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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, BPIP, FB
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
REQ	Reagent blank questioned (direct)
No Male	No male DNA detected
Low Male	Female:male ratio is 10:1 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 record the overall shape of the hair, whether or not a root is present and, where present, its shape and appearance, the basic features of the shaft, and medullary structure.

Hairs recovered in 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

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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 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
- hair shape or form

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, dyeing, 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.

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 autooxidation 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 primate) 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 one 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 Omnipoint™ 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 FFB 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 μ L works well for one full swab tip), 150 μ L 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 μ L 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 100 μ l 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 / Fingers and Hand Swabs*

Fingernail scrapings and fingers/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.
- 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.

Since reference samples are not extracted at this stage, it is not necessary to create a reagent blank for the reference samples.

Documentation:

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

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- 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 bench notes for each case file.

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Section 2 Biological Screening Report Writing and Review

Proficiency test reports will be written in a manner similar to casework reports.

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 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)

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

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P-30/ABA card positive	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. For more information please contact the laboratory's DNA Technical Manager, XXXXX XXXXX (269-XXXX) or (xxxxx.xxxxx@alaska.gov).
P-30/ABA card negative	No semen/PSA was detected by immunoassay on (item).

Speciation	Report
ABA card positive	Stain(s) present on (item), tested positive using an immunoassay test for the presence of human hemoglobin. This test is specific to human, higher primate and ferret blood.
ABA card negative	No human hemoglobin was detected by immunoassay.

Hair/Fiber Evidence	Report
Hairs/debris recovered/not recovered	(If a) (Animal) Hairs/debris were observed/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.

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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 6mm 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. nylon 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.

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

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.

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- 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.

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.
- Where incubations are required, they may be performed in either an incubator or in a thermomixer set at 900 rpm and appropriate temperature.
- Questioned and known samples shall be eluted in TE buffer.

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- 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 uL) 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.
 - 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.

3.2.1 Direct (non-differential) Extraction for Questioned samples

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)

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.

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

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.

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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.

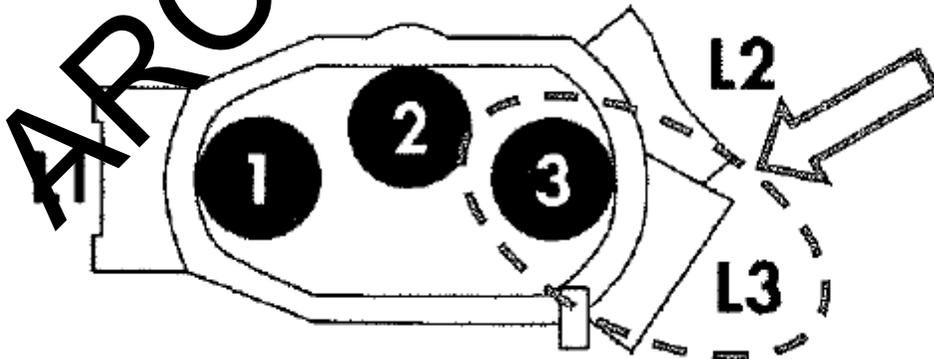
11. Aliquot Buffer G2 in 30 mL reagent bottle in Reagent Bottle Rack position 1. Do not fill above the fill line. Minimum volumes of G2 buffer are listed in the table below:

<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).



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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.

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 μ L 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 NSA 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

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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 poly-p-like structures, which are occasionally observed with yeast cells.

The presence or absence of sperm is documented in the bench notes. If one or no spermatozoa are observed, the result should be confirmed by a second qualified analyst. Verification is documented in bench notes.

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 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 fractions may be run by Large Volume protocol, but this must be documented in the bench notes and central log.

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EZ1 Extraction:

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

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.
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

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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.

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 ("Main") 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 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 samples.

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

References: ABI Prism 7000 Sequence Detection and Applied Biosystems 7500 Real Time PCR System User Bulletin and Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance 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 Duo 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. 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 “Q” may be added at 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 (~2 hours to complete a run).
 - Comments section may be used to add instrument number or other relevant information.
 - Under Plate Set Up, the default settings include Standards and NTC.
 - Under the Define Samples tab, add samples. These can be left as Sample 1, Sample 2, and so on, with Q or K codes manually written on printouts later; or they can be entered with Q or K codes.
 - Under the Assign Targets and Samples tab, highlight a box (or boxes) on the map, then check the box from the list on the left (Heading: Assign samples to the selected wells) for the corresponding reaction well(s). Data collected from wells that are assigned to the same sample will be averaged by the software.
 - Standard Curve wells are already correctly assigned. The template default includes one NTC reaction. Be sure that the final plate includes two NTC reactions.

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- The Quantifiler Duo report prints out results ordered by rows (i.e. A1, A2, A3, A4...). These reports are easier to read if replicates samples are placed in side-by-side wells.
- Selected samples are automatically assigned three targets: Duo Human, Duo IPC, and Duo Male.
- 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.

- Bring the reagents to room temperature before pipetting.
- Label eight sterile microcentrifuge tubes for standards 1 through 8.
- Dispense 30 uL of Quantifiler Duo DNA Dilution Buffer into Std 1 and 20 uL into each of the remaining tubes
- Prepare Standard 1:
 - Vortex the Quantifiler Duo DNA Standard for 3 – 5 seconds.
 - Using a new pipette tip, add 10 uL of Quantifiler Duo DNA Standard to the tube for standard 1.
 - Mix the dilution thoroughly.
- Prepare Standards 2 through 8:
 - Using a new pipette tip, add 10 uL 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 quantities of DNA in prepared Standards 1 through 8 are as follows:

Standard 1 \approx 50ng/ μ L
Standard 2 \approx 16.7ng/ μ L
Standard 3 \approx 5.56ng/ μ L
Standard 4 \approx 1.85ng/ μ L
Standard 5 \approx 0.62ng/ μ L
Standard 6 \approx 0.21ng/ μ L
Standard 7 \approx 0.068ng/ μ L
Standard 8 \approx 0.023ng/ μ L

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

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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™ Duo Primer Mix at 10.5µL per reaction.
 - Quantifiler™ Duo PCR Reaction Mix at 12.5µL per reaction.
- Vortex the Quantifiler Duo Primer Mix for 3-5 seconds and centrifuge briefly before opening the tube.
- Swirl the Quantifiler Duo PCR Reaction Mix gently before using. Do not vortex it.
- Pipette the required volumes of the components into a sterile tube. Vortex and centrifuge briefly.
- Dispense 23µL of reaction mix into each reaction well.
- Add 2µL of sample, standard or control to the applicable wells. 2µL 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 button 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

Both the human 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.

R^2 is a measure of the closeness of fit between the standard curve regression line and the individual data points of the quantification standard reactions. A value of 1.0 indicates a perfect fit between the regression line and the data points. Passing value for R^2 is ≥ 0.98 . It is

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acceptable to remove one or both of the Standard 8 data points in order to achieve a passing R^2 value. If, after removing up to two data points, the R^2 value is <0.98 , the plate must be re-run.

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

Y-intercept is the theoretical expected quantity for 1 ng/uL of DNA. Y-intercept is used to compare performance among different QD 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/uL will now quantify at 1 ng/uL. 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/uL will now quantify at 2 ng/uL. 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 accepted ranges for Y-intercept values are 28.8 – 29.2 for the human standard curve and 29.8 – 30.2 for the male standard curve. During QD kit verification, if either Y-intercept value falls outside the accepted range, the plate must be re-run. If the Y-intercept value(s) is reproducible, note the average Y-intercept values (human and male) both on the verification paperwork and the reagent storage container. Subsequent Y-intercept values for that QD kit lot number must fall within ± 0.5 of the verified Y-intercept values. Non-reproducible Y-intercept values, or values falling outside the accepted range, should be brought to the DNA Technical Manager 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 28 to 31. Values lower than 28 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 31, 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 do not 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 Duo Human and Duo Male is negative (C_T = undetermined). A detected quantity of DNA could indicate contamination of the master mix. If the quantity of either Duo Human or Duo Male is greater than zero, consult the DNA Technical Manager for a course of action.

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4.6.5 Checking the Initial Template Quantity

Quantities of DNA, including both total human and male DNA, 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 4.1.1. When STR analysis will not be performed based on the quantification results (as described below), this shall be reflected on the DNA Case Extraction Worksheet.

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 total human 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 <0.050 ng/uL, 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 10:1 female:male are likely to appear as single-source samples upon amplification, since any minor component present is very unlikely to be suitable for comparison.
- 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.

- Sample decision trees for this process (for consumed samples) are shown in Appendices D - H. 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 quant result of undetermined for both human and male DNA will not routinely proceed to amplification.
 - Extracts with no male DNA detected will be retained with no further testing. (Exceptions would include condom samples where a female profile may have probative value.)
 - Extracts with any detected male quantitation value but undetermined total human DNA are best suited for STR analysis only if the entire remaining evidence / extract can be consumed.

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- Extracts likely to contain single-source male DNA, where both human and male DNA are detected by quantitation, are suitable for STR analysis.
- Extracts with male DNA present at ratios greater than 10:1 female:male are not suitable for 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 with documented approval by the discipline supervisor or the DNA Technical Manager.
- Extracts with male DNA present in a ratio of 10:1 female:male or less, but with a total human DNA concentration less than 0.05 ng/uL, 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 10:1 female:male or less
 - total human DNA concentration equal to or greater than 0.10 ng/uL
 - male DNA concentration is equal to or greater than 0.05 ng/uL
- Not-consumed samples with likely mixtures: Extracts likely to contain mixtures are ready 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 only if the remaining sample is extracted and all the combined extract from the sample is consumed.
 - male DNA is present in a ratio of 10:1 female:male or less
 - total human DNA concentration equal to or greater than 0.05 ng/uL
 - male DNA concentration is equal to or greater than 0.005 ng/uL
- 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 Rationale for recommending consumption of extract:

In instances where quantitation results indicate that limited amounts of DNA (<0.10 ng/uL) are present, the best course of action depends in part on whether one or more sources of DNA are likely to be present in the extract. While single-source extracts may yield results suitable for comparison at very low template quantities, the same is not true for mixtures. The combined information from both human and male DNA quantitation results may indicate whether it is reasonable to expect the extract to contain a mixture. Furthermore, some sample types (such as gun swabs and contact swabs) are likely to contain DNA from more than one individual by their nature.

Interpretation guidelines in this manual recommend that low-level mixtures be amplified twice to assess reproducibility of peak heights and peak height ratios. Additionally, for mixtures, it is sometimes useful to target DNA at 1.0 ng rather than 0.5, in order to improve the chance of detecting a full profile. This is particularly true when there is an even or nearly even ratio of contributors. A total of 4 ng or more of DNA enables an analyst to amplify the sample twice, plus retain at least half of the extract. When less than 4 ng of total DNA is present in a sample (i.e. when a 40 µL extract has a total human DNA concentration of less than 0.1 ng/uL), it is not possible to optimize analysis while retaining half the extract.

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Section 5 DNA Amplification

References:

- PowerPlex® 16 System Technical Manual
- 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: pp16_320.c**

95°C for 11 minutes, then:

96°C for 1 minute, then:

ramp 100% to 94°C for 30 seconds

ramp 29% to 60°C for 30 seconds

ramp 23% to 70°C for 45 seconds

for 10 cycles, then:

ramp 100% to 90°C for 30 seconds

ramp 29% to 60°C for 30 seconds

ramp 23% to 70°C for 45 seconds

for 22 cycles, then:

60°C for 30 minutes

4°C hold

5.2 Amplification Set-up of Forensic Casework Samples

- If samples 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.

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

- Ensure that all kit components have thawed completely before use. Vortex reagents and centrifuge briefly to ensure uniform mixing and collection of tube contents.

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

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- Prepare a PCR master mix by adding the following volumes of reagents to an appropriately sized tube:
 - # of samples x 2.5µL Gold STR 10X Buffer
 - # of samples x 2.5µL PowerPlex® 16 10X Primer Pair Mix
 - # of samples x 0.8µL AmpliTaq Gold™ DNA Polymerase (5U/µL)

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

- Vortex the master mix and spin briefly. Transfer 5.8µL of master mix to each sample well of a 96-well amplification plate. Cover the entire well plate with Glad® Press 'n Seal (or equivalent).
- Prepare the samples to be amplified in individual 0.5ml tubes

Selection of amplification target values

- For any sample in a case with a named suspect where the original material has been consumed, at least half the extract must be retained. For example, for 40 µL of extract from a consumed sample, 2 µL are used for quantitation and at least 20 µL are to be retained, leaving up to 18 µL 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 Quantiler Duo results, and the rationale will be documented on the DNA worksheet.
- Forensic casework amplifications should be targeted between 0.5 ng – 1 ng, if sufficient extract is available. The amount of template DNA may be higher or lower depending on the sample (i.e. low-level samples that are likely to be mixtures may be targeted higher, while bloodstains likely to be single-source may be targeted lower). Add sterile de-ionized water to bring the sample to a final volume of 19.2µL.
- Samples that can be amplified at 0.5 ng-1.0 ng do not necessarily need to be amplified in duplicate. However, if subsequent analysis indicates that peak heights of a probative contributor fall below the stochastic threshold, replicate amplification will ensure that reproducibility and peak height ratios can be assessed more accurately.
- No sample extract may be amplified at a greater volume than its corresponding reagent blank. Reagent blanks amplification volumes should be chosen based on the largest volume used in the corresponding casework extracts.
- Low-level samples that are likely to be mixtures, for which written permission has been obtained to consume the sample, (concentration < 0.1 ng/µL) should be amplified at the maximum volumes possible in order to make best use of the extract.
- Single-source samples are more likely to yield results suitable for comparison at very low template quantities. Some rules of thumb for likely single source samples are:
 - A male quantitation result that is higher than the human quantitation result, where the case scenario does not indicate more than one likely male source of DNA.
 - A sample with no male DNA detected, where the case scenario does not indicate more than one likely source of female DNA.

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In the case of a single-source sample, better results may be obtained by performing a single amplification that is closer to the optimal target value of 0.5 ng, than by performing duplicate reactions at a lower template quantity.

- 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 19.2 uL sterile water and amplified.

Positive Amplification Control

Vortex and spin the tube briefly and add diluted positive control DNA to a 0.5 mL tube. Typically, 1-2uL will yield a full profile with minimal artifacts. Bring the volume up to 19.2uL with sterile, de-ionized water.

Negative Amplification Control

Add 19.2uL of sterile de-ionized water.

- Transfer the entire 19.2uL of the above prepared samples to the appropriate sample well, already containing the PCR master mix. During sample addition, the pipette tip is inserted through the Press 'n Seal.
- Once all samples have been added, remove the Press 'n Seal and cover the plate with a sheet of amplification tape.
- Transfer the plate to the PCR room and place directly into the thermal cycler. 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 and cathode buffer 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 expiration date for the 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 0.5µl ILS 600
 - # of samples x 9.5µl 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:
 - PPIex16_15sec
- 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 3500m 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

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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 **My Computer > data on 3500- > Applied Biosystems > 3500 > Data** on the laboratory "I Drive" 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 (3500_PowerPlex_16_Casework), and Size Standard (ILS_80-550_Adv) 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 R, 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 the 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. A range of Analysis Methods are available to analyze casework data. Options for Analysis Methods vary by analysis range. These methods shall not be modified. New methods shall only be created or modified with permission from the DNA Technical Manager.

3500CW-2400
3500CW-2800
3500CW-3200
3500 Blank - Casework

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7.4 Casework Allele Tab Settings

These settings are consistent within all casework methods.



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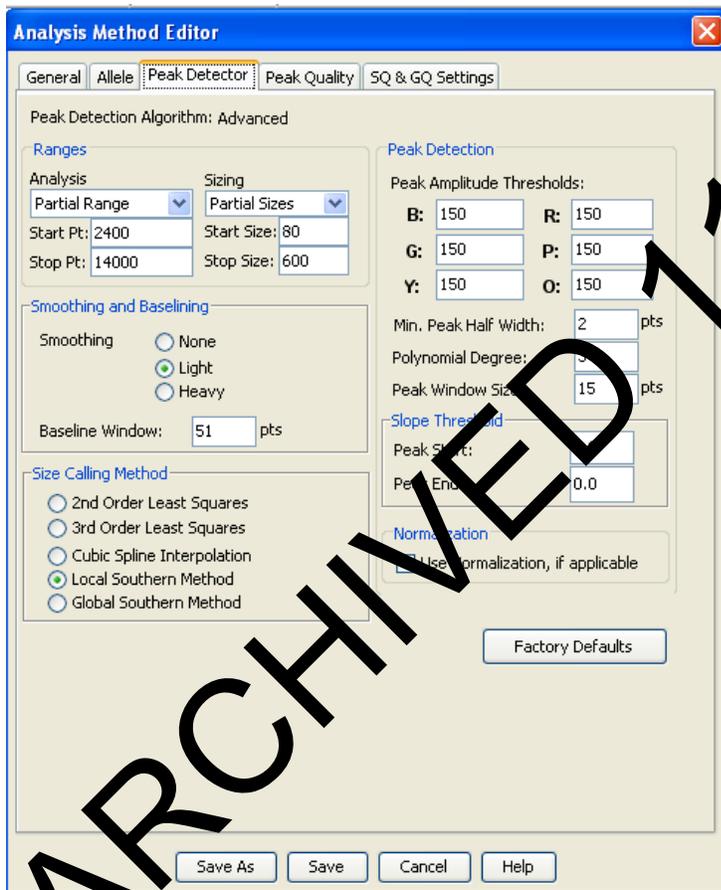
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7.5 Casework Peak Detector Tab Settings

The Analysis Range is the only setting that varies between the alternative Analysis Methods. The Analysis Range is selected to ensure capture of the 80bp ILS peak for all allelic ladders, positive controls, and questioned and known samples in the project. The Analysis Range is selected to capture the primer peaks for reagent blanks, negative amplification controls, and samples that do not yield at least a partial profile.

The Analytical Threshold for all analyses is 150RFU.

Example: 2400 Casework Analysis Method



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7.6 Casework Peak Quality Tab Settings

These settings do not vary among the casework methods and they are not relevant in analysis of any samples where the sample type is set to Negative Control.

Example: 2400 Casework Analysis Method



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7.7 Casework SQ and GQ Tab Settings

These settings do not vary among any of the analysis methods, and they 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)	0.7	Allele Number (AN)	1.0
Out of Bin Allele (BIN)	0.7	Low Peak Height (LPH)	0.3
Overlap (OVL)	0.7	Max Peak Height (MPH)	0.3
Marker Spike (SPK)	0.3	Off-scale (OS)	0.3
		Peak Height Ratio (PHR)	0.3

Control Concordance (CC) Weight = 1.0 (Only applicable to control)

SQ Weighting

Broad Peak (BD)	0.5
-----------------	-----

Allelic Ladder GQ Weighting

Spike (SSPK/SPK)	1	Off-scale (OS)	1
------------------	---	----------------	---

SQ & GQ Ranges

Pass range: Low Quality Range:

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

Reset Defaults

Save As | Save | Cancel | Help

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).

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- Electropherograms can be printed from the sample's plot window. Various plots have been created for different sample types. Commonly used plot displays include: Traditional Genotype Plot, Casework Blank, and Casework artifacts 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
D5Mut	D5 Mutation
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
A/TA	Artifact (i.e. stutter) or true allele
IF	Instrument failure
ILS	ILS failure
DB/T	Data below reporting threshold
ART	Artifact
TRI	Tri-allele
OBL	Obligate allele
UND	Undetermined

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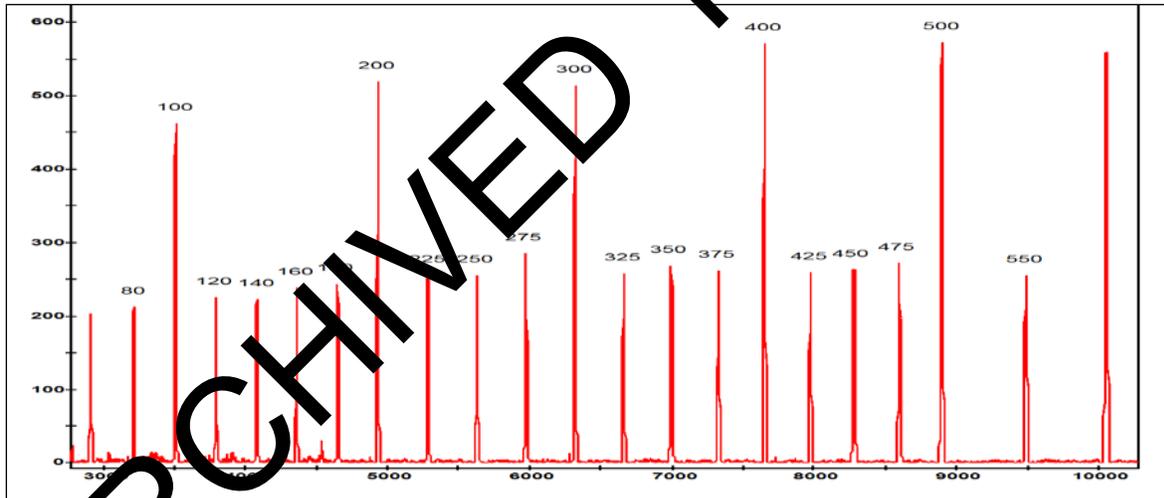
DNA Abbreviations and Definitions (continued)	
()	Minor component allele
[]	Major/Minor components not separated

8.1 Interpretation of Batch Controls

Prior to assessment of casework data, the analyst must first examine the batch control 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.

8.1.1 Internal Lane Standard (ILS)

The Promega PowerPlex16® System uses ILS 600. The analyst should verify that all peaks from 80-550 base pairs are present and labeled as shown.



The peak heights for the ILS peaks must be at least 150RFU (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.

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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 Promega PowerPlex16® System allelic ladder are shown below. 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 13 FBI STR core loci, the Penta D and Penta E loci, and Amelogenin. In addition, alleles not labeled in the allelic ladder (virtual alleles), may be detected and labeled 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 call 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.

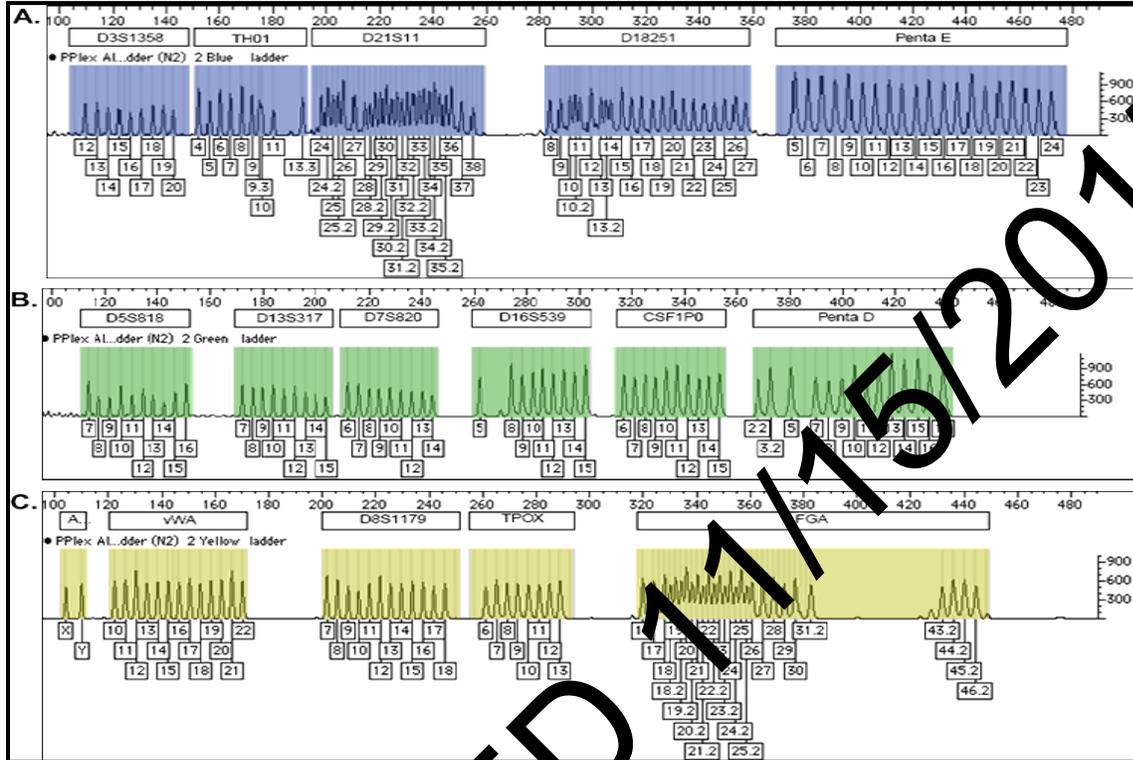
Additional information about the allelic ladder can be found in the Promega PowerPlex16® System Technical Manual.

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PowerPlex16® Allelic Ladder



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

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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.

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. Either standard DNA template 9947A or 2800M, included with the Promega PowerPlex16[®] System, may be used as a positive amplification control. A minimum of one positive amplification control must be amplified and processed concurrently with each casework amplification.

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.

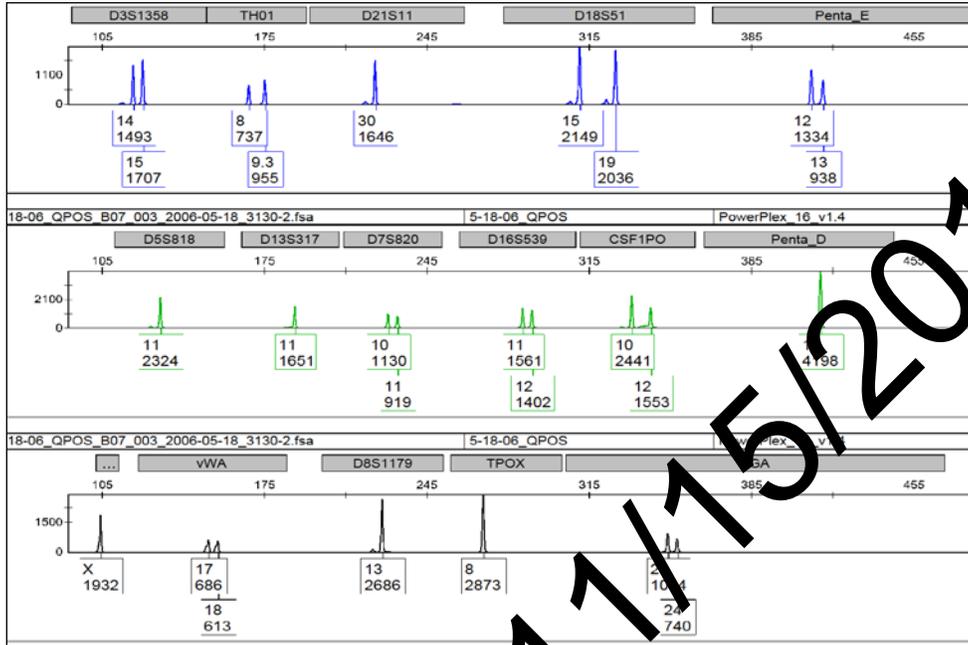
Alternatively, other samples in an amplification may serve as a positive control (e.g. duplicate offender/staff sample, offender confirmation sample, casework known sample with a verified profile, or a buccal swab freshly obtained from laboratory personnel whose genetic profile(s) was/were previously 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, 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 in either amplification/run may be used.

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STR Profile of 2800M



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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 water 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 80-550 base pairs, the analyst will determine if the peaks are artifacts (e.g. spike, pull-up) or true allele peaks.

The presence of peaks above the reporting threshold 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 documented on the electropherogram.

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 investigate possible causes and, if possible, will determine the source of the contaminating DNA. 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.

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

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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 STR table and 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.

For questioned samples amplified once, 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. Artifacts in questioned samples which do not bin as alleles and are called OL, OMR or spike, may be struck without a documented explanation. When questioned samples are amplified in duplicate, non-reproducibility of peaks (whether or not they bin) is sufficient justification for striking; no further written explanation is required. All artifacts in reference samples may be struck without documented explanation.

8.2.1 Baseline noise

The analytical threshold (AT) for analysis of casework samples is 150 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 150 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), 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. In the penta-nucleotide loci, the stutter peak is typically five bases shorter than the corresponding base peak.

The proportion of stutter product relative to the main allele peak is calculated by dividing the height of the stutter peak by the height of the main allele peak. The stutter percentage is fairly reproducible for a particular locus. The following table lists the values of the maximum expected percentage of N-4 (or N-5) stutter for the loci in the PowerPlex® 16 System when run on the Applied Biosystems 3500xl. The stutter values are based on data obtained by internal validation

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studies. These values, along with a universal N+4 stutter of 2%, are set as stutter filters used by the GeneMapper ID-X software.

Maximum Expected N-4 (N-5) Stutter Percentages for PowerPlex® 16 loci on the 3500xl (in single source samples)

D3S1358 14%	TH01 5%	D21S11 13%	D18S51 14%	Penta E 7%	
D5S818 13%	D13S317 10%	D7S820 11%	D16S539 11%	CSF1PO 11%	Penta D 8%
Amelogenin NA	vWA 14%	D8S1179 11%	TPOX 6%	FGA 11%	

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 % versus the expected, 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 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 150 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 $800 \times 0.13 = 104$. Thus, the maximum +4 / -4 expected stutter would be 284. Since the peak height of the 29 is 150 RFU, elevated stutter could be

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documented as follows: Max +4 = 180 Max -4 = 104 Total = 284 150 < 284

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 four different fluorescent dye colors into distinct spectral components. The four dyes (Fluorescein, JOE, TMR, and CFXR) 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 from another dye. This typically occurs at high RFU values, when an excess of template DNA has been amplified.

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.

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.

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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 bin 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 a double amplification of a single-source sample, or in multiple single source samples each amplified once and injected simultaneously, further amplifications are not necessary to verify the OL allele. The DNA Technical Manager must approve reporting an OL allele without re-amplification (e.g. limited amount of sample available for analysis).

Documentation of the OL allele must include an electropherogram with both the sample and the associated allelic ladder for the locus, showing the OL allele(s) in the locus, with the bins and base pair sizes. The OL allele designation is to be handwritten 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 (ATA).

If the same tri-allelic pattern is also observed in multiple samples (from the same case) but from different tissue sources – for example, blood / semen / saliva / hair, with the same STR profile,

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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. The same principle applies to control samples where no DNA should be detected.

8.3 Assessing interpretable alleles

Alleles are peaks that are detected and labeled by the Gen Mapper™ 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 alleles are examined and evaluated in the process of data interpretation.

In addition to the Analytical Threshold (AT) of 100 RFU, a Stochastic Threshold (ST) of 700 RFU is also used for casework analysis. When replicate amplifications are performed, only reproducible alleles above the AT will be relied upon for casework interpretation. All allelic peaks greater than or equal to the ST are reportable when only a single amplification is performed.

8.3.1 Alleles below the Stochastic Threshold

Refer to Appendix 1 for a flowchart of interpretation of low-level data.

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 700 RFU is based on validation studies and represents the RFU value below which stochastic effects are commonly observed. Alleles which fall between the AT and the ST are subjected to a higher degree of scrutiny due to the possibility of stochastic effects. Alleles in this range are noted with an asterisk on the electropherogram. If an electropherogram includes one or more loci where all alleles fall below the AT and may have allelic dropout, the electropherogram and STR Table (if applicable) should be noted as DBRT to account for the possibility of drop-out. DBRT is applicable on the STR table when there is possible dropout specific to the data on the table. If, for example, a minor component may have drop-out but is not included on the table, DBRT is not noted on the STR table.

Reported refers to alleles whose peak heights are equal to or above the stochastic threshold and are generally reproducible when re-amplified. Such alleles are routinely used in making

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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 stochastic threshold and/or alleles whose peak heights are greater than the analytical threshold, but that are not reproducible in replicate amplifications of the same DNA extract. Such alleles are not routinely included in the STR results table. Observed alleles may be considered for use when reporting the presence of multiple sources of DNA in a sample and/or when reporting the presence of mixed DNA. On occasion, depending on the quality of the data and the nature of the sample, such alleles may be used to generate a composite profile, with the documented approval of the DNA Technical Manager.

8.3.2 Reproducing Alleles Below ST for Interpretation

In most instances, allelic peaks below the ST must be demonstrated to be reproducible by re-amplification in order to be included on the STR table and deemed suitable for use in comparisons and statistical calculations. Non-reproducible alleles are struck and initialed by the analyst (or electronic equivalent). These alleles are not included on the STR table. When non-reproducible alleles (not attributable as artifacts) are present in duplicate amplifications, the samples will be noted as having Data Below Reporting Threshold (DBRT) on the electropherogram and the STR Table if applicable.

Questioned samples likely to be from more than one individual with a quantification value of $<0.05\text{ng}/\mu\text{l}$ and greater than undetermined, shall be routinely amplified in duplicate to ascertain reproducibility of alleles below the ST. Exceptions require advance approval by the Technical Manager.

Samples with human and male quantitation results of undetermined are not routinely amplified.

Questioned samples with a quantification value $>0.05\text{ng}/\mu\text{l}$ need not be amplified in duplicate. When a sample that was not initially amplified in duplicate yields a DNA mixture profile with the probative fraction being the minor or low-level component and/or contributor, re-amplification may be appropriate to assess allele reproducibility of the minor component and/or contributor.

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

8.3.3 Using Alleles Below ST Without Demonstrating Reproducibility

Ideally, any STR alleles below the ST should be demonstrated to be reproducible in order to be reported in the STR table and used for statistic calculations. However, in a few specific instances (ex. Amelogenin peaks), alleles below the ST may be included on the STR table and/or used in statistical calculations without demonstrating reproducibility.

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.

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 Assessing for Genotypes – Interpretation and Statistics

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.

Assessment of a questioned profile includes the determination of which loci are suitable for comparison and/or for the calculation of population statistics. Loci considered to be 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 either be suitable for exclusions only or not suitable for comparison, depending on the number of sources and the extent of the possible drop-out. A locus with no reproducible 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 with both alleles reproducible).

A locus where drop-out is a possibility is noted with NS on the electropherogram (and STR table, if the profile is charted), to indicate that the locus is not suitable for statistics. Examples of loci not suitable for statistics due to possible drop-out include:

- Non-reproducible alleles which occur outside of stutter positions
- In a single-source profile, a locus with only one allele, and the allele is below ST in all amps performed
- In a two-source mixture profile, a locus where there are fewer than four alleles and at least one of the alleles is below ST in all amps performed.
- In a two-source mixture profile with a minor component, a locus where a minor allele could be masked by the stutter filter adjacent to large major component peak

The NS at a given locus may apply to the locus as a whole, or only to the minor component. If the NS applies only to the minor component or mixture, this should also be noted on the electropherogram (and STR table, if charted).

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

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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 this dividend 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 0.5 to 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.

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 profile with complete information at three or fewer loci may be suitable for exclusions only.

A single source profile, partial profile, or minor component from any intimate sample which is solely attributable to an “owner” is not routinely included on the STR table.

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 reporting threshold

In general, a profile with more than two alleles at two 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 alleles/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.

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

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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.

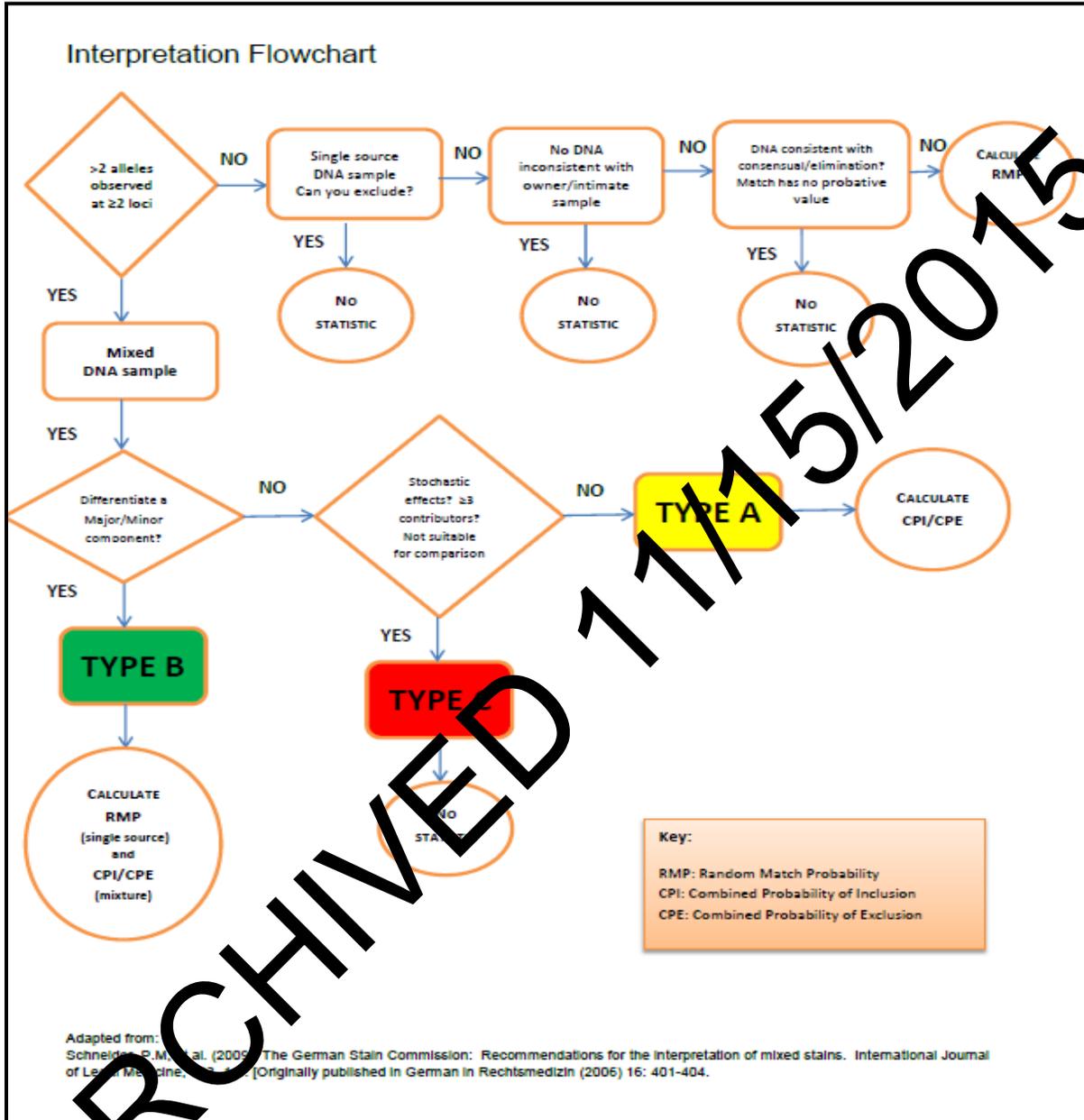
The classification of indistinguishable does not imply that the profile is not interpretable. Individuals may still be included or excluded as possible contributors to an indistinguishable mixture. However, a minimum of 4 complete loci (i.e. loci suitable for calculating population statistics) are required in order for an unresolved mixture to be suitable for comparisons.

Suitable for exclusions only: This type of mixture may be suitable for exclusions only when a two source mixture has only three complete loci.

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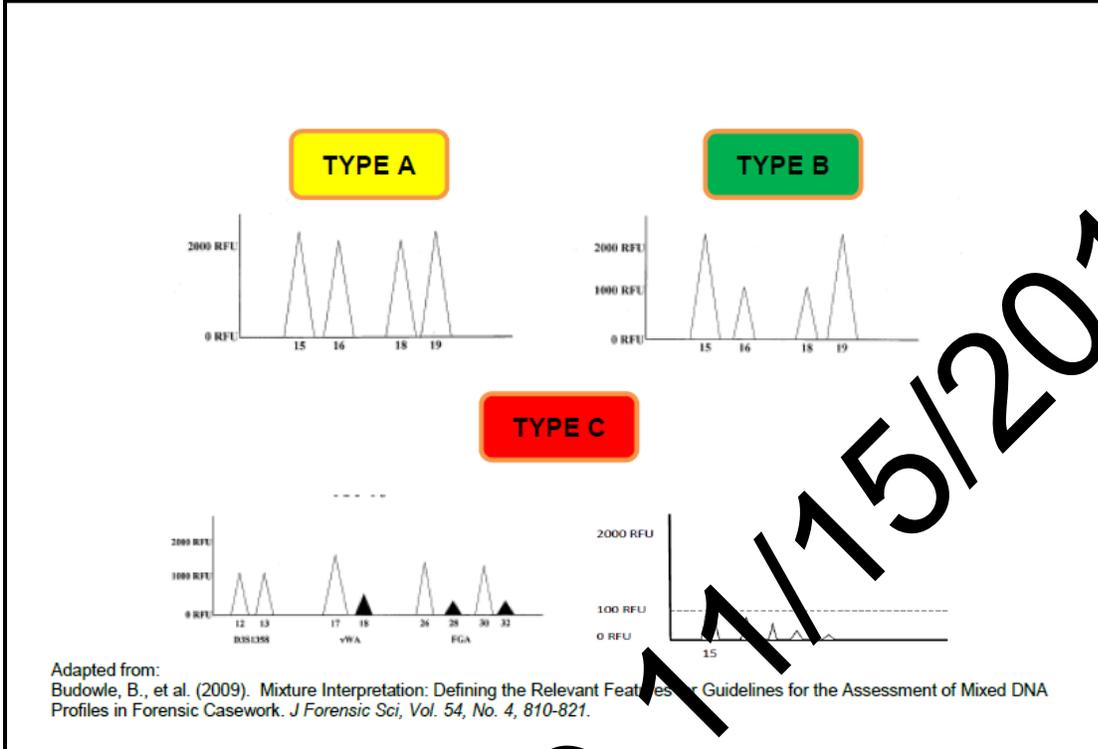
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TYPE B:

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.

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. This may also encompass mixture profiles that exhibit more than one major contributor.

Suitable for exclusions only: The minor component in a two-source Type B mixture would be suitable for exclusions only when two or three loci can be considered complete for the minor component. Alternatively, the minor may be suitable for exclusions only when reproducible minor alleles are detected at several loci outside of stutter positions, but fewer than four loci are suitable for statistics for the minor contributor.

TYPE C:

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

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

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

Suitable for exclusions only: A Type C mixture with several loci where all data is above ST may be suitable for exclusions only, particularly for intimate samples where one contributor is known.

8.4.2.2 Separating Major and Minor Contributors in DNA Mixtures

Refer to Appendix J for a flowchart of interpretation of low-level minor component of an indistinguishable mixture.

Separating a two-source mixture into major and minor components is easier when the number of loci exhibiting four alleles increases and the ratio of the major contributor to the minor contributor increases above a 5:1 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 should be used very conservatively in separation of major and minor components. Quantitative assessment of peak height ratios is not appropriate with the current platform; and qualitative information (e.g. assessment of which peaks at a given locus are consistently higher or lower) should be used very conservatively. Duplicate amplifications are recommended in order to allow an analyst to assess reproducibility of peak height ratios, as well as reproducibility of alleles.

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

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

When evaluating if a minor component is suitable for comparison the number of potential contributors and whether the peak falls into stutter position should be considered. A minor component is suitable for comparison if a complete minor profile is determined for at least 4 of 13 core loci. This is rarely possible when there is more than one contributor in the minor component. Minor component alleles often are comparable in peak height to stutter. Because of the possibility that a potential minor allele may be masked by being in a stutter position to a major allele, a minor component whose alleles are close in height to stutter peaks (i.e. about 14% of the heights of the major component alleles) can only be considered complete when heterozygote minor alleles are detected outside of stutter positions.

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When applicable, an “owner” reference may also be used to assist in separating the major and minor components (see section 8.4.2.3). Assessment of the major/minor components which includes deduction must be documented on a Mixture Interpretation Worksheet and included in the bench notes. Documentation includes a statement of any assumptions made, such as number of contributors, as well as a brief explanation of the rationale used to separate major and minor, such as subtracting an owner’s alleles or peak height ratio information.

In some instances, it may be appropriate to separate a two-source major component from additional minor contributors. In these instances, the mixture statistic is issued for only the major component. Mixture statistics should not be issued for the component of a profile that exhibits DBRT.

In instances when low-level contamination can be distinguished as a minor contributor, the major component is suitable for comparison.

8.4.2.2.1 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 sperm fraction may be detected as a minor contributor in the epithelial fraction. Because more than one profile is associated with the sample, some additional interpretational concerns apply specifically to instances of low-level differential carryover.

- When all minor component alleles are attributable to differential carryover, the analyst will add a note to the electropherogram: “Minor consistent with differential carryover”. If applicable, DBRT is noted on the electropherogram. Minor components which can be entirely attributed to differential carryover are not included on the STR table. They are included in the reported conclusions.
- When all minor component alleles are attributable to a combination of differential carryover and artifact/true alleles, the analyst will document that on the electropherogram. If applicable, DBRT is noted on the electropherogram. Such a minor component is not included on the STR table, but it is described in the reported conclusions.
- 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 yield 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.

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8.4.2.2.2 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 collected sample. When the minor component of an intimate sample is attributable to the owner, or the owner and artifact/true alleles, DBRT is noted on the electropherogram if appropriate. This minor component is not included on the STR table, but it is described in the reported conclusions (except for internal/cavity samples). Underwear and other clothing cannot be considered as intimate samples.

8.4.2.3 Deducing an Unknown Contributor in a DNA Mixture

Refer to Appendices K and L for flowcharts of deductions at 2-allele and 3-allele loci where the elimination sample is in the major component. Refer to Appendices M, N, and O for flowcharts of deductions at 2-allele and 3-allele loci where the elimination and unknown profiles are roughly equal.

When DNA consistent with being from two individuals is detected, 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 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. When fewer than nine loci can be deduced for a mixture from a body swab, both a mixture statistic for the profile as a whole (including all complete loci), and a single source statistic for the deduced contributor should be issued.

Several criteria should be taken into consideration when deducing an unknown contributor:

- 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 are only approximations. As such, loci with only two alleles are more likely to be unsuitable for deduction.
- Peak heights in relation to each other within a locus should be qualitatively reproducible in samples with multiple amplifications. Although specific peak heights and peak height ratios are not expected to be precisely replicated, peaks should be consistent in which is

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largest, which is smallest, etc within a locus. Loci where relative peak heights are markedly inconsistent between amplifications are not suitable for deduction unless the locus includes two obligate alleles for the unknown contributor.

- Peak height ratios may be used to compare different genotype possibilities. However, peak height ratios increase in variability with lower concentrations of DNA, and peak height ratios should be used with caution in interpreting data below the 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 2000 RFU or greater. At RFU levels below 2000 RFU, greater variability may be observed. Particularly in samples which are amp'd only once, it may not be appropriate to rely on PHR information for deducing when the peak heights are below 2000 RFU.
- When all 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 (reproducibly) detected.
- A large peak from a major contributor could potentially mask a minor allele in the stutter position. This is especially true for loci with a homozygous major contributor. If the maximum filtered stutter for a large peak is less than the peak heights of all or nearly all the minor component alleles, then stutter masking may be ruled out. If the maximum filtered stutter is greater than or close to the peak heights of the minor component alleles, the locus may not be suitable for deduction of the minor contributor.

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

8.4.2.4 Incorrect interpretation of major/minor assessment or mixture deduction

Despite making best use of available peak height ratio 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.

8.5 Comparison of DNA Results

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

- Exclusion
- Cannot exclude
- Inclusive
- 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 when 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.

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

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.

Insufficient DNA/Data:

Low amounts of DNA can result in allelic drop out, elevated stutter, and non-reproducibility of results. 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. As such, this data will not be included on the STR results table.

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

No Genetic Profile:

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

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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), a clear major component cannot be deduced, and/or a majority of the alleles are not reproducible when amplified in duplicate. Accordingly, the sample will be deemed not suitable for comparison. This is typically the case for indistinguishable mixtures with three or more contributors. While it is possible that a three-source mixture may be of sufficient quality and reproducibility to be suitable for comparison, such instances are exceptions that require approval from the DNA Technical Manager.

Close Biological Relatives:

In a mixture where two close biological relatives (parent-child or full siblings) cannot be excluded as contributors, a comparison can still be made and a mixture statistic issued if appropriate. However, the report must include an additional statement to clarify that a modified statistic that takes into account relatedness may be more appropriate. At this time, the Alaska Crime Laboratory does not perform such statistics; but upon request, the DNA Technical Manager can refer agencies to outside resources.

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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. Statistical analysis is not required when an individual is identified as a source of DNA in an intimate sample.

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. (Example: A stain from underwear yields a single source male profile from a sperm fraction; and the epithelial fraction includes a single source major component consistent with the owner of the underwear, plus a minor component consistent with differential carryover. If statistics are calculated for the single-source male profile obtained from the sperm fraction as well as the major component from the epithelial fraction, it is not necessary to also calculate a statistic for the minor component of the epithelial fraction that is attributable to differential carryover.)

In some instances, it may be appropriate to issue more than one statistic pertaining to one sample. For example, when a reference sample is used to deduce an unknown contributor from a non-intimate item (such as a piece of clothing), statistics are reported for both the mixture and the deduced profile.

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).

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

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

- Click the Calculate button or select Calculate from the Poststats 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.

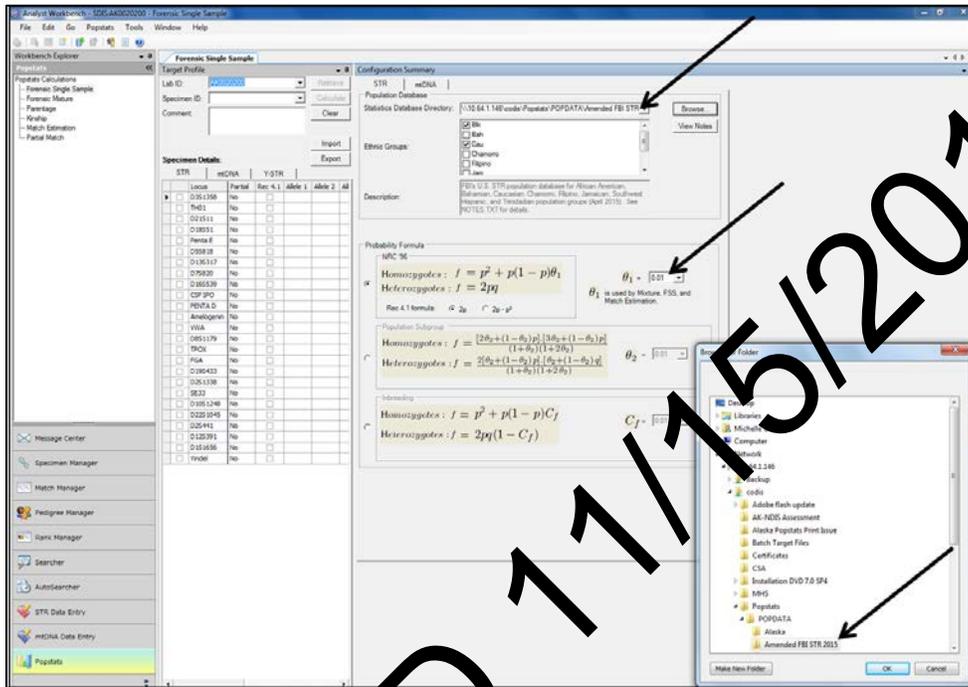
Note: The database and theta value must be changed in this order or the theta value will revert back.

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

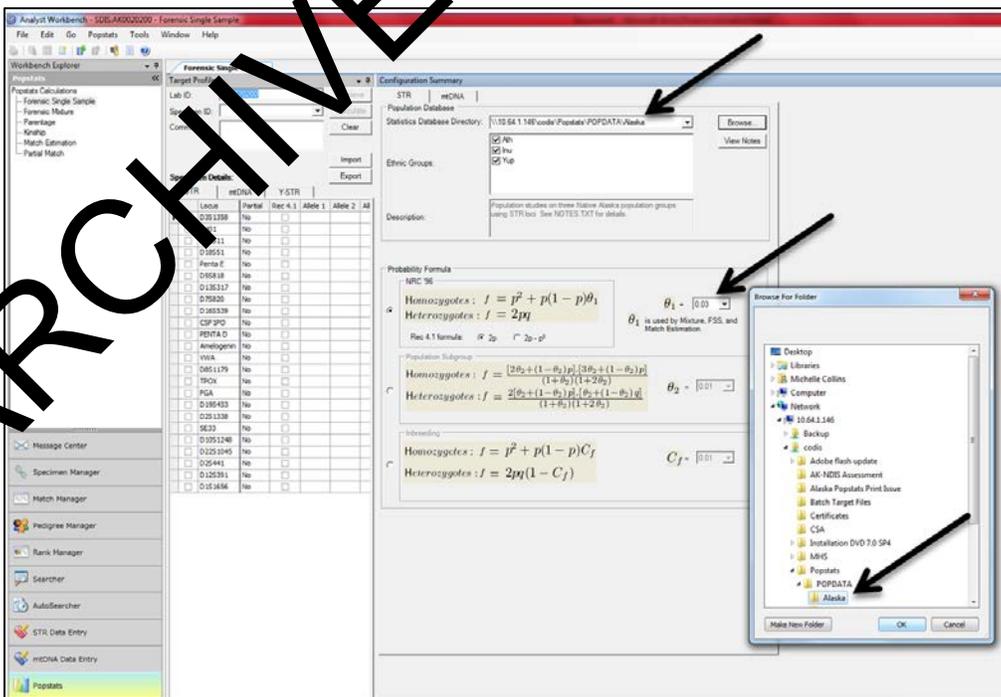
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Navigating to the FBI STR Caucasian and African-American Population Databases



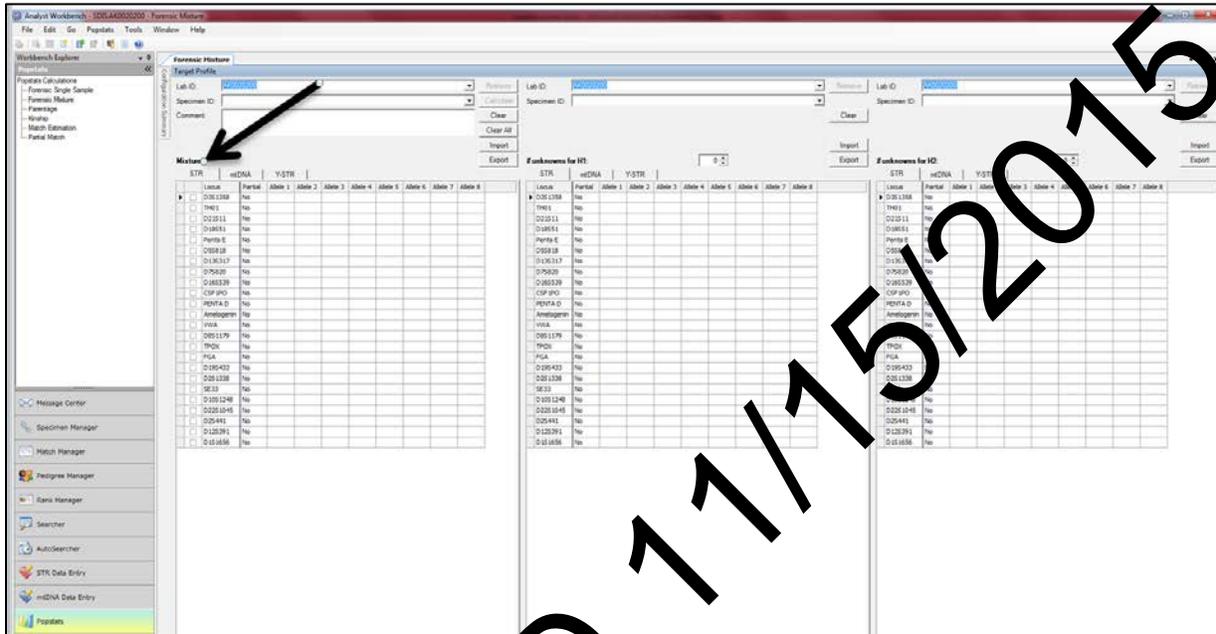
Selecting the Alaska Native Population Databases



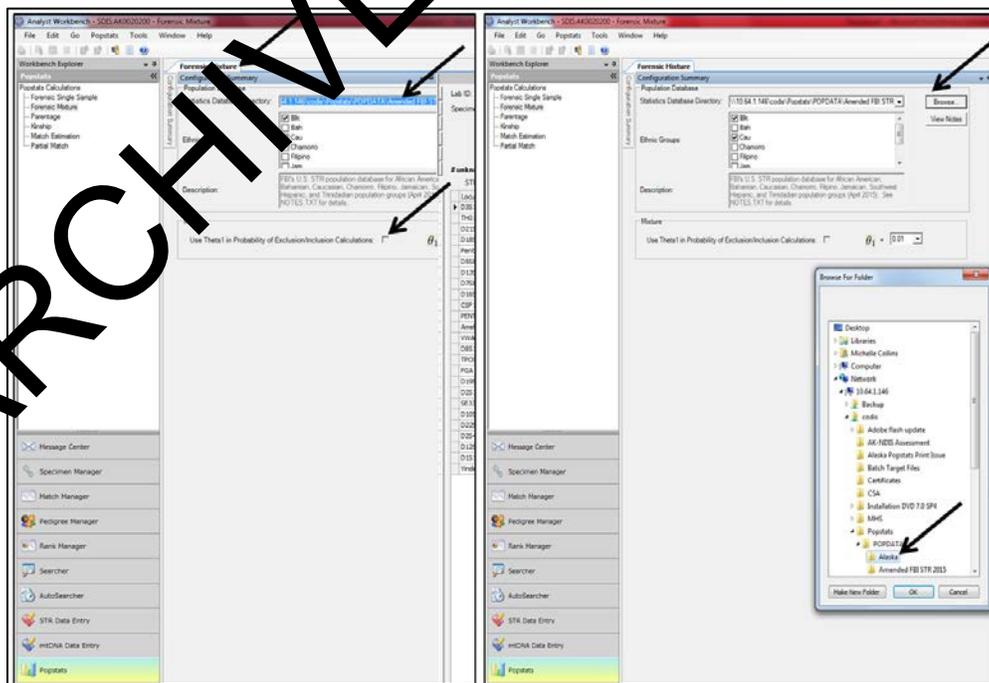
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Entering a Mixture Statistic



Changing the Population Database when Calculating Forensic Mixtures



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Section 10 DNA Screening and DNA Report Writing and Review

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

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, 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 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 Duo results	Report
No male DNA detected	Quantification results do not indicate the presence of male DNA. No further analysis was performed on this sample.
Female : Male ratio >10:1	<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>

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<p>Male DNA detected but human DNA quantity is undetermined</p> <p>Consumed sample - Not (effective) single-source</p> <p>Male DNA detected below 0.01 ng/uL OR human DNA concentration < 0.1 ng/uL</p> <p>Non-consumed sample - Not (effective) single-source</p> <p>Male DNA detected below 0.005 ng/uL OR human DNA concentration < 0.05 ng/uL</p>	<p>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.</p> <p>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</p>
<p>Human and male quantification results are both undetermined</p>	<p>Quantification results do not indicate the presence of amplifiable DNA. No further analysis was performed on this sample.</p>

All others	Report
<p>Samples suitable for STR analysis based on Quantifiler Duo 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 intimate samples (e.g. vaginal, rectal, and oral swabs), but do need to be reported for all sperm fractions and for all profiles from external body swabs.
 - 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
- 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 contained in the Methods section 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.

For samples not amplified based on Quantifiler Duo results	Report
No male DNA detected	Quantification results do not indicate the presence of male DNA. No further analysis was performed on this sample.
Female: Male ratio >10:1	<p>Quantification results do not indicate the presence of sufficient male DNA for Y-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>
Low-level mixture likely	<p>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.</p> <p>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</p>
Human and male quantification results are both undetermined	Quantification results do not indicate the presence of amplifiable DNA. No further analysis was performed on this sample.

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For single source samples	Report
Single Source: exclusion	XXX was excluded as the source of DNA detected in this sample.
Single Source: fail to exclude	XXX cannot be excluded as the source of DNA detected in this sample.
Same profile (for 2+ items)	The genetic profiles obtained from these samples were the same.
Single source statistic	Single Source formula The estimated frequency (13 core loci) of the genetic profile from the above samples 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 mixture	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.
No DNA inconsistent	No DNA inconsistent with XXX was detected in this sample.
DNA consistent	DNA consistent with XXX was detected in this sample
When cannot exclude but don't have all 13 core loci	DNA consistent with XXX was detected at XX of 13 core loci in this sample. Therefore, XXX cannot be excluded as a source of DNA detected in this sample. (change 13 if not all loci yielded data above reporting threshold)

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For mixtures	Report
More than 1 individual	DNA from more than one individual was observed in this sample.
To indicate # of individuals	DNA from at least XX individuals was observed in this sample. 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 and/or non-reproducible alleles may be present at the following loci: XXX. Therefore, population statistics are not reported for these loci.

Mixture statistic	<p>Mixture Formula</p> <p>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>
<p>Deduced Profile with Mixture and Single Source statistics</p>	<p>DNA consistent with being from two individuals was detected in this sample. XXX and YYY cannot be excluded as sources of DNA detected in this sample. No DNA inconsistent with XXX and YYY was detected in this sample.</p> <p>Data below the reporting threshold and/or non-reproducible alleles may be present at the following core loci: XXX. Therefore, population statistics are not reported for these loci.</p> <p>Mixture formula</p> <p>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.</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>

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Not Suitable for comparison	Report
Not suitable	The data obtained from this sample was not suitable for comparison (due to XXXXXX).
No profile	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.
Profile with contamination is inconclusive with respect to further comparisons	(In addition to above language) This profile is inconclusive for interpretation.

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

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10.2.3 Review of 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 of the ILS for all passing samples
- Review of all allelic ladders designated as such
- Review of at least one passing amplification positive control
- Review 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

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. 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

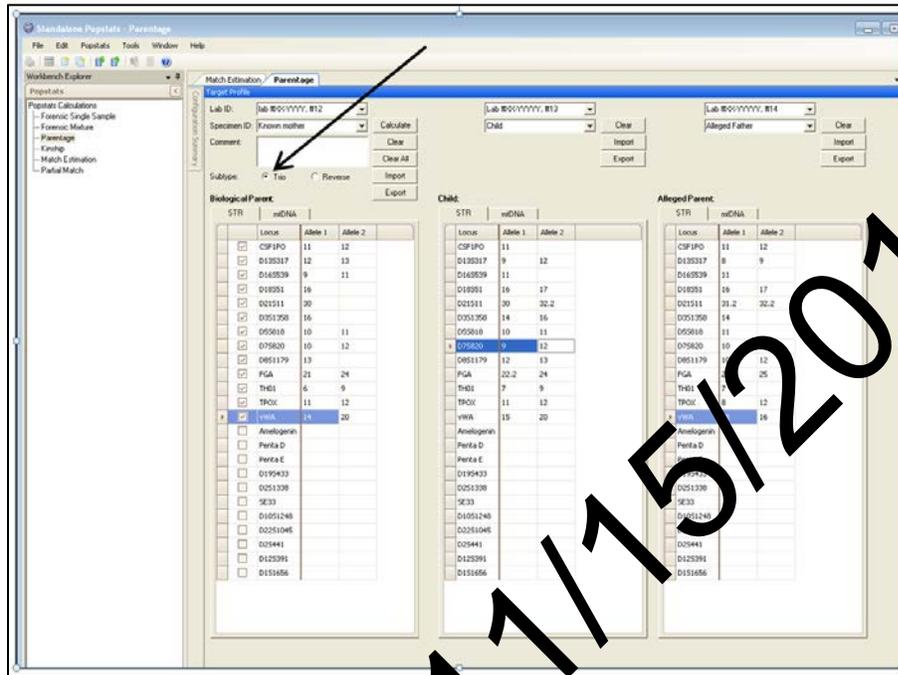
[**Note:** On occasion, especially when entering mutation rates or toggling between databases, the screen may not automatically refresh to show updated changes. If you do a Print Preview, you can confirm that changes have been applied.]

A. One-parent forward paternity (two):

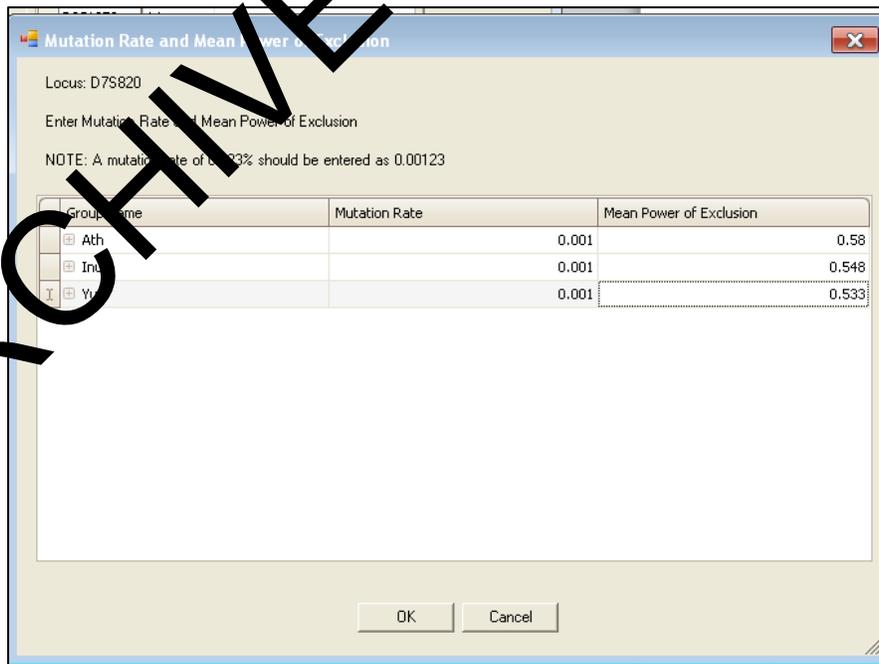
1. Open Popstats and choose Parentage from the menu on the left side of the screen.
2. Choose the Parentage tab at the top of the screen.
3. Use the Lab ID field to enter the lab number at least, item number space permitting. If the item numbers do not fit in the typed file they may be hand-written on 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 (mismatches) you will be prompted to enter the mutation rate and the mean power of exclusion (see following image).

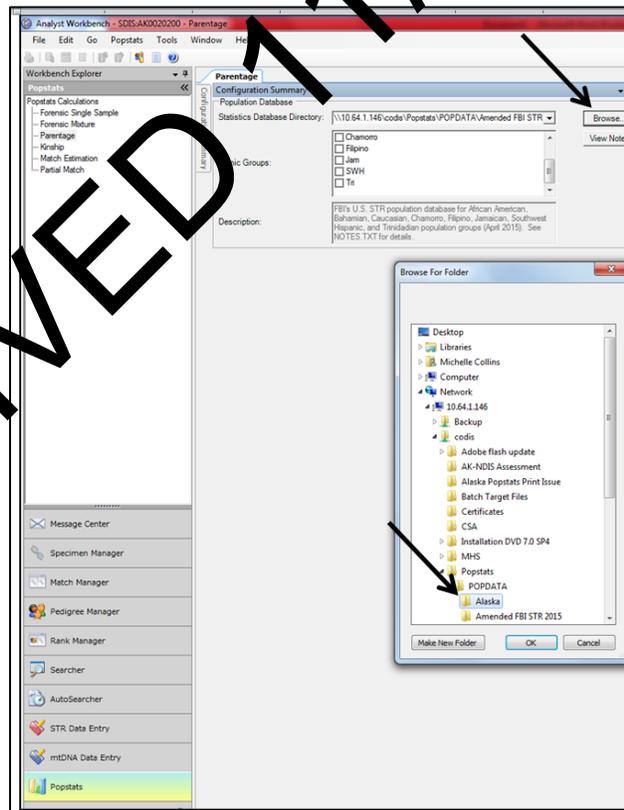


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8. Enter the locus-specific mutation rate from the NIST website. A printout of the relevant page is included in this manual.
9. Enter the locus-specific mean probability of exclusion (PE) from the published database references listed at the end of 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.
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.



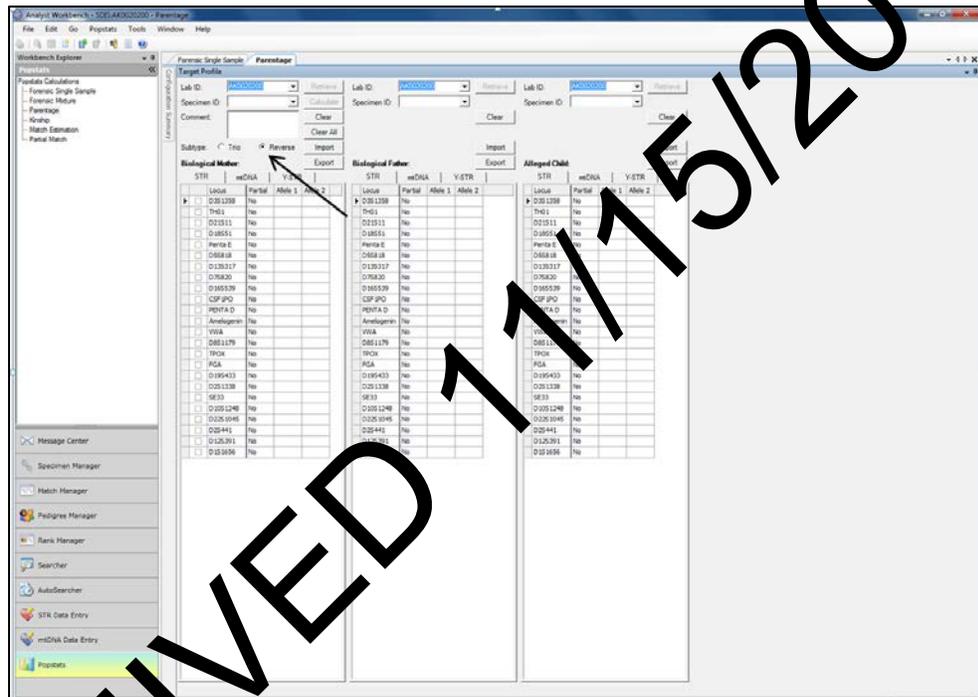
13. As before, if necessary, you will be prompted to enter mutation rate and mean power of exclusion. When finished, choose OK. Print report as described previously.

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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 Lab 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 1-13 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 reliability of Paternity calculations as well. Show work clearly.

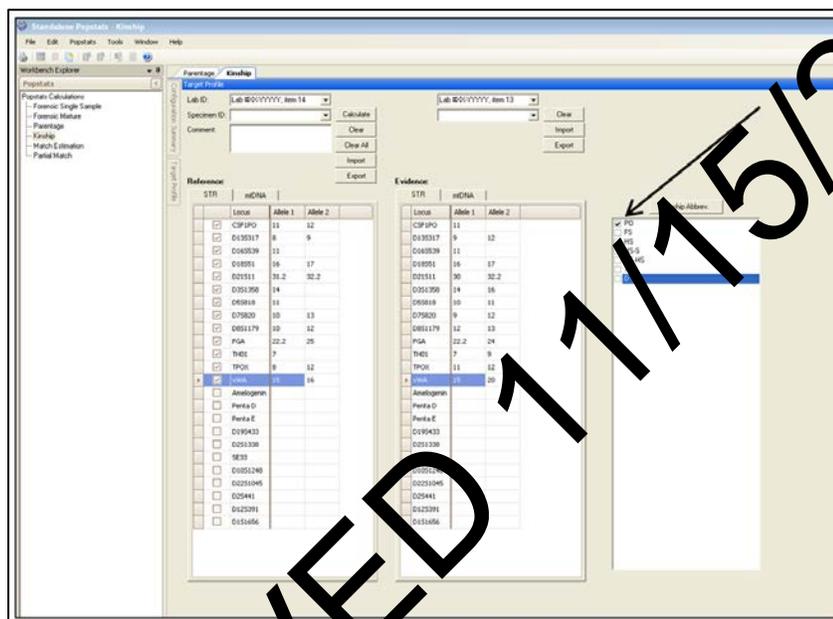
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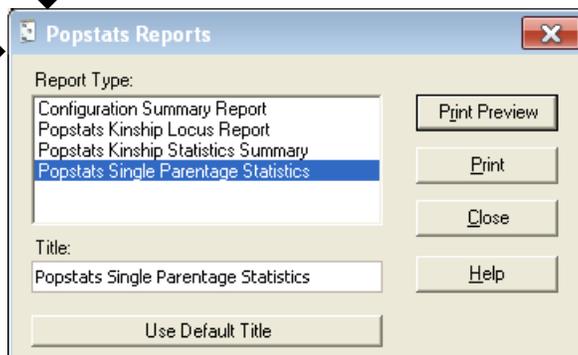
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C. Zero-parent forward:

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 Lab 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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11.4 References

Mean Power of Exclusion by locus:

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 extract available for analysis has been consumed, OR
 - b. Upon completion of the technical review of a case record.
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.
6. To recover dried samples, add up to 50 µl of sterile 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 unmarked (usually labeled as reagent blank replicate -2) is brought up in 19.2 µL of sterile water and amplified as well.

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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 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)			
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			
The date evidence was opened and/or sealed is documented			
Retained items created in LIMS; all retained and unexamined items HELD in LIMS			
The "FUTURE TECH" flag has been tripped for the case, 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 sample 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			
SCPs are linked to request in LIMS			
Chain of Custody for all tested items can be tracked through RLS and LIMS			
Assign DNA holding as appropriate			

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DNA Screening Review Checklist

Date: _____	Analyst: _____ Tech. Reviewer: _____ Admin. Reviewer: _____
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			
Q-PCR plate set-up is documented			
Q-PCR standard curve printouts: Results are acceptable.			
Q-PCR Initial Template Quantity is documented by 1500 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 LOCATION

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			
SAPS are linked to request in LIMS			
Chain of Custody for all tested items 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 #

Analyst:

Batch:

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

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STR Casework Review Checklist

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

Analyst:	15
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 items 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			
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			
PP16 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 LOCATION

Central Log Folder location documented in 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.			
ICS checked by Technical Reviewer			
Technical Reviewer checked Controls and Standards sheet: initialed and dated.			
Disposition of blank extracts documented; item created in LIMS (if appropriate)			
PP16 printouts for reagent blanks and negative control(s): Results are acceptable.			
PP16 printouts for positive control(s): correct DNA profile obtained.			
PP16 printouts for all appropriate Allelic Ladders.			
Worksheets contain all lot #s and expiration dates for all reagents used.			

List 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 Review Checklist for Vendor Lab

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

Tech Review: _____
 CODIS Review: _____

PP16 printouts for all appropriate Allelic Ladders: obtained expected ILS and allelic		
PP16 printouts for positive control: obtained expected ILS peaks and DNA profile		
PP16 printouts for negative control: results are acceptable (including ILS)		
PP16 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 (if any) on each page		

List of eligible profiles to be entered into CODIS (include specimen category and SDIS or NDIS)		
Specimen integrity 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		

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STR Single Offender Review Checklist

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

Analyt.	Tech. Review
---------	--------------

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		
Packaging / extraction procedure is documented on DNA worksheet		
Disposition of offender extract(s) documented		
Technical Reviewer checked DNA worksheet: initialed and dated		
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 LOCATION:

Central Log Folder location documented in LIMS		
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.		
Amplification volumes are documented.		
ICS checked by Technical Reviewer		
Technical Reviewer checked Controls and Standards meet: initialed and dated.		
Disposition of blank extracts documented when created in LIMS (if appropriate)		
PP16 printouts for reagent blanks and negative control(s): Results are acceptable.		
PP16 printouts for positive control(s): correct DNA profile obtained.		
PP16 printouts for all appropriate Allelic ladders.		
Worksheets contain all lot #s and expiration dates for all reagents used.		

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/analyst 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 2015 R0 Page	FBCP 2014 R1 Page	Location	Revision made
NA	NA	Throughout	Updated Document Structure, page numbering, and section numbering as appropriate.
14	14	1.10	Changed "Slides are made from all sperm pellets, but they do not need to be stained or read prior to issuing the screening report" to "Slides are made from all sperm pellets and stained, but they do not need to be read prior to issuing the screening report".
15	15	1.10	Added "from Department of Law" to mentions of requiring written permission for consumption of extract.
15	15	1.10	Added "Duplicate" to first sentence under Reagent Blanks heading.
18	18	2.2	Removed "No spermatozoa were observed microscopically; but" from conclusion for PSA positive result.
20	20	3.1.2	Changed second bullet point to clarify when swabs require tags with identifying information.
22-30	22-29	3.2	Removed options for questioned sample elution volumes other than 40 µL throughout section.
23	22-23	3.2	Added clarification on creation of reagent blanks to General Instructions.
31	30	4	Added clarification on number of quantification reactions required for different types of extract.
34	33	4.6.2	Changed assessment of IPC controls that fall outside expected range.
35	34	4.7.1	Added "then dividing by the male quantification value"
35	34	4.7.3	Added "Sample decision trees for this process (for consumed samples) are shown in Appendices D - H. 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."
35	34	4.7.3	Added "Extracts with a quant result of <u>undetermined for both human and male DNA</u> will not routinely proceed to amplification."
36	35	4.7.3	Added "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."

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36	35	4.7.4	Changed "Interpretation guidelines in this manual <i>recommend</i> that low-level mixtures be amplified twice to assess reproducibility of peak heights and peak height ratios. Additionally, for mixtures, it is <i>sometimes</i> useful to target DNA at 1.0 ng rather than 0.5, in order to improve the chance of detecting a full profile <i>This is particularly true when there is an even or nearly even ratio of contributors.</i> "
39	38	5.2	Removed: "This also applies to samples with an undetermined human DNA concentration; in those cases, a single amplification should be done at the largest volume permitted."
39	NA	5.2	Added directions on use of DNA Stable-d reagent blanks with consumed sample extracts.
43,48-49	NA	7.1, 7.2, and 7.8	Added these sections; numbering of other sections adjusted accordingly.
56	53	8.1.5	Added clarification on reagent blanks.
57	54	8.2	Added paragraph beginning "For questioned samples..."
58-59	NA	8.2.2.1	Added section on elevated +4 / -4 stutter
61	58	8.3.1	Added "Refer to Appendix I for a flowchart of interpretation of low-level data".
61	58	8.3.1	Added clarification on when it is appropriate to put DBRT on the STR table.
62	58	8.3.2	Changed: "Questioned samples <i>likely to be</i> from more than one individual with a quantification value of <0.05ng/μl and greater than undetermined, shall be routinely amplified in duplicate to ascertain reproducibility of alleles below the ST. <i>Exceptions require advance approval by the Technical Manager.</i> "
62	58	8.3.2	Changed "Samples with a quantitation result of undetermined do not require duplicate amplification" to "Samples with human and male quantitation results of undetermined are not routinely amplified."
62	58	8.3.3	Removed paragraph beginning with "Intimate samples:".
63	59	8.4	Added paragraph starting "A locus where drop-out is a possibility..."
64	60	8.4.1	Added "A single source profile, partial profile, or minor component from any intimate sample which is solely attributable to an "owner" is not routinely included on the STR table."
65,67,68	61,63,64	8.4.2.1	Added examples of profiles suitable for

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			exclusion only to each of the three mixture classes.
68	64	8.4.2.2	Added "Refer to Appendix J for a flowchart of interpretation of low-level minor component of an indistinguishable mixture."
69	NA	8.4.2.2.1	Added section on minor components arising from differential extraction
70	NA	8.4.2.2.2	Added section on minor components consistent with owner on intimate samples
70	65	8.4.2.3	Added references to Appendices K, L, M, N, and O.
71	65	8.4.2.3	Added bullet point that begins "As a general rule..."
71	66	8.4.2.3	Added bullet point that begins "A large peak from a major contributor..."
74	68	9	Added "Erratum to above article (2015) Journal of Forensic Science 60(4)". Added paragraph beginning "For differential samples..."
74-77	68-71	9.1	Changed from FBI STR database to Amended FBI STR 2015 database throughout section. Changed "The SWH and SEH populations" to "All populations except BLK and CAU". Changed images under headings <u>Navigating to the FBI STR Caucasian and African-American Population Databases</u> and <u>Changing the Population Database when Calculating Forensic Mixtures</u> to show Amended FBI STR 2015 database.
79	73	10.1.2	Added "Alternatively, this may be suitable for Y-STR analysis".
79	73	10.1.2	Added a conclusion for samples with undetermined human and male quantification results.
79	73	10.1.2	Added: "from the Department of Law" to the report statement regarding written permission for consumption of evidence.
82	76	10.2.2	Added a conclusion for samples with undetermined human and male quantification results.
82	76	10.2.2	Changed conclusions for low-level mixture likely to align with DNA screening report conclusions.
85	79	10.2.2	Changed "loci" to "core loci" in statements regarding statistics on deduced profiles.
86	80	10.2.2	Added conclusions section for consumed extracts
NA	82	11.1	Removed references to probability of paternity

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			and probability of exclusion.
90	84	11.2	Changed Part A Step 12 from FBI – STR database to Amended FBI STR 2015 database. Changed image in Note to show point to Amended FBI STR 2015 database.
92	86	11.2	Changed Part C step 7 from FBI – STR database to Amended FBI STR 2015 database.
96	90	11.4	Added “Erratum to above article (2015) Journal of Forensic Science 60(4)”.
97	91	12	Changed step 6 from “add 50 µl” to “add up to 50 µl”.
97	91	12	Added step 7 describing reconstitution of consumed extracts and their corresponding blanks.
98	92	Biological Screening Review Checklist	Changed “Assign DNA holding to DNA Supervisor” to “Assign DNA Holding as appropriate”
99	93	DNA Screening Review Checklist	Changed “Assign DNA holding to DNA Supervisor” to “Assign DNA Holding as appropriate” Added “Batch number is recorded in JT” to the admin review. Added space to record batch number on review checklist. Added check on elution volume, instrument and sample size to the tech review. Added (including reagent blanks) to check on items retained in LIMS.
101	95	STR Casework Review Checklist	Added “If applicable” to checks related to quantification documentation in central log. Added “theta values and databases are correct”.
111-115	104	Appendix D	Replaced Appendix D with Appendixes D - H
116-122	NA	Appendix I - O	Added Appendix I - O

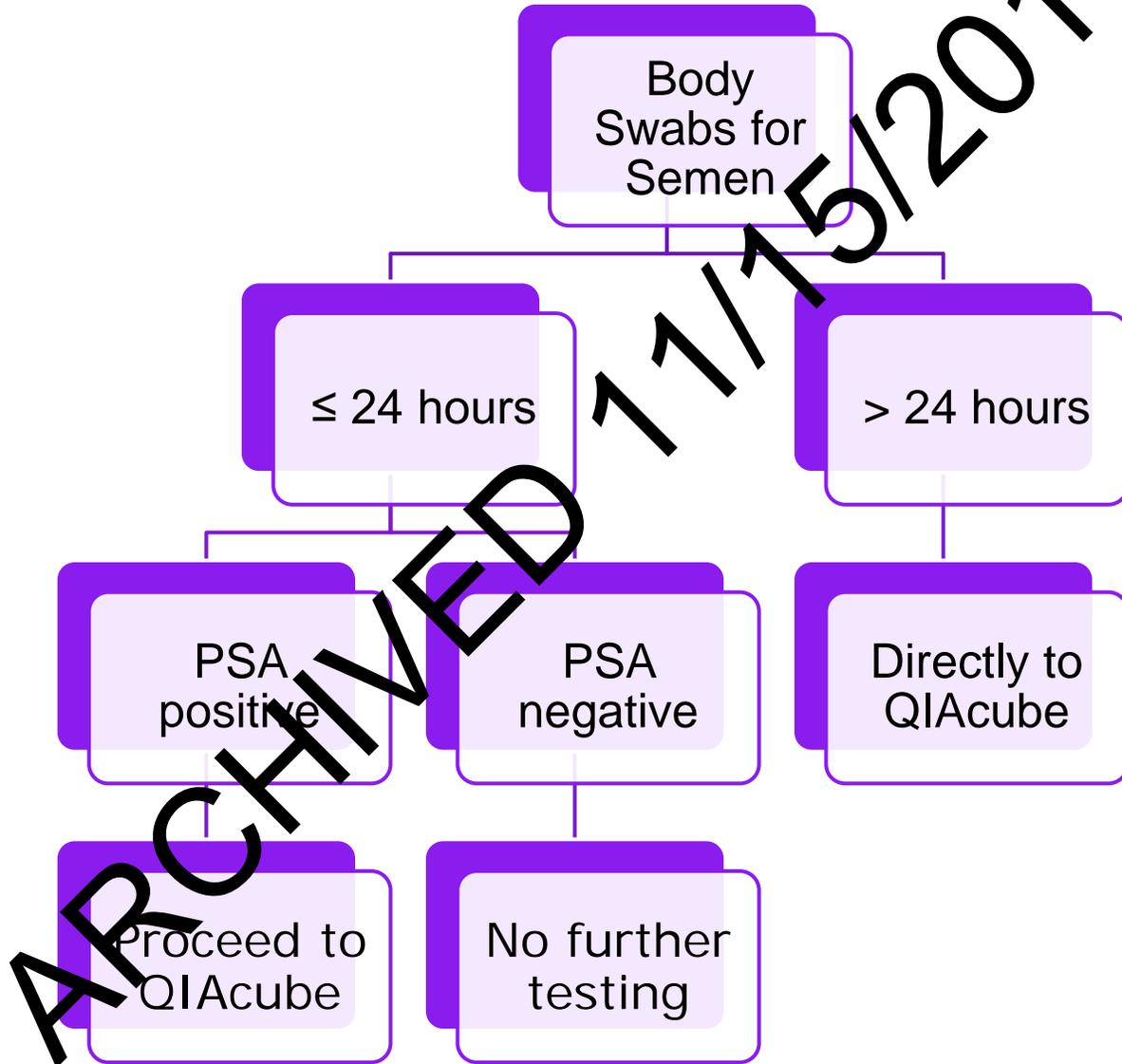
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Appendix B Biological screening of body swabs incorporating the QIAcube decision tree

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

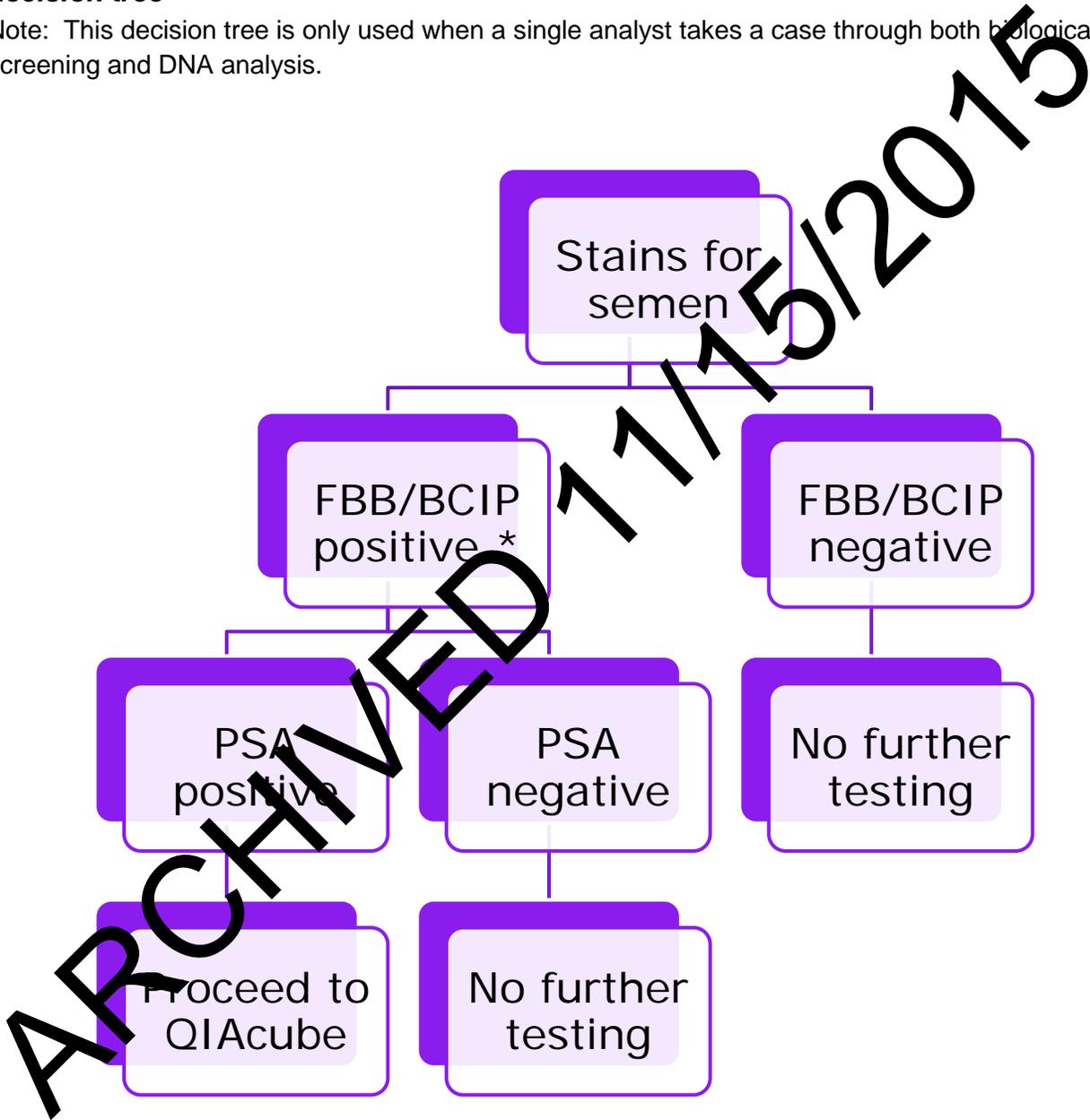


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Appendix C Biological screening of stains for semen incorporating the QIAcube decision tree

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



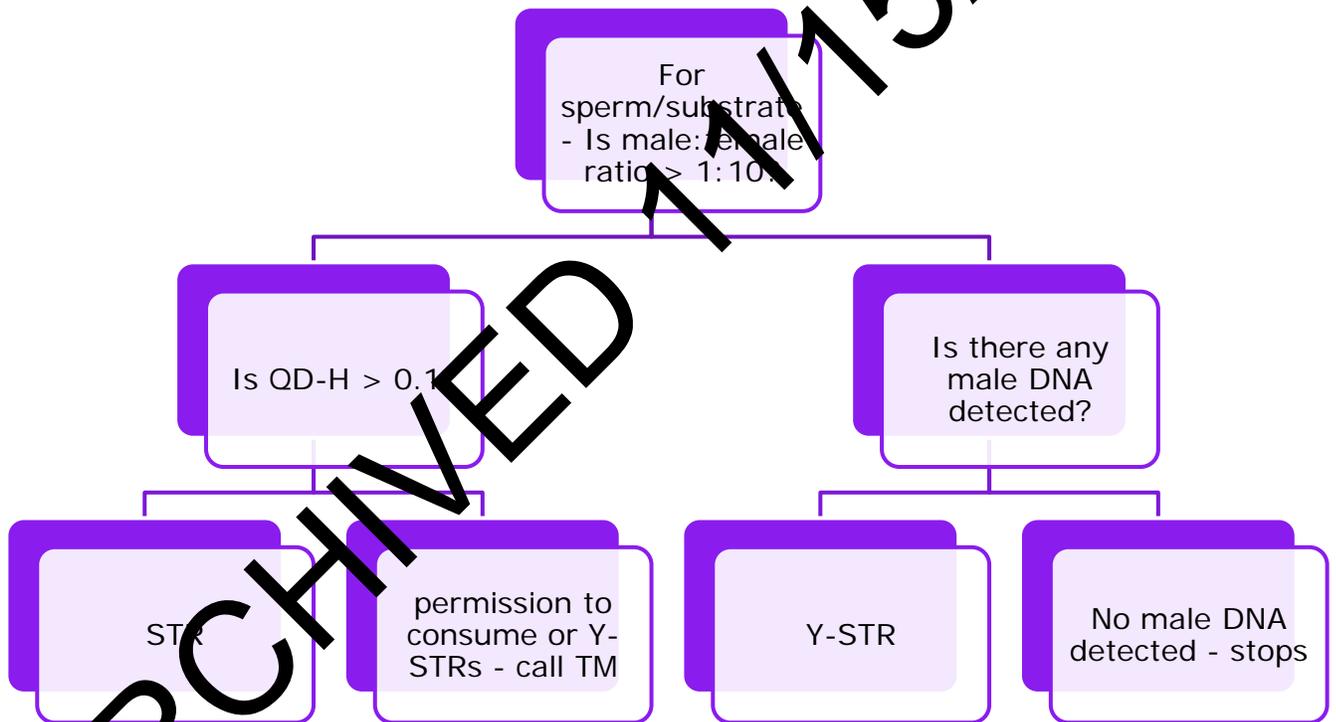
* Underwear not worn until 24 hours post-event can go directly to the QIAcube, without PSA testing

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Appendix D: Consumed sample quant decision tree for vaginal / oral / rectal swabs.
Epithelial is always worked unless QD-H is UND.

Yes = left; No = right



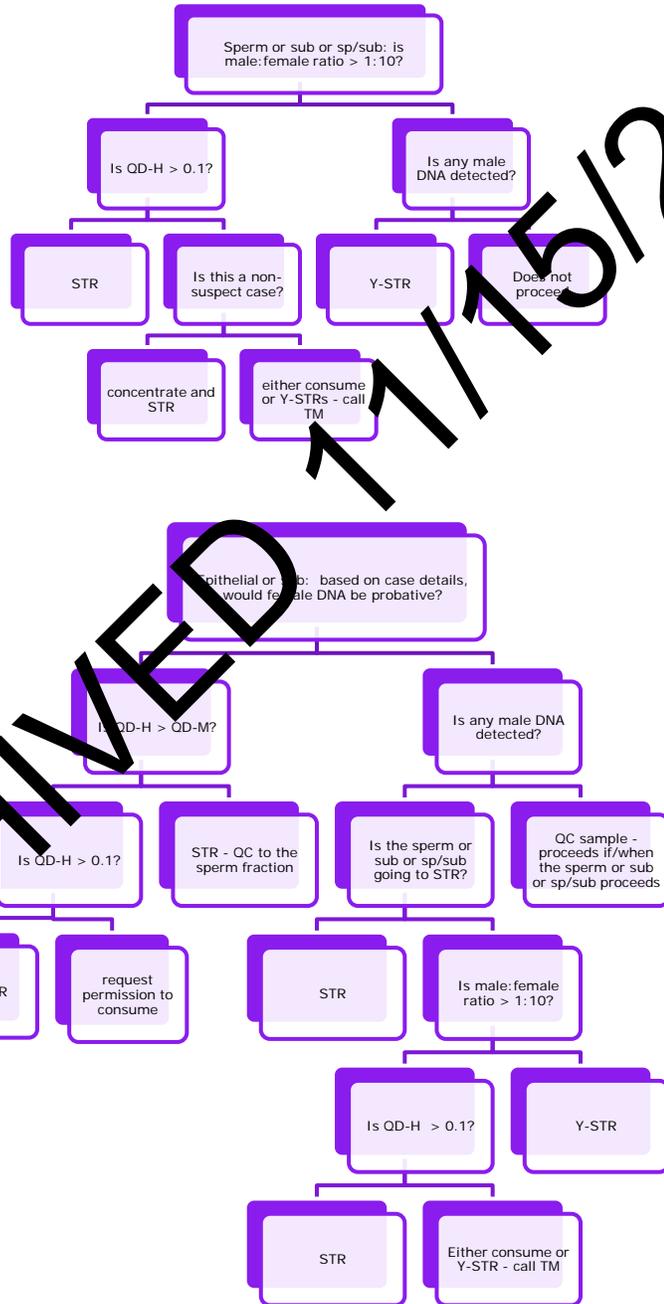
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Appendix E: Consumed sample quant decision tree for differential stains (condoms, clothing, etc.) – assumes male (or both) probative

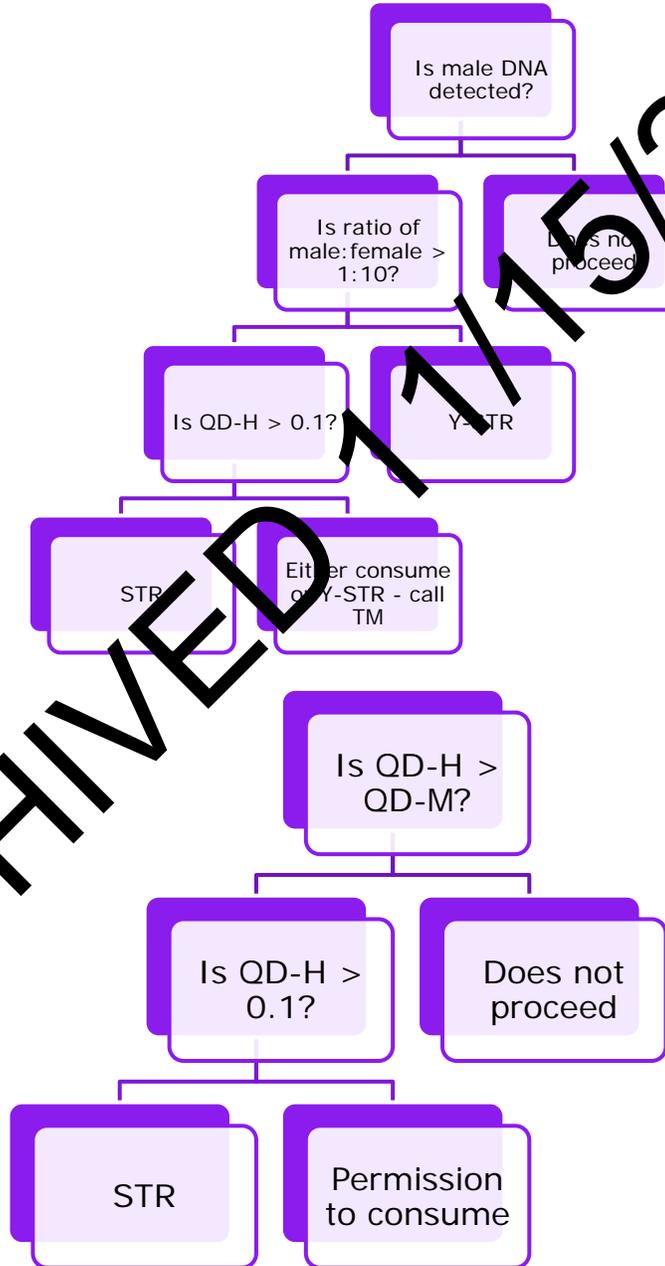
Yes = left; No = right



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Appendix F: Consumed sample quant decision tree for direct extraction - male probative (upper chart) or female probative (lower chart)

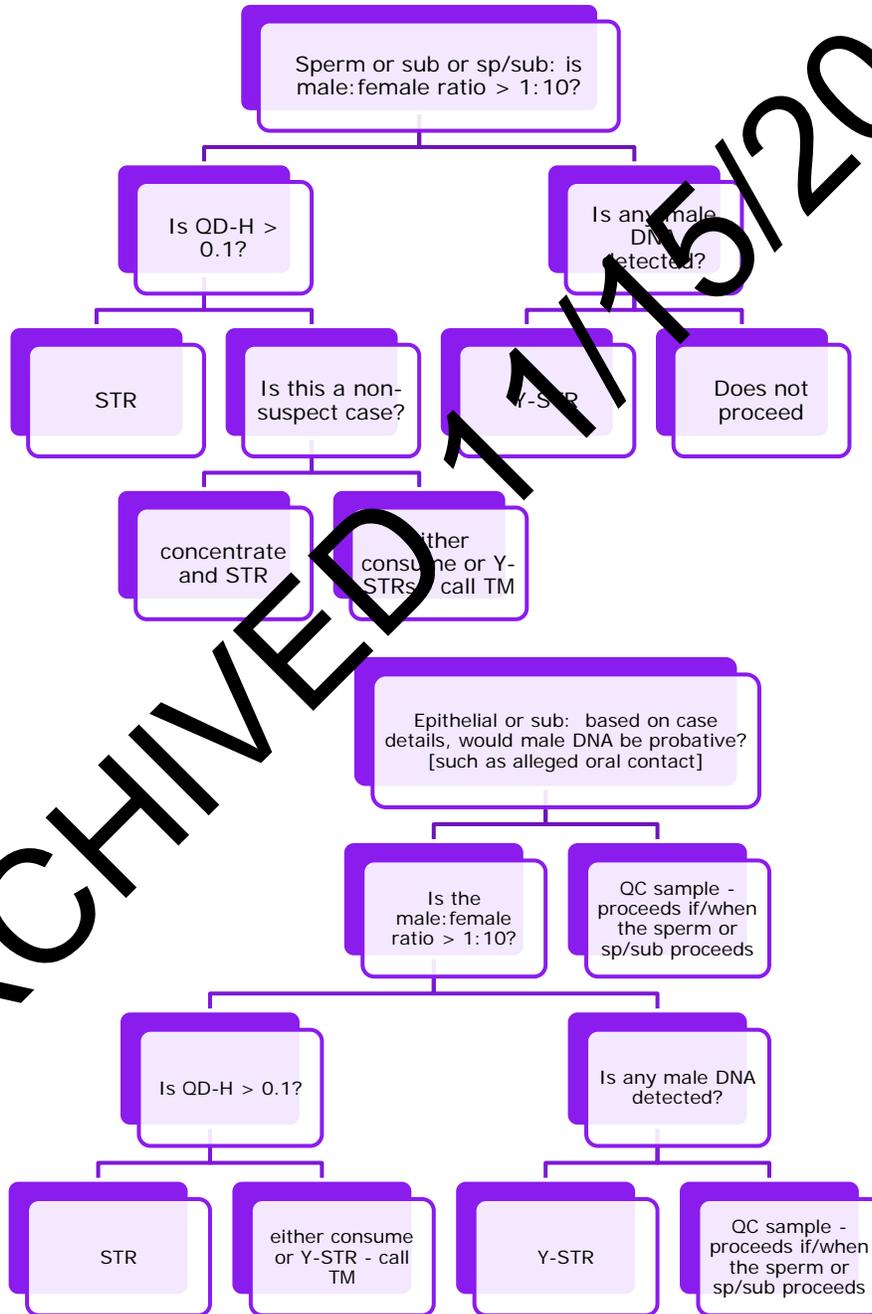
Yes = left; No = right



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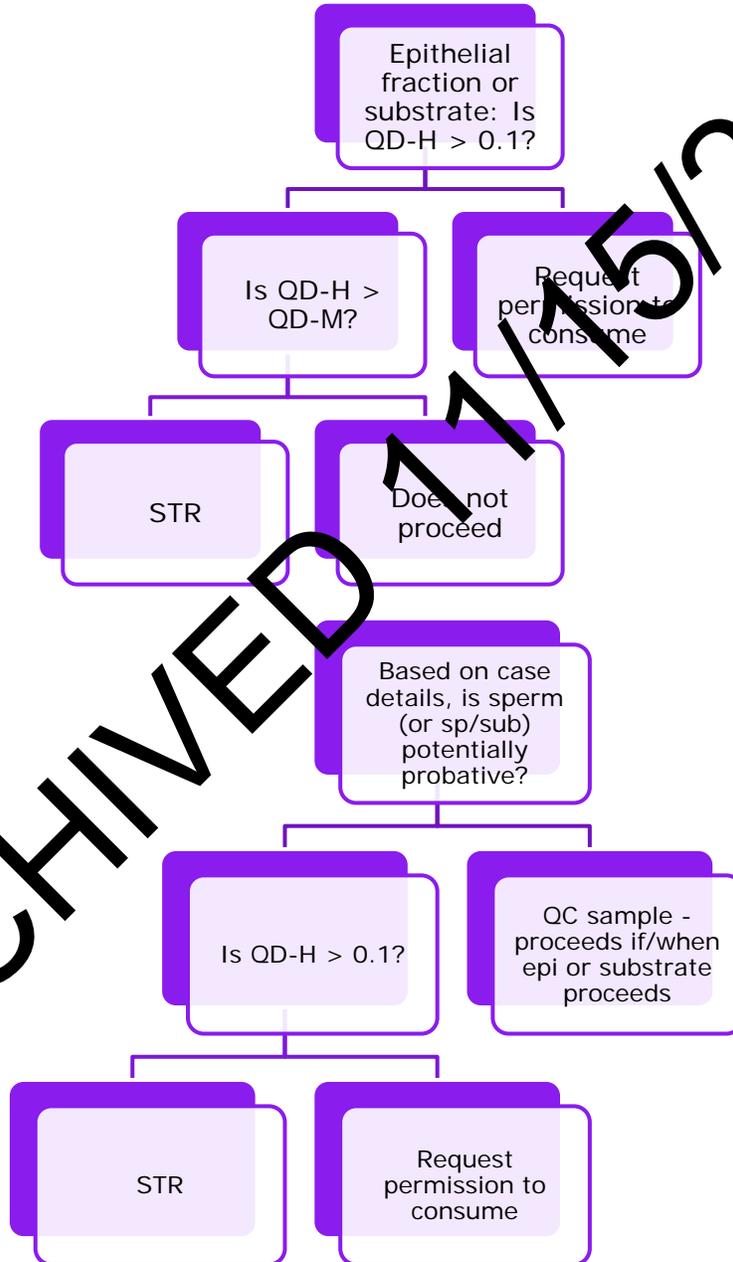
Appendix G: Consumed sample quant decision tree for external female body differentials (genitalia, thigh, etc.)

Yes = left; No = right



Appendix H: Consumed sample quant decision tree for penile/ scrotum / male body differential swabs

Yes = left; No = right



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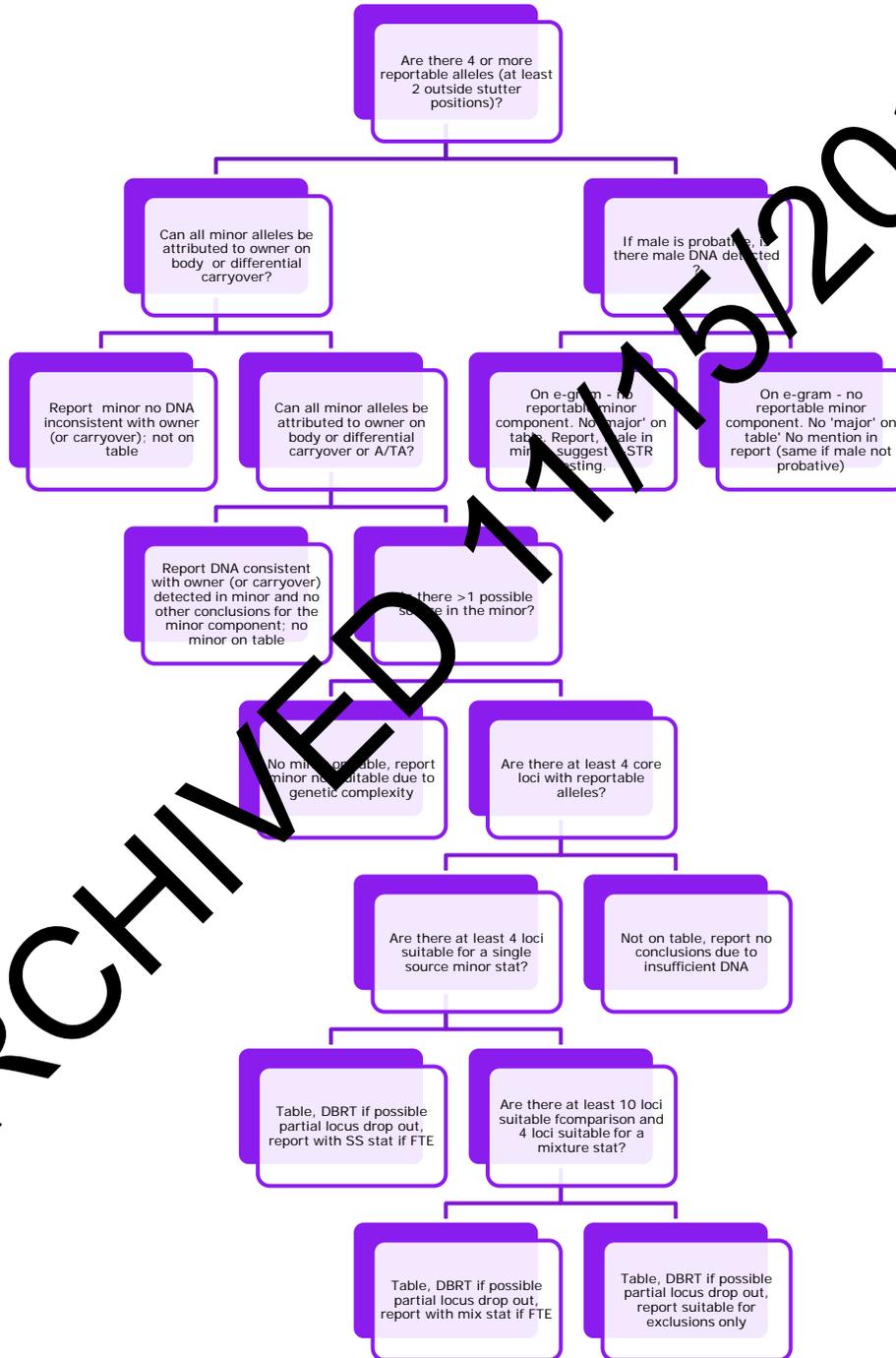
Appendix I: Interpretation of low-level data (data at any locus below ST), not the distinguishable minor component of a mixture Yes = left; No = right



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**Appendix J: Interpretation of low-level minor component of a distinguishable mixture.
Yes = left; No = right**



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Appendix K: Deduction at 2-Allele Locus where the elimination sample is the major contributor. Use allele calls to note obligate allele (if applicable).

Yes = left; No = right

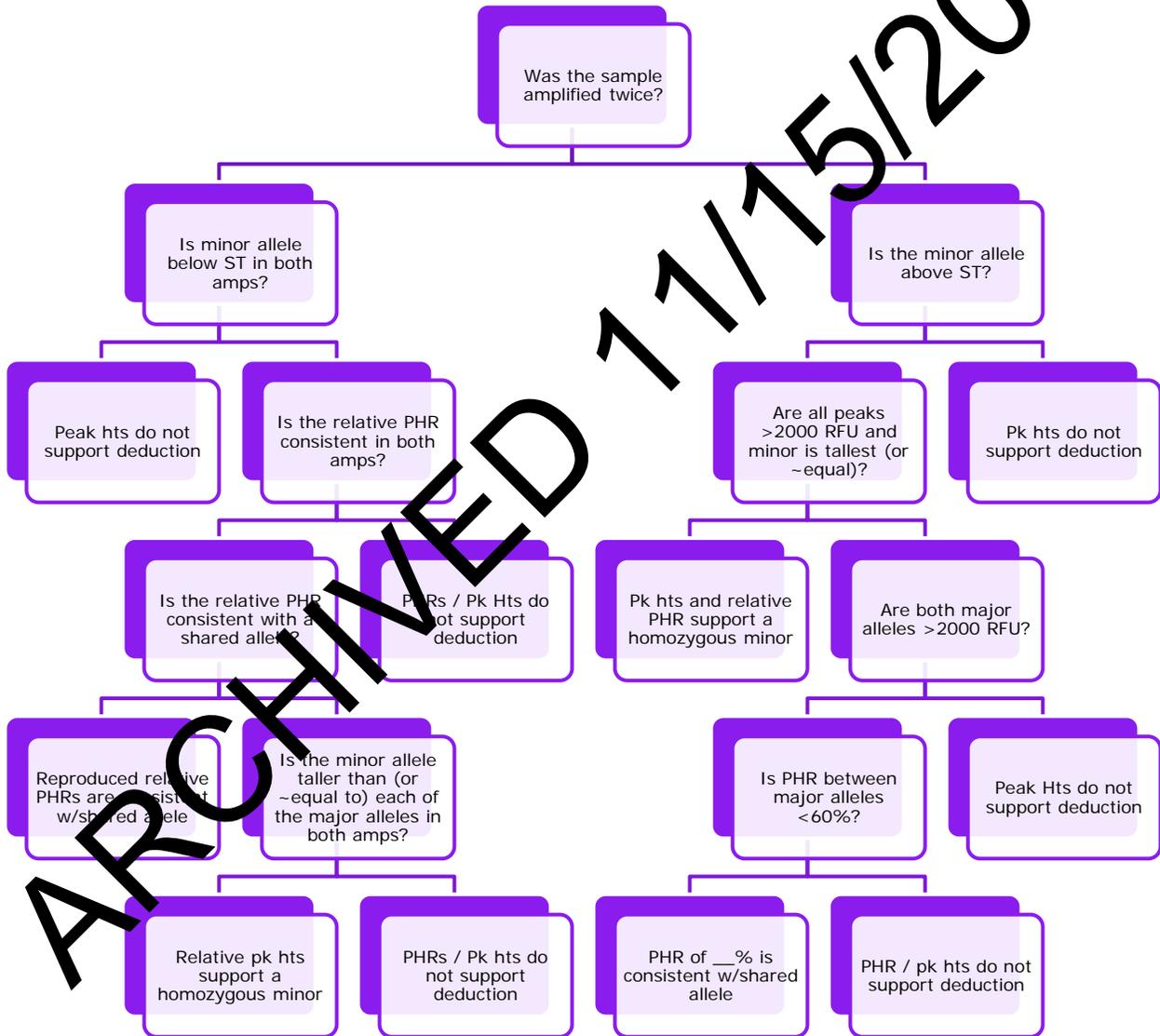


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Appendix L: Deduction at 3-Allele Locus where the elimination sample is the major contributor. Use allele calls to note obligate allele (if applicable).

Yes = left; No = right



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Appendix M: Deduction at 3-Allele Locus where the elimination sample and unknown are roughly even. Assumes all alleles at locus are above ST. Use allele calls to note obligate allele (if applicable).

Yes = left; No = right



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Appendix N: Deduction at 2-Allele Locus where the heterozygous elimination sample and unknown are roughly even. Assumes all alleles at locus are above ST. Use allele calls to note obligate allele (if applicable).

Yes = left; No = right



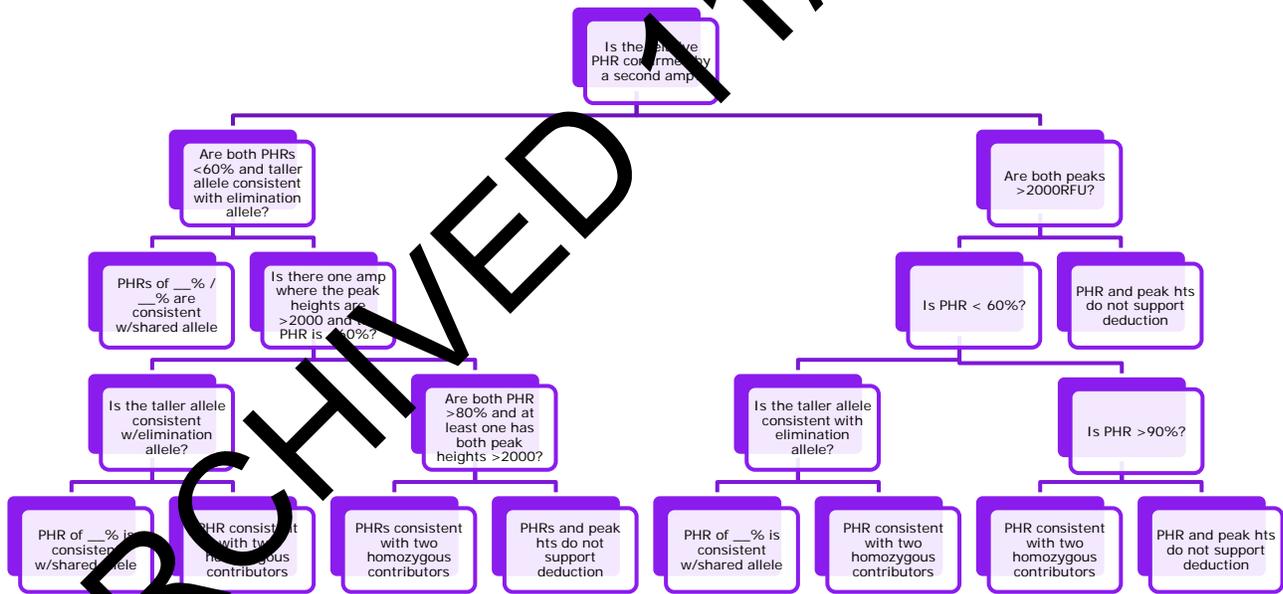
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Appendix O: Deduction at 2-Allele Locus where the homozygous elimination sample and unknown are roughly even. Assumes all alleles at locus are above ST. Use allele calls to note obligate allele (if applicable).

Yes = left; No = right



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Appendix P: 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	1,111/962,239	0.11%
D18S51	186/296,244 (0.06)	1,094/494,098 (0.22)	466	1,746/793,342	0.22%
D21S11	464/435,388 (0.11)	772/526,708 (0.15)	580	1,816/922,096	0.19%
Penta D	12/18,701 (0.06)	21/22,501 (0.09)	15	57/41,202	0.14%
Penta E	29/44,311 (0.065)	75/55,719 (0.135)	52	163/100,030	0.16%
D2S1338	15/72,830 (0.021)	157/82,310 (0.19)	90	262/225,140	0.12%
D19S433	38/70,001 (0.05)	103,460 (0.075)	71	187/173,490	0.11%
SE33 (ACTBP2)	0/330 (<0.30)	0/51,940 (0.04)	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)