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Section 1 Screening of Biological Evidence

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

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

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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, dying, or mechanical damage)
- Soft texture (pliable)
- little or no taper

Pubic Hairs

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

Suitable for Autosomal STR Analysis

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

DNA analysis of a hair consumes a portion of the hair, preventing subsequent examinations. If the analyst concludes that the hair(s) examined may be suitable for nuclear DNA analysis, a digital image of the hair's root will be included in the bench notes.

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1.2 Vaginal/Cervical, Rectal and Oral Swabs and Smears

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

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

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

As an alternative to processing as described below, these swabs may be processed via a QIAcube protocol, following the decision tree in Appendix B.

1.2.1 Microscopic Examination of Samples

This procedure is used for the examination of swab samples as well as for stains that test positive for the presence of acid phosphatase. The following procedure will provide an extract of the soluble substances and a pellet of the particulate material for analysis. This procedure is also used for extraction of swabs.

Microscope slides are packaged with the item after examination. Screening extracts are discarded after all analyses are completed.

Sample Extraction

- Label a sterile 2.0 mL tube for each sample to be extracted.
- Add 200µL of sterile, deionized water and a small cutting of the stain/swab(s) into each tube. Stains/swabs collected from the same area may be grouped.
- Soak for at least 30 minutes at 37°C. The extraction may also be accomplished overnight at 37°C or in the refrigerator at 4°C.
- Label a microscope slide for each sample.
- Agitate the material in each tube with a clean toothpick or a sterile pipette tip and then spot 3µL of the extract onto a labeled microscope slide.

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

- Agitate the cutting/sample using a clean toothpick for approximately one minute.
- Use a clean toothpick to transfer the cutting into a spin basket and insert into the 2.0mL tube containing the extract.
- Centrifuge for 1 minute (at speed sufficient to pellet the cellular material) and then discard the basket with the cutting.
- Re-suspend the pellet and then spot 3µL of the extract onto a labeled microscope slide. The 3µL may be withdrawn from the pellet, but this should be clearly stated in the bench notes.
- The extract is retained in the refrigerator, until completion of the case, for possible P30/PSA testing.

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.
- Slides may be examined microscopically prior to staining. Intact spermatozoa can often be detected. If intact spermatozoa are observed, staining is not required.

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

Interpretation of Results

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

If spermatozoa are detected, note if they are intact and score the number observed according to the scale below. Record the presence of nucleated epithelial cells (NECs) and non-nucleated cellular debris. NECs may also be graded using the scale given below.

<10 Few
1 + Hard to find
2 + Some in fields, easy to find
3 + Many or some in most fields
4 + Many in every field

If no spermatozoa are observed, one of the following should be performed to confirm the negative result:

- P30 analysis on the aqueous portion of the extract (supernatant)
- Preparation of a second slide (may occur after a 2nd epithelial digestion during DNA extraction)
- Verification of the negative slide by a second qualified analyst

1.2.2 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 after obtaining a positive presumptive test for seminal fluid (acid phosphatase) with negative sperm search results.

Procedure

- This procedure is done following extraction and a microscopic examination as previously described.
- Retrieve the extract prepared in the previous section.

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- Option: place the substrate into a spin basket and centrifuge the sample for 3-5 minutes.

- Remove the device and dropper from the sealed pouch. Label the PSA card with the case number, item number, your initials and the date.
- If refrigerated, allow the sample(s) to come to room temperature prior to testing.
- 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 results of the test are documented using digital imaging and included in the analyst's bench notes.

Interpretation of Results

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

Negative: The formation of only one pink line in the control area (I) indicates a negative test result. This may indicate that (a) No PSA is present above 4ng/ml or (b) presence of "high dose hook effect". Presence of "high dose hook effect" may give a false negative result due to the presence of high concentrations of PSA in the sample. If this is suspected, based on preliminary acid phosphatase test results, the sample should be retested using a 1:10 to a 1:1,000 fold dilution of the sample in question using the remaining 100µl of sample.

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

1.3 Miscellaneous Evidence Swabs

Suspected blood or semen samples may be examined using the screening methods described in this manual.

As an alternative to processing as described previously, these swabs may be 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.

Miscellaneous swabs suspected to contain foreign saliva or skin cells do not require biological screening before proceeding to DNA analysis.

1.4 External Genitalia Swabs

Swabs collected from the external genitalia area may be examined for the presence of semen/spermatozoa using the screening methods described in this manual. Penile swabs obtained from the victim/suspect within 24 hours of the offense may be examined for the

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presence of blood, epithelial cells or spermatozoa (useful in cases involving multiple suspects or to help establish recent contact).

As an alternative to processing as described previously, external genitalia swabs may be processed via a QIAcube protocol, following the decision tree in Appendix B. However, if utilizing the QIAcube protocol, penile/scrotum swabs may proceed directly to the QIAcube procedure without PSA testing.

1.5 Fingernail Scrapings / Finger and Hand Swabs

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

As an alternative to processing for semen as described previously, these samples may be processed via a QIAcube protocol, following the decision tree in Appendix B.

1.6 Condoms

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

- Document the condition of the condom. Imaging may be used as needed.
- Label one pair of sterile cotton swabs "outside". Moisten the swabs using sterile deionized water. Swab the "outside" surface of the condom, as received.
- Prepare a smear from the swabs collected.
- Repeat for the "inside" surface of the condom.
- Stain and grade the prepared smears as previously described in this manual.
- The swabs and prepared slides are retained as a new item of evidence.

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

As an alternative to processing as described previously, condom swabs may proceed directly to the QIAcube protocol without further screening.

1.7 Contact/Wearer Sources of DNA

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

- Document the item by 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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1.8 Semen Stain Analysis

The detection and identification of semen is achieved by visual examination, presumptive chemical testing for the presence of acid phosphatase, confirmation by microscopic identification of spermatozoa, and occasionally 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.

As an alternative, once a stain has been isolated, it may be processed via a PlAcure protocol, following the decision tree in Appendix C.

1.8.1 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.

1.8.1.1 Alternate Light Source Examination using the Omniprint™ 1000B

Reference: OmniprintTM 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 and microscopic confirmatory tests.

<u>General Safety Guidelines</u>

- 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.

<u>Proper Operation of the System</u>

- Check to see that both switches are in the “off” position.
- Plug the unit into a three-prong grounded outlet. If an extension cord is used, it must be a heavy duty grounded cord.

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- Turn on the power rocker switch (marked “Line”). The switch will light, and the fan will begin to operate. You may now turn the lamp switch on (marked “Lamp”). The lamp should light within a few seconds.
- You may hear a ticking noise and see brief flashes of light while the lamp is attempting to ignite. This is normal.
- If the lamp fails to ignite within two minutes, and you hear a ticking noise, turn the unit off. The lamp may have failed. Lamps are guaranteed for 500 hours of use, provided it has been used in the prescribed manner (periods of at least 15 minutes). Replace the lamp (see Lamp Changing Instructions provided with the manual). The lamp should be left running for periods of at least 15 minutes. The lamp must cool after it has been turned off and should not be restarted until it has fully cooled.
- Although the minimum suggested operating time is 15 minutes, it is important to note it is better to operate the lamp for continuous periods, rather than turning the lamp on and off. This procedure will increase lamp life.
- If you do not hear a ticking noise and the lamp does not light, this is an indication that the power supply is not functioning properly. Please contact Omnichrome for further instructions.
- Operation in high ambient temperatures – the Omniprint™1000B is equipped with over temperature protection. If the instrument is being operated at ambient temperatures exceeding 100° F, the over temperature protector may shut off the lamp. When the unit cools sufficiently, the lamp will come back on automatically.

Filter Selections

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

- 450nm band when used with yellow goggles or filters provides near UV excitation. Generally the optimum setting for the detection of physiological stains such as semen, saliva, urine and blood. Forensic odontologists and pathologists prefer this setting for bite mark and bruising detection and photographic documentation.
- Prior to the examination of casework evidence, a known semen stain control is examined with the wavelength set at 450nm. This ensures that the system is functioning properly. The stain must exhibit the expected fluorescence for the unit to be used in casework. This check is performed each day that the unit is in use and is documented in the bench notes.

Shutdown Procedure

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

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

1.8.2 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).

1.8.2.1 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 microscopic examination and/or a PSA test as previously described.

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 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 color and the negative control should not exhibit a color change. The controls must function as expected for the test results on questioned stains to be valid.

Additional Notes

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

1.8.2.2 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 microscopic exam and/or PSA test as previously described.

Procedure

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

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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.

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1.9 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.

1.9.1 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.

1.9.2 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.

1.9.2.1 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 (H_2O_2) is a presumptive positive test for the presence of blood. A pink color forming after one minute should not be considered as a positive result, as auto oxidation can occur in air and light.

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 H_2O_2 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.

1.9.2.2 Detection of Human Hemoglobin (hHb) using OneStep ABAcard HemaTrace

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

Procedure

- Place a portion of the suspected bloodstain or swab into an entire labeled tube of the extraction buffer provided.
- Allow the sample(s) to extract for 1 – 5 minutes.
- If refrigerated, allow the sample(s) to warm to room temperature.
- Remove the device/card and dropper from the sealed pouch and label the HemaTrace card with the lab number, item number, your initials and the date.
- Add 4-5 drops of the extract, using the dropper provided, into the sample region (S) on the card.
- Allow the sample to stand for 10 minutes. Positive results can be seen as early as 2 minutes depending on the hHb concentration.

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- Document the test results by digital imaging and include in the bench notes.

Interpretation of Results

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

Positive: The formation of two pink lines, one in the test area (T) and in the control area (I) is a positive result, indicating that the human hemoglobin concentration is at least 0.05µg/ml. Another analyst should confirm weak results. This verification will be documented in the bench notes.

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

- No human hemoglobin is present above 0.05µg/mL, or
- Presence of "high dose hook effect". Presence of "high dose hook effect" may give a false negative result due to the presence of high concentrations of human hemoglobin in the sample. If suspected, based on visual inspection of the stain, the sample should be retested using a 1:100 or 1:1,000 fold dilution of the sample.

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

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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

Sperm Findings	**Report**
Microscopic examination for spermatozoa	**Spermatozoa/few spermatozoa/no spermatozoa** were observed/detected (microscopically) in/on …
Blood Findings	**Report**
Phenolphthalein (Kastle-Meyer)	A stain(s) testing **positive/negative** with a presumptive test for blood was/were located/detected on (**item**). No blood was detected on (item) with a presumptive test for blood. This **stain/sample** tested **positive/negative** to a presumptive test for blood.
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 (no spermatozoa observed)	No spermatozoa were observed microscopically; but 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 spermatozoa observed)	No spermatozoa were observed microscopically and 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	(No) (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 3mm punch.
- Bloodstains may also be sampled by swabbing with a damp, sterile swab if the substrate is difficult to cut, or potentially contains PCR inhibitors (e.g. cigars or denim).
- Bloodstained swabs are usually sampled by cutting a portion of the stained area of the swab.

3.1.2 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 should be tagged with a label containing the case number and item number.
- Typically, known buccal swabs require only 1/3 of one swab or a small portion of two swabs.
- When the sampled swab material is too large for a single tube, it should be split into multiple tubes and the extracts ultimately recombined. The elution volume should be adjusted so that the final volume allows for at least half to be retained after analysis. Ensure that reagent blanks are treated similarly.

3.1.3 Fingernail Swabs/Scrapings

Samples are received either as swabs or debris that is scraped into a paper bindle. Swabs from under the nails are generally sampled by cutting the swab tips into the sample tube. However, as in the case of contact swabs, the entire swab may be used with at least half of the extract being retained for future use.

- When sampling debris in a paper bindle, a moistened swab is used to swab the scraper, visible debris and the inside of the paper bindle. Typically one swab is used per hand. The entire swab is then cut into the sample tube. When the bindle contains a large amount of debris, a representative amount may be sampled.
- Any extract remaining after analysis shall be retained since these samples may not be homogeneous.

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3.1.4 Fingernails or Fingernail Clippings

- Fingernails or fingernail clippings should be imaged prior to sampling.
- Swabs from the nail(s) may be collected for profiling of tissue and cellular debris external to the nail itself. The entire swab tips are cut into the sample tube.
- If surface material, i.e. blood or dirt, is observed on the nail, the nail may be washed by immersing in 200µL 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 10µL 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 200µL 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 10µL 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.
- The remaining portion of the hair is re-packaged with the evidence.
- DNA extracts from hairs will always be retained.

3.1.6 Other tissue samples

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

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.
- The Large Volume protocol requires an additional reagent blank because of the addition of Buffer MTL after digestion. When using the Large Volume protocol, be sure to aliquot enough dilute G2 to your reagent blank tube (≥700µl of dilute G2 buffer) before digestion to have enough post-digestion solution to extract two separate reagent blanks to accompany the Trace and Large Volume protocols, respectively. Alternatively, a second reagent blank tube can be set up prior to digestion and used exclusively as the reagent blank for Large Volume protocol sample(s).
- Where incubations are required, they may be performed in either an incubator or in a thermomixer set at 900 rpm and appropriate temperature.
- Questioned samples shall be eluted in TE buffer.
- Known samples may be eluted in water or TE buffer.
- Elution volumes are selected based on sample type and/or quantity:
    - Larger elution volumes (200µl) may be selected for most reference samples, large blood stains, 4+ sperm samples, 4+ epithelial fractions and reference samples
    - Smaller elution volumes (no less than 40 uL) are recommended for reference blood samples from decedents, previously extracted database samples, contact DNA samples and samples with few sperm or epithelial cells.
    - If the entire sample was consumed, the total elution volume shall be no less than 40 uL, and at least half of the extract volume shall be retained.
    - If more than one elution volume is used in a set of extractions, the corresponding reagent blank(s) should use the most stringent elution volume used in that set.

3.2.1 Direct (non-differential) Extraction

1. Prepare the pre-digest solution:
    - (Number of samples + 3) x 230 µl G2 buffer
    - (Number of samples + 3) x 230 µl sterile de-ionized water
    - (Number of samples + 3) x 10 µl Proteinase K

2. Add enough pre-digest solution to each sample cutting to allow for at least 200µl of free liquid in the tube after absorption by the substrate.

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

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3. Incubate at 56ºC. Known samples shall be incubated for at least one hour. Question samples will typically be incubated overnight. Exceptions will be noted in the bench notes.

4. Following incubation, transfer 200µl of the digest buffer into a sterile 1.5mL screw cap tube. If absorption by the swab is unusually great, transfer as much of the digest as possible, up to 200µl. (Optional: a spin basket in a 2 mL tube may be used to remove the substrate from the digest; this may be particularly helpful for recovery of DNA from samples expected to have low quantities of DNA.) For all casework questioned samples, add 1 µl carrier RNA solution to the transferred digest solution. It is not necessary to add carrier RNA to reference sample extracts.

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 500µl of the digest solution to an EZ1 sample tube, add 1µl carrier RNA solution, and add 400µl of Buffer MTL.*
- *Load the sample(s) onto the EZ1, select the Large-Volume Protocol, and elute in no less than 40µl TE buffer. An additional reagent blank with Buffer MTL added must be extracted with all Large-Volume Protocol samples.*

Option 2 – Large-Volume Protocol for Consumed Samples:

- *If the entire evidence sample has been consumed and digested, the analyst shall transfer the entire volume of digest solution to an EZ1 sample tube (or tubes – with no more than 500µl in each tube), add 1µl of carrier RNA solution and add 400µl of Buffer MTL.*
- *Run the Large-Volume protocol and elute in no less than 40µl 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.*
  ***NOTE**: If any sample is extracted using the Large-Volume protocol and eluted in a small volume (such as 40 or 50µl), then the reagent blank accompanying that sample will suffice as the reagent blank for a consumed sample extracted using the Large-Volume protocol and eluted in any larger volume (such as 100 or 200µl) If no other Large-Volume protocol samples are extracted, ensure that a reagent blank is extracted using the Large-Volume protocol and eluted in the lowest elution volume used.*

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 a protocol (Trace or Large-Volume), elution buffer and elution volume.

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

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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.

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 2ºC to 8ºC. Extracts should be dried down with DNAstable LD for long-term storage.

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

12. Clean the piercing unit after each use by selecting option 2 ("Man") on the main menu, then option 3 ("Clean"). Then wipe each piercing unit down with ethanol.

3.2.2 Differential Extraction with manual wash protocol

1. Prepare the 1:1 diluted G2 buffer:
   - (Number of samples + 3) x 1040µl G2 buffer
   - (Number of samples + 3) x 1040µl distilled water

2. Cut the sample and place in a 2.0ml tube.

3. Add at least 600µl of sterile de-ionized water to each of the tubes. The volume of water added should allow for full submersion of the swabs.

4. Vortex briefly. Incubate overnight at 37ºC. Pre-soaks/water soaks with less incubation time shall be performed only with documented pre-approval of the Forensic Biology Supervisor or DNA Technical Manager after evaluation of the sample.

5. Agitate the samples by vortexing or using a sterile pipette tip or a sterile toothpick to remove the cells from the substrate.

6. For each sample, transfer the substrate to a spin basket and place the basket back in the sample tube. Centrifuge the sample tubes for 5 minutes at approximately 14,500 RPM.

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7. Transfer approximately 550µl of the supernatant to a new, labeled 2.0mL tube. Also transfer the spin basket containing the substrate. Retain until the case has been completed.

8. Re-suspend the cell pellet and spot 3µl on a microscope slide. Stain and grade the slide (as described in Section 1).

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

OPTIONS:

- If few/no sperm/epithelial cells are observed, a second extraction may be performed by adding more of the original evidence item to the sample tube and repeating steps 2-8.

- Alternatively, the substrate may be added back to the sample tube for digestion or it may be digested separately.
    - If adding the substrate back to the sample tube, place substrate back in the tube and proceed to step 10.
    - If digesting separately, place substrate into a new 1.5mL tube and label it as "Q#sub"; analysis of the substrate resumes at step 17.

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

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

10. To the re-suspended cell pellet (may include substrate if added back), add up to 390µl of 1:1 diluted G2 buffer + 10µl of Proteinase K solution.

11. Vortex and spin briefly to force the material into the extraction fluid.

12. Incubate for at least two hours at 56ºC. During the incubation step label a new sterile tube for each sample, including the reagent blank. Label these as the epithelial fractions.

13. Centrifuge the sample tubes for 5 minutes at approximately 14,500 RPM. If the substrate was added back to the tube (step 9 option), using a sterile toothpick to place the substrate into a spin basket and centrifuge for 5 minutes at approximately 14,500 RPM. Remove as much as possible of the supernatant without disturbing the sperm pellet and transfer to the epithelial fraction tube. Analysis of the epithelial fraction resumes at step 19.

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

14. Wash the sperm pellet by adding approximately 500µl of dilute G2 buffer. Vortex briefly and centrifuge at approximately 14,500 RPM for 5 minutes.

15. Remove and discard the supernatant.

16. Repeat the wash two more times for a total of three washes. If few sperm were observed, the number of washes for the sperm fraction may be decreased.

17. Add 190µl of 1:1 diluted G2 buffer + 10µl Proteinase K solution + 10µl DTT.

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

18. Vortex and spin briefly to force the material into the extraction fluid.

19. Incubate overnight at 56ºC.

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

21. Turn on the EZ1 power switch on the back of the instrument.

22. Directions are displayed on the screen on the front of the instrument. Press the START button to select a protocol (Trace or Large-Volume), elution buffer and elution volume.

23. 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.

24. <u>For sperm fractions</u>:
    - Transfer 200µl of the digest to a 1.5mL screw cap tube, add 1µl carrier RNA solution, run Trace protocol and elute in 40µl to 200µl TE buffer.

    <u>For epithelial fractions</u>:

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- Transfer 200µl of the digest to a 1.5mL screw cap tube, add 1µl carrier RNA solution, run Trace protocol and elute in 40µl to 200µl TE buffer.

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

Option 1 – Large-Volume Protocol:

- *For samples that typically yield less DNA or if the sample required additional digest buffer for thorough cell lysis, the analyst may transfer up to 500µl of the digest solution to an EZ1 sample tube, add 1µl carrier RNA solution, and add 400µl of Buffer MTL to each tube(s).*

- *Load the sample(s) onto the EZ1, select the Large-Volume Protocol, and elute in no less than 40µl TE buffer. An additional reagent blank with Buffer MTL added must be extracted with all Large-Volume Protocol samples.*

Option 2 – Large-Volume Protocol for Consumed Samples:

- *If the entire evidence sample has been consumed and digested, the analyst shall transfer the entire volume of digest solution to an EZ1 sample tube (or tubes – with no more than 500µl in each tube), add 1µl of carrier RNA solution and add 400µl of Buffer MTL to the tube(s).*

- *Run the Large-Volume protocol and elute in no less than 40µl TE buffer.*

  ***NOTE**: If any sample is extracted using the Large-Volume protocol and eluted in a small volume (such as 40 or 50µl), then the reagent blank accompanying that sample will suffice as the reagent blank for a consumed sample extracted using the Large-Volume protocol and eluted in a larger volume (such as 100 or 200µl). If no other Large-Volume protocol samples are extracted, ensure that a reagent blank is extracted using the Large-Volume protocol and eluted in the lowest elution volume used.*

25. Upon completion of the EZ1 protocol, remove and cap the elution tubes containing the purified DNA.

26. The extracted DNA is now ready for quantification and amplification. DNA extracts can be stored for several weeks at 2ºC to 8ºC. Extracts should be dried down with DNAstable LD for long-term storage.

27. 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.

28. Clean the piercing unit after each use by selecting option 2 ("Man") on the main menu, then option 3 ("Clean"). Then wipe each piercing unit down with ethanol.

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3.2.3 Differential Extraction with QIAcube Automated Wash Protocol

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 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 PSA card extract (This section may be omitted for previously screened samples and those proceeding directly to extraction):

A. Place the sample in a 2.0 mL tube.

B. Add enough sterile water to the sample such that at least 250 uL can be easily removed later. (500 uL works well for one full swab tip.)

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. Pipette off water without disturbing the substrate. This extract may now be used with PSA cards, as described in Section 1.2.2. This should be done for items not previously screened for the presence of semen and includes relevant clothing and external body swabs, as well as orifice swabs collected in under 24 hours.

Sample preparation for differential extraction:

3. Add 480 uL G2 and 20 uL Proteinase K to each sample. Optional: a G2 buffer / Proteinase K master mix may be prepared with (number of samples +1) * 480 uL G2 and (number of samples + 1) * 20 uL Proteinase K; distribute 500 uL of master mix to each sample.

4. Incubate at 56° C for 1.5 – 2 hours at 900 rpm on a thermomixer.

5. Centrifuge briefly to remove liquid from inside the lid.

6. Transfer the substrate to a spin basket and replace in the tube.

7. Centrifuge at ~14500 rpm for 5 minutes.

8. Remove substrate in spin basket to a separate labeled tube.

9. Vortex to thoroughly re-suspend, and centrifuge briefly to remove liquid from inside the lid. 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 uL, wide-bore.

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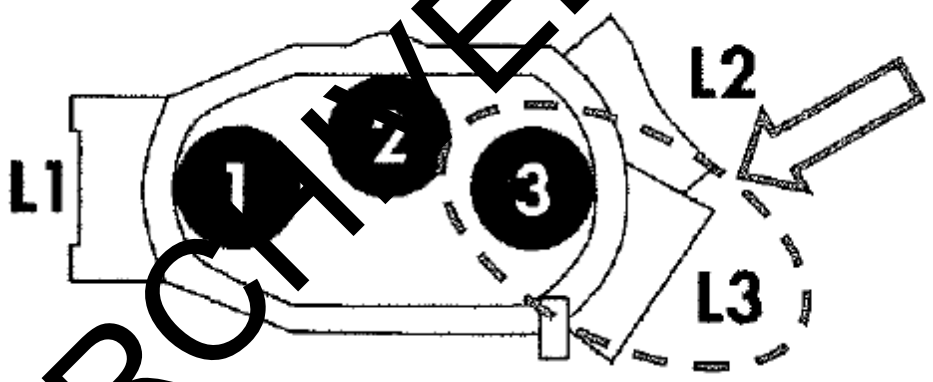
11. Aliquot Buffer G2 in 30 mL reagent bottle in Reagent Bottle Rack position 1. Do not fill above the fill line. Minimum volumes of G2 buffer are listed in the table below:

# samples	Buffer G2 (uL)
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. Refer to the QIAcube Loading Chart for correct tube position.

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



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

Running the automated wash protocol:

16. Remove G2 bottle cap.

17. Turn on the QIAcube by pressing the power button.

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

19. At the conclusion of “Separation and Lysis 12 A” protocol, refill the tip racks as needed and ensure that both the Buffer G2 reagent bottle and sterile water reagent bottle are in place and uncapped.

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. Remove 3 uL to a microscope slide and allow to air dry. 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.

23. Add the following reagents to each sperm fraction:

140 uL Buffer G2

20 uL Proteinase K

40 uL 1M DTT

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

(number of samples + 1) * 140 uL Buffer G2

(number of samples + 1) * 20 uL Proteinase K

(number of samples + 1) * 40 uL 1M DTT

Distribute 200 uL of master mix to each sample.

24. Vortex briefly. 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 uL warm Buffer MTL to the 2 mL sample tube, along with 1 uL cRNA. Run Large Volume extraction protocol with elution in TE buffer volume no less than 40 uL (see below).

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27. Sperm fraction: Add 1 uL cRNA. Run Trace protocol with elution in TE buffer volume no less than 40 uL (see below).

EZ1 Extraction:

Refer to Section 3.2.1 (Direct extraction) steps 5 – 12 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.

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Section 4 DNA Quantification

Questioned samples are routinely quantified in duplicate. When a consumed sample is eluted in 50 uL or less, a single quantification reaction is performed in order to preserve extract. Quantification is not mandatory for database samples or forensic known 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). 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).
  - 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).
      Standard Curve wells are already correctly assigned. The template default includes one NTC reaction. Be sure that the final plate includes two NTC reactions.
    - 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.

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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 ≈ 50ng/μL
Standard 2 ≈ 16.7ng/μL
Standard 3 ≈ 5.56ng/μL
Standard 4 ≈ 1.85ng/μL
Standard 5 ≈ 0.62ng/μL
Standard 6 ≈ 0.21ng/μL
Standard 7 ≈ 0.068ng/μL
Standard 8 ≈ 0.023ng/μL

4.3 Preparing the Reactions

- Bring the reagents to room temperature before pipetting.
- It is recommended that preparation of the reactions take place in a designated hood in order to minimize the introduction of contamination as well as dust or particulate matter.
- 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.

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- Pipette the required volumes of the components into a sterile tube. Vortex and centrifuge briefly.
- Dispense 23uL of reaction mix into each reaction well.
- Add 2uL of sample, standard or control to the applicable wells. 2uL of dilution buffer is added to each NTC well.
- Seal the plate with the Optical Adhesive Cover. Use an applicator to create a firm seal between each of the wells.
- Centrifuge the plate at 3000 rpm for at least 20 seconds to remove any bubbles.

4.4 Sample Loading

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

4.5 Starting the Run

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

4.6 Analyzing the Data

- When the run is complete and the quality checks described below in 5.6.1 through 5.6.4 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 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

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quantity. 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 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. The IPC C_T value for each reaction is expected to fall in the range of 28 to 31. When a C_T for an IPC is greater than 31, the sample should be assessed for possible amplification inhibition. 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.

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 5.1.1. 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.

4.6.5.1 Using female:male ratio to assess suitability for STR analysis

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. This ratio is documented on the relevant page of the Experiment Results Report.

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- 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.

Because of the low success rate of obtaining data suitable for probative comparisons for the minor contributor(s) in mixtures where the ratio of contributors is greater than 10:1, this ratio may be used to determine course of future use of the extract. Extracts for which the male DNA is the probative component, but for which the female:male ratio is greater than 10:1, should be recommended for Y-STR testing and retained with no 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.

4.6.5.2 Absence of male DNA where male DNA is probative

In cases such as sexual assaults with female victims and alleged male assailants, the absence of detectable levels of male DNA (as demonstrated by an undetermined Quant Duo Male result) in the presence of any female DNA means that no STR analysis will be performed on that sample.

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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*

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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: pp16-32cyc**

  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.

- 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 as follows (in individual 0.5mL tubes):

  Forensic Case Samples
  Add approximately 0.5ng to 1ng template DNA. The amount of template DNA may be higher or lower depending on the sample (i.e. low-level samples that are likely to be mixtures may be targeted higher, while bloodstains likely to be single-source may be targeted lower). Add sterile de-ionized water to bring the sample to a final volume of 19.2µL.

  Quantification Value <0.05ng/µl
  Samples with a quantification value <0.05ng/µl shall be routinely amplified in duplicate to ascertain reproducibility of alleles.

  Quantification Value >0.05ng/µl
  Samples with a quantification value >0.05ng/µl need not be routinely amplified in duplicate.

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

  Reagent Blank Sample(s)
  Add 19.2µL of the extraction reagent blank.

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

Negative Control Sample
Add 19.2µL of sterile de-ionized water.

- Transfer the entire 19.2µL of the above prepared samples to the appropriate sample well, already containing the PCR master mix. During sample addition, the pipette tip is inserted through the Press 'n Seal.
- Once all samples have been added, remove the Press 'n Seal and cover the plate with a sheet of amplification tape.
- Transfer the plate to the PCR room and place directly into the thermal cycler. 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.

- 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:
  - PPlex16_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 3500 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

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.

The panel for casework on the 3500xl is: 3500_PowerPlex_16_Casework.

7.1 Casework Analysis Methods

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

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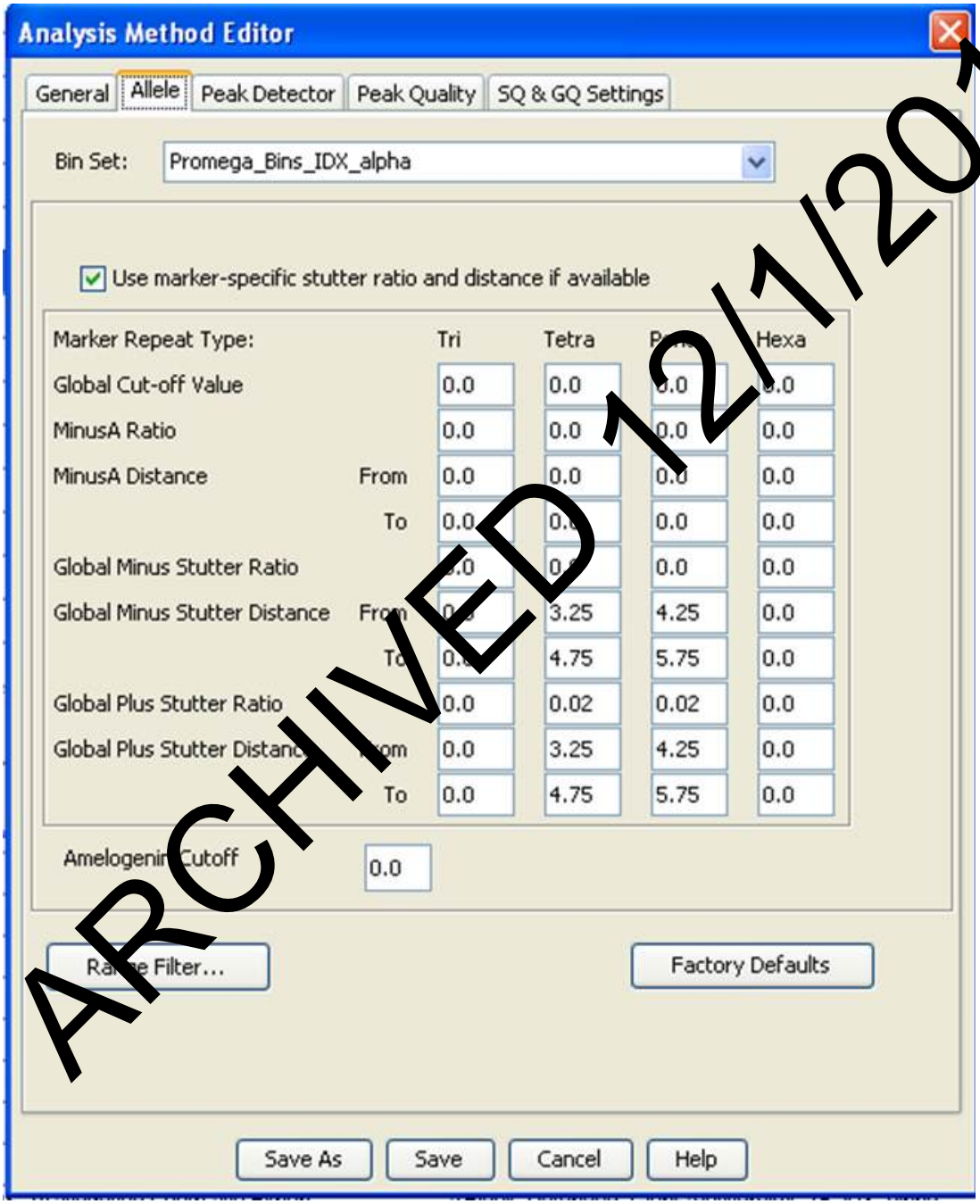
7.2 Casework Allele Tab Settings

These settings are consistent within all casework methods.



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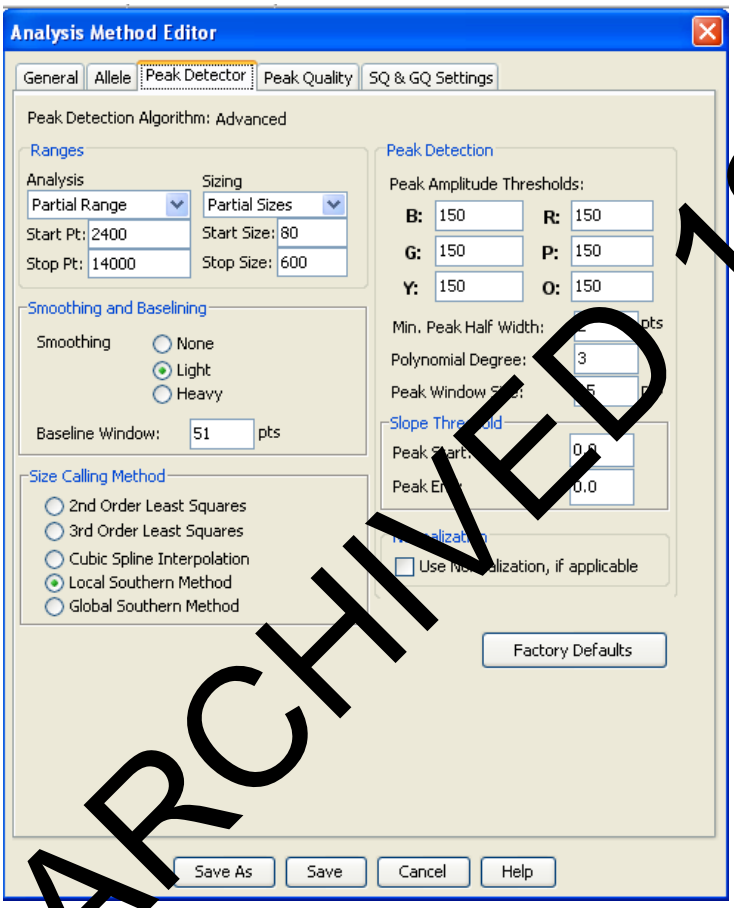
7.3 Casework Peak Detector Tab Settings

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.

<u>Example: 2400 Casework Analysis Method</u>



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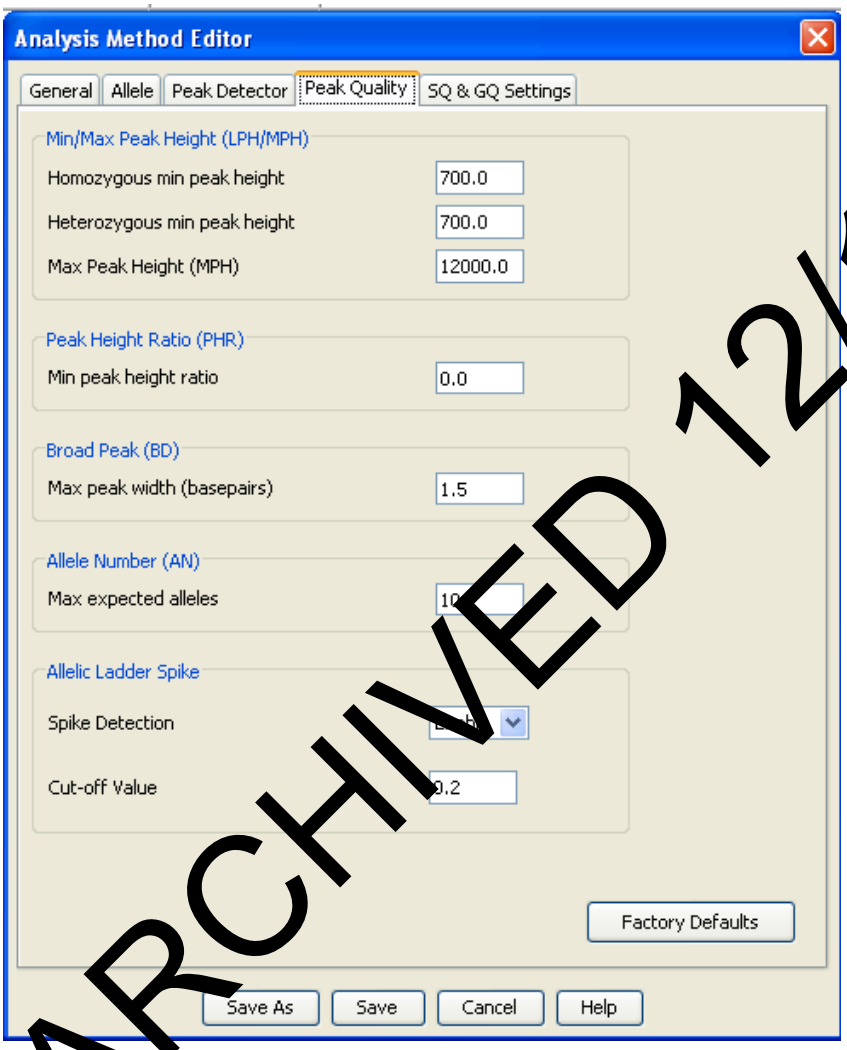
7.4 Casework Peak Quality Tab Settings

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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7.5 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.

<u>All Analysis Methods</u>

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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
DBRT	Data below reporting threshold
ART	Artifact
TRI	Tri-allele
OBL	Obligate allele
UND	Undetermined

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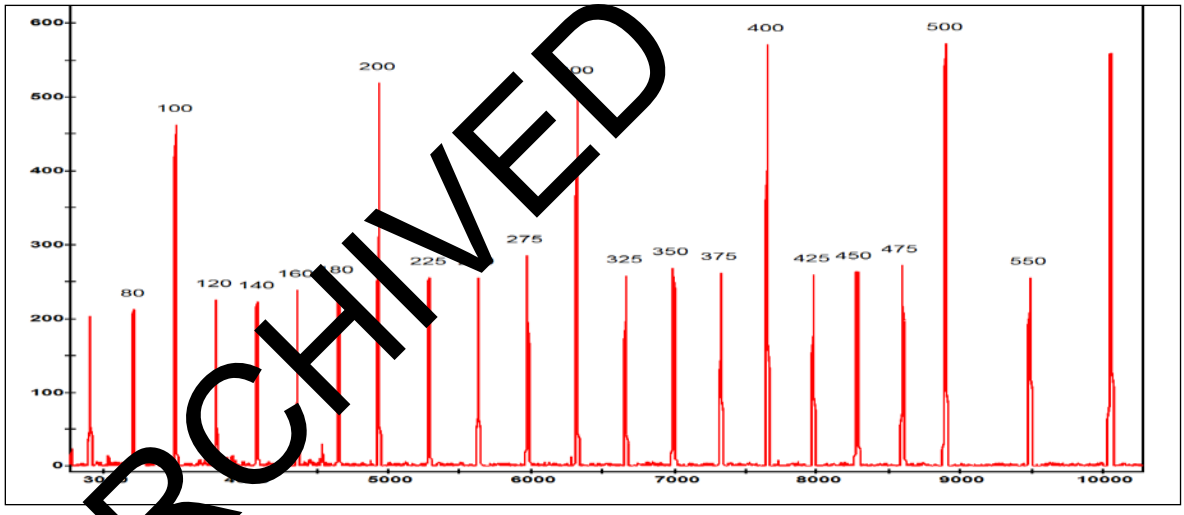
DNA Abbreviations and Definitions (continued)	
( )	Minor component allele
[ ]	Major/Minor components not separated

8.1 Interpretation of Batch Controls

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

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 CODIS 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 calls for the samples in the project.

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

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

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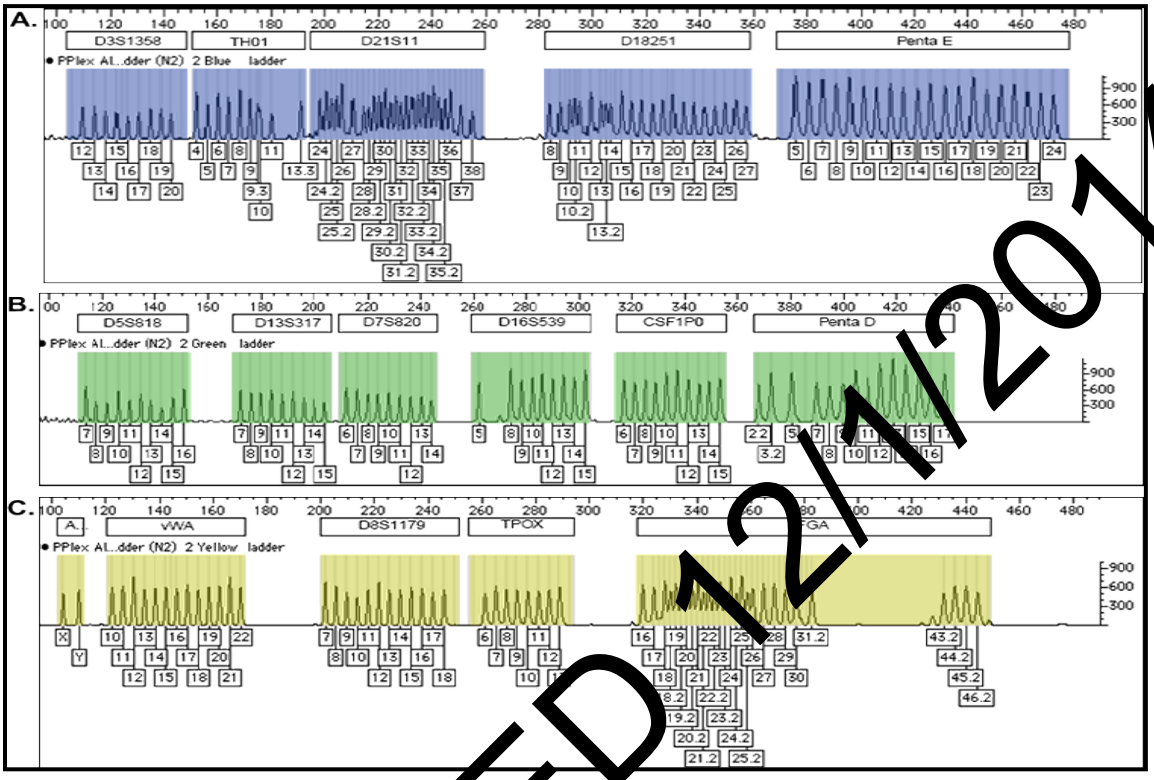
PowerPlex16® Allelic Ladder



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8.1.3 Internal Control Specimen (ICS)

An internal control specimen (ICS) is a designated, predetermined quality control sample whose expected profile is already known. The ICS is processed with an extraction batch and carried through the batch concurrently with the other samples. The purpose of this control is to demonstrate that all analytical processes are working correctly. An ICS may include any known sample, including those obtained from laboratory staff, where the individual has been previously typed to generate a genetic profile.

At least one ICS shall be processed with each casework batch, and will typically be extracted with the known reference samples. 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.

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 9947A

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.

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 peaks can be shown to be artifacts. Artifacts will be documented on the electropherogram.

When probable true allele peaks are detected above the reporting threshold, at several or all STR loci, the analyst will investigate possible causes. The Technical Manager will then be consulted to determine the appropriate course of action.

Since the failure of a negative control may indicate a problem at the extraction or amplification level, a Quality Review Form may need to be completed based on the nature of the discrepancy.

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

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.

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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 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 6%	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 17%	

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).

8.2.3 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 CXR) emit their maximum fluorescence at different wavelengths, but there is some overlap of the emission spectra. A spectral calibration is performed for a specific dye set to create a matrix that corrects for the spectral overlap.

Pull-up is the result of incomplete separation of the emission spectra and is typically observed as a non-allelic peak at the same base size as a peak in another dye. 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 call is determined by simultaneously examining the base sizes for the sample allele peak and the associated allelic ladder peaks in the relevant locus.

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

OL alleles considered to be true alleles may require re-amplification to confirm, particularly when they are only observed in a mixture. If the OL allele is observed in 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 (A/TA).

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,

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 GeneMapper™ ID-X software with a number and should lie within the range of the alleles in the ladder, and they are clearly distinguishable from artifacts. All alleles are examined and evaluated in the process of data interpretation.

In addition to the Analytical Threshold (AT) of 150 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

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 ST 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.

Reported refers 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 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.

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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 male 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 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.

Samples with a quantitation result of undetermined do not require duplicate amplification.

Questioned samples with a quantification value ≥0.05ng/µl 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.

Intimate samples: If all the alleles below ST can be attributed to a non-probative contributor (such as the owner on a body swab.), then those alleles may be included in the STR table and used for interpretation. Since they are attributable to an intimate source, statistical considerations do not apply.

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.

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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).

The assessment of a questioned sample profile takes place with 'sequential unmasking': initial assessment occurs before any probative reference samples are compared to that profile. However, an evidence item taken directly from an identified anatomical location (i.e., vaginal swab, penile swab, fingernail scrapings, etc.) and / or a piece of intimate apparel (i.e., undershirts, panties, bra, etc.) typically will yield DNA from the individual from which the evidence item was taken. In such circumstances, it is common to recover a DNA mixture which includes DNA consistent with that individual. In such cases it is useful to use the "owner's" (or any other non-probative) reference profile to assist in the interpretation of the questioned sample.

8.4.1 Single Source Samples

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

Peak height ratios are calculated by dividing the peak height (in RFUs) of the lower RFU allele by the peak height of the higher RFU allele, and then multiplying 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.

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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 allelic peaks (i.e., if at most five alleles are detected at a locus, the DNA results are consistent with having arisen from at least three individuals, provided that none of those peaks are consistent with being elevated stutter). However, phenomena such as tri-alleles, primer binding site mutations, or allele sharing between close relatives can complicate the determination of number of contributors.

8.4.2.1 DNA Mixture Type Categorization

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

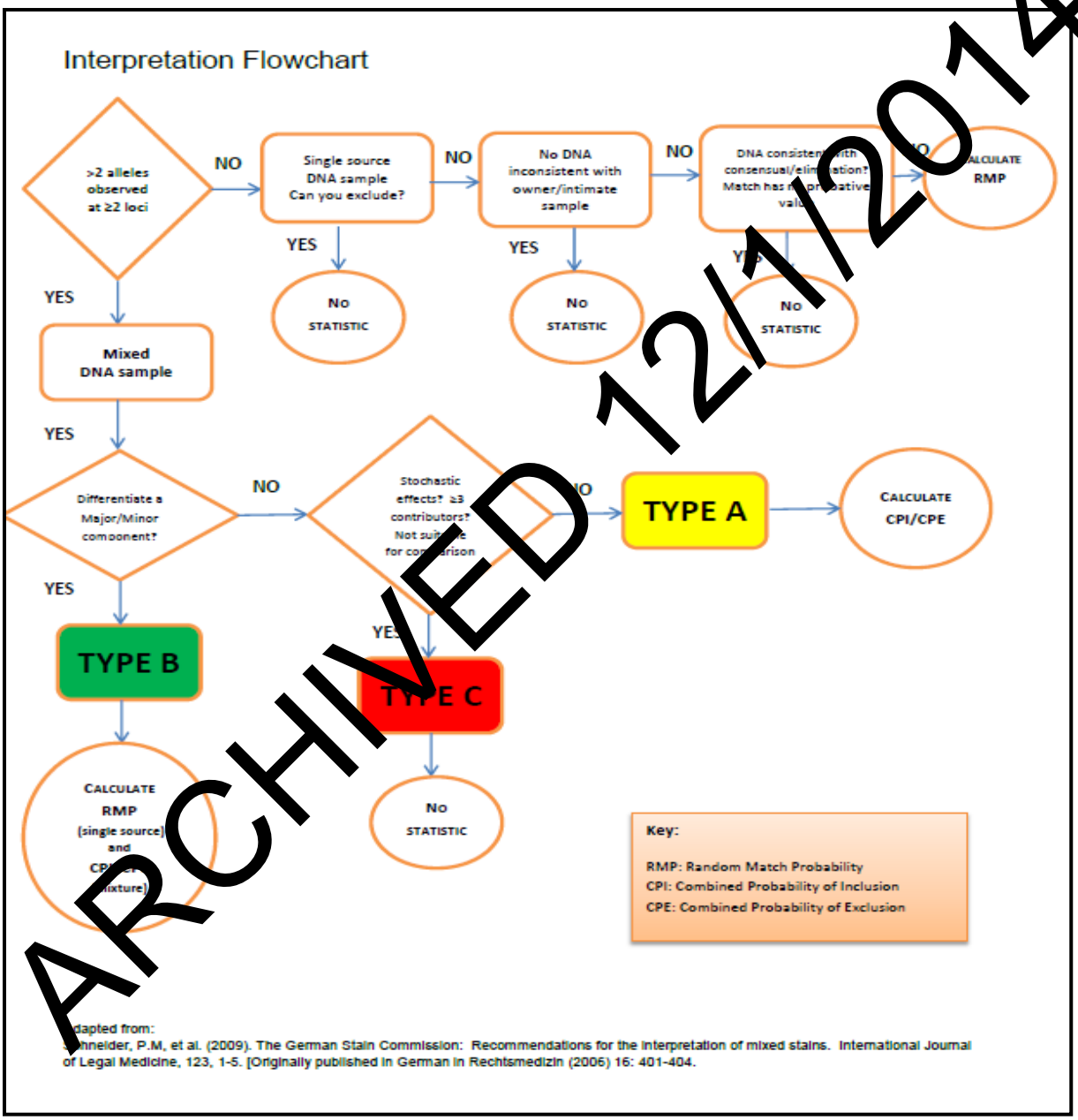
Three categories of mixed samples have been designated to guide general decision making as illustrated in the Interpretation Flowchart. Mixtures with a known contributor (i.e. intimate samples) are possible in each category:

- **TYPE A**: mixtures with indistinguishable contributors, including multiple major contributors
- **TYPE B**: mixtures with major/minor contributors
- **TYPE C**: indistinguishable mixtures containing DNA from at least three sources and/or mixtures that exhibit potential stochastic effects

TYPE A:

Description: If the amounts of biological material from multiple contributors are similar, it may not be possible to further refine the mixture profile. When major or minor contributors cannot be distinguished because of similarity in signal intensities (i.e. peak heights), the sample is considered to be an indistinguishable mixture. In two source mixtures where the “owner” can be assumed to be one contributor, the “owner” reference sample may be used to deduce a single source unknown contributor.

The classification of indistinguishable does not imply that the profile is not interpretable. Individuals may still be included or excluded as possible contributors to an indistinguishable mixture. 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.



Adapted from:
Schneider, P.M, et al. (2009). The German Stain Commission: Recommendations for the interpretation of mixed stains. International Journal of Legal Medicine, 123, 1-5. [Originally published in German in Rechtsmedizin (2006) 16: 401-404.

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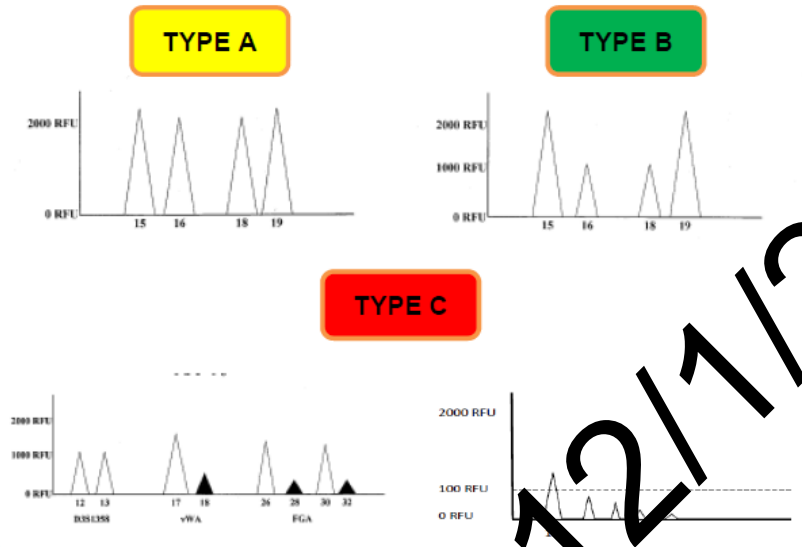

Adapted from:
Budowle, B., et al. (2009). Mixture Interpretation: Defining the Relevant Features for Guidelines for the Assessment of Mixed DNA Profiles in Forensic Casework. *J Forensic Sci, Vol. 54, No. 4, 810-821*

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.

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

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

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

8.4.2.2 Separating Major and Minor Contributors in DNA Mixtures

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.

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.

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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.

8.4.2.3 Deducing an Unknown Contributor in a DNA Mixture
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 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.

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- 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.

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

8.5 Comparison of DNA Results

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

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

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

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

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

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:

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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.

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.*
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 a sample collected from that person's own body. 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 and double click to select either Forensic Single Sample or Forensic Mixture from the menu.
- In the Specimen ID field, enter the lab #_Item#
- 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 Popstats pull-down menu.
- Forensic Single Source
  - Click the 1/f button.
  - Click the printer icon or select Print from the File pull-down menu. Select Broward Report and print.
- Forensic Mixture
  - The result should default to the Inclusion probability

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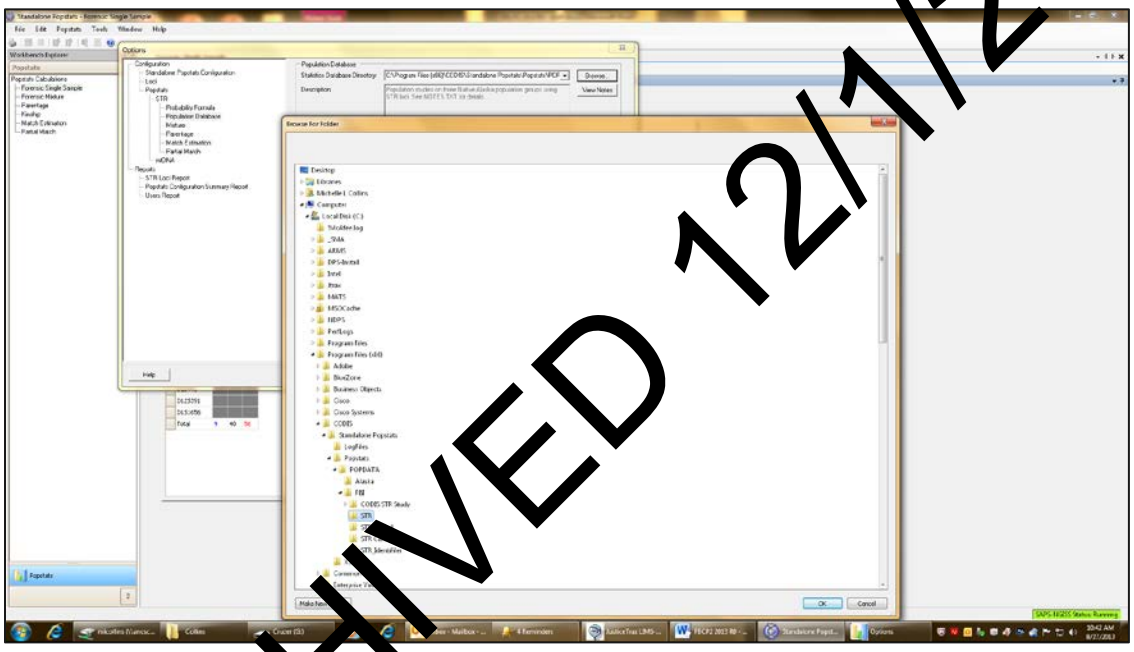
- Click the printer icon or select Print from the File pull-down menu. Select Probability of Inclusion (Short) and print.

Note: The above steps must be performed for both the Alaska Native database and the FBI STR database.

- To select a database, choose Options from the Tools pull-down menu.
- Click on Population Database and then Browse to navigate to the desired database.
- Select either the Alaska or STR folder. Click OK, Apply and OK.

Selecting the Caucasian and African-American Population Databases



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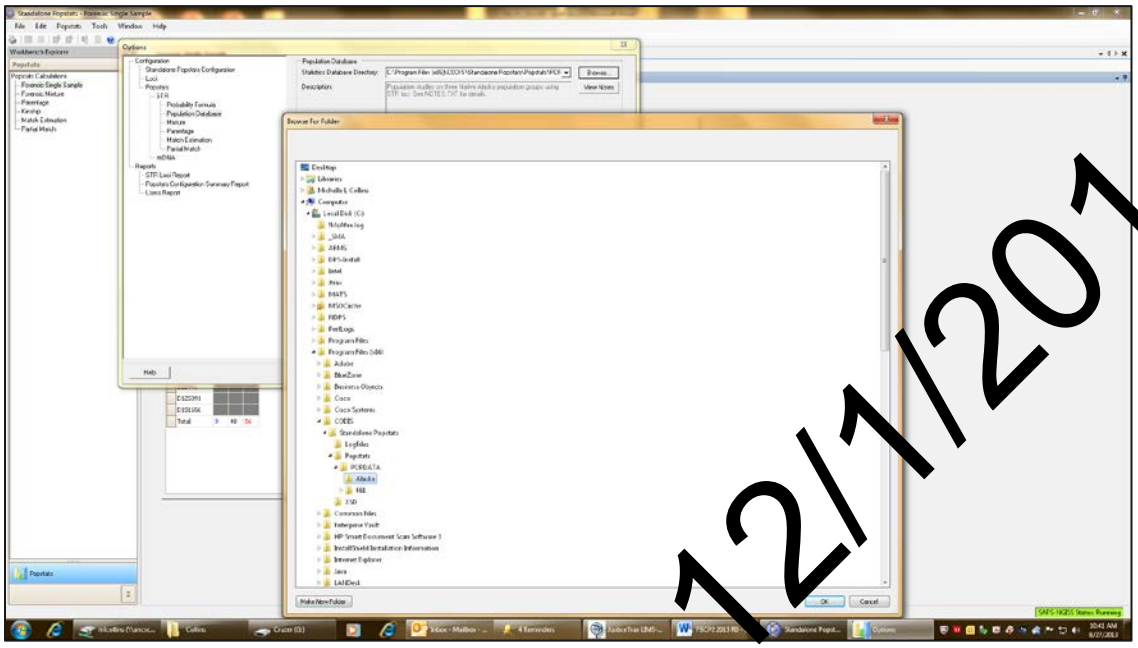
Selecting the Alaska Native Population Databases



Note: 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.

- To select a theta value, choose Options from the Tools pull-down menu.
- Click on Probability Formula and select the correct theta value from the pull down menu shown in the image below.
- Click Apply and OK.

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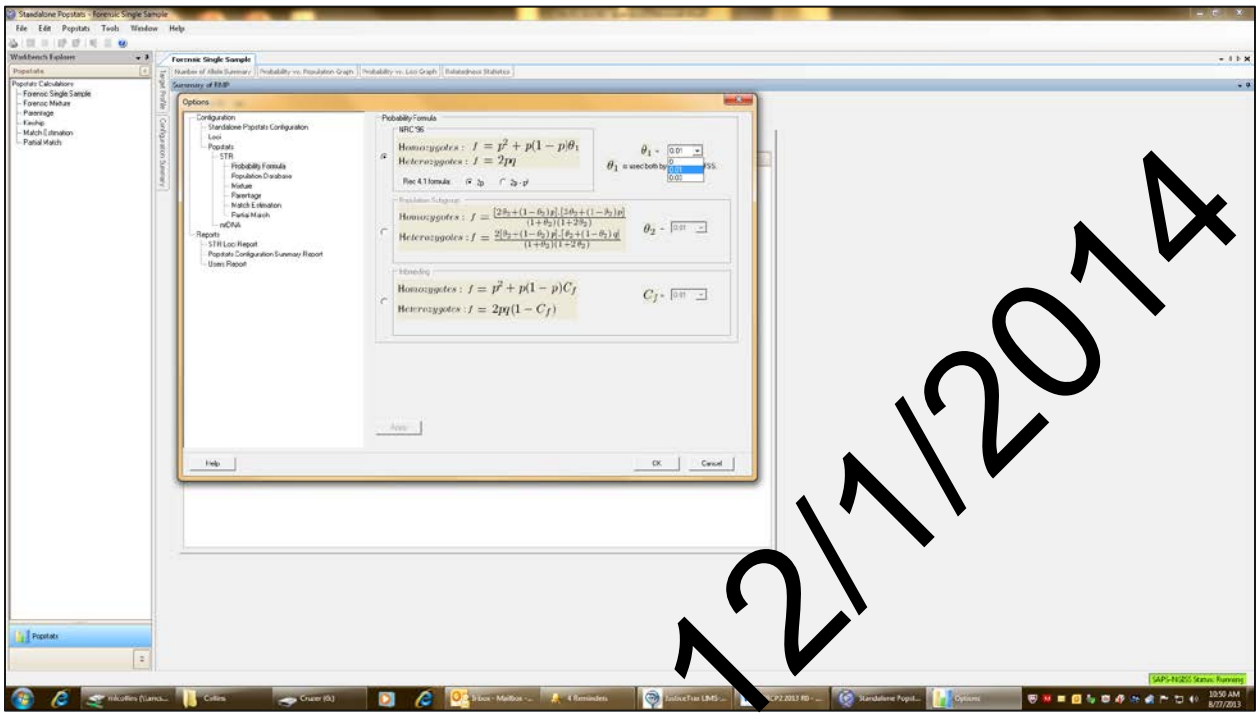

- Once the theta value is changed, it is necessary to close out the calculation window for the change to take effect. This is done by clicking the black X button near the top right of the window. Clicking the red X in the top right will close out the Popstats program entirely.
- Select Forensic Single Sample from the menu to repeat the process for the second population database.

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Section 10 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 Report 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.
- 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)

10.2 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	Quantification results do not indicate the presence of sufficient male DNA for STR analysis. No further analysis was performed on this sample. 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

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	**<u>Single Source formula</u>** The estimated frequency (13 core loci) of the genetic profile from the above sample(s) is approximately 1 in XXX quadrillion (Caucasian population), 1 in XXX quadrillion (African-American population), 1 in XXX quadrillion (Athabaskan population), 1 in XXX quadrillion (Inupiat population) and 1 in XXX quadrillion (Yupik population).

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For single source and/or mixtures	Report
Unknown Male DNA	A genetic profile from an unknown male individual was obtained from this sample.
Female present	The genetic profile obtained from 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	**Mixture formula:** 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).
Close relatives	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).

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Deduced Profile with Single Source statistics	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. XXX cannot be excluded as the source of the deduced genetic profile. No DNA inconsistent with XXX and YYY was detected in this sample. A single source genetic profile for the unknown contributor could not be deduced at the following loci: XXX, XXX and XXX. Therefore, these loci were not used to calculate population frequency statistics. **<u>Single Source Formula</u>** ……
Deduced Profile with Mixture and Single Source statistics	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. 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. **<u>Mixture formula</u>** …… 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. A single source genetic profile for the unknown contributor could not be deduced at the following loci: XXX, XXX and XXX. Therefore, these loci were not used to calculate population frequency statistics. **<u>Single Source Formula</u>** …….

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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.

10.3 Review of Casework Analyzed In-House

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

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

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- 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 or probability of paternity in such cases.
- Two or three loci with non-consistent genetic markers – these results are inconclusive. The CPI and Probability of Exclusion 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 and Probability of Exclusion will be calculated and reported.
- All loci have consistent genetic markers – the alleged parent cannot be excluded, and the CPI and Probability of Exclusion will be calculated and reported.

11.2 Using Standalone Popstats 7.0 for Parentage Statistics

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

A. One-parent forward paternity (trio):

1. Open Popstats and choose Parentage from the menu on the left side of the screen.
2. Choose the Parentage tab at the top of the screen.
3. Use the Lab ID field to enter the lab number at least, item number space permitting. If the item numbers do not fit in the typed filed 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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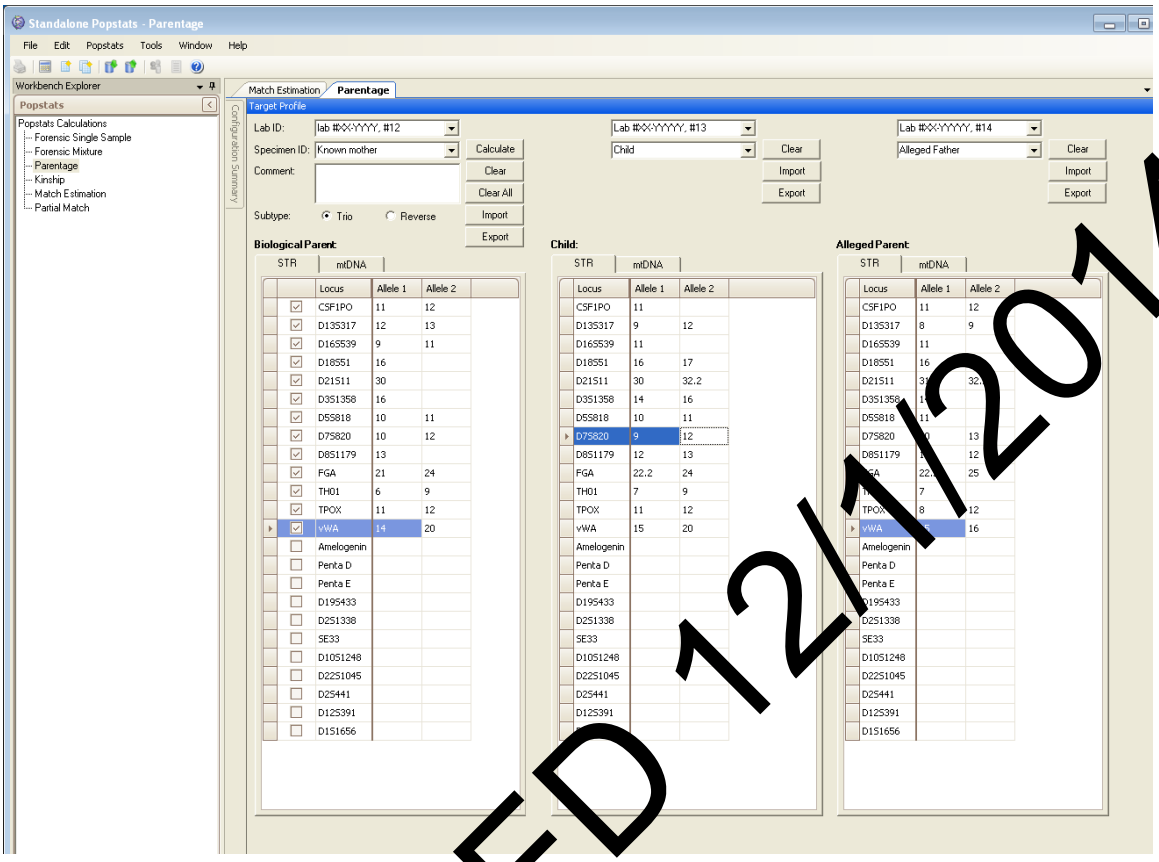

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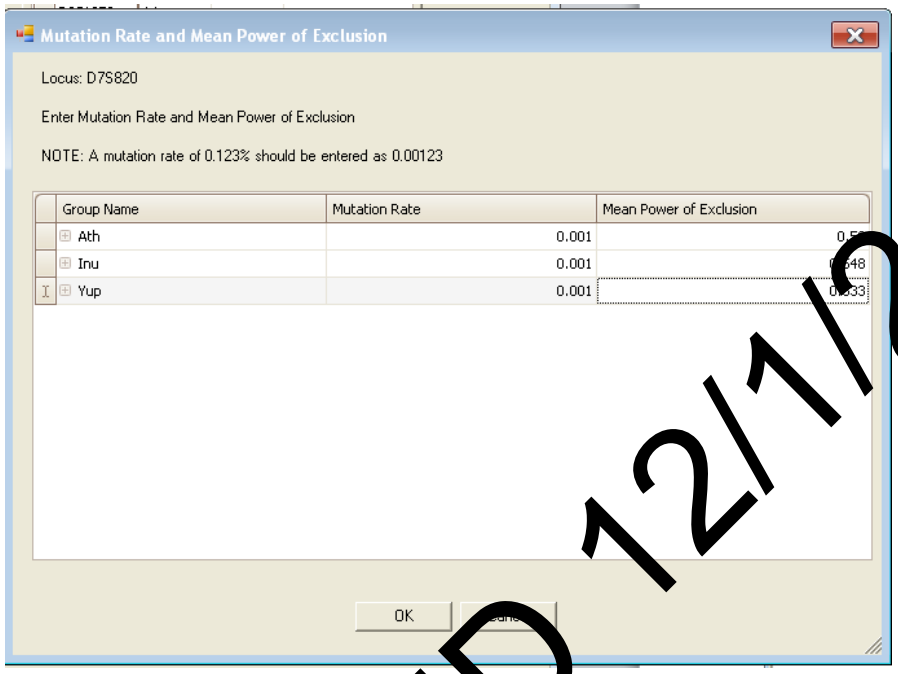
6. When the correct information has been entered, select the Calculate button (upper left).
7. If any loci have mutations (mismatched), you will be prompted to enter the mutation rate and the mean power of exclusion (see following image).



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 FBI – STR 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, click the Target Profile tab to return to the data entry screen, where you will again choose Calculate.
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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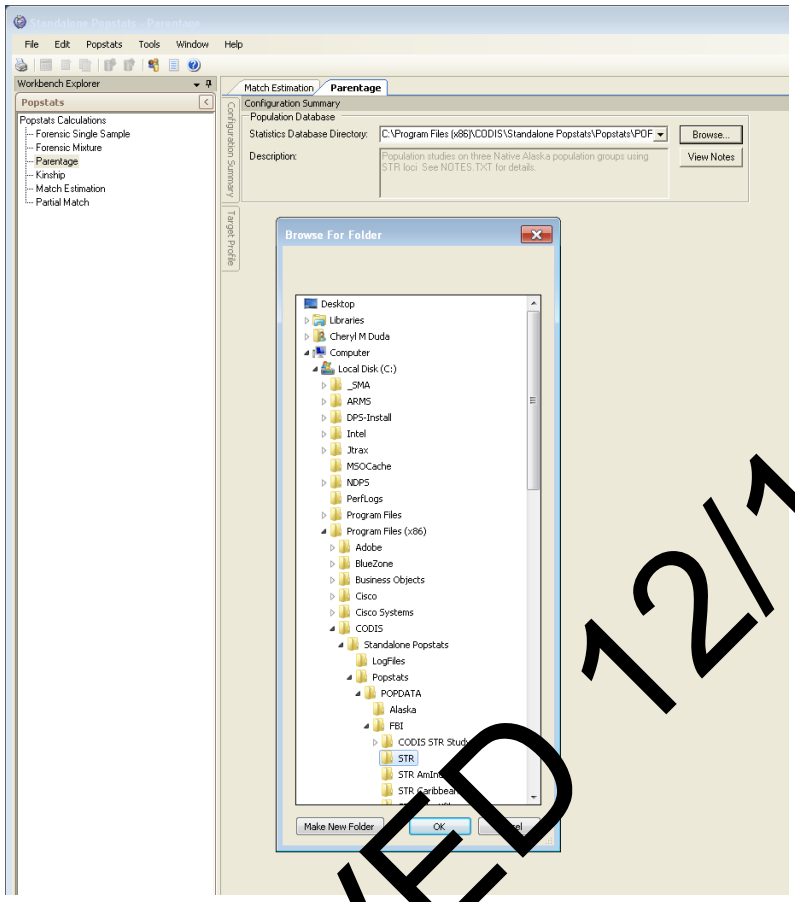

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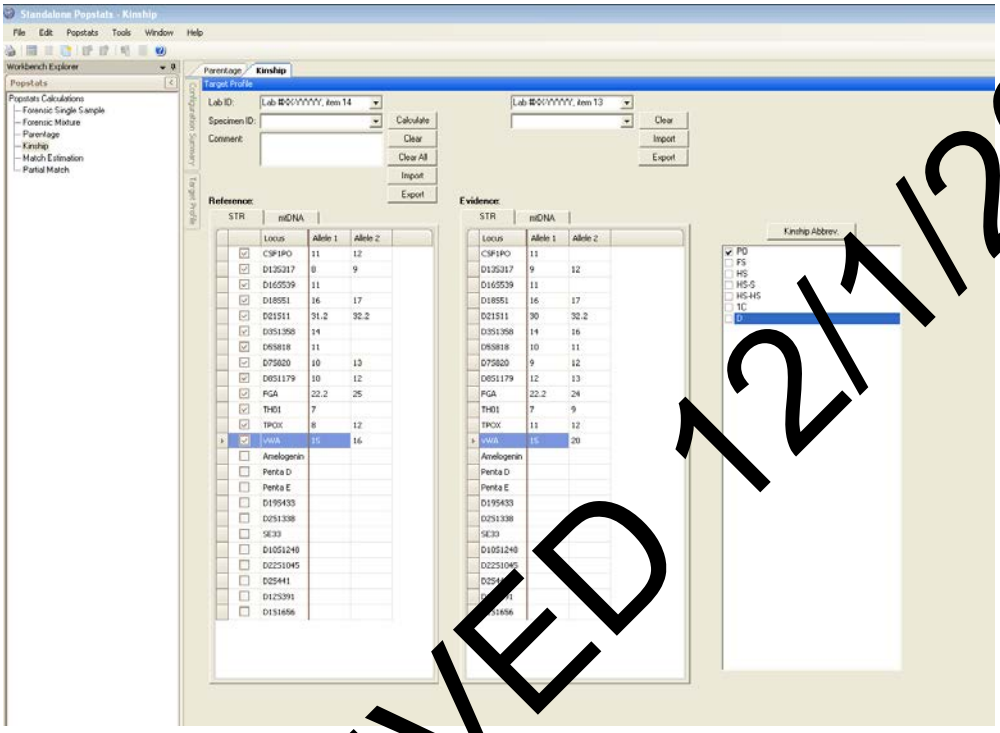
B. Reverse parentage (trio):
   1. Open Popstats and choose Parentage from the menu on the left side of the screen.
   2. Choose the Parentage tab at the top of the screen.
   3. Use the Lab ID field to enter the lab number at least, item number space permitting. If the item numbers do not fit in the typed filed they may be hand-written on the printout.
   4. Under Subtype, select the Reverse button (see following image)
   5. Repeat steps 5-12 from Section A.
   6. On reverse parentage trios, Popstats does not allow for the use of a locus with a mutation. In cases with a mutation, omit the locus with the mutation from the Popstats calculation. On the Popstats printout, manually calculate the PI for the locus with the mutation. Use this manual calculation to adjust the CPI and Probability of Paternity calculations as well. Show work clearly.

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 filed 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).

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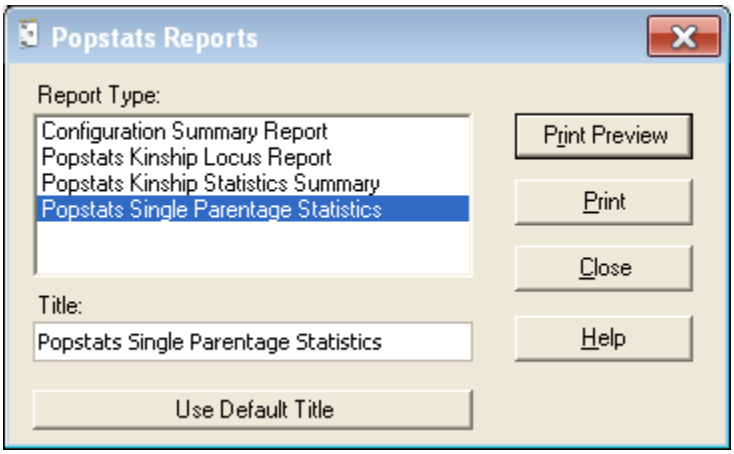

7. Print out statistic reports for the Caucasian and African-American databases (from the FBI – STR database file) and the Athabaskan, Inupiat, and Yupik databases (from the Alaska database file). Directions and image for toggling between databases are listed in Section A step 12.

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11.3 Sample Report Language

Sample report language for one parent forward paternity

Inclusion:

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

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

Population	Combined Paternity Index	Probability of Paternity
Caucasian		
African-American		
Athabaskan		
Inupiat		
Yupik		

Combined paternity index indicates how many times more likely the observed genetic evidence is if the alleged father is the true biological father of the tested child rather than an unrelated individual from each of the above populations.

Probability of paternity assumes a prior probability of 0.5 and is compared with an untested, 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, assuming MOTHER'S NAME is the biological mother of CHILD'S NAME.

Inconclusive:

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

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Sample report language for zero parent forward parentage

Inclusion:

Based on the DNA profiles obtained for the samples listed above (XX 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	Probability of Exclusion
Caucasian		
African-American		
Athabaskan		
Inupiat		
Yupik		

Combined parentage index indicates how many times more likely the observed genetic evidence is if FATHER'S NAME is the true biological father of CHILD'S NAME rather than an unrelated individual from each of the above populations.

Exclusion:

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

Inconclusive:

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

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Sample report language for reverse parentage

Inclusion:

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

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

Population	Combined Parentage Index	Probability of Exclusion
Caucasian		
African-American		
Athabaskan		
Inupiat		
Yupik		

Combined parentage index indicates how many times more likely the observed genetic evidence is if ALLEGED CHILD is the true biological child of FATHER and MOTHER rather than unrelated individuals from each of the above populations.

Exclusion:

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

Inconclusive:

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

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11.4 References

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.

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 upon completion of the technical and administrative reviews 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 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.

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Biological Screening Review Checklist

		Technical Review	Administrative Review
LAB NUMBER:			
ANALYST:			
Date Submitted for Review:			
Technical Review Started:	Reviewer:		
Administrate 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 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		
SOWs are linked to request in LIMS		
Chain of Custody for all tested items can be tracked through RLS and LIMS		
Assign DNA holding to DNA Supervisor		
VERIFICATION REVIEW	**Analyst:**	**Date:**
Semen/ABAcard® p30 Test		
Species/ABAcard® HemaTrace®		
Hairs - Stereoscopic		

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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					
TPOX					
FGA					

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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

STR Casework Review Checklist

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

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	Analyst:	Tech. Review:	Admin. Review:
DNA CASEWORK REPORT AND BENCH NOTES			
Pages are numbered correctly, case # and analyst initials are on each page, report dated			
Requesting agency, agency case #, crime lab case #, and officer's name are correct			
Chain of Custody for all tested 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 printouts are included in the case file			
Popstats DNA profile frequencies are consistent with those shown in 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			
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 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 of eligible profiles to be entered into CODIS (include specimen category and SDIS or NDIS)			
Specimen eligibility verified prior to CODIS entry (including match estimation, if applicable)			
Correct profile(s) and agency information entered in CODIS			
Appropriate specimen category selected			
Source ID updated if applicable			
Offender/arrestee laboratory comments updated in LIMS, if applicable			

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

NOTES:

STR Single Offender Review Checklist

Date:_____
Technical Review Started: _____

Lab Number: _____

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OFFENDER FOR DNA DATABASE OR CODIS CONFIRMATION

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

Appendix A Revision History

FBCP 2014 R0 Page	FBCP1 2014 R0 Page	Location	Revision made
5	11	**Section 1.2**	**Added** the sentence beginning "As an alternative…"
7	14	**Section 1.2.2**	**Added** to Interpreting negative results section "based on preliminary acid phosphatase test results"
7	14	**Section 1.3**	**Added** the sentence beginning "As an alternative…"
7	14	**Section 1.4**	**Added** the paragraph beginning "As an alternative…"
8	14	**Section 1.5**	**Added** the sentence beginning "As an alternative…"
8	15	**Section 1.6**	**Added** the sentence beginning "As an alternative…"
9	15	**Section 1.8**	**Added** the sentence beginning "As an alternative…"
15	22	**Section 1.9.2.2**	**Added** to Interpreting negative results section "based on visual inspection of the stain"
16	25	**Section 2.1**	**Revised** last bullet point to include technical reviewer
19	29	**Section 3.1**	**Added** *"Reference: EZ1 DNA Investigator Handbook"*
19	29	**Section 3.1.2**	**Revised** first and last bullet points.
21	31	**Section 3.2**	**Added** under General Instructions the sentence beginning "Where incubations are required…"
21	32	**Section 3.2.1**	**Removed** from step 2: (no less than 450µl)
22	32	**Section 3.2.1**	**Removed** the sentence beginning **"** If the reagent cartridges are being stored between 2ºC and 8ºC…" from step 3
22	32	**Section 3.2.1**	**Added** to step 4 the sentence beginning "(Optional: a spin basket may be used…"
23	34	**Section 3.2.2**	**Revised** section heading to "Differential extraction with manual wash protocol"
24	36	**Section 3.2.2**	**Replaced** "all but 50µl" with "as much as possible …without disturbing the sperm pellet"
25	36	**Section 3.2.2**	**Removed** the sentence beginning **"** If the reagent cartridges are being stored between 2ºC and 8ºC…" from step 19
27	n/a	**Section 3.2.3**	**Added** section: Differential Extraction with QIAcube Automated Wash Protocol
31	39	**Section 4**	**Revised** first paragraph

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98	n/a	**Appendix B**	**Added** Biological screening of body swabs incorporating the QIAcube decision tree
99	n/a	**Appendix C**	**Added** Biological screening of stains for semen incorporating the QIAcube decision tree
FBCP 2014 R0 Page	**FBCP2 2014 R0 Page**	**Location**	**Revision made**
39	5	**Section 6.2**	**Added** two sentences which each begin with "The expiration dates…"
39	5	**Section 6.2**	**Added** "to 60 °C" to pre-heat instructions.
57	n/a	**Section 8.2.6**	**Added** contamination check to the review process
58	25	**Section 8.3.3**	**Removed** reference to non-probative profiles
64	31	**Section 8.4.2.3**	**Revised** description of samples used for deduction
67	34	**Section 9**	**Revised** first paragraph, beginning at "Statistical analysis…"
75	42	**Section 10.2**	**Revised** report language for deduced profiles
76	n/a	**Section 10.2**	**Added** report language for contamination detected
91	47	**Forms**	**Added** to STR Casework Review Checklist "QC check of unidentified profile (if applicable)"
FBCP 2014 R0 Page	**FPM 2014 R0 Page**	**Location**	**Revision made**
78-87	5, 8-19	**Section 11**	**Moved** from Forensic Parentage Manual
98	20	**Appendix D**	**Moved** from Forensic Parentage Manual
FBCP 2014 R0 Page	**FBAM 2014 R0 Page**	**Location**	**Revision made**
57-58	27	**Section 8.3.1**	**Moved** definitions of *reported* and *observed*.
64	5	**Section 8.4.2.2**	**Moved** sentence beginning "In instances when low-level contamination…"
64	5	**Section 8.4.2.3**	**Moved** and **modified** sentence beginning "For profiles which contain contamination: if the source of

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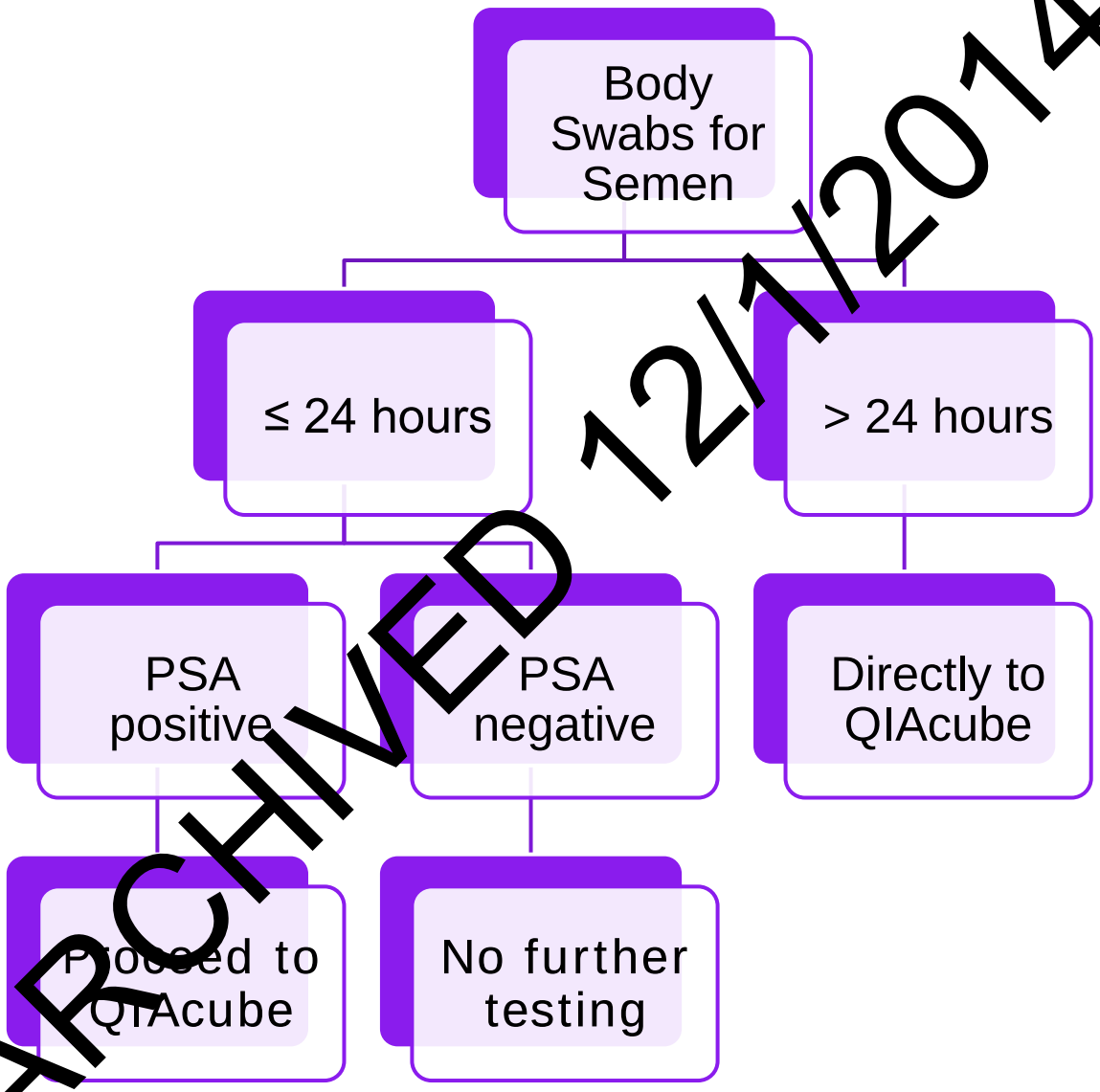
Appendix B Biological screening of body swabs incorporating the QIAcube decision tree

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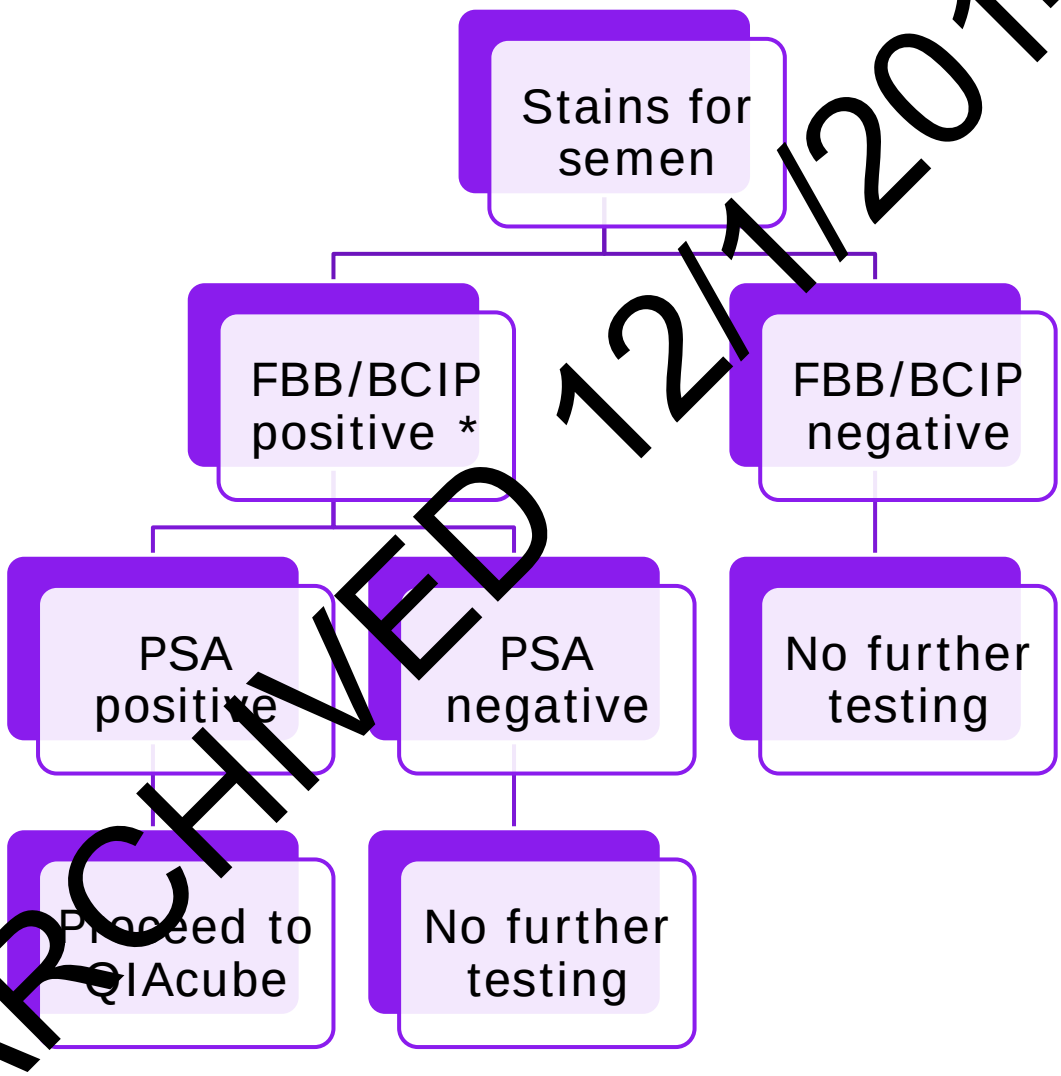
Appendix C Biological screening of stains for semen incorporating the QIAcube decision tree

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



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

Appendix D: 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.[illegible]%**
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	[illegible]259/89[illegible]7	**0.14%**
D13S317	192/482,136 (0.04)	881/621,146 (0.14)	485	[illegible]558/1,103,28[illegible]	**0.14%**
D16S539	129/467,774 (0.03)	540/494,465 (0.11)	[illegible]/2	1,04[illegible]59	**0.11%**
D18S51	186/296,244 (0.06)	1,094/494,098 (0.22)	[illegible]	1,[illegible]/790,342	**0.22%**
D21S11	464/435,388 (0.11)	772/526,708 (0.15)	580	1,816/962,096	**0.19%**
Penta D	12/18,701 (0.06)	21/2[illegible]1 (0.09)	[illegible]24	57/41,202	**0.14%**
Penta E	29/44,311 (0.065)	7[illegible]719 ([illegible])	59	163/100,030	**0.16%**
D2S1338	15/72,830 (0.021)	15[illegible]2,[illegible] (0.10)	90	262/225,140	**0.12%**
D19S433	38/70,00[illegible]	[illegible]/103,489 (0.075)	71	187/173,490	**0.11%**
SE33 (ACTBP2)	0/33[illegible] (<0.30)	330/51,610 (0.64)	None reported	330/51,940	**0.64%**

*Data used wi[illegible] permi[illegible]ion fr[illegible]an Association of Blood Banks (AABB) 2003 Annual Report.

Includes co[illegible]ilatio[illegible] 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 [illegible]nt paternity testing laboratories provided this STR mutation data.

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Data from the NIST webpage http://www.cstl.nist.gov/div831/strbase/mutation.htm (current as of 1/9/14)