed to a direct extraction with DTT. Swabs where semen is not suspected will proceed to a direct extraction.

For SAKs collected more than 48 hours after events, a Direct to DNA approach will be used: selected swabs will proceed straight to DNA extraction without biological screening. Samples likely to contain spermatozoa proceed to a direct extraction with DTT, while samples collected for possible saliva or contact (such as breast swabs and finger swabs) proceed to direct EZ1 extraction protocol.

Case-specific scenarios and forensic history information may impact the decision of which samples to process, as well as whether to use differential or direct extraction. Examples: specific allegations of digital penetration only, forensic exam showing rectal trauma, victim is deceased, pre-pubescent victim genitalia swabs (using direct extraction with DTT instead of differential extraction), etc. These decisions may be made at the discretion of the analyst, but the reasons for such exceptions must be described in the bench notes.

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Evidence guidelines for collection of SAKs are designed to collect as much potentially viable biological evidence as possible at the earliest possible opportunity. Sometimes the processing timelines described below for routine casework are narrower than the collection timeframes. At the discretion of the analyst, samples may be processed outside the recommended processing timeframes; but the reasons for the exceptions must be described in the bench notes.

Each sample typically includes up to two swabs worth of material. For many samples, this will consume all the swab material. All questioned extract remaining after analysis is retained, regardless of whether the swabs were consumed.

Samples typically extracted from SAKs include:

- a. Vaginal/cervical swabs with possible semen evidence
  - i. [Differential extraction](#) if within 48 hours and PSA positive; [direct with DTT](#) if PSA negative or over 48 hours
  - ii. Typically combined with all swabs sampled - these are only processed separately when the victim specifies recent prior consensual sex or when the victim specifies multiple vaginal assailants.
  - iii. In differential extractions, sperm and substrate fractions are worked separately.
  - iv. These are processed when collected up to seven days after the alleged event. Factors affecting length of time for viable testing include victim menstruation.
- b. "External" external female genitalia swabs (includes outer labia majora, mons, etc.) where semen OR semen and saliva suspected, miscellaneous semen swabs from non-genital areas
  - i. These are routinely processed when collected up to three days after the alleged event but may be worked when collected up to four days after, depending on case specific circumstances.
  - ii. [Differential extraction](#) if within 48 hours and PSA positive, otherwise [direct with DTT](#)
  - iii. All "external" external genitalia swabs are combined for a single extraction with all of each swab sampled. Exceptions are made for cases where saliva presumptive testing may be required based on case scenario; if so, only half the swab material is used.
  - iv. Sperm and substrate are worked as a combined fraction if differential
- c. "Internal" external female genitalia swabs (includes inner labia majora/labia minora/introitus/etc.) where semen suspected
  - i. These are routinely processed when collected up to three days after the alleged event but may be worked when collected up to four days after, depending on case specific circumstances.
  - ii. [Differential extraction](#) if within 48 hours and PSA positive, otherwise [direct with DTT](#)

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- iii. All “internal” external genitalia swabs are combined for a single extraction with all of each swab sampled.
  - iv. Sperm and substrate are worked as a combined fraction if differential
- d. Swabs with suspected saliva (includes breast swabs, bite marks, “external” external genitalia if specified in scenario)
  - i. Only processed if reason is indicated by forensic history
  - ii. Half of each of these swabs is routinely retained.
  - iii. Swabs originating from the same general area are combined for a single extraction
  - iv. These are only routinely processed if collected within two days of the alleged event but may be worked when collected up to four days after. Timeframes may be affected by circumstances described in forensic history, such as hygiene / showering, victim was deceased, etc.
  - v. Processed by direct extraction
- e. Miscellaneous contact swabs, vaginal swabs where digital penetration only is specified
  - i. Only processed if reason is indicated by forensic history
  - ii. Swabs originating from the same general area are combined for a single extraction
  - iii. All of swabs processed
  - iv. These are only routinely processed if collected within two days of the alleged event but may be worked when collected up to four days after. Timeframes may be affected by circumstances described in forensic history, such as hygiene / showering, victim was deceased, etc.
  - v. Proceed with direct extraction
- f. Finger/hand swabs, fingernail clippings/scrapings
  - i. Only processed if reason is indicated by forensic history
  - ii. Swabs from left and right hand are processed separately. Fingernail scrapings are usually not combined with finger/hand swabs.
  - iii. All of swabs are sampled.
  - iv. These are only processed if collected within two days of the alleged event but may be worked when collected up to four days after. Timeframes may be affected by circumstances described in forensic history, such as hygiene / showering, age of victim, etc.
  - v. Proceed with direct extraction
- g. External male genitalia swabs
  - i. Penile swabs are routinely processed when collected up to two days after the alleged event but may be worked when collected up to four days after, depending on case specific circumstances. Scrotum swabs are processed only if indicated by case scenario (such as condom use).
  - ii. Amount of swab samples will depend on allegations. If oral contact is alleged, half of the swab material should be sampled to allow for possible

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presumptive saliva testing, if required. Otherwise, all the swab material may be sampled.

- iii. [Differential extraction](#) or [direct extraction](#) performed, dependent on case scenario.
- iv. Sperm and substrate are worked as a combined fraction if differential
- h. Perineum swabs
  - i. Perineum swabs collected separately from other external genitalia areas are not routinely worked unless a rectal assault is alleged – in that case, they should be combined with anal swabs if present.
  - ii. These are processed when collected up to three days after the alleged event but may be worked when collected up to four days after, depending on case specific circumstances.
  - iii. Half of each of these swabs is routinely retained if case scenario suggests possible saliva and semen; otherwise all of swabs are sampled.
  - iv. [Differential extraction](#) if within 48 hours and PSA positive, otherwise [direct with DTT](#)
- i. Special Case: On occasion, permission is given by Department of Law to consume a sample for Y-STR analysis, where only half the sample has been extracted. A [protocol specific to this situation](#) can be found in the appendices.
- j. Reference samples
  - i. Sampling and processing of reference samples is described [later in this section](#).

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### 3.2.2 Questioned blood stains

- Typical bloodstains on fabric are sampled by cutting approximately 0.5sq cm of the stain using a sterile, disposable scalpel. The size of the cutting may vary depending on the size and condition of the stain. The entire stain may be sampled for very small or potentially degraded stains.
- Known bloodstains on thick fabric or filter paper may be cut or sampled using a 3mm 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.
- Typical extraction is by [questioned direct protocol](#)

### 3.2.3 Contact or saliva swabs/isolated samples

- Typically, swabs without visible staining will be sampled in their entirety, maximizing the chance of obtaining an interpretable DNA profile. Exceptions include:
  - Sexual assault kit swabs, where half the swabs are left un-sampled if saliva is expected.
  - If other case-specific circumstances suggest that saliva testing may be required in the future, then half the sample should be left untested.
  - If many swabs were collected from a single area, they may each be sampled in part due to size constraints on the amount of sample that fits in the extraction tube(s). Such swabs are cut lengthwise.
  - Cigarette butts are rarely swabbed and rarely sampled in their entirety. Instead, a small cutting (~1 cm<sup>2</sup>) of paper is removed from the filter end. Cigarette butts are digitally imaged prior to sampling.
- When the sampled swab material is too large for a single tube, it should be split into [multiple tubes](#) and the extracts ultimately recombined. Ensure that all reagent blanks from that extraction set are treated in the same way.
- Typical extraction is by [questioned direct protocol](#)

### 3.2.4 Fingernail Swabs/Scrapings

- Samples are received either as swabs or debris 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 with at least half of the extract being retained for future use.
- When sampling debris in a paper bindle, a moistened swab is used to swab the scraper, visible debris, and the inside of the paper bindle. Typically, one swab is used per hand. The entire swab is then cut into the sample tube. When the bindle contains a large amount of debris, a representative amount may be sampled.
- Typical extraction is by [questioned direct protocol](#)



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### 3.2.5 Fingernails or Fingernail Clippings

- Fingernails or fingernail 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 480µL of G2 buffer in a micro-centrifuge tube with gentle agitation and heat. 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 micro-centrifuge tube and digested by adding 20µL Proteinase K and continuing through the direct extraction process.
- Any clippings that remain after digestion are dried and re-packaged with the evidence.
- If the goal of extraction is to obtain a profile from any biological material on the nail that is foreign to the owner, extraction is by [questioned direct protocol](#)
- If the goal of the extraction is to obtain a profile of the person whose nail it was, use the [direct with DTT protocol](#).

### 3.2.6 Hairs

Possible hairs are not routinely extracted in the first round of testing.

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 substitute. Use a pipette to wash the mounting medium away with xylene substitute. 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, de-ionized water in a clean container.

**Note:** Hair extractions should be considered as their own extraction set, with their own hair ICS and reagent blanks. A reference (known) hair sample is processed in the batch alongside the questioned hair sample(s) as an internal control. Processing of the control hair should be performed similarly to the processing of the casework hair(s).

**Note:** Possible hairs are typically extracted individually. Exceptions may be made based on the nature of the evidence – for example, a clump of hairs attached with skin tissue may be worked in combination. However, these exceptions must be clearly documented in the bench notes.

- 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 480µL of G2 buffer in a micro-centrifuge tube with gentle agitation and heat. 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 micro-centrifuge tube and may be extracted by adding 20µL Proteinase K and continuing with the questioned direct extraction protocol.



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- Cut approximately 1cm of each end of the hair and combine for digestion. Using clean forceps, place the hair sample into a sterile 1.5mL tube.
- To minimize the number of transfers, hairs may be cut directly into and digested in a screw-cap tube. Alternatively, if a 1.5 mL Eppendorf tube is used, the cap can be cut off to be put directly onto the EZ1-XL.
- Hair extraction is performed using the [direct with DTT protocol](#).
- The remaining portion of the hair is re-packaged with the evidence.

### 3.2.7 Swabs other than body swabs for differential extraction (such as surface stains)

- Sample size is all of each swab unless there is reason to expect the possibility of saliva testing (unlikely for samples that are not body swabs). For isolated stains, sample size will depend on the size of the cutting.
- Substrates must be extracted, either by combining the substrate with the sperm pellet or extracting it separately.
- A flowchart titled "[Biological screening of stains for semen incorporating the QIAcube decision tree](#)" in the appendices summarizes the analysis decisions for semen stains.
- For samples which are likely to contain moderate or small amounts of epithelial DNA, such as less-intimate clothing stains, the substrate may be added back to the sperm pellet when it comes off the QIAcube.
- Extraction is performed using the [differential extraction protocol](#).

### 3.2.8 Isolated stains with possible semen

- For stains on items of clothing, bedding, etc., analysts follow the [flowchart](#) in the appendix of the FBCP to determine which samples are appropriate for extraction using the [differential extraction protocol](#).
- Adult underwear will be processed only if a kit was not collected within 48 hours and underwear were known to be those worn immediately post-event. For cases involving minors, it may be appropriate to analyze underwear even if less than 48 hours and/or unknown when worn. Other exceptions may be appropriate based on case scenario but must be documented in the bench notes.
- If an item has been consumed for analysis, the substrate must be extracted, either combined with the sperm pellet or worked separately:
  - Items likely to have very high amounts of epithelial cells, such as underwear cuttings from crotch area, should be processed without combining the sperm pellet with the substrate.
  - Items likely to have relatively lower amounts of epithelial DNA, such as cuttings from clothing other than underwear, may have the substrate added to the sperm pellet for a single extract.

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### 3.2.9 Condoms

- Condoms may contain evidence that can be isolated and compared to the DNA profiles of suspects or victims in sexual assault cases. Condoms should be sampled as follows:
  - Document the condition of the condom. Imaging may be used if necessary, to document unusual characteristics, such as extensive dirt, debris, tearing, etc.
  - Label one pair of sterile cotton swabs “outside”. Moisten the swabs using sterile deionized water. Swab the “outside” surface of the condom, as received.
  - Repeat for the “inside” surface of the condom.
  - If the samples are not proceeding immediately to DNA analysis, they are packaged and retained as a separate item of evidence.

**Note:** Acid phosphatase and PSA testing should not be conducted when screening condoms. The laboratory has previously encountered condoms which gave incorrect AP test results.

Condom swabs are routinely sampled in their entirety, with substrates combined with sperm pellets. Extraction is performed using the [differential extraction protocol](#).

### 3.2.10 Tampons and sanitary pads

- For screening a tampon:
  - image the item, then either
    - Swab and test for FBB – if positive, test small cutting(s) for PSA **or**
    - Go directly to PSA test
    - If FBB test is negative, no further analysis is required.
- For screening a sanitary pad:
  - Image the pad
  - ALS the pad. If there are distinct areas of fluorescence, swab and FBB test
    - If FBB positive, cut and test for PSA. Positive area(s) should be circled and noted on evidence as well as described in bench notes
    - If FBB negative, no further testing
  - Note: some brands of sanitary pad show background fluorescence. Therefore, even if the ALS is negative, the item should still be swabbed and FBB tested or cut and PSA tested. In such cases, it is useful to focus on areas that are bloody since blood can mask fluorescence. Some options for testing an ALS negative pad are:
    - Section the pad (into three or four sections), swab, and test each section for FBB activity. Areas that are positive can then be cut and combined for PSA as one stain (note in bench note documentation)
    - Swab areas with blood and FBB test – if FBB positive, cut for PSA. If FBB negative, no further analysis is required.
    - Cut directly for PSA.

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### 3.2.11 Fetal tissue

Fetal tissue may be received by the laboratory for DNA analysis in criminal paternity cases. The following are general guidelines for processing fetal tissue samples. The sample collection process should be documented and may vary from what is described on a case-by-case basis.

- Samples are generally received frozen in a specimen cup and should be completely thawed at room temperature prior to any processing.
- The thawed sample can be transferred in its entirety to a weigh boat (or similar consumable) for ease of viewing. If fetal parts are clearly visible, it is not necessary to transfer other non-fetal tissue that may be present.
  - If fetal tissue is clearly visible, use a sterile scalpel to cut a small portion (ex. a digit from a hand or foot) and place it in a sterile microcentrifuge tube.
  - If fetal tissue is not clearly visible, the analyst should assess all the tissue material and select 3-5 samples of possible fetal tissue. Each is placed in a separate microcentrifuge tube
- 2-3 washes of the tissue sample(s) should be performed to remove any possible maternal blood
  - Add ~500uL of sterile water to each sample
  - Vortex and centrifuge briefly
  - Remove wash using a pipette and discard as biohazardous material
  - Repeat 1-2 more times, as necessary until the wash is clear
- Proceed with [questioned direct protocol](#)

### 3.2.12 Other tissue samples

Refer to the EZ1 DNA Investigator handbook for instructions on the appropriate sample size based on tissue type. Consult with the Technical Manager before proceeding if you suspect that evidence may not be best analyzed using the above guidelines.

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### 3.2.13 Reference samples

- Typically, known buccal swabs require only 1/3 of one swab or a small portion of two swabs. However, if two or more swabs are available, all of one swab may be used.
- Typically, dried blood cards require ~0.5 cm<sup>2</sup> of stain for extraction.
- Typically, references are extracted using the [known direct extraction protocol](#).
- If one entire swab head is used, extraction may be by the Global Filer Express protocol as described in the Forensic Biology Database Manual, along with a corresponding reagent blank and internal control standard. Note: This method should only be attempted for samples collected within 3-4 months since the direct amplification procedure is less effective on older swabs. (Note: If this protocol is used, the technical reviewer must also be qualified in performing Global Filer Express analysis.
- If the reference sample is particularly old or potentially degraded (some database samples and references collected by the Medical Examiner), it may be more appropriate to use the [questioned direct extraction protocol](#), which is optimized for maximal DNA recovery. If more than one elution volume of used for an extraction set of reference samples, the reagent blank must be run using the smallest elution volume.

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#### **Section 4     DNA Quantification and Quant-Based Decisions**

Questioned samples and known samples not amplified by Global Filer Express are routinely quantified in single reactions to preserve extract. A single quantitation reaction is typically used for each questioned reagent blank but is not required if the extract is amplified. Quantitation is not mandatory for database samples or forensic known buccal samples amplified by Global Filer Express (QAS 9.4.1). As per QAS 9.4, quantitation is required for all questioned samples.

More than one batch of extracts may be included on the same quantitation plate. In such cases, the plate's name should include the initials of each analyst, and each analyst is responsible for documentation of their own samples.

The [procedure for setting up and running a quantification plate](#) using Quantifiler Trio on the 7500 is located in the appendices. As per QAS 9.5.2, a standard curve must be included with each quantification run.

DNA Stable LD may be added to extracts at any point after quantification. Since all questioned extracts are routinely either consumed or retained, it may be helpful to add the DNA Stable LD to all questioned extracts as soon as the samples have been quantified. However, this will affect sample concentrations; and re-calculations of the extract concentrations would be needed prior to amplification set-up. The [procedure](#) for drying down samples using DNA Stable LD is in the appendices.

##### *References:*

- *ABI Prism 7000 Sequence Detection and Applied Biosystems 7500 Real Time PCR System User Bulletin*
- *Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide*
- *Applied Biosystems Quantifiler HP and Trio DNA Quantification Kits User Guide*
  
- **This section covers the following topics:**
- [Criteria to evaluate quantification standards](#)
- [Assess the quality and potential probative value of each casework extract based on its QT results](#)
- [Identify casework extracts where analysis stops based on quantification results](#)
- [Choose appropriate samples for STR and Y-STR amplifications](#)

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#### 4.1 Criteria to evaluate quantification standards (QAS 9.6.1)

##### 4.1.1 Checking the Standard Curve

The large autosomal, small autosomal, and male DNA Standard curves need to be assessed for their  $R^2$  values, slopes, and Y-intercept values. No more than two data points may be removed from a standard curve to achieve passing quality metrics, and it is not acceptable to remove both end points from either end of the standard curve.

$R^2$  is a measure of the closeness of fit between the standard curve regression line and the individual data points of the quantification standard reactions. A value of 1.0 indicates a perfect fit between the regression line and the data points. Passing value for  $R^2$  is  $\geq 0.98$ . It is acceptable to remove up to two data points to achieve a passing  $R^2$  value. If, after removing up to two data points, the  $R^2$  value is  $< 0.98$ , the plate must be re-run.

**Slope** is an indicator of amplification efficiency. A slope value of -3.3 corresponds to true doubling of the amplicon at each cycle. Acceptable slope values fall in the range of -3.0 to -3.6. Slope values outside this range require approval by the DNA Technical Manager.

**Y-intercept** is the theoretical expected quantity for 1 ng/ $\mu$ L of DNA. Y-intercept is used to compare performance among different QT kit lot numbers. If an average Y-intercept value drops by one  $C_T$  value, the standard curve will shift to the left and samples that previously quantified at 2 ng/ $\mu$ L will now quantify at 1 ng/ $\mu$ L. This will cause an underestimation of DNA quantity, which may lead to higher peak heights and possible increased baseline artifacts in analysis of amplified samples. Conversely, if the average Y-intercept value increases by one  $C_T$  value, the standard curve will shift to the right and samples that previously quantified at 1 ng/ $\mu$ L will now quantify at 2 ng/ $\mu$ L. This will cause an overestimation of DNA quantity, which will lead to lower peak heights and possible stochastic effects in the analysis of amplified samples.

Typical ranges for Y-intercept values are 25.8 – 26.3 for the male standard curve, 24.9 – 25.6 for the large autosomal human standard curve, and 26.8 – 27.3 for the small autosomal human standard curve. During QT kit verification, the Y-intercepts for the three curves are noted in the 7500 logbook. Analysts will track subsequent Y-intercept values for all curves run in the 7500 logbook. If a Y-intercept falls farther than  $\pm 1$  from the verified value, the Technical Manager must be notified to determine a course of action.

##### 4.1.2 Checking the Internal PCR Control (IPC)

IPC values are documented in the Experiment Results Report for each reaction. As per the manufacturer, the IPC  $C_T$  value for each reaction is expected to fall in the range of 26 to 30. Values lower than 26 may be observed, but these do not require any notation since there is no expected impact on downstream processing. When a  $C_T$  for an IPC is greater than 30, the sample should be assessed for possible amplification inhibition. Such samples should be noted with the analyst's initials on the quantitation report printout to draw attention to the potential impact on amplification. Note that excessive quantities of DNA can exhibit excessive concentration inhibition.

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#### **4.1.3 Checking the Passive Reference (Mustang Purple)**

The passive reference signal flag (on the QC summary page of the Experimental Results Report, under the flag BADROX) indicates if any samples exhibited a bad passive reference signal. The results of this check are documented on the 1<sup>st</sup> page of the report and included in the central log. The Technical Manager is to be consulted when any samples fail this check.

#### **4.1.4 Checking the No Template Controls (NTC)**

In the Results Table of the Experiment Results Report, confirm that the quantity of DNA for both Trio Human (large and small) as well as Trio Male is negative ( $C_T = \text{undetermined}$ ). A detected quantity of DNA in both NTC wells could indicate contamination of the master mix. If the quantity of either Trio Human or Trio Male is greater than zero in both wells, consult the DNA Technical Manager for a course of action. Low-level results ( $<0.001$ ) in only one NTC well is not consistent with systemic contamination and does not require consultation.

#### **4.1.5 Checking the Initial Template Quantity**

Quantities of DNA, including large autosomal (T-L), small autosomal (T-S) and male DNA (T-Y) are listed for each reaction in the Results Table. The Quantity (Mean) column provides the average of any wells assigned to the same sample identifier in 3.1. When STR analysis will not be performed based on the quantification results (as described below), this shall be reflected in the bench notes for that sample.

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## 4.2 Quantifier Trio calculations for downstream assessment

A [summary](#) of the calculations below is in the appendices. In addition, these quant-based decision trees based on the information below can be found in the appendices:

- [Quant decision tree where male DNA is probative](#)
- [Y-STR Quant decision tree \(Questioned samples\)](#)
- [Consumed sample quant decision tree for non-suspect case extracts where male DNA is probative](#)
- [Quant decision tree for extracts where female DNA is probative](#)
- [Consumed sample quant decision tree for extracts where any profile \(male or female\) would be probative](#)

### 4.2.1 Degradation Index

#### 4.2.1.1 How to calculate Degradation Index (DI)

- Degraded or inhibited DNA samples may benefit from amplification at higher target input DNA amounts. Quantifier Trio results can be used to calculate a degradation index.

Small autosomal target / large autosomal target = degradation index (DI)

- DI may be calculated by hand and written on either the Quantifier Trio report printout or the amplification worksheet.
- Alternatively, the Quantifier Trio results can be exported into an Excel spreadsheet, which includes this calculation. If the exported results are used, a printout of the relevant data, including project name, sample names, T-L, T-S and DI, should be included with the central log documentation. Note: the Excel printout does not replace the Quantifier Trio report printout. As such, it is not necessary to include unaffected samples (such as standard curve and NTC wells) in the Excel printout.
- In addition, the DI may be recorded on the applicable bench note documentation, particularly for DI greater than 2.

#### 4.2.1.2 How Degradation Index impacts amplification decisions

- Throughout this section, quantitation cutoff values are used for stopping analysis and requesting permission to consume. For samples with a DI less than or equal to 1.2, there is no impact on quantification cutoff values.
- For samples with a DI from 1.2 to 2, the cutoff values for stopping analysis or requesting permission to consume will double.
- For samples with a DI greater than 2, the new cutoff value for stopping analysis or requesting permission to consume is the original cutoff multiplied by the DI.
- This applies to both STR and Y-STR analysis.



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#### **4.2.2 Assessing whether an extract is likely to be single source, either entirely or effectively, for the purposes of STR amplification:**

##### **4.2.2.1 How to assess likely single source based on quantification results**

- **NOTE:** either of the below situations relies upon the assumption that a sample is likely to have DNA from only one contributor of the gender in question. Some case scenarios (e.g. multiple possible assailants or the possible presence of a consent partner as well as an assailant) preclude this assumption.
- Single-source (and effectively single-source) female: Intimate samples from a female's evidence collection kit (that is, body swabs or underwear from an evidence kit) with no male DNA detected are likely to be single source. Alternatively, samples with detectable male DNA present in a ratio of greater than 4:1 female: male are likely to be effectively single source / suitable for comparison for the major (non-male) component. As such, they are typically better candidates for Y-STR analysis.
- Single-source male: Samples where the male DNA quantitation value is higher than the total human quantitation value are likely to only yield results suitable for comparison for the male contributor(s).

##### **4.2.2.2 How a single source sample may impact amplification decisions**

- Based on case allegations, it is sometimes possible to assess whether a fraction from a differential extraction is likely to have probative value. For instance, in a case where a female alleges a sexual assault by a male, any fraction which does not include detectable male DNA, as determined by quantification results, will not be probative to the allegation. In other instances, such as an assault with a male victim and an alleged male suspect, gender alone cannot be used to assess whether a fraction may have probative value.
- Intimate sample fractions are not routinely processed when quantification results indicate a gender different from the putative perpetrator's gender. For example, in a case where a female alleges sexual assault by a male, an epithelial fraction where no male DNA was detected would not be amplified. Although these often have robust quantities of DNA present, they are occasionally low in quantity. These samples would only be run as a QA check, and it is not necessary to request permission to consume them. If such an extract has a T-S concentration less than 0.004 ng/μL, the extract need not be amplified. If such an extract has a T-S value between 0.004 and 0.053 ng/μL, a full-volume amplification will be performed without drying down the extract if amplification is needed for QA purposes.

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#### 4.2.3 Ratio of female to male DNA detected in an extract. (minor male)

##### 4.2.3.1 How to calculate a ratio of female to male (minor male)

- When female DNA is in excess, an estimate of the ratio of female to male DNA can be made by subtracting the male quantification value from the small autosomal concentration to obtain the female contribution, then dividing by the male quantification value. This ratio is documented on the relevant page of the Experiment Results Report.
- When male DNA quantity is  $<0.020$  ng/ $\mu$ L, stochastic effects can limit the accuracy of assessment of the true female to male ratio.

##### 4.2.3.2 Determining suitability for future testing for probative male

- These are general guidelines and may not apply to the specific details of a given case. Analysts use case-specific information to make decisions and document their reasons for decisions that differ from the suggested guidelines. ***The guidelines below apply to typical samples from female victim evidence kits in which  $\frac{1}{2}$  of each of the relevant swabs was extracted. When half the original evidence remains untested, as in these examples, extracts may be fully consumed.***
- Extracts with no male DNA detected will be retained with no further testing. (Exceptions would include samples where a female profile may have probative value, such as swabs from a condom.)
- Extracts with male DNA present at ratios greater than 4:1 female: male are not suitable for routine STR analysis but may be suitable for Y-STR analysis. Exceptions might include non-suspect cases or event-specific scenarios; these extracts may proceed to STR testing when ratios are 5:1 female to male or less. Exceptions outside this 5:1 range require documented approval from the DNA Supervisor or Technical Manager.
- Extracts with male DNA present in a ratio of 4:1 female: male or less, but with a small autosomal DNA concentration less than 0.026 ng/ $\mu$ L, are best suited for STR analysis only if permission is granted to consume the entire remaining evidence and combine with the original extract to maximize yield. Note: The T-S cutoff of 0.026 ng/ $\mu$ L is raised in cases where quantitation results show degradation – see Appendices.
- Extracts with likely mixtures: Extracts likely to contain mixtures are suitable for STR DNA analysis when ALL three conditions listed below are met.
  - male DNA is present in a ratio of 4:1 female: male or less
  - small autosomal concentration DNA equal to or greater than 0.026 ng/ $\mu$ L
  - male DNA concentration is equal to or greater than 0.01 ng/ $\mu$ LIf total human DNA or male DNA concentration is below the minimum concentrations, the best course of action is to obtain permission to extract and consume the remaining evidence.
- Consumed evidence minimum concentrations: If permission is granted to combine and consume the remaining evidence, the following cut offs apply for no further analysis:
  - If T-Y  $< 0.0007$ , no further analysis
  - For DI of 1.2 to 2: T-S  $< 0.004$  for likely single source and 0.014 for likely mixtures
  - For DI above 2: T-L  $< 0.002$  for likely single source and 0.007 for likely mixtures

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#### 4.2.4 Ratio of female to male DNA (minor female)

Extracts which have a T-Y value that is greater than the T-S value can be reasonably assumed to have so much male DNA present that any female DNA will not be sufficient for comparisons to probative female reference samples. However, when the T-Y is less than T-S, the situation is not as obvious. Because Quantifiler Trio (QT) uses multi-copy targets, which may vary in number between individuals, the ratio of T-S to T-Y for a single-source DNA extract from a male individual may not be exactly 1:1. Based on validation studies, the T-Y reading can differ by +/- 20% from the T-S reading; but the ratio of T-S to T-Y remains consistent for each male individual, regardless of tissue type (blood, semen, etc.). When a reference sample is analyzed by QT, a correction factor can be calculated for T-Y, which then allows for a better prediction of detectable quantities of female DNA in a questioned sample collected from the male's body.

##### 4.2.4.1 How to calculate an approximate ratio of female to male (minor female)

1. Calculate  $Y_{\text{corr}}$  from male reference sample quant results:

$$Y_{\text{corr}} = T-S / T-Y$$

2. For casework extract:

$$T-Y_{\text{original}} * Y_{\text{corr}} = T-Y_{\text{new}}$$

3. Use the new corrected value for T-Y to calculate a male: female contributor ratio

$$[T-Y_{\text{new}} / (T-S - T-Y_{\text{new}})]$$

Note: a more detailed example of this calculation can be found [here](#).

##### 4.2.4.2 Determining suitability of future testing for probative female

- Samples with a male: female ratio of 4:1 or smaller are likely to have detectable amounts of female DNA and may proceed to amplification.
- Samples with a ratio between 4:1 and 5:1 have only a fair chance of having detectable amounts of female DNA and should be amplified only when no other samples are readily available.
- Samples with a ratio greater than 5:1 are unlikely to yield an interpretable minor component profile. These are only amplified under case-specific circumstances and with prior authorization from the Technical Manager or Biology Supervisor.

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#### **4.3 Identify casework extracts where analysis stops based on quantification results**

##### **4.3.1 Extracts from consumed samples that contain insufficient DNA for analysis**

Based on validation studies, an extract containing a single source of DNA has a limited chance of being suitable for comparison when the input template DNA is less than 0.063 ng. Based on the low chance of success, samples likely to be single-source (such as blood stains, hairs, and some sperm and epithelial fractions) with a T-S value of less than 0.002 ng/ $\mu$ L will either be reported as recommending Y-STR's (if appropriate) or that no further analysis will be performed on the sample. 0.002 ng/ $\mu$ L is the concentration where, if the extract were dried down with DNASTable and reconstituted with 15  $\mu$ L, input template DNA would be ~0.076 ng.

For two source mixtures, sample below 0.25 ng of input template DNA have a limited chance of being suitable for comparison. Based on the low chance of success, samples likely to contain mixtures (such as body swabs and contact DNA samples) with a T-S of less than 0.007 ng/ $\mu$ L will either be reported as suitable for Y-STR's (if appropriate) or that no further analysis will be performed on the sample. 0.007 ng/ $\mu$ L is the concentration where, if the extract were dried down with DNASTable and reconstituted with 15  $\mu$ L, input template DNA would be ~0.266 ng.

In case-specific circumstances, it may be appropriate to proceed with such extracts. Discipline management may choose to overrule these thresholds for rare exceptions.

NOTE: Extracts where T-S, T-L and T-Y are all 0.0003 or lower are considered inconclusive for the presence of human DNA.

##### **4.3.2 Extracts where consuming the previously un-extracted evidence is unlikely to yield sufficient DNA for amplification**

Sometimes the poor quality of a sample indicates that further extraction of the remaining evidence is highly unlikely to yield sufficient amplifiable DNA. This is typically indicated either by the apparent condition of the item of evidence (such as mold or water damage), by an inconclusive quantitation result (T-S of 0.0003 or lower), and/or a high Degradation Index. In such instances, no further analysis will be performed, with the reason documented in the bench notes.

##### **4.3.3 Extracts which may require permission to consume**

In instances where quantitation results indicate that limited amounts of DNA (<0.053 ng/ $\mu$ L for STR, or T-Y < 0.026 ng/ $\mu$ L for Y-STR, or higher if DI>1.2) are present, the best course of action depends in part on whether one or more sources of DNA are likely to be present in the extract. While single-source extracts may yield results suitable for comparison at very low template quantities, the same is not true for mixtures. The combined information from both human and male DNA quantitation results may indicate whether it is reasonable to expect the extract to contain a mixture. Furthermore, some sample types (such as gun swabs and contact swabs) are likely to contain DNA from more than one individual by their nature.

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For STR, a total of 2 ng or more of DNA enables an analyst to amplify the sample once at an optimal target concentration of 1 ng while retaining at least half of the extract. When less than 2 ng of total DNA is present in a sample (i.e. when a 40  $\mu$ L extract has a total human DNA concentration of less than 0.053 ng/ $\mu$ L), it is not possible to optimize analysis while retaining half the extract.

For Y-STR, a total of 1 ng or more of DNA enables an analyst to amplify the sample once at an optimal target concentration of 0.5 ng while retaining at least half of the extract. When less than 1 ng of total DNA is present in a sample (i.e. when a 40  $\mu$ L extract has a total male DNA concentration of less than 0.026 ng/ $\mu$ L), it is not possible to optimize analysis while retaining half the extract.

In a suspect case, if an extract exceeds the minimal amount of necessary DNA but does not have sufficient DNA present for replicate amplifications at optimal target value, then the analyst should request written permission from the Department of Law to consume the evidence in its entirety. Non-suspect case extracts may be consumed without written permission from Department of Law.

#### **4.3.4 Extracts in suspect cases where half the evidence was used to create the extract**

The same general principles apply in this situation. If the existing extract does not contain sufficient DNA to perform amplification (either STR or Y-STR) at optimal template target but additional original material remains, the analyst should request permission to consume before extracting the remaining evidence to combine with the original extract. This situation applies when the resulting final extract may not be consumed in its entirety, but it is likely that less than half of the total DNA would remain after amplification.

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#### **4.3.5 Extracts from the non-probative fraction of a differential extraction of an intimate sample**

Differential extractions from body swabs are intended to maximize separation of owner and non-owner DNA. Based on the quantification results, only the more/most probative fraction from body swabs is routinely amplified. Underwear contained inside an Evidence Collection Kit also fall into this category of intimate samples.

Case scenarios must be considered to determine whether gender can be used to assess the probative value of a given fraction. In cases where the suspect and victim are the same gender, the gender results from quantification cannot be used as justification for not amplifying an extract. Other considerations, such as amount of DNA and degradation index, may be used to assess which extract(s) should be amplified.

If examination of the amplified fraction(s) indicates possible sample switch or contamination issues, the other fractions may then be amplified to further assess the concern.

If the sperm and substrate fractions are each extracted and quantified separately for a given sample, it is only necessary to amplify one of them. The decision of which extract to amplify will be based on the Quantifiler Trio results. Note the reason why on the DNA worksheet if an extract is not amplified.

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#### 4.4 Choosing appropriate samples for STR and Y-STR amplifications

The number of samples chosen for amplification, as well as which samples are chosen will depend on a variety of factors. In this section, the selection process is described by general offense types, including sexual assault, other major crimes, and property crimes. This section provides general guidelines which may not be appropriate for every case.

##### 4.4.1 ICS Amplification

Each casework batch must include at least one amplified ICS.

- If a batch includes only STRs or both STRs and Y-STRs, then only an STR amplification of an ICS needs to be included.
- If the batch only includes Y-STR amplification, then a male ICS must be amplified by Y-STR.

Any ICS must be amplified if:

- T-S is less than 0.053 ng/ $\mu$ L,
- ICS is from a known female source with a T-Y reading above 0.02 (Exception: epithelial fractions from differential extractions may reasonably be expected to include some male carryover and do not require amplification based on a detectable T-Y reading.)
- ICS sperm (or sperm/substrate) fraction with no detected male DNA

[Criteria for assessing ICSs are included in the Interpretation and report writing section.](#)

##### 4.4.2 Sample selection in sexual assault cases

###### 4.4.2.1 How many samples to amplify in sex assault cases

Routine guidelines for number of amplifications per victim in a case:

- For cases where a female victim remembers events and only one (male) suspect is alleged, only one sample is amplified.
- Situations where it may be appropriate to amplify more than one sample:
  - i. Victim is male
  - ii. Multiple suspects
  - iii. Recent prior consensual sex
  - iv. Victim has no recollection of events
  - v. Multiple allegations of penetration (e.g. both oral and vaginal penetration alleged)
  - vi. Pre-pubescent or vulnerable victim who may not be able to accurately describe events
  - vii. Underwear collected outside a sexual assault kit, where it is necessary to demonstrate both “wearer” and possible alleged assailant



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#### 4.4.2.2 How to prioritize samples in sex assault cases

Choice of which sample(s) to amplify is based on results of DNA quantification. Priorities are:

- a. Likelihood of obtaining an STR profile from a victim SAK suitable for comparison to a suspect / entry in CODIS AND the item in question is consistent with penetration.
- b. Likelihood of obtaining an STR profile from a suspect SAK suitable for comparison to a victim AND the item in question is consistent with penetration.
- c. Likelihood of obtaining an STR profile from a victim SAK suitable for comparison to a suspect / entry in CODIS AND the item in question is NOT consistent with penetration.
- d. Likelihood of obtaining an STR profile from a suspect SAK suitable for comparison to a victim AND the item in question is NOT consistent with penetration.
- e. Likelihood of obtaining a Y-STR profile from a victim SAK suitable for comparison to a suspect AND the item in question is consistent with penetration
- f. Likelihood of obtaining a Y-STR profile from a victim SAK suitable for comparison to a suspect AND the item in question is NOT consistent with penetration

STR amplification is generally preferable to Y-STR amplification based on its greater power of discrimination, as well as its ability to distinguish between patrilineal male relatives (except for identical twins). However, Y-STR amplification is preferred for sex assault cases when:

- a. A high percentage of female DNA means that STRs are unlikely to yield a probative male profile, or
- b. The amount of available DNA is so small (less than 2 ng) that amplifying an optimal amount of DNA for STRs would consume over half the extract, and it is not possible to get permission to consume the extract for STRs.

In some rare cases, it is best to amplify using both STRs and Y-STRs:

- Amplifying some samples with STRs and others with Y-STRs in order to address specific allegations: for example, it may be possible to get a full CODIS-eligible STR profile from a neck swab, but also useful to amplify a vaginal swab for Y-STR to address an allegation of penetration
- Amplifying the same extract with both STRs and Y-STRs to address the number of male contributors in a complex mixture: For example, Y-STR amplification in addition to STR amplification may give a better idea of the minimum number of male contributors in a complex minor component.

All relevant submitted reference samples (victim, suspect, and/or elimination) are amplified in any case where questioned samples yield results suitable for comparison.



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#### **4.4.3 Sample selection in non-sex assault major crime cases**

##### **4.4.3.1 How many samples to select in non-sex assault major crime cases**

In most non-sex assault major crimes cases, one or several samples might be extracted; but typically, two to four samples are amplified. However, given the complex nature of some cases in this category, that number could vary substantially. Examples of criteria which go into assessment of number of amplifications include:

- Number of possible contributors
- Number of items of potentially probative value

##### **4.4.3.2 How to prioritize samples in non-sex assault major crime cases**

- Highest priority is obtaining an STR profile suitable for comparison. (Note: Y-STRs are rarely performed in these cases unless sex assault is also suspected). This considers both quantity and quality of DNA present.
- Particularly for non-suspect cases, obtaining a profile suitable for CODIS entry is a high priority.
- For complex cases, when possible, confer with a case management supervisor, the submitting officer, and / or Department of Law to gather input for sample prioritization.
- 

#### **4.4.4 Sample selection in property crime cases**

##### **4.4.4.1 How many samples to select in property crime cases**

In most property crime cases, one or several samples might be extracted, but typically only one is amplified. Exceptions are made for cases with more than one suspect, such as when:

- Submitting officer described a scenario with more than one suspect
- Quantification results indicate likely single source extracts including both female and male extracts
- Recovered evidence suggests more than one suspect (cigarette butts from two different brands, items in a stolen vehicle recovered from the driver and passenger sides)

##### **4.4.4.2 How to prioritize samples in property crime cases**

- Highest priority is obtaining an STR profile suitable for comparison. (Note: Y-STRs are never performed in property crime cases). This considers both quantity and quality of DNA present
- Higher priority is given to items closer to the crime scene (such as blood stain from inside the building rather than outside the building)
- Items such as steering wheels where the owner is very likely to be present are not processed unless an elimination sample from the owner is also submitted. Exceptions are made when the owner has not been in contact with an item for an extended period.

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## Section 5 Amplification and Genetic Analysis

### 5.1 Selection of amplification target values

- For any sample in a case with a named suspect where the original material has been consumed, at least half the extract must be retained. For example, for 40 µL of extract from a consumed sample, 2 µL are used for quantification and at least 20 µL are to be retained, leaving up to 18 µL available for amplification.
- For any sample where only half or less of the original material was extracted, the entire extract may be consumed by drying it down with DNA Stable LD and reconstituting with sterile dH<sub>2</sub>O to the appropriate volume (15 µL for STR or 17.5 µL for Y-STR)
- No sample extract may be amplified at a greater concentration than its corresponding reagent blank. (QAS 9.5.1.2 and 9.5.1.3)
  - Questioned reagent blanks are routinely amplified at their maximum possible concentration (38 µL elution is DNA Stable'd and rehydrated with 15 µL sterile water for STR or 17.5 µL sterile water for Y-STR analysis).
    - Exception: When a proficiency test is worked as a stand-alone, such that the questioned reagent blanks correspond ONLY to proficiency test extracts, and none of the proficiency test extracts require concentration, then the questioned reagent blank may be amplified at full volume without concentrating the extract. This exception does not require prior approval from the Technical Manager.
    - NOTE: For batches which include sex assault casework, both questioned reagent blanks are routinely DNA Stable'd and amplified: one by STR and one by Y-STR.
  - Known reagent blank amplification volumes are routinely 15 µL for STR or 17.5 µL for Y-STR; these are only DNA Stable'd and rehydrated if a corresponding reference sample has also been DNA Stable'd and rehydrated.
- On occasion, extracts may be so concentrated that they require a 1:10 or 1:100 dilution before an amplification reaction can be set up. In such cases, a portion of the sample extract is diluted with TE<sup>-4</sup> buffer, and the dilution is then used to set up the amplification reaction. Diluted samples are noted as such on the amplification worksheet.
- When the DI is greater than 1, it may indicate degraded DNA or the presence of PCR inhibitors; but a DI of less than 1.2 has minimal impact on amplification. Note that very low template DNA samples (T-S < 0.005 ng/µL) may also have a DI greater than 1, in which case the DI is not necessarily an indicator of degradation or inhibition.

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### 5.1.1 STR Amplification

- Amplification volumes are selected based on the Quantifiler Trio Small Autosomal (T-S) results. Forensic casework amplifications should be targeted at 1 ng if sufficient extract is available. Exceeding an input of 1 ng can lead to increased artifacts, which can in turn complicate interpretation. See Selection of Amplification Target Values above for exceptions based on degradation.
- Add TE<sup>-4</sup> buffer (manufacturer may label this as DNA Suspension Buffer) to bring the sample to a final volume of 15µL.
- Low-level samples (concentration < 0.053 ng/µL) appropriate to consume (non-suspect cases, or suspect cases where written permission has been granted by DOL) should be dried down with DNASTable LD and reconstituted with sterile water in order to make best use of the extract. This will typically be 15µL of sterile water but may be adjusted such that the target input is as close to 1 ng as possible.
- Amplifying casework extracts created and retained in previous batches: When extracts previously DNA Stable'd are reconstituted for amplification, the analyst must assess whether a corresponding reagent blank needs to be run.
  - If a corresponding reagent blank has previously been run at the same or higher concentration as the newly reconstituted casework extract, there is no need to amplify the remaining reagent blank replicate. Central log documentation must indicate the batch in which the reagent blank was originally amplified so the analyst and technical reviewer can confirm its results.
  - If a corresponding reagent blank has not previously been run at the same or higher concentration as the newly reconstituted casework extract, a retained corresponding reagent blank replicate is rehydrated with 15 µL sterile water and amplified along with the casework extract.
    - On occasion, no corresponding reagent blank remains for analysis. In such cases, the analyst must consult with the Technical Manager to determine an appropriate course of action.
- *If sufficient extract is available*, the following amplification modifications are recommended:
  - For questioned extracts with a DI of 1.2 to 2: target 2 ng of DNA, based on T-S.
  - For questioned and known extracts with a DI greater than 2: target 1 ng of DNA based on T-L instead of T-S.
  - Note: for known extracts with a DI of 1.2 – 2, it is not necessary to increase the target DNA, since these are single source samples which are likely to yield full profiles even with some degradation present.
- Internal Control Specimen: At least one ICS per batch must be amplified. The amplified ICS can be from either a questioned or known sample.
- Positive Amplification Control: Vortex Control DNA and spin the tube briefly. Add 10µL control DNA to 5µL of TE<sup>-4</sup> buffer in a 0.5mL tube.
- Negative Amplification Control: Add 15µL of TE<sup>-4</sup> buffer.

[Directions for performing an STR amplification and genetic analysis are in the appendices.](#)

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### 5.1.2 Y-STR Amplification

- Autosomal STR analysis is preferable to Y-STR analysis whenever possible, based on its much greater power of discrimination. Y-STR analysis is not performed if the suspect reference sample(s) has/have not been submitted (elimination reference sample(s) should at least be requested).
- Amplification volumes are selected based on the Quantifiler Trio Small Autosomal (T-S) results for reference samples, and for Male (T-Y) for questioned samples. Forensic casework amplifications should be targeted at 0.5 ng if sufficient extract is available. Exceeding an input of 0.5 ng can lead to increased artifacts, which can in turn complicate interpretation. See Selection of Amplification Target Values above for exceptions based on degradation.
- Low-level samples (concentration < 0.026 ng/μL) appropriate to consume (suspect cases where written permission has been granted by DOL) should be dried down with DNASTable LD and reconstituted with sterile water to make best use of the extract. This will typically be 17.5μL of sterile water but may be adjusted such that the target input is as close to 0.5 ng as possible.
- Amplifying casework extracts created and retained in previous batches: When extracts previously DNA Stable'd are reconstituted for amplification, the analyst must assess whether a corresponding reagent blank has previously been run.
  - If a corresponding reagent blank has not previously been run for Y-STRs at the same or higher concentration as the newly reconstituted casework extract, the DNA Stable'd corresponding questioned reagent blank replicate is rehydrated with 17.5 μL sterile water and amplified along with the casework extract.
    - On occasion, no corresponding reagent blank remains for analysis. In such cases, the analyst must consult with the Technical Manager to determine an appropriate course of action.
- *If sufficient extract is available*, the following amplification modifications are recommended:
  - For reference extracts with a DI greater than 2: target 0.5 ng based on T-L instead of T-S.
  - For questioned extracts with a DI of 1.2 – 2, target 1 ng based on T-Y
  - For questioned extracts with a DI greater than 2: target 0.5 ng \* DI, based on T-Y (Example: for a questioned extract with a DI of 3, the template target would be 0.5 ng \* 3 = 1.5 ng)
  - Note: for known extracts with a DI of 1.2 – 2, it is not necessary to increase the target DNA, since these are single source samples which are likely to yield full profiles even with some degradation present.
- Positive Amplification Control: Vortex Control DNA and spin the tube briefly. The 2800M control DNA is typically in 25 μL of 10 ng/μL solution. The first analyst to use the tube may dilute it to a 1 ng/μL solution by adding 225 μL of TE<sup>-4</sup> buffer. Note on the tube the new concentration of the solution. Amplify 0.3 - 0.5 ng of the positive control (Ex: 0.5 μL of a 1:10 diluted 2800M standard + 17 μL TE<sup>-4</sup> buffer).
- Negative Amplification Control: Add 17.5μL of TE<sup>-4</sup> buffer.

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- If an ICS has been amplified for the batch using STRs, it is not necessary to additionally amplify an ICS using Y-STRs. However, if the batch only includes Y-STR amplifications, then at least one ICS must be amplified by Y-STR. A male ICS must be used for this purpose.

[Directions for performing a Y-STR amplification and genetic analysis are in the appendices.](#)

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## **Section 6      *Interpretation***

Interpretation of casework samples is based on the guidelines contained in this section, as well as the qualified DNA analyst's training and experience, experimental results, and case specifics. In all cases, interpretations must be independently reviewed and verified based on established peer review procedures. The DNA Technical Manager may authorize the interpretation/reporting of data outside of the criteria defined herein and will document this approval with a Protocol Exception Form. Guidance specific to interpretation and report writing for proficiency tests can be found [here](#).

### **6.1      Interpretation of GlobalFiler STR data**

Under the guidelines included in this section, only single source and two source mixtures may be suitable for interpretation.

In this context, an interpretable profile may be:

- Single source profile (entire profile consistent with one person)
- Single source major component
  - Separated from a mixture of two or more individuals based solely on peak heights
  - Deduced from a two-source mixture where one source is known
  - (Could be achieved with a combination of these two approaches)
- Single source minor component from a mixture of two individuals
  - Separated from a mixture of two individuals based solely on peak heights
  - Deduced from a two-source mixture where one source is known
  - (Could be achieved with a combination of these two approaches)
- Two source mixture
  - Entire profile consistent with two sources where neither is known and cannot be separated as major and minor (indistinguishable)
  - Two source major component from a mixture of three or more individuals

This section covers the following topics:

- [Criteria to evaluate internal size standards, allelic ladders, and analytical controls](#)
  - [GlobalFiler 007 positive amplification control profile](#)
- [Criteria for the interpretation of non-allelic peaks, allelic peaks, and mixtures](#)
  - [GlobalFiler stutter percentages](#)
- [Interpretation of a STR single source profile](#)
- [Interpretation of a STR single source major / deduced component](#)
- [Interpretation of a STR single source minor / deduced component](#)
- [Interpretation of STR indistinguishable two source / two source major mixtures](#)
- [Comparison of STR results](#)
- [Performing STR population statistical frequency calculations](#)

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**STR - [Single source profile summary table](#)**

Single source questioned sample		Rule	Exception(s) / Clarification	Documentation
Minimum for comparison/stat		Four complete loci eligible for stats	For non-probative, can compare if: * alleles < ST; * at least one full heterozygous locus; and * all alleles consistent with owner	PHRs < 60% Note loci unsuitable for stats/comparison as NS
Criteria for treating a profile as a single source (vs. mixture)		Possible minor peaks all < ST <b>and</b> may be artifacts	Report does not refer to additional contributor	A/TA on e-gram for possible artifacts
		Possible peaks < ST are not artifacts but at < 4 loci	Report language says DNA from more than one source may be present but can be treated as single source	Possible minor peaks in ( )
		Only 1 or 2 possible peaks are > ST at a single locus	Report language says DNA from more than one source may be present but can be treated as single source	Possible minor peaks in ( )
Criteria for comparison/stat eligibility	Homozygous locus	Allele > ST	Y-Indel, Amel, DYS391 suitable for comparison < ST	Loci not eligible marked as NS on e-gram
	Heterozygous locus	2 alleles > AT		

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**STR – Single source major / deduced component summary table**

Single source major contributor		Rule	Exception(s) / Clarification	Documentation
Minimum for comparison/stat		Four complete loci eligible for stats		Note loci unsuitable for stats
Criteria for separating major component based on peak heights	Homozygous locus	*Major allele > IT, AND *Minor allele(s) < 20%		*PHRs where major not separated and [ ] locus *PHRs between 10-20%
	Heterozygous locus	*1 or 2 Major alleles > IT, major PHR >=60%, AND *Minor alleles < 30%		*PHRs where major not separated and [ ] locus *PHRs between 20-30%
Criteria for deducing based on obligate alleles		*Two source mixture where one source is known	Two obligate alleles > AT	*State assumptions (such as two sources, known contributor) *Note – 2 obligate alleles
Criteria for deducing a (major) contributor based on genotype assessment		*Two source mixture *One source known *Ratio of 1 unknown: 2 owner or high *Alleles > IT	Follow flowcharts in appendices and MIW	*State assumptions (such as two sources, known contributor) *Use MIW if deducing using flowcharts

**STR - Single source minor / deduced component summary table**

Probative/Non-probative	Rule		Exception/Clarification	Documentation
Probative – must assess whether minor component is complete at each locus	*Two source mixtures only *4 or more heterozygous loci are stat eligible *If fewer than 4 heterozygous minor loci, see Profiles for mixture stats table	Separated by peak heights	*Major alleles>IT, AND *Minor alleles<30%	*PHRs where major not separated and [ ] locus *PHRs between 20-30%
		Separated by obligate alleles	Two obligate alleles >AT	*State assumptions (such as two sources, known contributor) *Note-2 obligate alleles
		Separated by genotype assessment	Follow flowcharts in appendices and MIW	*State assumptions (such as two sources, known contributor) *Use MIW if deducing using flowcharts
Non-probative – owner, elimination, or differential carryover	*Two source mixtures only AND *4 or more loci fully detected, including at least one heterozygous locus AND *No DNA inconsistent with non-probative contributor		*Includes all minor alleles >AT *Low-level A/TA do not preclude this	*A/TAs noted on e-gram



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**STR Indistinguishable two source / two source major mixture summary table**

Mixture type	Rule	Exception/clarification	Documentation
Two-source indistinguishable mixture (~1:2 – 2:1 contributor ratio)	No more than four alleles per locus	A/TAs do not count	A/TAs noted on e-gram
	Peak heights above IT are logically consistent with two contributors	Degradation of one contributor may make this difficult to assess	If degradation impacts decisions, note on e-gram: Possible degradation
	For an indistinguishable mix, no alleles at locus below IT	Four allele loci (or loci with two obligate alleles) are considered complete	NA
Two source distinguishable mixture	To run a mix stat on <u>combined</u> major and minor, can only use loci where one can assume full detection	Calculate contributor ratio (CR) as (sum minor pk hts)/(sum major pk hts) from two 4-allele loci. Document CR and assessment for all loci with less than two minor alleles	If heterozygous major, multiply smaller major peak by CR – if answer is above IT, can assume no drop out due masked by allele stacking
			If homozygous major, multiply major peak by half the CR – if above IT, can assume no drop out masked by allele stacking
			At each locus, rule out the possibility of drop out due to stutter filters. As applicable, note loci on e-gram as NS – possible stutter masking.
Two source major component	Major alleles > IT	If major has one or two alleles, (larger) minor peak < 10% of (smaller) major peak	On e-gram, [ ] loci not separated and note as NS. Note PHR if major/minor are separated.
		If major has three or four alleles, (larger) minor peak < 20% of (smaller) major peak	

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### 6.1.1 Criteria to evaluate internal size standards, allelic ladders, and analytical controls

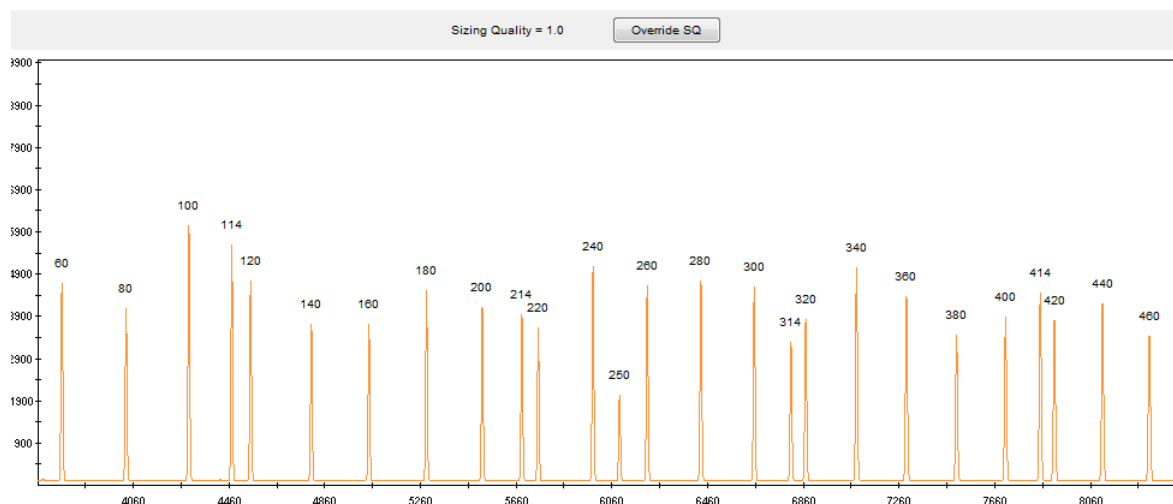
Prior to assessment of casework data, the analyst must first examine the batch controls to ensure that the extraction, amplification, and genetic analysis processes are functioning correctly. These include: Internal Lane Standard (ILS), allelic ladder(s), internal control specimen (ICS), positive and negative amplification controls, and extraction reagent blanks. All or part of an extraction, amplification and/or run may need to be repeated depending on the results of the batch controls. Any issues raised by the performance of a batch control must be addressed prior to the release of affected casework samples. Unresolved failure of a batch control requires the Technical Manager, or designated individual, to approve a course of action.

Review of batch controls is routinely performed by viewing the project in GMID-X. The analyst performing the review visually examines each of the batch controls (without relying solely on software quality flags). This electronic review is documented on the Central Log Checklist. Printouts of batch control documentation will be included in the Central Log only when the control in question requires further investigation, such as contamination assessment in a reagent blank.

#### Interpretation of Batch Controls (QAS 9.6.1)

##### Internal Lane Standard (ILS)

Global Filer is run with GeneScan 600 LIZ Internal Lane Standard (ILS). The analyst should verify that all peaks from 60-460 base pairs are present and labeled as shown. This verification may consist of confirming the sizing quality is greater than 0.5. If the sizing quality is 0.5 or lower, the analyst must confirm by visual inspection that each peak was correctly called. This check must be documented by the analyst and initialed by the technical reviewer; documentation is typically on the amplification worksheet.



The peak heights for the ILS peaks must be at least 160RFU (relative fluorescence units) to be identified and labeled by the GeneMapper™ ID-X software with a casework analysis method.

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Failure of an ILS can often be resolved by re-prepping and/or re-injecting the affected sample(s). If the ILS continues to fail after re-prepping and re-injecting, the analyst should consult the Technical manager for a course of action.

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

### **Allelic Ladder**

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 Global Filer allelic ladder are shown in the following image. The analyst should verify that all peaks from the allelic ladder(s) are present and labeled as shown.

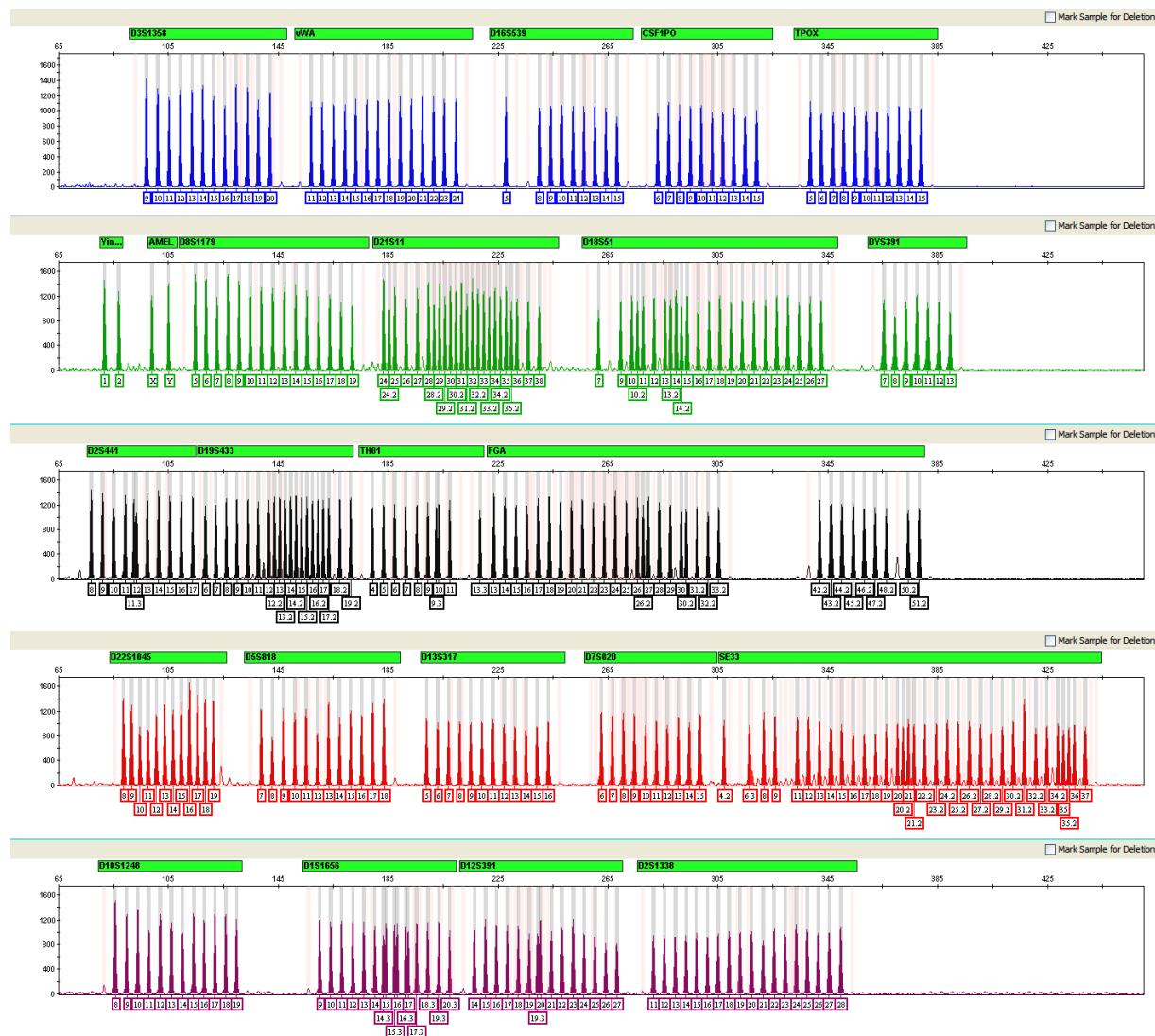
The allelic ladder contains the most common alleles determined for each of the following loci: D3S1358, vWA, D16S539, CSF1PO, TPOX, Y-INDEL, Amelogenin, D8S1179, D21S11, D18S51, DYS391, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, and D2S1338. In addition, alleles not labeled in the allelic ladder (virtual alleles), may be detected and labeled in some of the samples analyzed.

At least one allelic ladder must be included in a GeneMapper™ ID-X project with the sample type designated as “allelic ladder”. 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. 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 the locus shift and will impact the correct allele call / labeling of the samples in the project. Allelic ladders with such artifacts cannot be used to analyze data in the project. In such instances, re-injection is usually appropriate.

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**Global Filer Allelic ladder:**

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### Internal Control Specimen (ICS)

Definition of an Internal Control Specimen: An internal control specimen (ICS) is a designated, predetermined quality control sample whose expected profile is known based on prior amplification results. While reagent blanks are designed to assess the possibility of contamination in extraction reagents, the purpose of the ICS is to demonstrate that all analytical processes prior to quantification are working correctly to produce an extract with amplifiable DNA. The ICS is processed with an extraction batch and carried through at least quantification concurrently with the other samples. An ICS may include any known sample, including those obtained from laboratory staff, where the individual has been previously typed to generate a genetic profile.

Casework batches often contain multiple samples from previously typed individuals. Any one of these may be designated as the ICS for a set of known extractions. As good QA practice, the analyst confirms that all profiles from previously typed individuals yield expected profiles. This review is typically documented on the DNA worksheet for the appropriate case and is confirmed by the technical reviewer (see last box on checklist).

ICS extracts are discarded following completion of the DNA technical review of the batch.

When are ICSs created: At least one ICS shall be created with each set of casework extractions.

How are ICSs processed: All ICS samples are quantified to ensure that they contain sufficient amplifiable DNA (T-S greater than or equal to 0.053 ng/μL), and that the presence or absence of male DNA, as seen in T-Y, is consistent with expected results based on known sample type. Each ICS which meets these criteria is considered as passing and does not require amplification.

When are ICSs amplified: A minimum of one ICS per casework batch must be amplified and must yield a correct profile.

An ICS must be amplified if:

- T-S is less than 0.053 ng/μL,
- ICS is from a known female source with a T-Y reading above 0.02 (Exception: epithelial fractions from differential extractions may reasonably be expected to include some male carryover and do not require amplification based on a detectable T-Y reading.)
- ICS sperm (or sperm/substrate) fraction with no detected male DNA

ICS extracts may also need to be amplified in batches where troubleshooting is required, e.g. assessing the possibility of sample switching.

Interpretation of ICSs: ICSs are expected to yield full profiles that are consistent with expected typing results. However, a partial profile for an ICS does not necessarily invalidate the batch. If a minimum of fifteen complete CODIS core loci are detected for the ICS, with no inconsistent allele calls, the ICS is acceptable. The Technical Manager, or other designated individual, shall determine whether or not to approve the ICS when fewer than fifteen complete core loci are

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detected for the ICS. The data quality of other positive control samples in the batch and/or the quality of other concurrently extracted samples will be reviewed to determine the approval process.

The presence of artifacts in an ICS does not constitute a failed ICS, so long as the artifacts are not consistent with contamination. Artifacts are struck in the GMID-X project and checked during the electronic review of batch controls.

ICS failure and uninterpretable data: If one ICS is amplified and does not yield a sufficiently complete profile, another ICS from the same batch must be amplified. All batch data is considered uninterpretable until and unless the issue is resolved.

In any of these situations (or similar situations), the analyst will initiate a Quality Review Form and attempt to determine the cause of the discrepancy:

- An incorrect STR profile is obtained for the ICS in a casework batch
- No designated ICS yielded a sufficiently complete profile
- An ICS was omitted from an extraction set

### **Positive Amplification Control**

A positive control is defined as a single source sample whose genetic profile was previously determined and from which a full profile was developed; it is used to evaluate the performance of the amplification and typing procedures. DNA Control 007 is used as a positive control for amplification with Global Filer Kit. A minimum of two positive amplification controls are routinely amplified and processed concurrently with each casework questioned amplification. A minimum of one positive amplification control is routinely amplified and processed concurrently with each casework known amplification. (QAS 9.5.3.1)

Positive control failure and uninterpretable data: A positive control sample which yields no profile, an incorrect profile, or an incomplete profile is considered a failed positive control. All data from any amplification which does not have at least one passing positive control is considered uninterpretable until and unless the issue is resolved.

The presence of artifacts in a positive control does not constitute a failed positive control, so long as they are not consistent with contamination. Artifacts are struck in the GMID-X project and checked during the electronic review of batch controls.

It may be necessary to re-prep and/or re-inject the positive control if the cause of failure appears to be a failed electrophoretic injection. If the re-prepped and/or re-injected positive control types successfully, the positive control is considered successful.

Alternatively, other samples in an amplification may serve as a positive control (e.g. duplicate offender/staff sample, offender confirmation sample, casework known sample with a verified profile, or a buccal swab obtained from laboratory personnel whose genetic profile(s) was/were previously determined and documented). Use of an alternate positive control must have documented approval of the Technical Manager.

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All casework samples co-amplified will be re-analyzed if no positive control was successfully typed for that amplification. The laboratory will routinely include more than one positive control with every casework amplification reaction that includes questioned samples, in order to prevent reanalysis of samples that are limited in DNA content. If at least one of these positive control samples produces acceptable and expected results, the batch will not be reanalyzed.

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 from either (but not both) amplification/run may be used.

**DNA Control 007 amplified with Global Filer Kit (image can be found in [appendix](#)):**

D3S1358	15, 16
VWA	14, 16
D16S539	9, 10
CSF1PO	11, 12
TPOX	8
Y-INDEL	2
Amelogenin	X, Y
D8S1179	12, 13
D21S11	28, 31
D18S51	12, 15
DYS391	11
D2S441	14, 15
D19S433	14, 15
TH01	7, 9.3
FGA	24, 26
D22S1045	11, 16
D5S818	11
D13S317	11
D7S820	7, 12
SE33	17, 25.2
D10S1248	12, 15
D1S1656	13, 16
D12S391	18, 19
D2S1338	20, 23

#### **Negative Amplification Control and Extraction Reagent Blanks**

A reagent blank is carried through the entire analytical process as part of each extraction or type of extraction. It contains all the reagents - except DNA template - used during extraction, amplification and typing. For questioned extracts, each reagent blank is DNA-Stable'd. Only one of the blanks (either replicate -1 or the blank with the higher observed quantification reading) is rehydrated with 15 µL sterile water and amplified. Reagent blank replicates not amplified are created as items in the LIMS and retained.

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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 TE<sup>-4</sup> buffer used to dilute the DNA samples.

The purpose of the negative controls is to detect systemic DNA contamination that might occur from the reagents, the laboratory environment, between the samples being processed, and/or due to improper handling of the samples by the analyst. The negative controls must be run at the most stringent set of conditions for the batch. (QAS 9.5.3.1)

Verification of the presence of amplicon in the negative controls is performed by viewing the presence of unincorporated primer peaks. The negative controls should not yield any true STR allelic peaks above the analytical threshold. When peaks greater than or equal to the reporting threshold are present in the range between 60-460 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 the AT will not invalidate the sample as long as the data is at levels too low for interpretation, or the peaks can be shown to be artifacts. Artifacts will be struck in the project and checked in the electronic review of the batch controls.

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. If a low-level profile does not have at least four complete loci detected at the Troubleshooting threshold, it is not possible to determine the source and no further investigation or documentation is required.

Negative control failure and uninterpretable data: The failure of a negative control may indicate a problem at the extraction or amplification level. When probable true allele peak(s) are detected above the analytical threshold, the analyst will reanalyze the profile using the Global Filer troubleshooting analysis parameters (60 RFU). If the low-level profile has at least four complete loci detected at the troubleshooting threshold, this constitutes a failed negative control. All data from any amplification which does not have a passing negative amplification control is considered uninterpretable until and unless the issue is resolved. All samples which correspond to a failed reagent blank are considered uninterpretable until or unless the issue is resolved.

To resolve a failed negative control, the analyst will investigate possible causes of contamination and, if possible, will determine the source of the contaminating DNA. Such electropherograms are printed and included in the Central Log, with analysis parameters clearly noted. In some cases, the contamination may be attributable to a known source, such as an adjacent sample or a lab worker who was in close proximity to the extract.

- If the analyst can identify the source of the contamination, a Quality Review Form must be initiated to investigate the reason for the contamination. The DNA Technical Manager will then be consulted to determine the appropriate course of action.



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- If the source of the contamination cannot be identified by comparison to relevant staff profiles and concurrently analyzed samples, it may not be possible to determine the source of the contamination. When the source of the contamination cannot be identified, and the level of contamination does not interfere with interpretation of casework samples, the analyst will complete a Contamination Assessment Form. This form is submitted to the DNA Technical Manager for approval. Once signed by the DNA Technical Manager, it is included with the Central Log documentation. The DNA Technical Manager will maintain a log of Contamination Assessment Forms, and their respective profiles will be kept in GMID-X for comparison purposes.
- If the level of contamination has the potential to interfere with casework interpretation, whether or not its source is identified, the DNA Technical Manager must be notified to determine an appropriate course of action.

### ***6.1.2 Criteria for the interpretation of non-allelic peaks, allelic peaks, and mixtures***

#### **6.1.2.1 Criteria for the interpretation of non-allelic peaks (QAS 9.6.2)**

The initial assessment of a casework sample electropherogram separates interpretable alleles from artifacts. Artifacts can occur in data and much of their nature and origin have been determined and documented. Peaks determined to be artifacts are struck on the full view electropherogram. Peaks struck manually are also initialed by the analyst. When it is not possible to distinguish between an artifact and a true allele, this is indicated on the electropherogram as Artifact/True Allele (A/TA). If the presence of a definite or possible artifact at a locus impacts the suitability of that locus for either comparisons or statistical analysis, that is documented on the electropherogram. Artifacts are often associated with an overabundance of template DNA. If the presence of many artifacts complicates data analysis, it may be appropriate to re-amplify the sample with less template DNA.

On questioned samples, binned peaks which are called as alleles but determined to be artifacts must include an explanation of why the peak was determined to be an artifact (such as poor morphology, baseline noise associated with large peaks, pull-up, etc.). This explanation is documented on the electropherogram. Binned artifacts documented as pull-up also require an artifact view to show corresponding base pairs between large peak and the artifact in question. While these artifacts may not line up to have identical base pair sizes, they must be close (within 0.5 base pairs) and must correspond to a large peak (often off scale, but not necessarily) in another color. Artifacts in questioned samples which do not bin as alleles and are called OL, OMR or spike, may be struck without a documented explanation and without an artifact view printout. All artifacts in reference samples may be struck without documented explanation and do not require artifact view printouts. Stutter does not require artifact view printouts.

#### **Baseline noise**

The analytical threshold (AT) for analysis of casework samples is 160 RFU. Data below the AT is considered indistinguishable from baseline noise and not suitable for interpretation.

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Ideally, true allele peak heights should fall between 3000 and 12,000 RFU. Samples with peak heights near or above 12,000 RFU may include baseline noise that exceeds 160 RFU. Such baseline noise may be struck. However, if baseline noise is difficult to distinguish from possible low-level DNA, it may be more appropriate to re-amplify the sample with less template DNA.

### Stutter

The PCR amplification of tetranucleotide STR loci typically produces a minor product peak four bases shorter (N-4), two bases shorter (N-2, infrequently observed), eight base pairs shorter (N-8) or four base pairs larger (N+4) 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.

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 consistent for a particular locus. The following table lists the values of the maximum expected percentage of N-4 stutter for the loci in the Global Filer System when run on the Applied Biosystems 3500xl. The stutter values are based on data obtained by internal validation studies. These values, along with a universal N+4 stutter of 2%, are set as stutter filters used by the GeneMapper ID-X software. N-8 stutter has been observed rarely, typically in samples with more than 1 ng template DNA. As such, N-8 stutter is not included in the filters.

### Maximum Expected N-4 Stutter Percentages for Global Filer loci on the 3500xl (in single source samples)

D3S1358 11%	vWA 12%	D16S539 10%	CSF1PO 10%	TPOX 6%	
Y-INDEL NA	Amelogenin NA	D8S1179 10%	D21S11 11%	D18S51 13%	DYS391 8%
D2S441 9%	D19S433 11%	TH01 5%	FGA 13%		
D22S1045 (see below)	D5S818 10%	D13S317 10%	D7S820 10%	SE33 15%	
D10S1248 12%	D1S1656 13%	D12S391 14%	D2S1338 12%		

Additional max stutter percentages:

- D22S1045 (-3 BP) 17%
- D22S1045 (+3 BP) 7%
- SE33 (-2BP) 5%
- D1S1656 (-2 BP) 3%

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Peaks in the stutter position greater than the listed values may indicate the presence of DNA from more than one individual. However, elevated stutter is also associated with both excess template DNA and very low amounts of template DNA.

### **Elevated +4 / -4 stutter**

NOTE: In situations where a minor component is not suitable for comparison to probative reference samples, and that minor component includes peaks which may be elevated +4/-4 stutter, it is not necessary to perform the calculations described below.

When two large peaks at a given locus are eight base pairs apart, elevated stutter is commonly observed halfway between the large peaks. (For example, when large 28 and 30 peaks are detected at D21S11, it is common to observe a 29 peak above the maximum expected stutter.) This is likely due to the additive effect of +4 stutter from the first peak and -4 stutter from the second. Elevated +4 / -4 stutter may be documented in one of two ways:

1. An additional 2% is added to the above maximum expected stutter percentages for alleles in +4 / -4 stutter positions. On the electropherogram, the analyst calculates the -4 stutter percentage and can document as follows: “~X % < Y% + 2% Elevated +/- stutter”, where X is the calculated -4 stutter percentage and Y is the maximum expected stutter percentage for that locus.
2. Alternatively, the analyst may calculate and note the maximum expected +4 stutter (2% for all loci) and maximum expected -4 stutter (varies by locus; see previous table). This may be necessary when the peak contributing +4 stutter is significantly larger than the peak contributing -4 stutter. If the sum of these two calculations is greater than the peak height of the observed peak, then the observed peak can be noted as elevated +/- stutter.

Example: At D21S11, 28 has a peak height of 9000 RFU, 29 has a peak height of 190 RFU, and 30 has a peak height of 800 RFU. The maximum expected +4 stutter for the 28 would be  $9000 \times 0.02 = 180$ . The maximum expected -4 stutter for the 30 would be  $800 \times 0.11 = 88$ . Thus, the maximum +4 / -4 expected stutter would be  $180 + 88 = 268$  RFU. Since the peak height of the 29 is 190 RFU, elevated stutter could be documented as follows: Max +4 = 180; Max -4 = 88; Total = 268;  $190 < 268$   
Elevated +/- stutter

Note: In some cases, it may be useful to show this documentation as a way of confirming that a minor component is **not** attributable to elevated +/- stutter.

### **Assessment of stutter / true allele**

NOTE: In situations where a minor component is not suitable for comparison to probative reference samples, and that minor component includes peaks which may be stutter or true alleles, it is not necessary to perform the calculations described below.

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Striking a stutter peak is acceptable in the following situations:

- In questioned samples, when there is no indication of a mixture other than elevated stutter at four or fewer loci, the analyst will document the stutter as such on the electropherogram (including the calculated % and a notation that it was determined to be an elevated stutter peak).
- In questioned samples, when the +4 / -4 combined maximum expected stutter contribution is larger than the observed peak height in the stutter position, the analyst will document as described in the previous bullet.
- Reference samples are assumed to be single source. Therefore, any elevated stutter may be struck without further documentation.

Noting a stutter peak as indistinguishable artifact or true allele (A/TA) in questioned samples is acceptable in the following situations:

- When expected stutter contribution (either +4, -4, or +4 / -4) is subtracted from a peak in a stutter position and the resulting peak is below the analytical threshold (AT) of 160 RFU, the peak should be noted as A/TA.
- When possible elevated stutter peaks are observed at five or more loci in an otherwise apparent single-source questioned profile, and no minor peaks (or one minor peak) are observed outside of stutter positions, the elevated stutter peaks are documented as A/TA.

### **Subtracting stutter**

In mixture interpretation, many instances arise where one peak is in a stutter position to another. In some circumstances, it is appropriate to subtract stutter contribution from a peak in a stutter position to a larger peak. Since this requires manual calculations that must be checked by a technical reviewer, this should be done judiciously. Examples of instances when maximum stutter should be subtracted from the smaller peak height are:

- Calculating contributor ratios in a two-source mixture (If possible, it is better to use loci where stutter position is not a factor)
- Assessing whether or not a peak is above IT
- Assigning major and minor contributors
- Subtracting stutter impacts a decision in a mixture deduction flowchart

### **Amplification and Injection Artifacts**

#### **Injections with poor sizing quality**

Poor injections can lead to broad peaks, which in turn can adversely impact interpretation by increasing the possibility of allelic drop out. These issues usually can be resolved by reinjection. Any sample with a sizing quality of 0.5 or below, or any sample which has loci with broad peaks, is only acceptable for interpretation if it is a single-source sample, and if all loci with broad peaks are heterozygous (with the exception of DYS391, which only has one allele in a single source male sample).

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The following are examples of artifacts which may be struck and initialed on electropherograms. Re-prep and/or re-injection is not required if the artifact in question is clearly distinguishable and thus does not interfere with interpretation.

### **Pull-Up**

Multi-component analysis is the process that separates the six different fluorescent dye colors into distinct spectral components. The six dyes (6-FAM, VIC, NED, TAZ, SID, and LIZ) 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, often about 1.5% of the main peak. When 1 ng of DNA is targeted, this is most likely to be present above AT associated with large peaks, typically homozygotes. Documentation of a peak as pull-up includes a close up view of the locus in question as well as the corresponding color with the peak causing the pull-up, with base pairs included on the zoomed view electropherogram.

### **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 by re-injection.

### **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.

### **Incomplete 'A' nucleotide addition (-A or split peaks)**

While -A peaks are a known artifact, none of these were observed in the 3500xl validation studies. As such, any instances of -A require approval by the Technical Manager before the sample injection may be used for casework.

### **Persistent Kit Artifacts**

Occasionally, one or more lots of PCR kits may exhibit persistent artifacts. 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. Such artifacts will be noted in the verification process and acknowledged by the Technical Manager. Once documented in verification, these artifacts should be struck and initialed, and do not require TM acknowledgement when observed in casework.

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### **Tri-alleles**

Occasionally, a single-source sample may be observed to have three alleles at one (rarely more) locus/loci. Tri-allelic 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.

Samples exhibiting potential tri-alleles may be 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-amplified and found to be reproducible, the analyst may consider the tri-allele to be a reproducible observation without the A/TA designation. If the potential tri-allele is not reproducible, it will be labeled as an Artifact/True allele (A/TA).

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

### **Contamination**

The verification of reagents prior to use in casework is conducted in part to detect contamination which permeates an entire lot of a given reagent or set of reagents. However, such checks will not necessarily detect contamination arising from a single event (for example, cross-contamination between two adjacent samples).

For casework samples, whenever data suitable for comparison is not attributable to any reference samples associated with its case, the possibility that the profile was introduced via contamination must be considered. As a part of the review process, both the analyst and the technical reviewer must rule out laboratory staff working in direct proximity to the open evidence as well as cross-contamination within the batch of samples. This comparison may be done manually or via the Profile Comparison tool in GeneMapper ID-X. The same principle applies to control samples where no DNA should be detected.

#### **6.1.2.2 Criteria for the interpretation of allelic peaks**

##### **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. Peaks that are labeled as OL (off ladder) at a 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.

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

When a true 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



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by simultaneously examining the base sizes for the sample allele peak and the associated allelic ladder peaks in the relevant locus.

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 allele from the ladder that is closest in proximity to the OL allele.

OL alleles considered to be true alleles may require re-amplification to confirm, particularly when they are only observed in a mixture. If the OL allele is observed in multiple amplifications from a single item (such as two stains from the same item of clothing, or the sperm and epithelial fractions from a vaginal swab), further amplifications are not necessary to verify the OL allele. The DNA Technical Manager must approve reporting an OL allele seen only once in casework amplification (e.g. limited amount of sample available for analysis).

Documentation of the OL allele must include an electropherogram with both 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. The OL allele designation is to be written on the full view electropherogram.

#### **Assessing interpretable alleles (QAS 9.6.3)**

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, and they are clearly distinguishable from artifacts. All peaks are examined and evaluated in the process of data interpretation.

#### **Three types of thresholds used for data analysis and interpretation (QAS 9.6.4)**

- Analytical Threshold (AT) of 160 RFU: below this level, it is not possible to distinguish artifacts from true alleles. The detection limit set for data analysis is defined by the AT.
- Stochastic Threshold (ST) of 630 RFU: below the ST, there is a greater likelihood of drop-out of a sister allele at a heterozygous locus. When data is present below ST, allelic drop out must be a consideration.
- Interpretational Threshold (IT) of 1300 RFU: above the IT, peak height ratios are minimally affected by stochastic effects. Above the IT, sister alleles are expected to have a peak height ratio (PHR) of 60% or greater.

#### **Alleles below the Stochastic Threshold**

Stochastic effects result when limited DNA molecules are used to initiate PCR, and unequal sampling of the two alleles present from a heterozygous individual leads to peak height imbalance, elevated stutter, and/or allelic dropout. The ST of 630 RFU is based on validation studies and represents the RFU value below which stochastic effects can lead to drop out of a sister allele at a heterozygous locus. Alleles which fall between the AT and the ST are subjected to a higher degree of scrutiny due to the possibility of stochastic effects.

Note that when an allele is in a stutter position, the “stacking” of stutter with the peak can artificially inflate the peak height. Allele stacking must be considered when assessing whether a probative allele lies above or below the ST.



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**Reported** refers to alleles whose peak heights are equal to or above the ST and are generally reproducible if re-amplified. Such alleles are routinely used in making comparisons between known and questioned sample genetic profiles under the interpretation guidelines stated in this manual. These alleles may also be described as **detected** in report language.

**Observed** refers to data (peaks) below the ST. Alleles below ST may be considered in these routine circumstances:

- reporting the presence of multiple sources of DNA in a sample
- reporting the presence of male DNA
- reporting the presence of an “owner” on a sample such as body swabs or clothing.

On occasion, depending on the quality of the data and the degraded or inhibited nature of the sample, such alleles may be used to generate a composite profile, with the documented approval of the DNA Technical Manager.

In most instances, allelic peaks below the ST are not suitable for use in comparisons and statistical calculations.

**Alleles below the Interpretational Threshold and at or above the Stochastic Threshold**

Alleles in this range may be subject to stochastic effects, but not likely to the extent of sister allele drop out. Probative alleles in this range may be suitable for comparison and for statistics. However, they cannot be relied upon to have predictable peak height ratios. Interpretational cutoffs for deduction of a single- source contributor that rely on assumptions about peak height ratios are not appropriate when the alleles in question fall below IT.

Note that when an allele is in a stutter position, the “stacking” of stutter with the peak can artificially inflate the peak height. Possible stutter contribution should be subtracted out when considering whether a probative allele falls above the IT.

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### 6.1.2.3 Criteria for the interpretation of mixtures

#### **Distinguishing between multiple binned artifacts and a minor contributor**

The amplification template target of 1 ng is used in part because minimal artifacts, including stutter, are detected at that target value. However, even when 1 ng is amplified, occasional instances of elevated stutter, particularly N-8 stutter, N+4 stutter, and elevated +4/-4 stutter, may be observed. If an electropherogram has 4 or fewer such peaks, all of which can be attributed to attributable artifacts (such as stutter or pull-up), the profile may be considered as not having a low-level minor contributor; provided all such artifacts are documented as such on the electropherogram.

#### **Distinguishing a minor male contributor from possible artifact**

In order to report that male DNA was detected, a minor contributor must have male DNA detected at two or three of the following:

- Y peak at Amelogenin
- 1 or 2 detected at Y-Indel
- Any binned allele called at DYS391

An indication of male DNA at only one of these loci could be attributable to an artifact, and no conclusion is reported as to whether male DNA may be present.

#### **Profile Interpretation: Single Source versus Mixture**

Genetic profiles from questioned samples may be from a single individual (single source) or from multiple individuals (mixtures). Assessment of whether a profile is a single source or mixture, and whether a mixture can be separated out into single source components, impact subsequent interpretation. Documentation of interpretation, including assumptions, must be included in the report (either in the written body of the report or in the supporting bench note documentation).

For questioned samples that are assumed to be single source, it is not necessary to write out calculated percentages for peak height ratios when they are at or above 60%.

For questioned samples that are mixtures, it is not necessary to write out calculated percentages such as peak height ratios and stutter percentages when they are more than 10% above (or below) a minimum cutoff. For example, it is not necessary to note a peak height ratio of 80% (or 40%) if the minimum peak height ratio to call a major component is 60%. Documentation of calculations that are within 10% of a cutoff is very helpful during the review process for mixtures.

Because peak height ratios are of the greatest value in mixture assessments, it is not necessary to document peak height ratios in reference sample profiles unless the ratio is so extreme as to cast doubt as to whether the smaller peak is a true allele (20% or lower).

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### **Profile interpretation: Completeness of a locus**

Assessment of a questioned profile includes the determination of which loci are suitable for comparison and/or for the calculation of population statistics.

Statistics-eligible loci considered complete – that is, with no indication of possible drop-out, either for the entire locus or just for a resolved component of a mixture – are suitable for exclusions as well as for population statistics (if corresponding population frequency data is available). Loci where possible drop-out is evident may not be suitable for comparison, depending on the number of sources and the extent of the possible drop-out. A locus with no alleles above the ST can only be considered complete, and therefore suitable for statistics, when genotype analysis indicates that drop-out is not a consideration (e.g. a heterozygous probative contributor).

A statistics-eligible locus where drop-out is a possibility is noted with NS on the electropherogram to indicate that the locus is not suitable for statistics. Examples of loci not suitable for statistics due to possible drop-out include:

- In a single-source profile, a locus with only one allele, and the allele is below ST
- In a two-source indistinguishable mixture profile, a locus where there are fewer than four alleles and at least one of the alleles is below ST
- In a two-source mixture profile with a minor component, a locus where a minor allele could be masked by the stutter filter adjacent to large major component peak

The NS at a given locus may apply to the locus as a whole, or only to the minor component. If the NS applies only to the minor component or mixture, this should also be noted on the electropherogram as NS<sub>mix</sub>.

### **Profile interpretation: Sequential unmasking**

The assessment of a questioned sample profile takes place with 'sequential unmasking': initial assessment occurs before any probative reference samples are compared to that profile. However, 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. In such cases it is useful to use the "owner's" (or any other non-probative) reference profile to assist in the interpretation of the questioned sample.

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### **Mixture interpretation – assessment of number of contributors**

The same principles used to assess whether a sample was single source or a mixture can be applied to assessing number of possible contributors in a mixture.

The minimum number of contributors may be assessed by evaluating the loci that exhibit 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, provided that none of those peaks are consistent with being elevated stutter). However, phenomena such as tri-alleles, primer binding site mutations, or allele sharing between close relatives can complicate the determination of number of contributors.

A mixture may be distinguishable, meaning major and minor contributors can be separated; or it may be indistinguishable. A major component may consist of either one or two contributors.

Indistinguishable mixtures consistent with being from two individuals suitable for comparison display the following properties:

- No more than four alleles at a given locus
- When peak heights are above IT, peak height ratios are logically consistent with being from two contributors. (For example, a locus with four peaks with RFU heights of 2000, 2000, 2000 and 10,000 is not logically consistent with being from two individuals.)
- Alleles below ST can be accounted for completely, either by the presence of exactly four alleles or by comparison to a known “owner” profile.

Indistinguishable mixtures consistent with being from more than two individuals are not suitable for any comparisons.

Additional caution must be used when analyzing mixtures which indicate degradation in the Degradation Index and/or by a “ski slope” profile where alleles at small loci amplify noticeably better than alleles at larger loci. Particularly in mixtures where one contributor is more degraded than another/others, degradation may result in results that require modified interpretation (such as only interpreting at small loci, or only interpreting based on the presence of obligate alleles), or mixtures that are not suitable for interpretation. Reasons for restricted interpretation for degraded mixtures must be documented on mixture interpretation worksheets or electropherograms.

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### 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 possibilities that may be encountered by the forensic scientist, nor the conclusions that may be rendered from his/her interpretation of the results.

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:** indistinguishable mixtures containing DNA from at least three sources and/or mixtures that exhibit potential stochastic effects

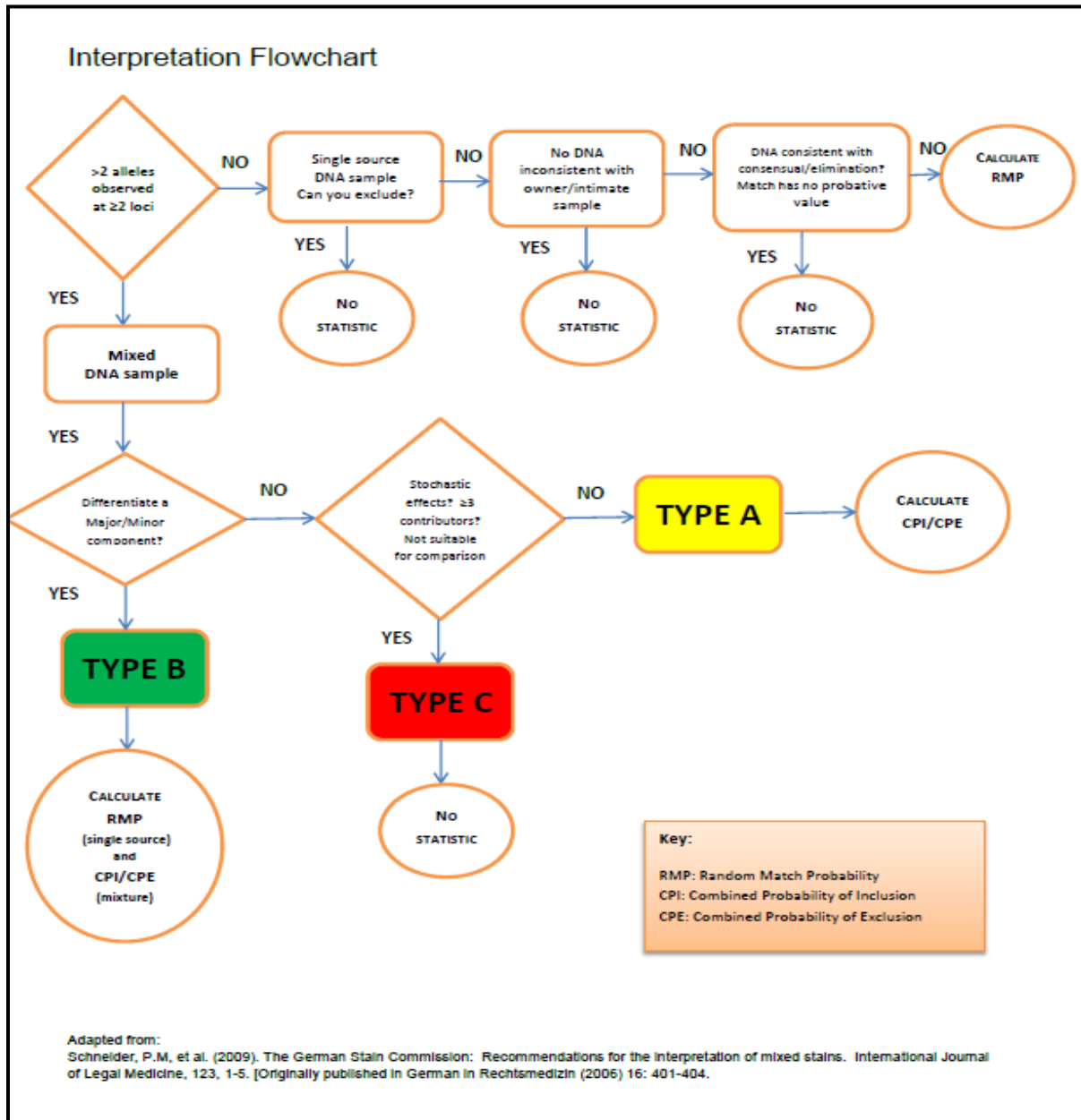
#### TYPE A:

*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. In two source mixtures where the “owner” can be assumed to be one contributor, the “owner” reference sample may be used to deduce a single source unknown contributor.

The classification of indistinguishable does not imply that the profile is not interpretable. Individuals may still be included or excluded as possible contributors to an indistinguishable mixture, provided the mixture is consistent with having no more than two contributors. However, a minimum of 4 complete loci suitable for calculating population statistics are required in order for an indistinguishable mixture to be suitable for comparisons. Refer to appendices for minimum requirements for interpretation of low-level data in an indistinguishable mixture.

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NOTE: Under TYPE B, only the more appropriate statistic is calculated, not both.





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**TYPE C:**

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

If no distinguishable major component is observed, this type of genetic profile is deemed unsuitable for probative comparisons, and no statistical analysis will be performed.

A sample may be deemed not suitable for probative comparisons if the majority of alleles in a sample are below ST 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. Note: it may be possible to compare a type C mixture to an “owner” on a sample such as a body swab or item of clothing.

**Incorrect interpretation of major/minor assessment or mixture deduction**

Despite making best use of available information, an analyst may incorrectly deduce an unknown contributor from a mixture, or incorrectly separate major and minor contributors. When such discrepancies are discovered, they should be brought to the attention of the Technical Manager, who will determine the impact on interpretation and the necessary documentation.

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### 6.1.3 Single Source Questioned Sample Interpretation

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 (in RFUs) of the lower RFU allele by the peak height of the higher RFU allele, and then multiplying by 100 to express the PHR as a percentage. Based on validation studies, the minimum expected PHR for single-source samples, where there is no indication of a mixture and 1 ng of template DNA is used, is 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.

#### **Minimum amount of data for a single source to be suitable for comparison**

**Criteria for interpretable versus uninterpretable data (QAS 9.6.5):** A single source profile must have complete information for at least 4 statistic-eligible loci to be deemed interpretable and suitable for all comparisons. This applies to profiles with a single source of DNA as well as to deduced contributors and major or minor components from mixtures. A partial profile which does not meet this minimum is uninterpretable, unless otherwise excepted as described below.

#### **Exception / Clarification: Using Alleles Below ST in non-probative comparisons**

A single source profile may be consistent with an “owner” on an intimate sample or on an item “owned” by that person. A single source profile, partial profile, or minor component from any intimate sample which is solely attributable to an “owner” is not used for statistical analysis, but is addressed in the report with a qualitative statement.

These are situations in which a given profile might reasonably be expected to be present on an item. These conclusions are reported with a qualitative statement in the report, provided the following minimum criteria are met:

- Fully detected at a minimum of four loci (below ST and non-core loci are acceptable for this purpose)
- Fully detected at one or more heterozygous loci
- No DNA inconsistent with the expected profile (and/or artifacts) was detected in the partial profile.

If these minimum criteria are not met, no qualitative statement can be made regarding the source of the DNA.

#### **Documentation:**

On electropherogram, note peak height ratios < 60%, and note loci unsuitable for statistics or probative comparisons as NS.

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### **Criteria for treating a profile as a single source vs. mixture**

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 ST, particularly outside of stutter positions

However, it can be difficult to determine whether or not an additional contributor is actually present when the peaks in question are low-level (that is, below ST) peaks in stutter positions and/or consistent with possible baseline artifacts. When all or most of the peaks that might suggest a possible contributor are consistent with possible elevated stutter and/or baseline artifacts, the profile cannot be definitely declared as a mixture (or, in some cases, as a mixture with more than two contributors).

### **Rules and Clarification for report language**

- When all peaks in question are below ST and may be attributable to artifacts, a mixture cannot be assumed.
  - In report, conclusions are made as to the reportable DNA – that is, the DNA which is not attributable to possible artifacts.
- When peaks in question are below ST are \*not\* attributable to artifacts but are seen at fewer than four loci, DNA from more than one source (or more than two sources) may be present in the sample. Alternatively, if only one or two peaks are seen above ST at a single locus (if applicable, subtracting out the possible contribution of stutter), then DNA from more than one source (or more than two sources) may be present in the sample.
  - Report language regarding number of contributors should include “may” (as in, DNA from more than one individual may be present in this sample) to indicate that the presence of an additional contributor is a possibility but not a certainty. Because it is not certain that a minor contributor is present, it is not necessary to refer to a major component.
- Alternatively, if only one or two peaks are seen above ST at a single locus (if applicable, subtracting out the possible contribution of stutter), then DNA from more than one source (or more than two sources) may be present in the sample.
  - Report language regarding number of contributors should include “may” (as in, DNA from more than one individual may be present in this sample) to indicate that the presence of an additional contributor is a possibility but not a certainty. Because it is not certain that a minor contributor is present, it is not necessary to refer to a major component.

A profile with two or more alleles above ST (not attributable to artifacts) at two or more loci, or alleles below ST (not attributable to artifacts) at four or more loci, is deemed to be a mixture.

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### **Documentation**

- Possible artifact or true alleles are noted as A/TA on the electropherogram. Parentheses are not required for A/TAs on these profiles.
- Possible peaks below ST which are not artifacts are marked with parentheses on the electropherogram

### **Criteria for probative comparisons and eligibility for statistics**

#### **Rules**

A locus must be deemed complete to be suitable for use in probative comparisons and population frequency calculations. Completeness for a locus in a single source profile is addressed as follows:

Homozygous loci: Detected allele must be above ST to be eligible for single source statistics and probative comparisons.

Heterozygous loci: Two detected alleles above AT and consistent with being sister alleles are eligible for single source statistics and probative comparisons.

In single source samples, the primary concern with using alleles below ST is the possibility of allelic dropout. An apparent homozygous allele below ST may in fact be a heterozygote with dropout of the sister allele. If both sister alleles of a heterozygote are present below the ST, dropout is not a possibility and therefore the alleles may be used for statistical calculations. Because this relies on the assumption of a single-source sample, the assumption must be documented in the report language (e.g. Assuming a single source profile...)

Any other instances of using alleles below ST without demonstrating reproducibility, including compositing alleles from multiple amplifications, require documented approval by the Technical Manager.

#### **Exceptions / Clarification**

In a few specific instances (Amelogenin peaks, Y-Indel, and DYS391), single alleles below ST may be used for comparisons.

### **Documentation**

Loci which are not eligible for probative comparisons and statistics are noted as NS on the electropherogram.

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#### **6.1.4 Single source major / deduced component interpretation**

##### **Minimum amount of data for a single source to be suitable for comparison**

**Criteria for interpretable versus uninterpretable data (QAS 9.6.5):** A single source profile must have complete information for at least 4 statistic-eligible loci to be deemed interpretable and suitable for all comparisons. This applies to profiles with a single source of DNA as well as to deduced contributors and major or minor components from mixtures. If a minimum of four loci cannot be separated, then separation should not be attempted. Note: while the profile may not be interpretable with respect to the single source contributor, it may be interpretable as an indistinguishable mixture – see indistinguishable mixture interpretation section

##### **Documentation**

Loci which are not eligible for probative comparisons and statistics are noted as NS on the electropherogram.

##### **Criteria for separating major component based on peak heights**

Separating a 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 4:1 ratio. Distinguishable mixtures of this type will result in a single source major component suitable for probative comparisons.

##### **Notes:**

- Since peak height ratios as low as 60% have been observed in single source samples with ideal concentrations of DNA, and even lower peak height ratios have been observed with lower concentrations of DNA, peak height ratio information can be used most effectively when alleles are above the IT.
- It may be necessary to factor out stutter when determining the ratios described below.
- The presence of more than two contributors means that greater caution must be used with respect to alleles sharing and peak height ratios.
- Whether the minor component is suitable for probative comparisons depends on the number of contributors. Refer to the section on interpretation of single source minor/deduced component
- Some mixtures where major and minor contributors could be separated are nonetheless treated as a whole. This is often the case for the non-probative fraction of a differential extraction. In those instances, the minor component does not need to be noted on the electropherogram.

##### **Rules**

##### **Proposed major component has one allele**

- Major component allele must be over IT
- Minor allele(s) peak height must be 20% or less of the major allele
- If these conditions are not met, locus cannot be separated

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**Proposed major component has two alleles**

- At least one of the major alleles must be over IT and PHR >60%
- Minor alleles must be 30% or less of the smaller major allele
- If both conditions are not met, locus cannot be separated

**Documentation**

- If a mixture can be successfully separated into major and minor components, and the minor component will be interpreted separately, the minor component alleles are designated on the electropherogram with parentheses.
- The loci where an unambiguous major or minor component is not distinguishable are marked in brackets (e.g. [10,11,12]) on the electropherogram.
- Peak height ratios are typically documented on the electropherogram or mixture interpretation worksheet (MIW) when they are within ~10% of the cutoff value.
- Peak height ratios may be noted on the electropherogram or mixture interpretation worksheet to document possible genotypes, if appropriate.

**Criteria for deducing potential contributors based on obligate alleles**

For some two source mixtures where one source is assumed, it may only be possible to separate based on the presence of two obligate alleles. Some examples of this are:

- a mixture with a contributor ratio less than 1:2 (unknown: owner)
- a mixture where one or both contributors are highly degraded
- a mixture with many or all peaks below IT (see [flowchart](#) in appendix for interpretation of low-level mixtures)

**Rule:**

For two source mixtures where one source is known, an “owner” profile may be used to deduce the profile of the foreign contributor based solely on the presence of two obligate alleles.

**Clarification**

Because this assumes the presence of two sources where one source is known, the two detected obligate alleles need to be >AT and consistent with being sister alleles.

**Documentation**

Because this kind of profile assessment relies on assumptions:

- report language refers to component as deduced (or deduced major, as applicable)
- report language must include assumptions relied upon (two source mixture, identity of one source is known)

Electropherogram must be noted to clearly indicate which loci are eligible for statistics:

- Loci are noted as 2 OBL on the electropherogram, with the two obligate alleles indicated.
- Loci which are not eligible for a single source statistic are noted on the electropherogram as ND (not deduced) or NS (not statistic-eligible)

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**Criteria for deducing a (major) contributor based on genotype assessment**

*Refer to Appendices for [flowcharts of deductions](#) at 2-allele and 3-allele loci.*

NOTE: The deduction flowcharts are designed for interpretation of most loci where all alleles are above IT. It may be possible to deduce a foreign contributor profile at a locus where one or both of the owner alleles is below IT but above ST, or a locus where all alleles are above IT but where the flowcharts lead to not deducing. To deduce such a locus, the analyst may use the MIW to document their assessment of all possible genotypes for the locus for each possible combination by using the contributor ratio to calculate PHRs for the possible genotypes. When this documented assessment clearly indicates only one genotype combination that is reasonable, given the contributor ratio for the profile as a whole, then the locus can be deduced. It is recommended that this process be used only when it has the potential to impact whether or not a mixture is distinguishable.

**Rule**

Mixtures which contain DNA from two individuals in a proportion of ~1/2 to 2/1 cannot readily be separated into major and minor contributors, due to the fairly even balance between contributors. In these cases, it may be possible to use an “owner’s” profile to deduce the genetic profile for an unknown contributor. For body swabs, the “owner” is the person from whose body the sample was collected. For non-intimate samples, the “owner” is a person whose DNA might reasonably be expected to be present on an item, based on information from the submitting agency. Examples of this would be a person’s own DNA detected on his/her clothing or property, described as belonging to that person on a Request for Laboratory Service form or evidence packaging. Assumptions relied upon for deduction must be documented in the case report.

**Explanations/Clarification**

For mixtures with a contributor ratio less than 1:2 (unknown: owner), an elimination sample can be used to deduce only based on alleles detected, not on peak height ratios.

For profiles which contain contamination: if the source of contamination can be identified, it may be possible to use its known profile to deduce a profile that is suitable for comparisons.

It may not be possible to deduce an unknown contributor at all loci; those loci which cannot be deduced must be labeled as such. The unknown contributor profile must be deduced at a minimum of four statistic-eligible core loci in order to be deemed suitable for comparison. A deduced profile (or partial profile) is treated as a single source profile for the purposes of drawing conclusions and issuing statistics.

Several criteria should be taken into consideration when deducing an unknown contributor using peak height ratio information:

- Deduction must include a genotype assessment based on the presence of obligate allele(s). All genotypes incorporating the obligate allele(s) must be considered in determining the deduced profile.
- In samples with high quantities of DNA, relative amounts of DNA from each contributor can be approximated by examining loci with four alleles. However, these approximations are only reliable when:



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- At least two loci with no allele sharing between contributors (preferably four-allele loci, but three allele loci will work if the known contributor is homozygous) are averaged together
  - No alleles below ST are present
  - SE33 is not included in the calculation
  - There is no minor, low-level third source in the sample
- Peak height ratios may be used to compare different genotype possibilities when alleles are above the IT. When deducing based on allele calls (e.g. a locus with two alleles foreign to the owner's profile), alleles do not need to be above IT or ST.
- Based on observations of casework data and validation studies, the following loci do not exhibit sufficient reliability of peak height ratios to support deduction which relies upon peak height ratios: D22S1045, SE33, and D2S1338. However, these loci can be deduced based on the presence of obligate alleles, if appropriate.
- As a general rule, based on validation studies, heterozygous alleles from a single contributor should have a peak height ratio (PHR) of 60% or higher when the peak heights of both alleles are above the IT. When peak heights are below the IT, greater variability may be observed.
- When any alleles at a locus are below the ST, the possibility of allelic drop-out must be considered. Therefore, such loci are only suitable for deduction if two obligate alleles are above the AT.

**Exceptions:**

- The flowcharts in the appendices were designed based on validation studies. However, on occasion, the flowcharts may lead to a conclusion which does not make sense in conjunction with the calculated contributor ratio. When an analyst believes the deduction protocol leads to a potentially incorrect conclusion in light of the contributor ratio, the analyst should not deduce the locus, and document the reason for that decision on the Mixture Interpretation Worksheet.

**Documentation**

- The rationale for deduction of the genetic profile for an unknown contributor when deduction is performed based on the flowcharts in the Appendices must be documented on a Mixture Interpretation Worksheet, which is included in the bench notes.
- Report language must state assumptions, such as two source mixture and identity of the known contributor.

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### **6.1.5 Single source minor / deduced component interpretation**

#### **Probative comparisons must assess whether minor component is complete at each locus**

Refer to Appendices for a [flowchart](#) of interpretation of low-level minor component of a distinguishable mixture.

#### **Rules**

- If a two source mixture can be successfully separated into major and minor components, the minor component can be considered complete single source at heterozygous loci only. A minimum of four heterozygous minor loci are required for interpretation, probative comparisons, and statistics.
- If the profile has fewer than 4 heterozygous minor loci, it may be interpretable as an indistinguishable mixture.

#### **Exception / Clarification and Documentation**

Refer to the clarification provided for separating a major component, including [separation by peak heights](#), [separation by obligate alleles](#), and [separation by genotype assessment](#). Bear in mind that, with respect to minor components, separation based on peak heights can only lead to an interpretable minor component with a two source mixture.

#### **Non-probative comparisons for a minor component**

#### **Rules**

Certain special cases are routinely encountered in forensic casework which lead to minor components for which probative comparisons and statistical analysis are not a necessary consideration. Two common examples of these situations are minor components arising from differential carryover and minor components consistent with an “owner” on an intimate sample. These are situations in which a given profile might reasonably be expected to be present on an item. These conclusions are reported with a qualitative statement in the report, provided the following minimum criteria are met:

- Two source mixtures only
- Non-probative profile is fully detected at a minimum of four loci (homozygous or heterozygous, below ST, and non-core locus SE33 are acceptable for this purpose)
- Non-probative profile is fully detected at one or more heterozygous loci
- No DNA inconsistent with the expected non-probative profile (and/or artifacts) is detected in the profile/partial profile/minor component.

If these minimum criteria are not met, no qualitative statement can be made regarding the source of the DNA.

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## Explanation / Clarification

### Minor components arising from differential carryover

The differential extraction protocol is designed to optimize the separation of spermatozoa DNA from epithelial cell DNA. However, this separation process is rarely perfect. As a result, carryover is often observed, where the major contributor to one fraction is observed as a minor contributor in a different fraction – for example, from a vaginal swab which was differentially extracted, the major contributor to the sperm fraction may be detected as a minor contributor in the epithelial fraction. Because more than one profile is associated with the sample, some additional interpretational concerns apply specifically to instances of low-level differential carryover.

- When all minor component alleles are attributable to differential carryover, the analyst will report that DNA consistent with differential carryover was observed in the minor component. This is reported if applicable for all sperm and sperm/substrate fractions, as well as epithelial fractions from non-internal cavity samples. Epithelial fractions from internal cavities, such as oral, rectal, or vaginal swabs, are run as a quality assurance check only and are not routinely included in reported conclusions. For epithelial fractions from internal cavities, the checking for differential carryover is a part of the review process and does not require further documentation.
- When all minor component alleles are attributable to a combination of differential carryover and artifact/true alleles, the analyst will describe results in the reported conclusions as described above.
- Minor components which are attributable to differential carryover do not require a separate statistic, if a statistic is being issued for its primary fraction. Example: A swab from a condom is differentially extracted. The sperm fraction yields a single-source male profile. The epithelial fraction yields a major component consistent with being from a single source female, and the minor component is consistent with carryover from the sperm fraction. In this case, a single source statistic would be issued for the sperm fraction and for the major component from the epithelial fraction. It is not necessary to run a statistic on the minor component of the epithelial fraction, since the minor is attributable to the sperm fraction from the same swab.

### Minor components consistent with an “owner” on an intimate sample

When a sample is collected directly from a person’s body, it is not unusual to detect DNA consistent with that person in the sample. When the minor component of an intimate sample is attributable to the owner, or the owner and artifact/true alleles, it is described in the reported conclusions (except for internal/cavity samples). Underwear and clothing collected directly from a person can be considered as intimate samples and may be reported with a qualitative conclusion instead of a statistic.

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### **6.1.6 Indistinguishable two source / two source major mixture interpretation**

#### **Two source indistinguishable mixture (~1:2 to ~2:1 contributor ratio)**

Interpretable data: A mixture must have sufficient information at a minimum of 15 statistics-eligible loci (or 75% of detected statistics-eligible loci, if profile is partial) to perform comparisons to probative reference samples. In a two-source mixture, the locus can be considered completely detected, interpretable, and suitable for mixture statistics when

- Four alleles (or a heterozygous minor component) are detected, or all detected alleles are above the IT AND it is reasonable to assume all alleles are fully detected
- Peak heights above IT are logically consistent with two contributors

In a two-source mixture, if a locus cannot be considered complete, it should be noted as not suitable for mixture statistics (NS<sub>mix</sub>) on the electropherogram.

Uninterpretable data (QAS 9.6.5): An indistinguishable mixture is uninterpretable when:

- Three or more sources are present
- Two or more sources are present in an indistinguishable minor component
- Less than 75% of the detected loci in a two-source mixture can be considered complete
  - In a severely degraded mixture, a minimum of four complete loci (out of six or fewer detected loci) are required for interpretation

#### **Clarification / Explanation**

To be suitable for statistical analysis, a locus must be deemed complete. As the quantity of DNA present becomes smaller, more concerns about possible drop out arise. Specific concerns include:

- Stochastic drop-out: when one allele is detected above AT but below ST, it is possible that a heterozygous sister allele has dropped out.
- Stutter masking: an allele may be effectively filtered out by the stutter filter associated with a large peak.
- Allele stacking: an allele shared by contributors may appear to be above the ST, when the portion of the allele contributed by the minor contributor is actually below the ST. In this case, drop out of a sister allele could happen even when the called sister allele is above ST. Therefore, the ST alone cannot predict the possibility of sister allele drop out.
- Complete drop-out: in instances where the major component is much larger than the minor component, the minor component may drop out entirely.

These concerns must be considered when assessing whether alleles at a given locus are completely detected. Given that the presence of multiple sources of DNA in a sample can make peak height ratio information potentially less reliable, the more conservative IT is applied here.

Degradation of one or both contributors may complicate the assessment of indistinguishable two source mixtures. If degradation impacts decisions made during interpretation, a note must be added to the electropherogram explaining the reasoning.

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## Two source distinguishable mixture

In order to assess when it is reasonable to assume complete detection in a distinguishable mixture:

1. Calculate the relative contributor ratio, based on at least two loci with four alleles above ST and not including SE33. Document this calculation on the electropherogram (Ex. Based on the ratios observed at D3 and D5, the relative contributor ratio is ~4/1) Note: if there are not at least two four-allele loci with all four alleles above ST, then it is only reasonable to assume full detection at loci with two heterozygous minor alleles.
2. Based on the peak heights of the alleles detected at the locus, would a *heterozygous minor* contributor have peaks below the IT? If one minor allele is present and above the IT, this is less of a concern, since complete drop out would not be expected when the sister allele is above IT. However, when no minor alleles are detected separately from the major component alleles, it may be due to either allele sharing with the major contributor or allelic drop out. When no minor alleles are detected separately from the major alleles, the contributor ratio can be used to calculate the predicted height of minor alleles at the locus, and thus to assess the possibility of drop out. It is important to keep in mind that the calculated contributor ratio assumes that either both the major and the minor are heterozygous, or both the major and the minor are homozygous.
  - a. If the major is heterozygous, multiply the smaller major peak by the contributor ratio. If the resulting hypothetical peak height is below IT, then the locus cannot be considered complete due to the possibility of stochastic drop out.
  - b. If the major is homozygous, multiply the major peak height by half the contributor ratio. This is necessary to compare a homozygous major with a heterozygous minor. For example, if the ratio of a homozygous minor allele to a homozygous major allele is 1/4, then the ratio of each heterozygous minor allele to the homozygous major allele would be 1/8. If the calculated comparison is below the IT, the locus cannot be considered completely detected due to possible drop out masked by allele stacking.
3. Based on the peak heights of the alleles detected, could a lower-level heterozygous contributor (calculated as in step 2) have peaks that could be filtered out by a stutter filter on one of the detected alleles? If so, the locus cannot be considered completely detected due to possible stutter masking.

Note: If close biological relatives potentially involved, four-allele loci may not be available to calculate contributor ratios, and an alternative means of determining a relative contributor ratio should be discussed with the Technical Manager.

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### **Two source major component**

Distinguishable mixtures of this type will result in a two source major component suitable for probative comparisons.

Separating a two-source major component will usually only be possible when the major mixture is well-balanced (~2:1 to 1:2) and when 1 ng of DNA is amplified. Separating out a minor third contributor is often further complicated by allele sharing between the major contributors as well as with the minor contributor. The minor component will not be suitable for comparisons based on the number of contributors, likelihood of allele sharing, and possible drop out.

- Major component alleles must be above IT
- At loci where the major component has one or two alleles, minor component allele(s) peak height must be below 10% of the smallest major allele
- At loci where the major component has three or four alleles, minor component allele(s) peak height must be below 20% of the smallest major allele.
- If these conditions are not met, the locus cannot be separated.
- If fewer than fifteen statistic-eligible loci (or 75% of detected statistic-eligible loci) are distinguishable, it is not appropriate to separate the profile into major and minor components

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### 6.1.7 Comparison of STR DNA Results

#### Criteria used for the formulation of conclusions:

When an analyst assesses a questioned sample profile to determine its suitability for making comparisons to reference samples and calculating population frequency statistics, there are several considerations to make for the profile as a whole:

- Probative versus non-probative: thresholds for comparison differ for some non-probative samples, such as intimate samples. These are described in more detail in the preceding sections. Also, probative comparisons require statistics, while non-probative comparisons do not.
- Degradation / inhibition: when extreme inhibitory or degradation effects are present, or when degradation impacts contributors in a mixture to differing degrees, the analyst must consider the impact on the interpretation of the whole profile.
- Degree of relatedness: some case scenarios which indicate the close relatedness of individuals in the case may prompt the analyst to interpret with greater caution and/or include report language to address the possibility of relatedness.
- Completeness of a locus: for probative comparisons, only complete loci are suitable for determining inclusions and exclusions
- Assumptions: when assumptions are used for deducing foreign contributors they must be documented in the case file. These typically include
  - Two source mixtures
  - Owner / known contributor
- Minimum thresholds for number of complete loci required for comparison, as well as the criteria for assessing completeness of each locus, are described previously in this section.

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

- Exclude
- Cannot exclude
- Foreign DNA detected
- Inconclusive
- Insufficient DNA / Data
- No genetic profile
- Complex mixture

**Note:** Comparisons and conclusions for Forensic Parentage cases are addressed specifically in the Forensic Parentage section.

#### **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.

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.



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**Cannot Exclude:**

For single source samples (including resolved mixtures): The donor of the known sample cannot be excluded as a source of the questioned sample because there are no significant differences between the allele designations obtained from these samples.

For unresolved 2-source mixtures: When alleles detected in a known/reference sample are also detected in at least 75% of the detected 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.

**Foreign DNA Detected:**

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 also detected in the sample.

**No Genetic Profile:**

No genetic profile is declared when there is reportable data at fewer than four loci. This includes fewer than four complete core loci in probative samples, as well as those which do not meet minimum requirements for intimate swabs consistent with owner or differential carryover.

**Inconclusive:**

If the STR results support neither inclusion nor exclusion, the comparison will be deemed inconclusive and reported as such. When a profile is deemed inconclusive, an explanation must be included for the reason. Common reasons for inconclusive results are insufficient DNA/data or genetic complexity of a mixture.

**Insufficient DNA/Data:**

Low amounts of DNA can lead to stochastic effects such as allelic drop out, elevated stutter, and peak height ratio variability. In many instances, incomplete loci cannot be appropriately used to make exclusions or inclusions. When fewer than four loci can be considered complete, this profile will be considered insufficient and will not be utilized for comparison.

**Complex Mixture:**

A complex mixture may be declared when DNA from multiple sources is detected in a sample, there is evidence of stochastic effects (i.e. drop out) at several or all loci (from limited DNA), and/or a clear major component cannot be deduced. Accordingly, the sample will be deemed not suitable for comparison. This is the case for indistinguishable mixtures with three or more contributors.

**Close Biological Relatives:**

In a mixture where two close biological relatives (parent-child or full siblings) cannot be excluded as contributors, a comparison can still be made and a mixture statistic issued if appropriate. However, the report must include an additional statement to clarify that a modified statistic that takes into account relatedness may be more appropriate. At this time, the Alaska

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Crime Laboratory does not perform such statistics; but upon request, the DNA Technical Manager can refer agencies to outside resources.

### **Non-probative associations**

Many situations arise in forensic casework where a positive association between a questioned sample and a known sample (inclusion) does not have probative value for the criminal investigation. Examples of non-probative comparisons would be:

- Owner on a body swab
- Owner on an item of clothing or piece of property where the attribution to the owner is not in question (e.g. owner on an item of clothing collected from the owner, or steering wheel swab from the owner's car)
- Victim's consent partner on victim's body swabs

In these cases, a qualitative statement describing the association is sufficient for reporting purposes; statistical analysis is not required for these associations. Examples of qualitative statements for non-probative associations are listed in the DNA reporting guidelines.

These associations rely on the assumption that the individual's DNA may reasonably be expected to be present. This assumption can be documented in the report in one of the following ways:

- Swabs collected from a person's body are identified as such in the Item Description section of the report.
- Clothing or property collected from a person are identified as such in the Item Description section of the report. Alternatively, a statement is added to the conclusion for that item, indicating that the item has been previously identified as belonging to the person.
- Elimination samples and consent partners are identified as such in the Item Description section of the report. Alternatively, a positive association between a questioned body swab or clothing and a known consent partner includes a statement in the conclusion that this individual has been previously identified as a consensual partner of the person in question.

### **Probative associations**

A positive association between a questioned sample and an individual whose DNA is not reasonably expected requires population statistics. If a profile is not of sufficient quality or quantity to calculate population statistics, then it is not suitable for performing comparisons to probative reference samples.

A single statistic may be calculated for multiple identical profiles. (Example: two blood stains on a shirt yield identical profiles, with a positive association to a probative reference sample).

If profiles from multiple items are positive associations to the same individual but are not fully identical profiles (typically due to partial drop out in at least one profile), statistics must be calculated for each of the items.

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It may be appropriate to issue multiple statistics for a single item, if those statistics reflect different associations made to that item. (Example: a swab from a boot yields a single source major component as well as a minor component which is suitable for comparisons as a mixture. If this profile has positive associations to two different people, it is appropriate to run both a single source statistic for the major component, and a mixture statistic for the minor component. If only one positive association is made, it is only necessary to calculate the relevant statistic for that association.)

Note: A sample which has undergone differential extraction yields sperm, substrate and/or epithelial profiles from the same item. Differential carryover is frequently observed in such samples. It is not necessary to calculate a separate statistic for the minor component of a fraction that is attributable to differential carryover if the probative contributor has been addressed in a different fraction. Likewise, if multiple fractions from one differential extraction yield profiles consistent with being from the same individual, it is only necessary to issue a statistic on one of those profiles, since all fractions originate from the same single sample.

#### **Data Interpretation for forensic parentage**

On occasion, reference samples may be of poor quality or degraded by environmental conditions. For such samples, loci not deemed complete (due to the possibility of partial or complete allelic drop-out) will not be considered exclusionary solely on the basis of incomplete information. All complete loci will be considered in assessing consistency between reference samples.

- Four or more loci with non-consistent genetic markers – the alleged parent(s) is/are excluded. It is not necessary to calculate Combined Parentage Index (CPI) in such cases.
- Two or three loci with non-consistent genetic markers – these results are inconclusive. The CPI will be calculated and reported, but further testing with additional markers will be recommended to the submitting agency.
- One locus with an inconsistent genetic marker within one repeat unit of the obligate allele – given the possibility of a genetic mutation, this will not be deemed an exclusion. In such a case, the alleged parent(s) cannot be excluded, and CPI will be calculated and reported.
- All loci have consistent genetic markers – the alleged parent cannot be excluded, and the CPI will be calculated and reported.

#### **Reinterpretation of data typed with a legacy amplification kit**

On occasion, the laboratory may be asked to revisit a case where analysis has been performed using a legacy amplification kit, defined as an amplification kit no longer covered by the current SOP (e.g. PowerPlex 16 or Profiler / CoFiler). Examples of this situation would include:

- Evaluation of a moderate stringency match in CODIS
- Submission of new questioned evidence in an old case
- Submission of new reference samples in an old case

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Legacy data is suitable for comparisons if it can be used “as is” – in other words, as originally interpreted by the analyst. Typically, this would include:

- Single source questioned profiles
- Single source major component profiles from questioned mixtures
- Single source deduced profiles
- Two-source indistinguishable mixtures where a stochastic threshold was in place
- Reference profiles
  - While most reference profiles will not need to be retyped, it may be beneficial to retype reference samples when complex mixture interpretation is required in the current amplification kit.

Reinterpretation of legacy data is not permitted. Reinterpretation includes assessing or evaluating allele calls or genotype calls (including potential for drop out), changing assumptions used, or removing loci from statistic calculations. Because of extensive changes in mixture interpretation policy over time, indistinguishable mixtures in legacy data with more than two sources as well as all indistinguishable mixtures interpreted without a stochastic threshold would require reinterpretation.

Exceptions may be possible if the analyst and technical reviewer have been proficiency tested in the legacy kit within two years of the reinterpretation request. However, this would require documented and technical manager approved review of relevant validation studies and legacy SOPs.

Consult with the Technical Manager first if a situation arises that involves legacy data requiring interpretation. If reinterpretation of legacy data is requested, the analyst should discuss options with the requesting agency, possibly including re-amplification of previously generated extract.

Most CODIS profiles generated by legacy kits do not require reinterpretation and can be used “as is”, as described above. However, some CODIS profiles from previously analyzed casework may require reinterpretation for comparison. When CODIS profiles that would require reinterpretation of legacy data are encountered, notify the CODIS Administrator or alternate CODIS Administrator. If the Administrator agrees that the profile would require reinterpretation for comparison, then the profile will be removed from CODIS.

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### 6.1.8 Statistical Analysis of STR Data

*References: Moretti, T. et.al, Population data on the expanded CODIS core STR loci for eleven populations of significance for forensic DNA analysis in the United States (2016) Forensic Science International: Genetics 25: 175 - 181.*

*Budowle, B. et al., Population studies on three Native Alaska population groups using STR loci. (2002) Forensic Science International 129: 51-57.*

*Expanded Loci Genotyping and Statistical Evaluation of Three Alaskan Native Populations (FBI correspondence, posted on lab website)*

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 positive association is to a probative reference sample. The statistic is calculated from the questioned profile and includes the statistics-eligible loci which are completely detected and eligible for population statistics. (Note: in the rare case that a known sample does not yield a full profile at all statistics-eligible loci, then the questioned profile loci which could not be compared to the reference sample are not included in the statistic.)

For differential samples, a separate statistical analysis is not required for differential carryover in a minor component, if statistics are calculated from the relevant individual(s) in a different fraction from the same item.

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. Combined Parentage Index calculations are performed as applicable for forensic paternity cases. 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). For frequencies more rare than 1 in 330 billion (approximately 1000 times the population of the United States), a ceiling value of 1 in 330 billion is reported.

The Alaska Scientific Crime Detection Laboratory routinely calculates the frequency/probability and reports the most common from among the following populations: Caucasian, African American, Athabaskan, Inupiat and Yupik.

Caucasian and African-American population frequency statistics are calculated using the Expanded FBI STR 2015 frequency database. Theta correction factor for these populations is 0.01.

Athabaskan, Inupiat, and Yupik population frequency statistics are calculated using the Alaska Expanded Core 2017 frequency database. Theta correction factor for these populations is 0.03.

[Protocols for performing statistical calculations on STR data are in the appendices.](#)

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## 6.2 Y-STR Data Interpretation

Interpretation of casework samples is based on the guidelines contained in this section, as well as the qualified DNA analyst's training and experience, experimental results, and case specifics. In all cases, interpretations must be independently reviewed and verified based on established peer review procedures. The DNA Technical Manager may authorize the interpretation/reporting of data outside of the criteria defined herein and will document this approval by initialing and dating comments on the relevant paperwork.

This section covers the following topics:

- [Criteria to evaluate internal size standards, allelic ladders and analytical controls](#)
- [Criteria for the interpretation of non-allelic peaks, allelic peaks, and mixtures](#)
  - [PowerPlex Y23 stutter percentages](#)
- [Interpretation of a Y-STR single source profile – probative inclusions](#)
- [Interpretation of a Y-STR single source profile – non-probative inclusions](#)
- [Interpretation of a Y-STR profile – exclusions](#)
- [Comparison of Y-STR results](#)
- [Performing Y-STR population frequency statistic calculations](#)

### Y-STR Interpretation Summary Table

**Note:** when a peak in a two-source mixture is determined to be a shared peak, that locus “counts” toward minimum distinguished loci.

**Note:** Contributor ratio (CR) calculated using 4 loci (both alleles > IT is possible)

Comparison	Rule	Minimum (distinguished) loci	Exception/Clarification
Probative inclusions	Single source profile	12 loci	Only DYS385 needs to be >ST to be considered complete
	Single source deduced contributor	12 loci	2 allele locus: probative allele > ST
			1 allele locus: probative allele > IT and CR used to rule out stutter masking
			DYS385: only separate if 2 obligate alleles
	Single source major or minor from a two source mixture	12 loci	Major > IT, PHR 60% or less, CR used to assess shared peak
	Single source major from a 3 or more source mixture	16 loci	Major > IT and PHR 60% or less
Non-probative inclusions	Single source profile or component	6 loci	All alleles accounted for by elimination
Exclusions	Single source or two source mixture	6 complete loci	Completeness in a mix locus means: *alleles(s)>ST *alleles in stutter position >ST after max stutter removed

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## 6.2.1 Criteria to evaluate internal size standards, allelic ladders, and analytical controls

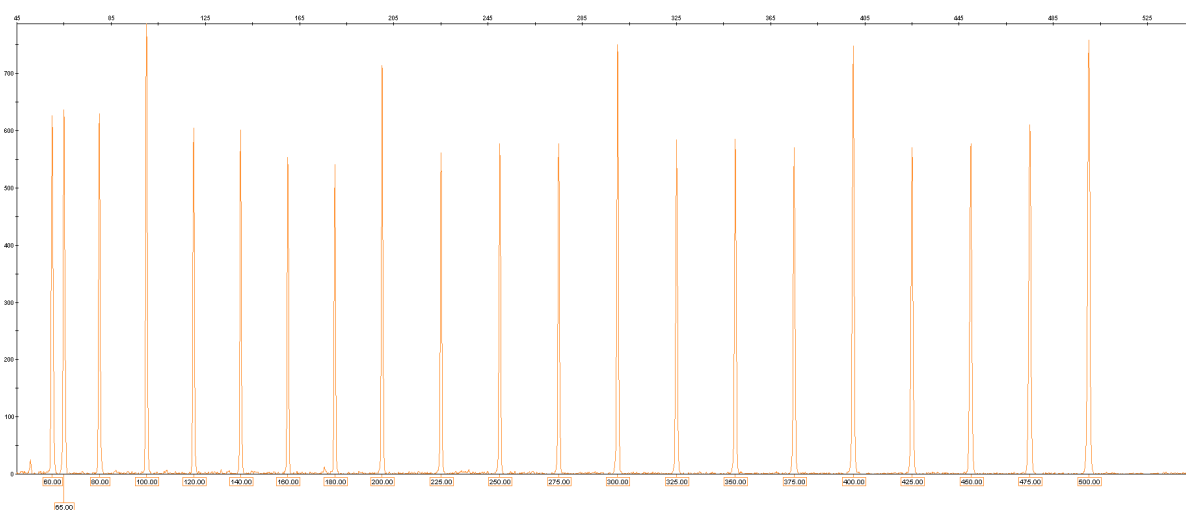
### **Interpretation of Batch Controls (QAS 9.6.1)**

Prior to assessment of casework data, the analyst must first examine the batch controls to ensure that the extraction, amplification and genetic analysis processes are functioning correctly. These include: Internal Lane Standard (ILS), allelic ladder(s), internal control specimen (ICS), positive and negative amplification controls, and extraction reagent blanks. All or part of an extraction, amplification and/or run may need to be repeated depending on the results of the batch controls. Any issues raised by the performance of a batch control must be addressed prior to the release of affected casework samples. Unresolved failure of a batch control requires the Technical Manager, or designated individual, to approve a course of action.

Review of batch controls is routinely performed by viewing the project in GMID-X. The analyst performing the review visually examines each of the batch controls (without relying solely on software quality flags). This electronic review is documented on the Central Log Checklist. Printouts of batch control documentation will be included in the Central Log only when the control in question requires further investigation, such as contamination assessment in a reagent blank.

### **Internal Lane Standard (ILS)**

PowerPlex Y23 is run with WEN ILS 500 Internal Lane Standard. The analyst should verify that all peaks from 60-500 base pairs are present and labeled as shown. This verification may consist of confirming the sizing quality is greater than 0.5. If the sizing quality is 0.5 or lower, the analyst must confirm by visual inspection that each peak was correctly called. This check must be documented on the electropherogram by the analyst and initialed by the technical reviewer for casework profiles, or in the central log documentation for control samples.





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

Failure of an ILS can often be resolved by re-prepping and/or re-injecting the affected sample(s). If the ILS continues to fail after re-prepping and re-injecting, the analyst should consult the Technical manager for a course of action.

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

### **Allelic Ladder**

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 PowerPlex Y23 allelic ladder are shown in the following image. The analyst should verify that all peaks from the allelic ladder(s) are present and labeled as shown.

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PowerPlex Y23 Allelic ladder:



The allelic ladder contains the most common alleles determined for each of the following loci: DYS576, DYS389 I, DYS448, DYS389 II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385, DYS456, and YGATAH4. In addition, alleles not labeled in the allelic ladder (virtual alleles), may be detected and labeled in some of the samples analyzed.

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

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particular locus, the remaining allele designations / labels at the locus shift and will impact the correct allele call / labeling of the samples in the project. Allelic ladders with such artifacts cannot be used to analyze data in the project. In such instances, re-injection is usually appropriate.

### **Internal Control Specimen (ICS)**

ICSs are usually addressed prior to Y-STR analysis and are only amplified for Y-STRs when recommended by the Technical Manager for a specific troubleshooting issue, or when the batch as a whole includes only Y-STR analysis. Refer to the STR section for guidance on addressing ICS assessment and ICS failure.

### **Positive Amplification Control**

A positive control is defined as a single source sample whose genetic profile was previously determined and from which a full profile was developed; it is used to evaluate the performance of the amplification and typing procedures. DNA 2800M is used as a positive control for amplification with PowerPlex Y23 Kit. A minimum of two positive amplification controls are routinely amplified and processed concurrently with each casework questioned amplification. A minimum of one positive amplification control is routinely amplified and processed concurrently with each casework known amplification. (QAS 9.5.3.1)

The presence of artifacts in a positive control does not constitute a failed positive control, so long as they are not consistent with contamination. Artifacts are struck in the GMID-X project and checked during the electronic review of batch controls.

Positive control failure and uninterpretable data: A positive control sample which yields no profile, an incorrect profile, or an incomplete profile is considered a failed positive control. All data from any amplification which does not have at least one passing positive control is considered uninterpretable until and unless the issue is resolved.

It may be necessary to re-prepare and/or re-inject the positive control if the cause of failure appears to be a failed electrophoretic injection. As long as the re-prepped and/or re-injected positive control types successfully, the positive control is considered successful.

Alternatively, other samples in an amplification may serve as a positive control (e.g. duplicate offender/staff sample, offender confirmation sample, casework known sample with a verified profile, or a buccal swab obtained from laboratory personnel whose genetic profile(s) was/were previously determined and documented). Use of an alternate positive control must have documented approval of the Technical Manager.

All casework samples co-amplified will be re-analyzed if no positive control was successfully typed for that amplification. The laboratory will routinely include more than one positive control with every casework amplification reaction that includes questioned samples, in order to prevent reanalysis of samples that are limited in DNA content. If at least one of these positive control samples produces acceptable and expected results, the batch will not be reanalyzed.

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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 from either (but not both) amplification/run may be used.

#### **The PowerPlex® Y23 System Allele Determinations for 2800M Control DNA.**

DYS576	18
DYS389I	14
DYS448	19
DYS389II	31
DYS19	14
DYS391	10
DYS481	22
DYS549	13
DYS533	12
DYS438	9
DYS437	14
DYS570	17
DYS635	21
DYS390	24
DYS439	12
DYS392	13
DYS643	10
DYS393	13
DYS458	17
DYS385a/b	13, 16
DYS456	17
Y-GATA-H4	11

#### **Negative Amplification Control and Extraction Reagent Blanks**

A reagent blank is carried through the entire analytical process as part of each extraction or type of extraction. It contains all the reagents - except DNA template - used during extraction, amplification and typing. For questioned extracts, each reagent blank is DNA-Stable'd. Only one of the blanks (either replicate -1 or the blank with the higher observed quantification reading) is rehydrated with 17.5 µL sterile water and amplified. Reagent blank replicates not amplified are created as items in the LIMS and retained.

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 TE<sup>-4</sup> buffer used to dilute the DNA samples.

The purpose of the negative controls is to detect systemic DNA contamination that might occur from the reagents, the laboratory environment, between the samples being processed, and/or due to improper handling of the samples by the analyst. The negative controls must be run at the most stringent set of conditions for the batch. (QAS 9.5.3.1)

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Verification of the presence of amplicon in the negative controls is performed by viewing the presence of unincorporated primer peaks. The negative controls should not yield any true STR allelic peaks above the analytical threshold. When peaks greater than or equal to the reporting threshold are present in the range between 60-500 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 the AT will not invalidate the sample if the data is at levels too low for interpretation, or the peaks can be shown to be artifacts. Artifacts will be struck in the project and checked in the electronic review of the batch controls.

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.

Negative control failure and uninterpretable data: The failure of a negative control may indicate a problem at the extraction or amplification level. When probable true allele peak(s) are detected above the analytical threshold, the analyst will reanalyze the profile using the Global Filer troubleshooting analysis parameters (60 RFU). If the low-level profile has at least four complete loci detected at the troubleshooting threshold, this constitutes a failed negative control. All data from any amplification which does not have a passing negative amplification control is considered uninterpretable until and unless the issue is resolved. All samples which correspond to a failed reagent blank are considered uninterpretable until or unless the issue is resolved.

To resolve a failed negative control, the analyst will investigate possible causes of contamination and, if possible, will determine the source of the contaminating DNA. Such electropherograms are printed and included in the Central Log, with analysis parameters clearly noted. In some cases, the contamination may be attributable to a known source, such as an adjacent sample or a lab worker who was in close proximity to the extract.

- If the analyst can identify the source of the contamination, a Quality Review Form must be initiated to investigate the reason for the contamination. The DNA Technical Manager will then be consulted to determine the appropriate course of action.
- If the source of the contamination cannot be identified by comparison to relevant staff profiles and concurrently analyzed samples, it may not be possible to determine the source of the contamination. When the source of the contamination cannot be identified, and the level of contamination does not interfere with interpretation of casework samples, the analyst will complete a Contamination Assessment Form. This form is submitted to the DNA Technical Manager for approval. Once signed by the DNA Technical Manager, it is included with the Central Log documentation. The DNA Technical Manager will maintain a log of Contamination Assessment Forms, and their respective profiles will be kept in GMID-X for comparison purposes.
- If the level of contamination has the potential to interfere with casework interpretation, whether or not its source is identified, the DNA Technical Manager must be notified to determine an appropriate course of action.

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## **6.2.2 Criteria for the interpretation of non-allelic peaks, allelic peaks, and mixtures**

### **6.2.2.1 Criteria for interpretation of non-allelic peaks (QAS 9.6.2)**

#### **Distinguishing Alleles from Artifacts**

The initial assessment of a casework sample electropherogram separates interpretable alleles from artifacts. Artifacts can occur in data and much of their nature and origin have been determined and documented. Peaks determined to be artifacts are struck on the full view electropherogram. Peaks struck manually are also initialed by the analyst. When it is not possible to distinguish between an artifact and a true allele, this is indicated on the electropherogram as Artifact/True Allele (A/TA). If the presence of a definite or possible artifact at a locus impacts the suitability of that locus for either comparisons or statistical analysis, that is documented on the electropherogram. Artifacts are often associated with an overabundance of template DNA. If the presence of many artifacts complicates data analysis, it may be appropriate to re-amplify the sample with less template DNA.

On questioned samples, binned peaks which are called as alleles but determined to be artifacts must include an explanation of why the peak was determined to be an artifact (such as poor morphology, baseline noise associated with large peaks, pull-up, etc.). This explanation is documented on the electropherogram. Binned artifacts documented as pull-up also require an artifact view to show corresponding base pairs between large peak and the artifact in question. While these artifacts may not line up to have identical base pair sizes, they must be close (within 0.5 base pairs) and must correspond to a large peak (often off scale, but not necessarily) in another color. Artifacts in questioned samples which do not bin as alleles and are called OL, OMR or spike, may be struck without a documented explanation and without an artifact view printout. All artifacts in reference samples may be struck without documented explanation and do not require artifact view printouts. Stutter does not require artifact view printouts.

#### **Baseline noise**

The analytical threshold (AT) for Y-STR analysis of casework samples is 100 RFU. Data below the AT is considered indistinguishable from baseline noise and not suitable for interpretation. Ideally, true allele peak heights should fall between 2000 and 10,000 RFU. Samples with peak heights near or above 10,000 RFU may include baseline noise that exceeds 100 RFU. Such baseline noise may be struck. However, if baseline noise is difficult to distinguish from possible low-level DNA, it may be more appropriate to re-amplify the sample with less template DNA.

#### **Stutter**

The PCR amplification of tetranucleotide Y-STR loci typically produces a minor product peak four bases shorter (N-4), two bases shorter (N-2, infrequently observed), eight base pairs shorter (N-8) or four base pairs larger (N+4) 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. Loci with repeating units of different lengths have corresponding stutter as well.

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 maximum expected percentage of stutter for the loci in the PowerPlex Y23 System when run on the Applied

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Biosystems 3500xl. The stutter values are based on data obtained by internal validation studies as well as developmental validation studies. These values are set as stutter filters used by the GeneMapper ID-X software. N-(2 repeat units) stutter has been observed rarely, typically in samples in excess of 1 ng template DNA. As such, N-(2 repeat units) stutter is not included in the filters.

**Maximum Expected Stutter Percentages for PowerPlex Y23 loci on the 3500xl (in single source samples)**

	REPEAT	N-1 UNIT	Stutter percentage	N+1 UNIT	Stutter percentage
DYS576	4	N-4	16.4	N+4	5
DYS389 I	4	N-4	8.2	N+4	5
DYS448	6	N-6	4.0	N+6	5
DYS389 II	4	N-4	15.2	N+4	5
DYS19	4	N-4	11.2	N+4	5
		N-2	10.2	N+2	3.8
DYS391	4	N-4	12.3	N+4	5
DYS481	3	N-3	29.8	N+3	5
DYS549	4	N-4	11.4	N+4	5
DYS533	4	N-4	10.7	N+4	5
DYS438	5	N-5	5.8	N+5	5
DYS437	4	N-4	8.4	N+4	5
DYS570	4	N-4	15.9	N+4	5
DYS635	4	N-4	13.0	N+4	5
DYS390	4	N-4	13.4	N+4	5
DYS439	4	N-4	10.7	N+4	5
DYS392	3	N-3	17.8	N+3	11
DYS643	5	N-5	3.9	N+5	5
DYS393	5	N-5	15.1	N+5	5
DYS458	4	N-4	16.3	N+4	5
DYS385	4	N-4	15.7	N+4	5
DYS456	4	N-4	15.0	N+4	5
YGATAH4	4	N-4	11.1	N+4	5

Peaks in the stutter position greater than the listed values may indicate the presence of DNA from more than one individual. However, elevated stutter is also associated with low amounts of template DNA. Many peaks which appear as elevated stutter are below the stochastic threshold, where increased variability in peak heights can be expected.



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### **Elevated + / - stutter, assessment of stutter / true allele, and amplification and injection artifacts**

Refer to the discussion in the [Global Filer interpretation section](#), for discussion of stutter artifacts, pull-up, spikes, dye blobs, -A, persistent kit artifacts, and off-ladder (OL) alleles.

### **Null alleles**

Primer binding site mutations may occasionally result in a null allele, where no allele is amplified at a given locus. A locus may be called as having a null allele without confirmation by re-amplification:

- only in single-source profiles,
- when all other alleles in the profile are greater than 1200 RFU, and
- based on quantitation results, there is no indication that the proposed null allele could be the result of degradation or inhibition.

A null allele that meets these conditions is designated by writing “Null allele” on the electropherogram at the locus. The null allele may be included for purposes of population frequency Y-STR database searches.

### **Duplications**

DYS385 a/b often has two alleles present in a single source profile. Occasionally, a single-source sample may be observed to have two alleles at one (rarely more) other locus/loci. In a single source profile, two alleles may be considered a true duplication without confirmation by re-amplification if both alleles are above 1200 RFU and have a peak height ratio of 60% or greater.

It may be possible to search a locus with a duplication in population statistic Y-STR database searches.

### **Contamination**

The verification of reagents prior to use in casework is conducted in part to detect contamination which permeates an entire lot of a given reagent or set of reagents. However, such checks will not necessarily detect contamination arising from a single event (for example, cross-contamination between two adjacent samples).

For casework samples, whenever data suitable for comparison is not attributable to any reference samples associated with its case, the possibility that the profile was introduced via contamination must be considered. As a part of the review process, both the analyst and the technical reviewer must rule out laboratory staff working in direct proximity to the open evidence as well as cross-contamination within the batch of samples. This comparison may be done manually or via the Profile Comparison tool in GeneMapper ID-X. The same principle applies to control samples where no DNA should be detected.

Validation studies have shown that it is possible for some female contributors to show some low-level peaks when amplified with PowerPlex Y23. In situations where an unidentified low-

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level male contributor is observed, it may be necessary to rule out low-level detection of a female contributor.

#### 6.2.2.2 Criteria for the interpretation of allelic peaks

##### Assessing interpretable alleles (QAS 9.6.3)

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, and they are clearly distinguishable from artifacts. All peaks are examined and evaluated in the process of data interpretation.

##### Three types of thresholds used for data analysis and interpretation (QAS 9.6.4)

- Analytical Threshold (AT) of 100 RFU: below this level, it is not possible to distinguish artifacts from true alleles. The detection limit set for data analysis is defined by the AT.
- Stochastic Threshold (ST) of 600 RFU: below the ST, there is a greater likelihood of drop-out of a sister allele at a heterozygous locus. When data is present below ST, allelic drop out must be a consideration. Additionally, data below ST may require extra caution in determining whether or not a peak is a true allele.
- Interpretational Threshold (IT) of 1500 RFU: above the IT, peak height ratios are minimally affected by stochastic effects. Below IT, and to a greater extent below ST, peak height ratios can vary significantly from predicted values.

##### Alleles below the Stochastic Threshold

Stochastic effects result when limited DNA molecules are used to initiate PCR, and unequal sampling of the two alleles present from a heterozygous individual leads to peak height imbalance, elevated stutter, and/or allelic dropout. The ST of 600 RFU is based on validation studies and represents the RFU value below which stochastic effects can lead to drop out of a sister allele at a duplication locus, typically DYS385. Peaks which fall between the AT and the ST are subjected to a higher degree of scrutiny due to the possibility of stochastic effects.

Note that when an allele is in a stutter position, the “stacking” of stutter with the peak can artificially inflate the peak height. Allele stacking must be considered when assessing whether a probative allele lies above or below the ST.

**Reported** refers to alleles whose peak heights are equal to or above the ST and are generally reproducible if re-amplified. Such alleles are routinely used in making comparisons between known and questioned sample genetic profiles under the interpretation guidelines stated in this manual. These alleles may also be described as **detected** in report language.

**Observed** refers to data (peaks) below the ST. Alleles below ST may be considered when reporting the presence of an elimination reference on a sample such as body swabs or clothing.

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On occasion, depending on the quality of the data and the degraded or inhibited nature of the sample, such alleles may be used to generate a composite profile, with the documented approval of the DNA Technical Manager.

**Alleles below the Interpretational Threshold and at or above the Stochastic Threshold**

Alleles in this range may be subject to stochastic effects that impact the reliability of peak height ratios between major and minor contributor alleles. As such, data below IT is less likely to be suitable for separating into major and minor components. Interpretation which relies on peak height ratios is typically only done when the larger allele is above IT.

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### 6.2.2.3 Criteria for the interpretation of mixtures

#### **NOTE: Null alleles and duplications**

The mixture interpretation guidelines for all Y-STR loci except DYS385 are predicated on the assumption that each contributor has one and only one allele per Y-STR locus. If one or more contributors have either a null allele or a locus with allele duplication, mixture interpretation may not be appropriate at that locus or loci. This determination may only be apparent after the reference sample profiles are generated. If a relevant reference sample is found to have a null allele or a duplication which has the potential to impact mixture interpretation of a corresponding questioned locus:

- At that locus/loci, the questioned profile should not be separated (major/minor or deduction)
- If interpretation changed, a comment must be added to the bench notes to explain why the questioned profile locus interpretation changed after examination of the reference profile(s)
- Questioned profiles at the non-separated locus/loci should still be compared for possible exclusions
- Unseparated loci are not eligible for calculation of population frequency statistics.

#### **Distinguishing between multiple binned artifacts and a minor contributor**

The amplification template target of 0.5 ng is used in part because minimal artifacts, including stutter, are detected at that target value. However, even when 0.5 ng is amplified, occasional instances of elevated stutter, particularly +4/-4 stutter, may be observed. In addition, pull-up between dye colors and instances of elevated baseline noise or “drop-in” have been observed, especially when peak heights exceed 10,000 RFU. Elevated stutter, both +4 and -4, is more common with low template DNA.

Validation studies indicate that most binned artifacts occur at peak heights below 600 RFU, including elevated stutter, pull-up, and baseline noise. As such, all peaks below 600 RFU that suggest a possible mixture / additional contributor must be interpreted with caution, and possible reasons that could lead to an artifact being called as a true allele must be ruled out.

#### When low level peaks are assessed as not an additional contributor:

If an electropherogram has 4 or fewer peaks below ST, all of which can be attributed to attributable artifacts (such as stutter or pull-up), the profile should be considered as not having a low-level minor contributor; provided all such artifacts are documented as such on the electropherogram. For interpretation purposes, the remaining profile is treated like a single-source profile, and it is not described as a major component.

#### When low level peaks are assessed as an additional contributor:

A profile is generally considered a mixture when two or more alleles above ST are present at two or more single-copy loci. In these cases, the low-level alleles are marked in parentheses, and the report references that a minor component was detected.

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When low level peaks are assessed as a possible contributor:

In between the situations described above are situations where a minor contributor may or may not be present. In these instances, the report language must reflect the uncertainty of whether there is a true minor component (e.g. DNA from an additional male source may be present)

Some common interpretation scenarios are described in the table below:

IF profile includes a contributor >IT plus...	AND the low-level peak(s)	THEN Report language for the high level	AND Report language for the low level
4 or fewer peaks below ST	<b>All</b> could be attributable to artifacts	Treat like a single source profile	Not mentioned
5 or more peaks below ST	(how many could be artifacts doesn't matter)	Describe as a major component	...more than one contributor may be present...
4 or fewer peaks below ST	<b>At least one cannot</b> be attributable to an artifact	Describe as a major component	...more than one contributor may be present...
1 or more low-level peaks	One is above ST <u>and</u> not attributable to an artifact	Describe as a major component	...more than one contributor may be present...
2 or more peaks >ST	(Artifacts are not routinely observed above ST, but the possibility should be ruled out)	Describe as a major component	Male DNA from more than one source was detected...

**Number of possible contributors**

Note: It is not possible to declare the number of contributors with absolute certainty, since more than one person could have the same Y-STR profile. The Y-STR interpretation guidelines in this manual were written for situations in which the case specifics, including both case scenario and the observed reference profiles, do not suggest the presence of close male relatives within the pool of possible contributors. When case specifics suggest the possibility of close male relatives, interpretation must consider the potential for a very high degree of allele sharing. Please consult with the Technical Manager in such cases to determine an appropriate course of action.

Genetic profiles from questioned samples may be from a single individual (single source) or from multiple individuals (mixtures). Assessment of whether a profile is a single source or mixture, a minimum number of contributors, and whether a mixture can be separated out into single source component(s), determines subsequent interpretation. Documentation of interpretation, including assumptions, must be included in the bench notes. Typically, this documentation is written on the electropherogram or included in the written report language.

Unlike autosomal STR analysis, Y-STR mixtures are not used for inclusions. However, in some circumstances, Y-STR mixtures may be used for exclusions. The guidelines for interpretation of a Y-STR profile are highly dependent on whether a single source profile can be drawn from the data.

Indistinguishable mixtures may be suitable for exclusionary purposes. Only single-source components isolated from mixtures are suitable for probative inclusions as well as exclusions.

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Mixtures might be distinguishable by deduction, major/minor separation, or occasionally by a combination of the two methods.

### **Y-STR Single Source Samples**

In a typical single source sample, loci are characterized by one labeled peak or allele. If two peaks/alleles are detected at DYS385, they should ideally be of equal intensity – that is, the peak height ratio (PHR) is approximately 1:1.

In a typical single source profile, DYS385 is the only locus where two peaks might be observed. Peak height ratios are calculated by dividing the peak height (in RFUs) of the lower RFU allele by the peak height of the higher RFU allele, and then multiplying by 100 to express the PHR as a percentage. Based on validation studies, the minimum expected PHR for a heterozygous locus, where there is no indication of a mixture and 0.5 ng of template DNA is used, is 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 at DYS385. If only one allele is detected at DYS385, and it is below ST, the locus may be suitable for exclusions only.

Note: Because DYS385 is the only locus where heterozygous alleles are routinely encountered, it is not necessary for alleles at other loci to be greater than ST in a single source sample to be considered complete and therefore suitable for inclusions and exclusions.

### **Y-STR mixtures**

A profile is interpreted as consistent with being from two individuals when no loci other than DYS385 have more than two alleles. Peaks which have been determined to be artifacts do not apply. Peaks which could be artifacts or true alleles do apply.

Note: When peaks are above IT but some loci have peak height ratios that vary widely (such as of 90% and 20%), it suggests the possibility of a third, likely closely related, contributor. Alternatively, large variance in peak height ratios when peaks are below ST could indicate degradation or low-level template DNA. Regardless of the cause, if no single copy loci have more than two alleles but the peak height ratios are not largely consistent, the profile should be reported as having at least two contributors.

Relying on the profile being a mixture from exactly two individuals, it is possible to assume the minor component is complete under the conditions specified in the following sections.

Above two contributors, profiles are reported as being from at least three individuals, at least four individuals, and so on. For each minimum number of contributors, there must be at least two loci with that number or more alleles above ST. For example, if least two single-copy loci have four alleles above ST, then DNA from at least four individuals was detected in the sample.

The assessment of a questioned sample profile takes place with 'sequential unmasking': initial assessment occurs before any probative reference samples are compared to that profile. However, in some cases an elimination sample (such as from a consent partner) is available for

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comparison. In such cases the profile from the elimination sample may be used to assist in interpretation of the questioned sample.

### 6.2.3 Interpretation of a Y-STR Profile for probative inclusions

#### Probative inclusions

A single source profile must be suitable for comparison at a minimum of 12 loci to be deemed suitable for probative inclusions. This applies to:

- profiles with a single source of DNA
- deduced contributors
- single-source major component from a mixture
- single-source minor component from an apparent two-source mixture

#### Deduced contributors

A Y-STR profile may be suitable for deduction when:

- Both an elimination sample and a probative reference sample(s) have been submitted for analysis
- The questioned profile is consistent with being from two individuals
- The questioned profile cannot be separated into major and minor components
- Little or no degradation effects are present in the questioned profile
- If deduction is not possible at a minimum of 12 loci, the deduction should not be performed

#### Rules for deduction

- Begin by calculating a contributor ratio by averaging the peak heights of elimination and probative alleles from at least four two-allele loci, using alleles above IT (above ST is acceptable when there are not four loci with both alleles above IT).

For loci with two alleles:

- The probative allele must be above ST. If the probative allele is in a stutter position, max stutter is subtracted before determining if the peak is above ST.
- If the locus is suitable, deduce the non-elimination allele as the probative allele.

For loci with one allele:

- The allele in question must be above IT.
- Based on the contributor ratio, determine whether the probative allele would be below ST or potentially masked by stutter filter. If the probative allele would be less than ST or potentially masked, the locus cannot be deduced.
- If the locus is suitable, deduce one allele shared by elimination and probative contributor

For DYS385:

- This locus can only be deduced when two obligate probative alleles are present.

Documentation of deduction is noted on the electropherogram.

- Assumptions are that the mixture is 2 sources only, and the elimination reference is one contributor. These must be stated in the report and do not need to be written on the electropherogram.
- Contributor ratio calculation is included on the electropherogram



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- Loci not deduced are noted as ND
- Elimination alleles are noted with an “E”
- Deduced probative alleles are noted with a “P”
- A deduced shared peak is noted with “SP”

#### **Rules for major/minor separation in mixture consistent with being from two sources**

- Major component allele must be above IT
- Peak height ratio must be 60% or less
- A major component must be separated at a minimum of 12 loci according to these rules, or major/minor should not be separated
- DYS385 cannot be separated into major and minor components if more than one allele is present. If only one allele is present and it is above IT, it may be included in the major component.
- Under these conditions, a single peak locus may be inferred to be a shared peak that includes both the major and minor contributor:
  - A contributor ratio can be calculated using four two-peak loci where both the major and minor alleles are above IT (ensures the consistency in the peak height ratios)
  - The minor: major contributor ratio is greater than 1:5 (ensures that the minor component will be above stutter percentages at all loci)
  - The peak at the single-allele locus is above IT
  - A single peak allele which is determined to be a shared peak is noted on the electropherogram as “SP”.
- The assumption of the mixture being consistent with being from two individuals must be included in the report language.
- If at least 12 minor alleles (not shared) are above ST, the minor component and the major component are each suitable for inclusions and exclusions.
- If at least 6 minor alleles (not shared) are above ST, the minor component is suitable for exclusions only and the major component is suitable for inclusions and exclusions.
- If fewer than 6 minor alleles (not shared) are above ST, the minor component is not suitable for comparisons and the major component is suitable for inclusions and exclusions.

#### **Rules for major/minor separation in a mixture of at least two sources**

- Major component allele must be above IT
- Peak height ratio must be 60% or less
- A major component must be separated at a minimum of 12 loci according to these rules, or major/minor should not be separated
- DYS385 cannot be separated into major and minor components if more than one allele is present. If only one allele is present and it is above IT, it may be included in the major component.
- Under these conditions, the major component is suitable for inclusions and exclusions, but the minor component is not suitable for comparisons due to the possibility of drop-out or multiple contributors.

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**Rules for major/minor separation in a mixture with at least three or more contributors**

- Major component allele must be above IT
- Peak height ratio must be 60% or less
- A major component must be separated at a minimum of 16 loci according to these rules, or major/minor should not be separated
- DYS385 cannot be separated into major and minor components if more than one allele is present. If only one allele is present and it is above IT, it may be included in the major component.
- Under these conditions, the major component is suitable for inclusions and exclusions, but the minor component is not suitable for comparisons due to its genetic complexity.

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#### **6.2.4 Interpretation of a Y-STR profile for non-probative inclusions**

A single source profile, partial profile, or minor component from any sample which is solely attributable to an elimination sample is not used for statistical analysis but is addressed in the report with a qualitative statement. Such statements may be used when alleles are suitable for comparison at a minimum of 6 loci are detected and the elimination sample profile can account for all the alleles in question.

#### **6.2.5 Interpretation of a Y-STR profile for exclusions**

A single source or mixture profile may be suitable for exclusions, even if it is not suitable for inclusions. A profile (or partial profile) is suitable for exclusionary purposes when:

- Locus (or component) can be considered complete
- For profiles with a mixture: allele(s) at locus (or component of locus) are above ST. For alleles in stutter positions, max stutter is subtracted, and the remaining peak height must be above ST to be considered for exclusions.
- At least six loci meet these criteria

Examples of profiles that would be suitable for exclusions only include:

- partial single source profile with fewer than 12 loci detected
- indistinguishable two-source mixture with no indication of drop-out
- distinguishable minor component in an apparent two source mixture, detected at 6-11 loci

Exclusions are based on the presence of an obligate allele above ST which does not match the reference profile. Exclusions in minor components or low-level samples may not be based on the absence of an allele, because low-level contributors may have data which falls below the detection threshold due to stochastic effects.

Low-level minor components where more than one contributor may be present and low-level indistinguishable mixtures of 3 or more sources are not suitable for exclusions.

#### **6.2.6 Comparison of Y-STR Results**

Refer to STR interpretation section for discussion of types of result comparisons for probative and non-probative samples.

In a case where Y-STR profiles suggest a close biological relationship between potential contributors (parent-child, siblings, or any individuals with Y-STR profiles that differ at no or very few loci), the ability to draw conclusions may be impacted. Reported conclusions must reflect any impact caused by potential relatedness among submitted references.

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### 6.2.7 Probative associations and statistical analysis

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 positive association is to a probative reference sample. The statistic is calculated from the questioned profile and includes the statistics-eligible loci which are completely detected (or have a declared null allele) and are eligible for population statistics. (Note: in the rare case that a known sample does not yield a full profile at all statistics-eligible loci, then the questioned profile loci which could not be compared to the reference sample are not included in the statistic.)

Statistical analyses are performed by searching the profile on the YHRD website.

- From the home page, select Search the database from the top menu
- Select Manually enter the haplotype/haplotypes to search for
- Select PowerPlex Y23 in order to rearrange data entry to the PowerPlex Y23 kit order.
- Enter data for all loci suitable for inclusions, then click Search
- In the blue bar, click Add features to this report
- From the dropdown menu, choose National Database (with subpopulations, 2014 SWGDAM-compliant)
- Once again, in the blue bar, click Add features to this report
- From the dropdown menu, choose Metapopulation
- In the result box that appears under National Database (with subpopulations, 2014 SWGDAM-compliant) result box, Click to change, then choose Eskimo Aleut form the dropdown menu.

The most common frequency among the Caucasian, African American, Native American, and Eskimo Aleut population groups is reported, using the profile probability with 95% confidence interval.

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## **Section 7     *Report Writing***

### **7.1     Required Content**

#### **Required Content for Biological Screening Reports**

All biological screening reports will contain the following:

- Date report was issued (automatically populates)
- Laboratory and agency case numbers (automatically populates)
- Name of submitting agency and case officer (automatically populates)
- List of all items for which analysis was requested in the assignment
  - 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 #
- Results of presumptive/confirmatory tests (in body of report)
- Results, conclusions, and opinions for all tested items (guidelines for reporting are provided later in this section and may be modified, as necessary, on a case by case basis)
- Explanation of why testing was stopped, if appropriate
- The disposition of all tested retained items
- Any known samples that are required for DNA analysis, when applicable
- 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; the analyst is required to scan their barcode and enter a pin when signing the report in LIMS)

#### **Forensic Biology report required content**

All Forensic Biology reports will contain the following (as applicable to the analysis performed):

- Date report was issued (automatically populates)
- Laboratory and agency case numbers (automatically populates)
- Name of submitting agency and case officer (automatically populates)
- List of all items for which analysis was requested in the assignment
  - Includes identification of which samples (such as stains, swabs, or other sub-items) were tested. In this context, tested means that an attempt was made to isolate DNA from the sample.
  - 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 #
  - elimination reference samples are noted as such in parentheses, such as (previously identified as a consent partner of Jane Smith)
  - items which belong to a specified individual are noted as such in parentheses, such as (previously identified as belonging to Jane Smith)
- 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.**"

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- Include the issuing laboratory, if other than the AK SCDL.
- Results of presumptive/confirmatory tests (in body of report) if not previously reported in a biological screening report
- Results, conclusions, and opinions, for all tested items, based on the DNA typing results where appropriate (guidelines for reporting are provided in section 9.3 and may be modified, as necessary, on a case by case basis)
- Results of any statistical analyses performed
- Explanation of why testing was stopped, if appropriate
- Statement of which profiles have been entered (or removed) in CODIS, if appropriate
- Description of analyses performed, including technology used and a list of STR loci amplified (this is contained in the Methods section of the report)
- Description of statistical analyses performed including the methods and loci used in the calculations (this is contained in the Methods section of the report)
- The disposition of the evidence (this is usually contained in the standard report template)
  - Consumed evidence is noted in the body of the report.
  - Retained sub items may be noted in the items analyzed section. For example, if two isolated stains were retained from item 123, then the 123 item description would also include “123-1 and 123-2 were retained as 123JLF”
- Any known samples that are required for (further) DNA analysis, when applicable
- Signatures of the reporting analyst and the technical reviewer (the analyst will electronically sign the report by setting the milestone to Draft Complete prior to submitting for technical review; only the analyst is required to scan their barcode and enter a pin when signing the report in LIMS)

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## **7.2 Items Received By Analyst – common report terminology**

<b>Sample type</b>	<b>Sample report notation</b>
Swabs from evidence collection kit	123-V Vaginal swab(s) under Items Received  Vaginal sample from Jane Smith under Results Conclusions and Opinions
Blood or buccal reference	Known DNA sample from John Smith
Stains or samples retained separately from original item	12-1 through 12-5 were retained as 12JLF
Item belongs to known person	Blouse (from Jane Doe)
Known consent partner	Known DNA sample from John Smith (identified as consent partner of Jane Doe)



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### 7.3 Results, Conclusions and Opinions – common report terminology for screening

Blood Findings	Report
Phenolphthalein (Kastle-Meyer) negative (chemically or visually)	Blood: (visually/chemically) negative (No further analysis was performed on this sample.)
Phenolphthalein (Kastle-Meyer) positive	Blood: presumptive positive
Phenolphthalein (Kastle-Meyer) inconclusive	A presumptive test for blood was inconclusive. Test results could not be observed because of interference from (colored) substrate material beneath the stain.
Seminal Fluid	Report
FBB negative; P-30/ABA card negative (chemically or by ALS)	Semen: (chemically / ALS) negative (No further analysis was performed on this sample.)
P-30/ABA card positive	Semen: presumptive positive
P-30/ABA card inconclusive	This PSA test was deemed inconclusive due to (repeated) failure of the internal control on the test card

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.
ABA card inconclusive	This Hematrace test was deemed inconclusive due to (repeated) failure of the internal control on the test card

Hair/Fiber Evidence	Report
Hairs/debris recovered/not recovered	(No) Possible hairs/debris were observed/recovered from (item).

Contact/wearer	Report
Cuttings or swabbing from items	This sample was collected for contact/wearer sources of DNA.

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**7.4 Results, Conclusions and Opinions – common report terminology for items stopping after quantification – no samples amplified**

For samples not amplified	Report
No male DNA (or T-Y < 0.0007 ng/μL) detected	Quantification results do not indicate sufficient DNA for further analysis.
Female: Male ratio >4:1 OR T-Y is 0.026 – 0.0007 ng/μL in a suspect case (at any female: male ratio)	Quantification results indicate sufficient DNA for Y-STR analysis (may be present if the sample is consumed.)  (No further analysis will be performed on this sample without written permission from the Department of Law for consumption of the sample in its entirety.)  (A reference sample from the suspect is required before Y-STR analysis can occur.)
T-Y is 0.026 - 0.0007 ng/μL in a non-suspect case AND female: male ratio is greater than 5:1 (cut-off changes if DI > 1.2)	Quantification results indicate sufficient DNA for Y-STR analysis may be present if the sample is consumed.  Should a suspect be developed, this sample can be analyzed further if written permission is obtained from the Department of Law for consumption of the sample in its entirety, and if a reference sample from the suspect is also submitted.
Consumed sample - T-S is < 0.053 ng/μL (cut off changes if DI > 1.2)  (If likely mixture, female: male ratio is 4:1 or less and T-Y is at least 0.026 ng/μL)	Based on the low quantity of DNA present in this sample, the recommended STR amplification procedure may consume the remaining sample in its entirety. Alternatively, this sample may be suitable for Y-STR analysis.  No further analysis will be performed on this sample without written permission from the Department of Law to either consume of the sample in its entirety for STR analysis or to

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	proceed with Y-STR analysis only.  (A reference sample from the suspect is also required before Y-STR analysis can occur.)
Extract where <b>female</b> DNA is probative <b>and</b> DNA screening indicates unlikely to yield female profile	Quantification results indicate that this sample is not likely to yield an interpretable female profile due to the high concentration of male DNA. No further analysis was performed on this sample.
T-S is 0.002 ng/μL or lower for a likely single source or 0.007 ng/μL for a likely mixture	Quantification results do not indicate sufficient DNA for further analysis.
Based on DNA Screening and/or DNA testing, not every extract proceeds to DNA analysis	Quantification results indicate sufficient DNA for (Y-STR) analysis.  However, based on the results obtained from other items, no further analysis will be performed on this sample.

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**7.5 Results, Conclusions and Opinions – common report terminology for items stopping after quantification – other samples amplified**

Extract suitable for analysis but not amplified for current report	Report
<p>T-Y is 0.026 ng/μL or higher (if male is probative) <b>and</b> T-S ≥ 0.053 ng/μL <b>and</b> female: male ratio is 4:1 or less</p> <p>Or for Y-STR analysis: T-Y is 0.026 ng/μL or higher (if male is probative) <b>and</b> female: male ratio is higher than 4:1</p> <p>Other samples suitable for STR analysis based on Quantifiler Trio results</p>	<p>Quantification results indicate sufficient DNA for (Y-STR) analysis.</p> <p>However, based on the results obtained from other items, no further analysis will be performed on this sample.</p>

Other possible samples for testing / screening	Report
<p>Submitted items such as clothing, bedding, or additional swabs were not tested for the issued report but could be viable options for a further round of testing. (This statement is <u>not</u> used regarding items not at the lab or items which would not be considered reasonable candidates for analysis.)</p>	<p>The submitted request for laboratory services (RLS) sought analysis beyond that already completed and reported above. Please contact the reporting analyst, after consulting with the Department of Law prosecutor assigned to this case, to discuss the need for additional testing.</p>

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### 7.5 *Results, Conclusions and Opinions – common report terminology for amplified items*

For single source samples – probative associations	Report
Single Source (include 'deduced' any time the profile relies on assumptions of two source mixture and one source known; include major or minor any time the profile relies on separation based solely on peak heights)	<p>A single source (partial) DNA profile from (the major/minor component of) this sample MATCHED the DNA profile from XXX. Therefore, XXX cannot be excluded as the source of DNA detected in this sample.</p> <p>[or, DID NOT MATCH and was excluded, if applicable]</p> <p>[previously identified as consent partner statement, if applicable]</p> <p>[if applicable: Due to possible drop out / Because the major and minor components could not be separated, the following loci are not suitable for calculation of population frequency statistics: XXX, YYY, and ZZZ.]</p> <p>[stat statement here, if applicable]</p> <p>[CODIS statement here, if applicable]</p> <p>[If applicable: Additional DNA (from at least one male individual) was observed in this sample, but it is too low-level for probative comparisons.]</p>
Same profile (for 2+ items)	The genetic profiles obtained from these samples were the same.
Single source statistic	The estimated frequency (XX loci) of the genetic profile from the above sample(s) is rarer than 1 in 330 billion (or, is 1 in at least XXX).

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Gender statements	Report
Unknown Male DNA	A (deduced) 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.
Male DNA present	DNA from (a OR at least one) male individual was observed in this sample. Note: this statement is usually not necessary if a male reference sample is included as a possible contributor.

For non-probative associations	Report
No DNA inconsistent	No DNA inconsistent with XXX (and YYY) was detected in this sample.
DNA consistent	DNA consistent with XXX (and YYY) was detected in (the minor component of) this sample
Differential carryover	DNA consistent with carryover from the differential extraction process was observed in (the minor component of) this sample.
Item belongs to owner (only if not mentioned in item description)	This item has previously been identified as belonging to XXX.
Association is to a consent partner (only if not mentioned in item description)	YYY has previously been identified as a consent partner of XXX.

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CODIS related	Report
Profile(s) entered	This (these) profile(s) have been entered into the Combined DNA Index System (CODIS), where regular searches will be performed. Notification will be issued if there is a hit in the database or if the profile(s) is/are removed from CODIS at any time in the future.
Profile removed – matched consent partner or elimination	The genetic profile from item XXX has been removed from CODIS because it can be attributed to a non-probative individual.
Profile removed – not suitable for comparison by current interpretation	<p>Based on changes to DNA interpretation guidelines since the XX/XX/XX DNA report by YYY was issued, the DNA profiles obtained from the following items and any comparisons previously reported were revisited.</p> <p>A review of the (partial) profile from item Z determined it (the minor component) is not suitable for probative comparisons due to (insufficient data, genetic complexity of the mixture, etc.). This profile has been removed from CODIS and will no longer be searched.</p>



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For mixtures	Report
More than 1 individual	DNA from more than one/two/three individual(s) was observed in this sample.
To indicate # of individuals	DNA from at least XX individuals was observed in this sample. OR DNA from two individuals was observed in this sample.
Two source mixtures	<p>DNA from two individuals was observed in this sample. XXX (and YYY) were INCLUDED as possible contributors to the DNA mixture from this sample (20 loci).</p> <p>No DNA inconsistent with XXX and YYY was detected in this sample.</p> <p>OR XXX was INCLUDED as a possible contributor to the DNA mixture from this sample (20 loci).</p> <p>OR ZZZ was EXCLUDED as a possible contributor to the DNA mixture from this sample.</p> <p>[consent partner statement, if applicable]</p> <p>[loci not used in mix stats and reason, if applicable] [mixture stat statement, if applicable]</p> <p>[CODIS statement, if applicable]</p> <p>[If applicable: Additional DNA (from at least one male individual) was observed in this sample, but it is too low-level for probative comparisons.]</p>
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 (or, the minor component), no meaningful comparisons can be made to known reference samples.
Mixture statistic	Data below the reporting threshold may be present at the following core loci: XXX. Therefore, population frequency statistics are not reported for these loci.
Mixture statistic	The estimated probability (XX loci) of selecting an unrelated individual at random that can be included as one of the possible contributors to the DNA profile obtained from this sample is rarer than 1 in 330 billion (or, is 1 in at least XXX).
Close relatives	The mixture statistic listed above applies to random and unrelated individuals. In cases where close biological

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	<p>relatives are known or suspected to be contributors, a modified statistic which takes into account relatedness may be more appropriate. Please submit known samples for any additional suspected contributors of DNA as soon as possible. For more information please contact the laboratory's DNA Technical Manager, XXXXX XXXXX (269-XXXX) or (xxxxx.xxxxx@alaska.gov).</p>
Deduced Profile with Single Source statistics	<p>A two source DNA profile was obtained from this sample. A single source profile for the unknown (male) contributor was deduced using the reference profile from XXX. The single source DNA profile deduced from this sample MATCHED the DNA profile from ZZZ. Therefore, ZZZ cannot be excluded as the source of the deduced profile.</p> <p>[or, DID NOT MATCH and was excluded, if applicable]</p> <p>A single source DNA profile for the unknown contributor could not be deduced at the following core loci: XXX, XXX and XXX. (Therefore, these loci were not used to calculate population frequency statistics.)</p> <p>[previously identified as consent partner/elimination statement, if applicable]</p> <p>[stat statement here, if applicable]</p> <p>[CODIS statement here, if applicable]</p> <p>[If applicable: Additional DNA (from at least one male individual) was observed in this sample, but it is too low-level for probative comparisons.]</p>

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Contamination detected	Report
Profile matches lab staff	A quality assurance review of the DNA data has indicated that (the major / minor component of) this profile can be attributed to a laboratory staff member who was involved in evidence processing. As a result, this (component of the) profile was determined to be unrelated to the case.
Contamination matches a non-lab staff source	A quality assurance review of the DNA data has indicated that (the major / minor component of) this profile can be attributed to contamination. As a result, this (component of the) profile was determined to be unrelated to the case.
Profile with contamination is inconclusive with respect to further comparisons	(In addition to above language) This profile is inconclusive for interpretation.

Evidence consumed	Report
All of evidence used to make extract; all of extract consumed in analysis.	This sample was consumed in analysis.

Not Suitable for comparison	Report
Not suitable	DNA: not suitable for comparisons.  The data obtained from this sample was not suitable for comparison (due to XXXXXX).
No profile (<four reportable loci)	No genetic profile was obtained from this sample.
Inconclusive	No conclusions can be made as to whether XXX contributed DNA to this sample due to insufficient reportable DNA.
Insufficient	No (other) conclusions are reported for this sample due to insufficient reportable DNA.

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Reference sample needed	Report
<b>Reference required (suspect in STR case; suspect or consent in Y-STR case)</b>	<p>A known buccal swab from __ (DOB: ) is required to proceed with DNA analysis. Please ensure that both the name of the individual and at least one identifier (such as date of birth, APSIN, AK driver's license number, etc.) are included on the outer packaging of the sample prior to submission to the laboratory.</p> <p>Please contact the Forensic Biology Supervisor, Michelle Collins (907-269-5620 or <a href="mailto:michelle.collins@alaska.gov">michelle.collins@alaska.gov</a>), if you are unable to obtain the required sample. If no response is received in 30 days, the request for DNA analysis may be suspended.</p> <p>Note: Supervisor name and contact information should be specific to case type.</p>
<b>Reference desirable (consent partner in STR case)</b>	<p>A known buccal swab from __ (DOB: ) is recommended to proceed with DNA analysis. Please ensure that both the name of the individual and at least one identifier (such as date of birth, APSIN, AK driver's license number, etc.) are included on the outer packaging of the sample prior to submission to the laboratory.</p> <p>DNA analysis is pending.</p>

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## 7.6 Results, conclusions, and opinions specific to Y-STR analysis

The following reporting language is specific to Y-STR results and conclusions. Refer to previous report writing guidelines for more generally applicable report language.

Circumstance	Sample report language
Any time Y-STR analysis is performed. (Autosomal STR is considered the 'default' DNA analysis method and does not need to be specified.)	Y-STR analysis was performed on this sample.
Number of contributors	(Same as STR reporting, but specifies male individuals, such as: DNA from at least three male individuals was observed in this sample.)
Exclusions or Suitable for exclusions only	<p>(The minor component of) this sample is suitable for exclusions only.</p> <p>EITHER: XXX was excluded as the source of DNA detected in (the minor component of) this sample. Assuming no mutations in the Y chromosome, all paternal male relatives of XXX are also excluded.</p> <p>OR: No conclusions are reported as to whether XXX could be a contributor to (the minor component of) this sample due to insufficient DNA.</p>
Inclusions with frequency statistic	<p>The single source (deduced, partial) Y-STR DNA profile from (the major/minor component of) this sample (if not all loci, at XX loci) MATCHED the Y-STR profile from XXX. Therefore, XXX cannot be excluded as the source of DNA detected in this sample. Assuming no mutations in the Y chromosome, all paternal male relatives of XXX also cannot be excluded.</p> <p>[or, DID NOT MATCH and was excluded, if applicable]</p> <p>A single source Y-STR profile for the unknown contributor could not be deduced (or major/minor could not be separated) at the following loci: XXX, XXX and XXX. Therefore, these loci were not used to calculate population frequency statistics.</p> <p>This (partial) Y-STR profile is not expected to occur more frequently than 1 in XXX male individuals.</p> <p>[If applicable: Additional DNA (from at least one male individual) was observed in this sample, but it is too low-level for probative comparisons.]</p>

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## **7.7 Results, conclusions, and opinions specific to forensic paternity**

### ***Sample report language for one parent forward paternity***

#### **Inclusion:**

Based on the DNA profiles obtained from the samples listed above (XX loci), ALLEGED FATHER cannot be excluded as the possible biological father of CHILD'S NAME, assuming MOTHER'S NAME is the biological mother of CHILD'S NAME.

[If applicable: A single genetic inconsistency is observed for the obligate allele at locus \_\_\_\_\_. This is indicative of a mutation or recombination event in which the allele inherited from the alleged father by the child has been altered.]

Combined paternity index (CPI) indicates how many times more likely the observed genetic evidence is if the alleged father is the true biological father of the tested child rather than an unrelated individual from each of the following populations: Caucasian (AAAA), African American (BBBB), Athabaskan (CCCC), Inupiat (DDDD), and Yupik (EEEE).

#### **Exclusion:**

Based on the DNA profiles obtained for the samples listed above (XX loci), ALLEGED FATHER is excluded as a possible biological father of CHILD'S NAME, assuming MOTHER'S NAME is the biological mother of CHILD'S NAME.

#### **Inconclusive:**

Based on the DNA profiles obtained for the samples listed above (XX loci), no conclusions are reported as to whether ALLEGED FATHER could be a possible biological father of CHILD'S NAME, assuming MOTHER'S NAME is the biological mother of CHILD'S NAME. It is recommended that further testing be performed to gain additional information from more genetic loci.

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***Sample report language for zero parent forward parentage***

**Inclusion:**

Based on the DNA profiles obtained for the samples listed above (XX loci), ALLEGED FATHER cannot be excluded as the possible biological father of CHILD'S NAME.

[If applicable: A single genetic inconsistency is observed for the obligate allele at locus \_\_\_\_\_. This is indicative of a mutation or recombination event in which the allele inherited from the alleged father by the child has been altered.]

Combined parentage index indicates how many times more likely the observed genetic evidence is if FATHER'S NAME is the true biological father of CHILD'S NAME rather than an unrelated individual from each of the following populations: Caucasian (AAAA), African American (BBBB), Athabaskan (CCCC), Inupiat (DDDD), and Yupik (EEEE).

**Exclusion:**

Based on the DNA profiles obtained for the samples listed above (XX loci), ALLEGED FATHER is excluded as a possible biological father of CHILD'S NAME.

**Inconclusive:**

Based on the DNA profiles obtained for the samples listed above (XX loci), no conclusions are reported as to whether ALLEGED FATHER could be a possible biological father of CHILD'S NAME. It is recommended that further testing be performed to gain additional information from more genetic loci.



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***Sample report language for reverse parentage***

**Inclusion:**

Based on the DNA profiles obtained for the samples listed above (XX loci), ALLEGED CHILD cannot be excluded as the possible biological child of FATHER and MOTHER.

[If applicable: A single genetic inconsistency is observed for the obligate allele at locus \_\_\_\_\_. This is indicative of a mutation or recombination event in which the allele inherited from the parent by the alleged child has been altered.]

Combined parentage index indicates how many times more likely the observed genetic evidence is if ALLEGED CHILD is the true biological child of FATHER and MOTHER rather than unrelated individuals from each of the following populations: Caucasian (AAAA), African American (BBBB), Athabaskan (CCCC), Inupiat (DDDD), and Yupik (EEEE).

**Exclusion:**

Based on the DNA profiles obtained for the samples listed above (XX loci), ALLEGED CHILD is excluded as a possible biological child of FATHER and MOTHER.

**Inconclusive:**

Based on the DNA profiles obtained for the samples listed above (XX loci), no conclusions are reported as to whether ALLEGED CHILD could be a possible biological child of FATHER and MOTHER. It is recommended that further testing be performed to gain additional information from more genetic loci.

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## **Section 8      Review**

All case reports issued by the Forensic Biology discipline will be subjected to a technical review and an administrative review. All supporting documentation that is part of the case record or the central log will be subjected to a technical review.

Technical reviews of casework will be conducted by a second qualified analyst, in accordance with the laboratory Quality Assurance Manual (current version) and the FBI QAS Guidelines (current version) as applicable.

Administrative reviews of casework will be conducted by an analyst qualified for casework within the Forensic Biology discipline. Any report which includes DNA profile(s) must be administratively reviewed by an analyst qualified in DNA analysis. Note: technical and administrative review for a case may be performed by the same analyst.

The analyst and the reviewer may consult a third qualified 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 discipline checklist is 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.

When a case has been through technical and administrative review, the report will be issued in accordance with laboratory policies and procedures.

### **8.1      Adding your bench notes to JT and Central Log to the lab network for review**

- For casework bench notes
  - When the case is ready for review and you are about to “Draft complete” the report in JT, the PDF of the bench notes will be added to JT (prior to draft complete)
    - In JT under the “Attachments” tab right click on the applicable request “Add new attachment”; choose applicable PDF; rename (If needed) as “lab number bench notes original”
- From JT the technical reviewer will download the “case number bench notes original” PDF and save it to the applicable folder on the lab network (see Appendix C of this document)
  - In JT under the Attachments tab, right click on the original bench notes PDF; choose “download original” and save as “In tech review case number TR initials” (ex. In review 20-xxxxx JLF). This should be saved to the correct case folder on the lab network
- Add the completed bench notes with the check list to the appropriate request in JT “case number bench notes final”
  - This FINAL version of the bench notes is what will be sent out with a LEVEL 2 discovery request

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## 8.2 Review of Casework Analyzed In-House

Technical review of Forensic Biology casework includes the following, at a minimum and as applicable, to ensure compliance with the interpretation guidelines contained within this document:

- Review of all worksheets contained within the bench notes and central log
- Review (usually electronic) of the ILS for all passing samples
- Review (usually electronic) of all allelic ladders designated as such
- Review (usually electronic) of at least one passing amplification positive control
- Review (usually electronic) of all reagent blanks and negative amplification controls
- Review of DNA typing data (used to draw conclusions) for questioned and known samples
- Verification that all results/conclusions in report are supported by data
- Review of all statistical analyses
- Verification of CODIS eligibility and review of entry for all entered profiles
  - Eligibility review must occur prior to entry
  - Eligibility review of a Forensic Unknown profile is based on the electropherogram
  - Eligibility review of a Forensic Mixture or Forensic Partial profile is based on the Match Estimation report provided by the analyst along with the report and bench notes. The Match Estimation report should include notes of any loci to be marked as partial, as well as any notations of obligate alleles. Since this content is reflected in the profile that is ultimately uploaded, it is not necessary to retain the Match Estimation report once the review process has been completed.

Administrative review of Forensic Biology, DNA and DNA Screening casework will be conducted by a second qualified analyst, in accordance with the FBI QAS Guidelines (current version) and includes the following, at a minimum, to ensure compliance with the interpretation guidelines contained within this document:

- Review of case file and final report
- Review of chain of custody and evidence disposition
  - The chain of custody review will typically include checking that all retained evidence and dried extracts have been repackaged and returned to their appropriate locations.
  - In extreme rush cases, it may not be expedient to wait for extracts to be DNA Stable'd and repackaged before distributing the final report. In such cases, it is okay for the administrative review to not include a chain of custody review of final evidence returns. However, in such cases, this must be clearly noted on the review checklist in the chain of custody review row (check box marked NA, and reason given).

After the administrative reviewer has confirmed that the report and documentation are complete and correct, the checklist and any other paperwork not yet in the LIMS are returned to the analyst. All documentation (including bench notes, forensic histories, checklists, CODIS specimen detail reports, etc. as case appropriate) is scanned into the LIMS by the analyst. The final check of the administrative reviewer is to confirm that all materials scanned to the case file

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are correct and complete. The completion of the full administrative review, including a check of scanned documentation, is documented by the administrative reviewer milestone in the LIMS.

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.

### **8.3 Review procedure for outsourced casework**

Outsourcing of DNA casework must follow applicable requirements of FBI QAS Standard 17 (current version). Because details may vary between outsource contracts, this section of the FBCP will be updated as needed to reflect the procedures specific to the outsource contract.

The SCDL takes ownership of outsourced casework only when it will enter and search a DNA profile in CODIS from data generated by a vendor laboratory, or when it will use samples, extracts, or materials from the vendor laboratory for the purposes of forensic testing. Technical and administrative review of DNA casework will be conducted by qualified DNA analysts, either at the AK SCDL or contract technical reviewers, in accordance with the FBI QAS Guidelines (current version) and following the checklists provided either in this FBCP or as separate controlled documents.

If the technical reviewer finds any issues with an outsourced case, those issues should be brought to the attention of the DNA Technical Manager or designee, who will coordinate efforts with the vendor laboratory to resolve the issues. Profiles may not be entered into CODIS until technical issues have been resolved.

Together with the vendor laboratory review checklists, this section serves as guidance for the review of vendor laboratory data, reports, and documentation specific to cases submitted for analysis to Bode Technology, and to Marshall University for additional technical review (if applicable), as a part of the Capital Project.

TM = Technical Manager

FB sup = Forensic Biology supervisor

CTR = Contract Technical Reviewer

AK SCDL analyst = qualified casework analyst at Alaska Scientific Crime Detection Laboratory

CJP = Criminal Justice Planner

MU = Marshall University, contract technical reviewing agency

An overview of this process is summarized in the appendices

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### 8.3.1 Initial Case Assessment

When Bode performs casework analysis, they group their returned results according to whether or not cases have potentially CODIS eligible profiles. As a part of the initial assessment of the returned cases, a FB sup or TM will check the following:

- All Bode documentation for cases, controls, and reviews is downloaded to the laboratory network.
- Bode issued a report for each submitted case
- Appropriate submitted evidence was analyzed for each submitted case
- If the case has potentially CODIS eligible data,
  - relevant elimination samples are requested from the submitting agency
  - The RLS is checked for the reason the kit was previously un-submitted. If the reason is No Crime Occurred / Unfounded, or if the reason was not determined by the submitting agency, then clarification must be sought from the submitting agency.
- If the case will not be sent to Marshall University for technical review, the reason the case is documented on the Capital Project tracking spreadsheet, which may be one of the following:
  - No male/foreign DNA detected
  - Insufficient male/foreign DNA detected
  - No analysis was performed (not a kit, condition of evidence, etc.)
  - Profile not from putative perpetrator (This may be determined through Bode's analysis of elimination sample(s), or by prior in-house analysis of a reference sample from an elimination individual)
  - Anonymous victim
  - Case verified as no crime occurred or case unfounded. If the case documentation is unclear as to whether or not a crime occurred (or is unfounded),
- The SCDL does not take ownership of outsourced casework that does not yield potentially CODIS eligible profiles. Cases identified as not having potentially CODIS eligible results will not be sent for secondary technical reviews. Following the above checks, these cases will be noted as completed on the tracking spreadsheet.
- If the case will be sent to Marshall University for technical review, a Capital Project Bode Casework Review Checklist will be started

Marshall University will be notified approximately monthly of which cases are ready for review.

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When all of an agency's reports are returned from Bode and have been through initial case assessment, the TM or CJP will send all Bode reports to the agency using a secure information transfer device, such as a password protected thumb drive.

### 8.3.2 Technical review by Contract Technical Reviewer (CTR)

1. Technical review of reports with possible CODIS profiles and their corresponding controls will be performed by CTR, who reviews report elements as listed on Capital Project Bode Control Review Worksheet and Capital Project Bode Casework Review Checklist, including:
  - a. General: Batch documentation includes a list of all applicable related cases, as well as review documentation by the vendor lab. Any troubleshooting or quality issues raised in the batch controls are appropriately documented.
  - b. Extraction: Each set of concurrently extracted samples includes at least one reagent blank. All reagent blanks are quantified. At least one reagent blank per extraction set is amplified. Any contamination issues with reagent blanks have been documented and satisfactorily investigated.
  - c. Quantitation: standard curves have appropriate  $R^2$  and slope values; NTC values are appropriate, and reagent blanks were quantitated.
  - d. CE: reagent blanks, allelic ladders, positive controls, and negative controls are amplified with expected results obtained; ILS is confirmed for all controls.
  - e. Case files: all tested items (or probative fractions) are addressed, the CTR agrees with the reported conclusions, the conclusions are supported by the associated data, and the agency case and item numbers are correct.
  - f. If CTR does not agree with or finds issues to be addressed in the report, CTR notifies TM and works with Bode to resolve the concern.
2. CTR notifies TM or FB sup of completed reviews and returns completed checklists via a secure web portal.

### 8.3.3 CODIS entry of eligible profiles

1. Upload of profiles is performed by a AK SCLD analyst who is qualified for CODIS entry. AK SCDL analyst checks the following items prior to CODIS upload
  - a. Case was not determined to be No Crime Occurred or Unfounded
  - b. Correct specimen category identified
  - c. If the profile to be uploaded is a Forensic Mixture or Forensic Partial, the original 13 core loci must be checked with Match Estimator to ensure the entry will not cause excessive spurious matches. (See CODIS manual for details on use of the Match Estimator tool.)
2. AK SCDL analyst uploads profile to CODIS (if appropriate).

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3. AK SCDL analyst completes their section of the case checklist. The checklist and the CODIS specimen detail report are submitted to a FB sup or TM for final case assessment.

#### **8.3.4 Final case assessment**

1. The final case assessment is typically performed by the TM or FB sup but can be performed by an AK SCDL CODIS qualified analyst, if designated. The final case assessment includes the following elements:
  - a. Review of CODIS entry, including the profile, specimen category, and NDIS/SDIS eligibility.
  - b. Scan the completed and reviewed CODIS specimen detail report to the case file in LIMS.
  - c. Update Capital Project spreadsheet
  - d. Copy Code reports and documentation to case file in LIMS
  - e. Create and assign any CODIS hit letter requests that arise from Capital Project CODIS entries. Hit letters will be released as they are completed, following the distribution of the case file to the law enforcement agency.
  - f. Scan the completed review documentation to the case file in LIMS

#### **8.3.2.5 Monitoring performance of external providers**

##### **Procedure for monitoring performance of Bode Technology analysis of SAKs**

- Prior to start of analysis, points of contact are established for Bode Technology and the SCDL. Questions between the agencies are routed through these points of contact.
- As a part of initial case review, all returned reports from Bode are checked to ensure
  - reports are issued for all requested cases
  - all appropriate items were analyzed
  - names and item numbers in Bode report are consistent with Request for Lab Services (checked for cases with possible CODIS profiles)
  - case files are submitted for each case, and include necessary documentation
- As a part of the technical review of case files with potential CODIS profiles, Bode reports undergo a secondary technical review which encompasses the elements captured in the outsourced technical review checklist (OLTRW, current version).
- The FBI conducted on-site visit to Bode Technology is reviewed and accepted by the TM annually; documentation is maintained on the laboratory network.
- As a part of the forensic biology annual quality review, the TM or designee will complete the Standard 17 documentation review checklist.



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Actions arising from performance monitoring:

- Any issues discovered during initial case review at SCDL or during technical review at MU will be routed through the TM to the technical point of contact at Bode, or from the technical reviewer to Bode with the TM also being notified. The TM will review Bode's responses to ensure that quality concerns are adequately addressed. If corrected reports are issued, either the TM or the CJP will distribute to the submitting law enforcement agency. If corrected case documentation or control documentation is provided, the TM will ensure that it is added to SCDL documentation archives, either in the LIMS or the lab network, as appropriate.

**Procedure for monitoring performance of Marshall University contract technical reviewers**

- Prior to start of analysis, points of contact are established between SCDL, Bode, and MU.
- As a part of the forensic biology annual quality review, the TM or designee will complete the Standard 17 documentation review checklist.
- As a part of the CODIS entry procedure, SCDL analysts will confirm that profiles identified by MU technical reviewers as suitable for CODIS upload meet laboratory-defined eligibility criteria prior to CODIS upload.
- As a part of the final case assessment, FB sup or TM will confirm that TR documentation is correct and complete for case reviews and control reviews.

Actions arising from performance monitoring:

- Any issues discovered following technical review at MU will be routed through the TM to the technical point of contact at Marshall University. The TM will review MU's responses to ensure that quality concerns are adequately addressed. If corrected documentation is issued, the TM will ensure that it is added to SCDL documentation archives, either in the LIMS or the lab network, as appropriate.

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***Appendix A Revision History***

<b>FBPM 2021 R1 Page</b>	<b>FBCP 2021 R0 Page</b>	<b>Location</b>	<b>Revision made</b>
<b>NA</b>	<b>NA</b>	<b>throughout</b>	<b>Corrections</b> to grammar, spelling, and page and section numbering; <b>References</b> to QAS standard numbers
<b>239</b>	<b>243</b>	<b>Appendix QQ</b>	<b>Added</b> components of performance monitoring program and passing criteria for assessment of them

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**Appendix B Sexual Assault Kit Routine Guidelines for Initial Processing**

- Always review RLS and Forensic History first for case-specific details affecting triage decisions; may need to contact law enforcement for clarification
- Guidelines below are routine, but exceptions will be encountered. Reasons for exceptions must be documented in bench notes.

**Female victim kits**

Sample type	Routinely sampled?	Timeframe	Sampling and processing
Internal body swabs with suspected semen	Y for vaginal/ cervical N for oral and rectal	Up to 48 hours for vaginal and/or cervical; up to 24 hours for oral; up to 72 hours for rectal	<ul style="list-style-type: none"> <li>• all of each swab</li> <li>• differential if PSA + / direct w/DTT if PSA negative</li> <li>• vaginal/cervical combined</li> <li>• substrate processed separately</li> </ul>
Internal body swabs with suspected semen	Y for vaginal/ cervical N for oral and rectal	>48 hours to 7 days	<ul style="list-style-type: none"> <li>• all of each swab</li> <li>• vaginal/cervical combined</li> <li>• direct extraction w/DTT</li> </ul>
External body swabs with suspected semen	Y	Up to 48 hours	<ul style="list-style-type: none"> <li>• all of each swab</li> <li>• all "internal" external genitalia combined (inner labia majora/labia minora/perineum/introitus/etc.); "external" extremal genitalia swabs combined (outer labia majora/mons/etc.)</li> <li>• anal and perineum swabs combined if alleged anal penetration</li> <li>• differential extraction if PSA +; direct with DTT if PSA negative</li> <li>• sperm and substrate combined</li> </ul>
External body swabs with suspected semen	Y	>48 hours to 3 (or 4) days	<ul style="list-style-type: none"> <li>• all of each swab</li> <li>• all "internal" external genitalia combined (inner labia majora/labia minora/perineum/introitus/etc.); "external" extremal genitalia swabs combined (outer labia majora/mons/etc.)</li> <li>• anal and perineum swabs combined if alleged anal penetration</li> <li>• direct extraction w/DTT</li> </ul>
Body swabs with suspected saliva	N	Up to 2 days	<ul style="list-style-type: none"> <li>• ½ of each swab</li> <li>• swabs from one area are combined</li> <li>• direct extraction</li> </ul>
Body swabs for contact DNA (bruise, strangulation, etc.)	N	Up to 2 days	<ul style="list-style-type: none"> <li>• all of each swab</li> <li>• swabs from one area are combined</li> <li>• direct extraction</li> </ul>
Hand/finger swabs, fingernail scrapings	N	Up to 2 days	<ul style="list-style-type: none"> <li>• All of swabs/scrapings</li> <li>• R/L separate</li> <li>• fingernail scrapings separate</li> <li>• finger/hand swabs may be combined by hand or limited (e.g. index only)</li> <li>• direct extraction</li> </ul>

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Known sample(s)	Y	NA	<ul style="list-style-type: none"> <li>• Portion of swab, or all of one if &gt;1 collected</li> </ul>
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**Male suspect kits**

Sample type	Routinely sampled?	Timeframe	Sampling and processing
Penile swabs	Y	Up to 2 days	<ul style="list-style-type: none"> <li>• ½ of each penile swab if oral alleged; otherwise all of swab(s)</li> <li>• Differential extraction with sperm and substrate combined</li> </ul>
Bite marks or suspected saliva	N	Up to 2 days	<ul style="list-style-type: none"> <li>• ½ of each swab</li> <li>• swabs from one area are combined</li> <li>• direct extraction</li> </ul>
Miscellaneous contact swabs	N	Up to 2 days	<ul style="list-style-type: none"> <li>• All of swabs</li> <li>• Direct extraction</li> </ul>
Hand/finger swabs, fingernail scrapings	N	Up to 2 days	<ul style="list-style-type: none"> <li>• All of swabs/scrapings</li> <li>• R/L separate</li> <li>• fingernail scrapings separate</li> <li>• finger/hand swabs may be combined by hand or limited (e.g. index only)</li> <li>• direct extraction</li> </ul>
Known sample(s)	Y	NA	<ul style="list-style-type: none"> <li>• Portion of swab, or all of one if &gt;1 collected</li> <li>• extraction and quant required at screening if victim is female (if applicable)</li> </ul>

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### Appendix C: Direct (non-differential) Extraction for Questioned samples

**Note:** Swabs or cuttings may be placed in either a 2.0 mL microfuge tube or a 1.5 mL QIAcube microfuge tube, since either of these will accommodate a spin basket. If a 2.0 mL tube is used, an additional transfer step will need to take place before the sample can be put on the EZ1-XL instrument.

1. Prepare the pre-digest solution:  
(Number of samples + 3) x 480 µl G2 buffer  
(Number of samples + 3) x 20 µl Proteinase K
2. Add enough pre-digest solution to each sample cutting to allow for at least 200µl of free liquid in the tube after absorption by the substrate.
3. Incubate at 56° C for 1.5 – 2 hours at 900 rpm on a thermomixer.
4. Centrifuge briefly to remove liquid from inside the lid.
5. Transfer the substrate to a spin basket and replace in the tube.
6. Centrifuge at ~14500 rpm for 5 minutes.
7. Remove substrate in spin basket to a separate labeled tube.
8. Vortex and spin down briefly.
9. Transfer up to 500 µl of the digest into a sterile 1.5mL or 2.0 mL screw-cap tube. Note: Qiagen 1.5 mL tubes which hold spin baskets do not require a transfer step. Instead, after the spin basket is removed and carrier RNA is added, the lid is cut off and the tube placed directly on the EZ1 instrument.
10. Add 1 µl carrier RNA solution and 400µl of pre-warmed (at least 10 minutes at 56° C) Buffer MTL to the transferred digest solution.
11. Proceed to [EZ1 protocol](#)

### Option 1 – Direct extraction with DTT

**WHEN TO USE:** This option should be used with samples that may contain semen but are not processed by differential extraction, due to length of time between event and sample collection or other case-specific circumstances. This option should also be used with hair samples and with fingernail clippings where the source or owner of the nail is to be determined.

All samples using this protocol constitute their own extraction set, with an ICS and appropriate reagent blanks. This protocol is the same as the above protocol for Direct Extraction for Questioned Samples, with these modifications:

- In step 1, 40 µl DTT is added to the pre-digest solution for each sample
- In step 3, incubation temperature is increased from 56° C to 70° C
- In step 9, up to 540 µL of digest are transferred.

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### Option 2 – Extracting large samples in multiple tubes

**When to use:** if it is necessary to extract a sample that consists of more than two swabs worth of substrate material, then use of more than one tube may be required to digest and extract the entire sample efficiently.

- Divide the swab or other substrate material among enough tubes such that the material in each tube can fit in a spin basket and can move somewhat freely when 500 µL of pre-digest solution are added to the tube.
- Each reagent blank in the set must be prepared using the same number of EZ1 channels. For example, if a set of swabs are extracted among three tubes and then combined into a single extract, then each reagent blank should also be made using three EZ1 tubes which are then combined into a single extract.

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#### **Appendix D: *Differential Extraction with QIAcube Automated Wash Protocol***

**Note:** Swabs or cuttings may be placed in either a 2.0 mL microfuge tube or a 1.5 mL QIAcube microfuge tube, since either of these will accommodate a spin basket. If a 2.0 mL tube is used, an additional transfer step will need to take place before the sample can be put on the QIAcube instrument.

##### **Before starting:**

1. Refer to QIAcube Loading Chart for correct loading of sample tubes in the Rotor Adaptor and Shaker
2. Heat a thermomixer to 56° C for the epithelial digest, and a thermomixer to 70° C for the sperm digest. (1.5 mL or 2 mL thermomixer should be chosen to correspond to the tube sizes to be placed in it.)

##### **Sample preparation for differential extraction:**

3. Add 480 µL G2 and 20 µL Proteinase K to each sample. Optional: a G2 buffer / Proteinase K master mix may be prepared with (number of samples +3) \* 480 µL G2 and (number of samples + 3) \* 20 µL Proteinase K; distribute 500 µL of master mix to each sample.
4. Incubate at 56° C for 1.5 – 2 hours at 900 rpm on a thermomixer.
5. Centrifuge briefly to remove liquid from inside the lid.
6. Transfer the substrate to a spin basket and replace in the tube.
7. Centrifuge at ~14500 rpm for 5 minutes.
8. Remove substrate in spin basket to a separate labeled tube.
9. If the sample is in a 2.0 mL tube, vortex to thoroughly re-suspend, and centrifuge briefly to remove liquid from inside the lid, then transfer all of tube contents to a 1.5 mL QIAcube tube.

##### **Loading the QIAcube:**

10. Fill tip racks with adequate supply of disposable filter tips, 1000 µL, wide-bore.
11. Aliquot Buffer G2 in 30 mL reagent bottle in Reagent Bottle Rack position 1. Do not fill above the fill line. Minimum volumes of G2 buffer are listed in the table below:

<u># samples</u>	<u>Buffer G2 (µL)</u>
7 or fewer	16920



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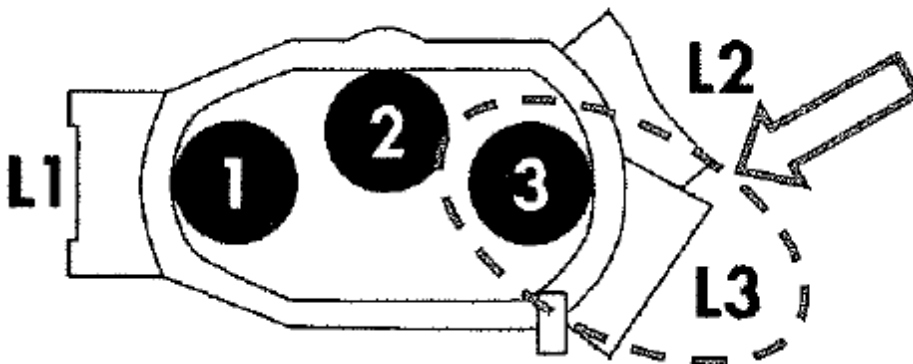
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8	18980
9	21040
10	23100
12	27220

12. Add sterile water to the fill line of the reagent bottle in position 2. This bottle may either be kept capped during part A, or not loaded until part B, of the QIAcube run.

13. Place clean, labeled, straight-sided 2 mL tubes (such as screw-cap EZ1 sample tubes, uncapped) in Shaker. These tubes will ultimately contain the epithelial fractions. Refer to the QIAcube Loading Chart for correct tube position. Each tube must also have a plug placed in its corresponding lid slot, since this is how the optical sensor detects that a tube is present.

14. Place lysed samples from step 9 in position 3 of the QIAcube rotor adaptor, with lid in position L3 (see image below of rotor adaptor).



15. Place rotor adaptors containing samples into QIAcube centrifuge. Refer to QIAcube Loading Chart for correct position.

#### **Running the automated wash protocol:**

16. Remove G2 bottle cap.

17. Ideally, QIAcube instruments should always be left on. If it isn't already on, turn on the QIAcube by pressing the power button.

18. Run QIAcube Separation and Lysis protocol, "Separation and Lysis 12 A". The run protocol can be located as follows: under DNA, select Pipetting; select Epithelial and Sperm Cell, select Lyse 12 A mod. Start and follow prompts.

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19. At the conclusion of "Separation and Lysis 12 A" protocol, refill the tip racks as needed and ensure that both the Buffer G2 reagent bottle and sterile water reagent bottle are in place and uncapped. Epithelial fractions are now located in the shaker and can be removed from the QIAcube at this point.

20. Run "Separation and Lysis 12 B".

21. After protocol "Separation and Lysis 12 B" is complete, each sperm fraction will be found in the 1.5 mL tube in rotor adaptor position 3.

**Sperm fraction processing:**

22. Re-suspend the sperm pellet by vortexing; centrifuge briefly to remove liquid from inside the lid.

23. Add the following reagents to each sperm fraction:

140 µL Buffer G2

20 µL Proteinase K

40 µL 1M DTT

Optional: these reagents may be prepared and distributed as a master mix.

(number of samples + 3) \* 140 µL Buffer G2

(number of samples + 3) \* 20 µL Proteinase K

(number of samples + 3) \* 40 µL 1M DTT

Distribute 200 µL of master mix to each sample.

Optional: If a substrate is being added to its sperm pellet, it is added after the sperm pellet digest reagents have been added to the tube.

Optional: If a substrate is being processed separately, the above reagents are added to a tube and vortexed briefly, followed by addition of the substrate.

24. Incubate at 70° C at 900 rpm in a thermomixer for at least 10 minutes.

**Preparing for EZ1 protocol:**

25. Pre-warm Buffer MTL at 56° C for at least 10 minutes.

26. Epithelial fraction: add 1 µL cRNA and 400 µL warm Buffer MTL to the 2 mL sample tube.

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27. Sperm (and/or substrate) fraction: if applicable, remove substrate by spin basket (as described in steps 5-7). Add 1  $\mu$ L cRNA and 400  $\mu$ L warm Buffer MTL to the sample tube.

28. Proceed to [EZ1 protocol](#)

**QIAcube clean-up:**

After running a protocol, perform the regular maintenance procedure:

- Wipe down platform with a Kimwipe moistened with ethanol and then distilled water.
  - Do not directly spray the inside of the QIAcube with water or ethanol.
  - Do not use bleach, which can react with the reagents.
- Empty the waste drawer.
  - If necessary, wipe down with a Kimwipe moistened with ethanol and then distilled water.
- Remove used disposable labware and unwanted samples and reagents from the worktable. Discard in biohazardous waste.
  - Plastic rotor adaptors are single use only.
- Replace the lids of reagent bottles and close tightly.
- Re-rack the tips if there are any partially used tip racks.

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## Appendix E: Guidelines for consuming a sample for possible Y-STR analysis

Routine practice in analysis of sexual assault kits from female victims includes sampling half of swabs that potentially contain saliva. However, previous routine practice was to sample half of swabs that potentially contain semen. These guidelines refer specifically to extracts where:

- half the original material remains
  - the original extracts have high female to male contributor ratios
  - the T-Y values for the original extracts are below 0.014
  - permission to consume has been granted by Department of Law
  - original extracts and their corresponding Y-STR reagent blank have been DNA Stable'd
    - If the original Y-STR reagent blank has already been DNA Stable'd and amplified with passing results, the below procedure may still be performed with the new Y-STR reagent blank amplified separately. If the original Y-STR reagent blank has already been DNA Stable'd and amplified with apparent DNA detected, consult the Technical Manager for appropriate course of action before proceeding.
1. The extraction of the remaining evidence, along with a corresponding reagent blank, is performed as a **direct extraction with DTT**, with elution in 40  $\mu$ L **water**.
  2. From the original extract fractions (if original was differentially extracted), choose the one with the highest T-Y value.
  3. Add the new extract to the DNA Stable'd best original extract, bringing the combined extracts to a volume of 40  $\mu$ L. Because the new extract is a water elution, there is no impact on amounts of buffer salts or DNA Stable LD components.
    - a. If the original corresponding Y-STR blank has not yet been amplified, add the new reagent blank extract to rehydrate the DNA Stable'd reagent blank.
    - b. If the original corresponding Y-STR blank has already been DNA Stable'd and amplified, the new Y-STR reagent blank should also be DNA Stable'd and amplified.
  4. Quantify the newly combined extracts for the sample and its corresponding reagent blank.
  5. Based on the quantification results, if 0.5 ng can be targeted without concentrating, then extracts can proceed directly to amplification. Otherwise, if the T-Y value is greater than or equal to 0.0007, the extract and its corresponding reagent blank should be dried down and consumed in a single amp. Extracts with T-Y below 0.0007 are dried down and retained.
    - a. Note: since the DNA Stable from the original extract is still present in the combined extract, it is not necessary to add any more DNA Stable reagent to the tube.
    - b. Note: Cutoff values should be adjusted as needed based on Degradation Index.

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#### **Appendix F: Direct Extraction for known samples**

1. Prepare the pre-digest solution:  
(Number of samples + 3) x 230 µl G2 buffer  
(Number of samples + 3) x 230 µl sterile de-ionized water  
(Number of samples + 3) x 10 µl Proteinase K
2. Add enough pre-digest solution to each sample cutting to allow for at least 200µl of free liquid in the tube after absorption by the substrate.
3. Incubate at 56°C for at least one hour. The incubation may be performed in either an incubator or in a thermomixer set at 900 rpm and appropriate temperature.
4. Following incubation, transfer 200µl of the digest buffer into a sterile 1.5mL or 2 mL screw-cap tube.
5. Proceed to [EZ1 protocol](#)

**Note:** In an effort to yield as much DNA as possible from a low-level or degraded reference sample, the questioned direct protocol may also be used.

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## Appendix G: EZ1 protocol

Insert the DNA Investigator card into the slot located on the front of the BioRobot EZ1 Advanced-XL.

Turn on the power switch on the back of the instrument.

Directions are displayed on the screen on the front of the instrument. Press the START button

For questioned samples select Large Volume protocol, TE elution buffer, and 40 µL elution volume. Exceptions documented in bench notes and/or central log as appropriate.

For known samples select Trace protocol, TE elution buffer, and 200 µL elution volume. Exceptions documented in bench notes and/or central log as appropriate.

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.

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

Upon completion of the protocol, remove the elution tubes containing the purified DNA and cap the tubes.

Clean the instrument by wiping down with ethanol, followed by distilled water, after each use. Do NOT use bleach, as it may react with the extraction reagents.

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, followed by distilled water.

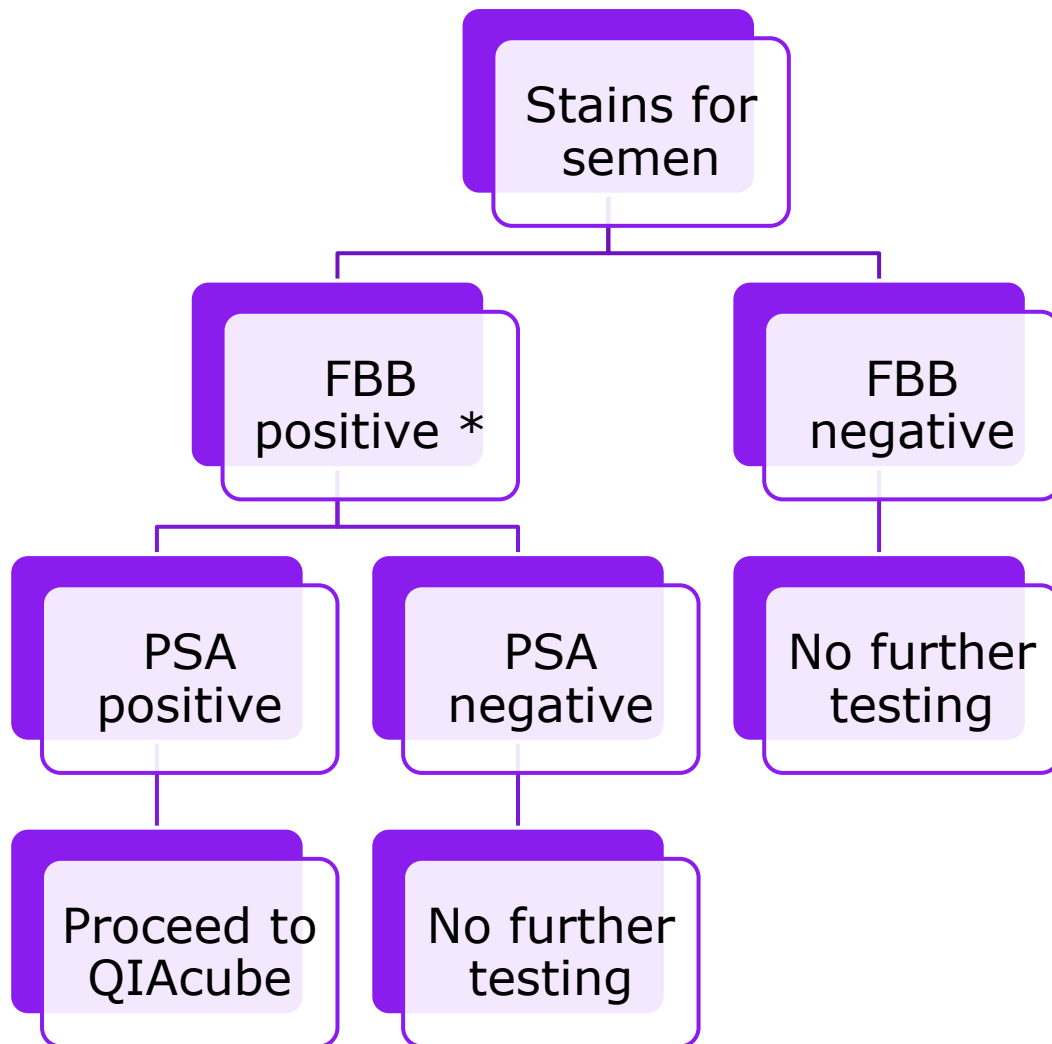
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**Appendix H Biological screening of stains for semen incorporating the QIAcube decision tree**

Note: This decision tree is only used when a single analyst takes a case through both biological screening and DNA analysis.



\* Underwear not worn until 24 hours post-event can go directly to the QIAcube, without PSA testing

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## Appendix I Procedure for Quantifiler Trio on the 7500

### Preparing the 7500 Real-Time PCR Instrument for a Run

- Open the HID Real-Time PCR Analysis software:
  - Log in under analyst name or initials
- Select the Quantifiler Trio icon on the left
- On the left is a menu with three parts: Experimental Properties, Plate Set Up and Run Method
  - Run method is correct as default – do not alter these settings.
  - Under Experimental Properties, enter a name for the project that begins with the batch name or quantification date. An additional designator such as Q or K can be added to distinguish between multiple quants in a single batch (ex. 13-1230MLC-Q). Also, the designator “QT” may be added to the beginning of the project name. Default settings below are correct and should not be changed (Instrument: 7500 (96 wells); Experiment Type: Quantitation – HID Standard Curve; Reagents: TaqMan Reagents; Ramp Speed: Standard (~1 hour to complete a run).
  - Comments section may be used to add instrument number or other relevant information.
  - Under Plate Set Up, the default settings include Standards and NTC.
    - Under the Define Samples tab, add samples. These can be left as Sample 1, Sample 2, and so on, with Q or K codes manually written on printouts later; or they can be entered with Q or K codes.
    - Under the Assign Targets and Samples tab, highlight a box (or boxes) on the map, then check the box from the list on the left (Heading: Assign samples to the selected wells) for the corresponding reaction well(s). Data collected from wells that are assigned to the same sample will be averaged by the software.
    - Standard Curve wells are already correctly assigned. As required in the plate set-up, the template default also includes two NTC reactions.
    - The Quantifiler Trio report prints out results ordered by rows (i.e. A1, A2, A3, A4...).
    - Selected samples are automatically assigned four targets: T.Large Autosomal (T-L), T.Small Autosomal (T-S), T.IPC, and T. Male (T.Y).
- Save the experiment in the experiments folder (Applied Biosystems > 7500 > experiments)



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### Preparing the DNA Quantitation Standards

**Note:** The volumes listed below are minimum volumes to optimize accuracy in pipetting. Larger quantities of standard curve may be prepared by scaling all volumes as needed. Prepared standard curves can be stored for up to two weeks at 2 to 8 °C with documentation of the date made, the expiration date, and the reagent lot numbers. Note: the directions below will make 30 µL of standard 1. The directions may be modified to create a different volume of standard 1 if desired (example: 40 µL Quantifiler THP DNA Dilution Buffer and 40 µL Quantifiler THP DNA Standard)

- Bring the reagents to room temperature before pipetting.
- Label five sterile microcentrifuge tubes for standards 1 through 5.
- Dispense 20 µL of Quantifiler THP DNA Dilution Buffer into Std 1 and 90 µL into each of the remaining tubes
- Prepare Standard 1:
  - Vortex the Quantifiler THP DNA Standard for 3 – 5 seconds.
  - Using a new pipette tip, add 20 µL of Quantifiler Trio DNA Standard to the tube for standard 1.
  - Mix the dilution thoroughly.
- Prepare Standards 2 through 5:
  - Using a new pipette tip, add 10 µL of the prepared standard 1 to the tube for the next standard.
  - Mix the standard thoroughly.
  - Repeat until you complete the dilution series.

The approximate concentrations of DNA in prepared Standards 1 through 5 are as follows:

Standard 1  $\approx$  50ng/µL  
Standard 2  $\approx$  5 ng/µL  
Standard 3  $\approx$  0.5 ng/µL  
Standard 4  $\approx$  0.05 ng/µL  
Standard 5  $\approx$  0.005ng/µL

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### Preparing the Reactions

- Bring the reagents to room temperature before pipetting.
- It is recommended that preparation and aliquotting of the master mix take place in a designated hood in order to minimize the introduction of contamination as well as dust or particulate matter. Addition of DNA to the plate may happen in the designated hood or at the analyst's bench.
- While preparing the reactions, keep the 96-well reaction plate in a base (not directly on the bench top) to protect the plate from scratches and particulate matter.
- Calculate the volume of each component needed to prepare a master mix, including a few additional reactions. This calculation is made automatically on the designated 96-well plate QPCR worksheet that is used for well mapping.
  - Quantifiler™ Trio Primer Mix at 8  $\mu$ L per reaction.
  - Quantifiler™ THP PCR Reaction Mix at 10  $\mu$ L per reaction.
- Vortex the Quantifiler Trio Primer Mix and PCR Reaction mix for 3-5 seconds and centrifuge briefly before opening the tube.
- Pipette the required volumes of the components into a sterile tube. Vortex and centrifuge briefly.
- Dispense 18  $\mu$ L of reaction mix into each reaction well.
- Add 2  $\mu$ L of sample, standard or control to the applicable wells. 2 $\mu$ L of dilution buffer is added to each NTC well.
- Seal the plate with the Optical Adhesive Cover. Use an applicator to create a firm seal between each of the wells. NOTE: Do not write on the optical film; if possible do not write on the plate at all prior to a run.
- Centrifuge the plate at 3000 rpm for at least 20 seconds to remove any bubbles.

### Sample Loading

- Push the tray door on the 7500 instrument to open it.
- Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder, with the notched A12 position in the top right of the tray.
- Close the tray door. Apply pressure to the right side of the tray door at an angle.

### Starting the Run

- Click the green box labeled START RUN in the upper left hand corner.

### Analyzing the Data

- When the run is complete and the quality checks described below in 3.6.1 through 3.6.4 have been completed, print the Experiment Results Report for documentation in the central log file.

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## Appendix J: Quantifiler Trio Calculation formulae

Degradation Index =  $T-S / T-L$

Female: Male ratio (minor male):  $(T-S - T-Y) / T-Y$

Ycorr calculations

1. Use QT data for the male reference sample to calculate a T-Y correction factor of T-S / T-Y

Example: A male reference sample has QT readings of T-S = 0.80 and T-Y = 0.70

$$Y_{\text{corr}} = T-S / T-Y = 0.80 / 0.70 = 1.14$$

2. For a questioned sample extract of body swabs from that male, multiply the T-Y by the Y correction factor.

Example: A left hand swab from the above male has QT readings of T-S = 1.60 and T-Y = 1.20. After multiplying by  $Y_{\text{corr}}$ , the new T-Y value is  $1.20 * 1.14 = 1.37$

$$[T-Y_{\text{original}} * Y_{\text{corr}} = T-Y_{\text{new}}]$$

3. Use the new corrected value for T-Y to calculate a male: female contributor ratio

Example: For the above sample, the ratio of male to female is  $1.37 / (1.60 - 1.37)$   
= 6:1  $[T-Y_{\text{new}} / (T-S - T-Y_{\text{new}})]$

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**Appendix K Long-Term Storage of DNA Extracts by DNASTable® LD**

1. All questioned extracts (except from proficiency tests) are routinely retained. Retained DNA extracts are dried down when the analyst has determined that no further work is required on the extract. DNA Stable LD may be added to extracts before or after amplification.
2. Spin down the DNA extract by centrifuging at approximately 14,500 rpm for 5 minutes.
3. For DNA extract volumes up to 100 µl, add 20 µl of DNASTable® LD directly to the DNA extract. Mix the sample thoroughly with gentle pipetting. Avoid dispensing bubbles into the sample.
4. Dry the DNA extract solution by placing the uncapped tube in a rack on the lab bench or in a laminar flow ventilation hood (recommended). The uncapped tube may be covered with a Kimwipe®. Approximate drying times are 48-64 hours for 100 µl volumes. Samples must be dried completely for optimal protection and stability when stored at room temperature.
5. Once dry, cap the tube and store in either (a) a dry storage cabinet at room temperature or (b) in a foil-lined, moisture barrier envelope with a silica gel desiccant packet added (also at room temperature). Typically, one extract is packaged per foil envelope. More than one extract derived from a single sample (such as sperm and epithelial fractions) may be packed in a single foil envelope if the envelope is as long as one desiccant packet per extract. Dried extracts are retained long-term in the original evidence packaging. Alternatively, if the foil envelope does not fit in the original packaging, or if the original item is to be returned to the submitting agency, a new item is created in the LIMS.
6. To recover dried samples, add sterile water. The maximum volume of added water can be as large as the volume of the sample prior to drying down. Up to 40 µL of extract eluted in TE buffer may be reconstituted in as little as 15 µL dH<sub>2</sub>O. However, if a larger volume of TE-eluted extract is dried down, the minimal volume for reconstitution must also be proportionally larger. For example, if 80 µL of extract eluted in TE is dried down with DNA Stable, it must be reconstituted in no less than 30 µL dH<sub>2</sub>O. This is specifically the case for extracts eluted in TE buffer, not in water. Incubate the sample at room temperature for 15 minutes to allow complete rehydration. Mix the sample thoroughly with vortexing. Store unused rehydrated samples at room temperature or at 4°C for up to 10 days. After ten days, or sooner if work is completed, unused samples can be re-dried as in steps 3-4 without appreciable DNA loss.
7. When a questioned sample is recovered and amplified, the corresponding reagent blank previously unworked (usually labeled as reagent blank replicate -2) is brought up in the same volume of sterile water and amplified as well.

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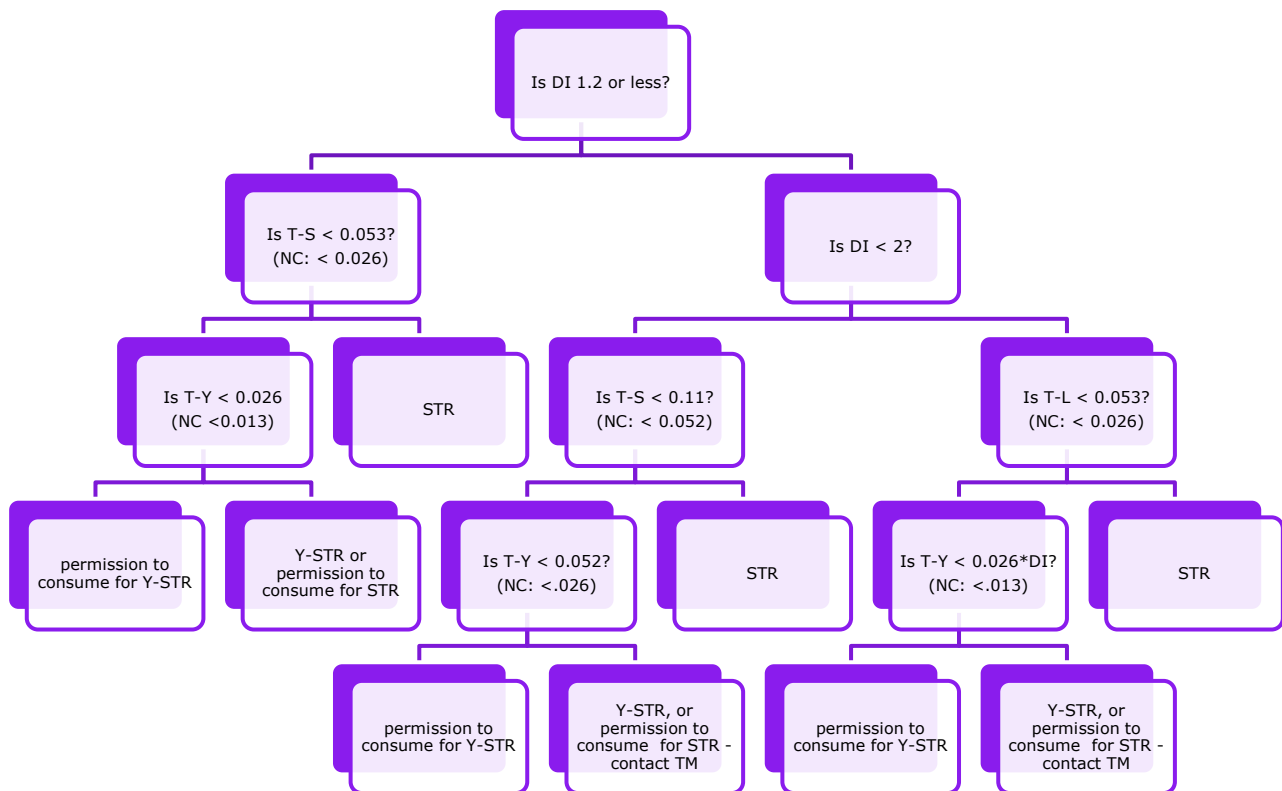
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**Appendix L: Quant decision tree where male DNA is probative**

• **Consumed sample extracts in suspect cases**

• **Non-consumed sexual assault kit swab extracts (suspect and non-suspect)**

- If T-Y < 0.0007 for a consumed sample extract, no further analysis.
- Y-STR analysis only performed in sexual assault and homicide cases
- Samples with a female: male ratio of 5:1 or greater are not eligible for STR analysis.
- For consumed sample extracts with DI of 1.2 or less: If T-S is <0.002 for likely single-source or 0.007 for likely mixtures, no further analysis. For DI 1.2 to 2, cutoffs are T-S < 0.004 and 0.014; for DI >2, cutoffs are T-L < 0.002 and 0.007
- Cut-offs specific to non-consumed sample extracts are noted as NC
- T-S and T-L cutoffs assume that up to 18 µL of extract will be DNA Stable'd and rehydrated to a volume of 15 µL
- Yes = Left; No = Right

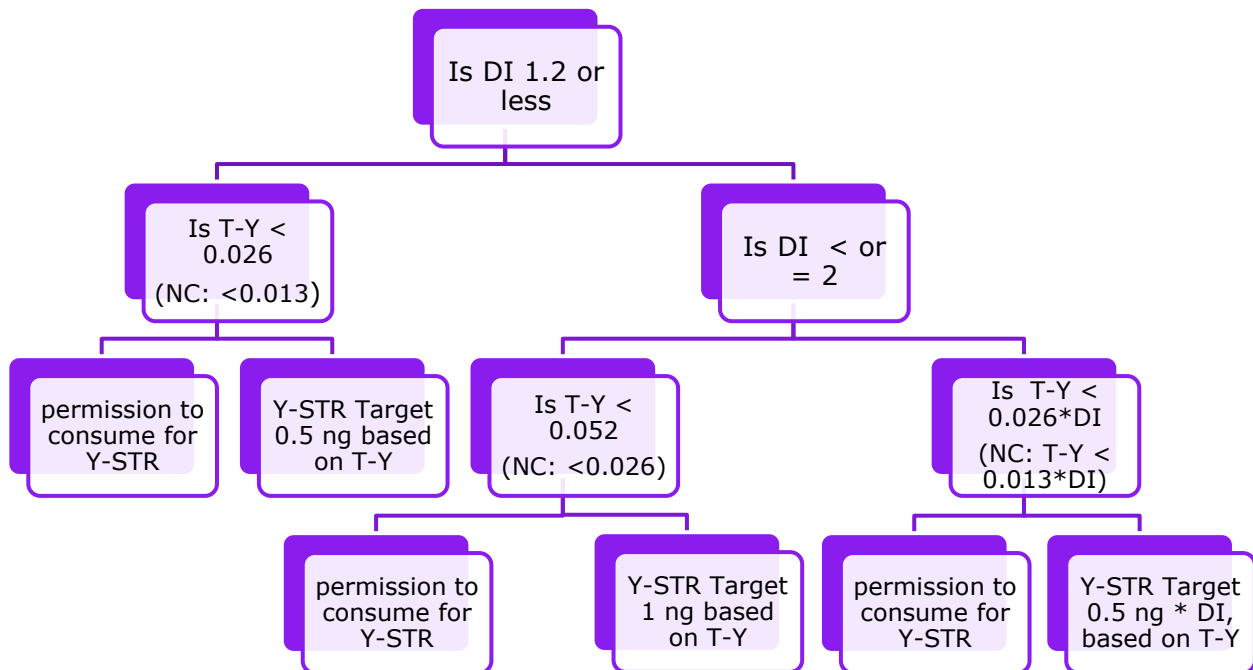


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**Appendix M: Y-STR Quant decision tree (Questioned samples)**

- If  $T-Y < 0.0007$  for a consumed sample extract, no further analysis.
- Y-STR analysis only performed in sexual assault and homicide cases
- Y-STR analysis is only performed when a suspect known, at a minimum, has been submitted
- If STR analysis is a possibility, it should be attempted first
- NC = non-consumed evidence. Values are for consumed evidence unless noted otherwise
- Yes = Left; No = Right

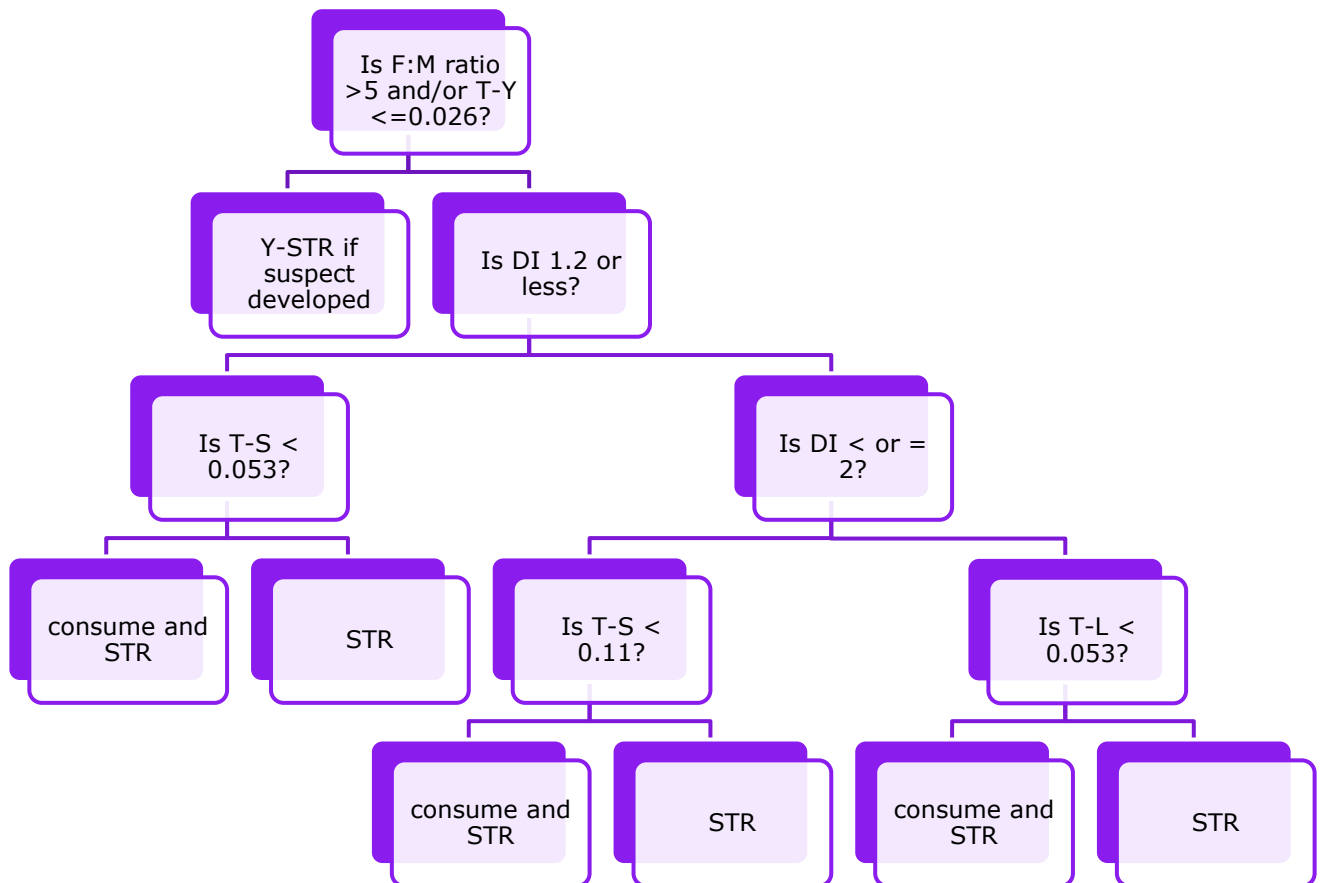


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**Appendix N: Consumed sample quant decision tree for non-suspect case extracts where male DNA is probative**

- This does not apply to swabs from non-suspect sexual assault kits.
- If evidence was not consumed and male DNA was detected, but T-S is  $< 0.053$  ng/ $\mu$ L, analyst will need to extract more evidence before proceeding. Report out as DNA analysis is pending.
- Y-STR analysis only performed in sexual assault and homicide cases
- If T-Y  $< 0.0007$ , no further analysis (consumed and not consumed evidence)
- T-S and T-L cutoffs assume that up to 18  $\mu$ L of extract will be DNA Stable'd and rehydrated to a volume of 15  $\mu$ L
- For DI of 1.2 or less: If T-S is  $< 0.002$  for likely single-source or 0.007 for likely mixtures, no further analysis (consumed and not consumed evidence)
  - For DI 1.2 to 2, cutoffs are T-S  $< 0.004$  and 0.014; for DI  $> 2$ , cutoffs are T-L  $< 0.002$  and 0.007
- Yes = Left; No = Right

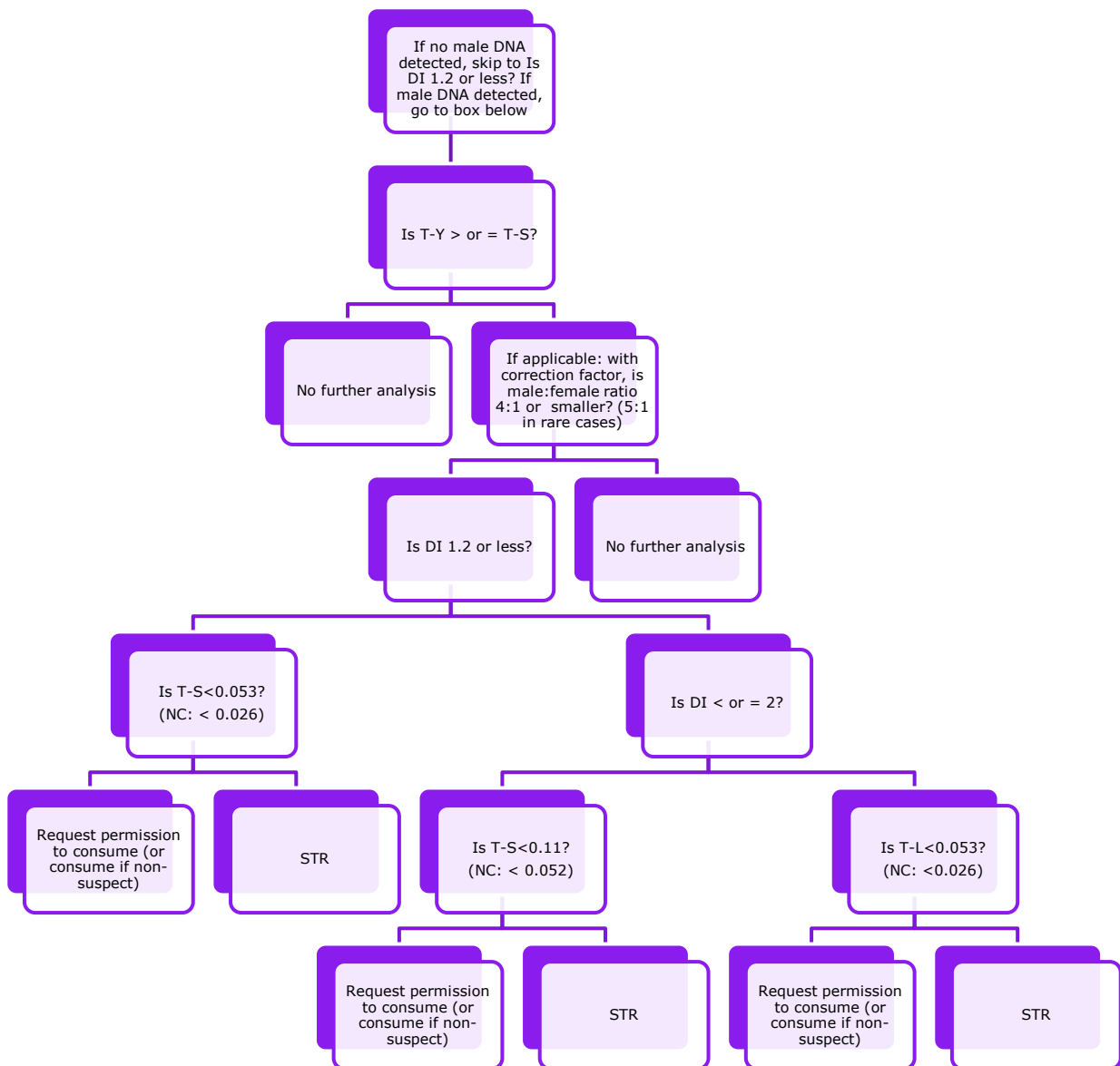


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### Appendix O: Quant decision tree for extracts where female DNA is probative

- For DI of 1.2 or less: If T-S is  $<0.002$  for likely single-source or  $0.007$  for likely mixtures, no further analysis (consumed evidence)
  - For DI 1.2 to 2, cutoffs are T-S  $< 0.004$  and  $0.014$ ; for DI  $>2$ , cutoffs are T-L  $< 0.002$  and  $0.007$
- Cut-offs for non-consumed evidence are noted NC in parentheses
- T-S and T-L cutoffs assume that up to  $18\ \mu\text{L}$  of extract will be DNA Stable'd and rehydrated to a volume of  $15\ \mu\text{L}$
- Yes = Left; No = Right



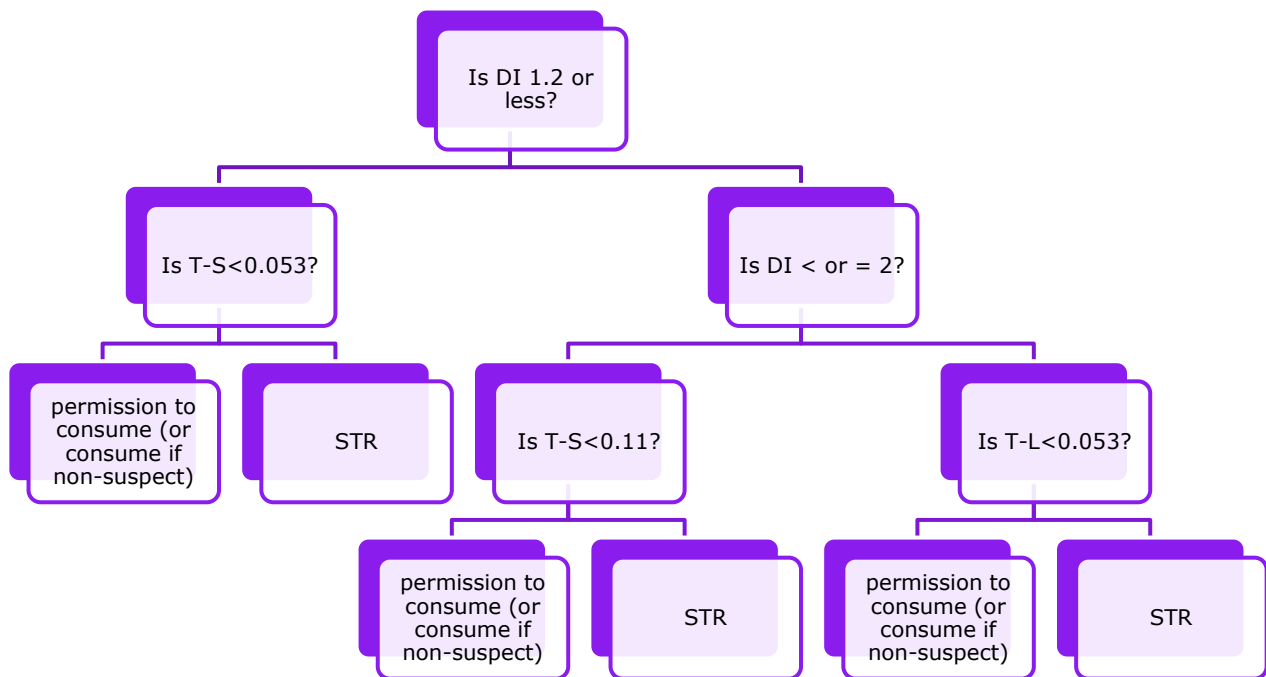


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**Appendix P: Consumed sample quant decision tree for extracts where any profile (male or female) would be probative**

- If evidence was not consumed but T-S is  $< 0.053$  ng/ $\mu$ L, analyst will need to extract more evidence before proceeding. Report out as DNA analysis is pending.
- T-S and T-L cutoffs assume that up to 18  $\mu$ L of extract will be DNA Stable'd and rehydrated to a volume of 15  $\mu$ L
- For DI of 1.2 or less: If T-S is  $< 0.002$  for likely single-source or 0.007 for likely mixtures, no further analysis (consumed and not consumed evidence)
  - For DI 1.2 to 2, cutoffs are T-S  $< 0.004$  and 0.014; for DI  $> 2$ , cutoffs are T-L  $< 0.002$  and 0.007
- Yes = Left; No = Right



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## **Appendix Q: Amplification and Genetic Analysis**

### **References:**

- *GeneAmp® PCR System 9700 96-Well Sample Block Module User's Manual*
- *Global Filer PCR Amplification Kit User Guide*
- *PowerPlexY23 for Use on the Applied Biosystems Genetic Analyzers – Technical Manual*
- *GlobalFiler™ Express PCR Amplification Kit User Guide – Applied Biosystems*

### **Amplification Cycling Parameters**

- Ensure AB GeneAmp® PCR System 9700 thermal cycler has been turned on to allow the instrument time to properly warm up.
- For GlobalFiler:
  - Confirm the cycling parameters for **user: alaska, program: gf-cswk**
  - **Max** ramping mode is used for amplification

95°C for 1 minutes, then:

ramp 100% to 94°C for 10 seconds  
ramp 100% to 59°C for 90 seconds  
for 29 cycles, then:

60°C for 10 minutes  
4°C hold

- For PowerPlex Y23
  - Confirm the cycling parameters for **user: alaska, program: Y23-30cyc**
  - **Max** ramping mode is used for amplification

96°C for 2 minutes, then:

ramp 100% to 94°C for 10 seconds  
ramp 100% to 61°C for 1 minute  
ramp 100% to 72°C for 30 seconds  
for 30 cycles, then:

60°C for 20 minutes  
4°C hold

Note: Amplification takes about 1 hour and 40 minutes.

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- For GlobalFiler express
  - Confirm the cycling parameters for **user: alaska, program: gfe26c**
  - Confirm that ramping mode is set to **Max**, and reaction volume is set to **15µL**.

HOLD  
95°C for 1 minute, then:

26 CYCLES  
94°C for 3 seconds  
60°C for 30 seconds

HOLD  
60°C for 8 minutes

HOLD  
4°C

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### Amplification Master Mix Set Up

- If amplification reagents have been frozen, allow them to warm to room temperature, then vortex for 3 seconds and centrifuge briefly before opening tubes. If Master Mix is in a bottle instead of a tube, swirl to mix. Thawing is only required during the first use of a tube. After first use, reagents are stored at 2-8°C
  - For the first use of the GlobalFiler Express kit only: Add the following volumes of Master Mix Additive to the master mix tube:
    - 200 reaction kit – 80µL Master Mix Additive
    - 1000 reaction kit – 390µL Master Mix Additive
  - Gently invert the Master Mix tube 10 times and centrifuge briefly. If Master Mix is in a bottle instead of a tube, swirl to mix.
  - Mark the cap of the Master Mix tube with a (+) to indicate that Master Mix Additive has been added.
  - Discard the Master Mix Additive tube.
  - Note the thaw date on the Master Mix and Primer tubes – the expiration date for the reagents is now the earlier date of the manufacturer's expiration date or six months from the date of the first thaw.
- Transfer the amplification reagents to the designated PCR set-up area.

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

**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:
  - For GlobalFiler:
    - # of samples x 7.5µL Global Filer Master Mix
    - # of samples x 2.5µL Global Filer Primer Set
  - For PowerPlex Y23:
    - # of samples x 5µL PowerPlex Y23 5X Master Mix
    - # of samples x 2.5µL PowerPlex Y23 10X Primer Pair Mix
  - For GlobalFiler Express
    - # of samples x 6.0µL Master Mix
    - # of samples x 6.0µL Primer Set
- Vortex the master mix and spin briefly.

**Note:** Prepare enough for a few extra reactions to allow for loss during pipetting. This is calculated automatically on the DNA amplification/electrophoresis worksheet.

**Note:** It is not essential to prepare a master mix for a small number of reactions.

**Note:** Preparation of the amplification reagents takes place in the PCR prep room. Once the reagents have been aliquoted into the plate, the plate, covered with Press and Seal, may be taken to the extraction lab for loading the samples or sample lysates.

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**Preparing a GlobalFiler amplification:**

- If casework extracts have been stored at 2-8°C, allow them to warm to room temperature, then vortex and spin briefly using a centrifuge.
- Prepare the samples to be amplified in individual tubes, using a total volume of **15µL**. Refer to [Amplification and Genetic Analysis section](#) for more details.
- Vortex the amplification master mix and spin briefly. Transfer **10µL** of master mix to each sample well of a 96-well amplification plate (optical skirted plate or non-skirted plate is acceptable).
- Cover the entire well plate with Glad® Press 'n Seal (or equivalent, such as aluminum foil or kim wipes).
- Transfer the entire **15µL** of the prepared samples to the appropriate sample wells containing the PCR master mix. During sample addition, the pipette tip is inserted through the Press 'n Seal (or through aluminum foil, or kim wipes are shifted immediately before addition of sample).

**Preparing a PowerPlex Y23 amplification:**

- If casework extracts have been stored at 2-8°C, allow them to warm to room temperature, then vortex and spin briefly using a centrifuge.
- Prepare the samples to be amplified in individual tubes, using a total volume of **17.5µL**. Refer to [Amplification and Genetic Analysis section](#) for more details.
- Vortex the amplification master mix and spin briefly. Transfer **7.5µL** of master mix to each sample well of a 96-well amplification plate (optical skirted plate or non-skirted plate is acceptable).
- Cover the entire well plate with Glad® Press 'n Seal (or equivalent, such as aluminum foil or kim wipes).
- Transfer the entire **17.5µL** 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 (or through aluminum foil, or kim wipes are shifted immediately before addition of sample).

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**Preparing a GlobalFiler Express direct amplification:**

- Prepare the samples to be amplified in individual tubes, as follows:
  - Label the appropriate number of 1.5mL tubes.
  - One entire swab tip is snapped off (or expelled) into the sample tube. It is not necessary to retain the swab stick.
    - For database samples, if only one swab was present, note that the sample was consumed on the swab envelope and transfer the sample to the "Consumed in Analysis" disposition in JT. The envelope is retained in the container.
    - For database samples, each batch of extractions must include randomly placed internal control samples, comprising approximately 5% of the batch. This is typically five samples for a full batch. These may be previously typed samples, staff duplicates, or offender duplicates for samples in the current run.
  - Each batch of extractions must include a reagent blank/negative amplification control consisting of Prep-n-Go buffer.
  - Add 400µL of Prep-n-Go buffer to each sample (and reagent blank/negative amplification control tube) and let stand for at least 20 minutes but ideally no more than 60 minutes at room temperature (about 20 to 25°C). NOTE: Incubation time can have a significant impact on the profile ultimately obtained for the sample.
  - Note: Following amplification set-up, transfer the tubes with lysates to short-term (less than 2 weeks) storage at 2 to 8°C
- Pipet 12µL of master mix into each well of a 96-well VWR amplification reaction plate. NOTE: on a full database plate, wells A1, A4, A7, A10 and any unused wells do not require master mix.
- Cover the plate with Glad Press and Seal.
- After sample incubation, transfer 3µL of lysate to the prepared amplification plate.
- To the reagent blank/negative amplification control well (typically well H12 on a full database plate): add 3µL of Prep-n-Go buffer from the reagent blank tube.
- To the positive amplification control well (typically well G12 on a full database plate): add 3µL of DNA Control 007.

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### Amplification on the AB GeneAmp® PCR System 9700 thermal cycler

- Once all samples have been added to the amplification plate, remove the Press 'n Seal (or other plate covering) and cover the plate with a sheet of amplification tape.
- Transfer the plate to the PCR room and place directly into the thermal cycler.
  - For skirted plates, place a MicroAmp compression pad on top of the plate to further prevent evaporation during thermal cycling.
- Close the heated cover.
- Double-check the selected program, ramp speed, and volume:
  - For GlobalFiler:
    - **user: alaska, program: gf-cswk**
    - **Max** ramping mode is used for amplification
    - Volume is **25 µL**
  - For PowerPlex Y23:
    - **user: alaska, program: Y23-30cyc**
    - **Max** ramping mode is used for amplification
    - Volume is **25 µL**
  - For GlobalFiler Express:
    - **user: alaska, program: gfe26c**
    - **Max** ramping mode is used for amplification
    - Volume is **15 µL**
- Plate should ideally be removed from the thermal cycler less than 24 hours after completion of the run.
- On completion of the run, the amplified DNA is stored at 2 to 8°C. All amplified products will be disposed of upon completion of the technical and administrative reviews.

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### **Data Collection by 3500xl Genetic Analyzer**

*References: Applied Biosystems 3500/3500xL Genetic Analyzer User Guide*

#### **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 have 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**
- Log in to the 3500 Series Data Collection Software

#### **Preparing the Instrument**

- Navigate to the Dashboard of the software
- Check consumables by clicking **Refresh** to update consumable status.
- Refer to the Forensic Biology General Lab Maintenance manual if any maintenance or instrument preparations are required prior to running samples on the instrument.
- The expiration dates for the anode buffer and cathode buffer are “hard stops” – if these reagents are expired, they must be replaced for the run to proceed.
- The seven-days-on-instrument stop for the POP-4 polymer and the number of injections on a capillary are “soft stops” – as long as the resulting data for the ILS meets the quality standards described below, these stops may be overridden.
- Click **Start Pre-heat** to 60 °C to warm the oven.
- Check the pump assembly for bubbles and run the Remove Bubble wizard if necessary.

#### **Sample Preparation for the 3500xl**

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

<b>Amplification Kit</b>	<b>Amount of Size standard per sample</b>	<b>Amount Hi-Di Formamide per sample</b>
GlobalFiler	0.4 µl GeneScan 600 LIZ	9.6µl
PowerPlex Y23	0.5µl WEN ILS 500 Y23	9.5µl
GlobalFiler Express	0.5 µl GeneScan 600 LIZ	9.5µl

**Note:** Prepare enough for a few extra reactions to allow for loss during pipetting.

**Note:** It is not necessary to prepare a master mix when only a few reactions are being prepared.

- Vortex the master mix and spin briefly. Transfer 10µl of master mix to appropriate sample wells of a 96 well plate.
- Add 10µl of Hi-Di Formamide to unused wells of a set of 24 (i.e. A1-H3).
- Add 1µl of allelic ladder and 1µl of each amplified sample to the appropriate wells. When all samples have been added, cover with plate septa.



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- 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.
- 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.

### Creating a Plate Record

#### 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. 10-1222KAL\_Q\_101224)
  - 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 the following Assay:
  - For GlobalFiler: AB\_GF\_POP4\_xl
  - For PowerPlex Y23: PPY23
  - For GlobalFiler Express: AB\_J6OSR\_LS\_POP4\_xl
- 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.

#### 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.
- 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. 10-1222KAL\_Q\_101224)
  - Select 96 for the number of wells
  - Select HID for plate type
  - Select 36cm for capillary length
  - Choose POP4 for polymer

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- 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.

#### 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 cleared and the run begins before walking away.

#### Viewing Data During a Run

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

#### Storage of data files

After the run has finished, transfer a copy of the data from the 3500 computer to the designated location on the laboratory network. As of the effective date of this manual, CE data is stored at I: Discipline Shares/Forensic Biology/CE data. Copying the files to the laboratory network ensures that the data is regularly backed up.

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

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### ***Data Analysis with GeneMapper™ ID-X***

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

#### **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.

#### **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 stored data as 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**.
- When all samples have been selected, 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.
  - For GlobalFiler:
    - Analysis method may be Global Filer Casework, Global Filer Blank, or Global Filer Troubleshooting (not used in routine casework)
    - Panel is GlobalFiler\_Panel\_v1
    - Size Standard is GS600\_LIZ(60-460)
  - For PowerPlex Y23:
    - Analysis Method is PowerPlex y23,
    - Panel is Alaska\_PowerPlexY23\_IDX\_v2.0
    - Size Standard is WEN\_ILS\_500\_IDX
  - For GlobalFiler Express:
    - Analysis Method is GFE
    - Panel is GlobalFiler\_Express\_v1.4.1X
    - Size Standard is GS600\_LIZ\_(60-460)
- The user will be prompted to name and save the project to the appropriate Security Group (GeneMapper ID-X Security Group). Projects are typically named with the Batch name, a Q or K, and the run date. Click OK.
  - Example: 15-0426CMD\_Q\_150428
- Analysis is complete when the green arrows in the Status column on the left are gone, and an Analysis Summary Screen appears.

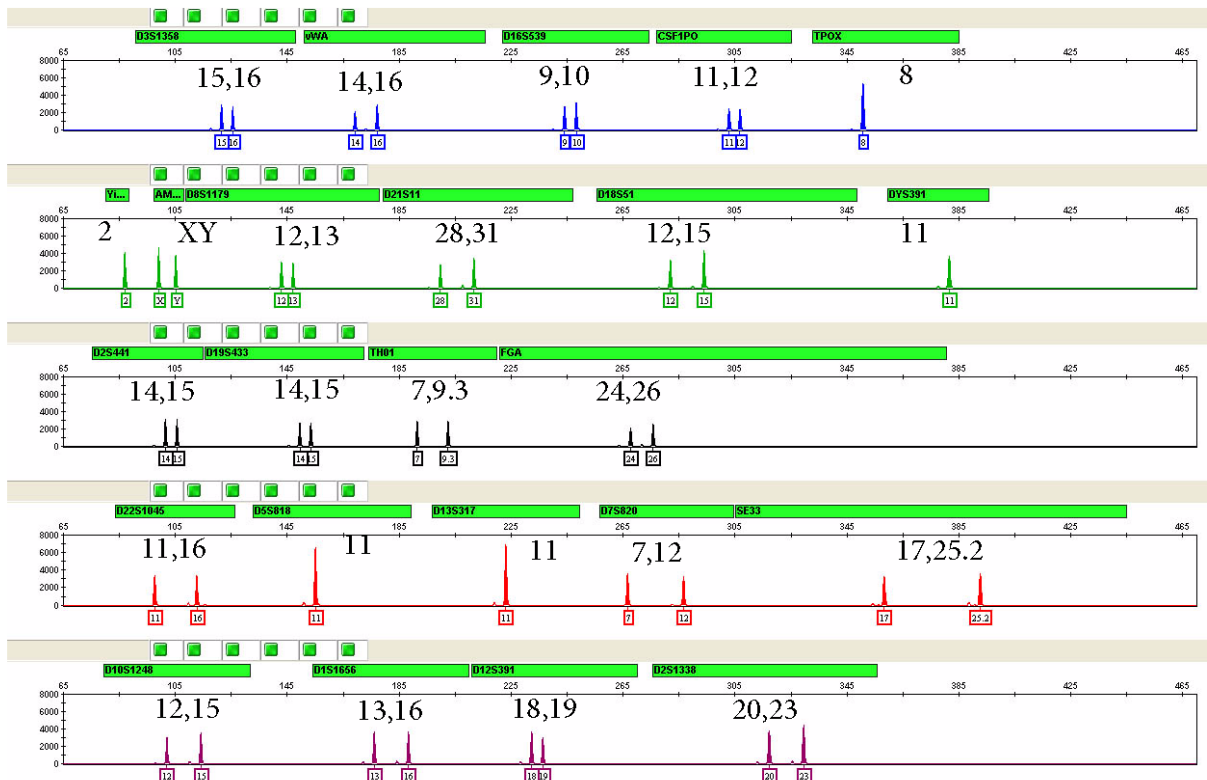
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### Viewing the Data

- The ILS is viewed by highlighting all samples and clicking on the Size Match Editor, the icon with the red peaks on the toolbar.
- 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).
- Electropherograms can be printed from the sample's plot window. Various plots have been created for different sample types. Commonly used plot displays include:
  - GF Casework
  - GF and GFE Blank
  - GF and GFE Artifact View
  - Traditional Genotype Plot
  - Casework Blank
  - Casework artifacts
  - 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.

### GlobalFiler Positive Control 007 Profile (also GlobalFiler Express)



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## Appendix R Analysis Method 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. These methods shall not be modified. New methods shall only be created or modified with permission from the DNA Technical Manager.

### GlobalFiler Analysis Methods

#### ***GlobalFiler Casework Allele Tab Settings***

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Allele' tab selected. The 'Bin Set' is 'AmpFLSTR\_Bins\_v6X'. A checkbox 'Use marker-specific stutter ratio and distance if available' is checked. Below this is a table for 'Marker Repeat Type' with columns for Tri, Tetra, Penta, and Hexa. The table contains settings for Global Cut-off Value, MinusA Ratio, MinusA Distance, Global Minus Stutter Ratio, Global Minus Stutter Distance, Global Plus Stutter Ratio, and Global Plus Stutter Distance. At the bottom, there is an 'Amelogenin Cutoff' field set to 0.0, and buttons for 'Range Filter...', 'Factory Defaults', 'Save As', 'Save', 'Cancel', and 'Help'.

Marker Repeat Type:		Tri	Tetra	Penta	Hexa
Global Cut-off Value		0.0	0.0	0.0	0.0
MinusA Ratio		0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Global Minus Stutter Ratio		0.0	0.0	0.0	0.0
Global Minus Stutter Distance	From	0.0	3.25	0.0	0.0
	To	0.0	4.75	0.0	0.0
Global Plus Stutter Ratio		0.0	0.02	0.0	0.0
Global Plus Stutter Distance	From	0.0	3.25	0.0	0.0
	To	0.0	4.75	0.0	0.0

Amelogenin Cutoff: 0.0

Buttons: Range Filter..., Factory Defaults, Save As, Save, Cancel, Help

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### GlobalFiler Casework Peak Detector Tab Settings

The Analytical Threshold for all GlobalFiler casework analysis is 160RFU.

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Detector' tab selected. The 'Peak Detection Algorithm' is set to 'Advanced'. The 'Ranges' section has 'Analysis' set to 'Full Range' and 'Sizing' set to 'All Sizes'. The 'Start Pt' is 0 and 'Stop Pt' is 10000. The 'Start Size' is 0 and 'Stop Size' is 1000. The 'Smoothing and Baseline' section has 'Smoothing' set to 'Light' and 'Baseline Window' set to 33 pts. The 'Size Calling Method' section has 'Local Southern Method' selected. The 'Peak Detection' section has 'Peak Amplitude Thresholds' set to 160 for B, R, G, P, Y, and O. The 'Min. Peak Half Width' is 2 pts, 'Polynomial Degree' is 3, and 'Peak Window Size' is 13 pts. The 'Slope Threshold' section has 'Peak Start' and 'Peak End' both set to 0.0. The 'Normalization' section has 'Use Normalization, if applicable' checked. A 'Factory Defaults' button is at the bottom right. At the very bottom of the dialog are 'Save As', 'Save', 'Cancel', and 'Help' buttons.

Analysis Method Editor

General Allele **Peak Detector** Peak Quality SQ & GQ Settings

Peak Detection Algorithm: Advanced

Ranges

Analysis: Full Range Sizing: All Sizes

Start Pt: 0 Start Size: 0

Stop Pt: 10000 Stop Size: 1000

Smoothing and Baseline

Smoothing: ☐ None ☒ Light ☐ Heavy

Baseline Window: 33 pts

Size Calling Method

☐ 2nd Order Least Squares

☐ 3rd Order Least Squares

☐ Cubic Spline Interpolation

☒ Local Southern Method

☐ Global Southern Method

Peak Detection

Peak Amplitude Thresholds:

B: 160 R: 160

G: 160 P: 160

Y: 160 O: 160

Min. Peak Half Width: 2 pts

Polynomial Degree: 3

Peak Window Size: 13 pts

Slope Threshold

Peak Start: 0.0

Peak End: 0.0

Normalization

☒ Use Normalization, if applicable

Factory Defaults

Save As Save Cancel Help

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### GlobalFiler Casework Peak Quality Tab Settings

These settings are not relevant in analysis of any samples where the sample type is set to Negative Control.

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Quality' tab selected. The dialog has a title bar with a close button (X) and a tabbed interface with the following tabs: General, Allele, Peak Detector, Peak Quality (selected), and SQ & GQ Settings. The 'Peak Quality' tab contains several settings:

- Min/Max Peak Height (LPH/MPH):**
  - Homozygous min peak height: 630.0
  - Heterozygous min peak height: 1300.0
  - Max Peak Height (MPH): 12000.0
- Peak Height Ratio (PHR):**
  - Min peak height ratio: 0.6
- Broad Peak (BD):**
  - Max peak width (basepairs): 1.5
- Allele Number (AN):**
  - Max expected alleles:
    - For autosomal markers & AMEL: 4
    - For Y markers: 2
- Allelic Ladder Spike:**
  - Spike Detection: Enable (dropdown)
  - Cut-off value: 0.2
- Sample Spike Detection:**
  - Spike Detection: Enable (dropdown)

At the bottom right of the dialog is a 'Factory Defaults' button. At the bottom of the dialog are four buttons: 'Save As', 'Save', 'Cancel', and 'Help'.

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### GlobalFiler Casework SQ and GQ Tab Settings

These settings are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.

The screenshot shows the 'Analysis Method Editor' dialog box with the 'SQ & GQ Settings' tab selected. The dialog has a close button (X) in the top right corner. The 'SQ & GQ Settings' tab is highlighted, and the following settings are visible:

Quality weights are between 0 and 1.

Sample and Control GQ Weighting

Broad Peak (BD)	0.8	Allele Number (AN)	0.3
Out of Bin Allele (BIN)	0.8	Low Peak Height (LPH)	0.3
Overlap (OVL)	0.8	Max Peak Height (MPH)	0.2
Marker Spike (SPK)	0.3	Off-scale (OS)	0.2
AMEL Cross Check (ACC)	0.0	Peak Height Ratio (PHR)	0.3

Control Concordance (CC) Weight = 1.0 (Only applicable to controls)

SQ Weighting

Broad Peak (BD)	0.5
-----------------	-----

Allelic Ladder GQ Weighting

Spike (SSPK/SPK)	1	Off-scale (OS)	1
------------------	---	----------------	---

SQ & GQ Ranges

	Pass Range:	Low Quality Range:
Sizing Quality:	From 0.75 to 1.0	From 0.0 to 0.25
Genotype Quality:	From 0.75 to 1.0	From 0.0 to 0.25

Reset Defaults

Save As Save Cancel Help



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### ***PowerPlex Y23 Analysis Method Settings***

### ***PowerPlex Y23 Casework Allele Tab Settings***

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Allele' tab selected. The 'Bin Set' is 'PowerPlexY23\_Bins\_IDX\_v2.0'. A checkbox 'Use marker-specific stutter ratio and distance if available' is checked. Below this is a table for 'Marker Repeat Type' with columns for 'Tri', 'Tetra', 'Penta', and 'Hexa'. The table contains settings for 'Global Cut-off Value', 'MinusA Ratio', 'MinusA Distance' (From/To), 'Global Minus Stutter Ratio', 'Global Minus Stutter Distance' (From/To), 'Global Plus Stutter Ratio', and 'Global Plus Stutter Distance' (From/To). All values are set to 0.0. Below the table is the 'Amelogenin Cutoff' set to 0.0. At the bottom are buttons for 'Range Filter...', 'Factory Defaults', 'Save As', 'Save', 'Cancel', and 'Help'.

Marker Repeat Type:		Tri	Tetra	Penta	Hexa
Global Cut-off Value		0.0	0.0	0.0	0.0
MinusA Ratio		0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Global Minus Stutter Ratio		0.0	0.0	0.0	0.0
Global Minus Stutter Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Global Plus Stutter Ratio		0.0	0.0	0.0	0.0
Global Plus Stutter Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0

Amelogenin Cutoff: 0.0

Buttons: Range Filter..., Factory Defaults, Save As, Save, Cancel, Help

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### PowerPlex Y23 Casework Peak Detector Tab Settings

The Analytical Threshold for all Y-STR casework analysis is 100RFU.

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Detector' tab selected. The 'Peak Detection Algorithm' is set to 'Advanced'. The 'Ranges' section has 'Analysis' set to 'Full Range' and 'Sizing' set to 'All Sizes', with 'Start Pt' at 0, 'Stop Pt' at 10000, 'Start Size' at 0, and 'Stop Size' at 1000. The 'Smoothing and Baseline' section has 'Smoothing' set to 'Light' and 'Baseline Window' at 51 pts. The 'Size Calling Method' section has 'Local Southern Method' selected. The 'Peak Detection' section has 'Peak Amplitude Thresholds' for B, G, Y, R, P, and O all set to 100. 'Min. Peak Half Width' is 2 pts, 'Polynomial Degree' is 3, and 'Peak Window Size' is 15 pts. The 'Slope Threshold' section has 'Peak Start' and 'Peak End' both at 0.0. The 'Normalization' section has 'Use Normalization, if applicable' unchecked. A 'Factory Defaults' button is at the bottom right. At the very bottom are 'Save As', 'Save', 'Cancel', and 'Help' buttons.

**Analysis Method Editor**

General | Allele | **Peak Detector** | Peak Quality | SQ & GQ Settings

Peak Detection Algorithm: Advanced

**Ranges**

Analysis: Full Range (dropdown)  
Sizing: All Sizes (dropdown)  
Start Pt: 0  
Stop Pt: 10000  
Start Size: 0  
Stop Size: 1000

**Smoothing and Baseline**

Smoothing: ☐ None ☒ Light ☐ Heavy  
Baseline Window: 51 pts

**Size Calling Method**

☐ 2nd Order Least Squares  
☐ 3rd Order Least Squares  
☐ Cubic Spline Interpolation  
☒ Local Southern Method  
☐ Global Southern Method

**Peak Detection**

Peak Amplitude Thresholds:  
B: 100 R: 100  
G: 100 P: 100  
Y: 100 O: 100

Min. Peak Half Width: 2 pts  
Polynomial Degree: 3  
Peak Window Size: 15 pts

**Slope Threshold**

Peak Start: 0.0  
Peak End: 0.0

**Normalization**

☐ Use Normalization, if applicable

Factory Defaults

Save As Save Cancel Help

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### PowerPlex Y23 Casework Peak Quality Tab Settings

These settings are not relevant in analysis of any samples where the sample type is set to Negative Control.

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Quality' tab selected. The dialog has a title bar with a close button (X). Below the title bar are five tabs: 'General', 'Allele', 'Peak Detector', 'Peak Quality' (selected), and 'SQ & GQ Settings'. The 'Peak Quality' tab contains several sections with input fields and dropdown menus. At the bottom right of the tab is a 'Factory Defaults' button. At the bottom of the dialog are four buttons: 'Save As', 'Save', 'Cancel', and 'Help'.

Section	Parameter	Value
Min/Max Peak Height (LPH/MPH)	Homozygous min peak height	600.0
	Heterozygous min peak height	750.0
	Max Peak Height (MPH)	8000.0
Peak Height Ratio (PHR)	Min peak height ratio	0.6
	Broad Peak (BD)	
	Max peak width (basepairs)	1.5
Allele Number (AN)	Max expected alleles:	
	For autosomal markers & AMEL	2
	For Y markers	1
Allelic Ladder Spike	Spike Detection	Enable
	Cut-off Value	0.2
Sample Spike Detection		
	Spike Detection	Enable

Factory Defaults

Save As Save Cancel Help

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### PowerPlex Y23 Casework SQ and GQ Tab Settings

These settings are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.

The screenshot shows the 'Analysis Method Editor' dialog box with the 'SQ & GQ Settings' tab selected. The dialog has a title bar with a close button (X). Below the title bar are five tabs: 'General', 'Allele', 'Peak Detector', 'Peak Quality', and 'SQ & GQ Settings'. The 'SQ & GQ Settings' tab is active and contains the following settings:

Quality weights are between 0 and 1.

Sample and Control GQ Weighting

Broad Peak (BD)	0.8	Allele Number (AN)	1.0
Out of Bin Allele (BIN)	0.8	Low Peak Height (LPH)	0.3
Overlap (OVL)	0.8	Max Peak Height (MPH)	0.3
Marker Spike (SPK)	0.3	Off-scale (OS)	0.8
AMEL Cross Check (ACC)	0.0	Peak Height Ratio (PHR)	0.3

Control Concordance (CC) Weight = 1.0 (Only applicable to controls)

SQ Weighting

Broad Peak (BD)	0.5
-----------------	-----

Allelic Ladder GQ Weighting

Spike (SSPK/SPK)	1	Off-scale (OS)	1
------------------	---	----------------	---

SQ & GQ Ranges

	Pass Range:	Low Quality Range:
Sizing Quality:	From 0.75 to 1.0	From 0.0 to 0.25
Genotype Quality:	From 0.75 to 1.0	From 0.0 to 0.25

Reset Defaults

Save As Save Cancel Help

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**GlobalFiler Express Analysis Methods:****GlobalFiler Express Allele Tab Settings**

Analysis Method Editor

General **Allele** Peak Detector Peak Quality SQ & GQ Settings

Bin Set: AmpFLSTR\_Bins\_v6X

☐ Use marker-specific stutter ratio and distance if available

Marker Repeat Type:		Tri	Tetra	Penta	Hexa
Global Cut-off Value		0.2	0.2	0.2	0.2
MinusA Ratio		0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Global Minus Stutter Ratio		0.0	0.0	0.0	0.0
Global Minus Stutter Distance	From	0.0	3.25	0.0	0.0
	To	0.0	4.75	0.0	0.0
Global Plus Stutter Ratio		0.0	0.0	0.0	0.0
Global Plus Stutter Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0

Amelogenin Cutoff 0.0

Range Filter... Factory Defaults

Save As Save Cancel Help

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Status: Active

### ***GlobalFiler Express Peak Detector Tab Settings***

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Detector' tab selected. The dialog is divided into several sections for configuring peak detection parameters.

**General** | **Allele** | **Peak Detector** | **Peak Quality** | **SQ & GQ Settings**

Peak Detection Algorithm: Advanced

**Ranges**

Analysis	Sizing
Full Range	All Sizes
Start Pt: 0	Start Size: 0
Stop Pt: 10000	Stop Size: 1000

**Smoothing and Baseline**

Smoothing: ☐ None ☒ Light ☐ Heavy

Baseline Window: 33 pts

**Size Calling Method**

☐ 2nd Order Least Squares  
☐ 3rd Order Least Squares  
☐ Cubic Spline Interpolation  
☒ Local Southern Method  
☐ Global Southern Method

**Peak Detection**

Peak Amplitude Thresholds:

B:	R:
175	175
G:	P:
175	175
Y:	O:
175	175

Min. Peak Half Width: 2 pts  
Polynomial Degree: 3  
Peak Window Size: 15 pts

**Slope Threshold**

Peak Start: 0.0  
Peak End: 0.0

**Normalization**

☐ Use Normalization, if applicable

Factory Defaults

Save As Save Cancel Help

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**GlobalFiler Express Peak Quality Tab Settings**

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Quality' tab selected. The dialog has a title bar with a close button (X). Below the title bar are five tabs: 'General', 'Allele', 'Peak Detector', 'Peak Quality' (selected), and 'SQ & GQ Settings'. The 'Peak Quality' tab contains several sections with input fields and dropdown menus. The 'Min/Max Peak Height (LPH/MPH)' section has three input fields: 'Homozygous min peak height' (350.0), 'Heterozygous min peak height' (175.0), and 'Max Peak Height (MPH)' (50000.0). The 'Peak Height Ratio (PHR)' section has one input field: 'Min peak height ratio' (0.5). The 'Broad Peak (BD)' section has one input field: 'Max peak width (basepairs)' (1.5). The 'Allele Number (AN)' section has two input fields: 'Max expected alleles: For autosomal markers & AMEL' (2) and 'For Y markers' (1). The 'Allelic Ladder Spike' section has two input fields: 'Spike Detection' (Enable) and 'Cut-off value' (0.2). The 'Sample Spike Detection' section has one input field: 'Spike Detection' (Enable). At the bottom right of the dialog is a 'Factory Defaults' button. At the bottom of the dialog are four buttons: 'Save As', 'Save', 'Cancel', and 'Help'.

Analysis Method Editor

General Allele Peak Detector **Peak Quality** SQ & GQ Settings

Min/Max Peak Height (LPH/MPH)

Homozygous min peak height 350.0

Heterozygous min peak height 175.0

Max Peak Height (MPH) 50000.0

Peak Height Ratio (PHR)

Min peak height ratio 0.5

Broad Peak (BD)

Max peak width (basepairs) 1.5

Allele Number (AN)

Max expected alleles:

For autosomal markers & AMEL 2

For Y markers 1

Allelic Ladder Spike

Spike Detection Enable

Cut-off value 0.2

Sample Spike Detection

Spike Detection Enable

Factory Defaults

Save As Save Cancel Help

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Version FBPM 2021 R1  
Status: Active

### ***GlobalFile Express SQ and GQ Tab Settings***

These settings do not vary and are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.

The screenshot shows the 'Analysis Method Editor' dialog box with the 'SQ & GQ Settings' tab selected. The dialog has a title bar with a close button (X). Below the title bar are five tabs: 'General', 'Allele', 'Peak Detector', 'Peak Quality', and 'SQ & GQ Settings'. The 'SQ & GQ Settings' tab is active and contains the following settings:

Quality weights are between 0 and 1.

Sample and Control GQ Weighting

Broad Peak (BD)	0.8	Allele Number (AN)	1.0
Out of Bin Allele (BIN)	0.8	Low Peak Height (LPH)	0.3
Overlap (OVL)	0.8	Max Peak Height (MPH)	0.3
Marker Spike (SPK)	0.3	Off-scale (OS)	0.8
AMEL Cross Check (ACC)	0.0	Peak Height Ratio (PHR)	0.3

Control Concordance (CC) Weight = 1.0 (Only applicable to controls)

SQ Weighting

Broad Peak (BD)	0.5
-----------------	-----

Allelic Ladder GQ Weighting

Spike (SSPK/SPK)	1	Off-scale (OS)	1
------------------	---	----------------	---

SQ & GQ Ranges

	Pass Range:	Low Quality Range:
Sizing Quality:	From 0.75 to 1.0	From 0.0 to 0.25
Genotype Quality:	From 0.75 to 1.0	From 0.0 to 0.25

Reset Defaults

Save As Save Cancel Help

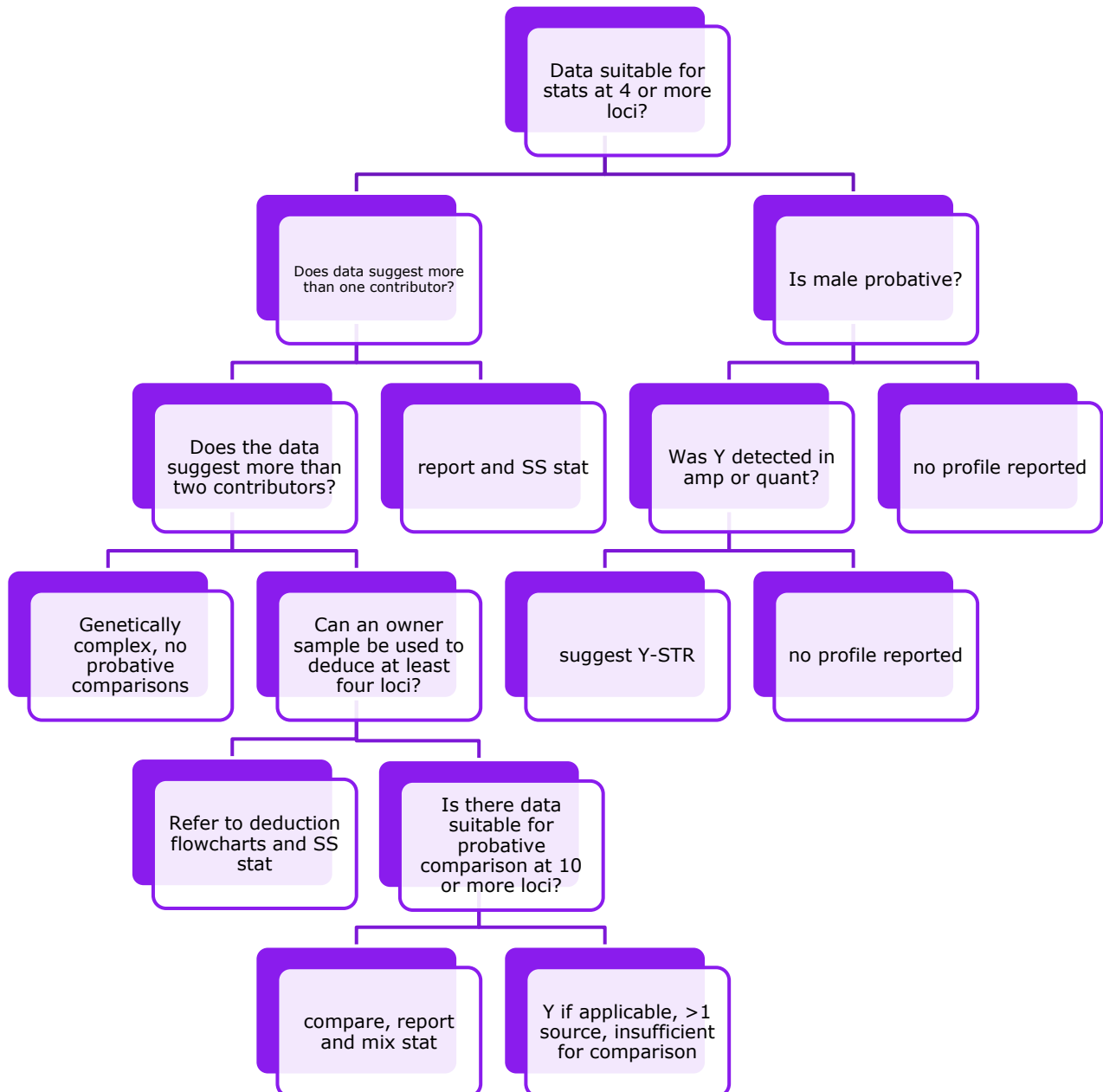


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**Appendix S: Interpretation of low-level data (data at any locus below ST), not the distinguishable minor component of a mixture. Does not apply to differential carryover or single-source owner on intimate sample. Y-STR analysis only performed in sexual assault and homicide cases**

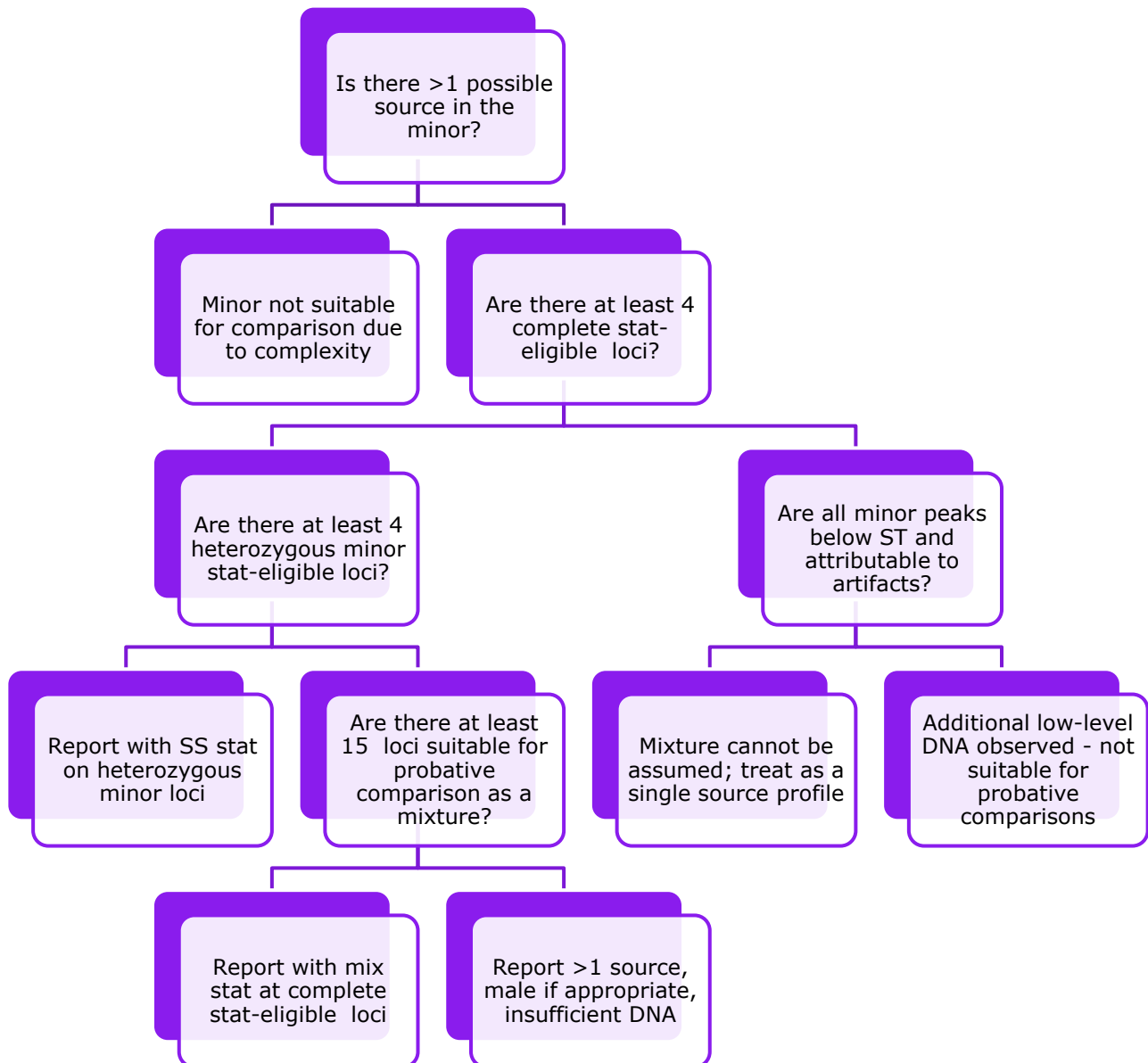
**Yes = left; No = right**



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Status: Active

**Appendix T: Interpretation of low-level minor component of a distinguishable mixture.**  
**Does not apply to differential carryover or single-source owner on intimate sample**  
**Yes = left; No = right**

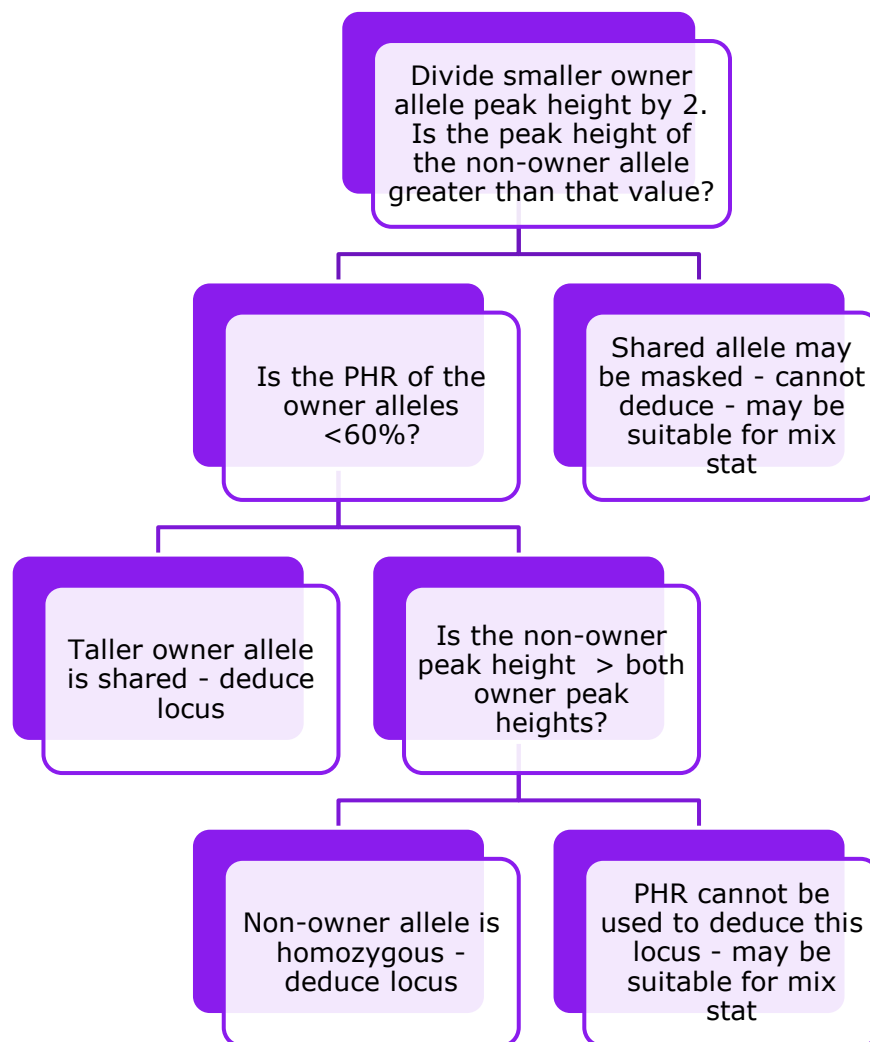


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### ***Appendix U: Deduction at 3-Allele Locus with a heterozygous elimination sample***

- All alleles at two-or three-allele deduced locus must be at or above IT
- Before beginning, assess possible drop out of the second contributor:
  - Use at least two four-allele loci with all alleles above ST (not SE33) to calculate an approximate contributor ratio. Ratio must be no less than 1:2 unknown to owner in order to deduce.
  - Given this ratio, confirm that a minor contributor peak would be above ST
- Yes = Left; No = Right

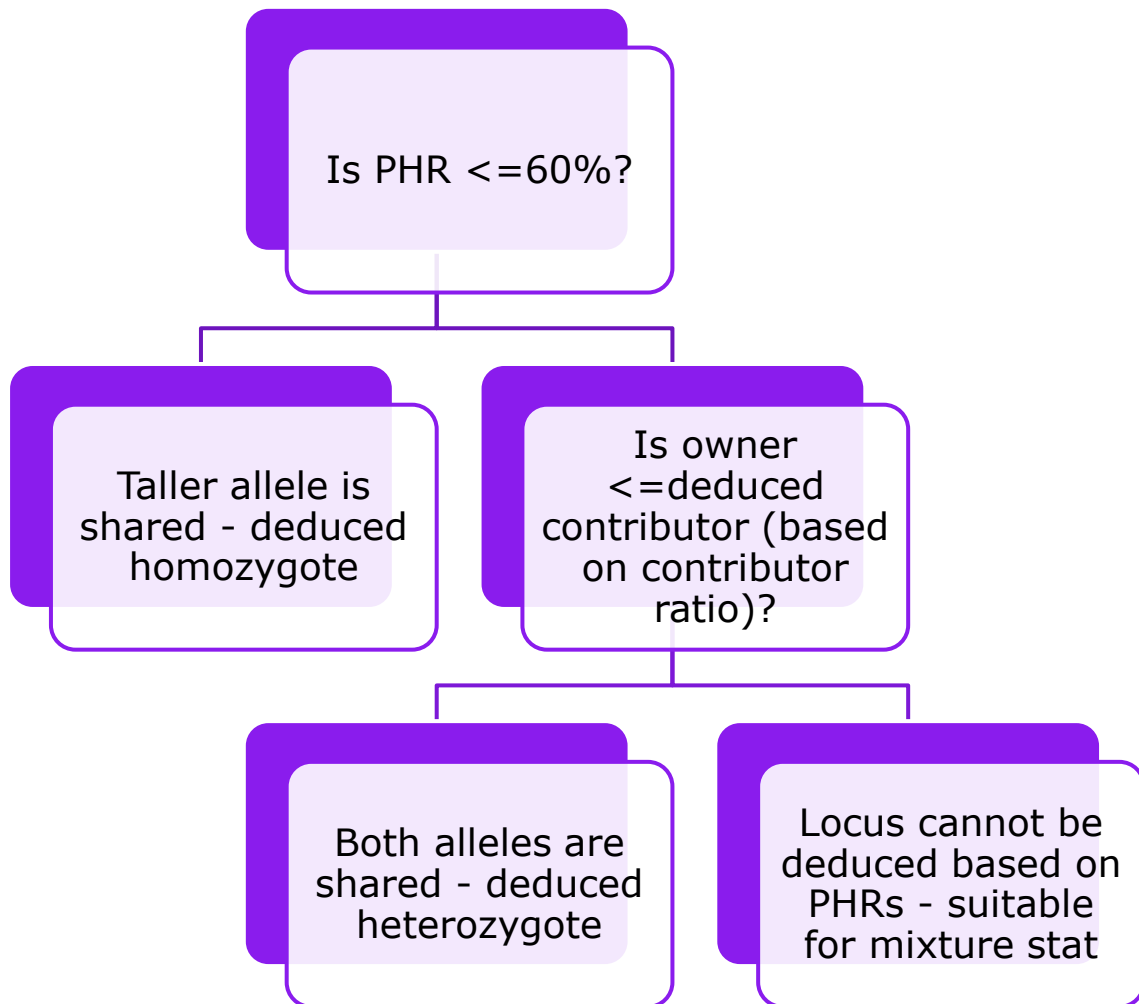


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Status: Active

***Appendix V: Deduction at 2-Allele Locus with a heterozygous elimination sample***

- All alleles at two-or three-allele deduced locus must be at or above IT
- Before beginning, assess possible drop out of the second contributor:
  - Use at least two four-allele loci with all alleles above ST (not SE33) to calculate an approximate contributor ratio. Ratio must be no less than 1:2 unknown to owner in order to deduce.
  - Given this ratio, confirm that a minor contributor peak would be above ST
- Yes = Left; No = Right

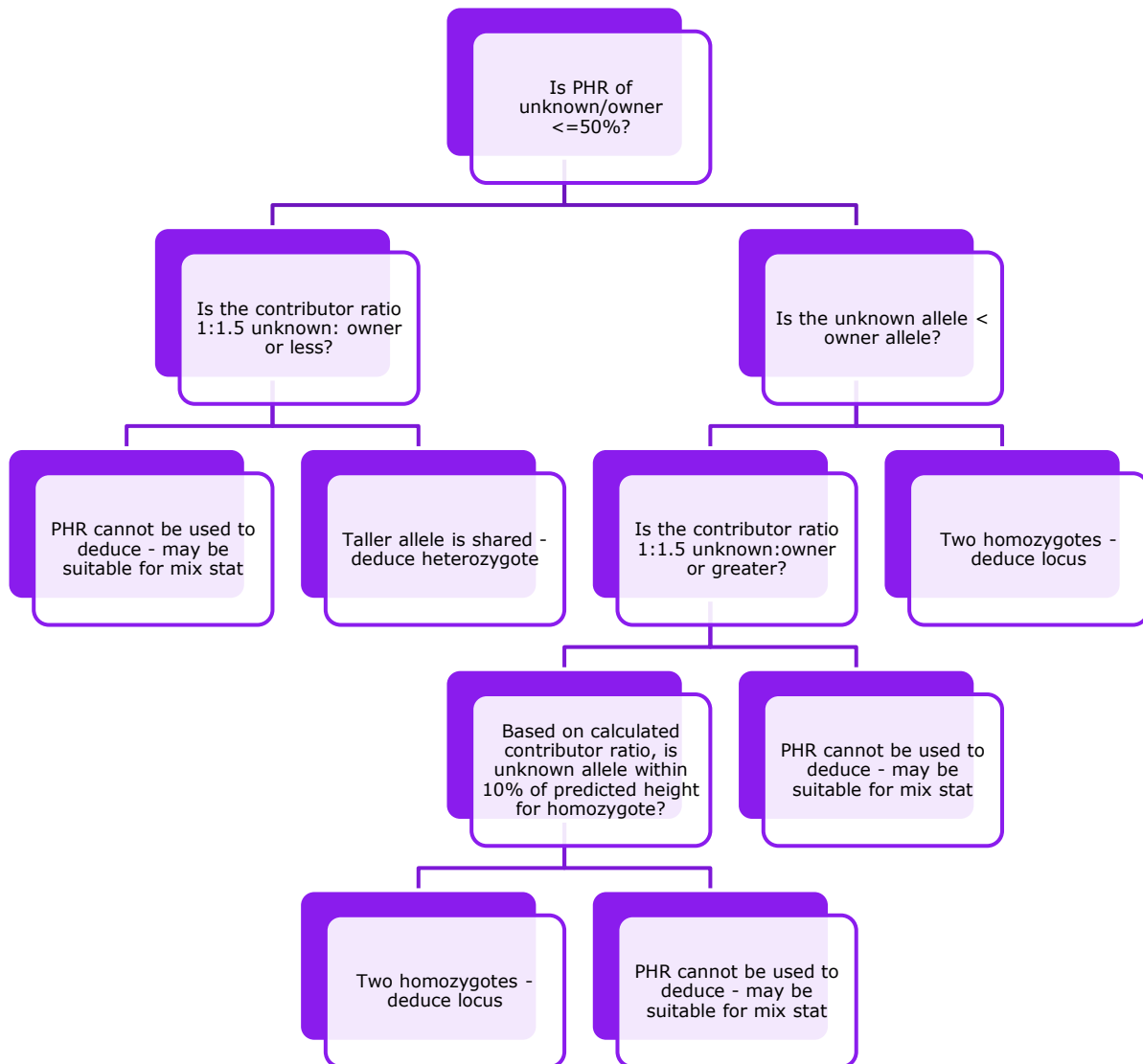


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Status: Active

### ***Appendix W: Deduction at 2-Allele Locus with a homozygous elimination sample***

- All alleles at two-or three-allele deduced locus must be at or above IT
- Before beginning, assess possible drop out of the second contributor:
  - Use at least two four-allele loci with all alleles above ST (not SE33) to calculate an approximate contributor ratio. Ratio must be no less than 1:2 unknown to owner in order to deduce.
  - Given this ratio, confirm that a minor contributor peak would be above ST
- Yes = Left; No = Right
- To calculate predicted unknown homozygote peak height (HPH):
  - owner HPH \* unknown/owner contributor ratio = predicted unknown HPH



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### Appendix X: NIST Mutation Rates at STR Loci

Apparent Mutations Observed at STR Loci in the Course of Paternity Testing\*

STR System	Maternal Meioses (%)	Paternal Meioses (%)	Number from either	Total Number of Mutations	Mutation Rate
<a href="#">CSF1PO</a>	95/304,307 (0.03)	982/643,118 (0.15)	410	1,487/947,425	<b>0.16%</b>
<a href="#">FGA</a>	205/408,230 (0.05)	2,210/692,776 (0.32)	710	3,125/1,101,006	<b>0.28%</b>
<a href="#">TH01</a>	31/327,172 (0.009)	41/452,382 (0.009)	28	100/779,554	<b>0.01%</b>
<a href="#">TPOX</a>	18/400,061 (0.004)	54/457,420 (0.012)	28	100/857,481	<b>0.01%</b>
<a href="#">VWA</a>	184/564,398 (0.03)	1,482/873,547 (0.17)	814	2,480/1,437,945	<b>0.17%</b>
<a href="#">D3S1358</a>	60/405,452 (0.015)	713/558,836 (0.13)	379	1,152/964,288	<b>0.12%</b>
<a href="#">D5S818</a>	111/451,736 (0.025)	763/655,603 (0.12)	385	1,259/1,107,339	<b>0.11%</b>
<a href="#">D7S820</a>	59/440,562 (0.013)	745/644,743 (0.12)	285	1,089/1,085,305	<b>0.10%</b>
<a href="#">D8S1179</a>	96/409,869 (0.02)	779/489,968 (0.16)	364	1,239/899,837	<b>0.14%</b>
<a href="#">D13S317</a>	192/482,136 (0.04)	881/621,146 (0.14)	485	1,558/1,103,282	<b>0.14%</b>
<a href="#">D16S539</a>	129/467,774 (0.03)	540/494,465 (0.11)	372	1,041/962,239	<b>0.11%</b>
<a href="#">D18S51</a>	186/296,244 (0.06)	1,094/494,098 (0.22)	466	1,746/790,342	<b>0.22%</b>
<a href="#">D21S11</a>	464/435,388 (0.11)	772/526,708 (0.15)	580	1,816/962,096	<b>0.19%</b>
<a href="#">Penta D</a>	12/18,701 (0.06)	21/22,501 (0.09)	24	57/41,202	<b>0.14%</b>
<a href="#">Penta E</a>	29/44,311 (0.065)	75/55,719 (0.135)	59	163/100,030	<b>0.16%</b>
<a href="#">D2S1338</a>	15/72,830 (0.021)	157/152,310 (0.10)	90	262/225,140	<b>0.12%</b>
<a href="#">D19S433</a>	38/70,001 (0.05)	78/103,489 (0.075)	71	187/173,490	<b>0.11%</b>
<a href="#">SE33 (ACTBP2)</a>	0/330 (<0.30)	330/51,610 (0.64)	None reported	330/51,940	<b>0.64%</b>

\*Data used with permission from [American Association of Blood Banks \(AABB\) 2003 Annual Report](#).

Includes compilation of multiple years. Information also available on population and allele-specific mutation rates (see <http://www.aabb.org/sa/facilities/Pages/relationshipreports.aspx>). A total of 44 different paternity testing laboratories provided this STR mutation data.

Data from the NIST webpage <http://www.cstl.nist.gov/div831/strbase/mutation.htm>  
(current as of 1/9/14)

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**Appendix Y: Overview of Capital Project Work Flow****FB Supervisor or DNA TM**

- \* Scan cases for anonymous/no crime
- \* Notify tech reviewer cases are ready
- \* Send list of requested eliminations to LE agency while tech reviews are in progress
- \* Asks LE agency for clarification on No Crime cases, as needed

**Criminal Justice Planner or DNA TM**

- \* Sends all Bode case file documentation to LE agency via secure transfer device

**Contract tech reviewer(s)**

- \* Perform technical reviews
- \* Identify cases with CODIS eligible profiles
- \* Notify AK lab when reviews complete

**AK SCDL qualified analyst**

- \* Enter eligible CODIS profiles

**FB Supervisor or DNA TM**

- \* Review CODIS entry (may be done by a second qualified analyst)
- \* For cases: copy completed review form and Bode documentation into LIMS
- \* For batches: ensure control data saved on I: drive
- \* Notify LE agency of which cases have profiles entered into CODIS
- \* Update spreadsheet
- \* As hits occur, assign follow up to AK qualified analysts

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## ***Appendix Z: Protocols for STR population frequency statistic calculations***

### **Random Match Probability Formula – for single source profiles**

**Note:** Only data from complete, single source loci are used to calculate statistics with this method. Uninterpretable loci (those with possible allelic dropout), or loci where a single source contributor could not be determined, are not to be used in this calculation.

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

Heterozygotes	$2pq$
Homozygotes	$p^2 + p(1-p)\theta$

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

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 expected frequency of a DNA profile in a population is the inverse of the multiple locus genotype frequency.

### **Combined Probability of Inclusion (CPI; Mixture Formula)**

**Note:** Only data from complete, interpretable loci are used to calculate statistics with this method. Uninterpretable loci (those with possible allelic dropout or loci where a two-source major component could not be determined), are not to be used in this calculation.

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.



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## Using Popstats to calculate population frequency statistics

### *Calculating Frequencies/Probabilities Using Popstats*

- Open Popstats (in the CODIS software) and choose either Forensic Single Sample or Forensic Mixture from the menu.
- In the configuration summary, verify that the database is the Expanded FBI STR 2015 database and the theta value is set to 0.01 (see image below).
- All populations except African American and Caucasian can be unchecked as these data are not reported.
- In the Specimen ID field, enter the lab #\_Item#

**Note:** When entering a Forensic Mixture, be sure to enter the profile in the left most available fields (labeled "Mixture" in the image below). The middle and right columns are for use only in calculating likelihood ratios.

- In the Comments field, add any additional info (i.e. sperm fraction, major profile)
- Enter the alleles deemed appropriate for statistical analysis (see STR Data Interpretation section).

**Note:** It is only appropriate to enter alleles for loci where statistics can be calculated for all required populations.

- Click the Calculate button or select Calculate from the Popstats pull-down menu.
- Forensic Single Source
  - Click the 1/f button.
  - Click the printer icon or select Print from the File pull-down menu. Select Broward Report and print.
- Forensic Mixture
  - The result should default to the Inclusion probability
  - Click the printer icon or select Print from the File pull-down menu. Select Probability of Inclusion (Short) and print.

**Note:** The above steps must be performed for both the Alaska Expanded Core 2017 database and the Expanded FBI STR 2015 database. The theta correction factor (0.01 for the STR database and 0.03 for the Alaska database) must be specifically selected for single-source statistic calculations when toggling between databases. Theta values are not used for calculation of mixture statistics. Ensure the check box is unmarked in the configuration summary.

- To change to the Alaska Expanded Core 2017 database, click Browse (in the configuration summary tab) to navigate to the Alaska Expanded Core 2017 folder and click OK (see image below).

**Note:** It may be necessary to expand the configuration summary window to reveal the Browse button when calculating Forensic Mixture statistics (see image below).

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- For single source statistics, change the theta value to 0.03 using the drop down menu in the configuration summary tab.

**Note:** The database and theta value must be changed in this order or the theta value will revert back.

- Click the Calculate button and repeat the above steps for printing the resulting statistics

### Navigating to the FBI STR Caucasian and African-American Population Databases

The screenshot displays the Analyst Workbench software interface for a forensic single sample analysis. The 'Configuration Summary' tab is active, showing various settings for the analysis. The 'Population Database' is set to 'Expanded FBI STR'. The 'Ethnic Groups' section has 'African American' and 'Caucasian' selected. The 'Probability Formula' section shows the 'NRC '96' formula with a theta value of 0.01. A 'Browse For Folder' dialog box is open, showing the file system structure with 'Expanded FBI STR 2015' selected. Purple arrows point to the 'Population Database' dropdown, the 'theta' value, and the 'Expanded FBI STR 2015' folder.

**Configuration Summary**

Lab ID: AK0020200  
Specimen ID:   
Comment:   
Retrieval:   
Calculate:   
Clear:   
Import:   
Export:   
STR:   
mtDNA:   
Y-STR:   
Population Database:   
Statistics Database Directory: \\10.64.1.146\codin\Popstats\POPDATA\Expanded FBI STR   
Browse...   
View Notes:   
Ethnic Groups:   
African American   
Apache   
Caucasian   
Chamorro   
Filipino   
Navaho   
Description:   
FBI's U.S. STR population database for African American, Apache, U.S. Caucasian, Chamorro, Filipino, Navajo, Southeast Hispanic, Southwest Hispanic, and Tenejapan population groups (February 2015). See NOTES.TXT for details.

**Probability Formula**

NRC '96  
Homozygotes:  $f = p^2 + p(1-p)\theta_1$   
Heterozygotes:  $f = 2pq$   
Rec 4.1 formula:  $2p$   $2p - p^2$   
 $\theta_1 = 0.01$   
 $\theta_1$  is used by Mixture, FSS, and Match Estimation.

**Population Subgroup**

Homozygotes:  $f = \frac{(2\theta_2 + (1-\theta_2)p)[3\theta_2 + (1-\theta_2)p]}{(1+\theta_2)(1+2\theta_2)}$   
Heterozygotes:  $f = \frac{2\theta_2 + (1-\theta_2)p[\theta_2 + (1-\theta_2)q]}{(1+\theta_2)(1+2\theta_2)}$   
 $\theta_2 = 0.01$

**Inbreeding**

Homozygotes:  $f = p^2 + p(1-p)C_f$   
Heterozygotes:  $f = 2pq(1-C_f)$   
 $C_f = 0.01$

**Browse For Folder**

- Batch Target Files
- Certificates
- CSA
- Installation DVD 7.0 SP7
- MAV
- MHS
- Popstats
  - POPDATA
    - Alaska
    - Alaska Expanded Core 2017
    - Amended FBI STR 2015
    - Expanded FBI STR 2015
    - FBI
  - Troubleshooting
  - upgrade files
  - netlogon
  - sysvol

Make New Folder OK Cancel

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## Selecting the Alaska Native Population Databases

Analyst Workbench - SDIS-AK0020200 - Forensic Single Sample

File Edit Go Popstats Tools Window Help

Workbench Explorer

Popstats Calculations  
Forensic Single Sample  
Forensic Mixture  
Parentage  
Kinship  
Match Estimation  
Partial Match

MM:Default Forensic Single Sample

Target Profile  
Lab ID: AK0020200  
Specimen ID:  
Comment:

Retrieval  
Calculate  
Clear  
Import  
Export

Specimen Details:

STR	mtDNA	Y-STR
Locus	Partial	Rec 4.1
D3S1358	No	
VWA	No	
D16S539	No	
CSF1PO	No	
TPOX	No	
Yindel	No	
Amelogenin	No	
D8S1179	No	
D21S11	No	
D18S51	No	
D2S441	No	
D19S433	No	
TH01	No	
FGA	No	
D22S1045	No	
D5S818	No	
D13S317	No	
D7S820	No	
SE33	No	
D10S1248	No	
D1S1656	No	
D12S391	No	
D2S1338	No	
Penta E	No	
PENTA D	No	

Configuration Summary

STR mtDNA Y-STR

Population Database  
Statistics Database Directory: \\10.64.1.146\code\Popstats\POPDATA\Alaska Expanded Core 2017  
Browse...  
View Notes

Ethnic Groups:  
☒ Ath  
☒ Iu  
☒ Yup

Description:  
Population studies on three Native Alaska population groups using STR loci. See NOTES.TXT for details.

Probability Formula

NRC '96  
Homozygotes:  $f = p^2 + p(1-p)\theta_1$   
Heterozygotes:  $f = 2pq$   
Rec 4.1 formula:  $\theta_1 = 0.03$   
 $\theta_1$  is used by Mixture, FSS, and Match Estimation.

Population Subgroup  
Homozygotes:  $f = \frac{[2\theta_2 + (1-\theta_2)p][3\theta_2 + (1-\theta_2)p]}{(1+\theta_2)(1+2\theta_2)}$   
Heterozygotes:  $f = \frac{2[\theta_2 + (1-\theta_2)p][\theta_2 + (1-\theta_2)q]}{(1+\theta_2)(1+2\theta_2)}$   
 $\theta_2 = 0.01$

Inbreeding  
Homozygotes:  $f = p^2 + p(1-p)C_f$   
Heterozygotes:  $f = 2pq(1-C_f)$   
 $C_f = 0.01$

Browse For Folder

10.64.1.146  
codis  
AK-NDIS Assessment  
Alaska Popstats Print Issue  
Batch Target Files  
Certificates  
CSA  
Installation DVD 7.0 SP7  
MAV  
MHS  
Popstats  
POPDATA  
Alaska  
Alaska Expanded Core 2017  
Amended FBI STR 2015  
Expanded FBI STR 2015  
FBI

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## Entering a Mixture Statistic

The screenshot shows the Analyst Workbench - Forensic Mixture interface. It consists of three main panels for entering mixture statistics. Each panel has a 'Lab ID' field, a 'Specimen ID' field, and a 'Comment' field. Below these are checkboxes for 'STR', 'mtDNA', and 'Y-STR'. The main area of each panel is a table with columns for 'Locus', 'Partial', and 'Allele' (Allele 1 through Allele 8). The 'Mixture' section at the bottom of each panel has a '# unknowns for H1' or '# unknowns for H2' field. A black arrow points to the 'Mixture' section of the left panel.

## Changing the Population Database when Calculating Forensic Mixtures

The screenshot shows the Analyst Workbench - Forensic Mixture Configuration Summary dialog box. The 'Population Database' dropdown is set to '10.64.1.146-popdata-popdata-expanded-fbi-str'. The 'Ethnic Groups' section shows 'African-American', 'Apache', 'Cherokee', 'Filipino', and 'Hispanic' selected. The 'Description' field contains text about the FBI's U.S. STR population database. The 'Mixture' section has a 'Use Theta1 in Probability of Exclusion/Inclusion Calculations' checkbox and a 'theta1' field set to 0.01. A 'Browse For Folder' dialog box is open, showing the file structure of the '10.64.1.146' folder, with 'POPDATA' selected. Purple arrows point to the 'Population Database' dropdown, the 'Ethnic Groups' section, the 'theta1' field, and the 'POPDATA' folder in the 'Browse For Folder' dialog.

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### Relationship Testing Terminology

These are standard definitions, included here for ease of access. For purposes of convenience, many of these definitions are phrased in terms of an alleged father, since that is the most common scenario encountered in forensic paternity work.

- **Likelihood Ratio:** the ratio of two probabilities of the same event under different hypotheses. For example, in the case of one-parent paternity testing, the likelihood ratio compares the support of the genetic evidence for the hypothesis that the alleged man is the true biological father, against the support of the biological evidence that a random and unrelated man is the true biological father.
- **Paternity Index (PI):** A likelihood ratio at a single genetic locus that compares the probability of the observed genotypes (DNA profiles) if the tested man is the true biological father, to the probability of the observed genotypes (DNA profiles) if a random untested man is the true biological father.
- **Combined Paternity Index (CPI):** Because the genetic information at each of the loci is inherited independently, paternity indexes can be multiplied together to get a combined paternity index. The CPI is a measure of the strength of the genetic information from several loci. It indicates whether the hypothesis that the tested man is the father or the hypothesis that a random untested man is the father is more supported by the genetic evidence.
- **Combined Paternity Index Ranges:** In theory, for a CPI less than one, the genetic evidence is more consistent with non-paternity than paternity. In theory, for a CPI greater than one, the genetic evidence supports the hypothesis that the tested man is the father: "It is XXX times more likely to see the genetic results if the tested man was the true biological father than if an untested random man was the father."
- **Probability of Paternity:** A calculation based on both the paternity index and the prior odds. This probability should, in theory, include all evidence in the case, including both the non-genetic information and the genetic information from the DNA paternity test; as such, it is a measure of the weight of all the evidence. In practice, it is common to assume a prior probability of 0.5, or 1:1, when calculating and reporting probability of paternity.

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- **Mutation rate:** the rate at which a genetic marker mutates or changes over time. It is the number of mutations per hundreds of generations expressed as a decimal value or a percentage. It indicates how often, on average, one expects a random man in the population, unrelated to the child whose parentage is in question, to appear as if he is the biological father.
- **Mean power of exclusion:** the average probability that a random person would have a pattern of genetic information inconsistent with paternity at a particular locus. It indicates how often, on average, one expects a random person in the population, who is unrelated to the child whose parentage is in question, to be correctly excluded as a biological parent.
- **One-parent Paternity:** A scenario that includes biological reference samples from a child, one known parent and one alleged parent.
- **Zero-parent Paternity:** A scenario that includes biological reference samples from a child and one alleged parent (with no reference from a known biological parent).
- **Exclusion:** The obligate parental alleles in the child do not match the alleles in the alleged father in at least four loci. The alleged father is excluded from being the biological father of the child being tested.
- **Inclusion (Cannot Exclude):** The obligate parental alleles in the child match the alleles in the alleged father at all (or all but one) of the loci. The alleged father cannot be excluded from (or is consistent with being) the biological father of the child being tested.
- **Reverse parentage:** A scenario in which a missing person's reference sample is compared to samples from a pair of alleged biological parents.
- **Parentage:** Refers to either paternity or maternity; paternity and parentage are often used interchangeably in genetic testing terminology.

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## Significance Estimations in Relationship Testing

As with ASCDL casework, calculations will be performed and reported for the following populations: Caucasian, African American, Athabaskan, Inupiat, and Yupik. Calculations are performed using the U. S. Department of Justice, FBI Popstats Standalone program, version 7.0. The resultant values may be truncated for reporting but should never be rounded up.

Some assumptions underlying the statistical calculations include:

- Hardy-Weinberg equilibrium
- In a false trio - the biological father is from the same population
- In a false trio – the biological father is unrelated to the mother and child
- In a false trio – the biological father is the same genotype as the tested father
- PI for a mutation is not allele dependent

For one-parent forward calculations, 0.5 is used for the prior probability.

For all calculations at a locus with possible mutation, Paternity Index = mutation rate for that locus / mean power of exclusion for that locus. Calculations will use mutation rates by locus as reported by NIST, and mean powers of exclusion from the published articles for the respective databases (references listed at the end of this section).

Paternity Index (PI) is calculated for each locus, according to the formulas listed in the tables below. In those tables, capital letters refer to the allele(s) present in each individual tested, and the small letters refer to the frequency of the allele(s).

After PI is calculated (as below) for each locus, the Combined Paternity Index (CPI) is calculated by multiplying together all the individual PIs.

Assuming a prior probability of 0.5, probability of paternity (expressed as a percentage) is calculated as  $(CPI/(CPI+1)) \times 100$  and reported to three decimal places (XX.xxx %).

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## Forward one-parent testing:

Known Parent	Child	Alleged Parent	Paternity Index
BD	AB	AC	$1/2a$
BC	AB	AC	$1/2a$
BC	AB	AB	$1/2a$
BC	AB	A	$1/a$
B	AB	AC	$1/2a$
B	AB	AB	$1/2a$
B	AB	A	$1/a$
AB	AB	AC	$1/[2(a+b)]$
AB	AB	AB	$1/(a+b)$
AB	AB	A	$1/(a+b)$
AB	A	AC	$1/2a$
AB	A	AB	$1/2a$
AB	A	A	$1/a$
A	A	AC	$1/2a$
A	A	A	$1/a$

## Forward zero-parent testing:

Child	Alleged Parent	Paternity Index
AB	AC	$1/4a$
AB	AB	$(a+b)/4ab$
AB	A	$1/2a$
A	AC	$1/2a$
A	A	$1/a$



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Reverse parentage testing:

Alleged Parent - 1	Child	Alleged Parent -2	Paternity Index
BD	AB	AC	1/8ab
BC	AB	AC	1/8ab
BC	AB	AB	1/8ab
BC	AB	A	1/4ab
B	AB	AC	1/4ab
B	AB	AB	1/4ab
B	AB	A	1/2ab
AB	AB	AC	1/8ab
AB	AB	AB	1/4ab
AB	AB	A	1/4ab
AB	A	AC	1/4a <sup>2</sup>
AB	A	AB	1/4a <sup>2</sup>
AB	A	A	1/2a <sup>2</sup>
A	A	AC	1/2a <sup>2</sup>
A	A	A	1/a <sup>2</sup>

### ***Calculating Frequencies/Probabilities Using Popstats – Paternity***

**[Note:** On occasion, especially when entering mutation rates or toggling between databases, the screen may not automatically refresh to show updated changes. If you do a Print Preview, you can confirm that changes have been applied.]

#### **A. One-parent forward paternity (trio):**

1. Open Popstats and choose Parentage from the menu on the left side of the screen.
2. Choose the Parentage tab at the top of the screen.
3. Use the Specimen ID field to enter the lab number at least, item number space permitting. If the item numbers do not fit in the typed file they may be hand-written on the printout.
4. Under Subtype, select the Trio button (see following image)
5. Enter STR information from all complete loci (If a locus has partial information, it will not be used for statistics – this includes not only the reference with the partial information, but the same locus in the other references as well).

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The screenshot shows the 'Standalone Popstats - Parentage' window. The 'Match Estimation' tab is active, and 'Parentage' is selected. The 'Lab ID' is 'Lab #00000000, #12'. The 'Specimen ID' is 'Known mother'. The 'Subtype' is 'Two'. The 'Calculate' button is highlighted with an arrow. Below the 'Calculate' button are three tables: 'Biological Parent', 'Child', and 'Alleged Parent'. Each table has columns for 'Locus', 'Allele 1', and 'Allele 2'. The 'Biological Parent' table has a 'TPOL' entry highlighted. The 'Child' table has a 'TPOL' entry highlighted. The 'Alleged Parent' table has a 'TPOL' entry highlighted.

6. When the correct information has been entered, select the Calculate button (upper left).
7. If any loci have mutations (mismatched), you will be prompted to enter the mutation rate and the mean power of exclusion (see following image).

The screenshot shows the 'Mutation Rate and Mean Power of Exclusion' dialog box. The 'Locus' is 'D7S820'. The text 'Enter Mutation Rate and Mean Power of Exclusion' is displayed. Below this is a table with the following data:

Group Name	Mutation Rate	Mean Power of Exclusion
Ath	0.001	0.58
Inu	0.001	0.548
Yup	0.001	0.533

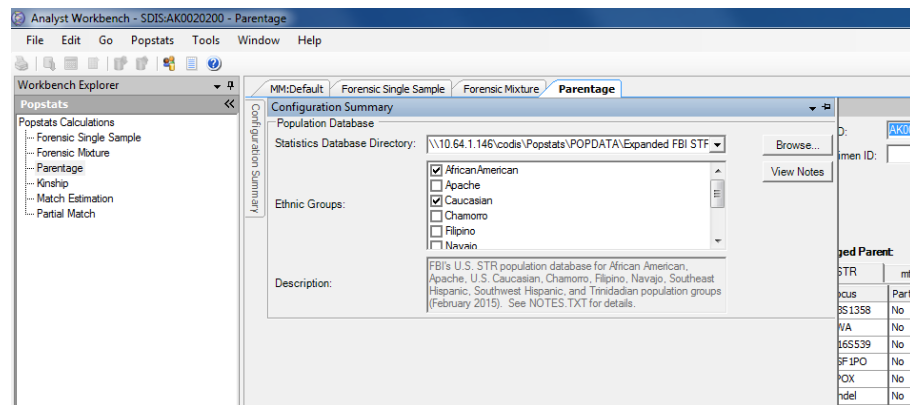
At the bottom of the dialog box are 'OK' and 'Cancel' buttons.

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8. Enter the locus-specific mutation rate from the NIST website. A printout of the relevant page is included in an appendix of this manual.
9. Enter the locus-specific mean probability of exclusion (PE) from the published database references listed at the end of this section of the manual. Please note that these values are NOT the default values included in the Popstats software.
10. Once these values are entered, click OK, then click Calculate.
11. Printing: Under the File tab, select Print, then select Parentage Trio Calculations, then select Print.
12. Print out statistic reports for the Caucasian and African-American databases (from the Expanded FBI STR 2015 database file) and the Athabaskan, Inupiat, and Yupik databases (from the Alaska Expanded Core 2017 database file). In order to toggle between databases, click on the Configuration Summary tab on the left side. Choose Browse to switch between the Alaska and FBI databases (see following image). Once the new database has been selected, the software will return to the Target Profile tab, where you will again choose Calculate.

**Note:** It may be necessary to expand the configuration summary window to reveal the Browse button when changing the population database.



13. As before, if necessary, you will be prompted to enter mutation rate and mean power of exclusion. When finished, choose OK. Print report as described previously.

**B. Reverse parentage (trio):**

1. Open Popstats and choose Parentage from the menu on the left side of the screen.
2. Choose the Parentage tab at the top of the screen.
3. Use the Specimen ID field to enter the lab number at least, item number space permitting. If the item numbers do not fit in the typed file they may be hand-written on the printout.
4. Under Subtype, select the Reverse button (see following image)

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The screenshot shows the Analyst Workbench software interface. The main window is titled "Forensic Single Sample - Parentage". It contains three columns for "Biological Mother", "Biological Father", and "Alleged Child". Each column has a table with headers: "Locus", "Partial", "Allele 1", and "Allele 2". The tables are populated with various loci such as D2S1338, TH01, D1S11, D18S51, Penta E, D2S18, D13S317, D7S820, D16S539, CSF1PO, PENTA D, Amelogenin, VWA, D8S1179, TPOX, PGA, D19S433, D2S1338, SE33, D10S1248, D2S1045, D2S441, D12S391, and D151656. The interface also includes a sidebar with various tools like Popstats, Match Manager, and Pedigree Manager.

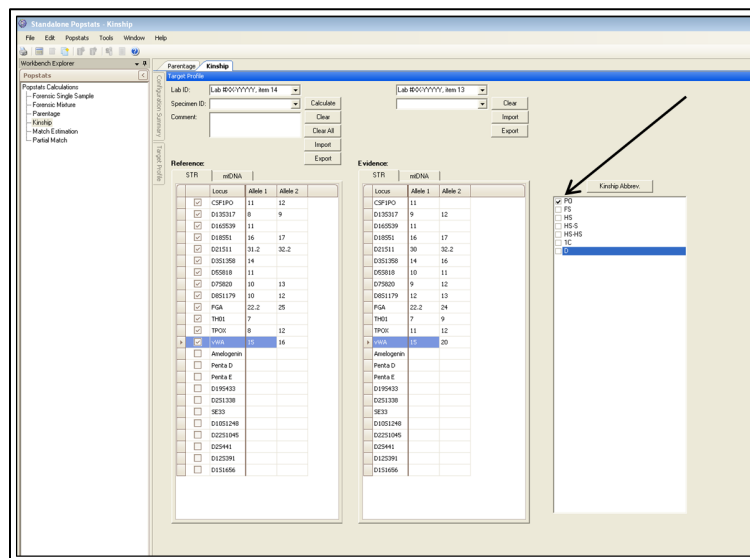
5. Repeat steps 5-13 from Section A.
6. On reverse parentage trios, Popstats does not allow for the use of a locus with a mutation. In cases with a mutation, omit the locus with the mutation from the Popstats calculation. On the Popstats printout, manually calculate the PI for the locus with the mutation. Use this manual calculation to adjust the CPI and Probability of Paternity calculations as well. Show work clearly.

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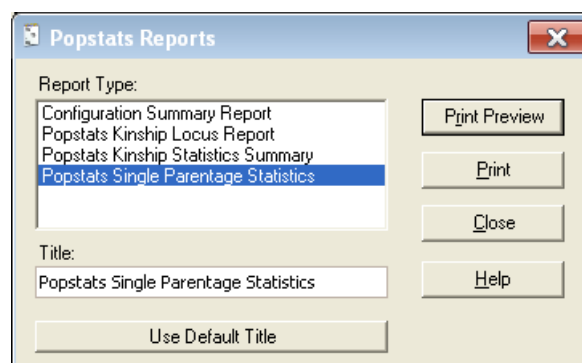
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C. Zero-parent forward:

1. Open Popstats and choose Kinship from the menu on the left side of the screen.
2. Choose the Kinship tab at the top of the screen.
3. Use the Specimen ID field to enter the lab number at least, item number space permitting. If the item numbers do not fit in the typed file they may be hand-written on the printout.
4. On the right side of the screen, make sure that only the PO (Parent-Offspring) box is checked (see following image).



5. Repeat steps 5-10 from section A.
6. Printing: Under the File tab, select Print, then select Popstats Single Parentage Statistics, then select Print (see following image).



Print out statistic reports for the Caucasian and African American databases (from the Expanded FBI STR 2015 database file) and the Athabaskan, Inupiat, and Yupik databases (from the Alaska Expanded Core 2017 database file). Directions and image for toggling between databases are listed in section earlier in this appendix.

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## Appendix AA CODIS Sample Accessioning

CODIS samples come into the laboratory through the evidence section, typically via the United States Postal Service. Samples are either logged by evidence staff or retrieved from evidence and taken to the CODIS storage room for accessioning. Alternatively, samples may be placed in an analyst's mailbox for retrieval.

Each sample is opened and inspected prior to entry in LIMS.

- Verify that the submitting agency is listed on the inside of the card. If not, write in the agency name [from the box or outer envelope] on the appropriate line. The outer envelope may be thrown away.
- If the inner swab envelope is not sealed, place evidence tape across the opening to secure it, initial & date the evidence tape, and make a note on the card that it arrived unsealed.
- Bundle the cards [in sets of approximately 20] and place them in a designated cabinet until they are ready to be entered into LIMS.

**NOTE:** Entry of CODIS samples requires APSIN access. Any individual training to enter samples must first receive APSIN clearance.

### LIMS entry

- a. CODIS samples are entered using the LIMS-APSIN Interface. The log in for this is the second username/password and the operator ID used for APSIN.
- b. Click Change Printer toward the bottom of the window. Select current barcode printer.
- c. Enter the APSIN number from the card and click Search APSIN. It may take a moment for the information to pull up.  
**NOTE:** If the APSIN number is less than 7 digits long, add leading 0's to make it 7 digits. For example, 123456 would be entered as 0123456.
- d. Ensure the information pulled up by the APSIN search is the same as the information on the card (name, DOB, etc.).
- e. If all information is concordant, select the Agency Name. The fastest way to do this is to begin typing it. All agency names used for CODIS cards begin with an X. For example, Anchorage Correctional Complex (ACC) is listed as X ANCHORAGE CORRECTIONAL COMPLEX.  
**NOTE:** Duplicate samples will not allow you to change the submitting agency or individual type; in this instance skip steps e and g.
- f. Select the agency representative from those available in the drop down (often simply Agency Rep or Records Sergeant).
- g. Select the individual type. If no type is listed, default to Arrestee.  
**NOTE:** Juvenile cards do not include options for the individual type. Select "juvenile offender" for all blue cards.
- h. Click "Save". This will create the offender record in LIMS, print 2 barcodes, and show a pop up stating the record was created/updated.

**NOTE:** If there is an error, the barcodes will still print but the case will not be created/updated; make sure to read the pop up. If there is an error and the case isn't created/updated, start back at step c and discard the barcodes.

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- i. Separate the print card from the side with the swab attached. Swabs are the “a” evidence (1a, 2a, etc.) and the print cards are the “b” evidence (1b, 2b, etc.). When applying barcodes to the “a” evidence, ensure the barcode is on the swab envelope, not the card, in case the envelope becomes detached from it.
- j. Click “Clear” before entering the next card. A prompt will ask if you’re sure you want to clear the screen. Click yes.
- k. When you are finished entering cards, transfer them to the appropriate containers in LIMS and then physically put them there. Older print cards and swabs do not need to be containerized in LIMS. Each box is labeled on the front with the first and last case numbers that are present in the box.

**NOTE:** If the sample belongs in a container that is in an analyst’s possession, leave the card in the designated location on the counter.

### Troubleshooting

- If the information pulled up in the APSIN search does not match the information on the card, pull up the offender in the APSIN program and check for aliases (F9 once you get to the profile). If the information on the card is that of the offender’s alias, enter the case then make a note on the card and add the additional information under “Individuals” in the LIMS case with the word “ALIAS” in the Company field.
- If the card does not list an APSIN number or lists an incorrect APSIN number, search for the offender by name in APSIN (with and without DOB if listed on the card). If unable to locate the offender in APSIN, contact the submitting agency to find out the APSIN number and verify the information on the card.
- Any corrections or notes on the CODIS cards must be accompanied by the initials of the person making the correction and the date the correction was made. The date and initials should be placed near the APSIN line on the left edge of the card.
- If unable to resolve any issues that arise, contact the CODIS administrator for assistance.

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## Appendix BB Database Batch Set-up

In LIMS, view the database samples available for analysis

- On top toolbar select Analysis
  - Assign Requests for Analysis
    - Department: Biology/DNA
      - Refine list
        - Service: DNA Database
        - Reason: All
        - Suspect: All

Request Assignment

Lab LAB Department Biology/DNA

Cannot unassign request

Total 3270 Selected 4 Refine List

Lab Case #	Req #	Evid. Count	Reason	Requested	Due By	Name	Service
13-04180	0001	2		08/20/2013	11	Agent	DNA Database
13-04181	0001	2		08/20/2013	11	Supervisor	DNA Database
13-04182	0001	2		08/20/2013	11	Supervisor	DNA Database
13-04183	0001	2		08/20/2013	11	Agent	DNA Database
13-04184	0001	2		08/20/2013	11	Agent	DNA Database
13-04185	0001	2		08/20/2013	11	Liu	DNA Database

Analyst DeBoer, Veronica ZCH2N2GR05 Total 6122

Lab Case #	Req #	Service	Reason	Assigned	Due By
06-2166	0001	DNA Database		12/08/2009	11
09-0166	0001	DNA Database	Rush	03/18/2009	11
09-0750	0001	DNA Database		04/09/2009	11
09-0751	0001	DNA Database		04/09/2009	11
09-0752	0001	DNA Database		04/09/2009	11
09-0753	0001	DNA Database		04/09/2009	11

Select All Clear Close

For a full database batch, select the first 85 samples. To do this you may click each individual box or use the space bar and downward arrow key on the keyboard. Assign the samples to yourself. This is done by selecting your name under “Analyst” (NOT the version that begins with APSIN-) and then clicking the down arrow. You may now close the window.

Create the database batch worksheet

Exporting LIMS sample list to create an Excel spreadsheet

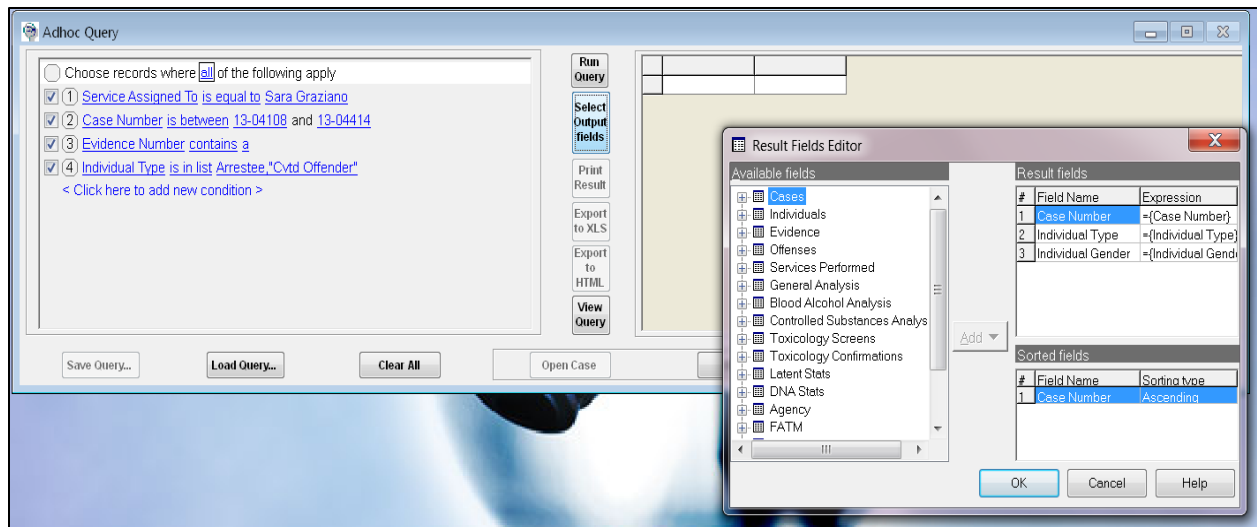
- Open Microsoft Excel
- In JTRAX select “Search”
  - Ad Hoc Query
    - Load Query
    - DNA new database assignments (public, not in alpha/numeric order)
      - Select yourself as individual
      - Select the date service assigned is between \_\_\_\_ and \_\_\_\_ for the newly assigned samples
      - (A private query may be created. Use the output fields as listed below)
    - Select output fields
      - Case Number



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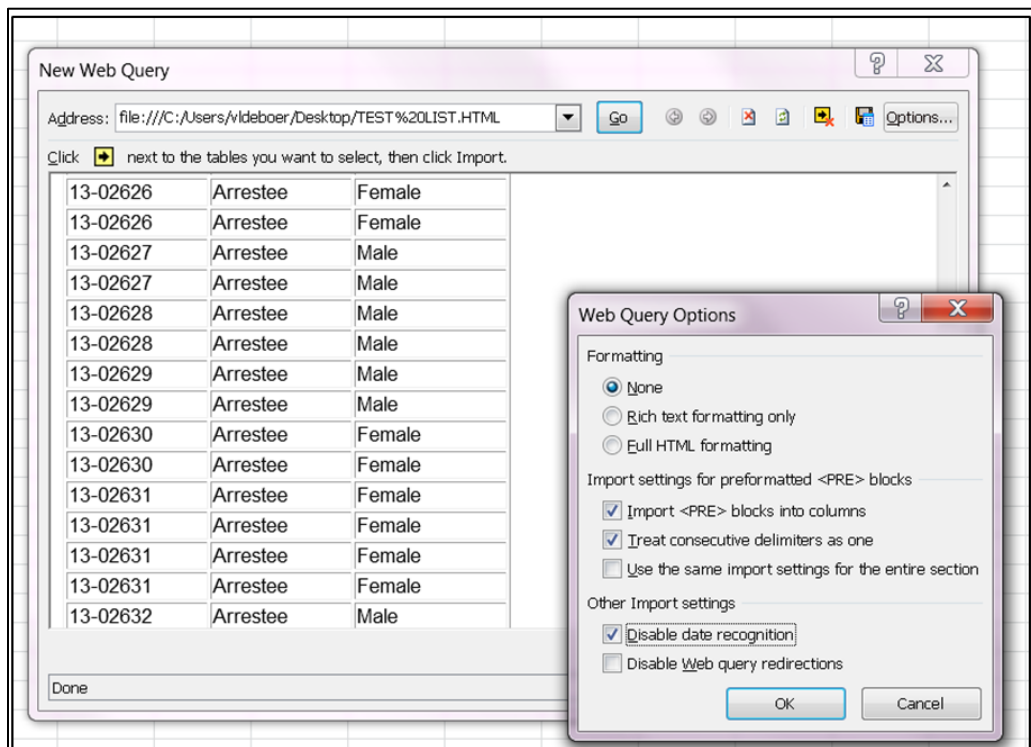
- Individual Type
- Individual Gender
- Sort by ascending case number
- Select “OK”
- Run Query



- Chose “Export to XML” option and save query to your chosen location.
- Open Microsoft Excel
  - Go to “Data”
    - “From Other Sources”
      - “From XML Data Import” – now navigate to the location where you saved your list (select “all files types” – to the right of file name to see your file). Double click on file
      - This will open a “New Web Query” box
        - Click on “Options” – click on the “Disable Date Recognition” within the Web Query Options. Leave others as is. Select “OK”
        - The above bullet may no longer be applicable. Historically imports had trouble with 08-xxx and 09-xxx samples regarding dates.

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- Select Import. You will be prompted “Where do you want to put the data”  
– Leave it as default (existing worksheet A1)

From this master list, groups of sample ids and offender types can be copied and pasted into the database batch worksheet. This can also be used to track samples that require good faith efforts, etc.

Review the assigned samples to see if any lab number has been duplicated. This often indicates that a -2a or -3a sample exists. These can be used as part of the 5% quality control samples for a batch but are not easily copied/pasted because they should not be positioned directly next to their duplicate. Additionally, duplicates do not have to be in the same batch as their corresponding sample.

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#### Adding samples to the database batch worksheet

- Open the current GFEWS (located in I:\Quality Assurance Program\Controlled Documents\International Program\Discipline Procedure Manuals\Biology\Controlled Forms (not part of a manual))
- Batch name is typically DBYY-MMDD followed by the analyst's initials. If doing multiple batches in a single day, add an additional identifier. For example DB16-0720RT\_A.
- Enter the sample names (lab case number) and codes (ex. A1, A2 .....)
  - This can be done by copying from the spreadsheet created in section 2.3.1
  - Remember that wells A1, A4, A7 and A10 (of a full 96 well amplification plate) will be for the allelic ladder. It is only necessary to run one ladder per injection when running a partial plate. The last two wells (G12 and H12 on a full plate) are for the reagent blank/negative and positive amplification controls.
  - Make sure the worksheet contains 5 duplicate samples – 1 per injection, 1 injection will have 2 duplicates but don't place them right next to each other.
- To assist with QC checks, check the appropriate box on the worksheet for female samples.
- Save the worksheet

In LIMS, transfer the necessary boxes to your custody.

- Evidence Transfer
  - From: (leave blank)
  - To: scan your badge; enter your PIN
  - Evidence to Transfer: scan your box(es). **Important:** say "NO" when prompted if you want to empty your container(s). If you click yes, you will need to re-containerize all of the samples in the box.
  - Click Apply – say "yes" to "record evidence transfer" prompt.

Open LIMSEvidenceQueue (Active) and log in with your LIMS username/password.

- Click on "requested by" to sort.
- Highlight all of the database samples assigned to you
- Click the single down arrow
- Click the image of an envelope with a red circle in front of it

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## **Appendix CC**

### ***STR Data Interpretation***

Interpretation of database samples is based on the guidelines contained in this section, as well as the qualified DNA analyst's training and experience. In all cases, interpretations must be independently reviewed and verified based on established peer review procedures. The DNA Technical Manager may authorize the interpretation/reporting of data outside of the criteria defined herein and will document this approval by initialing and dating comments on the relevant paperwork.

The minimum peak height acceptable for database sample STR loci alleles is 175RFU. Lower threshold limits for hit confirmations or ILS (Internal Lane Standard) may be authorized by the DNA Technical Manager.

#### **Interpretation of Batch Controls**

Prior to assessment of data, the analyst must first examine the batch controls to ensure that the extraction, amplification, and genetic analysis processes are functioning correctly. These include: Internal Lane Standard (ILS), allelic ladder(s), internal control specimen (ICS), positive amplification control, and negative amplification control (also serves as reagent blank). All or part of an extraction, amplification and/or run may need to be repeated depending on the results of the batch controls. Any issues raised by the performance of a batch control must be addressed prior to the release of affected database samples. Unresolved failure of a batch control requires the Technical Manager, or designated individual, to approve a course of action.

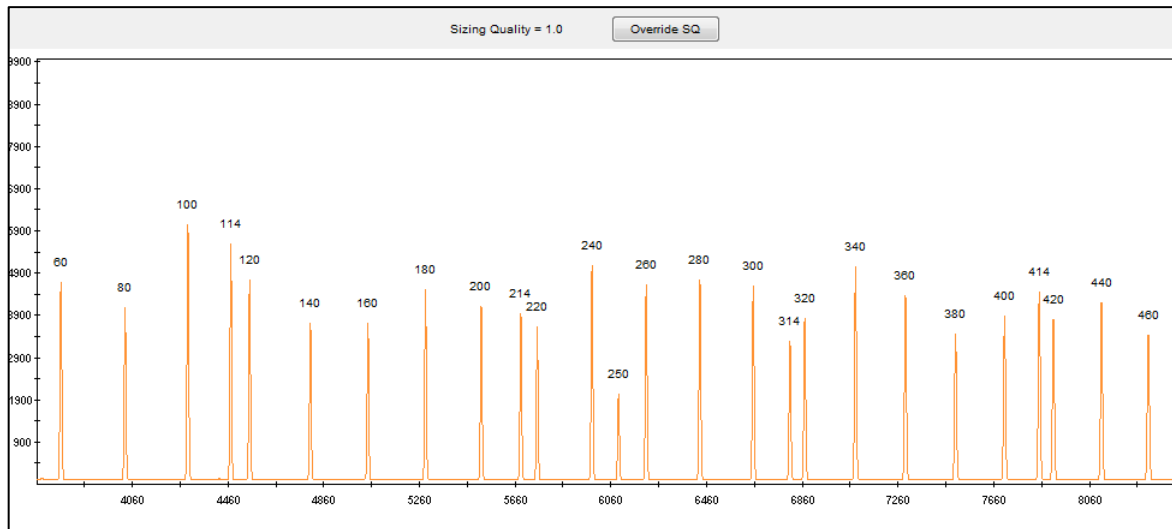
#### **Internal Lane Standard (ILS)**

GlobalFiler Express uses GeneScan 600 LIZ as an internal lane standard. The analyst should verify that all peaks from 60-460 base pairs are present and labeled as shown.

This verification consists of confirming that the sizing quality is greater than 0.5. If the sizing quality is 0.5 or lower, the analyst must confirm by visual inspection that each peak was correctly called. Once verified, the analyst should override the sizing quality to reflect that the ILS has been manually reviewed. In addition, samples with broad peaks and a Sizing Quality of 0.5 or less are more susceptible to allelic drop out.

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The peak heights for the ILS peaks must be at least 175RFU (relative fluorescence units) to be identified and labeled by the GeneMapper™ ID-X software with the database method.

Failure of an ILS can often be resolved by re-prepping and/or re-injecting the affected sample(s). If the ILS continues to fail after re-prepping and re-injecting, the analyst should consult the Technical manager for a course of action.

### Allelic Ladder

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 analyst should verify that all peaks from the allelic ladder(s) are present and labeled as shown.

The allelic ladder contains the most common alleles determined for each of the 20 FBI STR core loci, Amelogenin, DYS391, SE33 and a Y indel. In addition, alleles not labeled in the allelic ladder (virtual alleles), may be detected and labeled in some of the samples analyzed.

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

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

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(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 the locus shift and will impact the correct allele call / labeling of the samples in the project. Allelic ladders with such artifacts cannot be used to analyze data in the project. If other allelic ladders in the run are successful, it may be possible to change the sample type for the failed ladder from Allelic Ladder to Sample. In that case, the failed ladder is not averaged in with the other ladders. If omitting the failed allelic ladder does not lead to successful analysis, re-injection is usually appropriate.

Additional information about the allelic ladder can be found in the GlobalFiler Express PCR Amplification Kit User Guide.



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### ***Positive Amplification Control***

A positive control is defined as a single source sample whose genetic profile was previously determined and from which a full profile was developed; it is used to evaluate the performance of the amplification and typing procedures. The standard DNA template 007 is currently used as the positive amplification control for the GlobalFiler Express Amplification Kit.

Generally, approximately 5% of the samples in a database batch will be quality control samples such as a duplicate offender sample or a staff duplicate sample. One of these samples may also serve as the positive amplification control when the kit control fails. Failure to obtain the expected profile for at least one positive control sample requires re-amplification of the entire plate.

Another option for a failed positive control is to re-prepare and/or re-inject the positive control if the cause of failure appears to be a failed electrophoretic injection. As long as the re-prepped and/or re-injected positive control types successfully, the positive control is considered successful.

When an incorrect STR profile is obtained for one or more of the positive control samples in a batch, other profiles in the 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 a case by case basis.



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**DNA Control 007 (image can be found in [appendix](#)):**

<b>D3S1358</b>	<b>15, 16</b>
<b>VWA</b>	<b>14, 16</b>
<b>D16S539</b>	<b>9, 10</b>
<b>CSF1PO</b>	<b>11, 12</b>
<b>TPOX</b>	<b>8</b>
<b>Y-INDEL</b>	<b>2</b>
<b>Amelogenin</b>	<b>X, Y</b>
<b>D8S1179</b>	<b>12, 13</b>
<b>D21S11</b>	<b>28, 31</b>
<b>D18S51</b>	<b>12, 15</b>
<b>DYS391</b>	<b>11</b>
<b>D2S441</b>	<b>14, 15</b>
<b>D19S433</b>	<b>14, 15</b>
<b>TH01</b>	<b>7, 9.3</b>
<b>FGA</b>	<b>24, 26</b>
<b>D22S1045</b>	<b>11, 16</b>
<b>D5S818</b>	<b>11</b>
<b>D13S317</b>	<b>11</b>
<b>D7S820</b>	<b>7, 12</b>
<b>SE33</b>	<b>17, 25.2</b>
<b>D10S1248</b>	<b>12, 15</b>
<b>D1S1656</b>	<b>13, 16</b>
<b>D12S391</b>	<b>18, 19</b>
<b>D2S1338</b>	<b>20, 23</b>

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### Negative Controls

A reagent blank/negative amplification control is carried through the entire analytical process as part of each extraction or type of extraction. It contains all the reagents - except DNA template - used during extraction, amplification and typing. For GlobalFiler Express, the control consists of Prep-n-Go buffer and the PCR master mix (reagents used to prepare the PCR amplification mixture).

The purpose of the negative control is to detect DNA contamination that might occur from the reagents, the laboratory environment, between the samples being processed, and/or due to improper handling of the samples by the analyst. The negative control must be run at the most stringent set of conditions for the batch.

Verification of the presence of amplicon in the negative control is performed by viewing the presence of unincorporated primer peaks.

The negative control should not yield any true STR allelic peaks above the reporting threshold. When peaks greater than or equal to the reporting threshold are present in the range between 60-460 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 the reporting threshold will not invalidate the sample as long as the peaks can be shown to be artifacts. Artifacts will be documented in the GeneMapper ID-X project.

When probable true allele peaks are detected above the reporting threshold, at several or all STR loci, the analyst will investigate possible causes. The Technical Manager will be consulted to determine the appropriate course of action.

Since the failure of a negative control may indicate a problem at the extraction or amplification level, a Quality Review Form may need to be completed based on the nature of the discrepancy.

**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.

### Quality Control Check

Offender profiles are compared against the profiles of relevant staff members to ensure that contamination by laboratory staff is not included in uploaded profiles. This can be accomplished using the Profile Comparison tool in GeneMapper ID-X or by manual comparison. Staff member

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buccal swabs may be used intentionally as quality control samples; and these are expected to yield profiles concordant with the profiles on record. However, the unexpected presence of a laboratory staff profile indicates a problem at the extraction or amplification stage and requires a Quality Review Form.

### ***Interpretation of Samples***

Typically, each locus is characterized by one or two labeled peaks or alleles of approximately equal intensity.

Allele peak heights should typically fall between 1000 and 6000RFU. 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 350 RFU may occasionally exhibit peak height imbalance due to stochastic effects caused by low template copy number.

Peak height ratios (PHR) are calculated by dividing the peak height (in RFUs) of the lower RFU allele by the peak height of the higher RFU allele, and then multiplying this dividend by 100 to express the PHR as a percentage. The GeneMapper™ ID-X software is set to flag samples with a PHR below the expected minimum of 50%. PHR imbalance (in a single source sample) may occur with low quantities of template DNA, degraded template DNA or in the presence of PCR inhibitors. Severe imbalance may result in allelic dropout, where one or more alleles occur below the detection threshold or are not visible at all.

The presence of more than two peaks or severe PHR imbalance at a locus may be explained by a tri-allele, primer binding site mutation, variant allele, or other biological or procedural artifact. The presence of these at several loci is indicative of a mixed sample.

### **Tri-alleles**

Tri-allelic patterns (three alleles at a single locus) are known to occur 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.

Samples exhibiting tri-allelic patterns with all alleles of approximately equal intensity, or with the 3<sup>rd</sup> allele having a peak height greater than or equal to 50% of the second-largest peak, are entered as such in CODIS. When tri-allelic patterns are exhibited at two or more loci, the sample is entered as a multi-allelic offender.

For samples exhibiting possible tri-allelic patterns where the 3<sup>rd</sup> allele is less than 50% the height of the second-largest peak, only the two largest alleles will be entered into CODIS. The analyst will note that there is a possible tri-allele at the locus in question on both the batch worksheet and the comments section in the CODIS entry.

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### **Off-Ladder (OL) Alleles**

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

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

True OL alleles will exhibit peak morphology similar to the other alleles in the sample and the PHR should be appropriate relative to the other allelic peaks in the sample. When the overall sample quality is poor, the analyst should consider re-amplification prior to designating an OL peak as a true allele.

When a true 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.

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 allele from the ladder that is closest in proximity to the OL allele.

True OL alleles are documented on the batch worksheet.

### **Low-Level DNA Samples**

Stochastic effects, including extreme peak height imbalance, are typical of samples with very low levels of DNA. One risk associated with such samples is the possibility that a true heterozygote will appear to be a homozygote due to a sister allele below the analytical threshold. To minimize this risk, a homozygote threshold (HT) of 350 RFU must be used for interpretation of database samples analyzed by GlobalFiler Express. The homozygote threshold is analogous to the GlobalFiler 630 RFU stochastic threshold employed in casework analysis, but specifically addresses the concern of partial drop out in a low-level database sample. A homozygous locus may only be considered complete if the peak height of the allele is equal to or greater than 350 RFU.

Any homozygous locus with an allele peak height below 350 RFU must be considered as potentially a partial profile at that locus.

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**NOTE:** The homozygote threshold of 350 RFU does not apply to profiles with broad peaks and sizing quality of 0.5 or less. Injections with broad peaks and sizing quality of 0.5 or less are only acceptable when it can be demonstrated that drop out could not have occurred.

- Samples which have been previously typed, such as QA samples and positive controls, and which can be shown to be complete by their previous results, are acceptable with broad peaks.
- Database profiles where all loci with broad peaks are heterozygous, which confirms that drop out could not have occurred at the locus, are acceptable.
- All other profiles with broad peaks must be re-injected and/or re-analyzed until a profile without broad peaks is achieved.

If allelic dropout, or possible drop-out based on the homozygous threshold, is observed in one or more core STR loci, the analyst will make a “good faith effort” (see Section 9) to re-process the sample in order to generate a complete genetic profile.

- It is not necessary to proceed with “good faith efforts” on QC samples if at least four of them yield complete data for at least ½ of the core loci. If not, attempt re-analysis on all QC samples that were not complete at the 20 core loci.

If the “good faith effort” also fails to generate a complete genetic profile, several possibilities exist:

- A profile with data (partial or complete) at all core loci and no more than three core loci with possible dropout (i.e. a homozygous peak below the HT) is eligible for upload to NDIS.
- A profile with complete information for at least 10 STR core loci is acceptable for upload to SDIS.
- Consult the DNA Technical Manager or a CODIS Administrator for any profile which does not meet the above criteria after “good faith” efforts.

When incomplete typing results are possible at one or more STR loci, indicate this by selecting “Yes” in the Partial Profile field in CODIS for the applicable loci.

Data may be composited from multiple amplifications/injections of the same lysate/extract of a database sample in order to obtain a more complete profile at the core loci.

Composite profiles of offender samples, created by combining the typing results from separate extracts, are acceptable for entry and upload to NDIS. Prior to creating this type of composite profile, the biographical information and overlapping loci shall be verified as being concordant.

### Artifacts

Artifacts are data peaks resulting from the analytical process and are not attributable to an individual contributor to the sample.

When artifacts are not pervasive throughout the sample and do not interfere with data interpretation, the sample does not require re-analysis. When an artifact is present within the size

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range of the alleles in a core locus and the presence of the artifact interferes with interpretation of the data in that locus, or when a sample exhibits artifacts in several loci, interpretation of the sample becomes more challenging and it may be necessary to re-process the sample. Artifacts may be resolved merely by re-injecting a sample; or they may require re-prepping with less amplification product, re-amplification or re-extraction to resolve. If the interfering artifact is located in one of the non-CODIS-core loci, the analyst may choose to enter the profile, omitting all data from the affected locus.

Samples uploaded into CODIS must not contain any labeled artifacts or OL alleles. The analyst may remove the artifact label. Artifact or OL peaks are either deleted or re-labeled by right clicking on the peak and adding a comment ('microvariant allele', 'artifact' etc.). Any labeled artifacts deemed true alleles should be noted on the batch worksheet.

#### **Incomplete 'A' nucleotide addition (-A or split peaks)**

Platinum Taq™, 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 final step of the STR amplification process is a terminal extension step to promote complete non-template 'A' nucleotide addition. The "-A" form is the peak that represents the actual template length. The "+A" peak will be the predominant form, with the "-A" peak typically not being detected.

Failure to attain complete terminal nucleotide addition (typically due to an excess of template DNA) results in "split peaks", two peaks above threshold that are one base apart. If the labeled "-A" peaks are not pervasive throughout the sample and the sample appears to otherwise be a single source sample, the labels may be deleted in the project and the reason documented electronically.

#### **Pull-up**

Multi-component analysis is the process that separates the six different fluorescent dye colors into distinct spectral components. The six dyes (6-FAM, VIC, NED, TAZ, SID, and LIZ) 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. This typically occurs at high RFU values, when an excess of template DNA has been amplified.

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.

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If the pull-up is prevalent enough to interfere with data interpretation, the sample may need to be re-injected. Otherwise, the labels may be deleted in the project and the reason documented electronically.

### **Stutter**

The PCR amplification of tetranucleotide STR loci typically produces a minor product peak four bases shorter than the corresponding base peak. This artifact is the result of slippage of the Taq polymerase and is referred to as the stutter peak or stutter product. Stutter products four bases longer, or two bases shorter, than the corresponding base peak are less frequently observed. Two base pair stutter products are so rare that peaks in these positions are not filtered out in the analysis parameters.

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 GeneMapper™ ID-X database analysis methods have a global stutter filter of 20%. 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 elevated stutter labels may be deleted in the project and the reason documented electronically.

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.

### **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, usually appearing in more than one color at the same base size, and not reproducible by re-injection.

Peaks labeled as such by the GeneMapper™ ID-X software do not interfere with upload to CODIS and no re-analysis is required if the spike does not interfere with interpretation of the sample.

### **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.

If the artifact is labeled but does not interfere with data interpretation, the dye blob label(s) may be deleted in the project and the reason documented electronically.

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### **Persistent Kit Artifacts**

Occasionally, PCR kits may exhibit persistent artifacts that may or may not appear as true alleles. Typically, these artifacts are observed and documented during kit verification process. As with other artifacts, if the artifact does not interfere with data interpretation, the labels may be deleted in the project and the reason documented electronically.

### **Contamination**

The verification of reagents prior to use in the analysis of database batches is conducted in part to detect contamination which permeates an entire lot of a given reagent or set of reagents. However, such checks will not necessarily detect contamination arising from a single event (for example, cross-contamination between two adjacent samples).

For database samples, whenever data suitable for comparison indicates that DNA from more than one individual is present in a sample, the sample must be re-processed in an effort to obtain a single source profile. In the meanwhile, no samples from the affected batch are to be uploaded without approval from the DNA Technical Manager.

Furthermore, an assessment must be conducted (using the form provided in FBPM) in an attempt to establish the source of the contamination. Corrective Action may be pursued depending on the results of the root cause analysis.



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## **Appendix DD      Review of Data**

Database projects are routinely reviewed electronically, with the technical reviewer looking at the controls/samples in GeneMapper ID-X rather than printed electropherograms. The analyst may choose to submit printed electropherograms to facilitate documentation and review of artifacts, true OL alleles, tri-alleles, concordance checks on re-analyzed samples, etc.

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 (as Convicted Offender, CO Duplicate, Arrestee, Staff Duplicate). This is done by selecting the appropriate Specimen Category while in GeneMapper ID-X. Samples not exported to CODIS are marked "no export".

### ***Review of Offender Database Samples Analyzed In-House***

Genetic profiles and all supporting documentation generated in the course of analysis will be subjected to a technical review. The DNA analyst will submit a packet containing the database batch worksheet and any printed electropherograms to another qualified database analyst for technical review. This review will be conducted in accordance with the FBI QAS Guidelines and includes the following, at a minimum, to ensure compliance with the interpretation guidelines contained within this document:

- Review of the ILS for all passing samples
- Review of all allelic ladders designated as such
- Review of all amplification positive controls and quality control samples
- Review of all reagent blank/negative amplification controls
- Review of all DNA typing data for all passing samples
- Verification of eligibility for all profiles marked for export, to include selection of an appropriate specimen category
- Concordance with previous results for re-processed samples

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. Upon completion of the technical review, the analyst may import the passing samples to SDIS. Another qualified analyst or a CODIS Administrator will then complete an administrative review, to include reconciling the results of the offender duplicate and casework autosearches.

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Upon completion of the technical review, the GMID-X project will be exported (using GeneMapper Manager) to the discipline share.

Upon completion of the technical and administrative reviews, NDIS eligible samples are uploaded by a CODIS Administrator. Additional information on CODIS procedures is contained in the CODIS Administrative Manual. Upon completion of the upload, the following paperwork is archived as a .pdf in the LIMS (Laboratory Information Management System):

- Database batch worksheets
- STR Offender Batch Review Checklist
- SDIS Import Reconciliation Report

#### **Review of Outsourced Offender Database Samples**

The laboratory does not currently use a vendor laboratory for DNA analysis. Should the laboratory enter into a contract for DNA analysis of database samples with a vendor laboratory, a procedure for the in-house review of outsourced samples will be created before analysis begins.

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## Appendix EE “Good Faith Efforts” at Recovery of Low-level Samples

As mentioned in section 7.2.3, if allelic dropout, or possible drop-out based on the homozygous threshold, is observed in one or more core STR loci, as per the NDIS Operation Procedure, the analyst will make a “good faith effort” to obtain a complete genetic profile. **A “good faith effort” may include any appropriate combination of the following options used to obtain a full interpretable profile at all CODIS core loci.** Good faith efforts described in this section must be completed for all samples within a batch before any portion of the batch is submitted for technical review. Alternatively, a LIMS request may be created for any samples requiring re-extraction [as in section 9.3], and the batch may be submitted after all re-injections, re-preparations and re-amplifications are completed.

If good faith efforts are unsuccessful and a duplicate sample is available, it shall be processed in a new batch of database samples using GlobalFiler Express.

Good faith efforts always require a comparison between the original profile (if any is obtained) and the follow-up profile to ensure that no incorrect transfer(s) occurred and that results are consistent.

- For samples that are re-injected, re-prepped with diluted amplification product, or re-amplified, the new data may be added to the original GMID-X project for a verification comparison within the project. If the differences between allelic ladders are significant enough to cause sizing issues in the combined project, then it will be necessary to keep the runs in separate projects.
- Re-extracted samples are always analyzed in separate projects. For re-extracted samples where the original GFE analysis yielded interpretable/complete data for at least 50% of the core loci, the analyst shall submit a printout of the original amplification to the technical reviewer for a concordance check.

For all concordant data resulting from a second extraction, the analyst will note that the profile has been confirmed in the case synopsis in JT.

### Re-prepping Samples Over Targeted with GlobalFiler™ Express

Samples that produce DNA profiles with an extensive amount of pull up may be diluted at a 1:10 ratio on the original amplification plate and re-prepped for CE analysis. This must be done within one week of the original amplification date.

To dilute the samples:

- Replace the amp tape with Glad Press n Seal and circle the affected well(s).
- Add a sufficient amount of deionized formamide (dF) to the affected well(s) and gently pipette up and down five times to mix the contents. Typically, the well will contain 14µL of amplified product after the initial prep, and 126µL dF will be added.

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- Place an additional layer of Glad Press n Seal over the top of the amplification plate
- From this point, refer to section 5.3 to preparing a CE plate.
- Re-seal the amplification plate with adhesive tape and store at 2-8°C.

### **Re-amplification**

The incubation time of less than 60 minutes at room temperature is optimized for a majority of whole-swab samples. Samples containing less DNA may benefit from prolonged incubation time at 2 – 8 °C. As a second attempt to gain a full profile for CODIS entry, the lysate may be incubated at 2 – 8 °C for up to two weeks and amplification re-attempted. In this case, the only change to the amplification protocol is the length of the incubation time.

### **Extraction by EZ1-XL and Amplification by GlobalFiler**

If re-amplification is not successful and additional untested swab material remains (i.e. a second swab within the original item), extraction shall be attempted. The remaining swab may be extracted using either the protocol for Direct (non-differential) Extraction for Questioned samples or the Known Direct Extraction protocol (FBCP, current version). The resulting extracts will then be quantified and amplified using GlobalFiler.

Controls and offender reference samples will be analyzed according to the parameters set forth for casework reference samples. When multiple offenders are analyzed in a casework batch, the analyst may document the analysis of the samples electronically and upload the profiles as a .cmf file (as described in this manual). When batch uploading offenders from a casework batch, the offender samples are documented using the sample list page of GFEWS (current version). The casework batch name is used as the database batch name on the top of the form. A separate review checklist is provided [in this manual] for offender samples being batch processed in casework. The offender review checklist and sample list worksheet will be retained in the casework central log.

CODIS confirmations may be uploaded if they meet the eligibility requirements in section 7.2.3. One of the specimens will later be changed to offender duplicate as described in this manual. Offenders that are not going to be batch uploaded to CODIS are documented using the CEW and single offender review checklist (current versions).

If the database analyst is performing extraction and amplification as part of the database batch, analysis should be conducted as soon as is practicable; before beginning a new set of GFE database projects or before beginning a new casework batch.

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## **Appendix FF Upload of projects to CODIS**

### **Incomplete profiles**

- a. If SDIS only – designate as such on the batch worksheet and mark for upload to SDIS with the project. The sample must be unmarked at SDIS to prevent it from going to NDIS. If a subsequent sample yields a more complete profile, the initial sample will be changed to a duplicate.
- b. NDIS eligible – designate the appropriate specimen category on the batch worksheet and mark for upload to SDIS/NDIS with the initial project. If a subsequent sample yields a more complete profile, the initial sample may be changed to a duplicate. If the original sample was already sent to NDIS, it will automatically come back out once the specimen category is changed.
- c. If the initial sample does not yield a complete profile at the 20 core loci and a second swab or duplicate sample exists, it will be processed in a subsequent batch of samples.

### **Export batch from ID-X to CODIS**

Batches of offender samples analyzed in-house are exported from GeneMapper ID-X as CMF 3.2 (.xml) files and then imported into SDIS as follows. When exporting, AK0020200 should be entered in the fields for both the source and destination labs.

- a. Open Specimen Manager from the Analyst Workbench
- b. Select Import Specimens under the Specimen Manager pull down menu or click on the Import icon on the toolbar.
- c. Select the appropriate CMF file (.xml) and click Open. When prompted, the CODIS User importing the file should select Data Import and their User ID and click OK.
- d. A message will appear indicating that the import file was successfully imported; click OK.
- e. Open the Message Center and select the Import STR Files tab; double click on the bold file to validate the file.
- f. Select the Import Reports tab; double click on the bold file.
- g. The reconciliation report details the samples that will be imported. The CODIS User should check to see that all profiles can be successfully uploaded and identify any problem samples.
- h. Once any problems are corrected, the analyst can select the file within the Import STR Files tab and execute using the icon in the toolbar. The reconciliation report (for the import) will be printed and retained with the offender batch paperwork.
- i. Edit any samples with (possible) partial locus dropout to set the partial profile flag to yes at the applicable loci.

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**Note:** Marking a locus as partial will automatically trip the flag for the sample. A sample for which there is only complete locus dropout does not require a partial flag to be marked yes.

- j. Un-mark any profiles not suitable for upload to NDIS.
- k. A CODIS Administrator or another qualified analyst may be consulted to troubleshoot problems with importing offender batches.
- l. Administrative review is completed by a CODIS Administrator or qualified database analyst and includes reconciliation of the matches resulting from the duplicate offender and AK new searches. These searches are scheduled to occur automatically Monday-Friday at 7pm. Data must be searched prior to an NDIS upload. Additional information on CODIS searches is contained in section 3.3 of the CODIS Administrative manual.
- m. Upload of NDIS eligible profiles is completed by a CODIS Administrator.
- n. Once the samples have been successfully uploaded to NDIS, the paperwork will be returned to the analyst to be scanned into the LIMS.

### Offender Duplicate Samples

Once two offender samples are verified as duplicates, the specimen category of one of the samples is changed to CO Duplicate.

- If samples have the same specimen category (i.e. both arrestees or both convicted offenders), the specimen category of the sample with the most complete profile remains as is and the other is changed to CO Duplicate.
  - If both profiles are complete, either one may be changed to CO Duplicate.
- When the match is between a convicted offender sample and an arrestee sample, the specimen category for the arrestee is changed to CO Duplicate.
  - If the convicted offender sample is incomplete at the original 13 core CODIS loci or a less complete profile overall, it can be changed to CO duplicate and the arrestee sample changed to Convicted Offender.
  - If the original sample was not at NDIS, it will be necessary to manually mark it for upload.

If a sample already at NDIS is changed to CO Duplicate, the sample is automatically unmarked and removed from the NDIS level upon the next upload from the state laboratory. This will be indicated on the next reconciliation report from NDIS.

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## **Appendix GG      Follow-up for Incomplete Profiles**

When an offender profile fails to generate a complete profile for all 20 core loci (no profile or partial at 1 or more core loci) after good faith efforts, and the lab does not possess a duplicate offender sample for the individual, the analyst (or designee) shall complete the following steps.

1. Remove the APSIN # (State ID) from the case record in JT and adds either "SDIS only", "partial NDIS" or "no profile" to the "DL No." field in JT. This ensures that any new sample for the offender will result in a new request for analysis.
2. Locate print card. Indicate (and highlight) one of the following on the card (preferably down the right side of the card front):
  - a. No DNA profile obtained; need new sample
  - b. Partial DNA profile obtained; need new sample
3. Refile the print card.

On occasion, it may be appropriate to modify an offender entry based on subsequent typing of another sample for the same individual. For example, an offender sample may yield only a partial profile, but a sample submitted later in casework yields a complete profile. The offender entry may be updated with data obtained from the subsequent sample. Typically, this will be done only when the initial entry was incomplete at the original 13 core CODIS loci.

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## **Appendix HH      Transferring Print Cards to and from R&I**

### Transferring print cards to R&I division of DPS

1. Print cards are transferred to R&I as a bundled set of cards (usually ~170) and only after DNA analysis has been completed for all the samples in the container.
2. Obtain the next unverified set of cards from the CODIS storage room and transfer custody to oneself and subsequently to R&I using the LIMS.
3. Deliver the set of cards to DPS headquarters.

### Receiving print cards from R&I

1. Transfer custody of the received set of cards from R&I to oneself and then back to CODIS Storage.
2. Place the set of cards at the end of the completed prints row.
3. Inventory the set of cards to verify that all cards are accounted for. Notify the CODIS State Administrator if any cards were not returned in the set.
4. Pull all cards that were flagged by R&I as having an incorrect APSIN number or an APSIN merge. Band or clip them together and provide to a CODIS Administrator.
5. For all cards with a deceased flag, add the note "deceased as per R&I" in the *Synopsis* window in the offender record in LIMS.
6. Flags indicating "no prints on file" or on cards with no prints may be removed from the cards. No further action is required.
7. Pull all cards that were flagged by R&I as having poor quality prints that could not be verified. Band or clip them together and provide to the Latent Supervisor.



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## **Appendix II Follow-up for CODIS hits to offenders**

### Database analyst

1. Print match details short report.
2. If forensic case is 2009 or later, look up in JT to determine DNA analyst.
  - a. If current analyst, provide them with match detail report for follow-up
  - b. If former analyst, provide match detail report to a CODIS administrator for follow-up.
3. If forensic case is before 2009, provide match detail report to a CODIS administrator for follow-up.

### Casework analyst

1. Print match details short report, if not provided by the database analyst.
2. If match is less than high stringency, examine data to confirm match. A second qualified analyst must also confirm and both must date and initial the match details report.
  - a. Once confirmed, change the source ID of the forensic specimen to yes and the hit disposition to offender/arrestee hit.
3. E-mail a scan of the offender print card to the current designee to request verification of a qualifying offense. The response is saved to the case activity log in LIMS. It is not necessary to wait for the response before continuing to step 4.
  - a. If advised that the hit cannot be reported, change the hit disposition to Investigative Information and include a note as to why in the hit notes field. Advise a CODIS Administrator, who will determine what further action may be required.
4. Determine if the offender has previously been confirmed.
  - a. If yes, create hit letter request in forensic case (request date = match date) and draft hit letter.
  - b. If no, create a CODIS confirmation request in the offender case (request date = match date) in addition to the hit letter request above.
5. Put the match details report and the offender individual report (from LIMS) in the confirmation box on the office counter. Include a note indicating who the packet should be returned to once confirmed.
6. Latents verified (note in case synopsis in LIMS):
  - a. The presence of initials and date next to the APSIN number on cards that have been to R&I indicates that the latents were verified.
  - b. If latents could not be verified by R&I or the card has not been sent yet, transfer print card to a latent examiner to verify.

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**Offender Batch - Review Checklist**

Date Submitted (end date): \_\_\_\_\_

Technical Review Started: \_\_\_\_\_

Administrative Review Started: \_\_\_\_\_

Database Batch Number: \_\_\_\_\_

Analyst:	Tech. Review:	Admin. Review:
----------	---------------	----------------

Database batch # and analyst's initials on each page			
Reagent worksheet complete: analysis dates, instruments, # samples, reagent lot # and expiration dates, raw data folder(s), and project name(s)			
Batch worksheet complete			
Technical Reviewer checked batch worksheet (inc. reagents): initialed and dated each page and initialed each sample and control			
ILS labeled correctly for all passing samples			
Allelic Ladder(s): correct allele calls obtained <sup>1</sup>			
Positive control(s): correct DNA profile obtained <sup>1</sup>			
Reagent blank(s)/negative control(s): results are acceptable <sup>1</sup>			
Check for consistency between NO EXPORT samples on paperwork and in project			
Confirm appropriateness of NO EXPORT status			
Technical review of passing samples: ≤3 problem core loci, RFU 175, homozygotes > 350 RFU appropriate allele edits, OL allele documentation			
Correct specimen categories are assigned			
Confirm specimen category is concordant between paperwork and project			
Samples for SDIS only are flagged on paperwork			
Profiles checked against relevant staff profiles			
Profiles checked for concordance with prior results for any previously typed samples			
Any samples still requiring re-extraction have assignments created in LIMS			

<sup>1</sup> includes verification of ILS and controls for re-worked samples

Eligible profiles uploaded into SDIS (in the Offender Index)			
Reconciliation report: #samples uploaded consistent with batch worksheet			
Problem samples corrected and documentation attached			
Edits completed in CODIS for composited profiles			
Notations made on print cards for incomplete profiles			
Duplicate offender search performed and all high stringency matches reconciled			
AK new moderate stringency search performed (only after previous step completed)			
Project(s) exported to discipline share			

**Performed by CODIS Administrator**

Upload to NDIS performed			
Reconciliation report received; consistent with # samples uploaded			

**For Items with a LIMS assignment (CODIS confirmations or re-extracts)**

Results entered (e.g. Profile Verified) and assignment signed in LIMS			
Batch number noted in LIMS			

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### **Batch Upload of Offenders in Casework Batch - Review Checklist**

Central Log / Batch Number: \_\_\_\_\_  
Sample Cut Date(s): \_\_\_\_\_  
Date Analysis Completed: \_\_\_\_\_  
Technical Review Started: \_\_\_\_\_

Analyst:	Tech. Review:
----------	---------------

#### **OFFENDERS RUN IN CASEWORK BATCH – PROFILE UPLOAD OR CODIS CONFIRMATION**

Offender number(s) and analyst initials are on worksheet		
Start and end dates of examination/analysis are documented		
Consumed samples documented on worksheet and in LIMS		
Batch worksheet complete; technical reviewer initialed all samples		
ILS labeled correctly for all passing samples		
Technical review of all manual calculations, if applicable		
Check for consistency between NO EXPORT samples on paperwork and in project		
Confirm appropriateness of NO EXPORT status		
Results (i.e. profile verified) given for each tested item		
Correct specimen categories are assigned		
Confirm specimen category is concordant between paperwork and project		
Samples for SDIS only are flagged on paperwork		
Profiles checked against relevant staff profiles		
Profiles checked for concordance with prior results for any previously typed samples		
SOPs are linked to request in LIMS		

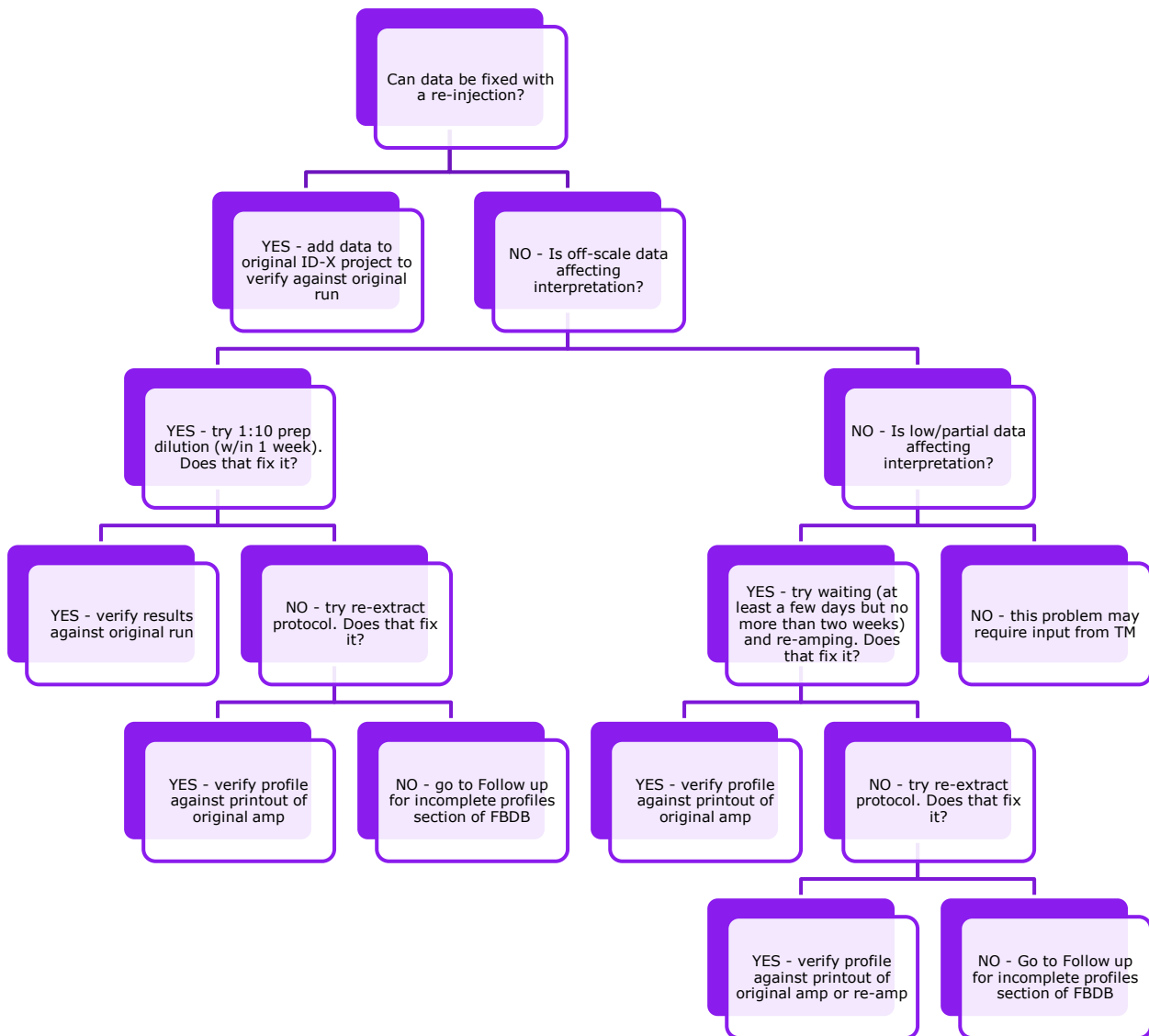
Eligible profiles uploaded into SDIS (in the Offender Index)		
Reconciliation report: #samples uploaded consistent with batch worksheet		
Problem samples corrected and documentation attached		
Edits completed in CODIS for composited profiles		
Notations made on print cards for incomplete profiles		
Duplicate offender search performed and all high stringency matches reconciled		
AK new moderate stringency search performed (only after previous step completed)		
Project(s) exported to discipline share		

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**Appendix LL: Good faith efforts for addressing profiles incomplete at CODIS core loci**



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***Appendix MM Forensic Biology Discipline Locker Key Policy***

- Evidence lockers in the Forensic Biology discipline are self-assigned. An analyst may choose any locker(s) for storing evidence. When lockers are not in use, keys are stored in the locks.
- When a locker is being claimed by an analyst for long term use, the analyst shall take custody of the key in the LIMS.
- A master set of locker keys is stored in the discipline supervisor's office. These may only be used with permission from the discipline supervisor or designated individual.
- If one of the locker keys is lost, the discipline supervisor shall be notified.

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## ***Appendix NN Forensic Biology Literature Review Policy***

Any member of the Forensic Biology discipline may put forward an article of scientific literature for members of the discipline to read, as appropriate to their areas of competency. Literature reviews are included as a part of regular discipline meetings. In general, analysts will be expected to present one article per year in a discipline meeting.

Analysts document their literature reading in Sharepoint in the discipline meeting minutes. In addition to the check boxes for attendance, for each meeting where literature was reviewed, the meeting minutes will have a list where analysts will record the date by which they completed the assigned reading.

A spreadsheet is maintained on the lab network which tracks scientific literature put forward to the Forensic Biology discipline.

Literature review documentation is monitored annually by the DNA Technical Manager.

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## ***Appendix OO    Extended Absence Policy***

When an analyst is away from the laboratory for an extended period (three months or longer), he/she will be required to successfully complete an internal competency test before resuming casework analysis. The scope of the competency test and authorization to resume casework are the responsibility of the DNA Technical Manager and/or the discipline supervisor

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## ***Appendix PP Forensic Biology Case Management***

### Incoming requests for service

Requests for biology services are received from the evidence discipline and are usually handled by the discipline supervisor(s).

New requests are evaluated using the following criteria:

- Ability of laboratory to perform requested service
- Evidence requiring examination has been submitted
- Required reference samples submitted
- Items requiring analysis in multiple disciplines marked as such
- Information in LIMS consistent with Request for Laboratory Services (RLS) form
- Adequate case information provided to make analytical decisions

If the laboratory has received sufficient evidence and information to proceed, requests are made available to be assigned in the LIMS.

- All cases will be completed in Justice Trax. Evidence received in Themis will be reported under the Justice Trax request. All items that were received in Themis have now been created in Justice Trax and the chain of custody will be maintained in Justice Trax going forward.

If the laboratory requires additional evidence and/or information to proceed, the laboratory will contact the submitting agency via telephone or e-mail. The agency may be informed that the request will be suspended if the case is not remedied within 30 days. Communications are retained in the LIMS. The case manager may retain a hard copy of the RLS to monitor the case.



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#### Case prioritization

Requests for service are generally prioritized as follows:

- Cases with upcoming trial certain dates
- Sexual Assault of a Minor/Homicides with significant public safety threat
- Sexual Assault/Homicides not included above
- Non-sexual assault
- Property Crime

#### Cancelled biology requests

Requests may be cancelled for a variety of reasons (based on results of previous examinations, probative results previously obtained, notification from agency or Department of Law that analysis no longer required, case adjudicated prior to testing).

It is incumbent on the individual who cancels a request to ensure that the reason is documented in the LIMS and that the requesting agency is informed.

The individual who cancels a request should also ensure that the intended disposition of relevant items is correct (in Justice Trax), as per the guidelines in the following section.

If analysis has already been started, completed worksheets/data will be submitted for a technical review and DNA metrics recorded

If samples were amplified, electropherograms will be interpreted with appropriate notations. Deductions are not required, if not yet completed.

The analyst will enter findings in LIMS, indicating only that no report is being issued and the reason.

The report and documentation are subject to technical and administrative review.

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### Forensic Biology evidence retention

Items submitted for Forensic Biology examinations are routinely triaged for processing, with the items most likely to yield relevant, interpretable results being given priority. Additional testing will not typically occur once probative results are obtained. More details on item selection policies are provided on the crime lab webpage.

The following guidelines will apply to most cases. Evidence may be returned to an agency at their request.

- Sexual assault kits will be retained by the laboratory indefinitely
- Stains/samples isolated (by laboratory personnel) from larger items will be retained indefinitely
- Non-consumed questioned DNA extracts will be retained
- Stains/samples isolated by law enforcement (or laboratory staff at the crime scene) and submitted as swabs for analysis will only be retained by the laboratory if they are tested by the DNA unit.
- Untested questioned samples/items will be returned to the submitting agency after completion of the case
- Control swabs/stains are not typically processed and will be returned
- Reference samples are typically retained indefinitely
  - o These items may be returned if no other items are being retained in a case.

### Forensic Biology Case Report Close-Out

Once a forensic biology report has been completed, the following administrative tasks must be completed. These tasks may be completed by the analyst or an administrative designee, but it is the responsibility of the analyst to confirm that all the above tasks have been completed correctly.

- DNA Central Log files retained in a designated location on the lab network or Sharepoint.
- Report sent to submitting agency (and prosecutor, if required). This may become automated in the LIMS.
- Release of report documented in LIMS.
- The bench notes must accompany the report unless analysis dates are included in the report. If the bench notes are being distributed, the distribution e-mail must state the total number of attachments comprising “the report” and must be retained in the case activities or case log.

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## ***Appendix QQ     Annual Forensic Biology Quality Review and Performance Monitoring***

In fulfillment of QAS standard 3.3, an annual review of the quality system in the Forensic Biology discipline will occur concurrently with the lab wide annual quality system review.

The quality review of the Forensic Biology discipline will be approved by the DNA Technical Manager.

At a minimum, the quality review of the Forensic Biology discipline will include the following:

- Audit (internal and/or external) of the Forensic Biology discipline
- Summary of performance monitoring for Forensic Biology discipline members
- Review of a sample of case files, in fulfillment of QAS 3.4. While The scope of this review will be modified annually by the Technical Manager, in advance of the review. At a minimum it must include a representative sample of cases worked, specifically including cases which demonstrate the elements required for the performance monitoring plan outlined later in this section.
- Inventory of long-term biological evidence storage
- Collection of feedback from discipline members regarding improvements to discipline manuals
- Review and updates to manuals
- Review of all verifications, validations, and performance checks. Follow-up as required.
- Review of all performance monitoring conducted in the previous year
- Conduct an overview of all Forensic Biology CARs and QRFs for the preceding year
- Assessment of discipline-wide adherence to literature review policy

Documentation of the Forensic Biology quality system annual review will be by memo to the lab QA Manager.

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### Performance Monitoring

In addition to monitoring reagents, equipment, and protocols, the casework and database functions of analyst responsibilities are monitored through a performance monitoring program. The program ensures that a representative sample of qualified analysts are monitored through a lab accreditation cycle in each of the topics listed below:

Task	Method of performance monitoring
<b>Casework / Database Analysis</b>	
Swabbing for contact/wearer DNA	In-house ground truth performance check
Locating blood stains	Second analyst witness on first ten negative cases for newly qualified analysts and the first negative case for each analyst each calendar year
Kastle-Meyer presumptive test	PT - screening
ABAcad HemaTrace	In-house ground truth performance check
Locating semen stains, including ALS (clothing, bedding, pads/tampons, condoms)	Second analyst monitors on first ten negative cases for newly qualified analysts and the first negative case for each analyst each calendar year
Fast Blue B	PT - screening
Abacus ABA card	PT - screening
Microscopic sperm search	In-house ground truth performance check of slide making and/or slide reading
Triage practices by case/evidence type	Annual case review
From swab (blood, saliva, contact, reference samples)	PT – DNA (FA test + database analyst)
From object (cigarette butt, isolated stain)	PT – DNA (CTS)
From human tissue from decedents (fetal tissue, human remains)	Witness by a second qualified analyst
From other human samples (fingernail scrapings/clippings, hair)	In-house ground truth performance check
Differential – from swab (from SAKs or condoms)	PT – DNA (FA test)
Differential – from object (isolated semen stain)	PT – DNA (CTS)
Direct	PT – DNA and database
Differential	PT - DNA
Quantifiler Trio	PT - DNA
Triage based on quant results	Annual case review
Direct amp (GlobalFiler Express)	PT – database
STR (GlobalFiler)	PT - DNA
Y-STR (PowerPlex 16)	PT - DNA
Evaluation of controls / central log documentation	Annual case review
STR and Y-STR	In-house ground truth performance check
Database - passing	PT – database; Annual case review

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Database - failing	Annual case review
STR – number of contributors	Annual case review
STR – single source - full	PT - DNA
STR – single source - partial	Annual case review
STR – single source - inconclusive	Annual case review
STR – single source major	Annual case review
STR – two source major	Annual case review
STR – single source minor	Annual case review
STR - deduction	Annual case review
STR – 2-source indistinguishable	PT – DNA; Annual case review
STR - inconclusive	Annual case review
Y-STR – number of contributors	Annual case review
Y-STR – single source - full	PT - DNA
Y-STR – single source - partial	Annual case review
Y-STR – single source - inconclusive	Annual case review
Y-STR – single source major	Annual case review
Y-STR – single source minor	Annual case review
Y-STR - deduction	Annual case review
Y-STR - inconclusive	Annual case review
STR – single source statistic	Annual case review
STR – mixture statistic	Annual case review
Y-STR statistic	Annual case review
Report writing	Annual case review
Documentation and archiving of reports and notes	Annual case review
Report distribution	Supervisor checks at least quarterly
CODIS	
CODIS Database entry – bulk upload	Recon reports indicate success of upload and are included in admin review process
Evaluating casework profiles for CODIS suitability	Annual case review
CODIS Manual profile entry	Annual case review
CODIS Expungement	Checklist requires witnessing and double initials on checklist
CODIS Profile removal	When profiles have gone to NDIS, deletions get a double check when removed and are reviewed and archived annually with recon reports
CODIS Keyboard searches	Recorded in CODIS maintenance log and reviewed and archived annually
CODIS Match dispositioning	Moderate stringency casework matches are reviewed by CODIS administrator in each monthly match report; monthly match reports are reviewed and archived annually. Analysts must complete an in-house quiz on match dispositioning annually.
All CODIS confirmations (latent, qual check,	Annual case review

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moderate stringency matches, profile confirmation) performed and/or documented correctly	
Issuing CODIS communications	Annual case review
CODIS Upload to NDIS	Recon reports received from NDIS indicate success of upload
<b>Maintenance</b>	
Reagent verification – DNA Stable	TM sign off required
Reagent verification – GF or PPY23 kit	TM sign off required
Reagent verification - other	Review at end of calendar year
EZ1 – in use	Review at end of calendar year
EZ1 – monthly	Review at end of calendar year
EZ1 – semi-annual performance checks	Review at end of calendar year – passing PC indicates success of instrument maintenance
QIAcube – in use	Review at end of calendar year
QIAcube - monthly	Review at end of calendar year
QIAcube – biannual maintenance	Review at end of calendar year
QIAcube – performance check	Review at end of calendar year – passing PC indicates success of instrument maintenance
Thermomixer - biannual	Review at end of calendar year
7500 – in use	Review at end of calendar year
7500 - monthly	Review at end of calendar year
7500 - semiannual	Review at end of calendar year
7500 – annual	Review at end of calendar year
7500 – performance check	Review at end of calendar year – passing PC indicates success of instrument maintenance
9700 - monthly	Review at end of calendar year
9700 – performance checks	Review at end of calendar year – passing PC indicates success of instrument maintenance
3500 – in use	Review at end of calendar year
3500 - monthly	Review at end of calendar year
3500 – capillary change	TM sign off required; review at end of calendar year
3500 performance check	Review at end of calendar year – passing PC indicates success of instrument maintenance
Pipettes – annual calibration	Review at end of calendar year
Other instrument logs (incubators, heat blocks)	Review at end of calendar year
Other calibrations (balances, probes)	Review at end of calendar year
Cleaning/housekeeping	Review at end of calendar year

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Assessment of performance monitoring:

- Performance of instrumentation is monitored via ground truth positive and negative controls as well as regular ground truth performance checks for robotic instrumentation.
  - Passing criteria for each performance check are defined in appropriate manuals, and 100% compliance is expected.
  - A review of maintenance documentation is performed annually.
- Performance of reagents is monitored by verification prior to use in casework/database, as well as evaluation of ground truth positive and negative controls.
  - Passing criteria for each verification are defined in the Forensic Biology General Laboratory Maintenance Manual, and 100% compliance is expected.
  - A review of verification documentation is performed annually.
- Performance of analyst lab techniques including blood and semen presumptive tests, extraction, quantification, amplification, and capillary electrophoresis is monitored semiannually by ground truth proficiency tests created and graded by vendor providers.
  - Passing criteria are full concordance with expected vendor results, with exceptions made for differences resulting from lab-specific policies that are clearly documented.
  - Proficiency test results are monitored by lab QA Manager, DNA Technical Manager, and CODIS administrator as soon as results are available.
- Lab technique potentially leading to contamination is monitored using the staff database in the genotyping software for comparison to amplified casework and database samples.
  - Passing criteria is that no interpretable profile (or part of a profile, such as a major or minor component) is 100% consistent with and attributable to a staff member or a known DNA source handled by an analyst (identifiable cross-contamination) when analyzed with the Profile Comparison Tool in GeneMapper ID-X. Note that coincidental matches of up to 90% are often observed in casework complex mixtures of three or more sources
  - Contamination is monitored through GMID-X Profile Comparison Tool for all generated casework and database analysis. Contamination assessment forms or QRFs are used to document investigation of contamination that is sufficient in quantity for comparison.
- Performance of analyst adherence to interpretation and comparison protocols is monitored through a 100% technical review of all casework and database analysis. In addition, the annual case file review required under FBI QAS includes a further technical review of a range of technical case and database records.
  - Passing criteria for the annual case file review are adherence to laboratory protocols, with exceptions pre-approved and documented, fully correct reported interpretations, and minimal administrative errors (fewer than one per case on average).
- Performance of screening and sampling techniques not addressed by proficiency tests will be monitored by administering a set of ground truth mock casework evidence for



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processing. These samples will be extracted, quantified, and amplified (if necessary) to assess analyst success at the given task.

- Passing criteria are test-specific but generally require accurate results as compared to ground truth, consistent results between participants, correct DNA profiles, and no introduced contamination, as applicable
  - Specific answer keys are required for all in-house prepared tests and include the range of acceptable answers, including when results must be positive or negative as well as when inconclusive may be appropriate if applicable
- Performance monitoring related to CODIS software is achieved through a combination of methods.
  - Supplemental forms submitted at the same time as analyst proficiency tests will address decision-making related to CODIS suitability as well as the calculation of population frequency statistics.
    - Passing criteria will be identical statistic results for analysts with identical profiles, identical CODIS Forensic Unreviewed entries for analysts with identical profiles, and full agreement in CODIS eligibility with the determination made by the CODIS administrator or alternate.
  - Batch uploads, NDIS uploads, and profile deletions are monitored directly via the CODIS software use of Recon reports
    - Passing criteria will be 100% concordance between the expected upload/deletion and the Recon report results. Recon reports are regularly monitored by the CODIS administrator and/or alternate.
- In accordance with OSAC recommendations, performance of mixture interpretation protocols will be monitored by administering a set of ground truth mixtures to a subset of analysts for interpretation.
  - Passing criteria will be that all analysts arrive at the same ultimate reported conclusion, that analysts perform and document their interpretation with high consistency (no more than two minor discrepancies per analyst per mixture), and that the conclusion is not incorrect with respect to the ground truth (inconclusive results may be appropriate).
- Performance elements for some laboratory techniques are not suitable for mock casework/database ground truth samples. For example, it would not be appropriate to maintain human remains for mock casework ground truth reference material. Where ground truth solutions are not possible or practicable, witnessing by a second qualified analyst will be the preferred method of performance monitoring.
  - Passing criteria for direct observations of lab protocols, such as processing human remains, will be that correct protocols are followed, correct documentation is compiled, and any exceptions requiring deviations from protocol are approved in advance.
  - Passing criteria for direct observations of negative screening results for blood or semen will be that the primary analyst does not overlook or incorrectly observe test results as negative for any areas subsequently identified by the observing analyst as testing positive.



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## ***Appendix RR Continuing Education Records***

All DNA personnel (except for technicians) annually receive a minimum of 8 cumulative hours of continuing education, in accordance with the FBI QAS requirements.

Continuing education is documented in the analysts training record in LIMS. The record is reviewed and approved by the discipline supervisor and the DNA technical manager. The following information is required in the record:

- Course title
- Documentation of attendance (may include certificates, agenda/syllabus, etc.). Shall include an attendance list for internal training
- Training date(s) and number of continuing education hours
- Evaluation of course (content, instruction, relevance, etc.)

Additionally, the following is required for continuing education provided by lab personnel:

- A record of the presentation
- The curriculum vitae of the presenter

Programs based on multimedia or internet delivery require written documentation of approval by the DNA technical manager.

Documentation required specifically for internal and/or multimedia education is retained in the Forensic Biology discipline share.

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## ***Appendix SS Contingency Plan for DNA Technical Manager***

Pursuant to the FBI QAS, the laboratory must have a documented contingency plan if the technical manager position is vacated. The plan will be as follows:

- If a current staff member is qualified to serve as DNA Technical Manager, that individual will be appointed as an interim technical manager.
  - If the laboratory has more than one qualified individual, the discipline supervisor will coordinate with top management to appoint an interim technical manager. This individual will serve until a permanent replacement is hired.
  - The laboratory may continue to do work and issue reports under this scenario.
- If no current staff members are qualified to serve as the DNA technical manager, the DNA Technical Leader from another laboratory will be hired to serve in an interim capacity until a suitable replacement can be found.
  - The laboratory may not begin new casework until an interim technical manager is in place.

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## ***Appendix TT      Proficiency Tests***

This section provides additional guidance on handling of proficiency tests, where it varies from the handling/procedures of routine casework.

### **Casework Proficiency tests**

#### **Analysis**

- All questioned items in the test case, including both epithelial and sperm (or sperm/substrate) fractions if differentially extracted, must be carried all the way through amplification and reported with the test results.
- For Y-STR qualified analysts, Y-STR analysis is required in at least one proficiency test per year and must be performed on all male items in the proficiency test. For differentially extracted items, only one fraction per item needs to be Y-STR amplified.
  - There are two situations where it is acceptable to defer Y-STR analysis to the second proficiency test in a calendar year:
    - The first proficiency test does not have any detectable male DNA in either questioned sample, OR
    - Verified Y-STR reagents are not available at the time of the first proficiency test
    - Any other exception requires prior approval by DNA Technical Manager or supervisor.
  - If Y-STR analysis was successfully completed in the first proficiency test of the calendar year, it is not necessary to perform Y-STR analysis on the second proficiency test.
- Items will be reported as per the test provider's instructions when these vary from normal reporting procedures. However, they must be examined as per lab protocol.
- Test takers may consult with a similarly qualified peer during their examinations if that person is not assigned the same test.
  - Consulting another qualified examiner is required when specified as per the laboratory procedure.
- When a scenario suggests a possible sexual assault, even if ALS results

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are negative or inconclusive, all questioned samples must be FBB tested. This rule does not apply for proficiency tests where the screening results are provided.

- If necessary, evidence may be consumed without requesting permission to do so. If possible, original material should be retained.
- Prior experience with Collaborative Testing Services (CTS) has shown that some fabric substrates interfere with EZ1 extraction. Specifically, questioned blood samples might yield better results if the blood is swabbed off the substrate instead of using a direct cutting of the fabric. While this handling method is not required for a first attempt, it will be required for a second attempt if the first attempt does not yield a full profile.
- At least one quantification plate, including standard curve and NTC wells, must be set up by the analyst performing the proficiency test. (It is okay to use a standard curve prepared by another analyst, and to include casework samples from another analyst.)
- Every effort should be made to obtain a full profile at all loci. This may entail re- amplification or re-extraction of samples.

#### Documentation

- The applicable DNA conclusion page provided by the internal/external test provider (that is, the ones filled out with lab results and conclusions) will become part of the DNA analyst's bench notes and is typically the first numbered page. The page must include the lab number, date, and analyst initials.
- The LIMS report need only state that the case is a proficiency test and no report is being issued. Calculation of population frequency statistics is only required when specifically requested by the test provider.
- STR tables from the external provider will be filled out according to manufacturer's directions.
  - All data suitable for comparison is included on the table, including carryover from differential extraction.
  - Because the test provider assumes that all data reported in the table is suitable for comparison, any data which is not suitable for comparison under current FBCP guidelines must be clearly indicated as such in the test provider's comment section.
  - Any findings that have the potential to impact the interpretation (such as possible drop-out, artifact or true allele, etc.) should be noted in the

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test provider's comment section. Comments are not required for data determined to be artifacts and not included on the table (such as elevated stutter).

- Because no contributors are assumed on proficiency test samples, mixtures are not deduced.
- Major and minor contributors should be separated, as appropriate. Follow the test manufacturer's directions for noting minor alleles.
- Upon completion of the test, test materials are stored in the designated location in the discipline rather than being returned to one of the laboratory's secure evidence storage locations.

#### Review and submission

- For DNA online data entry, review of the electronic submission form is completed by the administrative reviewer.
- After completion and electronic submission of the test, the test taker scans a printout from the test portal which includes submission date and time, as well as submitted results, into the LIMS case file.
- Submission of external test results
  - FTS (Forensic Testing Services)
    - The laboratory quality manager will provide the analyst with the FTS test information and the laboratory case #
    - The analyst logs in to the FTS site to retrieve the test result form, enter results and save to the FTS system
    - The analyst provides the technical reviewer with a printed copy of the completed results form for review; corrections are made as required
    - The analyst notifies the laboratory quality manager when the test is completed, report released in LIMS and notes complete in case images
  - CTS (Collaborative Testing Services) – the procedure for CTS tests is provided in the laboratory quality manual
    - If the test taker is not able to submit the results to CTS, this task must be performed by a reviewer.
  - FA (Forensic Assurance) – the procedure for FA tests is provided in the laboratory quality manual

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## Database proficiency tests

### Analysis

- Good faith efforts extend to all loci, not just core loci.
- Analysts who perform database analysis exclusively must include at least one sample per proficiency test which is analyzed both with direct amplification and with extraction, quantification, and GlobalFiler amplification.
- For convenience of review, printouts of the database sample electropherograms may be included with the paperwork submitted for technical review. However, these pages are not included with the completed documentation.

### Documentation

- Batch paperwork and review checklist are scanned to the appropriate folder in the Forensic Biology folder on the lab network.

### Review and submission

- Technical and administrative review may be performed together by the same reviewing analyst.
- Review includes confirming that the electronic data forms are filled in completely and correctly.
- The procedure for CTS tests is provided in the laboratory quality manual
  - If the test taker is not able to submit the results to CTS, this task must be performed by a reviewer.
- After completion and electronic submission of the test, the test taker scans a printout from the test portal which includes submission date and time, as well as submitted results, into the LIMS case file.

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**Appendix UU      Outsourcing and On-Site Visits**

**A. Procedure for vendor laboratory performing forensic casework**

- *The on-site visit must happen prior to the vendor lab beginning casework, and annually thereafter for the life of the contract.*
- *If available, the laboratory shall review and evaluate the most recent (within one year) on-site visit performed by a designated FBI employee.*
- *Documentation for the on-site visit must include:*
  - *The date the on-site visit was performed*
  - *A summary of the visit*
  - *The personnel who performed the on-site visit*
  - *Acceptance by the Technical Leader*
- *FBI on-site visits have previously been available for the vendor laboratory used by this laboratory.*
  - *If no FBI on-site visit is available for review for a future contract, an on-site visit conducted by another NDIS laboratory using the same technology, platform, and typing amplification test kit may be sought for review.*
  - *If no alternative is available, the Technical Leader will design and document an on-site visit protocol prior to performing an on-site visit.*

**B. Procedure for vendor laboratory performing technical reviews only (not performing casework analysis)**

- *If the vendor laboratory is only providing technical review services, a full on-site visit is not required.*
- *Prior to the vendor laboratory performing technical reviews, the Technical Leader will assess and document acceptance of the following criteria:*
  - *Where the technical review services will be performed*
  - *What security precautions are in place to safeguard the confidentiality of the information being reviewed*
  - *How the vendor laboratory ensures that only authorized persons have access to casework information, if such information is taken outside the controlled laboratory environment*