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Section 1 Screening of Biological Evidence

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

Biological Screening Abbreviations and Definitions	
Phenolphthalein/Kastle-Meyer	PH, Pheno, or KM
Fluorescence/Alternate Light Source	F or ALS
Acid Phosphatase activity	AP, BCIP, 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 (=)
CIDI	Case,item,date & initials

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

RBS	Reagent blank sperm
RBSS	Reagent blank sperm + substrate
RBE	Reagent blank epithelial
RBsub	Reagent Blank substrate
RBQ	Reagent blank questioned (direct)
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.
STR	Quantitation indicates that the extract is suitable to proceed to STR testing.

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1.1 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.

1.1.1 Screening Hairs

An intact hair will have a root portion and a shaft portion. It is important to start with visual examination followed by macroscopic examination of the morphology of individual hairs. An initial examination at a magnification of 5-10X, followed by an examination at 30X or higher, will enable the analyst to observe the hair, whether or not a root is present and, where present, its shape and appearance, the basic features of the shaft, and medullary structure.

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

- Is the hair Animal or Human in origin
- If Human, is the hair suitable for nuclear DNA analysis

The analyst will also document the observed characteristics of the hair that were used to make the above described determinations. The analyst should also document characteristics

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indicative of a specific somatic region, although this determination is not being conclusively made. These characteristics may include the following:

Animal vs. Human

- color(s) of the hair
- medullary structure
- root shape
- gross structure of the hair

Head Hairs

- length of hair
- moderate shaft diameter and diameter variation.
- medulla absent to continuous and relatively narrow when compared with its structure in hairs from other body areas.
- often with cut or split tips
- may show artificial treatment (solar bleaching, dying, or mechanical damage)
- Soft texture (pliable)
- little or no taper

Pubic Hairs

- length of hair
- shaft diameter coarse with wide variations and buckling
- medulla relatively broad and usually continuous when present
- follicular tag often present on the root
- tips usually rounded or abraded
- stiff texture (wiry)

Suitable for Autosomal STR Analysis

- anagen or early catagen growth phase
- visible tissue/sheath material
- follicular tag

DNA analysis of a hair consumes a portion of the hair, preventing subsequent examinations. If the analyst concludes that the hair(s) examined may be suitable for nuclear DNA analysis, a digital image of the hair's root will be included in the bench notes. Inconclusive hair results must be confirmed by a second qualified analyst; this confirmation is documented in the bench notes.

1.2 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).
- The isolated sample(s) are packaged and retained as a separate item of evidence.

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1.3 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 a number of methods, including visual and stereoscopic examination, the use of alternate light sources and chemical presumptive testing.

1.3.1 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 an infrared hand held scope, video-imaging system using an infrared filter, or Omniprint™ 1000B (as previously described) may assist in the location of possible bloodstains. Use of these sources will provide the contrast needed to search for stains that are not visible otherwise.

Bloodstains on clothing 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 microscopically.

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

1.3.2 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.

1.3.2.1 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 sufficient 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.

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Procedure

- Record all lot numbers and expiration dates.
- 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.

Interpretation of Results

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

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.

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 H₂O₂ may be due to the presence of chemical oxidant. Fruit and vegetable peroxidases react similar to blood but slower and more weakly. 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.

A negative result is indicative of the absence of detectable quantities of heme or its derivatives.

1.3.2.2 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.
- Remove the device/card and dropper from the sealed pouch and label the HemaTrace card with the lab number, item number, your initials and the date.
- Add 4-5 drops of the extract, using the dropper provided, into the sample region (S) on the card.

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- 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 the bench notes.

Interpretation of Results

The control line in the control area (I) 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 (I) 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 I 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 (I) of the card. Repeat the test and re-examine the test procedure carefully.

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1.4 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 a number of methods, including careful visual examination, the use of tactile senses, fluorescence under alternate light sources, and chemical presumptive testing.

1.4.1 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.

1.4.1.1 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. Label goggles for the specific wavelength. Do not allow use of inappropriate or incorrect goggles. Some goggles offer only a narrow band of protection.
- Remove all unnecessary reflective surfaces from the area 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.

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- 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 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 bench notes.

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

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kinked, stepped on, bent, or frozen. Damage may not be evident, but there will be a decrease in output power (brightness).

1.4.2 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 (or BCIP for challenging substrates).

1.4.2.1 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.

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 one drop of Solution #1 (α -Naphthyl Phosphate).
- Add one drop of Solution #2 (Fast Blue B).

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.

1.4.2.2 Detection of Acid Phosphatase using BCIP

This is a presumptive test for the detection of seminal fluid. Samples with a positive result are further analyzed by performing a PSA test.

Procedure

- Place 200 μ L of BCIP substrate solution in labeled glass test tubes; one test tube for each sample. (Prepare a test tube for a positive control, a negative control, and for each Q sample).

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

Interpretation of Results

A positive test result is an aqua (blue-green) color. The appearance of an aqua color indicates the presence of acid phosphatase activity. The positive control should give an aqua color and the negative control should not exhibit a color change. The controls must function as expected for the test results on Q samples to be valid.

Additional Notes

- The BCIP procedure is not specific for semen.
- The BCIP procedure is 99% accurate in predicting a true negative stain, i.e. one out of 100 negative BCIP tests may be false.
- The BCIP procedure has not been found to be useful in screening latex condoms. The laboratory has encountered condoms containing numerous spermatozoa that yielded negative BCIP results.
- This test should not be performed on body cavity swabs since spermatozoa may be found on vaginal swabs which do not test positive to the BCIP reagent.

1.5 Vaginal/Cervical, Rectal and Oral Swabs

Samples collected within the following time frame should be analyzed for the presence of semen:

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

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

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1.5.1 Detection of PSA by the Abacus ABACard®

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 body swabs collected within 24 hours.

Procedure

- A. Place the sample in a 2.0 mL tube or a 1.5 mL QIAcube microcentrifuge tube.
- B. Add enough sterile water to the sample to just cover the material (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. Pipette off 150 µL for the PSA card. The remainder of the liquid must be removed, without disturbing the pellet, and the liquid discarded after the PSA test is completed.
 - Remove the device and dropper from the sealed pouch.
 - 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 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.

Interpretation of Results

Positive: The formation of two pink lines, one in the test area (T) and in the control area (I) 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 (I) 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 100µl of sample.

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

E. After the PSA test has been completed:

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- If the PSA test is positive, return the substrate to the pellet in the tube in order 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, the substrate must be air-dried and repackaged.
- If the PSA test for a non-consumed sample is negative, the substrate may be discarded.

1.6 Miscellaneous Evidence Swabs

Suspected blood or semen samples may be examined using the screening methods previously described.

Swabs collected after 24 hours that may contain semen are processed via a QIAcube protocol, following the decision tree in Appendix B.

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

1.7 External Genitalia Swabs

Swabs collected from the external genitalia area within 24 hours may be examined for the presence of semen by PSA as previously described. Penile swabs obtained from the victim/suspect within 24 hours of the offense may be examined for the presence of blood, dependent on the case scenario.

External genitalia swabs collected after 24 hours, as well as penile and/or scrotum swabs, proceed directly to the QIAcube protocol without PSA testing, following the decision tree in Appendix B.

1.8 Fingernail Scrapings / Finger and Hand Swabs

Fingernail scrapings and finger/hand swabs do not routinely require biological screening. Screening may be appropriate if the presence of blood or semen would be pertinent to the investigation.

If semen is detected, or if the presence of spermatozoa is suspected, these samples may be processed via a QIAcube protocol, following the decision tree in Appendix B.

1.9 Condoms

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

- Document the condition of the condom. Imaging may be used 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.
- The swabs are retained as a new item of evidence.

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

Condom swabs proceed directly to the QIAcube protocol, without further screening.

1.10 DNA Screening: selection, sample size, and extraction

Protocols for DNA extraction are found in the DNA Extraction section of the manual.

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. For samples with one or two swabs, the swabs are routinely sampled in their entirety. For samples with larger numbers of swabs, up to two swabs worth of total volume is routinely used – for example, half of each of four swabs. Elution volumes of 40 µL are routinely used for all questioned extracts, as sample size permits.

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

- Both the bench notes and the sample (noted in red on tube) must clearly indicate that the sample has been consumed.
- 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.

Semen-containing items (differential extraction):

- Analysts follow the two flowcharts in the appendices of the FBCP to determine which samples are appropriate for differential extraction using the QIAcube automated wash protocol.
- If an item has not been consumed in its entirety, extraction of the substrate is optional
- 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 vaginal swabs, cervical swabs, rectal swabs, or underwear cuttings from crotch area should be processed without combining the sperm pellet with the substrate.

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- Items likely to have relatively lower amounts of epithelial DNA, such as external genital swabs, condom swabs, and cuttings from clothing other than underwear, may have the substrate added to the sperm pellet for a single extract.
- Slides are made from all sperm pellets and stained, but they do not need to be read prior to issuing the screening report. Slides are repackaged with original evidence.

Non-semen-containing items (direct extraction):

- Analysts use case-specific information to choose stains testing positive to a presumptive test for blood, breast swabs, and other miscellaneous swabs most likely to have probative value, based on case scenario.
- Hairs are not routinely extracted in the first round of testing.

Reference samples:

- Reference samples are cut into tubes, but not extracted during the screening process.
- Reference sample cutting tubes may be stored with extracts created from the same kit if STR analysis is pending, or repackaged with the original evidence if no further analysis is pending.

Reagent blanks:

- Duplicate reagent blanks are created for each set of questioned extracted samples taken through the same extraction protocol (e.g. sperm/substrate, epithelial and/or direct) on the same day by the same analyst. Exception: when only one questioned sample corresponds to the reagent blank, it is not necessary to make two reagent blanks.
- Reagent blanks are named by extraction type, date, analyst, and replicate 1 or 2 (RBS 14-1025CD-1 and RBS 14-1025CD-2).
- Both reagent blanks are quantified.
- Blank replicate -1 proceeds directly to amplification (if appropriate). Blank replicate -2 proceeds directly to DNA-Stable LD. Exception: if blank replicate -2 has a quant result that is NOT undetermined, blank -2 proceeds to amplification and blank replicate -1 proceeds directly to DNA-Stable LD.
- Since reference samples are not extracted at this stage, it is not necessary to create a reagent blank for the reference samples.

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Items which are sampled must include the following information documented in the bench notes:

- Extraction date (or cut date if not extracted)
- Approximate amount of evidence sampled
- EZ1-XL instrument used
- QIAcube instrument used
- Elution volume

Upon Completion of DNA Screening:

- When any sample from a case with a named suspect is used in its entirety, the remaining substance of the evidence must be retained.
 - If the sample is extracted for DNA analysis, at least half of the extract must be retained and ultimately repackaged with the original evidence; swab material which has been through an extraction protocol is routinely discarded.
 - If a sample is not extracted, such as swabs with a negative result for PSA, then the sample must be air-dried and repackaged with the original evidence. Swabs must be repackaged in a breathable packaging (such as a manila coin envelope) in case any residual moisture remains in the swabs.
- Upon completion of DNA screening, evidence is transferred from the DNA screening analyst to a DNA analyst if further analysis is pending or to evidence storage if no further analysis is pending.
- Extracts and un-extracted reference sample cuttings which are destined for DNA analysis are all stored in a designated freezer until DNA STR analysis is completed for the case. After the case is completed, the DNA analyst is responsible for either discarding or archiving remaining extracts, as appropriate.
- Following quantitation, all extracts for which STR DNA analysis is not pending must be dried down using DNASTable LD. Extracts pending analysis may be stored in an extract refrigerator (for less than one week) or extract freezer.
- Copies of standards and controls documentation, as well as quantitation documentation, are included in the DNA Screening Central Log.

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Section 2 Biological Screening Report Writing and Review

2.1 Guidelines for Biological Screening Examinations / 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 analyzed
 - include the agency item # in () if the item was re-numbered at the laboratory
 - this is not necessary if the laboratory number contains the original agency item #
- Results of presumptive/confirmatory tests (in body of report)
- Results, conclusions and opinions for all tested items (guidelines for reporting are provided in the tables below and may be modified, as necessary, on a case by case basis)
- Explanation of why testing was stopped, if appropriate
- The disposition of all tested retained items
- Any known samples that are required for DNA analysis, when applicable
- Statement regarding Y-STR testing, 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)

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2.2 Results and Conclusions for Biological Screening Examinations

Blood Findings	Report
Phenolphthalein (Kastle-Meyer)	<p>A stain(s) testing positive/negative with a presumptive test for blood was/were located/detected on (item).</p> <p>No blood was detected on (item) with a presumptive test for blood.</p> <p>This stain/sample tested positive/negative to a presumptive test for blood.</p>
Seminal Fluid	Report
FBB/BCIP negative	No semen was detected chemically (FBB/BCIP) on (item)
FBB/BCIP positive	Positive results are not reported
P-30/ABA card positive	<p>The presence of the human prostate specific antigen (PSA/p30), found in seminal fluid, was detected by immunoassay on (item) and the item may be suitable for Y-STR analysis.</p> <p>For more information please contact the laboratory's DNA Technical Manager, XXXXX XXXXX (269-XXXX) or (xxxxx.xxxxx@alaska.gov).</p>
P-30/ABA card negative	No semen/PSA was detected by immunoassay on (item).

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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.

Hair/Fiber Evidence	Report
Hairs/debris recovered/not recovered	(No) (Animal) Hairs/debris were observed/recovered from (item).
If origin is undetermined	Hair and/or hair fragments of undetermined origin (human vs. animal) were recovered from item #.
If human hairs.....	A human hair(s)/hair fragment(s) was/were recovered from (item) and is not/may be suitable for nuclear DNA analysis.

2.3 Review of Biological Screening Reports

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

Technical and administrative reviews of biological screening casework will be conducted by a second qualified analyst, in accordance with the laboratory Quality Assurance Manual (current version).

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.

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Section 3 DNA Extraction

3.1 Evidence Sampling

Reference: EZ1 DNA Investigator Handbook

3.1.1 Bloodstains

- 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.
- 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.

3.1.2 Contact or saliva swabs

- Typically, swabs without visible staining will be sampled in their entirety, maximizing the chance of obtaining an interpretable DNA profile. Exception – a large number of swabs collected from a single area 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. When an entire sample is extracted, at least half of the extract must be retained for future use.
- Any swab(s) that is sampled unequally (i.e. a portion of only one of two swabs) should be tagged with a label containing the case number and item number. 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.
- Typically, known buccal swabs require only 1/3 of one swab or a small portion of two swabs.
- When the sampled swab material is too large for a single tube, it should be split into multiple tubes and the extracts ultimately recombined. The elution volume should be adjusted so that the final volume allows for at least half to be retained after analysis. Ensure that reagent blanks are treated similarly.

3.1.3 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.
- Any extract remaining after analysis shall be retained since these samples may not be homogeneous.

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3.1.4 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 200 μ L of dilute G2 buffer (1:1 solution of H₂O:G2 buffer) in a microcentrifuge tube with gentle agitation. This may be appropriate when attempting to obtain a DNA profile of the surface material separately from the nail itself.
- The washing is then transferred to a new microcentrifuge tube and digested by adding 10 μ L Proteinase K.
- Any clippings that remain after digestion are dried and re-packaged with the evidence.

3.1.5 Hairs

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

For mounted hairs, score the edge around the cover slip with a sterile scalpel. Remove the cover slip by prying it off or by soaking the slide in xylene 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: 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 include shaft and root samples.

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

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3.1.6 Swabs for differential extraction

- Sample size is dependent on the amount of starting material. For samples with one or two swabs, the swabs are routinely sampled in their entirety. For samples with larger numbers of swabs, up to two swabs worth of total volume is routinely used – for example, half of each of four swabs. However, if microscopic analysis was previously conducted and indicates that many spermatozoa are present (2+ or greater), a smaller sample size may be appropriate.
- For consumed samples undergoing differential extraction, substrates must be extracted, either by combining the substrate with the sperm pellet or extracting it separately.
- Extraction of the substrate is optional for samples that are not consumed.
- Internal cavity swabs, such as oral, rectal, vaginal and cervical swabs, as well as cuttings from the crotch area of underwear, are likely to have large amounts of epithelial DNA present. These samples should have the substrate processed in its own tube. Likewise, samples where the epithelial fraction is probative, such as penile swabs, should have the substrate processed in its own tube.
- For samples which are likely to contain moderate or small amounts of epithelial DNA, such as external body swabs or less-intimate clothing stains, the substrate may be added back to the sperm pellet when it comes off the QIAcube.

3.1.7 Other tissue samples

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

3.2 Qiagen BioRobot EZ1 Advanced-XL DNA Extraction

Reference: EZ1 Advanced XL User Manual

General Instructions:

- Use the Trace or Large Volume protocols for all questioned and known forensic casework samples.
- Questioned and known samples shall be eluted in TE buffer.
- Elution volumes are selected based on sample type and/or quantity:
 - Larger elution volumes (200µl) may be selected for most reference samples.
 - Smaller elution volumes (no less than 40 µL) are recommended for reference blood samples from decedents, previously extracted database samples, and all questioned samples.
 - If the entire sample was consumed, the total elution volume shall be 40 uL, and at least half of the extract volume (20 uL) shall be retained.
 - If more than one elution volume is used in a set of known sample extractions, the corresponding reagent blanks should use the most stringent elution volume used in that set.
- Duplicate reagent blanks are created for each set of questioned extracted samples taken through the same extraction protocol (e.g. sperm/substrate, epithelial and/or direct) on the same day by the same analyst.

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- Exception: if there is only one questioned sample to which the blank corresponds, it is not necessary to make duplicate blanks for a single sample.
- Reagent blanks are named by extraction type, date, analyst, and replicate 1 or 2 (RBS 14-1025CD-1 and RBS 14-1025CD-2).
- Both reagent blanks are quantified.
- If both blanks have undetermined quantification results, blank replicate -1 proceeds directly to amplification (if appropriate). Blank replicate -2 proceeds directly to DNA-Stable LD. Otherwise, amplify whichever blank had the higher quantification results and DNA-Stable the other.
- Direct extraction blanks – duplicate blanks are created for each EZ1 protocol performed (Trace and/or Large Volume), using the highest digest volume within the set.
- Differential extraction blanks – duplicate epithelial blanks and duplicate sperm blanks are always created. Separate duplicate substrate reagent blanks are only created if a different digest volume from the sperm blanks or a different EZ1 protocol from the sperm blanks is used.
- A single reagent blank is created for a set of known extractions, and it is made using the most stringent elution volume from the corresponding samples.
- Casework buccal swabs may be extracted, quantified and amplified. Alternatively, when at least two swabs are included with a reference, one of the swabs may be used for direct amplification using GlobalFiler Express, following the protocols in the Forensic Biology Database Manual.

3.2.1 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 QIAcube instrument.

1. Prepare the pre-digest solution:
 - (Number of samples + 1) x 480 µl G2 buffer
 - (Number of samples + 1) 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.

Note: for hair samples and nail clippings*, also add 10 µl DTT.
(* when the source or owner of the nail needs to be determined) This will also require additional corresponding reagent blank(s).

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. Transfer up to 500 µl of the digest into a sterile 1.5mL or 2.0 mL screw-cap tube. Add 1 µl carrier RNA solution to the transferred digest solution.

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Option 1 – Large-Volume Protocol:

- *For samples that typically yield less DNA or if the sample required additional digest buffer for thorough cell lysis, the analyst may transfer up to 500 μ l of the digest solution to an EZ1 sample tube, add 1 μ l carrier RNA solution, and add 400 μ l of pre-warmed (10 minutes at 56° C) Buffer MTL.*
- *Load the sample(s) onto the EZ1, select the Large-Volume Protocol, and elute in 40 μ l TE buffer.*

Option 2 – Large-Volume Protocol for Consumed Samples:

- *If the entire evidence sample has been consumed and digested, the analyst shall transfer the entire volume of digest solution to an EZ1 sample tube (or tubes – with no more than 500 μ l in each tube), add 1 μ l of carrier RNA solution and add 400 μ l of pre-warmed (10 minutes at 56° C) Buffer MTL.*
- *Run the Large-Volume protocol and elute in 40 μ l TE buffer.*
- *On occasion, use of more than one tube may be required to digest and extract the entire sample efficiently. In such situations, the EZ1 extracts obtained from all such digests may be combined in one tube and proceed to the quantitation step.*

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

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

11. Directions are displayed on the screen on the front of the instrument. Press the START button to select Large Volume protocol (Trace protocol may be used for samples requiring less digest buffer. If used, corresponding reagent blanks must also use Trace protocol, and Trace protocol must be documented in bench notes and central log), TE elution buffer and elution volume (typically 40 μ L, exceptions documented in bench notes and central log)

12. 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.

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

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14. The extracted DNA is now ready for quantification (optional for known samples) and/or amplification. DNA extracts can be stored for several weeks at 2°C to 8°C. Extracts should be dried down with DNAstable LD for long-term storage.
15. 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.
16. 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.

3.2.2 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 2 mL or 1.5 mL tube thermomixer to 56° C for the epithelial digest, and a 1.5 mL tube thermomixer to 70° C for the sperm digest

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 +1) * 480 µL G2 and (number of samples + 1) * 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. Vortex to thoroughly re-suspend, and centrifuge briefly to remove liquid from inside the lid. If the sample is in a 2.0 mL tube, 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.

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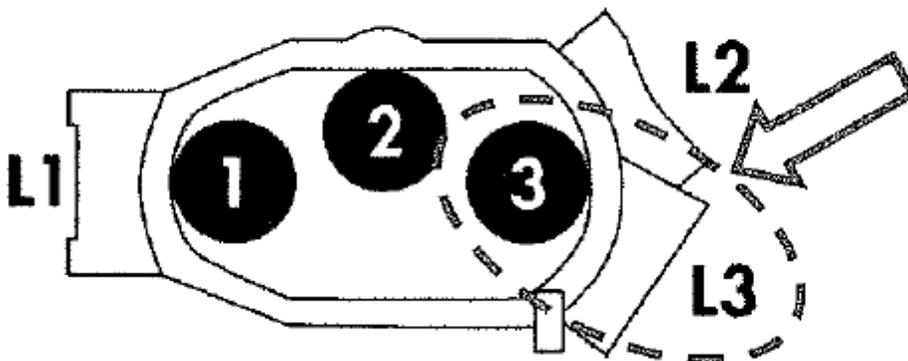
11. Aliquot Buffer G2 in 30 mL reagent bottle in Reagent Bottle Rack position 1. Do not fill above the fill line. Minimum volumes of G2 buffer are listed in the table below:

<u># samples</u>	<u>Buffer G2 (µL)</u>
7 or fewer	16920
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. Turn on the QIAcube by pressing the power button.

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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.

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. For all samples, remove 3 uL to a microscope slide and proceed to stain. For samples not previously examined microscopically, all slides will be retained in original packaging. Typically, for samples not previously examined microscopically, slides will be examined and results reported only when a probative, interpretable male profile is ultimately obtained from the sample and there is no presumptive PSA positive test result for semen.

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 gently flaming or 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, one of three options may be taken to verify this absence:

- Using the same extract, re-spot the slide and perform a second microscopic examination

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- A second analyst may 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.
- If the sample was collected within 48 hours of the assault, an immunoassay test may be performed to confirm the presence of seminal fluid.

23. Add the following reagents to each sperm fraction:

140 μ L Buffer G2

20 μ L Proteinase K

40 μ L 1M DTT

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

(number of samples + 1) * 140 μ L Buffer G2

(number of samples + 1) * 20 μ L Proteinase K

(number of samples + 1) * 40 μ L 1M DTT

Distribute 200 μ L 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 10 minutes.

Preparing for DNA Extraction:

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

26. Epithelial fraction: add 400 μ L warm Buffer MTL to the 2 mL sample tube, along with 1 μ L cRNA. Run Large Volume extraction protocol with elution in 40 μ L TE buffer.

27. Sperm (and/or substrate) fraction: if applicable, remove substrate by spin basket (as described in steps 5-7). Add 1 μ L cRNA. Run Trace protocol with elution in 40 μ L TE buffer. Optional: sperm (and/or substrate) fractions may be run by Large Volume protocol, but this must be documented in the bench notes and central log.

EZ1 Extraction:

Refer to Section 3.2.1 (Direct extraction) steps 9 – 16 for instructions on EZ1 extraction and clean-up.

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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.

3.2.3 Direct (non-differential) Extraction for known samples

1. Prepare the pre-digest solution:
 - (Number of samples + 3) x 230 μ l G2 buffer
 - (Number of samples + 3) x 230 μ l sterile de-ionized water
 - (Number of samples + 3) x 10 μ l Proteinase K
2. Add enough pre-digest solution to each sample cutting to allow for at least 200 μ l 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 200 μ l of the digest buffer into a sterile 1.5mL or 2 mL screw-cap tube.
5. Insert the DNA Investigator card into the slot located on the front of the BioRobot EZ1 Advanced-XL.
6. Turn on the power switch on the back of the instrument.
7. Directions are displayed on the screen on the front of the instrument. Press the START button to select Trace protocol, TE elution buffer, and elution volume (typically 200 μ L).
8. Follow the instructions displayed on the screen to set up the reagent cartridges, digest tubes, tip holders, and elution tubes.

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

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

9. Upon completion of the protocol, remove the elution tubes containing the purified DNA and cap the tubes.
10. The extracted DNA is now ready for quantification (optional for known samples) and/or amplification. DNA extracts can be stored for several weeks at 2°C to 8°C.
11. 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.
12. 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.

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Section 4 DNA Quantification

Questioned samples and known samples not amplified by GlobalFiler Express are routinely quantified in single reactions in order to preserve extract. A single quantitation reaction is typically used for each questioned reagent blank, but is not required as long as the extract is amplified. Quantitation is not mandatory for database samples or forensic known buccal samples amplified by GlobalFiler Express.

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.

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*

4.1 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.

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- Standard Curve wells are already correctly assigned. The template default includes one NTC reaction. Be sure that the final plate 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)

4.2 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: 50 µL Quantifiler THP DNA Dilution Buffer and 50 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 ≈ 50ng/µL
Standard 2 ≈ 5 ng/µL
Standard 3 ≈ 0.5 ng/µL
Standard 4 ≈ 0.05 ng/µL
Standard 5 ≈ 0.005ng/µL

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4.3 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 μ L per reaction.
 - Quantifiler™ THP PCR Reaction Mix at 10 μ 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 μ L of reaction mix into each reaction well.
- Add 2 μ L of sample, standard or control to the applicable wells. 2 μ 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.
- Centrifuge the plate at 3000 rpm for at least 20 seconds to remove any bubbles.

4.4 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.

4.5 Starting the Run

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

4.6 Analyzing the Data

- When the run is complete and the quality checks described below in 4.6.1 through 4.6.4 have been completed, print the Experiment Results Report for documentation in the central log file.

4.6.1 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 in order to achieve passing quality metrics, and it is not acceptable to remove both end points from either end of the standard curve.

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R² 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 R² is ≥ 0.98 . It is acceptable to remove up to two data points in order to achieve a passing R² value. If, after removing up to two data points, the R² 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/ μ 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/ μ L will now quantify at 1 ng/ μ 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/ μ L will now quantify at 2 ng/ μ 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 24.9 – 25.6 for the large autosomal human standard curve, 26.8 – 27.3 for the small autosomal human standard curve, and 25.8 – 26.3 for the male 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 + / - 1 from the verified value, the Technical Manager must be notified to determine a course of action.

4.6.2 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.

4.6.3 Checking the Passive Reference (ROX)

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

4.6.4 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 = undetermined). A detected quantity of DNA could indicate contamination of the master mix. If the quantity of either Trio

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Human or Trio Male is greater than zero, consult the DNA Technical Manager for a course of action.

4.6.5 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 4.1. 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.

4.7 Using Quantitation results for DNA Screening

4.7.1 Determining a ratio of female to male DNA detected in an extract.

- 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/ μ L, stochastic effects can limit the accuracy of assessment of the true female to male ratio.
- The calculation of minor female DNA in the presence of excess male DNA is not accurate and should **not** be used to assess the suitability for analysis.

4.7.2 Assessing whether or not an extract is likely to be single-source, either entirely or effectively, for the purposes of STR amplification:

- **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.
- 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).

4.7.3 Determining suitability for future testing for probative male

- Sample decision trees for this process (for consumed samples) are shown in Appendices D - F. 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:
- Extracts with a T-S result of lower than 0.002 ng/ μ L (if likely to be single source) or lower than 0.007 ng/ μ L (if likely to be a mixture) will not routinely proceed to amplification.
- 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.)

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- Extracts with any male quantitation value of 0.02 ng/μL or lower are recommended for Y-STR analysis in cases with a known suspect. These may proceed to STR amplification in non-suspect cases if the female: male ratio is less than 5:1.
- 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 0.07 ng/μL, are best suited for STR analysis only if the entire remaining extract is consumed.
- Consumed samples with likely mixtures: Extracts likely to contain mixtures are ready for STR DNA analysis when ALL three conditions listed below are met. If total human DNA or male DNA concentration is below the minimum concentrations, the extract is best suited for STR analysis only if the entire remaining extract is consumed.
 - male DNA is present in a ratio of 4:1 female: male or less
 - small autosomal concentration DNA equal to or greater than 0.07 ng/μL
 - male DNA concentration is equal to or greater than 0.02 ng/μL
- Not-consumed samples with likely mixtures: Extracts likely to contain mixtures are suitable for STR DNA analysis when ALL three conditions are met. If total human DNA or male DNA concentration is below the minimum concentrations, the extract is best suited for STR analysis when more of the remaining sample is extracted /or the extract is dried down with DNASTable LD and reconstituted in a smaller volume.
 - male DNA is present in a ratio of 4:1 female: male or less
 - total human DNA concentration equal to or greater than 0.05 ng/μL
 - male DNA concentration is equal to or greater than 0.02 ng/μL
- NOTE: When Y-STRs are recommended or when low DNA is reported, extracts are retained regardless of whether or not the original evidence was consumed.

4.7.4 Determining suitability for future testing for probative female

This situation arises most often with male sexual assault suspect kits, where the question is whether female DNA may be present. The Quantifiler Trio Kit is optimized for determining a ratio of female to male DNA when the female DNA is in excess. Variability in copy number for the Y target results in much lower accuracy in predicting successful amplification of a minor female contributor. However, when T-Y is equal to or greater than T-S, the chance of obtaining probative results becomes highly unlikely.

- Extracts with a T-S result of lower than 0.002 ng/μL (if likely to be single source) or lower than 0.007 ng/μL (if likely to be a mixture) will not routinely proceed to amplification.
- Extracts with T-Y greater than or equal to T-S are not suitable for routine analysis. Exceptions may be made on a case-specific basis and require documented approval from the DNA Supervisor or Technical Manager.

4.7.5 Rationale for recommending consumption of extract:

In instances where quantitation results indicate that limited amounts of DNA (<0.07 ng/μL) are present, the best course of action depends in part on whether one or more sources of DNA are

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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.

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 μ L extract has a total human DNA concentration of less than 0.07 ng/ μ L), it is not possible to optimize analysis while retaining half the extract.

4.7.6 Determining suitability for future testing for non-probative / QA amplifications

Samples such as the epithelial fraction from a differential body swab are routinely processed as a QA check to the extraction and sample handling process. Although these often have robust quantities of DNA present, they are occasionally low in quantity. Since these samples are run as a QA check, it is not necessary to request permission to consume them.

When a non-probative fraction from a differential extraction has a T-S concentration less than 0.004 ng/ μ L, the extract need not be amplified. When a non-probative fraction from a differential extraction has a T-S value between 0.004 and 0.07 ng/ μ L, a full-volume amplification will be performed without drying down the extract.

4.7.7 Rationale for stopping analysis of a sample based on low concentration

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/ μ 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/ μ L is the concentration where, if the extract were dried down with DNASTable and reconstituted with 15 μ 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/ μ L will either be reported as recommending Y-STR's (if appropriate) or that no further analysis will be performed on the sample. 0.007 ng/ μ L is the concentration where, if the extract were dried down with DNASTable and reconstituted with 15 μ 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. Extracts not amplified due to insufficient DNA are routinely retained.

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Section 5 DNA Amplification

References:

- *GlobalFiler PCR Amplification Kit User Guide*
- *GeneAmp® PCR System 9700 96-Well Sample Block Module User's Manual*

5.1 Amplification Cycling Parameters

- Ensure AB GeneAmp® PCR System 9700 thermal cycler has been turned on to allow the instrument time to properly warm up.
- 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

5.2 Amplification Set-up of Forensic Casework Samples

- If amplification reagents have been frozen, allow them to warm to room temperature, then vortex for 3 seconds and centrifuge briefly before opening tubes. Thawing is only required during the first use of a tube. After first use, reagents are stored at 2-8°C
- If reagents have been stored at 2-8°C, allow them to warm to room temperature, then vortex and spin briefly using a centrifuge.
- Transfer the amplification reagents to the designated PCR set-up area.
- 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.

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:
 - # of samples x 7.5µL GlobalFiler Master Mix
 - # of samples x 2.5µL GlobalFiler Primer Set

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

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- Vortex the master mix and spin briefly. Transfer 10 μ L of master mix to each sample well of an 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).
- Prepare the samples to be amplified in individual tubes

5.3 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 μ L of extract from a consumed sample, 2 μ L are used for quantification and at least 20 μ L are to be retained, leaving up to 18 μ L available for amplification.
- If the sperm and substrate fractions are both extracted and quantitated 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 on the DNA worksheet if an extract is not amplified.
- 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.
- Add TE⁻⁴ buffer (manufacturer may label this as DNA Suspension Buffer) to bring the sample to a final volume of 15 μ L.
- No sample extract may be amplified at a greater volume than its corresponding reagent blank. Reagent blanks amplification volumes are routinely 15 μ L. At a minimum, volumes should be chosen based on the largest volume used in the corresponding casework extracts.
- Low-level samples (concentration < 0.07 ng/ μ L) 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 15 μ L of sterile water, but may be adjusted such that the target input is as close to 1 ng as possible.
- When extracts previously DNA Stable-d are reconstituted for amplification, the corresponding designated reagent blank (typically designated as reagent blank replicate -2) is reconstituted in the same volume of sterile water and amplified.
- Positive Amplification Control: Vortex Control DNA and spin the tube briefly. Add 10 μ L control DNA to 5 μ L of low TE buffer in a 0.5mL tube.
- Negative Amplification Control: Add 15 μ L of TE⁻⁴ buffer.

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5.3.1 Degradation and selection of amplification target values

Degraded or inhibited DNA samples may benefit from amplification at higher target input DNA amounts. Quantifiler 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 the Quantifiler Trio report printout. Alternatively, the Quantifiler 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 Quantifiler Trio report printout. As such, it is not necessary to include unaffected samples (such as standard curve and NTC wells) in the Excel printout.

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.

If sufficient extract is available, the following amplification modifications are recommended:

For extracts with a DI of 1.2 to 2: target 2 ng, based on T-S.

For extracts with a DI greater than 2: target 1 ng based on T-L instead of T-S.

5.4 Loading an amplification plate

- Transfer the entire 15μL 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).
- Once all samples have been added, 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. If a skirted optical 96 well plate is use, place a compression pad on top of the plate. Start the run.
- Store amplified products at 2-8°C. All amplified products will be disposed of upon completion of the technical and administrative reviews of the case.

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Section 6 Data Collection by 3500xl Genetic Analyzer

References: Applied Biosystems 3500/3500xL Genetic Analyzer User Guide

6.1 Start the Software and Instrument

- Turn the computer on and log into the computer
- Turn on the 3500xl Genetic Analyzer. Wait for the green status light to turn on.
- Ensure both the Daemon and Service Monitor 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

6.2 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, cathode buffer, and capillary are “hard stops” – if these reagents are expired, they must be replaced in order 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.

6.3 Sample Preparation for the 3500xl

- Prepare a loading master mix by adding the following volumes of reagents to an appropriately sized tube:
 - # of samples x 0.4µl GeneScan 600 LIZ Size Standard
 - # of samples x 9.6µl of Hi-Di Formamide

Note: Prepare enough for a few extra reactions to allow for loss during pipetting.

- 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.
- 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.

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- 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.

6.4 Creating a Plate Record

6.4.1 Create a Plate Record from the Data Collection Software

- In the dashboard click **Create New Plate**
- In the Define Plate Properties screen:
 - Enter a plate name (i.e. 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:
 - AB_GF_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.

6.4.2 Create a Plate Record from an Export File

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

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6.5 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.

6.6 Viewing Data During a Run

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

6.7 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 16 November 2015, 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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Section 7 Data Analysis with GeneMapper™ ID-X

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

7.1 Logging in to GeneMapper™ ID-X

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

7.2 Creating a Project

- To add samples from the collection software at a workstation, go to the edit menu and select **Add Samples to Project**. Navigate to 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 (see section 7.3), Panel GlobalFiler_Panel_v1 and Size Standard GS600_LIZ(60-460) for each sample and click the green arrow on the tool bar to analyze the samples.
- 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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7.3 Casework Analysis Methods

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 Troubleshooting is not for use in routine casework.

GlobalFiler Blank
GlobalFiler Casework
GlobalFiler Troubleshooting

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7.4 Casework Allele Tab Settings

Analysis Method Editor

General **Allele** Peak Detector Peak Quality SQ & GQ Settings

Bin Set: AmpFLSTR_Bins_y5X

Use marker-specific stutter ratio and distance if available

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

Range Filter... Factory Defaults

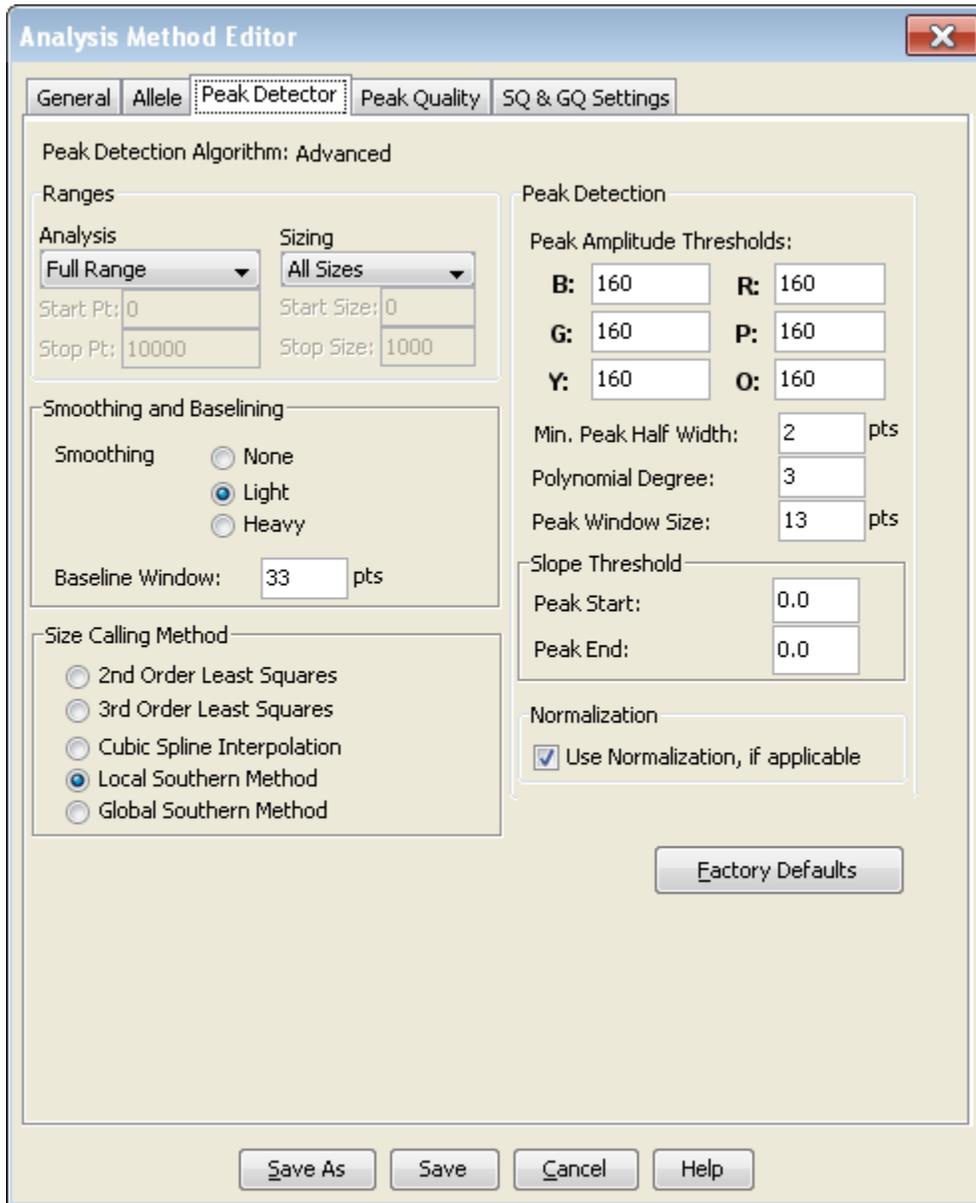
Save As Save Cancel Help

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7.5 Casework Peak Detector Tab Settings

The Analytical Threshold for all casework analysis is 160RFU.



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7.6 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', and 'SQ & GQ Settings'. The 'Peak Quality' tab is active and contains several sections of settings:

- Min/Max Peak Height (LPH/MPH)**
 - Homozygous min peak height: 630.0
 - Heterozygous min peak height: 1050.0
 - 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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7.7 Casework SQ and GQ Tab Settings

These settings are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.

All Analysis Methods

Analysis Method Editor

General | Allele | Peak Detector | Peak Quality | **SQ & GQ Settings**

Quality weights are between 0 and 1.

Sample and Control GQ Weighting

Broad Peak (BD)	0.8	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: (Green background) **Low Quality Range:** (Red background)

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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7.8 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.
- 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.

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Section 8 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.

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

DNA Abbreviations and Definitions	
BL	Noisy baseline
PHR	Peak Height Ratio
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
DBRT	Data below reporting threshold
ART	Artifact
TRI	Tri-allele
OBL	Obligate allele
UND	Undetermined

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DNA Abbreviations and Definitions (continued)	
NS _{mix}	Data not used for mixture statistic
()	Minor component allele
[]	Major/Minor components not separated

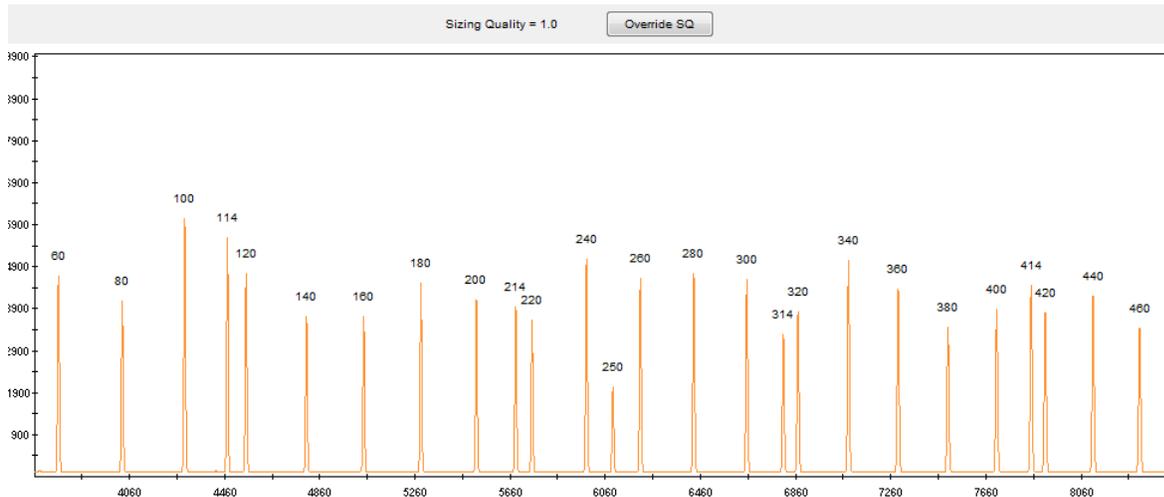
8.1 Interpretation of Batch Controls

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.

8.1.1 Internal Lane Standard (ILS)

GlobalFiler 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.



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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.

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.

8.1.2 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 GlobalFiler 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.

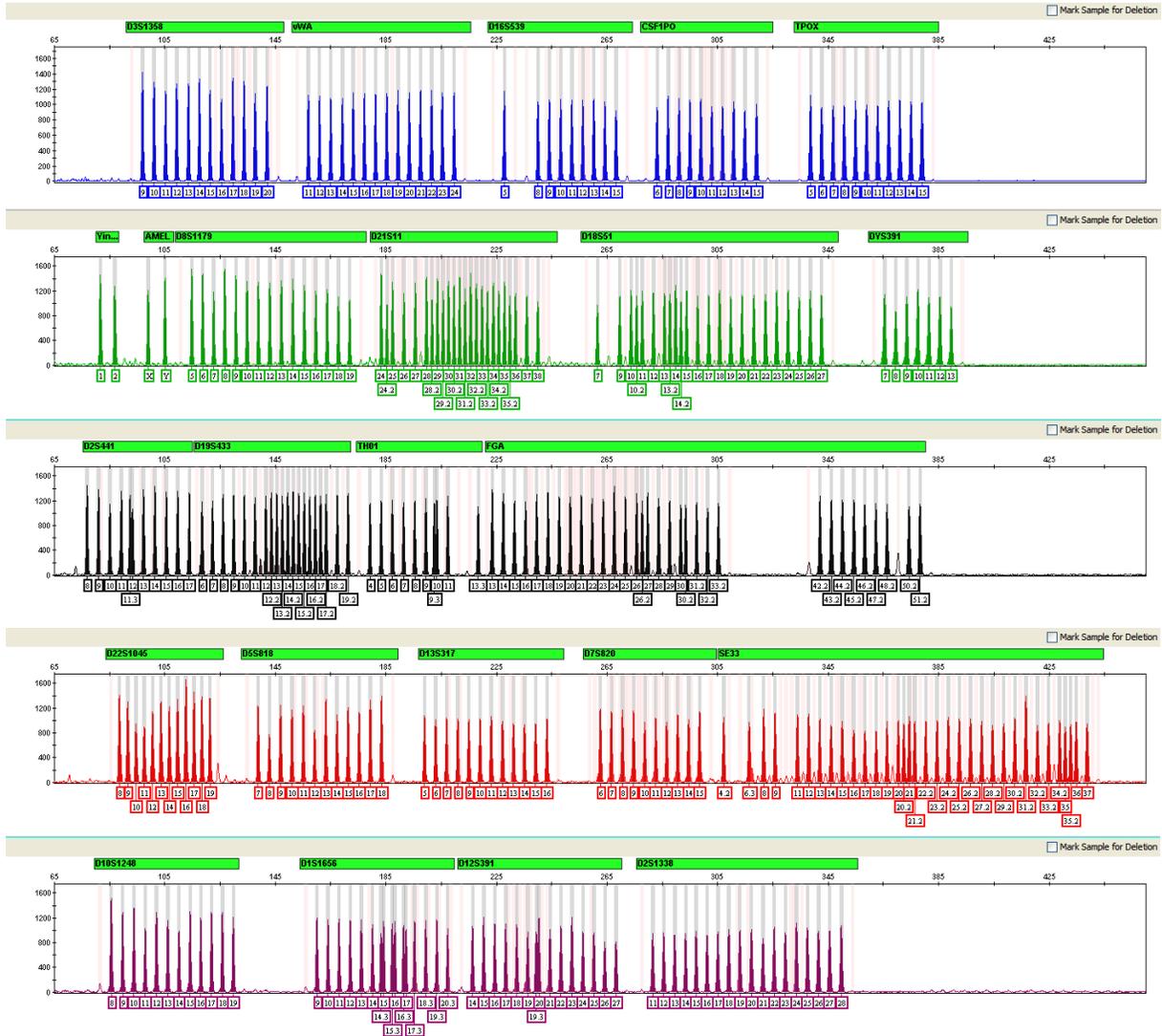
At least one allelic ladder must be included in a GeneMapper™ ID-X project with the sample type designated as “allelic ladder”. All the peaks in the allelic ladder must be labeled correctly for the software to assign the correct allele calls to samples in the run. When more than one ladder in a project 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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GlobalFiler Allelic ladder:



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8.1.3 Internal Control Specimen (ICS)

An internal control specimen (ICS) is a designated, predetermined quality control sample whose expected profile is already known. The ICS is processed with an extraction batch and carried through the batch concurrently with the other samples. The purpose of this control is to demonstrate that all analytical processes are working correctly. 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.

At least one ICS shall be processed with each casework batch, and will typically be extracted with the known reference samples. A printed electropherogram of the successfully typed ICS shall be included in the central log folder.

An ICS with a partial profile or no interpretable profile does not invalidate the batch, provided other positive controls in the batch yield the correct results. If a minimum of ten complete 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 ten complete core loci are detected for the ICS. The data quality of other positive control samples in the batch and/or the quality of known /reference samples will be reviewed to determine the approval process.

If an incorrect STR profile is obtained for the ICS in a casework batch, the analyst will attempt to determine the cause of the discrepancy. The discrepancy will be documented and corrective and future preventive actions may be taken as deemed necessary by the Technical Manager.

NOTE: Casework batches often contain multiple samples from previously typed individuals. Any one of these may be designated as the ICS for the batch. 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).

8.1.4 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 GlobalFiler 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.

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.

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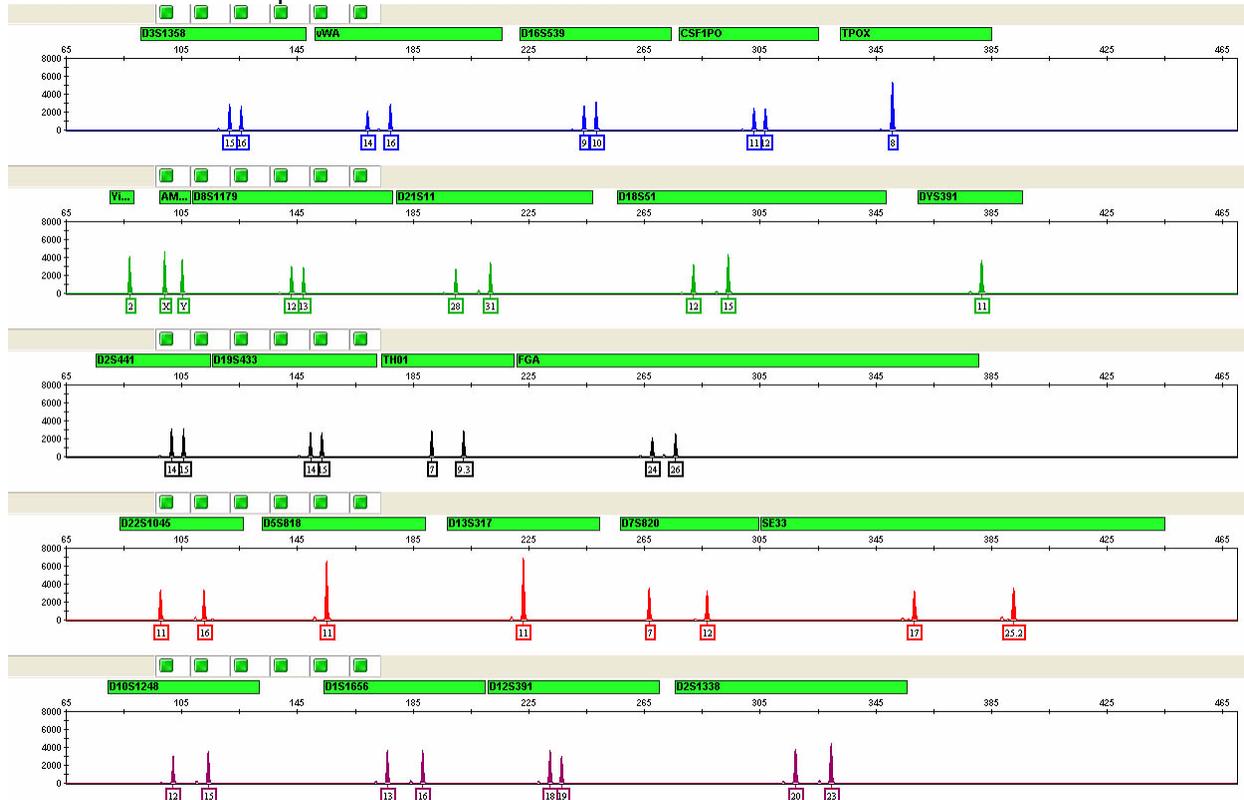
It may be necessary to re-prep and/or re-inject the positive control if the cause of failure appears to be a failed electrophoretic injection. As long as the re-prepped and/or re-injected positive control types successfully, the positive control is considered successful.

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.

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 GlobalFiler Kit:



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8.1.5 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 proceeding directly from quantitation to amplification, the reagent blank without DNA-Stable (typically replicate -1) is used. Any of this reagent blank extract remaining after amplification will be retained if any corresponding samples were also 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 low TE 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.

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.

The failure of a negative control may indicate a problem at the extraction or amplification level. When probable true allele peaks are detected above the reporting threshold at an interpretable level, the analyst will re-examine the sample using the GlobalFiler troubleshooting analysis parameters (60 RFU). If the low-level profile is not suitable for interpretation, it is not possible to perform root cause analysis and no further investigation is required. If the low-level profile is suitable for interpretation while using the troubleshooting threshold, 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 root cause of 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.

8.2 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 that are determined to be artifacts are documented by printing a zoomed in view of the artifact that includes base pair sizing. 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 STR results table and 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, 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. 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.

8.2.1 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.

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.

8.2.2 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.

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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 N-4 stutter for the loci in the GlobalFiler 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 in excess of 1 ng template DNA. As such, N-8 stutter is not included in the filters.

Maximum Expected N-4 Stutter Percentages for GlobalFiler 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 17%	D5S818 10%	D13S317 10%	D7S820 10%	SE33 15%	
D10S1248 12%	D1S1656 13%	D12S391 14%	D2S1338 12%		

Additional max stutter percentages:

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

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. When there is no indication of a mixture other than elevated stutter, 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).

8.2.2.1 Elevated +4 / -4 stutter

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

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- 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.

8.2.3 Amplification and Injection Artifacts

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.

8.2.3.1 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.

8.2.3.2 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.

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8.2.3.3 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.

8.2.3.4 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.

8.2.3.5 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.

8.2.4 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 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.

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.

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).

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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.

8.2.5 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 include the locus with the tri-allele in the STR results table. If the potential tri-allele is not reproducible, it will be reported as an Artifact/True allele (A/TA).

If the same tri-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 and may be included on the STR results table without the A/TA designation. No re-amplification of samples would be required in such instances.

8.2.6 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.

8.2.7 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.

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8.3 Assessing interpretable alleles

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.

8.3.1 Three types of thresholds used for data analysis and interpretation

- 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 1050 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.

8.3.1.1 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.

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.

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8.3.1.2 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.

8.4 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 impacts subsequent interpretation. Documentation of interpretation, including assumptions, must be included in the bench notes. This documentation may be written on the electropherogram or on a Mixture Interpretation Worksheet.

Note: 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.

Assessment of a questioned profile includes the determination of which loci are suitable for comparison and/or for the calculation of population statistics. Because population frequency databases are not universally available beyond the 13 FBI CODIS core loci, it is not necessary to document suitability of loci for statistics for loci not included in the FBI CODIS core loci.

Core 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. 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 core 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

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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_{mix}.

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.

8.4.1 Single Source Samples

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

Peak height ratios are calculated by dividing the peak height (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.

8.4.1.1 Minimum amount of data for a single-source to be suitable for comparison

A single source profile must have complete information for at least 4 of the 13 core loci to be deemed 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 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.

8.4.1.2 Using Alleles Below ST in probative comparisons and statistics in single-source samples

A locus must be deemed complete to be suitable for use in probative comparisons and population frequency calculations. In a few specific instances (ex. Amelogenin peaks), alleles below the ST may be used for comparisons and/or used in statistical calculations.

Single-source profiles with heterozygous alleles below ST: 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

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both sister alleles of a heterozygote are present below the ST, dropout is not a possibility and therefore the alleles may be included in the STR table and used for statistical calculations. Because this relies on the assumption of a single-source sample, the assumption must be documented on the electropherogram and in the report.

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

8.4.2 DNA Mixtures

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

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).

- When peaks in question are below ST and may be attributable to artifacts, a mixture cannot be assumed. 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 peak is seen above ST (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.
- A profile with more than two 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.

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.

Mixtures 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.

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Mixtures consistent with being from two individuals may be suitable for interpretation if the sample is of sufficient quality and quantity to yield complete information at a minimum of four loci. 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.

8.4.2.1 DNA Mixture Type Categorization

It is not possible to anticipate the nature of all potential DNA results, or the nature of the evidentiary samples from which they may be obtained. These categories do not exhaust the 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

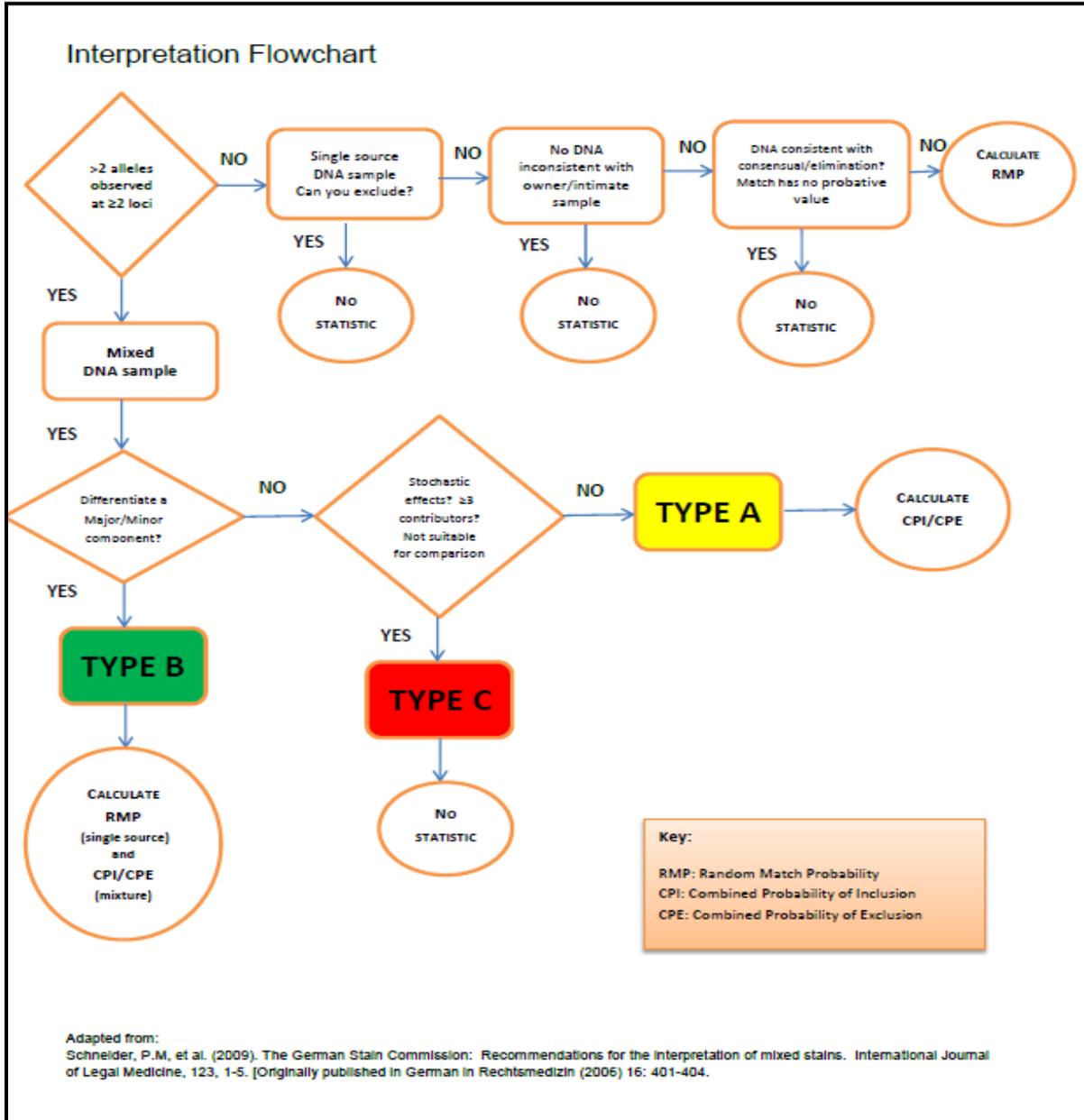
TYPE A:

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 (i.e. loci suitable for calculating population statistics) are required in order for an indistinguishable mixture to be suitable for comparisons. Refer to Appendix G for interpretation of low level data in an indistinguishable mixture.

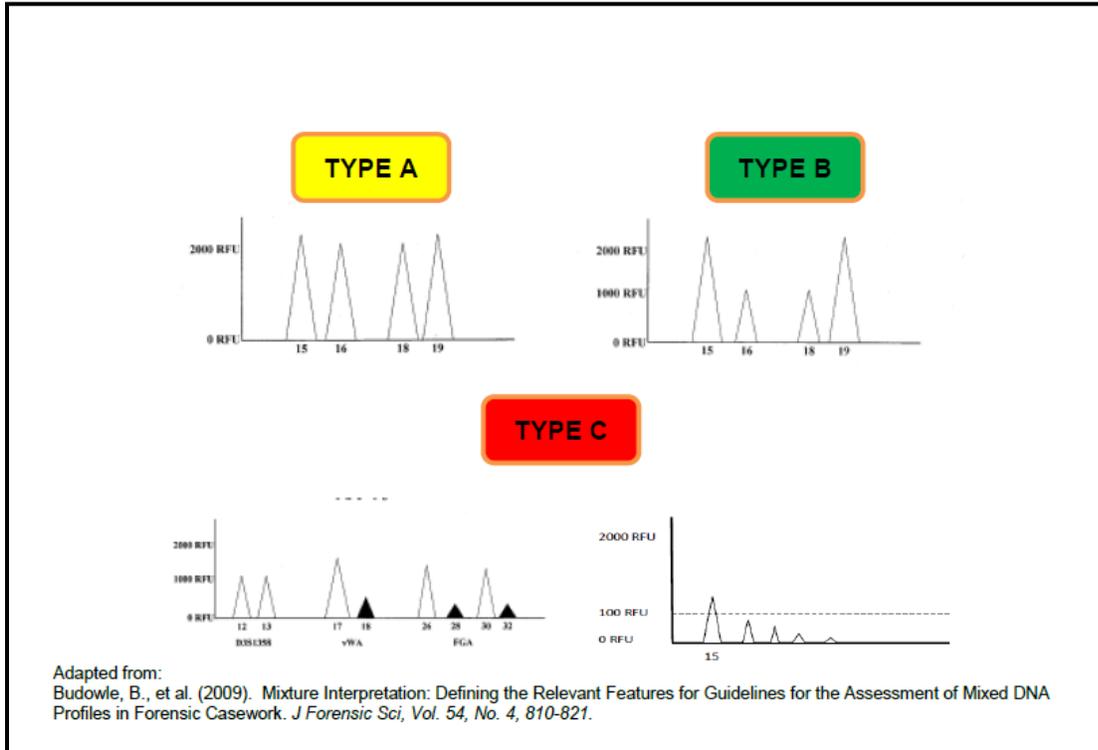
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TYPE B:

Description: If the amounts of biological material from multiple contributors are dissimilar, it may be possible to further refine the mixture profile. When major or minor contributors can be distinguished because of differences in signal intensities (i.e. peak heights), the sample is considered to be a distinguishable mixture. The difference is evaluated on a locus-by-locus basis. It is not necessary for all loci to be distinguishable. A minimum of four core loci must be separated into major and minor components for the profile to be considered distinguishable.

If the sample demonstrates a clear reproducible major component at a minimum of 4 of 13 core STR loci a conclusion as to the source of the major contributor DNA may be reported and will be included in statistical analysis, if appropriate.

Alternatively, the distinguishable major component may include two contributors. In that case, the profile as a whole is a distinguishable mixture, while the major component consists of an indistinguishable two-source mixture. Under current mixture interpretation guidelines, it is not possible to distinguish a mixture into three or more distinct contributors.

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.

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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.

8.4.2.2 Separating Major and Minor Contributors in DNA Mixtures

Refer to Appendix H for a flowchart of interpretation of low-level minor component of a distinguishable mixture.

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. 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.

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

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

8.4.2.2.1 Separating major and minor contributors in a two source mixture

Distinguishable mixtures of this type will result in a single-source major component suitable for probative comparisons. The minor component may or may not be suitable for probative comparisons. It may be necessary to factor out stutter when determining the ratios described below.

8.4.2.2.1.1 Proposed major component has one allele

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

8.4.2.2.1.2 Proposed major component has two alleles

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

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8.4.2.2.1.3 Using an “owner” profile to assist in separating major and minor

Additional information about an “owner” profile may be used to deduce the major and minor profiles from body swabs or an owner’s clothing. Deduction must be documented using a Mixture Interpretation Worksheet (MIW). (Note: loci separated based on peak heights alone may simply be noted as “major” on the MIW, and do not require further explanation).

8.4.2.2.2 Separating major and minor contributors with more than two sources – single major contributor

Distinguishable mixtures of this type will result in a single source major component suitable for probative comparisons. The minor component will not be suitable for comparisons based on the number of contributors. It may be necessary to factor out stutter when determining the ratios described below. Note that the presence of more than two contributors means that greater caution must be used with respect to alleles sharing and peak height ratios.

8.4.2.2.2.1 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

8.4.2.2.2.2 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

8.4.2.2.3 Separating major and minor contributors with more than two sources – two sources in major

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 ten core loci are distinguishable, it is not appropriate to separate the profile into major and minor components

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8.5 Deducing an Unknown Contributor in a Indistinguishable DNA Mixture (Type A)

Refer to Appendices I, J, and K for flowcharts of deductions at 2-allele and 3-allele loci.

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. 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 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:
 - At least two four-allele loci 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.
- 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.

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The rationale for deduction of the genetic profile for an unknown contributor must be documented on a Mixture Interpretation Worksheet.

8.6 Assessing which loci in a mixture are suitable for probative comparisons and statistics

8.6.1 Separating single sources out of a mixture

As described previously, a single source may be separated out from a mixture, either by identifying a major component, or by deducing an unknown contributor from an indistinguishable mixture. In instances when low-level contamination can be distinguished as a minor contributor, the major component may be suitable for comparison.

8.6.1.1 Minimum amount of data for a single-source to be suitable for comparison

A single source profile derived from a mixture, be it a major component, minor component, or deduced unknown profile, must have complete information for at least 4 of the 13 core loci to be deemed suitable for all comparisons.

8.6.1.2 Minor components for non-probative comparisons

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:

- 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 profile/partial profile/minor component.

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

8.6.1.2.1 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.

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- 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.

8.6.1.2.2 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.

8.6.1.3 Minimum quality of data for a single source to be suitable for probative comparisons and statistics

A locus must be deemed complete to be suitable for use in probative comparisons and population frequency calculations. Major components and deduced profiles separated using the criteria described above can be considered complete.

In a two-source mixture, the minor component can be considered complete and suitable for a single-source statistic only when two minor component alleles are detected.

8.6.1.4 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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8.6.2 Assessing loci in an indistinguishable two source mixture for suitability for probative comparisons and statistics

8.6.2.1 Minimum amount of data for a mixture to be suitable for comparison

A mixture must have sufficient information at a minimum of 10 of 13 core loci (or 75% of detected core loci) to perform comparisons to probative reference samples. Possible drop out of "owner" alleles does not necessarily preclude comparison, but minimum of four core loci must be complete and suitable for statistics in order for the profile to be suitable for comparison to probative reference samples.

8.6.2.2 Minimum quality of data for a mixture to be suitable for probative comparisons and statistics

In order 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.

In a two-source mixture, the locus can be considered completely detected 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

In a two-source mixture, if a locus cannot be considered complete, it should be noted as not suitable for mixture statistics on the electropherogram.

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.

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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.

8.7 Comparison of DNA Results

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.

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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.

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

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statistic that takes into account relatedness may be more appropriate. At this time, the Alaska Crime Laboratory does not perform such statistics; but upon request, the DNA Technical Manager can refer agencies to outside resources.

8.7.1 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 in Section 10.

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.
- 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.

8.7.2 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.

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Section 9 Statistical Analysis of STR Data

References: Budowle, B. et al., Population data on the thirteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. (1999) Journal of Forensic Science, 44(6): 1277-1286.

Erratum to above article - (2015) Journal of Forensic Science, 60 (4).

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

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 core loci which are completely detected. (Note: in the rare case that a known sample does not yield a full profile at all core loci, then the questioned profile core 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. 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).

The Alaska Scientific Crime Detection Laboratory routinely reports the frequency/probability for the following populations: Caucasian, African-American, Athabaskan, Inupiat and Yupik.

9.1 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 Amended FBI STR 2015 database and the theta value is set to 0.01 (see image below).
- All populations except Blk and Cau 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)

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- Enter the alleles deemed appropriate for statistical analysis (see STR Data Interpretation section).

Note: It is only necessary to enter alleles for CODIS core STR loci, as population data for other loci are not contained in the database.

- 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 Native database and the Amended 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 Native database, click Browse (in the configuration summary tab) to navigate to the Alaska 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).

- For single source statistics, change the theta value to 0.03 using the drop down menu in the configuration summary tab.

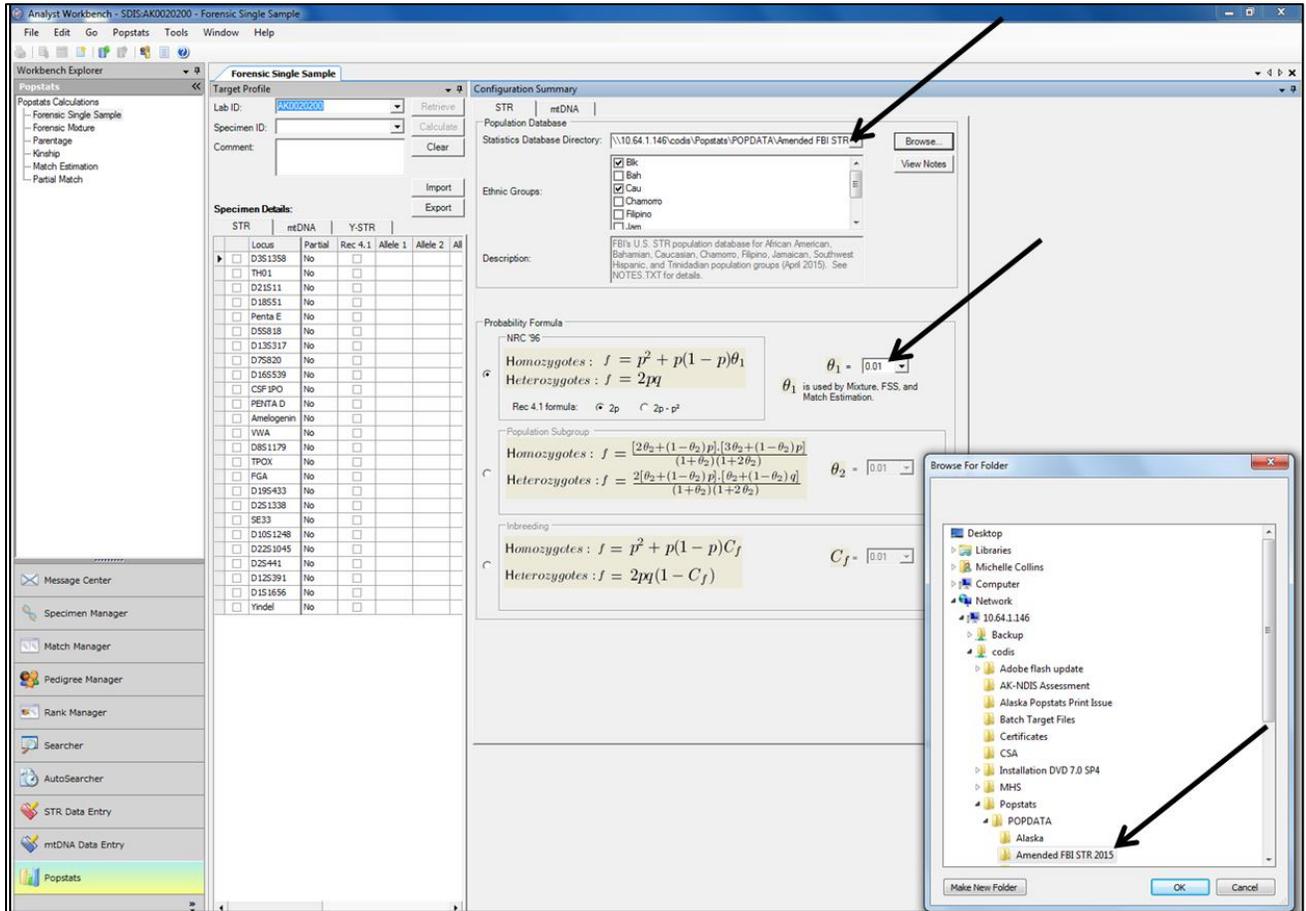
Note: The database and theta value must be changed in this order or the theta value will revert back.

- Click the Calculate button and repeat the above steps for printing the resulting statistics

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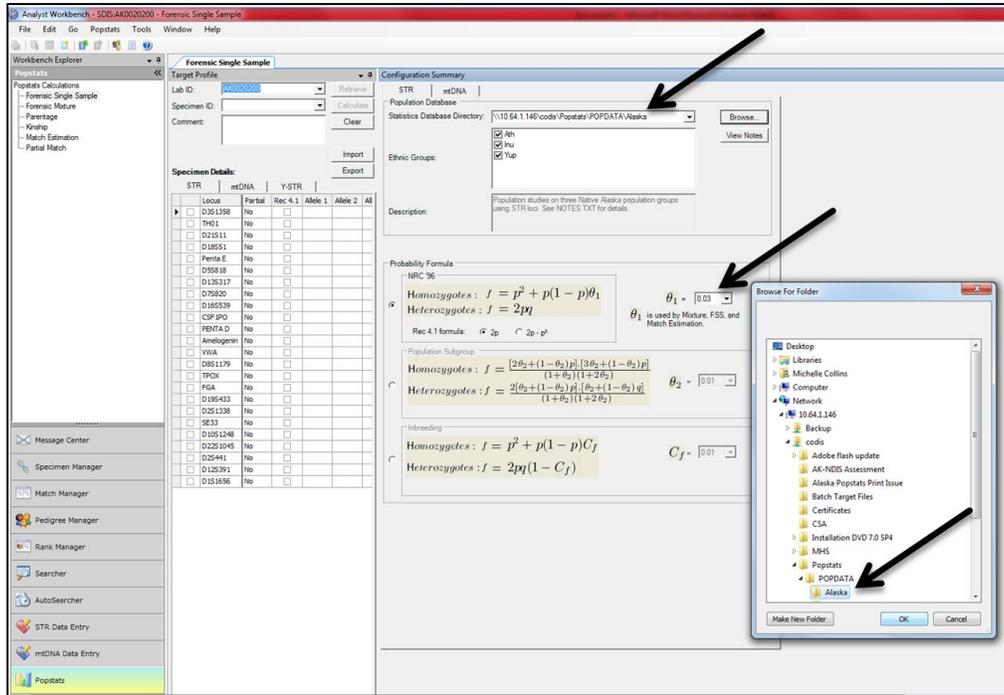
Navigating to the FBI STR Caucasian and African-American Population Databases



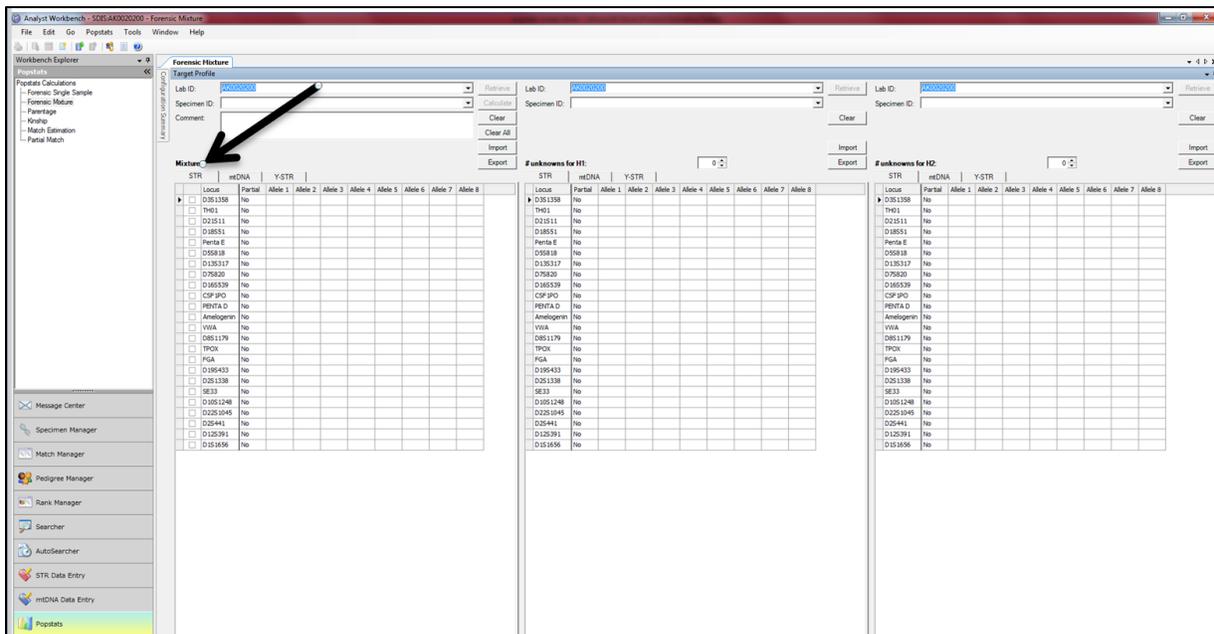
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Selecting the Alaska Native Population Databases



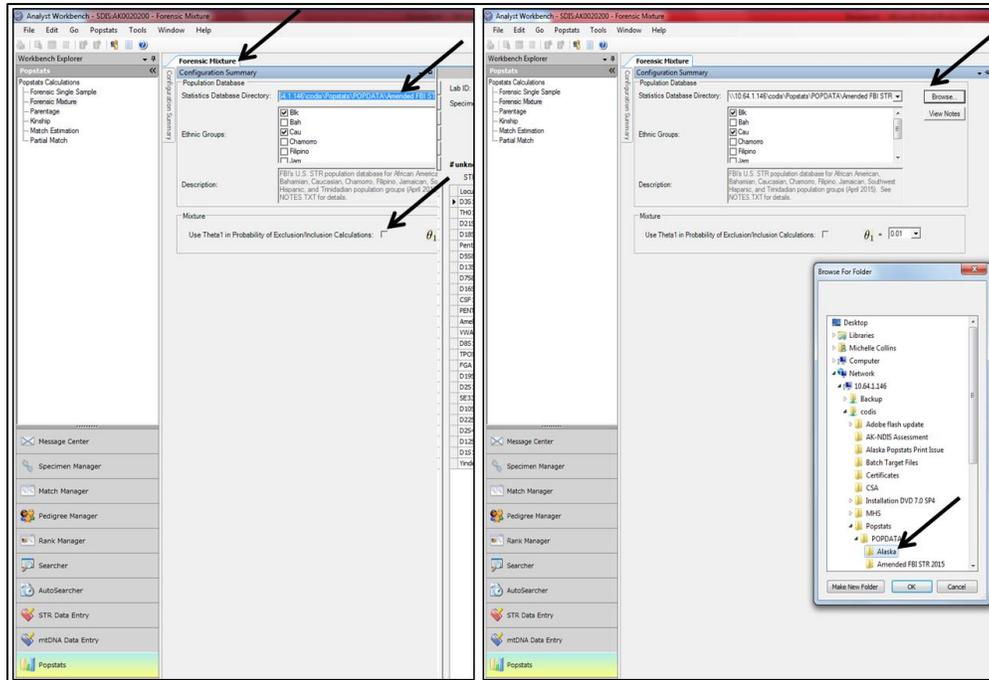
Entering a Mixture Statistic



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Changing the Population Database when Calculating Forensic Mixtures



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Section 10 DNA Screening and DNA Report Writing and Review

10.1 DNA Screening Reports

10.1.1 DNA Screening report required content:

The guidelines for Biological Screening reports, as listed previously in the Biological Report Writing and Review section (Section 2), also apply to DNA Screening reports. DNA Screening reports contain additional conclusions based on quantitation results.

10.1.2 Report Wording

Refer to the previous section on Biological Screening Report Writing and Review (Section 2) for report language specific to Biological Screening procedures. The following tables contain samples of results, conclusion and opinions appropriate for reporting various case results specific to DNA Screening. These are not all inclusive and may be modified slightly on a case by case basis.

For samples not amplified based on Quantifiler Trio results	Report
No male DNA (or T-Y < 0.0007 ng/μL) detected	Quantification results do not indicate the presence of amplifiable male DNA. No further analysis was performed on this sample.
Female: Male ratio >4:1	<p>Quantification results do not indicate the presence of sufficient male DNA for STR analysis. No further analysis was performed on this sample.</p> <p>This sample may be suitable for Y-STR analysis. For more information, please contact the laboratory's DNA Technical Manager, XXXX XXXX (269-XXXX) or XXX.XXX@alaska.gov</p>
T-Y is 0.02 – 0.0007 ng/μL in a suspect case (at any female: male ratio)	<p>Quantification results do not indicate the presence of sufficient male DNA for STR analysis. No further analysis was performed on this sample.</p> <p>This sample may be suitable for Y-STR analysis. For more information, please contact the laboratory's DNA Technical Manager, XXXX XXXX (269-XXXX) or</p>

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	XXX.XXX@alaska.gov
T-Y is 0.02 - 0.0007 ng/μL in a non-suspect case AND female: male ratio is greater than 5:1	Quantification results indicate the presence of male DNA. Should a suspect be developed, this sample may be suitable for Y-STR analysis. For more information, please contact the laboratory's DNA Technical Manager, XXXX XXXX (269-XXXX) or XXX.XXX@alaska.gov
Consumed sample - T-S is <0.07 ng/μL (If likely mixture, female: male ratio is 4:1 or less and T-Y is at least 0.02 ng/μL)	Quantification results indicate the presence of male DNA. Based on the low quantity of DNA present in this sample, the recommended 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 for consumption of the sample in its entirety. For more information, please contact the laboratory's DNA Technical Manager, XXXX XXXX (269-XXXX) or XXX.XXX@alaska.gov
Extract where female DNA is probative and T-Y is greater than or equal to T-S	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 the presence of sufficient amplifiable DNA. No further analysis was performed on this sample.

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All others	Report
<p>T-Y is 0.02 ng/μL or higher (if male is probative) and T-S >= 0.07 ng/μL and female: male ratio is 4:1 or less</p> <p>Other samples suitable for STR analysis based on Quantifiler Trio results</p>	<p>Quantification results indicate the presence of male DNA. This sample is suitable for further analysis.</p>

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10.1.3 Review of DNA Screening Reports

Technical review of DNA 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:

- 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 analyzed
 - include the agency item # in () if the item was re-numbered at the laboratory
 - this is not necessary if the laboratory number contains the original agency item #
- Results of presumptive/confirmatory tests (in body of report)
- Documentation of reagents and controls (in central log)
- Documentation of extraction parameters such as date and instruments used (in central log and bench notes)
- Documentation of quantitation analysis (in central log)
- Results, conclusions and opinions for all tested items (guidelines for reporting are provided and may be modified, as necessary, on a case by case basis)
- Explanation of why testing was stopped, if appropriate
- The disposition of all retained items
- Any known samples that are required for DNA analysis, when applicable
- Statement regarding Y-STR testing, when applicable
- 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 and technical reviewer are required to scan their barcode and enter a pin when signing the report in LIMS)

Administrative review of DNA Screening reports 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 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.

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10.2 DNA Reports

10.2.1 DNA report required content

All DNA discipline 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 analyzed
 - include the agency item # in () if the item was re-numbered at the laboratory
 - this is not necessary if the laboratory number contains the original agency item #
- When DNA analysis is reported for samples retained by another discipline in the laboratory, or comparisons are made to profiles reported by another laboratory, the report should reference the previously issued report
 - i.e. “Reference **biological screening** report dated **Month Day, Year** by **Analyst.**”
 - Include the issuing laboratory, if other than the AK SCDL.
- Results of presumptive/confirmatory tests, if not previously reported in a biological screening report
- Results, conclusions and opinions, for all tested items, based on the DNA typing results
 - Conclusions do not need to be reported for the epithelial fractions from internal intimate samples (e.g. vaginal, rectal, and oral swabs), but do need to be reported for all sperm fractions and for all other epithelial fractions.
 - Conclusions do not need to be reported for substrate fractions if the substrate serves as a quality control, i.e. if all interpretable alleles are consistent with both the sperm and epithelial fractions.
- 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 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 Methods section of the report). Consumed evidence is noted in the body of the report.
- 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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10.2.2 Report Wording

The following tables contain samples of results, conclusion and opinions appropriate for reporting various case results. These are not all inclusive and may be modified slightly on a case by case basis. DNA reports may include DNA screening results regarding decisions about which samples do not proceed to amplification. Refer to section 10.1.2 for DNA Screening report language.

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

For single source and/or mixtures	Report
Unknown Male DNA	A genetic profile from an unknown male individual was obtained from this sample.
Female present	The genetic profile obtained from 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.
When cannot exclude but don't have all 13 core loci	DNA consistent with XXX was detected at XX of 13 core loci in this sample. Therefore, XXX cannot be excluded as a source of DNA detected in this sample. (change 13 if not all loci yielded data above reporting threshold)

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For 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 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	This item has previously been identified as belonging to XXX.
Association is to a consent partner	YYY has previously been identified as being a consent partner of XXX.

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	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. 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.

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For mixtures	Report
More than 1 individual	DNA from more than one individual was observed (may be present) in this sample.
To indicate # of individuals	DNA from at least XX individuals was observed in this sample. OR DNA consistent with being from two individuals was observed in this sample.
Mixture: exclusion	XXX was excluded as a source of DNA detected in this sample.
Mixture: fail to exclude	XXX cannot be excluded as a source of DNA detected in this sample.
Foreign DNA present	DNA inconsistent with XXX was also detected in this sample.
Complex Mixture	Due to the complexity of the genetic profile obtained from this sample, no meaningful comparisons can be made to known reference samples.
Mixture statistic	Data below the reporting threshold may be present at the following loci: XXX. Therefore, population statistics are not reported for these loci.

Mixture statistic	<p><u>Mixture formula</u> The estimated probability (13 core loci) of an individual from each of the following population groups contributing to the DNA detected in the above sample(s) is as follows: approximately 1 in XXX quadrillion (Caucasian population), 1 in XXX quadrillion (African-American population), 1 in XXX quadrillion (Athabaskan population), 1 in XXX quadrillion (Inupiat population) and 1 in XXX quadrillion (Yupik population).</p>
Close relatives	<p>The mixture statistic listed above applies to random and unrelated individuals. In cases where close biological 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>

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<p>Deduced Profile with Single Source statistics</p>	<p>DNA consistent with being from two individuals was detected in this sample. Assuming the presence of XXX, a single source genetic profile for the unknown (male) contributor was deduced from the DNA mixture obtained from this sample. YYY cannot be excluded as the source of the deduced genetic profile. No DNA inconsistent with XXX and YYY was detected in this sample.</p> <p>A single source genetic 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>Single Source Formula</p>
--	--

Not Suitable for comparison	Report
Not suitable	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.

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.

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Profile with contamination is inconclusive with respect to further comparisons	(In addition to above language) This profile is inconclusive for interpretation.
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Evidence consumed	Report
All of evidence used to make extract; all of extract consumed in analysis.	This sample was consumed in analysis.

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10.2.3 Review of DNA Casework Analyzed In-House

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 review of DNA 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 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

Administrative review of DNA 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
 - Note: Extracts created during DNA Screening may be worked by DNA analysts before or without the kit (or similar parent item) being in the custody of the DNA 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.

Discipline checklists are used to document completion of the individual components of the technical and administrative reviews. The completion of the final laboratory report, technical review and administrative review are tracked electronically in LIMS.

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.

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Section 11 Forensic Parentage

11.1 Data Interpretation

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 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.

11.2 Using Popstats for Parentage Statistics

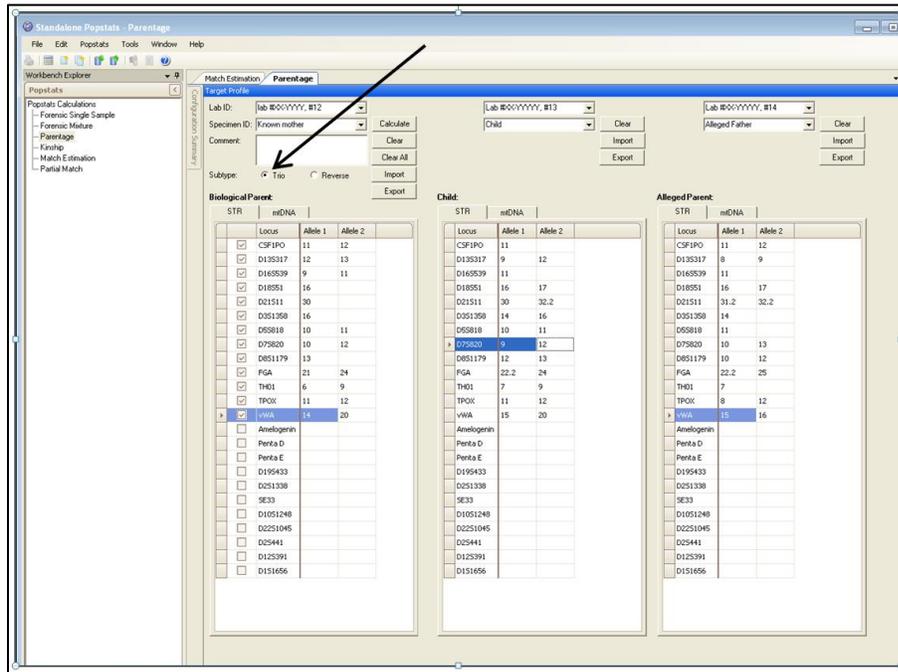
[Note: On occasion, especially when entering mutation rates or toggling between databases, the screen may not automatically refresh to show updated changes. If you do a Print Preview, you can confirm that changes have been applied.]

A. One-parent forward paternity (trio):

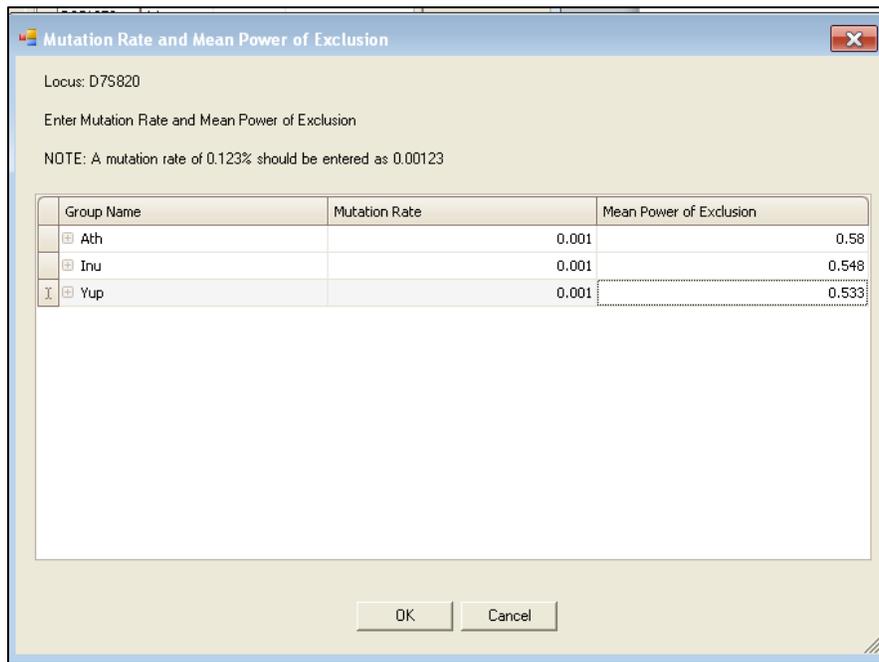
1. Open Popstats and choose Parentage from the menu on the left side of the screen.
2. Choose the Parentage tab at the top of the screen.
3. Use the Specimen ID field to enter the lab number at least, item number space permitting. If the item numbers do not fit in the typed file they may be hand-written 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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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).

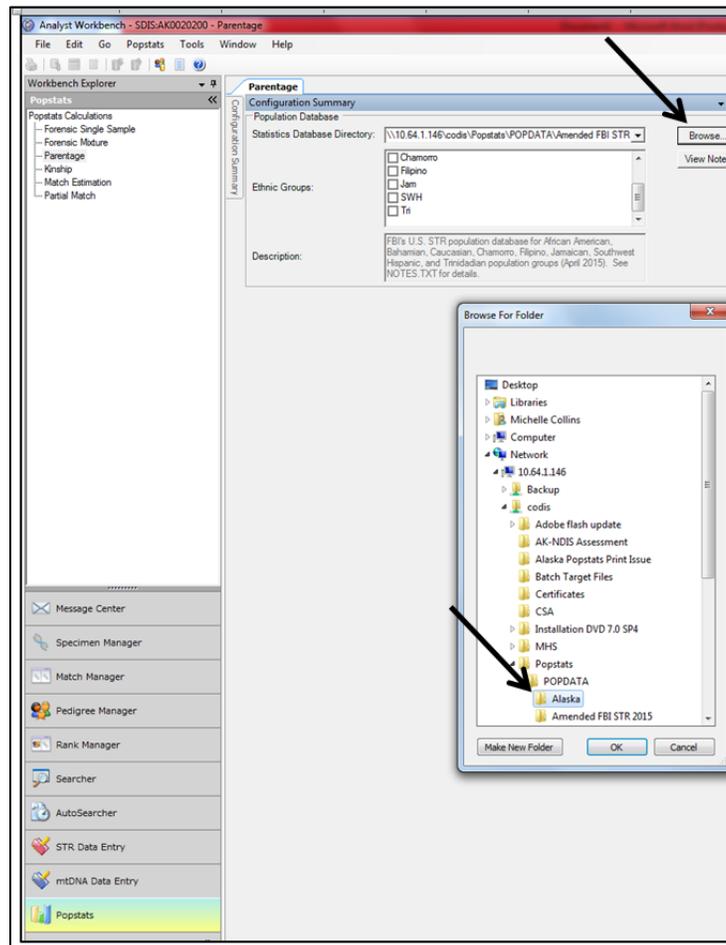


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8. Enter the locus-specific mutation rate from the NIST website. A printout of the relevant page is included in an appendix of this manual.
9. Enter the locus-specific mean probability of exclusion (PE) from the published database references listed 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 Amended FBI STR 2015 database file) and the Athabaskan, Inupiat, and Yupik databases (from the Alaska 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.



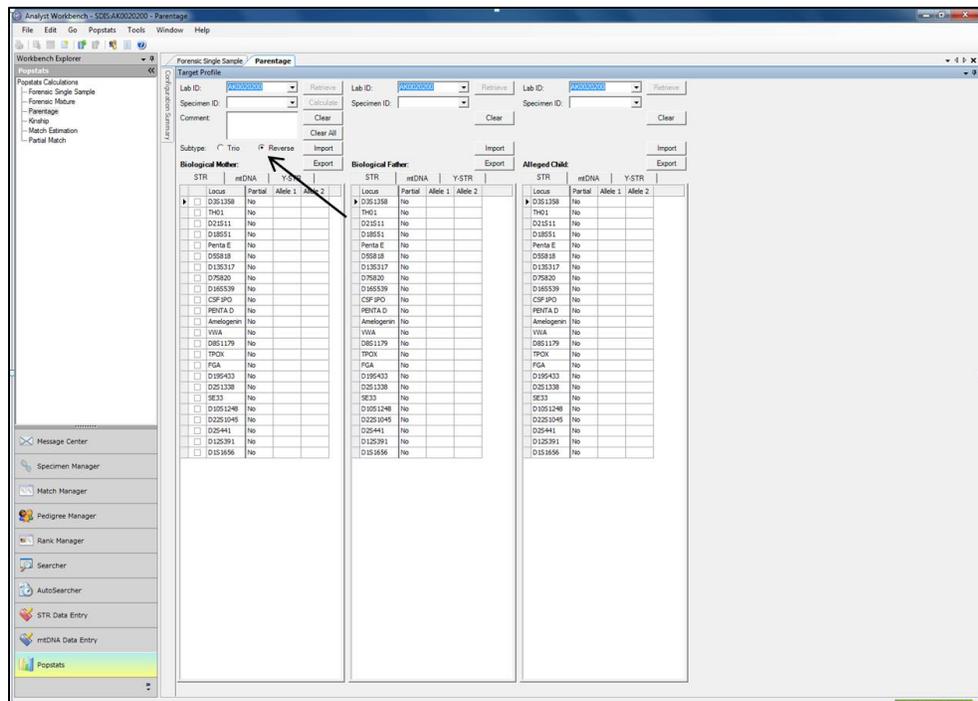
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13. As before, if necessary, you will be prompted to enter mutation rate and mean power of exclusion. When finished, choose OK. Print report as described previously.

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)



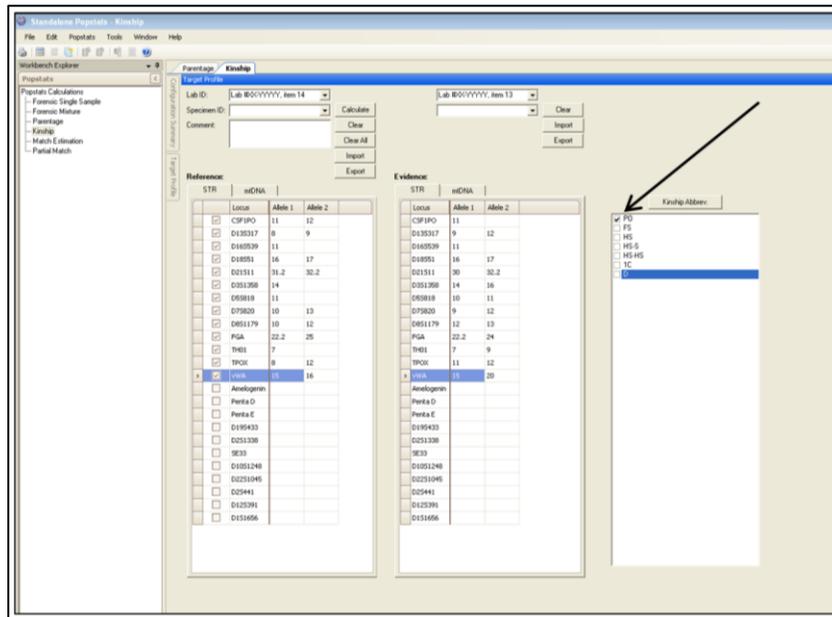
5. Repeat steps 5-13 from Section A.
6. On reverse parentage trios, Popstats does not allow for the use of a locus with a mutation. In cases with a mutation, omit the locus with the mutation from the Popstats calculation. On the Popstats printout, manually calculate the PI for the locus with the mutation. Use this manual calculation to adjust the CPI and Probability of Paternity calculations as well. Show work clearly.

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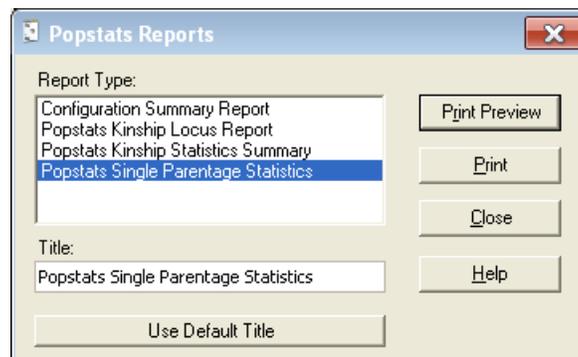
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C. Zero-parent forward:

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).



7. Print out statistic reports for the Caucasian and African-American databases (from the Amended FBI STR 2015 database file) and the Athabaskan, Inupiat, and Yupik databases (from the Alaska database file). Directions and image for toggling between databases are listed in section A, step 12.

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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 core 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.]

Population _____ Combined Parentage Index

Caucasian

African-American

Athabaskan

Inupiat

Yupik

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 above populations.

Exclusion:

Based on the DNA profiles obtained for the samples listed above (XX core 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 core 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 core 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.]

Population _____ Combined Parentage Index

Caucasian

African-American

Athabaskan

Inupiat

Yupik

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 above populations.

Exclusion:

Based on the DNA profiles obtained for the samples listed above (XX core 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 core 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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11.4 References

Budowle, B. et al. Population data on the thirteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S.Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. (1999) *Journal of Forensic Science*, 44 (6): 1277-1286.

Erratum to above article (2015) *Journal of Forensic Science* 60(4).

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

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Section 12 Long-Term Storage of DNA Extracts by DNASTable® LD

1. Retained DNA extracts are dried down:
 - a. When all the evidence available for analysis has been consumed, OR
 - b. When the analyst has determined that no further work is required on the extract
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). Only one extract may be packaged per foil envelope. 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 up to 50 µl of sterile water. Up to 40 µL of extract eluted in TE buffer may be reconstituted in as little as 15 µL dH₂O. 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 dH₂O. 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 gentle pipetting. Avoid dispensing bubbles into the sample. 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.

Alaska Scientific Crime Detection Laboratory

Forensic Biology Casework Procedures

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Biological Screening Review Checklist

	Technical Review	Administrative Review
LAB NUMBER:		
ANALYST:		
Date Submitted for Review:		
Technical Review Started: _____ Reviewer: _____		
Administrative Review Started: _____ Reviewer: _____		
Pages are numbered correctly, lab case #, item # and analyst initials are on each page		
Requesting agency, agency case #, lab case #, and officer's name are correct		
Item numbers / packaging / descriptions on report / notes are consistent with RLS/LIMS		
The types of examination (visual, stereoscopic, ALS) and testing performed are documented in notes		
Item descriptions are consistent with clothing/evidence images present (if applicable)		
The location of all chemical testing performed is documented in the notes (if applicable)		
All isolated stains/samples are documented and numbered correctly (if applicable)		
Verification reviews conducted are documented in the notes (if applicable)		
The location and disposition of all trace evidence is documented		
Worksheets contain all lot #s and expiration dates for all reagents used		
Start and end dates of examination/analysis are documented		
Retained items created in LIMS; all retained and unexamined items HELD in LIMS		
The "FUTURE TECH" flag has been tripped, if applicable (samples suitable for Y-STR)		
Check grammar/spelling/punctuation in report		
Results/conclusions/opinions are given for each item tested		
Conclusions/opinions drawn from results comply with laboratory guidelines		
Conclusions/opinions drawn from results are supported by documentation in the notes		
Known samples requested, if appropriate		
Report signed in LIMS		
All case related notes and attached/scanned documents are present		
Technical reviewer is in review history for each page of the bench notes		
SOPs are linked to request in LIMS		
Chain of Custody for all tested evidence can be tracked through RLS and LIMS		
Assign DNA holding as appropriate		

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Forensic Biology Casework Procedures

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DNA Screening Review Checklist

Date: _____	Analyst:	Tech. Review:	Admin. Review:
Technical Review Started: _____			
Administrative Review Started: _____			
Lab Number: _____			

Pages are numbered correctly, lab case #, item # and analyst initials are on each page			
Requesting agency, agency case #, lab case #, and officer's name are correct			
Item numbers / packaging / descriptions on report / notes are consistent with RLS/LIMS			
The type of examination (visual, stereoscopic, ALS) and testing performed is documented in notes			
Item descriptions are consistent with clothing/evidence images present (if applicable)			
The location of all chemical testing performed is documented in the notes (if applicable)			
All isolated stains/samples are documented and numbered correctly (if applicable)			
Elution volumes, instrument numbers, and sample size recorded (if applicable)			
Evidence consumed to create extract is noted as such in bench notes			
Verification reviews conducted are documented in the notes (if applicable)			
The location and disposition of all trace evidence is documented			
Worksheets contain all lot #s and expiration dates for all reagents used			
Start and end dates of examination/analysis are documented			
Q-PCR plate set-up is documented			
Q-PCR standard curve printouts: Results are acceptable.			
Q-PCR Initial Template Quantity is documented by 7500 printout.			
Retained items (including reagent blanks) created in LIMS; all retained and unexamined items HELD in LIMS as appropriate			
The "FUTURE TECH" flag has been tripped for the case, if applicable (samples suitable for Y-STR)			

CENTRAL LOG FOLDER NAME:

Check grammar/spelling/punctuation in report			
Results/conclusions/opinions are given for each item tested			
Conclusions/opinions drawn from results comply with laboratory guidelines			
Conclusions/opinions drawn from results are supported by documentation in the notes			
Known samples requested, if appropriate			
Report signed in LIMS			
All case related notes and attached/scanned documents are present			
Technical reviewer is in review history for each page of the bench notes			
SOPs are linked to request in LIMS			
Chain of Custody for all tested evidence can be tracked through RLS and LIMS			
Batch number is recorded in JT			
Assign DNA holding as appropriate			

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DNA STR Results Table

Laboratory Case #
Batch:

Analyst:

Item #					
Description/ Locus					
D3S1358					
TH01					
D21S11					
D18S51					
Penta E^{NS}					
D5S818					
D13S317					
D7S820					
D16S539					
CSF1PO					
Penta D^{NS}					
Amelogenin					
vWA					
D8S1179					
TPOX					
FGA					

Notes:

1. The Amelogenin locus indicates gender: X = Female; XY = Male
2. "NS" indicates data not used in statistical analysis
3. "NR" indicates data not reported
4. "DBRT" indicates data below reporting threshold
5. () indicates minor component alleles
6. [] indicates Major/Minor components not separated
7. "A/TA" indicates artifact (e.g. stutter) or true allele
8. Genetic data unsuitable for comparison may not be included in the table
9. "NA" indicates locus was not amplified

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Forensic Biology Casework Procedures

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STR Casework Review Checklist

Date: _____
Analysis Start Date: _____
Technical Review Started: _____
Administrative Review Started: _____
Lab Number: _____

Analyst:	Tech. Review:	Admin. Review:

DNA CASEWORK REPORT AND BENCH NOTES

Pages are numbered correctly, case # and analyst initials are on each page, report dated			
Requesting agency, agency case #, crime lab case #, and officer's name are correct			
Chain of Custody for all tested evidence can be tracked through RLS and LIMS			
Item numbers and descriptions on report / DNA worksheet are consistent with RLS/LIMS			
Packaging / extraction procedure used for all items is documented on worksheets			
Start and end dates of examination/analysis are documented			
Screening results on DNA worksheets are accounted for in the report, if appropriate			
Disposition of sample extracts/slides documented			
Technical Reviewer checked DNA worksheet: initialed and dated			
Technical review of all electropherograms: initialed and dated			
ILS checked for all questioned and reference samples			
Printouts for all questioned and reference samples are present in bench notes			
QC check of unidentified profiles (if applicable)			
Peak patterns on the electropherograms are consistent with the STR results table (if applicable)			
The correct DNA profile and descriptive information is entered into Popstats			
Popstats theta values and databases are correct; printouts are included in the case file			
Popstats DNA profile frequencies are consistent with those shown on the report			

Check Grammar/Spelling in DNA report			
Results/conclusions/opinions are given for each tested item			
Conclusions/opinions drawn from results comply with laboratory guidelines			
Initials and date of technical review(er) are on each page of the bench notes			
Verify content of Methods and Disposition sections of report			
Stats are recorded in LIMS			
SOPs are linked to request in LIMS			
Future tech flag is set if appropriate			
Disposition of evidence in LIMS is appropriate (HOLD or not)			

CENTRAL LOG FOLDER NAME:

Central Log Folder name documented in LIMS			
Central Log Review is complete			

CODIS

List of eligible profiles to be entered into CODIS (include specimen category and SDIS or NDIS)			
Specimen eligibility verified prior to CODIS entry (including match estimation, if applicable)			
Correct profile(s) and agency information entered in CODIS			
Appropriate specimen category selected			
Source ID updated if applicable			
Offender/arrestee laboratory comments updated in LIMS, if applicable			

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STR Central Log Review Checklist

Date: _____
Technical Review Started: _____

Analyst:	Tech. Review:
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CENTRAL LOG FOLDER NAME:

Central Log Folder name is documented in LIMS in each related case		
All applicable case numbers are listed		
Raw data file names for genetic analysis are listed		
Screening control results are documented (if appropriate)		
Worksheets contain all lot #s and expiration dates for all reagents used.		
Packaging / extraction procedure used for all blanks are documented		
Disposition of blank extracts documented; created in LIMS (if appropriate)		
Chain of Custody for all retained blanks can be tracked through LIMS		
Q-PCR plate set-up is documented (If applicable)		
Q-PCR standard curve printouts: Results are acceptable. (If applicable)		
Q-PCR Initial Template Quantity is documented by 7500 printout. (If applicable)		
Amplification volumes are documented.		
Amplification worksheets contain all lot #s and expiration dates for all reagents used.		
QC check of unidentified profiles (if applicable)		
ICS electropherogram: Results are acceptable		
Reagent blanks and negative control(s): Results are acceptable.		
Positive control(s): correct DNA profile obtained.		
Allelic ladder(s): correct alleles called		
ILS checked for allelic ladders, amplification controls, ICS, and reagent blanks		
Initials and date of technical review(er) are on each page of the printed notes		

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STR Review Checklist for Vendor Lab

Date: _____
 SCDL Lab Number: _____
 Vendor Laboratory: _____
 Vendor Lab Number: _____
 Agency: _____
 Agency #: _____
 Technology: _____
 Platform: _____
 Amplification kit: _____

Tech Review:	CODIS Review:
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Printouts for all appropriate Allelic Ladders: obtained expected ILS and allelic peaks		
Printouts for positive control: obtained expected ILS peaks and DNA profile		
Printouts for negative control: results are acceptable (including ILS)		
Printouts for reagent blanks: results are acceptable (including ILS)		

Screening results on DNA worksheets are accounted for in the report, if appropriate		
Raw or analyzed data provided for each tested item		
Technical review of all electropherograms (including ILS); initialed and dated by		
Peak patterns on the electropherograms are consistent with the table (if applicable)		

Results/conclusions/opinions are given for each tested item		
Conclusions/opinions drawn from results are supported by the genetic data		
Initials and date of technical review(er) are on each page		

List of eligible profiles to be entered into CODIS (include specimen category and SDIS or NDIS)		
Specimen eligibility verified prior to CODIS entry (including match estimation, if applicable)		
Correct profile(s) and agency information entered in CODIS		
Appropriate specimen category selected		
Source ID updated if applicable		

NOTES:

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STR Single Offender Review Checklist

Date: _____
Date Analysis Started: _____
Technical Review Started: _____
Lab Number: _____

Analyst:	Tech. Review:
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OFFENDER FOR DNA DATABASE OR CODIS CONFIRMATION

Offender number(s) and analyst initials are on each page		
Offender number(s) and name(s) on DNA worksheet are consistent with LIMS		
Start and end dates of examination/analysis are documented		
Packaging / extraction procedure is documented on DNA worksheet		
Disposition of offender extract(s) documented		
Technical Reviewer checked DNA worksheet: initialed and dated		
ILS checked		
Technical review of all electropherograms: initialed and dated		

Results (i.e. profile verified) given for each tested item		
Initials and date of technical review(er) are on each page of the bench notes		
SOPs are linked to request in LIMS		

CENTRAL LOG FOLDER NAME:

Central Log Folder location documented in LIMS		
Central Log Review is complete		

List of eligible profiles to be entered into CODIS (include specimen category and SDIS or NDIS)		
Specimen eligibility verified prior to CODIS entry (including match estimation, if applicable)		
Correct profile(s) and agency information entered in CODIS		
Appropriate specimen category selected		
Source ID updated if applicable		
Offender/arrestee laboratory comments updated in LIMS, if applicable		

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Contamination Assessment Form

Analyst: _____

Batch: _____

Sample with detected contamination: _____

Extraction instrument(s): _____

Quantitation result (if performed): _____

Have relevant staff and other samples analyzed concurrently been ruled out as possible sources of the contamination? _____

Was contamination observed in any other samples in this batch? _____

Root cause analysis: based on the available data, discuss possibilities for the point at which contamination was introduced, including specific plastic ware or consumables:

Acceptance by DNA Technical Manager Acceptance: _____

Date: _____

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Appendix A Revision History

FBCP 2016 R0 Page	FBCP 2015 R1 Page	Location	Revision made
NA	NA	throughout	Spelling, grammar corrections, and updates to numbering as needed
2	2	1	Changed Low male ratio from 10:1 to 4:1
17	17	2.1	Added "tested" to eighth bullet point; removed the technical reviewer from the last bullet point.
22	22	3.2	Removed second bullet point; removed exception in third bullet point
29	29	3.2.3	Added detail to step 3.
32	32	4.2	Changed "...the directions below will make 20 μ L..." to "...the directions below will make 30 μ L..."
33	33	4.3	Changed swirling master mix to vortexing.
34	34	4.6.2	Changed "do not" to "can" in last sentence
39-40	39-40	5.3 and 5.4	Split section 5.3 into 5.3 and 5.4; added section 5.3.1
48	48	7.6	Removed reference to choice of casework parameters.
52	52	8.1.1	Clarified use of sizing quality in verifying ILS
57	57	8.1.5	Changed 70-550 base pairs to 60-460 base pairs in fourth paragraph
58	58	8.2	In second paragraph, changed requirements for when artifact view printouts are required in DNA casework.
64	64	8.4	Added second paragraph; Added "indistinguishable" to second bullet point in fifth paragraph
66	66	8.4.2	Added clarification on when to call a profile a mixture
70	70	8.4.2.2.1.2	For separating a major component with two alleles, changed first bullet point from both major component alleles to at least one major component allele must be over IT
71	71	8.4.2.2.2.2	For separating a major component with two alleles, changed first bullet point from both major component alleles to at least one major component allele must be over IT
71	71	8.4.2.2.3	Updated rules for separating a two-source major component
72	72	8.5	Clarified contributor ratio below 1:2 (unknown:owner) cannot be used to deduce based on peak heights

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Forensic Biology Casework Procedures

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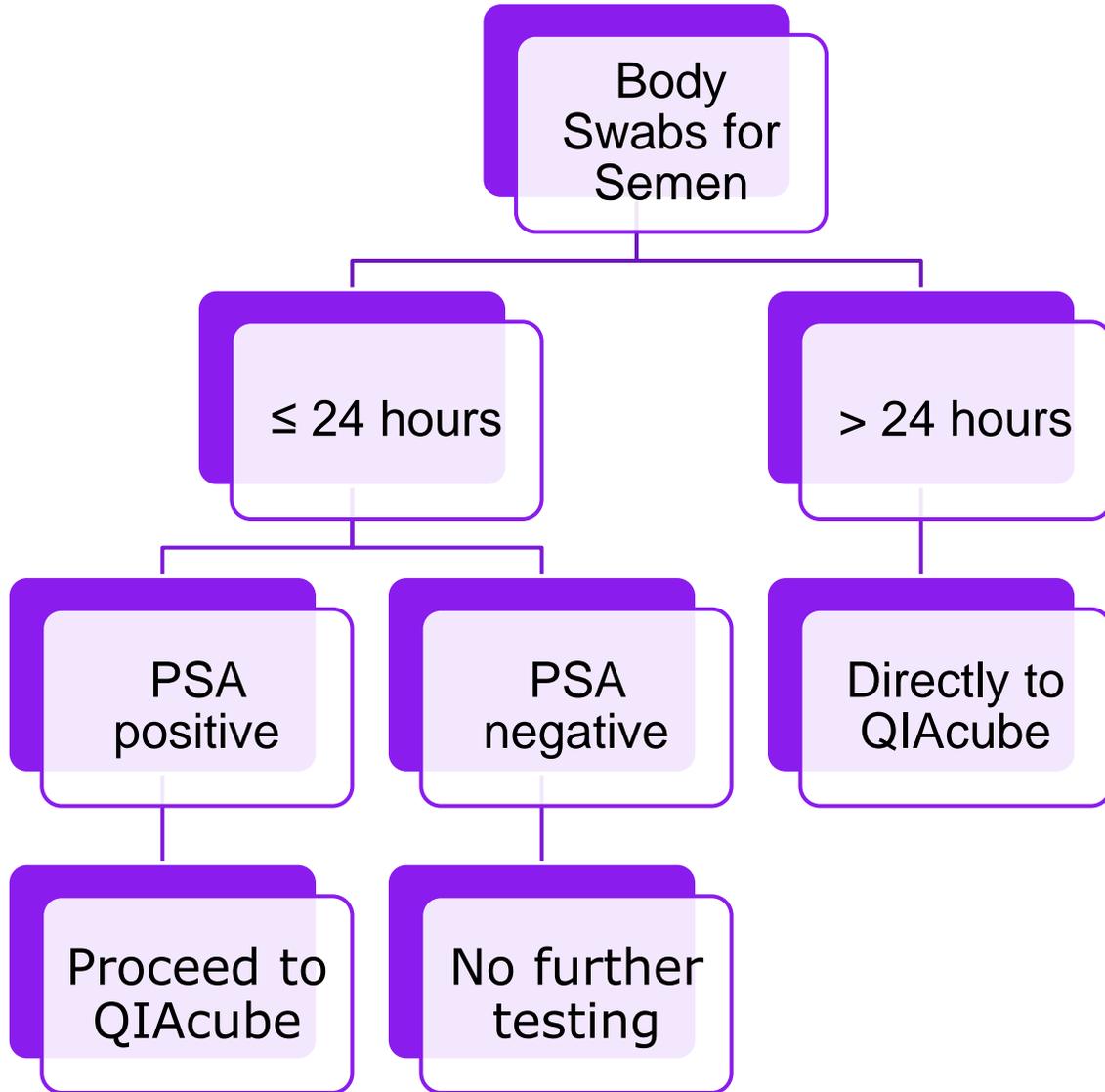
81	81	9.1	Changed first Note on page to reflect CODIS core loci instead of PowerPlex 16 loci
85-86	85-86	10.1.2	Changed No male DNA detected to include T-Y < 0.0007 ng/μL; changed T-Y is between 0.02 – 0.0007 ng/μL for recommending Y-STR analysis
89	89	10.2.1	Added “Consumed evidence is noted in the body of the report” to next to last bullet point. Added “Statement of which profiles have been entered (or removed) in CODIS, if appropriate”
91	91	10.2.2	Added sample CODIS language for DNA reports.
95	95	10.2.3	Added clarification regarding chain of custody on DNA Screening extracts
105	105	12	In Step 5, removed “evidence” from the last sentence, added “in the LIMS”.
106	106	Biological Screening Review Checklist	Changed “The date evidence was opened and/or sealed is documented” to “Start and end dates of examination/analysis are documented”
107	107	DNA Screening Review Checklist	Added “Start and end dates of examination/analysis are documented”; changed location to name for central log folder
109	109	DNA Casework Review Checklist	Added “Start and end dates of examination/analysis are documented”; added space for analysis start date.
110	110	STR Central Log Review Checklist	Removed “checked by Technical Reviewer from review of ICS; added ILS check
112	112	STR Single Offender Review Checklist	Added “Start and end dates of examination/analysis are documented”; added ILS check; changed location to name for central log folder; added blank for Date Analysis Started
118	119	Appendix D	Changed T-Y cutoffs as appropriate.
120	121	Appendix F	Changed 0.009 to 0.007 for likely mixtures in second bullet point
123-125	124-126	Appendixes I through K	Updated flowchart language for better clarity.

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Appendix B Biological screening of body swabs 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.

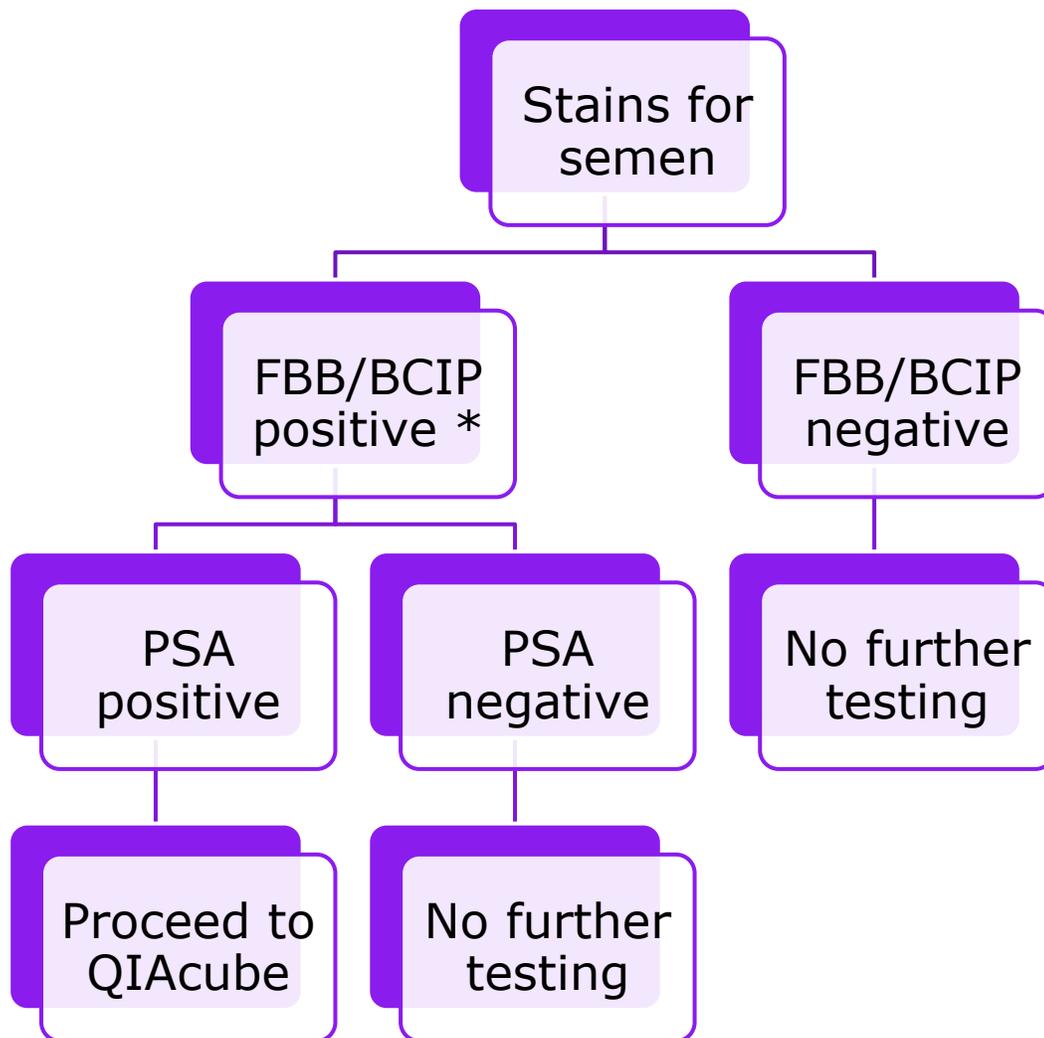


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Appendix C Biological screening of stains for semen incorporating the QIAcube decision tree

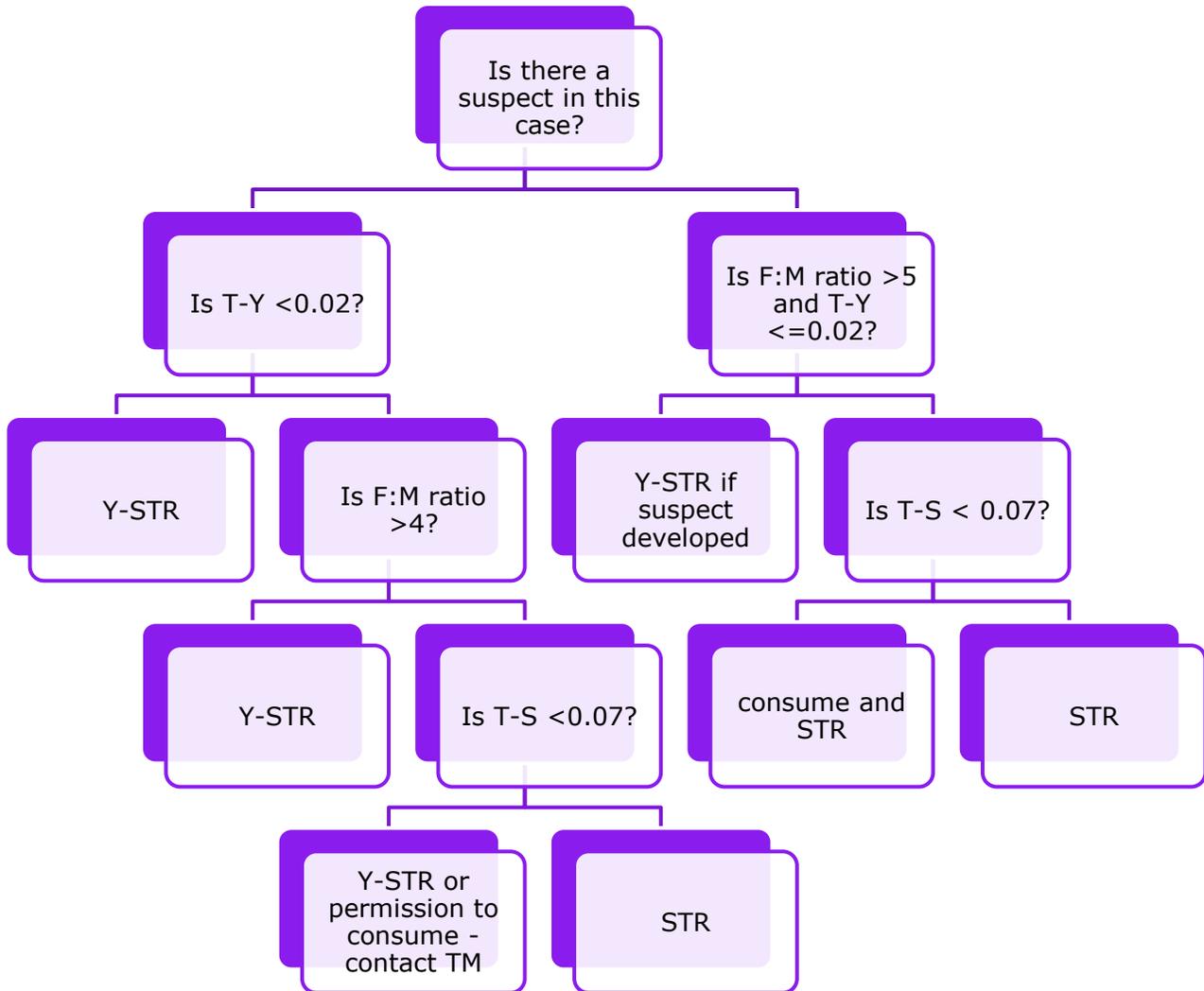
Note: This decision tree is only used when a single analyst takes a case through both biological screening and DNA analysis.



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

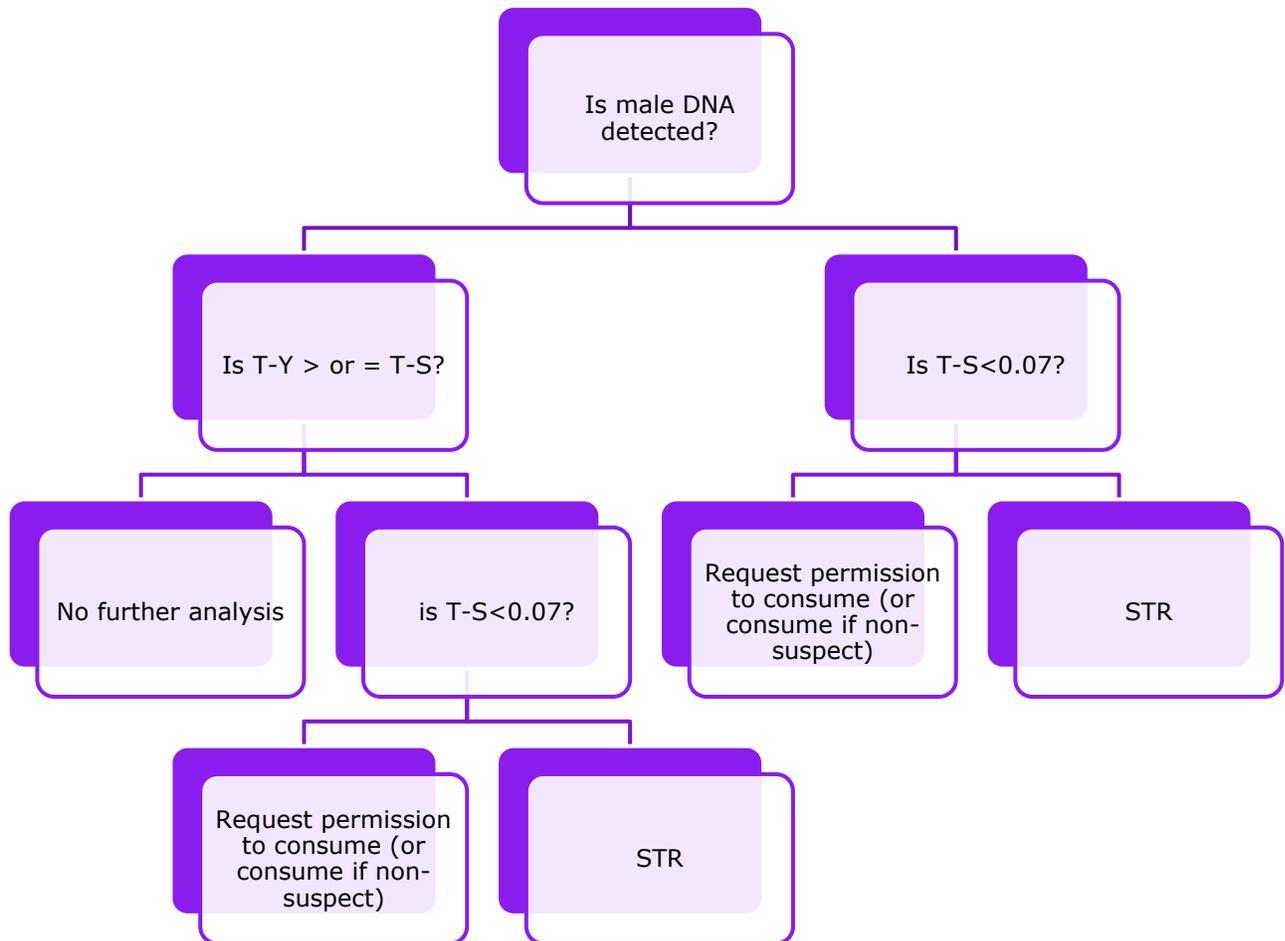
Appendix D: Consumed sample quant decision tree for extracts where male DNA is probative

- If evidence was not consumed and male DNA was detected, but T-S is < 0.07 ng/uL, analyst will need to extract more evidence before proceeding. Report out as DNA analysis is pending.
- If T-Y < 0.0007, no further analysis (consumed and not consumed evidence)
- If T-S is <0.002 for likely single-source or 0.007 for likely mixtures, no further analysis (consumed and not consumed evidence)
- Yes = Left; No = Right



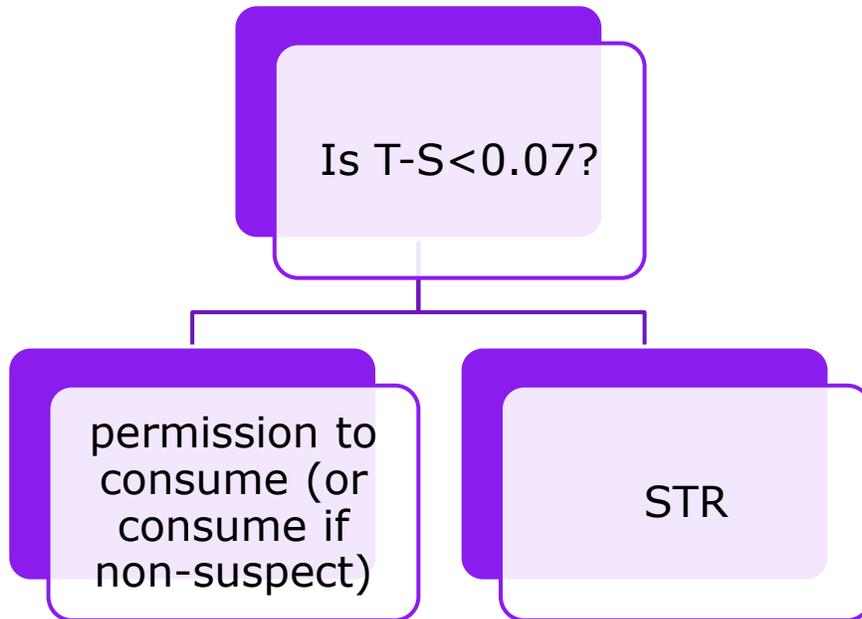
Appendix E: Consumed sample quant decision tree for extracts where female DNA is probative

- If evidence was not consumed, but T-S is < 0.07 ng/uL, analyst will need to extract more evidence before proceeding. Report out as DNA analysis is pending.
- If T-S is <0.002 for likely single-source or 0.007 for likely mixtures, no further analysis (consumed and not consumed evidence)
- Yes = Left; No = Right

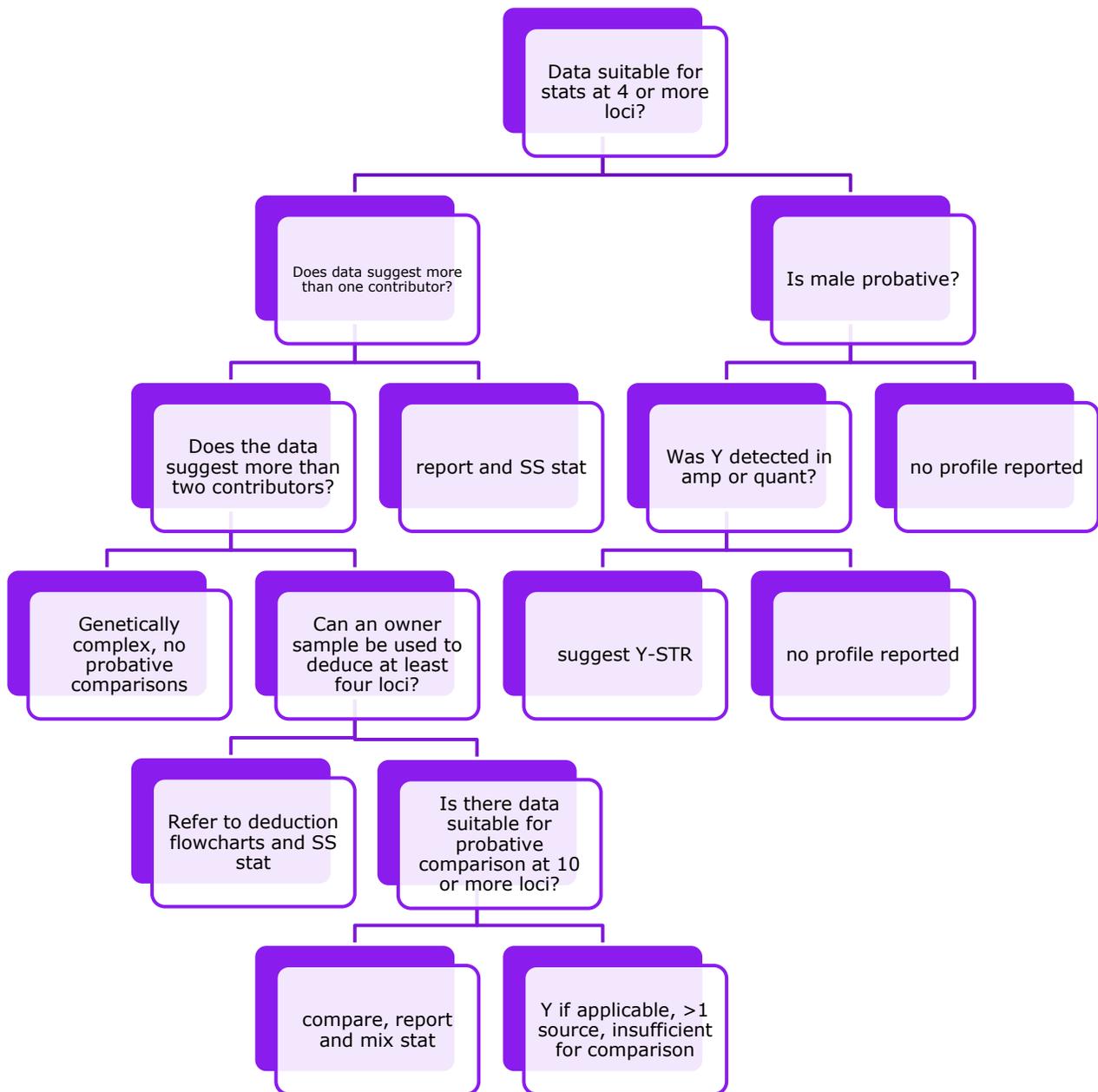


Appendix F: Consumed sample quant decision tree for extracts where any profile (male or female) would be probative

- If evidence was not consumed but T-S is < 0.07 ng/uL, analyst will need to extract more evidence before proceeding. Report out as DNA analysis is pending.
- If T-S is < 0.002 for likely single-source or 0.007 for likely mixtures, no further analysis (consumed and not consumed evidence)
- Yes = Left; No = Right



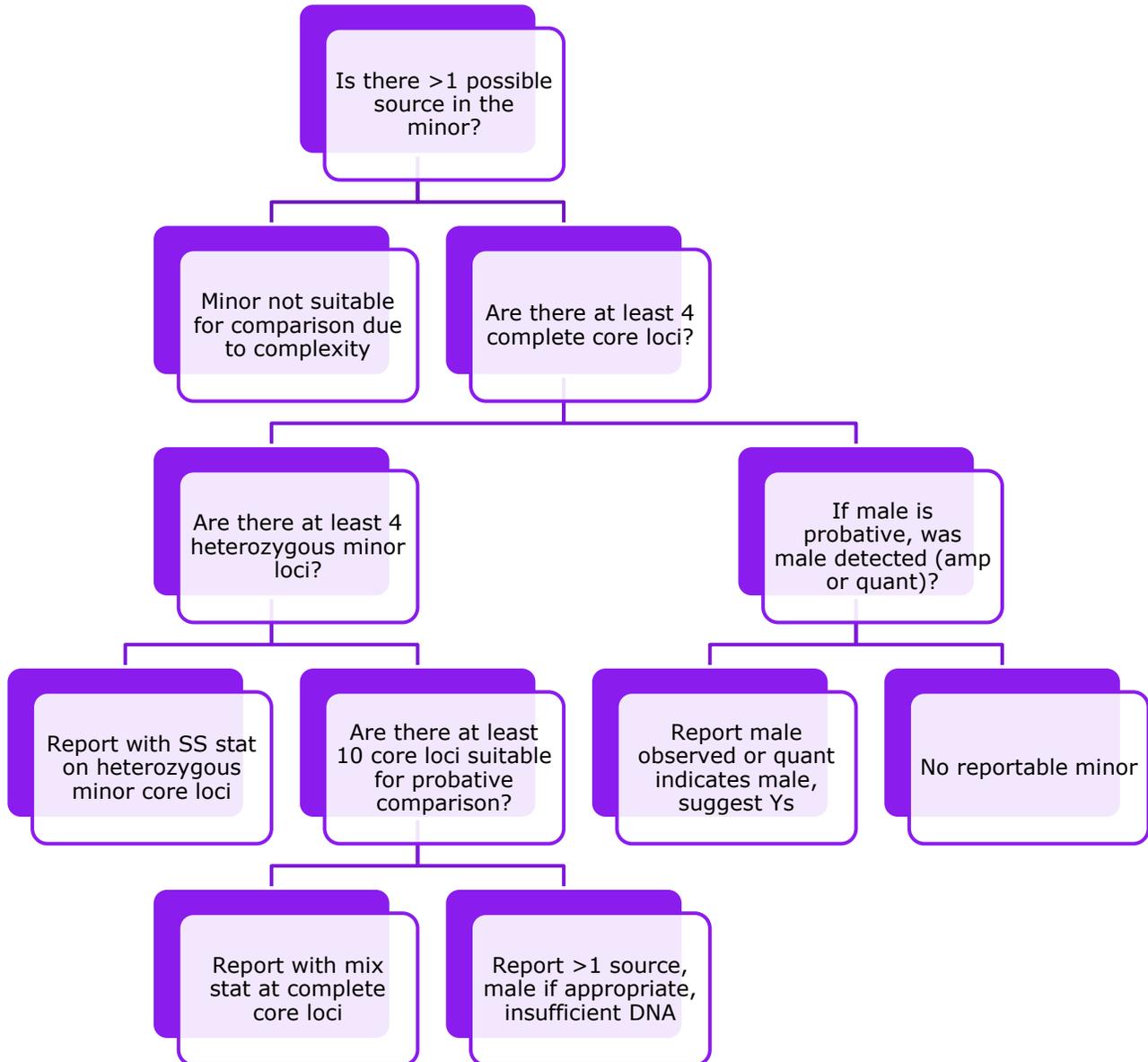
Appendix G: Interpretation of low-level data (data at any locus below ST), not the distinguishable minor component of a mixture. Does not apply to differential carryover or single-source owner on intimate sample
Yes = left; No = right



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**Appendix H: 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**

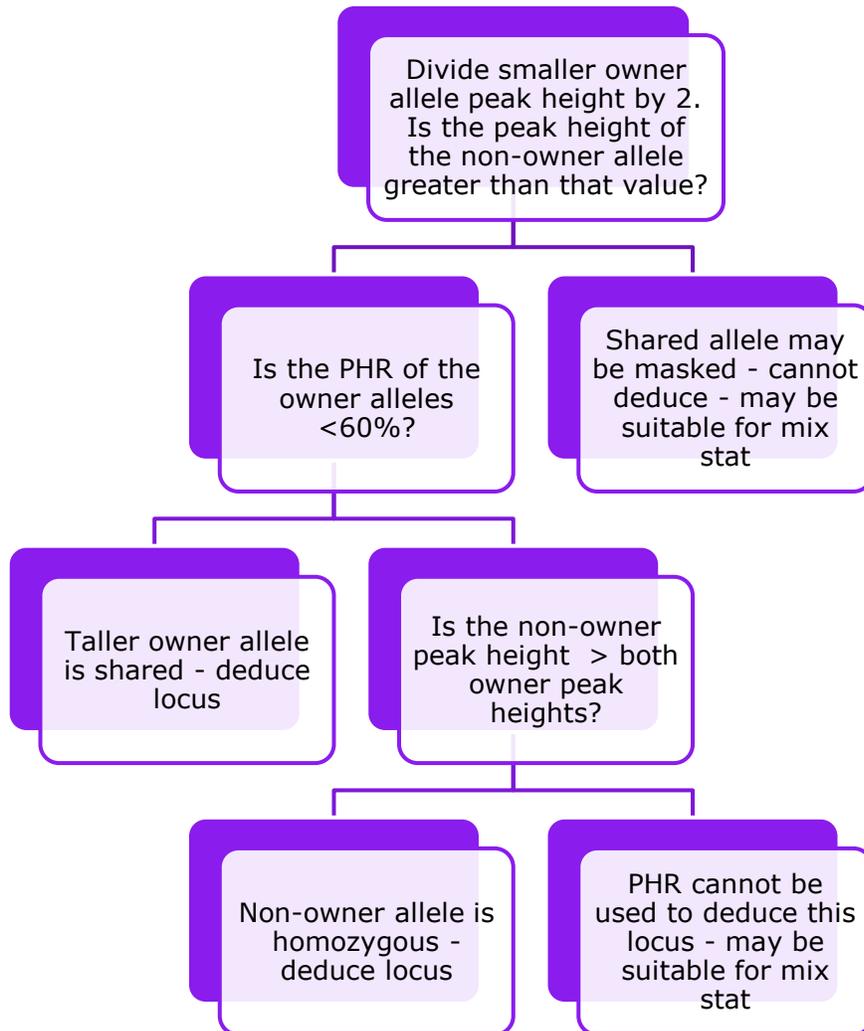


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Appendix I: Deduction at 3-Allele Locus with a heterozygous elimination sample

- 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
- Before beginning, if the non-owner allele is smaller and is in a stutter position to a larger (owner) peak, subtract out the max stutter contribution from the adjacent peak.
- Yes = Left; No = Right

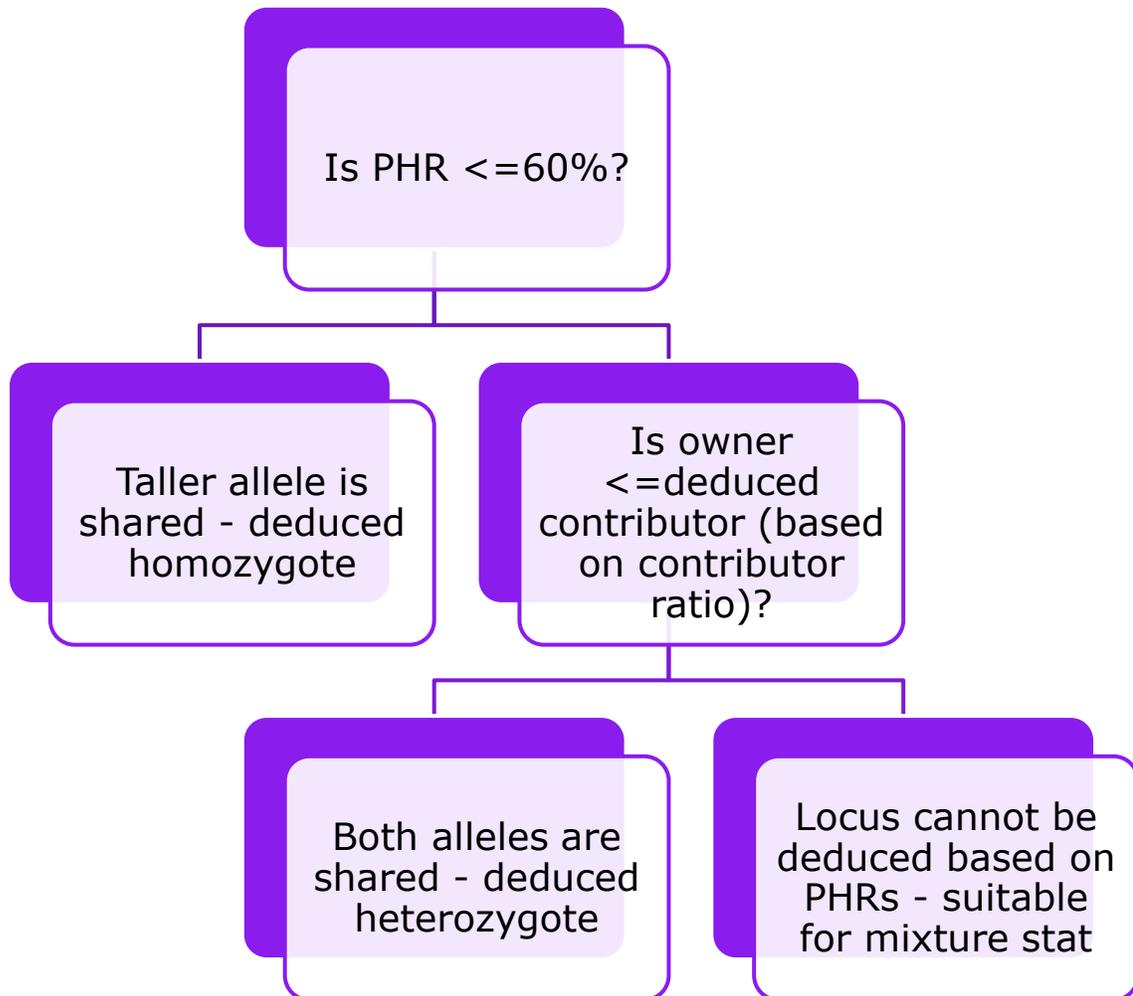


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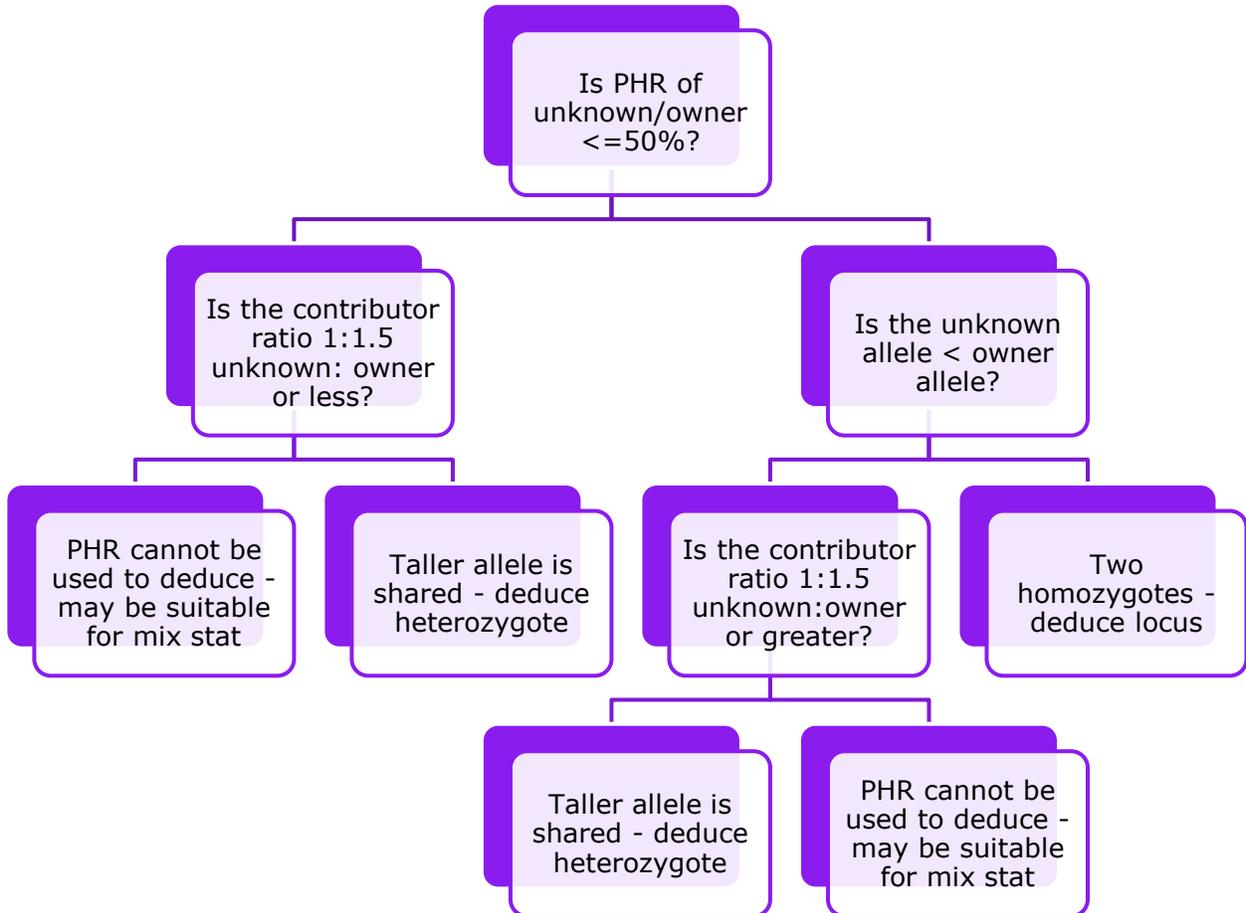
Appendix J: Deduction at 2-Allele Locus with a heterozygous elimination sample

- 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



Appendix K: 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



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Appendix L: 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
CSF1PO	95/304,307 (0.03)	982/643,118 (0.15)	410	1,487/947,425	0.16%
FGA	205/408,230 (0.05)	2,210/692,776 (0.32)	710	3,125/1,101,006	0.28%
TH01	31/327,172 (0.009)	41/452,382 (0.009)	28	100/779,554	0.01%
TPOX	18/400,061 (0.004)	54/457,420 (0.012)	28	100/857,481	0.01%
VWA	184/564,398 (0.03)	1,482/873,547 (0.17)	814	2,480/1,437,945	0.17%
D3S1358	60/405,452 (0.015)	713/558,836 (0.13)	379	1,152/964,288	0.12%
D5S818	111/451,736 (0.025)	763/655,603 (0.12)	385	1,259/1,107,339	0.11%
D7S820	59/440,562 (0.013)	745/644,743 (0.12)	285	1,089/1,085,305	0.10%
D8S1179	96/409,869 (0.02)	779/489,968 (0.16)	364	1,239/899,837	0.14%
D13S317	192/482,136 (0.04)	881/621,146 (0.14)	485	1,558/1,103,282	0.14%
D16S539	129/467,774 (0.03)	540/494,465 (0.11)	372	1,041/962,239	0.11%
D18S51	186/296,244 (0.06)	1,094/494,098 (0.22)	466	1,746/790,342	0.22%
D21S11	464/435,388 (0.11)	772/526,708 (0.15)	580	1,816/962,096	0.19%
Penta D	12/18,701 (0.06)	21/22,501 (0.09)	24	57/41,202	0.14%
Penta E	29/44,311 (0.065)	75/55,719 (0.135)	59	163/100,030	0.16%
D2S1338	15/72,830 (0.021)	157/152,310 (0.10)	90	262/225,140	0.12%
D19S433	38/70,001 (0.05)	78/103,489 (0.075)	71	187/173,490	0.11%
SE33 (ACTBP2)	0/330 (<0.30)	330/51,610 (0.64)	None reported	330/51,940	0.64%

*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)