

FORENSIC BIOLOGY SECTION



CASEWORK TEST METHODS

**INDIANA STATE POLICE
FORENSIC BIOLOGY SECTION
TEST METHODS**

FOREWORD

The Forensic Biology Section in the Forensic Services Division of the Indiana State Police (ISP) conducts tests on various body fluids, body fluid stains, and human hair for criminal justice agencies. DNA analysis is performed as needed on various biological materials. The Forensic Services Division reserves the right to evaluate and prioritize the items submitted and limit the total number to expedite service and to manage available resources. The accuracy and specificity of test results are ensured by running known controls with each set of tests.

The analysts of the Forensic Biology Section shall have a minimum of a baccalaureate or an advanced degree in a natural science or a closely related field. Analysts should have successfully completed college coursework covering subject areas such as genetics, biochemistry, molecular biology, and statistics. All analysts undergo a formalized training program covering forensic techniques and instrumentation. Successful completion of the Training Program is required before analysis of evidence is performed. Additionally, all analysts participate in external proficiency testing from an accredited vendor.

A technician shall follow the same guidelines and procedures as an analyst in all methods in which they are authorized.

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1. Serology Methods:

- 1.1. **Scope:** This test method is designed for the guidance of Biology Section personnel who assist investigations where body fluids or stains occur as physical evidence in a variety of alleged criminal activities. The scope of this type of evidence includes but is not limited to the following: identification of human blood, semen, and human hair. This test method may be expanded or altered as techniques and/or new genetic analyses are found applicable and validated.
 - 1.1.1. Trace evidence including but not limited to hair, fibers, glass, or paint may be examined. Trace evidence shall be retained in the original packaging unless value is immediately apparent. Appropriate notes shall be taken on the trace evidence collected. When appropriate, hairs may be evaluated for the possibility of human origin and the potential for future analysis.
 - 1.1.1.1. If evidentiary value is immediately apparent, microscopic hair identification or DNA analysis may be performed. If no microscopic examination is performed, both ends of one hair shall be cut into the same tube for extraction.
 - 1.1.2. A search is made of submitted items for the presence of biological material.
 - 1.1.3. Previously retained items may be released at the prosecutor's request. The request shall be documented in the case record. A valid court order shall circumvent this procedure.

1.2. Precautions/Limitations:

- 1.2.1. Evidence
 - 1.2.1.1. Liquid items such as blood standards shall be refrigerated not frozen. Stained materials may be frozen, refrigerated or stored at room temperature in a dry environment. Items to be analyzed for latent prints should not be frozen or refrigerated.
 - 1.2.1.2. Stains that are still moist shall be air dried in a secure area before submission to a Regional Laboratory.
 - 1.2.1.3. Clean paper or cardboard containers shall be used to package dried evidence. Plastic containers shall not be used for packaging because they hold moisture which often leads to putrefaction of biological materials. Exceptions to this rule would include blood tubes, body tissue samples and condoms. Each item should be placed in separate containers from the point of collection.
 - 1.2.1.4. If multiple items are placed in one container at time of collection, they may be submitted in the single, original package.
 - 1.2.1.5. Specific examinations performed on items of serological evidence shall be determined by Forensic Services Division personnel.

1.3. Related Information:

- 1.3.1. Appendix 1 Flow Charts
- 1.3.2. Work Sheet Manual

1.4. Instruments:

- 1.4.1. Balances – An analytical balance used for preparation of analytical reagents and buffers.
- 1.4.2. Centrifuges – A serofuge capable of operating at 3,400 rpm and a microcentrifuge capable of greater than 15,000 rcf, are used for separation of solid components from fluids.
- 1.4.3. Alternate light source (ALS) – A light source to aid in the location of stains and trace evidence by use of various wavelengths of light.

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- 1.4.4. Crosslinker - Preprogrammed ultraviolet exposure unit which is factory set to ~120,000 microjoules per cm².
- 1.4.5. Digital camera – Used to document evidence and/or the packaging.
- 1.4.6. Microscopes – A light microscope with magnification up to 400X, a stereoscope for general screening of items, and a phase contrast microscope for the identification of spermatozoa.
- 1.4.7. Miscellaneous Laboratory Equipment – Supportive laboratory equipment including ovens, incubators, pipettors, rotators, stirring/heating plates, vortex mixers, vacuum pumps, UV lights, and refrigerators/freezers for storing of reagents, buffers, and evidence.
- 1.4.8. pH meter – An instrument capable of manual or automatic temperature compensation and reading +/- 0.01 pH units. Used for preparation of buffers and reagents.

1.5. **Reagents/Materials:** **See Reagent Preparation Manual for instructions and logs.**

- 1.5.1. Alpha Naphthyl Phosphate Solution
- 1.5.2. Acid phosphatase Acetate Buffer
- 1.5.3. Acid phosphatase Dye Solution
- 1.5.4. Glucose, saturated
- 1.5.5. HemDirect Hemoglobin Cassettes
- 1.5.6. HemDirect Hemoglobin Buffer
- 1.5.7. 3% H₂O₂
- 1.5.8. Phenolphthalein Stock Solution
- 1.5.9. Phenolphthalein Working Solution
- 1.5.10. 10% Sodium Hydroxide

1.6. **Hazards/Safety:**

- 1.6.1. All chemicals shall be handled in a safe method as referenced in the specific SDS.
- 1.6.2. Preparation of the Phenolphthalein Stock Solution shall be performed in a chemical fume hood.
- 1.6.3. Universal Precautions shall be in use whenever biological materials are being handled.
- 1.6.4. Extreme caution shall be used when handling liquid body fluid samples.
- 1.6.5. Biological waste shall be disposed of in the appropriate waste receptacle.

1.7. **Reference Materials/Controls/Calibration Checks:**

- 1.7.1. HemDirect Hemoglobin tests shall be performance checked before use in casework.
 - 1.7.1.1. Each new lot of HemDirect Hemoglobin Tests shall be analyzed with a 1:10 dilution of blood dried on filter paper or a 1:500 dilution of liquid human blood. A positive result as described in the HemDirect test method shall be obtained before use in casework. The worksheet used to record the results shall be retained.
 - 1.7.1.2. If the lot fails, the quality control test it may be retested. If it fails a second time the lot shall not be used for casework. The Technical Leader shall be informed within one business day of the failure.
- 1.7.2. Running known controls with each test ensures the accuracy and specificity of the test results. See the specific test procedure for the appropriate controls to be run.

1.8. **Procedures/Instructions:**

1.8.1. **Examination Procedure**

1.8.1.1. **Standards for Comparison**

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- 1.8.1.1.1. Appropriate standards, such as whole blood or buccal swabs, from those individuals involved should be submitted along with the unknown materials to be analyzed.
- 1.8.1.1.2. The whole blood standard should be submitted in a purple top tube that contains ethylene-diamine-tetra-acetic acid (EDTA). Prior to examination, while in the custody of the ISP Forensic Services Division, this tube shall be refrigerated.
- 1.8.1.1.3. A stain card may be prepared from at least one whole blood standard from each individual.
- 1.8.1.1.4. Buccal swab standards containing multiple swabs from an individual may be labeled and treated as one item.
- 1.8.1.1.5. Oral swabs in a sexual assault kit with no identified standard may be used as a standard if a single source profile is obtained.
- 1.8.1.1.6. The analyst's notes shall indicate the presence of hair standards. In sexual assault kits where a blood or buccal swab standard is present the hair standards do not need to be opened or examined, just noted.
- 1.8.1.1.7. DNA profiles shall be generated from suspect standards in all cases and from victim and elimination standards in crimes against person cases. DNA profiles may be developed for victim and elimination standards in property crimes; however it is not required if no unknown profiles are developed for comparison. If any analysis is performed (e.g. extraction through quantification), then the DNA profile data should be generated.
- 1.8.1.1.8. In cases where a secondary standard is needed, the item to be used as a secondary standard shall be clearly identified as such in the item description section of the case report.
 - 1.8.1.1.8.1. The use of a blood stained article of clothing may be appropriate if stains from the person wearing the clothing can be definitively identified (i.e., blood from around the wound area).
 - 1.8.1.1.8.2. In the case of a missing person, personal items such as toothbrush, hairbrush, razor or other item may be submitted.
 - 1.8.1.1.8.3. When a contributor requests items to be used as secondary standards during the submission process, it should be indicated on the Request for Laboratory Examination form.
 - 1.8.1.1.8.4. Items collected as surreptitious standards or from trash pulls to be used for comparison shall be clearly identified within the body of the report. If a statistic is calculated, it shall be reported as "purported to belong to" or "contributor of the DNA profile from item #."
 - 1.8.1.1.8.4.1. If items within the trash pull generate profiles from different individuals of the same sex as the person of interest, no assumptions of profile ownership shall be done and no statistics shall be calculated.
 - 1.8.1.1.8.4.2. Mixtures from trash pulls shall not be utilized for comparison.
- 1.8.1.2. **Trace Evidence**
 - 1.8.1.2.1. A clean piece of paper shall be used under each item as it is examined. The exam paper should be retained in the original package. After returning the item to its

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package, fold the exam paper, tape it closed (making sure all trace evidence is secure within the bundle) and place it in the package with the item.

- 1.8.1.2.2. Items may be tape lifted to collect evidence. Where tape lifting is not appropriate, tweezing and/or scraping may be substituted. Items involving multiple examination requests shall only be tape lifted or scraped after consultation with analysts from the other disciplines.
- 1.8.1.2.3. All collected trace evidence shall be stored with original item unless value is immediately apparent.
- 1.8.1.2.4. Analysts shall document in the notes the presence of hair, fibers, plant material, glass, paint, etc. Such trace evidence shall be reported out only when it is deemed to have value. In those cases where the material appears to be extraneous debris of no significance, the trace statement may be omitted from the report.
- 1.8.1.2.5. Hair combings need not be opened or examined, just their presence noted. If opened, the approximate number and quality of hairs shall be documented.
- 1.8.1.2.6. In cases where there is other DNA evidence of investigative value that has been tested and profiles obtained, no DNA testing of the collected hair shall be performed without the approval of a Biology Unit Supervisor or Laboratory Manager.
- 1.8.1.2.7. When appropriate, examination of the hair collected may be performed to determine if hair consistent with human origin is present.
- 1.8.1.2.8. Human hairs can be submitted for DNA analysis when evidentiary value is immediately apparent.
- 1.8.1.2.9. If microscopic examination is not performed prior to DNA analysis, both ends of possible hairs shall be cut into one tube for DNA analysis.
- 1.8.1.3. **Stain Evaluation – Sexual Assault kits**
 - 1.8.1.3.1. No serological testing shall be performed on samples within a kit.
 - 1.8.1.3.2. Exceptions require supervisor notification documented in the case record.
 - 1.8.1.3.3. Acid Phosphatase testing and sperm searches may be done at the request of a Prosecutor with proper justification and documentation in the case record.
- 1.8.1.4. **Stain Evaluation – All other**
 - 1.8.1.4.1. Visual Inspection – A visual inspection of the item shall be conducted to determine the location, if present, of unknown stains. An ultraviolet light or an alternate light source may be used to help locate stains.
 - 1.8.1.4.2. The case record shall document physical descriptions of staining, when present, using descriptive notes, drawings, and/or photographs. This may include descriptions such as, but are not limited to the size, color, apparent concentration, and/or location of the staining.
 - 1.8.1.4.3. Presumptive testing should be performed if indicated by the appearance of the staining at the discretion of the analyst. Confirmatory testing for semen and human blood is at the discretion of the analyst.
 - 1.8.1.4.4. If a stain is found to be possible blood or semen, the analyst shall determine if DNA testing is performed based on an evaluation of the stain. Things that may be considered include, but are not limited to, the amount, size, concentration, location, and condition of the stain.

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1.8.1.4.5. If no possible blood or semen is identified on the item, potential for further DNA testing shall be evaluated by the analyst based on the evidence type and case circumstances.

1.8.1.4.6. No serological testing shall be performed on M-Vac filters.

1.8.1.5. Item designation of multiple items within submitted items (such as clothing, shoes, sheets, etc.)

1.8.1.5.1. Each distinct item within a submitted item should have a unique identifier.

1.8.1.5.2. If serological testing is performed on any of the subitems within a submitted item, each item shall have a unique identifier.

1.8.1.6. Collection and labeling of swabs

1.8.1.6.1. If serological testing is performed on any of the swabs within a submitted item, each swab shall have a unique identifier. If different testing results are obtained between swabs in the same item, each result shall be reported.

1.8.1.6.1.1. Swabs that have different presumptive serological testing results should not be combined for DNA extraction. When combined, documented reasoning shall be in the notes.

1.8.1.6.2. Swabs may be used as a sampling method for DNA analysis. If no serological testing is performed and the swabs are being treated as one sample (i.e., portions of all swabs are combined together for DNA analysis), the swabs do not need to be distinguished in the report or described as "combined".

1.8.1.6.2.1. If the swabs are treated differently for DNA analysis (i.e., one swab is tested while the other is not), the samples shall have unique identifiers and be reported separately.

1.8.2. Sampling of items that may contain unknown or dangerous drugs

1.8.2.1. Caution should be exercised when opening and examining evidence with unknown powders and liquids or known dangerous powders or liquids. Every effort should be made to prevent exposure to potentially hazardous materials. If there is any concern or knowledge about a specific hazard, analysts may use a fume hood for processing.

1.8.2.2. Narcan (or similar product) should be available and in a clearly marked location during analysis.

1.8.3. Phenolphthalein (Kastle-Meyer Reagent), Presumptive Test for Blood

1.8.3.1. **Principle:** Phenolphthalein is an oxidizable organic molecule, which can be oxidized by free hydroxyl ions liberated by peroxidase-like action. The heme group of hemoglobin possesses a peroxidase-like activity, which may catalyze the breakdown of hydrogen peroxide to form free hydroxyl radicals. Phenolphthalin (reduced form) is oxidized by the free hydroxyl ions to phenolphthalein (oxidized form), producing a pink color.

Phenolphthalein (abbreviated Phe on controlled worksheets) is a presumptive test for the presence of blood. Confirmatory testing shall be performed for conclusive identification. Phenolphthalein has been shown to give false positives or weak reactions with various oxidizing agents, plant material, etc.

1.8.3.2. Procedure

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- 1.8.3.2.1. Rub the suspected bloodstain with a piece of filter paper or a cotton swab or make a small cutting of the suspected bloodstain and place on white filter paper or in a white spot plate well.
- 1.8.3.2.2. Add one to three drops of the phenolphthalein working solution to the stain.
- 1.8.3.2.3. Wait 10-15 seconds, assuring no pink color develops at this time.
- 1.8.3.2.4. Add one to three drops of 3% Hydrogen Peroxide.
- 1.8.3.2.5. Upon addition of Hydrogen Peroxide an immediate pink color is indicative of the possible presence of blood.
- 1.8.3.3. Reagents shall be tested with positive and negative controls and the results recorded in the case notes. This shall be performed and recorded daily when in use and prior to use on evidence. Lot numbers and/or preparation dates of reagents shall also be recorded.
- 1.8.4. **HemDirect Hemoglobin Test, Confirmatory Test for Human Blood**
 - 1.8.4.1. **Principle:** Human blood reacts with an anti-human hemoglobin antibody, which forms a red line in the "test" region of the HemDirect cassette.
 - 1.8.4.2. **Procedure**
 - 1.8.4.2.1. Place a ~2mm² cutting, a few flakes, a few drops of liquid blood diluted to 1:500, or ~1/8 of a swab in the tube of supplied extraction buffer. The cutting shall remain in the buffer solution for 5 minutes. A maximum extraction time of 10 minutes may be utilized for inhibited samples.
 - 1.8.4.2.2. Add three drops of the extraction buffer/sample mix with the provided pipette to the sample well of the cassette.
 - 1.8.4.2.3. A positive result may be recorded as soon as red lines are observed in both the "C" (control) and "T" (test) regions.
 - 1.8.4.2.4. Negative results (a red line in only the "C" region; no line in the "T" region) should be confirmed after 10 minutes.
 - 1.8.4.2.5. If a line in the "C" region does not appear after 10 minutes, test results are invalid and shall be repeated.

Each new lot of HemDirect kits shall be performance checked before use. Additional testing of reagents with positive and negative controls before use on evidence is not required. Lot numbers of reagents and the result of the internal positive control shall be recorded.
- 1.8.5. **Acid Phosphatase, Presumptive Test for Semen**
 - 1.8.5.1. **Principle:** Seminal fluid is typically found to contain acid phosphatase in levels 400 -1000 times those found in other body fluids. This test uses the ability of acid phosphatase to hydrolyze a phosphate ester substrate to release a free alcohol, which in turn reacts with a diazonium salt to give a brilliantly colored end product.

Acid Phosphatase (abbreviated AP on controlled worksheets) is a presumptive test for the presence of semen. Conclusive identification of semen can only be done by performing confirmatory testing. Possible sources of acid phosphatase other than semen include vaginal secretions, urine, sweat or fecal material.

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- 1.8.5.2. **Procedure:** Prepare substrate and dye solution immediately prior to testing. These solutions will be stable for 1-2 hours at room temperature. Alternatively, use previously aliquoted and frozen solutions.
- 1.8.5.2.1. Weigh out about 20 µg of alpha naphthyl phosphate and place in shell vial or small test tube.
 - 1.8.5.2.2. Weigh out about 20 µg of fast blue or fast red dye (shall be in diazonium salt form) and place in second shell vial or small test tube.
 - 1.8.5.2.3. Add about 1 ml of acetate buffer to each and mix.
 - 1.8.5.2.4. Cut a small (approximately 2 mm x 2 mm) portion of the stained material and place it on a small piece of filter paper, in a white ceramic spot plate well or in a small test tube. An extract can be used.
 - 1.8.5.2.5. Wet the cutting with the alpha naphthyl phosphate solution. Wait about 1 minute.
 - 1.8.5.2.6. Add 1-2 drops of the dye solution to the cutting.
 - 1.8.5.2.7. The development of color (depending on dye used) in less than 15 seconds is indicative of the presence of acid phosphatase. Color soaked into the filter paper under the sample is also indicative of a positive test.
 - 1.8.5.2.8. A slow development of color could be an indication of acid phosphatase from sources other than semen. This may be indicated on the worksheet as a slight color change (SCC). A SCC result shall be reported as a negative result.
 - 1.8.5.2.9. The interpretation of the results shall be reported by the analyst.
- 1.8.5.3. Reagents shall be tested with positive and negative controls and the results recorded in the case notes. This shall be performed and recorded daily when in use and prior to use on evidence. Lot numbers and/or preparation dates of reagents shall also be recorded.

1.8.6. Extraction of Body Fluid Stains

- 1.8.6.1. **Principle:** Evidentiary stains can be extracted and the extracts used for body fluid stain identification.
- 1.8.6.2. **Procedure**
- 1.8.6.2.1. Prepare samples (and backgrounds, if used) for testing by eluting a cutting of the stain (approximately 3 mm by 3 mm) in an appropriate amount of distilled water in a microcentrifuge tube overnight in the refrigerator. Alternatively, the sample can be eluted in a 37°C oven for a minimum of 30 minutes or at room temperature for a minimum of 1 hour.
 - 1.8.6.2.2. After incubation, the cutting should be removed, placed into the cap with holes for drainage or a spinex basket, and the extract spun down.

1.8.7. Confirmation of Seminal Material

- 1.8.7.1. **Principle:** The visual identification of spermatozoa is a scientifically accepted method of positively identifying the presence of seminal material.
- 1.8.7.1.1. The observations should be rated using the following system:

- POS (+) Only a few sperm or sperm heads on the entire slide.
- 1+ One sperm or sperm head per several fields of view.
- 2+ One sperm or sperm head in about half of all fields of view.
- 3+ One sperm or sperm head in most fields of view.

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4+ Several sperm or sperm heads in most fields of view.

1.8.8. Phase Contrast Microscopy for the Visual Observation of Spermatozoa

1.8.8.1. **Principle:** Phase contrast microscopy is used to differentiate the cellular appearance. Spermatozoa are round to ovoid with an acrosomal cap, midpiece, and tail. When the tail and midpiece are not present, the distinctive flattened profile of turned spermatozoa should be observed.

1.8.8.2. Procedure

1.8.8.2.1. Place ~10 µl of the pellet from the bottom of a previously extracted sample on a microscope slide and cover.

1.8.8.2.2. Alternatively, a small (1 mm x 1 mm) cutting may be eluted directly onto the slide with a drop of distilled water, agitating with forceps. Remove the cutting and cover the extract with a cover slip.

1.8.9. Christmas Tree Stain (Kernechtrot-Picroindigocarmine Differential Stain) (Optional procedure to help visualize spermatozoa)

1.8.9.1. **Principle:** The Christmas tree stain is used to assist in scanning slides for the presence of spermatozoa. Red and green colored stains are utilized to enhance the appearance of spermatozoa and epithelial cells in biological samples.

1.8.9.2. Procedure

1.8.9.2.1. Pipette ~5-10 µl of the pellet from the bottom of extracted sample tube and place onto microscope slide. Previously prepared slides from the evidence collection kit may go directly to heat fix and staining procedures.

1.8.9.2.2. Alternatively, a small (1 mm x 1 mm) cutting may be eluted directly onto the slide with a drop of distilled water, agitating with forceps. Remove the cutting and cover the extract with a cover slip.

1.8.9.2.3. Also prepare a slide from a 1:50 dilution of a sperm positive semen stain to use as a positive control for the staining procedure.

1.8.9.2.4. Heat fix the sample. Pre-heat a hot plate to approximately 200°C. Place the prepared slides on the hot plate for approximately 30 seconds. Ensure that the sample is dry. Alternatively, a lower temperature with a longer time can be used and adjusted, as needed, to adequately fix the sample (e.g. 100 – 140°C heated for 5 – 20 minutes).

1.8.9.2.5. After the slide has cooled, place on a level surface and add several drops of the SERI Christmas Tree Stain A (red stain) to the slide. Stain for about 10 to 15 minutes.

1.8.9.2.6. Rinse stain from the slide by gently running distilled water over the slide.

1.8.9.2.7. Return slide to a level surface and add several drops of the SERI Christmas Tree Stain B (green stain) to the slide. Stain for about 10 seconds.

1.8.9.2.8. Rinse stain from the slide by gently running 95 to 100% ethanol over the slide.

1.8.9.2.9. Allow slide to air dry or dry in 56°C oven. After drying, slide is ready to view microscopically applying bright field procedure.

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1.8.9.3. Reagents shall be tested with a positive control and the results recorded in the case notes. This shall be performed and recorded with each batch of staining. Lot numbers and/or preparation dates of reagents shall also be recorded.

1.8.9.4. Interpretation

1.8.9.4.1. Spermatozoa will appear as small red/pink structures and have the following staining characteristics:

Acrosomal cap	clear to pink
Post acrosomal cap	dark pink to red
Midpiece	green to blue
Tail	green to blue

1.8.9.4.1.1. Nucleated epithelial cells will appear as green rhomboid-like structures with a red/pink nucleus. The cell's appearance can vary in shape, often being folded or overlapping and are normally larger than the spermatozoa heads.

1.8.9.4.2. **NOTE:** The smear slides contained in evidence collection kits shall be viewed using phase contrast microscopy before proceeding to the Christmas tree stain procedure.

1.8.10. Visual Observation of Human Hair

1.8.10.1. **Principle:** Before DNA analysis is performed on hair collected as evidence, a determination of possible human origin and the identification of the root may be performed.

1.8.10.1.1. Alternatively, both ends of a hair may be cut into one tube for DNA analysis.

1.8.10.2. Procedure for Identification of Human Hair

1.8.10.2.1. When the unknown hairs are on a tape lift, the lift should be examined using a stereomicroscope to eliminate obvious hair fragments, fibers, and animal hairs. All hairs of possible human origin with sufficient root material should be removed from the lift and placed in a paper bindle. A wet mount should be prepared when further examination is required to determine human origin and/or presence of root material. Unknown hairs are placed on a microscope slide with a drop of distilled water, covered with a cover slip, and examined with a light microscope. The number and general appearance of human hairs should be noted when practical. If additional hairs/fibers are present in the original package, they should be placed in a separate bindle and retained in the package.

1.8.10.2.2. Observation should begin with the presence of scales (imbricate in humans and some animals). Human hairs demonstrate a wide variety in color and pigment distribution among individuals. The color in the cortex should be fairly uniform throughout the length and breadth of the hair. An exception would be dyed or bleached hairs, which could demonstrate a sudden or gradual color change at the dye line. (Animal guard hairs demonstrate a characteristic color banding). The medulla (the amorphous central core) can be continuous, discontinuous, fragmentary, or not present in human hair. Medullary index should be one-third or less the total diameter of the hair. There is considerable variation in the general appearance of human hairs depending on the body area. In general, there is less variation in diameter along the length in human hairs than in animal hairs. The appearance of the root is very important both in determining origin and in determining the area

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to sample for DNA. The appearance of a large skin tag is not necessarily an indication of success in extraction of DNA.

- 1.8.10.2.3. Examination documentation shall be of sufficient detail so that an equally qualified analyst is provided enough information in the case notes so as to reach the same conclusion. In the case of hair, when a “human hair” conclusion is reached the notes shall include observations regarding scale type, medulla, coloring/pigment distribution and any other pertinent observations.

1.8.11. Crosslinker Procedure

- 1.8.11.1. Consumables (pipette tips, microtubes, columns, etc.) may be exposed in the crosslinker for 2 to 15 minutes before use in casework. Time in excess of 15 minutes shall have the approval of the Technical Leader documented in the case record.

- 1.8.11.2. The use of the crosslinker may reduce the presence of amplifiable DNA.

1.9. Records:

- 1.9.1. If a worksheet is utilized, the examination of sexual assault kits and suspect collection kits shall be recorded on an approved worksheet.
- 1.9.2. Results of testing of body fluids shall be recorded on the worksheets designed for that purpose, or alternatively be recorded in the case notes within the description of the item as long as all required controls are documented appropriately.
- 1.9.3. All photos and electronically created documentation shall be stored as part of the case record. See [Appendix 9](#) for instructions.
- 1.9.4. The technical review of the case record shall be recorded on the worksheet provided for that purpose.

1.10. Interpretations of Results:

- 1.10.1. The quality and quantity of a sample shall determine which tests are performed. Conclusions reached are based on the actual tests performed in each case. See specific tests for interpretation guidelines.
- 1.10.2. The analyst shall report out any identified body fluid stains present and list the number of samples tested for DNA in the report.

1.11. References:

- 1.11.1. Forensic Science Handbook. ed. Richard Saferstein. 1982. Prentice-Hall. New Jersey.
- 1.11.2. Forensic Science Handbook, Volume I. ed. Richard Saferstein. 2002. Prentice-Hall. New Jersey.
- 1.11.3. Forensic Science Handbook, Volume II. ed. Richard Saferstein. 1988. Prentice-Hall. New Jersey.
- 1.11.4. Forensic Science Handbook, Volume III. ed. Richard Saferstein. 1993. Prentice-Hall. New Jersey.
- 1.11.5. Gaensslen, R.E. 1983. Sourcebook of Forensic Serology, Immunology and Biochemistry. United States Government Printing Office, Washington D.C.
- 1.11.6. Jungreis, Ervin. 1997. Spot Testing Analysis. John Wiley & Sons, New York.
- 1.11.7. Laux, D. L. 1992. Detection of bloodstains; A Training Manual. Richfield Ohio.

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- 1.11.8. Jeyendran, R. S. 2000. Interpretation of Semen Analysis Results, a Practical Guide. Press Syndicate of the University of Cambridge, Cambridge, United Kingdom.
- 1.11.9. Deedrick, D. W. and Koch, S. L. Microscopy of Hair, Part 1: A Practical Guide and Manual for Human Hairs. *Forensic Science Communications*. January 2004. Volume 6(1).
- 1.11.10. Deedrick, D. W. and Koch, S. L. Microscopy of Hair, Part 2: A Practical Guide and Manual for Animal Hairs. *Forensic Science Communications*. July 2004. Volume 6(3).
- 1.11.11. Practical Aspects of Rape Investigation: A Multi Disciplinary Approach. Third Edition. eds. Robert R. Hazelwood and Ann Wolbert Burgess. Elsevier, New York.
- 1.11.12. Saferstein, R. 1995. Criminalistics, An Introduction to Forensic Science. Fifth Edition. Prentice Hall, New Jersey.
- 1.11.13. Instructions for use: SERATEC® HemDirect (Cat No: HBF07). Rev: 02.2016

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2. DNA METHODS:

2.1. Scope: This test method is designed for the guidance of Biology Section personnel who assist investigations where the specificity of DNA testing is needed. Evidence amenable to DNA testing includes blood, seminal fluid, saliva, bone, tissue, and hair. This manual may be expanded or altered as new techniques and/or genetic systems are found applicable.

2.1.1. The DNA is first extracted from the biological sample, quantified, amplified to produce many fluorescently tagged copies of specific regions of the DNA and finally processed to separate and detect a DNA profile(s). The analyst compares the DNA profile identified in the sample with the DNA profile from a reference standard from an individual to determine similarities or dissimilarities. In addition, the analyst may provide likelihood ratio statistic to give weight to the strength of an inclusion or exclusion.

2.1.2. Return of Evidence

2.1.2.1. Stain cards from whole blood samples, cuttings from body fluid stains, appropriate DNA extracts, and other selected DNA evidence may rarely be retained by the Forensic Services Division for any possible future testing.

2.1.2.2. All extracts are considered work product. Extracts should only be retained if needed for pending additional analysis, such as Y-STR, NGS or outsourced analysis. Extracts shall be discarded after administrative review when analysis is complete.

2.1.2.3. Subitems or extracts may be retained in long-term storage with supervisor notification. Special storage conditions may be required for long-term retention of extracts.

2.1.2.3.1. Retained extract tubes shall be labeled with the case number and unique sample designation.

2.1.2.3.2. Unresolved unidentified human remains cases or cold case violent crimes against persons cases where additional testing is likely should be considered for retention.

2.1.2.4. Any retained items may be released at the prosecutor's request. The request shall be documented in the case record. A valid court order shall circumvent this procedure.

2.2. Precautions/Limitations:

2.2.1. Evidence

2.2.1.1. Liquid items such as blood standards shall be refrigerated.

2.2.1.2. Stains which are still moist shall be air dried in a secure area before submission to the Forensic Services Division.

2.2.1.3. Clean paper or cardboard containers shall be used to package dried evidence. Plastic containers shall not be used for packaging because they hold moisture which often leads to putrefaction of biological materials. Exceptions to this rule would include blood tubes, body tissue samples and condoms.

2.2.1.4. Specific examinations performed on items for DNA analysis shall be determined by Forensic Services Division personnel.

2.3. Related Information:

2.3.1. Worksheet Manual

2.3.2. [Appendix 6](#) PowerPlex® Fusion 6C Stutter Table

2.4. Instruments:

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- 2.4.1. Applied Biosystems® 3500 Genetic Analyzer Capillary Electrophoresis Instrument – Simultaneously separates and detects multiple amplified DNA samples by size by capillary electrophoresis using fluorescent tagged primers.
- 2.4.2. Applied Biosystems® 7500 Real Time PCR System – Measures accumulation of amplification product over time to determine total DNA present by the use of a fluorescent reporter molecule.
- 2.4.3. Autoclave - An instrument that uses heat and steam to sterilize solutions, contaminated equipment and waste.
- 2.4.4. Balance - An analytical balance used for preparation of analytical reagents and buffers.
- 2.4.5. BIOMEK i5 Automation Platform – Robotic liquid handling system.
- 2.4.6. Centrifuges - A microcentrifuge capable of operating at greater than 15,000 rcf and a clinical serofuge capable of operating at a maximum of 3,400 rpm; used for the purification of DNA in evidence samples and the separation of solid components from liquids, respectively.
- 2.4.7. Crosslinker - Preprogrammed ultraviolet exposure is factory set to 120,000 microjoules per cm². Preset ultraviolet time exposure is factory set to 2 minutes.
- 2.4.8. Laminar Flow Hood – An air purifying biohazard cabinet that maintains a nominal inflow velocity of 80 fpm which prevents contaminants from entering or escaping the work area.
- 2.4.9. Maxwell® FSC, Maxwell® RSC – Robotic extraction system.
- 2.4.10. Microscope - A light microscope with magnification up to 400X, a stereoscope for general screening of items, and a phase contrast microscope for the identification of spermatozoa.
- 2.4.11. Miscellaneous Laboratory Equipment - Supportive laboratory equipment including ovens, incubators, pipettors, rotators, stirring/heating plates, vortex mixers, vacuum pumps, UV lights, and refrigerators/freezers for storing of reagents, buffers and evidence.
- 2.4.12. pH Meter – An instrument capable of manual or automatic temperature compensation and reading +/- 0.01 pH units. Used for preparation of buffers and reagents.
- 2.4.13. Thermal cycler - An instrument that can be programmed to rapidly cycle between high and low temperatures. This process is used to make many fluorescently tagged copies of specific regions on a DNA strand(s).
- 2.4.14. Water Purification System - An apparatus that routes water through a series of filtering devices to produce high quality, uncontaminated water used in buffer preparation and DNA typing methods.

2.5. Reagents/Materials: See Reagent Preparation Manual for instructions and logs where appropriate. Reagents critical to the DNA analysis process are listed in the Critical Reagent Manual.

- 2.5.1. Bone Demineralization Buffer
- 2.5.2. Digest/Wash Buffer
- 2.5.3. DTT 0.39M
- 2.5.4. DTT 1M
- 2.5.5. EDTA 0.5M
- 2.5.6. Nuclease Free Water (NFH₂O)
- 2.5.7. Phenol/Chloroform/Isoamyl Alcohol
- 2.5.8. Proteinase K (Pro K) 10 mg/ml
- 2.5.9. Proteinase K (Pro K) 18 mg/ml
- 2.5.10. Proteinase K (Pro K) 20mg/ml
- 2.5.11. Sarkosyl 20% w/v

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- 2.5.12. SDS 20% w/v
- 2.5.13. Stain Extraction Buffer Stock Solution
- 2.5.14. Stain Extraction Buffer with DTT
- 2.5.15. Stain Extraction Buffer for Automation
- 2.5.16. TE⁻⁴
- 2.5.17. TRIS/EDTA/NaCl Solution
- 2.5.18. TRIS-HCl pH 7.5, 1M
- 2.5.19. TRIS-HCl pH 8.0, 1M

2.6. Hazards/Safety:

- 2.6.1. All chemicals shall be handled in a safe method as referenced in the specific Safety Data Sheets (SDS).
- 2.6.2. The addition of phenol/chloroform/isoamyl alcohol during extraction, vortexing of the extracts, and transfer to Microcon® shall be performed in a chemical fume hood.
- 2.6.3. The manual preparation of samples for electrophoresis by the addition of Hi-Di™ formamide shall be confined to a chemical fume hood. **Caution:** Formamide is an irritant and teratogen; therefore, universal precautions and a fume hood shall be utilized when manually working with formamide to avoid inhalation and contact with the skin.
- 2.6.4. Universal Precautions shall be in use whenever biological materials are being handled.
- 2.6.5. Extreme caution shall be used when handling liquid body fluid samples.
- 2.6.6. Biological waste shall be disposed of in the appropriate waste receptacle.

2.7. Reference Materials/Controls/Calibration Checks:

- 2.7.1. The accuracy and specificity of test results are ensured by running known DNA controls and reagent controls at the same time as evidence samples. See the specific test for the appropriate controls to be run and the interpretation of the results.

2.8. Procedures/Instructions:

2.8.1. Examination Procedure

2.8.1.1. Standards for Comparison

- 2.8.1.1.1. Appropriate standards, preferably whole blood or buccal swabs, from individuals involved, should be submitted along with the questioned materials.
- 2.8.1.1.2. Whole blood standards should be submitted in a purple top tube that contains ethylene-diamine-tetra-acetic acid (EDTA). This tube shall be refrigerated.
- 2.8.1.1.3. Alternately a swabbing taken from the inside cheek of an individual may be collected, dried, and placed in a sealed envelope to be used as a standard.
- 2.8.1.1.4. When supplied, a secondary standard shall be clearly marked as such and treated as a standard.
 - 2.8.1.1.4.1. Surreptitious standards or items collected from trash pulls shall be clearly marked and may be extracted separately from both unknowns and standards, depending on the sample type.

2.8.1.2. DNA Analysis

- 2.8.1.2.1. If more than one swab from an area/stain is present within an item the analyst must indicate whether a single swab, multiple swabs, or a combined sample of

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two or more swabs are being analyzed in the case notes. Combined samples in which not all samples within the item were combined shall be tracked in the case notes by a unique identifier.

- 2.8.1.2.1.1. If an analyst is sampling an item by swabbing and the swabs are combined into one extract, this can be documented in the notes and report as a single sample.
- 2.8.1.2.1.2. If an analyst is sampling an item by swabbing and the swabs are extracted separately, these are considered separate samples. They shall be tracked in the case notes by a unique identifier and the number of samples tested shall be indicated in the report.
- 2.8.1.2.2. Sexual assault kit processing will depend on case scenario and requires analyst discretion. In order to expedite traditional SAK workflow, an optional tier-based process is allowed for situations with a female victim and a single male assailant.
- 2.8.1.2.3. Sexual Assault kits (female victim/single male assailant): A minimum of four samples/body areas may be chosen for the first tier of analysis based on the priority list below. If a foreign contributor (suitable for comparison) with information at eight or more loci is not obtained from first tier testing, the remaining samples present in the kit shall be tested in a second tier of testing. Analysts may choose to test all samples in the first tier. Not testing additional samples if no informative results are obtained requires permission of a supervisor with documentation in the case record.
 - 2.8.1.2.3.1. If the kit contains the samples listed below, these samples shall be selected for testing utilizing differential extraction methods. Regular extractions may be utilized for these samples for cases in which only digital penetration or fondling is indicated. Supervisor notification is required in these instances. If meaningful results are not obtained and quantification results indicate a differential extraction could be beneficial, the analyst should repeat the extraction as a differential.
 - 2.8.1.2.3.1.1. Either vaginal/cervical swabs or vaginal wash (choice to pick one is only for first tier priority testing. If all samples are required to be tested, both shall be tested, if present.)
 - 2.8.1.2.3.1.2. Anal/rectal swabs
 - 2.8.1.2.3.1.3. External genital swabs
 - 2.8.1.2.3.2. Additional samples shall be chosen based on the following suggested priority list developed based on internal studies of success rates by sample type. Selection of samples is dependent on case information provided and other samples that are present in the kit or already being tested.
 - 2.8.1.2.3.2.1. Breast swabs
 - 2.8.1.2.3.2.2. Dried secretions/bitemark swabs (including other body area swabs)
 - 2.8.1.2.3.2.3. Internal genital swabs
 - 2.8.1.2.3.2.4. Underwear
 - 2.8.1.2.3.2.5. Speculum swabs

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2.8.1.2.3.2.6. Bra

2.8.1.2.3.2.7. Oral swabs

- 2.8.1.2.4. In sexual assault kits, differential extractions shall be performed on samples most likely to contain seminal material (e.g., internal genital swabs, oral swabs, or underwear). If a sample is selected that is likely targeting skin cells and not seminal material (e.g., dried secretions/bitemark swabs or fingernail scrapings), the analyst may choose to perform a regular extraction.
- 2.8.1.2.4.1. The analyst may choose to perform a regular extraction for any samples tested as part of a second tier of analysis (as directed above), regardless of sample type.
 - 2.8.1.2.4.2. If a sperm fraction is amplified, the corresponding non-sperm fraction may be amplified regardless of the FTMR and amount of male DNA present; however, amplification of the non-sperm fraction is not required when the corresponding sperm fraction has sufficient male DNA.
 - 2.8.1.2.4.3. Swabs (up to 4) from each selected envelope/body area shall be combined for one extraction and evaluated for the presence of male DNA. The total sample must be completely covered by the specified reagent volume without needing to be adjusted/increased. Supervisor notification is required for multiple extractions of swabs from a single envelope/body area.
 - 2.8.1.2.4.4. At a minimum, DNA analysis shall be performed on the crotch area of underwear, either by swabbing the entire crotch or by taking multiple cuttings from the area to be extracted as one extract. Analyst discretion (based upon case scenario and visual examination) may be used to sample multiple areas (and perform multiple extractions) from a single pair of underwear. Supervisor notification is required if more than two areas are sampled for DNA.
 - 2.8.1.2.4.5. Smear slides, fingernail scrapings, debris, hairs, clothing other than undergarments and other items not listed above are never required but may be extracted at analyst's discretion, depending on the contents and results of other items in a kit.
 - 2.8.1.2.4.6. Case results should be carefully evaluated to determine if additional testing or technologies could provide meaningful information. For example, if only dried secretion samples demonstrate foreign alleles, any genital samples that qualify shall be submitted for Y-STR analysis.
- 2.8.1.2.5. Underwear and other samples normally collected in a SAK which are submitted outside of a kit shall be evaluated for stains as appropriate. No serological testing is required if no staining is observed; however, at a minimum, DNA analysis shall be performed on a sample of the crotch area. Staining that is distinctly separate or stains that have different presumptive testing results should not be combined. When combined, documented reasoning shall be in the notes.
- 2.8.1.2.6. A quantification procedure shall be performed to estimate the quantity of extracted DNA, human and male.

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- 2.8.1.2.7. To be used for comparison with evidentiary samples, any standard (primary, secondary, or trash pull standard) shall demonstrate a single source profile. Any exception to this rule shall only be made with approval of the Technical Leader and documented in the case record.
- 2.8.1.2.8. A portion of the amplified DNA is removed and used in the typing method. The remaining amplified DNA, considered work product, shall be destroyed after the case has been administratively reviewed.
- 2.8.1.2.9. The amount of sample available for testing, the recoverability of DNA from the sample, and the quality of the DNA shall affect which tests are performed. The conclusions reached are based upon the analysis performed.

2.8.1.3. General Rules

- 2.8.1.3.1. If one sample/cutting needs additional processing after quantitation (clean-up or concentration) and another sample/cutting from the same item has an appropriate quantity for analysis without additional processing; it shall be left to the analyst's discretion as to whether both samples or just the one sample will be typed.
- 2.8.1.3.2. Multiple reagent blanks (RB) may be extracted as part of a sample batch. Any samples proceeding for additional testing such as Y-STR analysis shall have more than one reagent blank if there are two or more samples associated with the blank. All reagent blanks shall at a minimum be carried through quantification. Amplification shall be performed on the reagent blank demonstrating the highest small autosomal quantification value. If no small autosomal value is detected, the highest large autosomal or Y value shall be analyzed. One reagent blank may be reserved without additional treatment to be used with those samples in the batch not requiring additional treatment. A batch is defined as all samples before a reagent blank(s) with no intervening reagent blanks.
- 2.8.1.3.3. Any additional treatment to a sample shall be done to the corresponding reagent blank. This would include but not be limited to additional Microcon® purifications, use of Centri-sep columns, amplifying more than 5 µl, or increased injection parameters. Reagent blanks shall not be diluted unless performed during the Biomek robotic processing of samples.
- 2.8.1.3.4. The extraction of unknown and reference samples shall be performed separately (separated by time and/or space). This shall include a separate reagent blank for reference and unknown samples and, when practical, use of separate extraction worksheets, separate columns of the same extraction worksheet, or clear indication of which samples are unknown and which are references. Unknown and reference samples shall not be extracted in the same Maxwell® run.
 - 2.8.1.3.4.1. An assumed reference standard (for any type of case) may be included within the same extraction batch as the associated unknowns on the large scale (Beckman) automated platform only as long as:
 - 2.8.1.3.4.1.1. All pre-robot processing steps were performed separately (separated by time and/or space); AND
 - 2.8.1.3.4.1.2. There is no additional evidence within the batch where the reference cannot be assumed. (For example: If

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sexual assault kit samples are submitted to the robot along with a penile swab from the suspect, the assumed victim standard cannot be included on that robot batch plate.)

- 2.8.1.3.5. Extraction and typing of background samples is often not beneficial, and is therefore not required, but may be done at the discretion of the DNA analyst to aid in the interpretation of case sample results. If a background sample is extracted, it shall be typed if DNA was detected during the quantification step. The background sample shall be concentrated only if the sample it is the background for is concentrated.
- 2.8.1.3.6. If during sampling for extraction, an evidence swab or cutting is dropped on the floor or other unclean area, a background swab of the area the evidence touched shall be collected and processed. The DNA results obtained from the background swab shall be utilized to evaluate the results from the evidence. The Technical Leader shall provide guidance on interpreting the data if DNA results are obtained from the background swab.

2.8.2. Crosslinker Procedure

- 2.8.2.1. Consumables (pipette tips, microtubes, columns, etc.) may be exposed in the crosslinker for 2 to 15 minutes before use in casework. Time in excess of 15 minutes shall have the approval of the Technical Leader documented in the case record.
- 2.8.2.2. The use of the crosslinker may reduce the presence of amplifiable DNA.

2.8.3. Sampling Protocols

- 2.8.3.1. Sample size may vary from indicated values depending on many factors including but not limited to total sample available and serology test results.
- 2.8.3.2. The analyst should consume only as much of the available sample as is needed. Generally, not more than half of likely body fluid samples (e.g., cigarette butts, blood or semen) will be used for testing.
- 2.8.3.3. In order to obtain usable results, epithelial cell swabs should be consumed. The Forensic Services Division shall receive permission to consume samples from the prosecutor or investigator prior to analysis of epithelial cell swabs or samples. This may be done at the time of submission, or the analyst may request it after sample evaluation. No analysis shall be performed on epithelial cell swabs or samples without permission to consume documented in the case record.
- 2.8.3.4. **Sampling Suggestions for Regular Extraction**
 - 2.8.3.4.1. **Blood:** Sample approximately 3 X 3 mm from a blood stain or 3 µl of whole blood.
 - 2.8.3.4.2. **Envelope:** Sample approximately 1 X 1 cm of a gummed envelope flap or stamp. Cut sample into smaller pieces before placing in extraction tube. Alternatively, may swab gummed area or wash with three 50 µl rinses and place each rinse into the extraction tube.
 - 2.8.3.4.3. **Cigarette:** Sample ½ to all of paper approximately 5 mm down from end or ½ of the paper and filter approximately 5 mm down. Filter not to be used when extracting with DNA IQ™ chemistry. Cut sample into smaller pieces before placing in extraction tube.

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- 2.8.3.4.4. **Epithelial Swabs:** The entire (swab/surface/area) used to collect epithelial cells should be consumed. The total sample must be completely covered by the specified reagent volume without needing to be adjusted/increased.
- 2.8.3.4.5. **Hair:** Rinse root end of hair in 100% ethanol and then dH₂O before sampling 1 cm of root end. If no microscopic examination was performed, 1 cm of both ends of the hair may be sampled and combined.
- 2.8.3.4.6. **Tissue:** Cut a piece of tissue approximately 3 X 3 mm. If tissue is immersed in liquid nitrogen, it shall also be crushed into a fine powder. Transfer the sample to a microcentrifuge tube. Larger samples than indicated may be used, as needed, with the amounts of stain extraction buffer with DTT and Pro K increased proportionally.
- 2.8.3.4.7. **Firearms:** Swab the grip, slide/sight, and trigger areas with separate swabs consumed for individual extractions. If magazine is present, an additional single swab should be utilized to swab the loading area and end where the magazine is handled. Additional areas may be sampled depending on the type of firearm.
- 2.8.3.4.8. **M-Vac filters:** For regular extraction (organic, Maxwell® and Biomek) and differential extraction for Biomek submission: Half of the filter should be used per extraction tube due to liquid volume. If not already cut, cut the filter in half. Cut that half in three strips, stack them (reduces static) and either 1) cut a few more times the other direction OR 2) roll the strips with tweezers. Place all of the pieces in a microcentrifuge tube (required for differential extraction) or lyse and spin basket for incubation. Use of tweezers and a static gun is recommended. For differential extraction on Maxwell® or organic: Up to two thirds of the filter may be used due to greater liquid volume. If permission to consume the sample has been obtained, it is recommended to extract both halves of the filter as the DNA distribution across the filter may not be equal.

If it is indicated that a funnel was used with the M-Vac concentration the sample should be only in the center of the filter. Cut out about 1.5 cm diameter circle from the center of the filter using static gun and tweezers. Further cut the center sample into smaller pieces and test as above. This will consume the sample.

2.8.3.4.9. **Combining extracts:**

- 2.8.3.4.9.1. Two or more swabs/samples may be extracted in separate extraction tubes, when using an organic extraction, combining at step 2.8.8.2. Multiple completed automation extracts may be combined before concentrating at step 2.8.8.2. The number and volume of the associated RBs shall be the same as the samples.

2.8.3.5. **Sampling for Semen (Differential Extraction)**

- 2.8.3.5.1. **Swabs:** A sample from at least one swab contained in an envelope shall be taken and combined into one tube. Samples from multiple swabs may be placed in one tube. The total sample addition must be covered by the specified reagent volume without needing to be adjusted/increased.
- 2.8.3.5.2. **Clothing:** Sample approximately a 5 X 5 mm cutting.
- 2.8.3.5.3. **Smear Slides:** Use a moistened swab and swab entire smear, including underside of coverslip. Sample swab as above.
- 2.8.3.5.4. **Vaginal Wash:** Spin down vaginal wash and collect pellet on one or more swabs. Sample swab(s) as above.

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2.8.3.6. Sampling for Bone, Teeth and Fingernails Used as Standards

- 2.8.3.6.1. Sample cleaning/preparation methods (e.g., scraping, heating, etc.) that best address the variability of sample quality may be used, as necessary.
 - 2.8.3.6.1.1. Bone marrow and soft tissue may be extracted using the Maxwell® or organic protocol. Bone, teeth and fingernails may be extracted by organic, demineralization followed by large-scale organic or the Promega Bone DNA Extraction kit followed by the Maxwell® procedure.
- 2.8.3.6.2. Remove and separate any soft tissue adhering to sample with a sterilized scalpel or other appropriate tool.
- 2.8.3.6.3. Clean the sample or sampling area to remove dirt/debris and reduce the presence of exogenous DNA. A dilute bleach solution is recommended.
 - 2.8.3.6.3.1. A small brush or toothbrush may be used to gently remove any dirt/debris from the outer surfaces and exposed inner surfaces of sample.
 - 2.8.3.6.3.2. Small bone samples with difficult-to-reach surfaces may be cleaned by sealing the sample in a 50 ml conical tube containing distilled water, then vortexing briefly, changing out the solution, and repeating, as necessary.
 - 2.8.3.6.3.3. If necessary, the specimen can be further cleaned by immersion in ethanol and ethyl ether for about 15 minutes each.
 - 2.8.3.6.3.4. If surface staining is present, it is recommended to remove the outer table of bone by scraping or sanding.
- 2.8.3.6.4. Rinse sample in distilled water and allow the sample to dry.
- 2.8.3.6.5. Pulverize sample into a fine powder by manual mechanical crushing or by use of a freezer/mill. Alternatively, bone samples may be collected using a powered rotary tool or a saw. Bone samples may require multiple freezing/crushing steps. Larger samples (e.g., bones and adult teeth) may need to be initially reduced in size to accommodate the method of pulverization. Note: any medical/dental/restorative work on a bone or tooth sample shall be removed prior to pulverization.
 - 2.8.3.6.5.1. **Manual Mechanical Crushing with Tissue Pulverizer**
 - 2.8.3.6.5.1.1. Larger samples may initially need to be reduced to ~0.5 in. fragments or smaller to accommodate the physical size of the pulverizer.
 - 2.8.3.6.5.1.2. Crush bone sample using the pulverizer and mallet. Optionally, sample may be immersed in liquid nitrogen for ~1–10 minutes to aid in crushing.
 - 2.8.3.6.5.2. **SPEX SamplePrep® 6770/6775 Freezer/Mill**
 - 2.8.3.6.5.2.1. Sample must initially be reduced to ~0.25 in. fragments (width of a pencil eraser) or smaller to accommodate the requirements of the mill. Larger fragments will decrease pulverization efficiency and may damage the mill.
 - 2.8.3.6.5.2.2. Insert a sterilized blunt end cap into a sterilized grinding cylinder.
 - 2.8.3.6.5.2.3. Add the sample to the cylinder. Do not add sample in excess of 1/3 total volume of the cylinder.

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- 2.8.3.6.5.2.4. Insert a steel impactor into the cylinder and seal the cylinder with a sterilized flanged end cap.
- 2.8.3.6.5.2.5. Slowly add liquid nitrogen to the freezer/mill until it reaches the fill mark. An initial amount of liquid nitrogen will quickly boil off as the tank cools. When the liquid settles, add additional liquid nitrogen up to the fill mark. Initial cooling of the instrument will consume ~4–5 L of liquid nitrogen, and each sample pulverization will require an additional ~1 L liquid nitrogen.
 - 2.8.3.6.5.2.5.1. Always handle liquid nitrogen containers or any item exposed to liquid nitrogen (including grinding cylinders) with cryogenic gloves.
- 2.8.3.6.5.2.6. Load assembled sample vial into the freezer/mill chamber (blunt end first). Optionally, up to two additional samples may also be prepared in vials and placed in the pre-cooling chamber above the freezer/mill chamber.
- 2.8.3.6.5.2.7. Close the freezer/mill and pulverize the sample using the following protocol: Cycles: 4, Pre-cooling: 10 min, Run: 2 min, Cool: 2 min, CPS (cycles per second): 10. Regularly check the control panel on the freezer/mill to ensure that sufficient liquid nitrogen is remaining in the tank. If “LOW LN LEVEL” is displayed, pause the protocol by pressing “PAUSE”, open the freezer/mill, and add liquid nitrogen to the fill mark, then close the freezer/mill and resume the protocol.
- 2.8.3.6.5.2.8. When the program is complete, open freezer/mill and remove vial from freezer/mill chamber. To conserve liquid nitrogen, any subsequent samples should be immediately loaded into the mill and pulverized as described above. If additional samples were loaded in the pre-cooling chamber during the initial sample run, they may be pulverized with the following protocol (omitting the pre-cooling phase): Cycles: 4, Pre-cooling: 0 min, Run: 2 min, Cool: 2 min, CPS: 10.
- 2.8.3.6.5.2.9. Allow vial to warm up for ~5–10 minutes before using the cap extractor tool to open the vial. If the sample was “sticky” or “tacky” prior to pulverization, it may be necessary to open the vial earlier to aid removal. Transfer the contents of the vial into a sterilized weigh boat and separate the impactor from the sample. A sterilized spatula may be used to aid sample removal.
- 2.8.3.6.6. Transfer the pulverized sample into one or more tubes. Manual mechanical crushing typically generates small sample fragments accompanied by some powder, while a freezer/mill typically converts an entire sample to a very fine powder.
- 2.8.3.6.7. For a regular organic extraction, fill one or more microcentrifuge tubes approximately halfway with powder. The amounts of extraction reagents may be increased proportionately as needed for a regular organic extraction. A sample size of approximately 0.5 – 1.0 grams of powder is recommended for the bone demineralization procedure.

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2.8.4. Regular Organic Extraction with Microcon® Concentration Of All Sample Types Including Blood, Hair, Tissue And Bone. (Microcon® 100 or Fast Flow)

- 2.8.4.1. Place the sample in a 1.5 ml microcentrifuge tube. Qiagen® Investigator® Lyse&Spin tubes shall not be used. Each group of samples being extracted shall include a reagent blank as the last sample in the batch.
- 2.8.4.2. To the sample add 300 µl of stain extraction buffer with DTT and 7.5 µl Proteinase K (Pro K) solution (10 mg/ml). Vortex and spin briefly in a microcentrifuge or tap the tube to force the cutting into the extraction fluid. The analyst may increase the amounts of stain extraction buffer with DTT and Pro K proportionally for larger samples. The same treatment shall be performed to any associated reagent blanks.
- 2.8.4.3. Incubate the tube at 56°C overnight (18 hours minimum/24 hours maximum). An extraction time of no less than 2 hours may be used for standards (blood or buccal swabs). A shortened extraction time may only be used with the approval of the Technical Leader (documented in case record) for any other sample types.
- 2.8.4.4. Spin briefly in a microcentrifuge to force the condensate into the bottom of the tube. For hairs, bones, or tissue it may be preferable to transfer supernatant to a clean microcentrifuge tube before proceeding to 2.8.4.6. Using a wooden applicator stick, remove the cutting and proceed to 2.8.4.6 or
- 2.8.4.5. Transfer the cutting into a spin basket insert. Place the basket insert into the tube containing the stain extract. Spin in a microcentrifuge at approximately 14,000 rcf (relative centrifugal force) for about 5 minutes. Remove and discard the basket insert.
- 2.8.4.6. In a fume hood, add 300 µl of phenol/chloroform/isoamyl alcohol to which buffer has been added (p/c/i) to the stain extract and vortex the mixture at low speed (in the fume hood) to attain a milky emulsion.
- 2.8.4.7. Spin in a microcentrifuge until completely separated (for at least 3 minutes) at approximately 14,000 rcf. Multiple p/c/i extractions may be performed to further clean up an extract if desired. If larger volumes are used in step 2.8.4.2, equivalent volumes of p/c/i shall be used. The same treatment shall be performed to any associated reagent blanks.
- 2.8.4.8. See 2.8.8 for Microcon® procedure.

2.8.5. Demineralization and Organic Extraction of Bones, Teeth and Fingernail Samples with Amicon® Ultra-4 30 kDa Concentration

- 2.8.5.1. Place the sample in a 50 ml conical or other appropriately sized container. Each group of samples being extracted shall include a reagent blank as the last sample in the batch.
- 2.8.5.2. To the sample add 4.5 ml of bone extraction buffer and 300 µl Proteinase K (Pro K) solution (20 mg/ml). Vortex and spin briefly to force the sample into the extraction fluid. The same treatment shall be performed to any associated reagent blanks.
 - 2.8.5.2.1. An alternative method including the addition of 2.0 ml Lysis Buffer containing Guanidinium Thiocyanate found in the Maxwell® DNA IQ™ Casework Kit to the demineralization buffer may be considered at the analyst's discretion.
- 2.8.5.3. Incubate the samples in an orbital shaker at 56°C and 150 rpm overnight (18 hours minimum/24 hours maximum). with an addition of an extra 300 µl Proteinase K (Pro K) solution (20 mg/ml) during the last 1-8 hours of incubation. A shortened extraction time

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may only be used with the approval of the Technical Leader (documented in case record).

- 2.8.5.4. Spin samples briefly at high speed. It may be preferable to transfer supernatant to a clean tube before proceeding to 2.8.5.5.
- 2.8.5.5. In a fume hood, add an equal volume (e.g., 5.1 ml for the standard demineralization protocol or 7.1 ml for the alternative protocol) of phenol/chloroform/isoamyl alcohol to which buffer has been added (p/c/i) to the extract and vortex the mixture (in the fume hood) to attain a milky emulsion.
- 2.8.5.6. Spin in a centrifuge until completely separated (for about 5 minutes at maximum speed).
- 2.8.5.7. In a fume hood, transfer approximately one-third to one-half of the aqueous layer to an Amicon® Ultra-4 30 kDa column (do not UV crosslink Amicon® Ultra-4 columns).
- 2.8.5.8. Place the Amicon® Ultra-4 columns in the centrifuge with one filter facing the outer wall. Centrifuge samples at max speed (up to 7500 x g (rcf) on a fixed angle rotor). Continue spinning until approximately 250 µl of sample remains.
 - 2.8.5.8.1. Note: Do not allow columns to become completely dry. Spin times may be adjusted per sample to address variability.
- 2.8.5.9. In a fume hood, discard the flow through and add the remaining aqueous layer to the column.
- 2.8.5.10. Repeat 2.8.5.8 until approximately 250 µl of sample remains. Discard the flow through.
- 2.8.5.11. Wash sample with 2 ml of nuclease free water. Spin (maintaining column orientation) at max speed until approximately 100 µl of sample remains.
- 2.8.5.12. Transfer the sample to a clean microcentrifuge tube.
- 2.8.5.13. Wash the column with an additional 100 µl of nuclease free water by pipetting up and down. Combine with extract.
- 2.8.5.14. Store refrigerated or proceed to QIAquick® purification.
- 2.8.5.15. **Combining extracts:** Two or more samples may be extracted in separate extraction tubes, combining at step 2.8.5.7. The number and volume of the associated RBs shall be the same as the samples.

2.8.6. QIAquick® Purification for Bone, Teeth and Fingernail Samples

- 2.8.6.1. QIAquick® purification may be performed at any time. The same treatment shall be performed on the associated reagent blank (or a portion of) for each sample batch.
- 2.8.6.2. Optionally, UV crosslink columns for 15 minutes.
- 2.8.6.3. Add 5 volumes of Buffer PB to 1 volume of sample (example for 200 µl of sample add 1000 µl Buffer PB). Place up to 750 µl into the spin column.
- 2.8.6.4. Spin 30 seconds at 17,900 x g (rcf); 13,000 rpm. If liquid remains in column, spin another 30 seconds.
- 2.8.6.5. Discard the flow through.
- 2.8.6.6. Repeat until all sample has been applied to the column.
- 2.8.6.7. Wash the sample by adding 750 µl of Buffer PE.

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- 2.8.6.8. Spin 30 seconds at 17,900 x g (rcf); 13,000 rpm. If liquid remains in column, spin another 30 seconds.
- 2.8.6.9. Discard the flow through.
- 2.8.6.10. Spin 1 minute at 17,900 x g (rcf); 13,000 rpm.
- 2.8.6.11. Place the column into a clean microcentrifuge tube (not provided in kit). To elute DNA, add 30-100 µl Buffer EB to the center of the membrane and let stand at least 1 minute.
- 2.8.6.12. Spin 1 minute at 17,900 x g (rcf); 13,000 rpm. Discard the column.
- 2.8.6.13. Extracts can be stored refrigerated or frozen prior to quantification/amplification. Prior to use after storage, the samples shall be vortexed and spun briefly in a microcentrifuge.
- 2.8.6.14. **Combining extracts:** Two or more completed extracts may be combined at step 2.8.6.3, adjusting the volume of Buffer PB appropriately for the total volume of the extracts. The number and volume of the associated RBs shall be the same as the samples.

2.8.7. Differential Organic Extraction with Microcon® Concentration of Semen Stains (Microcon® 100 or Fast Flow)

- 2.8.7.1. Place the sample in a 1.5 ml microcentrifuge tube. Qiagen® Investigator® Lyse&Spin baskets shall not be used for differential extractions. Each group of samples being extracted shall include reagent blanks (sperm and non-sperm fraction) as the last samples in the batch.
- 2.8.7.2. To the sample add:
 - 400 µl Tris/EDTA/NaCl
 - 25 µl 20% Sarkosyl
 - 75 µl NFH₂O
 - 5 µl Pro K (10 mg/ml)
- 2.8.7.3. Vortex and spin briefly in a microcentrifuge or tap the tube to force the cutting into the extraction fluid.
- 2.8.7.4. Incubate at 37°C for 2 hours.
- 2.8.7.5. Spin briefly in a microcentrifuge to force the condensate into the bottom of the tube.
- 2.8.7.6. Using a wooden applicator stick, remove the cutting and spin in a microcentrifuge for about 5 minutes at approximately 14,000 rcf.
- 2.8.7.7. Alternately transfer the cutting into a spin basket insert. Place the basket insert into the tube containing the stain extract. Spin in a microcentrifuge at approximately 14,000 rcf for about 5 minutes. Remove and discard the basket insert.
- 2.8.7.8. While being very careful to not disturb any pelleted material, remove the supernatant fluid from the extract and place it into a new, labeled tube. This supernatant is the non-sperm fraction. Analysis of the non-sperm fraction resumes at 2.8.7.15. The pellet remaining in the tube is the sperm fraction.
- 2.8.7.9. Wash the sperm pellet by resuspending it in 500 µl digest/wash buffer, vortexing the suspension briefly, and spinning the tube in a microcentrifuge at approximately 14,000 rcf for about 5 minutes. Remove and discard the supernatant fluid, being careful not to disturb the pellet.
- 2.8.7.10. Repeat 2.8.7.9 two additional times for a total of three washes of the sperm pellet.

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- 2.8.7.11. To the tube containing the washed pellet add:
150 µl Tris/EDTA/NaCl
50 µl 20% Sarkosyl
150 µl NFH₂O
10 µl Pro K (10 mg/ml)
40 µl 0.39M DTT
- 2.8.7.12. Close the tube cap, vortex and spin briefly in a microcentrifuge to force all fluid and material to the bottom of the tube.
- 2.8.7.13. Incubate at 37°C for 2 hours.
- 2.8.7.14. Spin briefly in a microcentrifuge to force the condensate into the bottom of the tube.
- 2.8.7.15. In the fume hood, add 400 µl phenol/chloroform/isoamyl alcohol (p/c/i) to which buffer has been added to both the tube containing the sperm pellet and to the tube containing the non-sperm fraction. Vortex the mixture briefly at low speed (in the fume hood) to attain a milky emulsion.
- 2.8.7.16. Spin in a microcentrifuge until completely separated (for at least 3 minutes) at approximately 14,000 rcf. Multiple p/c/i extractions may be performed to further clean up an extract if desired. The same treatment shall be performed to any associated reagent blanks.
- 2.8.7.17. See 2.8.8 for Microcon® procedure.

2.8.8. Microcon® Concentration of Extracted DNA (Microcon® 100 or Fast Flow)

- 2.8.8.1. To a Microcon® Concentrator add 100 µl TE⁻⁴.
- 2.8.8.2. For Microcon® concentration immediately after organic extraction, transfer the aqueous phase (top layer) to the concentrator in the fume hood. Avoid pipetting organic solvent from the tube into the concentrator. For Microcon® concentration of other DNA extracts, transfer the extract(s) to the concentrator.
- 2.8.8.3. Close the cap on the concentrator and spin in a microcentrifuge at 500 rcf for at least 10 minutes. Microcon® 100 may be spun up to 1,200 rcf.
- 2.8.8.4. Optional Step: Carefully remove the concentrator unit from the assembly and discard the fluid from the filtrate cup. Return the concentrator to the top of the filtrate cup.
- 2.8.8.5. Remove the cap and add 200 µl TE⁻⁴ to the concentrator. Replace the cap and spin the assembly in a microcentrifuge at 500 rcf for at least 10 minutes. Microcon® 100 may be spun up to 1,200 rcf.
- 2.8.8.6. Remove the cap and add a measured volume of TE⁻⁴ (40 µl is the recommended amount however, varied amounts can be added in some circumstances) to the concentrator. Remove the concentrator from the filtrate cup and carefully invert the concentrator into a new, labeled retentate cup. Discard the filtrate cup.
- 2.8.8.7. Spin the concentrator in a microcentrifuge at 1,000 rcf for at least 3 minutes. The Microcon® 100 should be spun 500-1,200 rcf for at least 5 minutes.
- 2.8.8.8. Discard the concentrator. If desired, the sample can be brought up with additional water by adding it to the retentate cup. Cap the retentate cup.
- 2.8.8.9. Extracts can be stored refrigerated or frozen prior to quantification/amplification. Prior to use after storage the samples shall be vortexed and spun briefly in a microcentrifuge.

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- 2.8.8.10. Multiple Microcon® concentration steps may be performed to further clean up or concentrate any extract. The same treatment shall be performed on the associated reagent blank (or a portion of) for each sample batch.

2.8.9. Centri-sep Clean-Up of Extracts

- 2.8.9.1. Clean-up of extracts with Centri-sep columns may be performed at any time. The same treatment shall be performed on the associated reagent blank (or a portion of) for each sample batch.
- 2.8.9.2. Remove the top column cap and reconstitute the column by adding 800 µl of nuclease free water (NFW). Leave the column end stopper in place so the column can stand up by itself. Replace the column cap and hydrate the gel by shaking and inverting the column or vortexing briefly.
- 2.8.9.3. Allow the column to hydrate for at least 30 minutes at room temperature. Reconstituted columns may be refrigerated overnight. Allow to warm up to room temperature before proceeding.
- 2.8.9.4. Remove the air bubbles from the column gel by inverting the column and sharply tapping the column, allowing the gel to slurry to the opposite end of the column. Stand the column up and allow the gel to settle while in a microtube rack.
- 2.8.9.5. First remove the column cap and then remove the column end stopper from the bottom.
- 2.8.9.6. Allow the excess column fluid to drain into a 2 ml wash tube. If the fluid does not begin to flow immediately through the column apply gentle pressure to the top of the column to force the fluid to start through the column filter. This can be accomplished by snapping the cap on briefly. Discard this fluid.
- 2.8.9.7. Spin the column and wash tube in a microcentrifuge at 750 rcf for 2 minutes. **NOTE:** It is important to keep track of the position on the column using the orientation mark molded into the column.
- 2.8.9.8. Optionally: discard eluate and rinse column with 150-200 µl NFW and spin for 2 minutes at 750 rcf.
- 2.8.9.9. Blot any drop at the end of the column. Discard the wash tube. Do not allow the gel material to dry excessively.
- 2.8.9.10. Transfer the sample extract to the top of the column, without disturbing the gel surface. Keep track of amount of sample added to column (less than 50 µl is recommended).
- 2.8.9.11. Place the column into the sample collection tube (1.5 ml) and place both into the microcentrifuge. Maintain proper column orientation. The highest point of the gel media in the column should always point toward the outside of the rotor. Spin the column and the collection tube at 750 rcf for 2 minutes.
- 2.8.9.12. Discard the spin column and cap the sample collection tube.
- 2.8.9.13. Extracts can be stored refrigerated or frozen prior to quantification/amplification. Prior to use after storage, the samples shall be vortexed and spun briefly in a microcentrifuge.

2.8.10. Demineralization of Bones, Teeth and Fingernails Used as Standards with the Promega Bone DNA Extraction Kit

The kit contains all the components necessary for the preprocessing method prior to Maxwell® automated extraction.

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- 2.8.10.1. Place 100 – 200 mg of crushed bone, tooth or fingernail into a microcentrifuge tube. Each batch of samples being extracted shall include a reagent blank as the last sample in the batch. Use the maximum weight of crushed sample if less than 100 mg.
 - 2.8.10.1.1. Exceeding 200 mg of crushed sample decreases the supernatant yield (2.8.10.6) and does not increase the final DNA yield. As an alternative to increased powder weight, multiple samples may be extracted; however, these samples shall not be combined until after the Maxwell® purification step.
- 2.8.10.2. Prepare the bone lysis cocktail A master mix by adding 400 µl Demineralization buffer, 40 µl Proteinase K (PK) solution and 10 µl 1-thioglycerol for each sample. 1-thioglycerol is viscous; pipet slowly. Add approximately 2 extra samples to account for pipetting error.
- 2.8.10.3. Add 400 µl of the bone lysis cocktail A to each sample and vortex for 10 seconds.
- 2.8.10.4. Incubate with shaking for 56°C at max speed for at least 2.5 hours. Alternatively, samples may be incubated overnight.
- 2.8.10.5. Remove from shaker and vortex for 10 seconds. Centrifuge the tubes at ~13,000 x g for 5 min.
- 2.8.10.6. Transfer the supernatant to a new microcentrifuge tube and discard the bone powder pellet.
- 2.8.10.7. Prepare bone lysis cocktail B master mix by adding 990 µl lysis buffer and 10 µl 1-thioglycerol for each sample.
- 2.8.10.8. Add 800 µl of bone lysis cocktail B to each sample and vortex for 10 seconds.
- 2.8.10.9. Proceed to Maxwell® FSC (2.8.11.3) or RSC 48 (2.8.11.4) Instrument Automated DNA Purification Methods.
 - 2.8.10.9.1. When adding the sample to the Maxwell® cartridge, pipette to mix the sample with the Maxwell® reagents.

2.8.11. Maxwell® Protocol

- 2.8.11.1. **Maxwell® Preparation of Samples for Any Cellular Material (except bone powder):**
 - 2.8.11.1.1. For each sample, combine 350 µl Incubation Buffer with 10 µl Pro K (18 mg/ml) and 40 µl DTT (1M). Alternatively, Stain Extraction Buffer for Automation (SEBA) may be substituted for Incubation Buffer. *Example: for 16 samples, combine 5,600 µl Incubation Buffer (or SEBA) with 160 µl Pro K and 640 µl DTT.*
 - 2.8.11.1.2. Place sample at the bottom of a 1.5 ml microcentrifuge tube or a Qiagen® Investigator® Lyse&Spin basket with 2 ml collection tube. Each group of samples being extracted shall include a reagent blank as the last sample in the batch. A batch can be no larger than the capacity of the Maxwell Unit being utilized. For a Maxwell® FSC it is 15 samples (plus 1 reagent blank). For a Maxwell® 48 it is 47 samples (plus 1 reagent blank). The reagent blank associated with each batch shall be run in the same Maxwell® run as the associated samples.
 - 2.8.11.1.3. To the sample add 400 µl Master Mix. Vortex and spin briefly in a microcentrifuge or tap the tube to force the cutting into the extraction fluid.
 - 2.8.11.1.4. Incubate the samples at 56°C for at least one hour. Alternatively, samples may be incubated overnight.
 - 2.8.11.1.5. **For samples lysed in a 1.5 ml microcentrifuge tube:**

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- 2.8.11.1.5.1. Spin briefly in a microcentrifuge to force the condensate into the bottom of the tube. (For hairs it may be preferable to transfer supernatant to a clean microcentrifuge tube before proceeding).
- 2.8.11.1.5.2. Optionally, 400 µl of a direct amp lysate may be placed in a 1.5 ml microcentrifuge tube.
- 2.8.11.1.5.3. Add 200 µl of Lysis Buffer to each sample.
- 2.8.11.1.5.4. Vortex samples briefly and spin down.
- 2.8.11.1.5.5. Using a wooden applicator stick, remove the cutting and proceed to 2.8.10.1.7 or
- 2.8.11.1.5.6. Transfer the cutting into a spin basket insert. Place the basket insert into the tube containing the stain extract. *Optionally, the Lysis Buffer may also be transferred to the spin basket.* Spin in a microcentrifuge at approximately 14,000 rcf for about 5 minutes. Remove and discard the basket insert.
- 2.8.11.1.6. **For samples lysed in a Qiagen® Investigator® Lyse&Spin basket:**
 - 2.8.11.1.6.1. Spin in a microcentrifuge at 10,000 – 14,000 rcf for at least 1 minute. Remove and discard the basket insert.
 - 2.8.11.1.6.2. Optionally, 400 µl of a direct amp lysate may be placed in a Qiagen® Investigator® Lyse&Spin tube.
 - 2.8.11.1.6.3. Add 200 µl of Lysis Buffer to each sample.
- 2.8.11.1.7. Save the extract until ready for automated DNA extraction. Do not refrigerate or freeze sample. Leave processed sample at room temperature (~22-25°C) overnight, if necessary.
- 2.8.11.1.8. Proceed to 2.8.10.3 or 2.8.10.4 for Maxwell® automated extraction.
- 2.8.11.2. **Maxwell® Preparation of Samples for Differential Extractions:**
 - 2.8.11.2.1. For each sample, combine 400 µl Tris/EDTA/NaCl, 10 µl 20% Sarkosyl, 90 µl NFH₂O and 5 µl Proteinase K (10 mg/ml).
 - 2.8.11.2.2. Place the sample in a 1.5 ml microcentrifuge tube. Qiagen® Investigator® Lyse&Spin baskets shall not be used for differential extractions. Each group of samples being extracted shall include reagent blanks (sperm and non-sperm fraction) as the last samples in the batch. A batch can be no larger than the capacity of the Maxwell Unit being utilized. For a Maxwell® FSC it is 15 samples (plus 1 reagent blank). For a Maxwell® 48 it is 47 samples (plus 1 reagent blank). The reagent blank associated with each batch shall be run in the same Maxwell® run as the associated samples.
 - 2.8.11.2.3. To the sample add 505 µl of the Master Mix. Vortex and spin briefly in a microcentrifuge or tap the tube to force the cutting into the extraction fluid.
 - 2.8.11.2.4. Incubate the sample at 37°C for 2 hours.
 - 2.8.11.2.5. Spin briefly in a microcentrifuge to force the condensate into the bottom of the tube.
 - 2.8.11.2.6. Using a wooden applicator stick, remove the cutting and spin in a microcentrifuge for about 5 minutes at approximately 14,000 rcf.

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- 2.8.11.2.7. Alternately transfer the cutting into a spin basket insert. Place the basket insert into the tube containing the stain extract. Spin in a microcentrifuge at approximately 14,000 rcf for about 5 minutes. Remove and discard the basket insert.
- 2.8.11.2.8. While being very careful to not disturb any pelleted material, remove the supernatant fluid from the extract and place it into a new, labeled tube. This supernatant is the non-sperm fraction. Analysis of the non-sperm fraction resumes at 2.8.10.2.11. The pellet remaining in the tube is the sperm fraction.
- 2.8.11.2.9. Wash the sperm pellet by resuspending it in 500 µl digest/wash buffer, vortexing the suspension briefly, and spinning the tube in a microcentrifuge at approximately 14,000 rcf for about 5 minutes. Remove and discard the supernatant fluid, being careful not to disturb the pellet.
- 2.8.11.2.10. Repeat step 2.8.10.2.9 two times for a total of three washes of the sperm pellet.
- 2.8.11.2.11. Lysis buffer step
 - 2.8.11.2.11.1. For each sample pair (sperm and non-sperm fraction) combine 600 µl of Lysis Buffer with 6 µl of 1M DTT.
 - 2.8.11.2.11.2. Add 400 µl of the Lysis Buffer master mix to each sperm fraction sample and 200 µl to each non-sperm fraction sample.
- 2.8.11.2.12. Vortex sample and spin down briefly.
- 2.8.11.2.13. Save until ready for automated DNA extraction. Do not refrigerate or freeze sample. Leave processed sample at room temperature (~22-25°C) overnight, if necessary.
- 2.8.11.2.14. Proceed to 2.8.10.3 or 2.8.10.4 Maxwell® automated extraction.
- 2.8.11.3. **Maxwell® FSC Instrument Automated DNA Purification:**
 - 2.8.11.3.1. Place the number of cartridges to be used into the cartridge preparation rack - one per sample. Ensure front and back of each cartridge is pushed down and snapped in. Remove the seals.
 - 2.8.11.3.2. Place one plunger into well #8 of each cartridge (closest well to elution tube).
 - 2.8.11.3.3. Transfer entire sample into well #1 of respective cartridge (furthest well from elution tube).
 - 2.8.11.3.4. Place an appropriately labeled 0.5ml elution tube into the elution tube slot associated with each sample, respectively. Ensure the elution tube is pressed all the way down into the holding well. Add 50µl of Elution Buffer to each elution tube.
 - 2.8.11.3.5. Gloves should not be worn when touching the tablet. A stylus should be used while wearing gloves. Use the power button on the far left top edge of the tablet to wake up the tablet.
 - 2.8.11.3.6. Swipe up on the tablet screen to select "Analyst" account on the tablet. Touch "Sign In" (no password necessary).
 - 2.8.11.3.7. Double tap the Maxwell® FSC icon on the tablet to open the instrument software. The instrument will perform a self-test and ask to start a sanitization cycle. This will take approximately 1 minute. Touch "Sanitize" to run the sanitization cycle. Each time the software is opened, the sanitization cycle needs to be run. If you do

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not run it upon opening of software it will force you to run before your samples are processed. Optionally this sanitization cycle may be performed between sample runs. After removing cartridge rack, from the home screen touch “Sanitize” then “Start”.

- 2.8.11.3.8. To begin a sample run select “Start” from the Maxwell® software home screen.
- 2.8.11.3.9. Using the barcode scanner attached to the instrument, scan the QR code at the top of the kit label of the Maxwell® FSC DNA IQ™ Casework Kit. Alternatively, you may touch the appropriate kit listed on the screen. This will highlight the appropriate kit on the screen. Touch “Proceed” on the right side of the highlighted line.
- 2.8.11.3.10. Touch “Proceed” at the bottom center of the screen. If the software window needs to be minimized to allow the proceed button to be visible, double tap the top center of the software window and move, as necessary.
- 2.8.11.3.11. “The door will now open” box will appear. Touch “OK”.
- 2.8.11.3.12. Load cartridge rack with cartridges, plungers, samples and **open** elution tubes. Touch “Start”.
- 2.8.11.3.13. If a run needs to be aborted, touch “Abort” then “Open Door”. A report will populate the screen. Touch the home icon in the upper left corner. To remove plungers, run a clean-up as described in 2.8.10.4.16.
 - 2.8.11.3.13.1. If a run is aborted by the analyst or by the instrument, Technical Leader notification is required.
- 2.8.11.3.14. When the run is complete touch “Open Door”. A report will populate the screen. Wait for the report to populate, then touch the home icon in the upper left corner.
- 2.8.11.3.15. If all plungers have been properly ejected from the bar into the cartridges, proceed to 2.8.10.4.17. If plungers have not properly ejected from the bar, you **MUST** run a clean-up. Do not remove cartridge rack from instrument.
- 2.8.11.3.16. To run a clean-up, touch “Settings” from the home screen. Then select “Clean Up” and touch “Start”. A report will populate the screen when finished. Touch the home icon in the upper left corner. You will then need to open the instrument door with the door icon (rectangle) in the upper right corner next to the question mark.
- 2.8.11.3.17. Remove cartridge rack from instrument.
- 2.8.11.3.18. Touch the door icon in the upper right to close the instrument door.
- 2.8.11.3.19. Close all elution tubes and remove from cartridge rack.
- 2.8.11.3.20. Remove all plungers from cartridges and dispose.
- 2.8.11.3.21. Remove all cartridges from cartridge rack and dispose.
- 2.8.11.3.22. Clean Maxwell® instrument and cartridge rack with 70% ethanol – never bleach! Tablet screens may be wiped with microfiber screen cleaning cloths.
- 2.8.11.3.23. Close out of the Maxwell® FSC software using the X icon in the upper left corner then touch “Yes”.
- 2.8.11.3.24. Using the Windows icon in the lower left corner of the tablet, sign out of the tablet by touching the person icon in the circle then “Sign out”.
- 2.8.11.3.25. DO NOT turn off the instrument.

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2.8.11.4. Maxwell® RSC 48 Instrument Automated DNA Purification:

- 2.8.11.4.1. Place the number of cartridges to be used into the cartridge preparation rack - one per sample. Ensure front and back of each cartridge is pushed down and snapped in. Remove the seal.
- 2.8.11.4.2. Place one plunger into well #8 of each cartridge (closest well to elution tube).
- 2.8.11.4.3. Transfer entire sample into well #1 of respective cartridge (furthest well from elution tube).
- 2.8.11.4.4. Place an appropriately labeled 0.5ml elution tube into the elution tube slot associated with each sample, respectively. Ensure the elution tube is pressed all the way down into the holding well. Add 50 µl of Elution Buffer to each elution tube.
- 2.8.11.4.5. Gloves should not be worn when touching the tablet. A stylus should be used while wearing gloves. Use the power button on the far left top edge of the tablet to wake up the tablet.
- 2.8.11.4.6. Swipe up on the tablet screen to select "Analyst" account on the tablet. Touch "Sign In" (no password necessary).
- 2.8.11.4.7. Double tap the Maxwell® RSC 48 icon on the tablet to open the instrument software. The instrument will perform a self-test and ask to start a sanitization cycle. This will take approximately 1 minute. Touch "Sanitize" to run the sanitization cycle. Each time the software is opened, the sanitization cycle needs to be run. If you do not run it upon opening of software it will force you to run before your samples are processed. Optionally this sanitization cycle may be performed between sample runs. After removing cartridge racks, from the home screen touch "Sanitize" then "Start".
- 2.8.11.4.8. To begin a sample run, select "Start" from the Maxwell® software home screen.
- 2.8.11.4.9. Using the barcode scanner attached to the instrument, scan the QR code at the top of the kit label of the Maxwell® FSC DNA IQ™ Casework Kit. Alternatively, you may touch the appropriate kit listed on the screen. This will highlight the appropriate kit on the screen. Touch "Proceed" on the right side of the highlighted line.
- 2.8.11.4.10. "The door will now open" box will appear. Touch "OK".
- 2.8.11.4.11. Load cartridge rack with cartridges, plungers, samples and elution tubes. Rack labeled "Front" must go in the front and rack labeled "Back" must go in the back of the instrument. Touch "Start". The instrument will automatically detect placement of the sample cartridges, if plungers are present, if elution tubes are present and if elution tubes are opened. If anything is wrong an error will pop up and ask you to take a closer look at your sample preparation before proceeding.
- 2.8.11.4.12. If a run needs to be aborted, touch "Abort" then "Open Door". A report will populate the screen. Touch the home icon in the upper left corner. To remove plungers, run a clean-up as described in 2.8.11.5.15.
 - 2.8.11.4.12.1. If a run is aborted by the analyst or by the instrument, Technical Leader notification is required.
- 2.8.11.4.13. When the run is complete touch "Open Door". A report will populate the screen. Wait for the report to populate, then touch the home icon in the upper left corner.

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- 2.8.11.4.14. If all plungers have been properly ejected from the bar into the cartridges, proceed to 2.8.10.5.16. If plungers have not properly ejected from the bar, you MUST run a clean-up. Do not remove cartridge rack from instrument.
- 2.8.11.4.15. To run a clean-up, touch "Settings" from the home screen. Then select "Clean Up" and touch "Start". A report will populate the screen when finished. Touch the home icon in the upper left corner. You will then need to open the instrument door with the door icon (rectangle) in the upper right corner next to the question mark.
- 2.8.11.4.16. Remove cartridge rack from instrument.
- 2.8.11.4.17. Touch the door icon in the upper right to close the instrument door.
- 2.8.11.4.18. Close all elution tubes and remove from cartridge rack.
- 2.8.11.4.19. Remove all plungers from cartridges and dispose.
- 2.8.11.4.20. Remove all cartridges from cartridge rack and dispose.
- 2.8.11.4.21. Clean Maxwell® instrument and cartridge rack with 70% ethanol – never bleach! Tablet screens may be wiped with a microfiber screen cleaning cloth.
- 2.8.11.4.22. Close out of the Maxwell® RSC 48 software using the X icon in the upper left corner then touch "Yes".
- 2.8.11.4.23. Using the Windows icon in the lower left corner of the tablet, sign out of the tablet by touching the person icon in the circle then "Sign out".
- 2.8.11.4.24. DO NOT turn off the instrument.

2.8.12. Rehydration of a Sample after Storage

- 2.8.12.1. Evaluate the stored sample and associated reagent blank for approximate volume.
- 2.8.12.2. If enough liquid is present in the sample and the reagent blank, they can be used for amplification.
 - 2.8.12.2.1 The sample and reagent blank shall be quantified and treated as normal.
- 2.8.12.3. If the volume is low or none, determine if the sample and/or the reagent blank extract was concentrated and consumed or almost consumed from the case record.
 - 2.8.12.3.1. If the reagent blank was concentrated and consumed after the issue of the 2009 DNA QAS audit document no further testing should be done.
 - 2.8.12.3.2. If the case was completed before the issue of the 2009 DNA QAS audit document and the reagent blank was consumed consult with the Technical Leader about reagent blanks.
- 2.8.12.4** If the volume is low and the loss of volume is from dehydration, the sample can be rehydrated.
 - 2.8.12.4.1 To the sample add 400 µl of TE⁻⁴.
 - 2.8.12.4.2 Add TE⁻⁴ to the reagent blank as needed. The volume added should not exceed the least amount added to any associated sample.
 - 2.8.12.4.3 The final volume of the reagent blank should not exceed the volume of any associated sample.

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- 2.8.12.4.4 If the reagent blank did not dehydrate, an additional reagent blank with the same volume of TE⁻⁴ as added to the sample shall be created and tested with the sample.
- 2.8.12.4.5 Briefly vortex the samples.
- 2.8.12.4.6 Incubate at least 2 hours to 24 hours at 4°C, vortexing periodically.
- 2.8.12.4.7 Concentrate the sample and associated reagent blank(s) to approximately 50 µl each with a Microcon®.
- 2.8.12.4.8 Quantify the sample and reagent blank(s) after rehydration.

2.8.13 Quantifiler® Trio DNA Quantification

2.8.13.1 Introduction

- 2.8.13.1.1 The Applied Biosystems® Quantifiler® Trio DNA Quantification Kit utilizes multi-copy target loci to simultaneously quantify an 80 bp small autosomal target (sm. auto), a 214 bp large autosomal target (lg. auto), and a 75 bp male Y-chromosome target (Y) to enable estimation of the amount of human DNA, male DNA, and level of degradation. The Internal PCR Control (IPC) enables inhibitor detection in the sample extract prior to STR amplification.
- 2.8.13.1.2 Each assay target consists of PCR primers and TaqMan® probes labeled with both a reporter dye and non-fluorescent quencher. During PCR, the TaqMan® probes anneal to a specific location between the targeted forward and reverse primers. Taq DNA polymerase cleaves the hybridized probes separating the reporter dye from the quencher, resulting in increased fluorescence.
- 2.8.13.1.3 The 7500 Real-Time PCR System measures the fluorescent signal of all four target reporter signals (VIC®, ABY®, FAM™, and JUN®) as well as a passive reference dye (Mustang Purple®) to help normalize the signal between samples. The signals for each sample are compared to that of a standard curve of known quantity to estimate DNA concentrations.
- 2.8.13.1.4 The Quantifiler® Trio Primer Mix and the Quantifiler® THP PCR Reaction Mix shall be protected from excessive exposure to light.
- 2.8.13.1.5 The preparation of the quantification plate shall be performed in the PCR amplification set-up area.

2.8.13.2 Standard Curve Preparation (may be set up in the PCR Amplification set-up area)

- 2.8.13.2.1 Label four microcentrifuge tubes “Std 1” through “Std 4”.
- 2.8.13.2.2 Dispense the appropriate volume of Quantifiler® THP Dilution Buffer to each tube using the appropriate option from the table below.
- 2.8.13.2.3 Briefly vortex the Quantifiler® THP DNA Standard [100 ng/µl stock].
- 2.8.13.2.4 Add the calculated amount of Quantifiler® THP DNA Standard to the tube labeled “Std 1” and mix thoroughly.
- 2.8.13.2.5 Using a new pipette tip, add 4 µl of “Std 1” to “Std 2” and mix thoroughly.
- 2.8.13.2.6 Complete the dilution series by adding 4 µl of “Std 2” to “Std 3” and “Std 3” to “Std 4”, mix thoroughly and use a new pipette tip with each step.
- 2.8.13.2.7 The standard curve is stable for up to 2 weeks when stored at 2 to 8°C.

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Standard	Concentration (ng/μl)	Dilution Buffer	DNA Standard	Dilution Factor
Std 1	50	15 μl dilution buffer (Option 1) 25 μl dilution buffer (Option 2) 35 μl dilution buffer (Option 3)	15 μl DNA stock (Option 1) 25 μl DNA stock (Option 2) 35 μl DNA stock (Option 3)	2X
Std 2	2.0	96 μl dilution buffer	4 μl Std 1	25X
Std 3	0.08	96 μl dilution buffer	4 μl Std 2	25X
Std 4	0.0032	96 μl dilution buffer	4 μl Std 3	25X
Note:	Option 1 – sufficient volume for 6 quant plates; Option 2 – sufficient volume for 11 quant plates; Option 3 – Sufficient volume for 16 quant plates			

2.8.13.3 Quantification Reaction Preparation

- 2.8.13.3.1** Each quantification plate shall contain a standard curve series run in duplicate in column 12 and a Non-Template Control (NTC) in well H11.
- 2.8.13.3.2** Determine the number of samples to be quantified, including standards and NTC, and add ~3 reactions to compensate for the loss that occurs during pipetting.
- 2.8.13.3.3** Using the Quantifiler® Trio Plate Record Worksheet, calculate the required amount of each component of the master mix.

PCR Master Mix Component	Volume per Reaction (μl)
Quantifiler® Trio Primer Mix	8
Quantifiler® THP PCR Reaction Mix	10
Total Volume	18

- 2.8.13.3.4** Vortex the Primer Mix 3 to 5 seconds, then centrifuge briefly.
- 2.8.13.3.5** Gently vortex the PCR Reaction Mix.
- 2.8.13.3.6** Pipette the required volume of each component into a microcentrifuge tube. Total volumes exceeding 1300 μl may be split into 2 tubes equally.
- 2.8.13.3.7** Vortex the Master Mix 3 to 5 seconds, then centrifuge briefly.
- 2.8.13.3.8** Dispense 18 μl of the Master Mix into each reaction well of a 96-well optical plate.
- 2.8.13.3.9** Add 2 μl of sample, standard, or Non-Template Control (water or dilution buffer) to the appropriate wells for a total reaction volume of 20 μl.
- 2.8.13.3.10** Seal the reaction plate with an Optical Adhesive Cover using the cover applicator.
- 2.8.13.3.11** Centrifuge at 3000 rpm for approximately 10 seconds or spin using a salad spinner to remove any bubbles and force samples into the bottom of each well.
- 2.8.13.3.12** Load the plate on the 7500 Real-Time PCR instrument ensuring proper orientation.

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2.8.13.4 Quantification Experiment Setup

2.8.13.4.1 Turn on the computer.

2.8.13.4.2 Turn on the 7500 Real-Time PCR instrument.

2.8.13.4.3 Open the HID Real-Time PCR Analysis Software v1.2.

2.8.13.4.4 Select the **Quantifiler® Trio** icon on the Home screen.

2.8.13.4.5 Enter the experiment name: First case on plate_date_plate# (i.e., 00A1234_01Jan16_01).

2.8.13.4.6 Ensure the following experiment properties are selected:

Instrument:	7500 (96 Wells)
Experiment Type:	Quantitation – HID Standard Curve
Reagents:	TaqMan® Reagents
Ramp speed:	Standard (~1 hour to complete a run)

2.8.13.4.7 Select **Plate Setup** from the left navigational panel.

2.8.13.4.8 Sample names may be entered manually or imported from a DNA Workbook into the HID Software. All samples shall be uniquely identified by case number and sample identifier.

2.8.13.4.8.1 Sample Name manual entry:

2.8.13.4.8.1.1 Select **Add New Sample** to enter each sample name.

2.8.13.4.8.1.2 Sample Type shall be designated as “Unknown” for casework samples.

2.8.13.4.8.1.3 Select the **Assign Targets and Samples** tab to enter the sample information into each well. Unused wells shall be left blank.

2.8.13.4.8.1.4 Verify that the standard curve and NTC information is correct.

2.8.13.4.8.2 Sample Name import:

2.8.13.4.8.2.1 In the DNA Workbook, navigate to the “Quant” tab, ensure that the sample information is filled out, and click **Create Quant Import File**. Copy the .txt file to the “IMPORT” folder on the 7500 Real-Time computer desktop.

2.8.13.4.8.2.2 In the HID Software, select **File** → **Import**, navigate to and select the .txt file that was created by the workbook.

2.8.13.4.8.2.3 The associated samples and standards will populate within their respective wells. Verify that the samples, standard curve, and NTC information is correct.

2.8.13.4.9 Assay targets, standard curve quantities, assay parameters, and export criteria are automatically assigned when using the Quantifiler® Trio template.

2.8.13.4.10 Save the plate document to the “CASEWORK” folder on the D: drive.

2.8.13.4.11 Click 

2.8.13.4.12 The Quantifiler® Trio assay will take ~1 hour.

2.8.13.5 HID Real-Time PCR Analysis Software set-up See [Appendix 2](#)

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2.8.13.6 Experiment Analysis

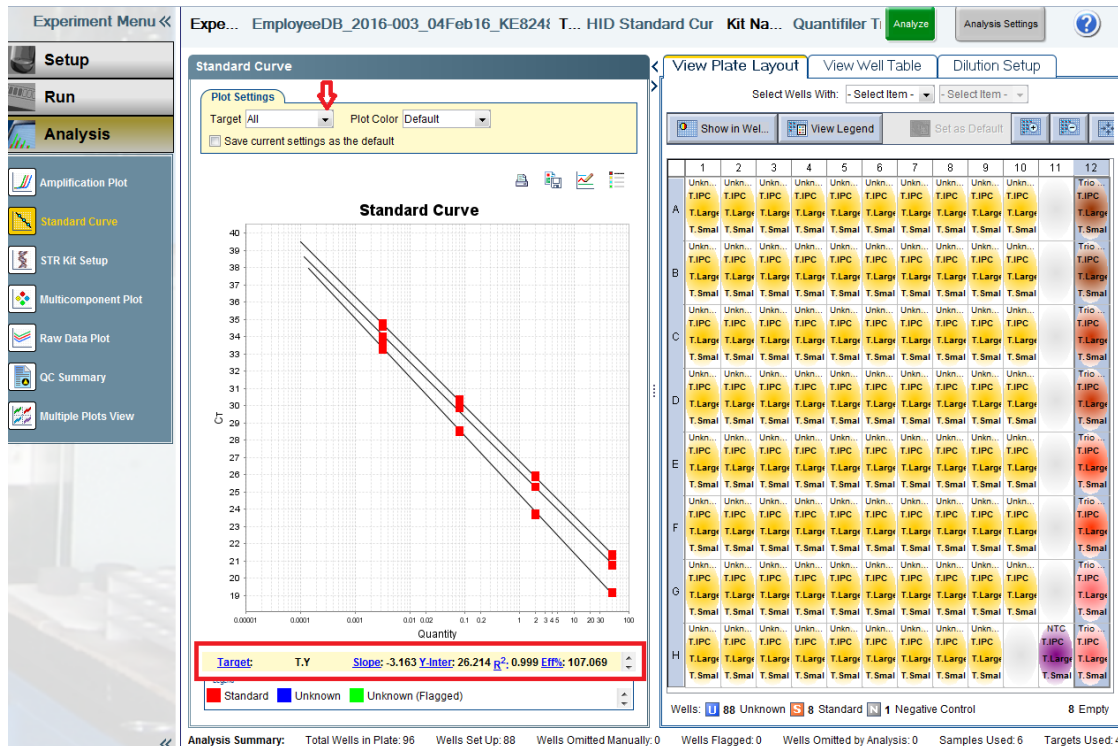
2.8.13.6.1 Analyze the experiment by clicking

Analyze

2.8.13.6.2 Evaluate the slope, Y-intercept, and R^2 values of all three standard curves as described under **2.8.13.7 Interpretation Guidelines**.

2.8.13.6.2.1 Up to 2 points from each curve may be deleted by right-clicking on the well, selecting **omit**, and selecting the appropriate target(s) or well. The results must be reanalyzed to incorporate the changes.

2.8.13.6.2.2 Omission of more than 2 points in any standard curve requires documented approval by a supervisor and notification to the Technical Leader.



2.8.13.6.3 Export the .txt data file to the “CASEWORK” folder on the D: drive using the previously established export properties.

2.8.13.6.4 Import the .txt data file to the DNA Workbook **Quant** tab.

2.8.13.6.5 View and retain the **Quant Results** tab. The case notes shall contain the quantification values, IPC C_T values, degradation indices, and all three standard curve slope, R^2 , and y-intercept values.

2.8.13.6.6 The .eds run file and .txt data file shall be saved to each associated laboratory case number and request folder located in the analyst’s folders on the server.

2.8.13.6.7 Quantification values from the small autosomal target shall be used for amplification of autosomal STRs.

2.8.13.6.8 Quantification values from the male target shall be used for amplification of Y-STRs.

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2.8.13.6.9 The quantification plate shall be discarded after the analysis is complete.

2.8.13.7 Interpretation Guidelines

2.8.13.7.1 Standard Curve: Evaluate the Small Autosomal, Large Autosomal, and Y standard curves individually or simultaneously.

2.8.13.7.1.1 Slope: Indicates the PCR amplification efficiency for the assay. A slope of -3.3 indicates 100% amplification efficiency. The analyst shall obtain documented Technical Leader approval to use data when a slope is outside the acceptable range. Scientific rounding rules to one decimal place apply.

Target	Acceptable slope range	Average slope
Large Autosomal	-3.1 to -3.7	-3.4
Small Autosomal	-3.0 to -3.6	-3.3
Y	-3.0 to -3.6	-3.3

2.8.13.7.1.2 Y-intercept: The expected C_T value for a 1 ng/μl sample. Y-intercept averages may vary by instrument, kit lot, and pipetting differences. The analyst shall obtain documented Technical Leader approval to use data when a Y-intercept value is outside the acceptable range. Scientific rounding rules to one decimal place apply.

Target	Acceptable Y-intercept range (C_T)
Large Autosomal	23.0 to 27.0
Small Autosomal	25.0 to 29.0
Y	25.0 to 29.0

2.8.13.7.1.3 R^2 value: A measure of the closeness of fit between the standard curve regression line and the individual C_T data points of the standard curve. A value of 1.00 indicates a perfect fit between the regression line and the data points. This value shall be ≥ 0.980 . If the R^2 value is <0.98 the experiment is inconclusive and shall be repeated with notification to the Technical Leader documented in the case record. Scientific rounding rules to two decimal places apply.

2.8.13.7.2 Internal PCR Control (IPC): Differentiates between a sample containing no human DNA and reactions affected by the presence of PCR inhibitors, assay setup, and chemistry or instrument failure.

2.8.13.7.2.1 An elevated IPC C_T value (relative to quantitation standards) is an indication of the presence of an inhibitor or high concentration of human template DNA.

2.8.13.7.2.2 If inhibition is suspected, the analyst may re-Microcon® and/or Centri-sep the sample prior to amplification and typing of the sample.

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- 2.8.13.7.2.3 If high concentration of human DNA is present, the analyst may make dilutions of the sample and re-quantify.
- 2.8.13.7.2.4 The analyst shall make reasonable efforts to rectify samples in which the IPC is undetected. If a DNA profile is not developed from a sample with an undetected IPC, it shall be reported as inconclusive.

Autosomal or Y targets	IPC	Interpretation	Suggestions / Options
Undetected	Detected	Negative result; No human DNA	No further analysis of sample
Undetected	Undetected	Invalid result; possible inhibition	Re-Microcon® and/or Centri-sep; Re-extract; or dilute and re-quantify
Detected (Low C _T)	Undetected or Elevated	Inconclusive IPC result	Dilute and re-quantify (optional)
Detected (High C _T)	Undetected or Elevated	Partial PCR Inhibition	Re-Microcon® and/or Centri-sep; Re-extract; or dilute and re-quantify

- 2.8.13.7.3 Non-Template Control (NTC):** Contains PCR reagents with water or dilution buffer in place of template DNA and is therefore expected to be undetected (i.e., value of 0.00) for all three quantification values. However, background fluorescence or contamination may occur resulting in quantification values with one or more targets.
- 2.8.13.7.3.1 A NTC demonstrating quantification value(s) ≥ 0.001 ng/μl requires documented notification to the Technical Leader and shall be considered a failed control. The quantification shall be repeated.
- 2.8.13.7.3.2 A NTC demonstrating quantification value(s) < 0.001 ng/μl may be due to background fluorescence. The analyst may proceed with amplification and typing of the samples using caution with interpretation of any profiles obtained from the samples.
- 2.8.13.7.4 Dynamic range:** The quantitation standard curve ranges from 50 to 0.0032 ng/μl. Quantitation values are most reliable when they fall near the middle of the standard curve and less reliable as they approach the ends and beyond.
- 2.8.13.7.4.1 If the quantity of small autosomal DNA in a sample is < 0.01 ng/μl, no additional autosomal STR analysis of that sample shall be performed.
- 2.8.13.7.4.2 If the quantity of male DNA in a sample is < 0.006 ng/μl, no additional Y-STR analysis of that sample shall be performed.
- 2.8.13.7.4.3 The analyst should make a reasonable effort to target ≤ 50 ng/μl during extraction. If a sample quantifies > 50 ng/μl and is suitable for amplification, a dilution shall be made and quantified.
- 2.8.13.7.5 Female To Male Ratio (FTMR):** In instances where it is valuable to identify a male contributor from a female contributor (i.e., sexual assault cases), the FTMR can assist in prioritizing samples and determining which are suitable for autosomal STR analysis and which are suitable for Y-STR analysis. The female DNA contribution

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is determined by subtracting the quantity of male DNA from the quantity of small autosomal DNA. The FTMR is determined by dividing the female contribution by the quantity of male DNA (equation: $[(\text{sm. auto-Y})/Y]$). The selection of samples to continue DNA analysis may be prioritized at analyst discretion by using the FTMR and quantity of male DNA input as described below. Priority 1 samples and Priority 2 and 3 samples with male DNA concentrations $\geq 0.006 \text{ ng}/\mu\text{l}$ should be amplified if no other samples have provided informative results.

- 2.8.13.7.5.1 Priority 1: Samples with FTMR $\leq 5:1$ are generally expected to yield interpretable information from male contributor with autosomal STR analysis.
- 2.8.13.7.5.2 Priority 2: Samples with FTMR between $5:1$ and $20:1$ may yield interpretable information from the minor male contributor with autosomal STR analysis if the concentration of male DNA is $\geq 0.006 \text{ ng}/\mu\text{l}$. If the male DNA concentration is less than $0.006 \text{ ng}/\mu\text{l}$, no further analysis should be performed.
- 2.8.13.7.5.3 Priority 3: Samples with FTMR between $20:1$ and $50:1$ are generally expected to yield minimal information from the minor male contributor with autosomal STR analysis if the concentration of male DNA is $\geq 0.006 \text{ ng}/\mu\text{l}$. If the male DNA concentration is less than $0.006 \text{ ng}/\mu\text{l}$, no further analysis should be performed.
- 2.8.13.7.5.4 Priority 4: Samples with FTMR $>50:1$ are generally not expected to yield interpretable information from the minor male contributor with autosomal STR analysis, therefore no further autosomal analysis should be performed. Y-STR analysis may be recommended if the concentration of male DNA $\geq 0.006 \text{ ng}/\mu\text{l}$.
- 2.8.13.7.6 **Degradation Index:** A value to assist in the identification of degradation prior to amplification. The degradation index is calculated by dividing the small autosomal by the large autosomal quant values. An index >1 may indicate that the analyst should increase the template DNA for autosomal STR amplification to generate interpretable data from the larger molecular weight loci.
- 2.8.13.7.7 **Reagent blank:** Should not have associated DNA and is therefore expected to be undetected (i.e., value of 0.00) for all three quantification values. However, background fluorescence or contamination may occur resulting in quantification values with one or more targets.
 - 2.8.13.7.7.1 A reagent blank that demonstrates quantification value(s) $\geq 0.001 \text{ ng}/\mu\text{l}$ requires documented notification to the Technical Leader. It shall be considered a failed control unless re-quantification and/or amplification demonstrate no contamination. It is recommended that the analyst evaluate the results for possible sample switching and requantify if that is suspected.
 - 2.8.13.7.7.1.1 If the reagent blank is amplified and no peaks above analytical threshold are observed in the electropherogram, the associated samples may be interpreted with caution.
 - 2.8.13.7.7.2 A reagent blank that demonstrates quantification value(s) $<0.001 \text{ ng}/\mu\text{l}$ may be due to background fluorescence or low level contamination.

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- 2.8.13.7.7.2.1 If all associated samples fail to demonstrate a sufficient quantity of DNA for amplification (i.e., <0.01 ng/ μ l for autosomal STRs; <0.006 ng/ μ l for Y-STRs), they shall be reported as such.
- 2.8.13.7.7.2.2 If the reagent blank is amplified and no peaks above analytical threshold are observed in the electropherogram, the associated samples may be interpreted normally.
- 2.8.13.7.7.3 A reagent blank that demonstrates peaks in the electropherogram shall be considered a failed control.
- 2.8.13.7.7.4 Any sample associated with a failed reagent blank, but not amplified due to an insufficient quantity of DNA (i.e., <0.01 ng/ μ l for autosomal STRs; <0.006 ng/ μ l for Y-STRs) shall be reported as failed to demonstrate a sufficient quantity of (male) DNA for further/autosomal STR analysis.
- 2.8.13.7.7.5 Samples associated with a failed reagent blank shall not be submitted for Y-STR analysis.

2.8.14 PowerQuant® DNA Quantification

2.8.14.1 Introduction

- 2.8.14.1.1 The PowerQuant® System is a five-dye, four-target hydrolysis probe-based qPCR multiplex that amplifies multicopy targets to quantify the total human and human male DNA present in a sample. The system also amplifies an additional multicopy target to assess the degree of DNA degradation. Additionally, the PowerQuant® System includes an internal PCR control (IPC) to detect inhibitors in an amplification reaction. The PowerQuant® System achieves levels of sensitivity and inhibitor tolerance comparable to those of newer STR systems (e.g., PowerPlex® Fusion and PowerPlex® Y23 Systems).
- 2.8.14.1.2 Each assay target consists of PCR primers and TaqMan® probes labeled with both a reporter dye and non-fluorescent quencher. During PCR, the TaqMan® probes anneal to a specific location between the targeted forward and reverse primers. Taq DNA polymerase cleaves the hybridized probes separating the reporter dye from the quencher, resulting in increased fluorescence.
- 2.8.14.1.3 The QuantStudio™ 5 Real-Time PCR System measures the fluorescent signal of all four target reporter signals (FAM™, CAL Fluor® Gold 540, TMR, Quasar® 670) as well as a passive reference dye (CXR) to help normalize the signal between samples. The signals for each sample are compared to that of a standard curve of known quantity to estimate DNA concentrations.
- 2.8.14.1.4 The PowerQuant® 2X Master Mix and the PowerQuant® Primer/Probe/IPC Mix shall be protected from excessive exposure to light.
- 2.8.14.1.5 The preparation of the quantification plate shall be performed in the PCR amplification set-up area.

2.8.14.2 Preparation of CXR Reference Dye

- 2.8.14.2.1 A separate 100 μ l tube of CXR Reference Dye will be supplied with each PowerQuant kit ordered.
- 2.8.14.2.2 One tube of CXR should be stored frozen within the kit it is associated with until the kit is ready for use.

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2.8.14.2.3 Upon thawing the PowerQuant® kit for use, the CXR reference dye must be diluted with the amplification grade water supplied in the kit.

2.8.14.2.3.1 Vortex the CXR thoroughly.

2.8.14.2.3.2 Add 20 µl of CXR to 1380 µl of amp grade water in four separate microcentrifuge tubes.

2.8.14.2.3.3 Store the original CXR tube as well as the four tubes of CXR/water in the respective PowerQuant® kit.

2.8.14.3 Standard Curve Preparation (may be set up in the PCR Amplification set-up area)

2.8.14.3.1 Label four microcentrifuge tubes “Std 1” through “Std 4”.

2.8.14.3.2 Dispense the appropriate volume of PowerQuant® Dilution Buffer to each tube using the volumes from the table below.

2.8.14.3.3 Briefly vortex the PowerQuant® Male gDNA Standard. This undiluted standard (50 ng/µl stock) is used as Std 1.

2.8.14.3.4 Add the calculated amount of PowerQuant® Male gDNA Standard to the tube labeled “Std 2” and mix thoroughly.

2.8.14.3.5 Using a new pipette tip, add 4 µl of “Std 2” to “Std 3” and mix thoroughly.

2.8.14.3.6 Complete the dilution series (using a new pipette tip) to add 4 µl of “Std 3” to “Std 4”, mix thoroughly.

2.8.14.3.7 The standard curve is stable for up to 2 weeks when stored at 2 to 8°C.

Standard	Concentration (ng/µl)	Dilution Buffer	DNA Standard	Dilution Factor
Std 1	50	N/A	PowerQuant® Male gDNA Standard	N/A
Std 2	2	96 µl dilution buffer	4 µl PowerQuant® Male gDNA Standard	25X
Std 3	0.08	96 µl dilution buffer	4 µl Std 2	25X
Std 4	0.0032	96 µl dilution buffer	4 µl Std 3	25X

2.8.14.4 Quantification Reaction Preparation

2.8.14.4.1 Each quantification plate shall contain a standard curve series run in duplicate in column 12 and a Non-Template Control (NTC) in well H11.

2.8.14.4.2 Determine the number of samples to be quantified, including standards and NTC, and add ~1-3 reactions to compensate for the loss that occurs during pipetting.

2.8.14.4.3 Using the PowerQuant® Plate Record Worksheet, calculate the required amount of each component of the master mix.

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PCR Master Mix Component	Volume per Reaction (µl)
PowerQuant® 2X Master Mix	10
PowerQuant® 20X Primer/Probe/IPC Mix	1
CXR + Amp Grade Water	7
Total Volume	18

2.8.14.4.4 Vortex the Master Mix, Primer Mix, and Water/CXR to mix.

2.8.14.4.5 Pipette the required volume of each component into a microcentrifuge tube and vortex to mix thoroughly. A 2 ml Dolphin tube will accommodate the volume needed for a full plate; otherwise, the total volume exceeding 1300 µl may be split into two 1.5 ml tubes equally.

2.8.14.4.6 Dispense 18 µl of the Master Mix into each reaction well of a 96-well optical plate.

2.8.14.4.7 Add 2 µl of sample, standard, or Non-Template Control (dilution buffer) to the appropriate wells for a total reaction volume of 20 µl.

2.8.14.4.8 Seal the reaction plate with an Optical Adhesive Cover using the cover applicator.

2.8.14.4.9 Centrifuge at 3000 rpm for approximately 10 seconds or spin using a salad spinner to remove any bubbles and force samples into the bottom of each well.

2.8.14.5 Quantification Setup with QuantStudio™ Design & Analysis Software v1.6.1

2.8.14.5.1 Turn on the computer and log on using the INSTR-ADMIN account.

Password: INSTR-ADMIN

2.8.14.5.2 Turn on the QuantStudio™ 5 Real-Time PCR instrument using the switch on the back right, above the power cord.

2.8.14.5.3 When the main menu appears on the instrument screen, open the plate drawer.

2.8.14.5.3.1 Ensure the area in front of the drawer is clear.

2.8.14.5.3.2 Press the open/close icon on the top right of the screen:



2.8.14.5.4 Place quant plate in the drawer ensuring proper orientation. Press the same icon to close the drawer.

2.8.14.5.5 Double click on “PowerQuant_v1_19AUG26.edt” on the desktop. The QuantStudio™ Design & Analysis Software v1.6.1 screen will appear.

2.8.14.5.5.1 NOTE: Do not open the QuantStudio™ Design & Analysis Software directly by clicking on the shortcut or program. You must open it from

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the saved Experiment named above. This Experiment is “Read-Only” so you will not be able to save over the template.

2.8.14.5.6 Once the Experiment has opened, type your plate name in the Name field under the **Properties** tab at the top. It currently says “PowerQuant” in the box. All other properties will remain the same.

2.8.14.5.6.1 Experiment name format: First case on plate_date_plate# (i.e., 00A1234_01Jan16_01).

QuantStudio™ Design & Analysis Software v1.6.1

File Edit Analysis Tools Help

Properties Method Plate Run Results Export

Experiment Properties

Name PowerQuant

Barcode NA

User name NA

Instrument type QuantStudio™ 5 System

Block type 96-Well 0.2-mL Block

Experiment type Standard Curve

Chemistry TaqMan® Reagents

Run mode Standard

Manage chemistry details

Type in plate name!

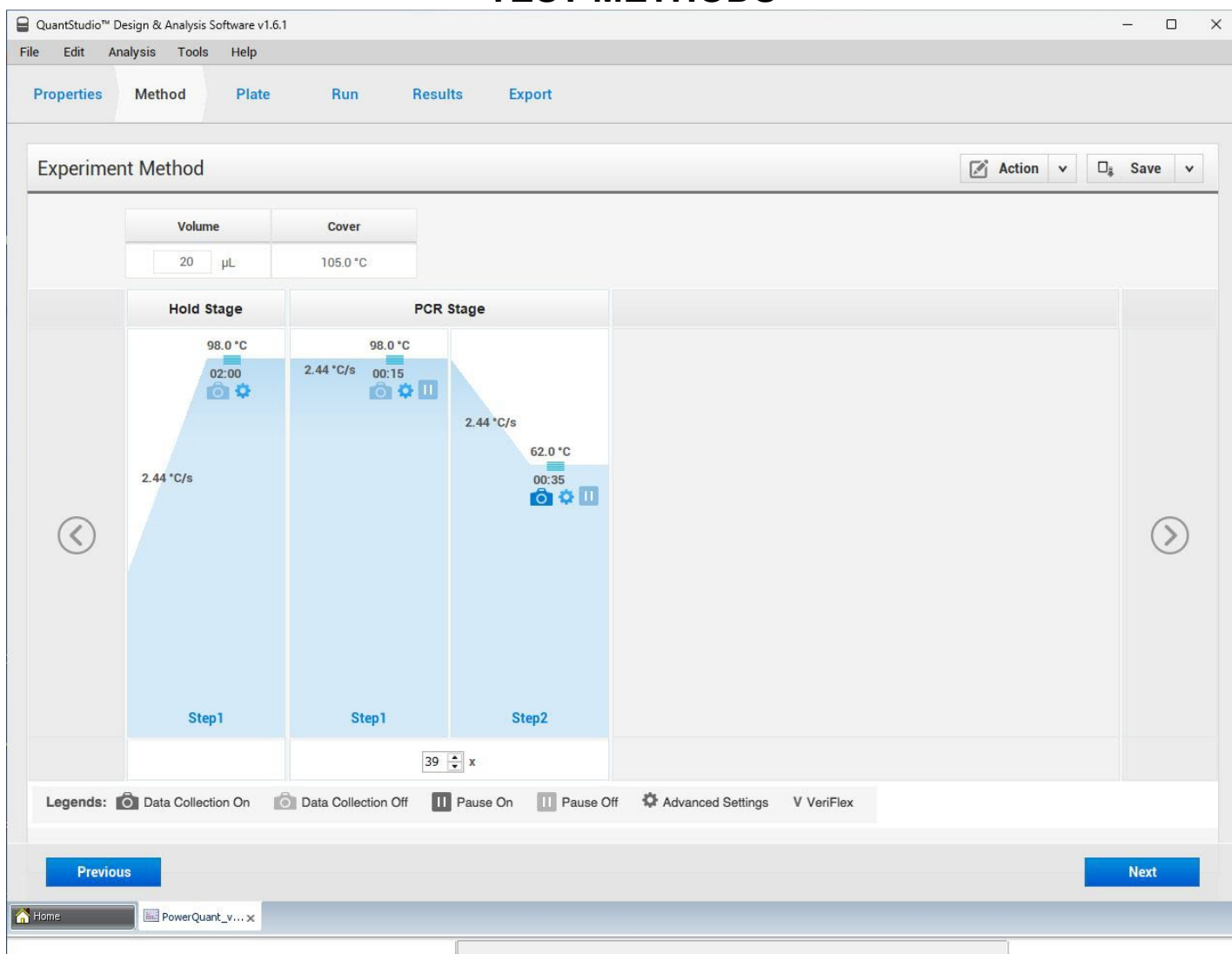
Save

Next

Home PowerQuant_v... x

2.8.14.5.7 The **Method** tab includes cycling information (as displayed) and shall not be adjusted from default (shown below).

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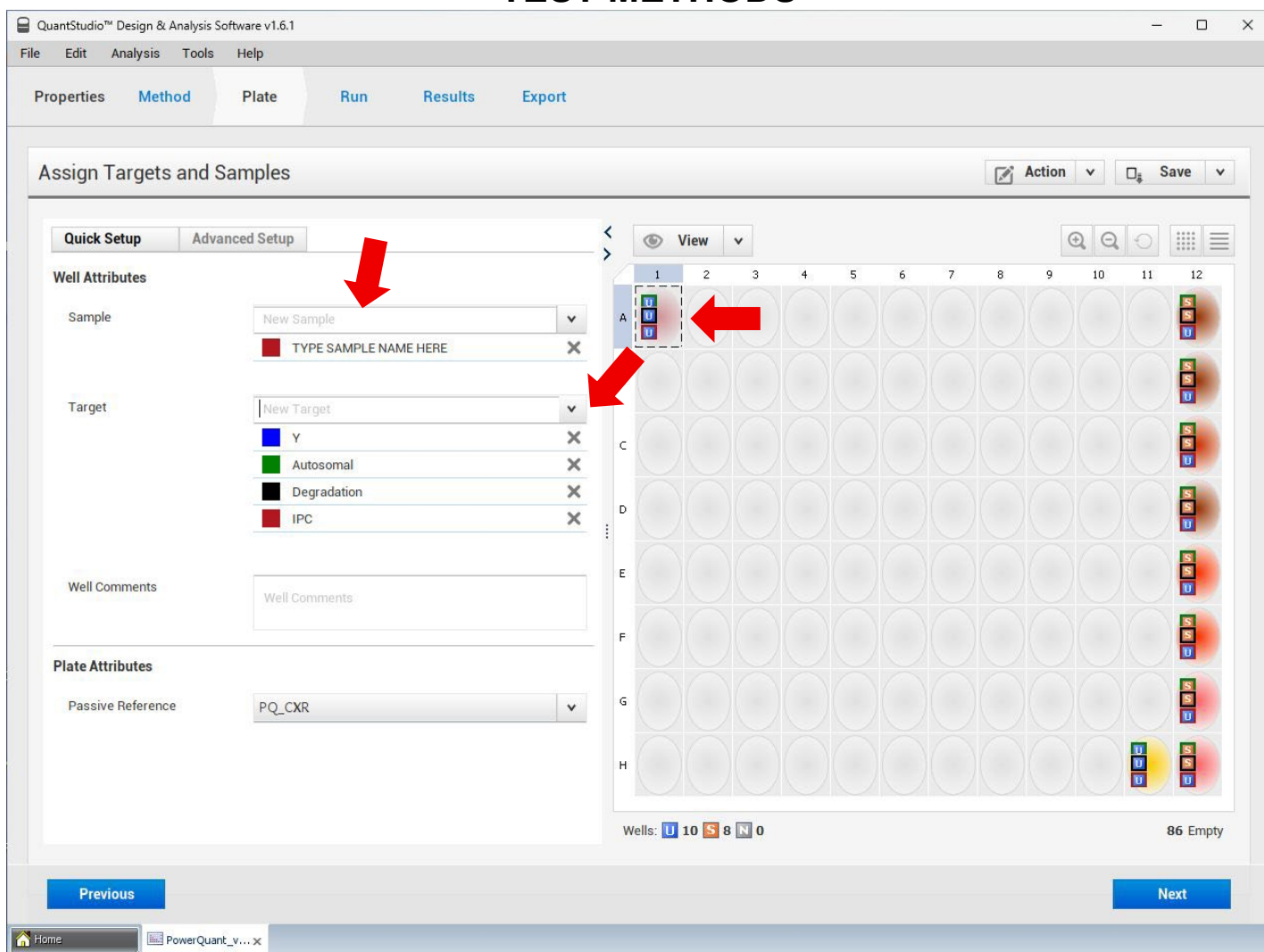


2.8.14.5.8 Select the **Plate** tab from the top. Note that sample names may be entered manually or imported from a DNA Workbook into the HID Software. All samples shall be uniquely identified by case number and sample identifier.

2.8.14.5.8.1 Sample Name manual entry:

- 2.8.14.5.8.1.1 Click on the well in the plate map where the respective sample was loaded.
- 2.8.14.5.8.1.2 Add the sample name in the Sample field (i.e. case number and item number).
- 2.8.14.5.8.1.3 From the drop-down menu next to Target, select all four dyes. Unused wells shall be left blank.
- 2.8.14.5.8.1.4 Verify that the passive reference is set to PQ_CXR and that the standard curve and NTC information is correct.

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2.8.14.5.8.2 Sample Name import:

- 2.8.14.5.8.2.1 In the DNA Workbook, navigate to the “Quant” tab, ensure that the sample information is filled out, and click **Create Quant Import File**. A copy of the import .txt file will be automatically saved to the desktop. Move this .txt file to a flash drive or DNAWorking drive.
- 2.8.14.5.8.2.2 On the QuantStudio™ 5 Real-Time PCR System computer, click on File > Import Plate Setup.
- 2.8.14.5.8.2.3 Click on Browse, then find the .txt file you wish to import (typically from a USB flash drive or a networked drive such as DNAWorking). Click Select, then Apply, and Yes to confirm imported plate setup.
- 2.8.14.5.8.2.4 You should now see your plate setup in the Plate tab. Confirm your samples are correct.

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2.8.14.5.9 Select the **Run** tab and click the blue “START RUN” button near the top right. The instrument serial number (SN) will appear in a drop-down box. Click on that SN.

2.8.14.5.9.1 You will be prompted to save your experiment. The file name will be the plate name and “.eds” at the end. The destination should automatically be set to go to the “CASEWORK” folder on the Desktop.

2.8.14.5.9.2 NOTE: If you saved before this step (e.g., after typing in the plate name), there will be an EDT file saved in the CASEWORK folder, which is a template file, in addition to the EDS file.

2.8.14.5.9.3 Click Save. The instrument run will begin. If it says it isn’t connected to the instrument, click “START RUN” again.

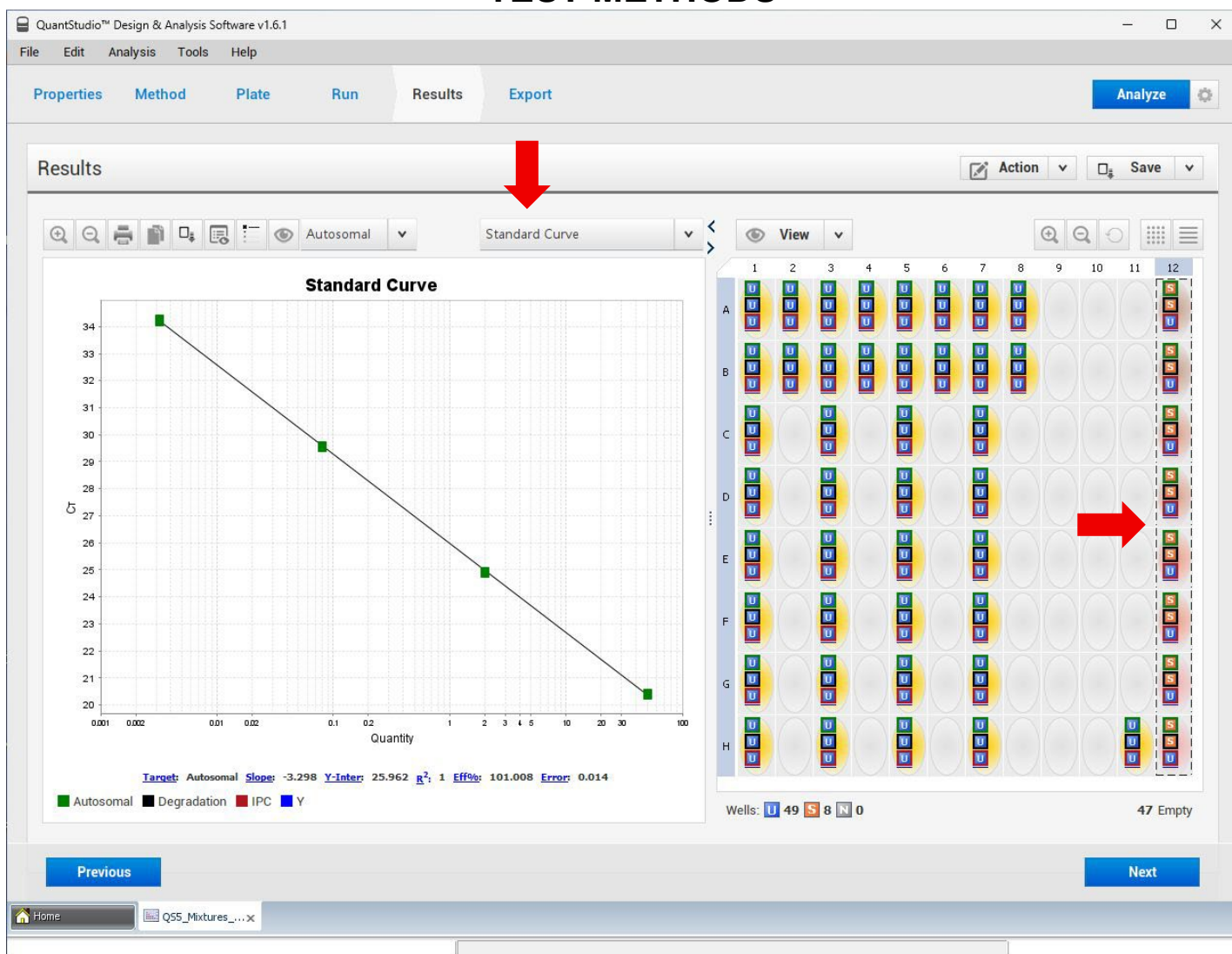
2.8.14.5.10 The PowerQuant assay will take ~1 hour.

2.8.14.6 Real-Time PCR Analysis Software set-up See [Appendix 2](#)

2.8.14.7 Real-Time Quantification Data Analysis

2.8.14.7.1 Once the run is complete evaluate the slope, Y-intercept, and R^2 values of all three standard curves as described under the **Quantification Interpretation Guidelines** section. The standard curve and values can be viewed by highlighting just the eight standards in column 12 and changing the graph view to Standard Curve.

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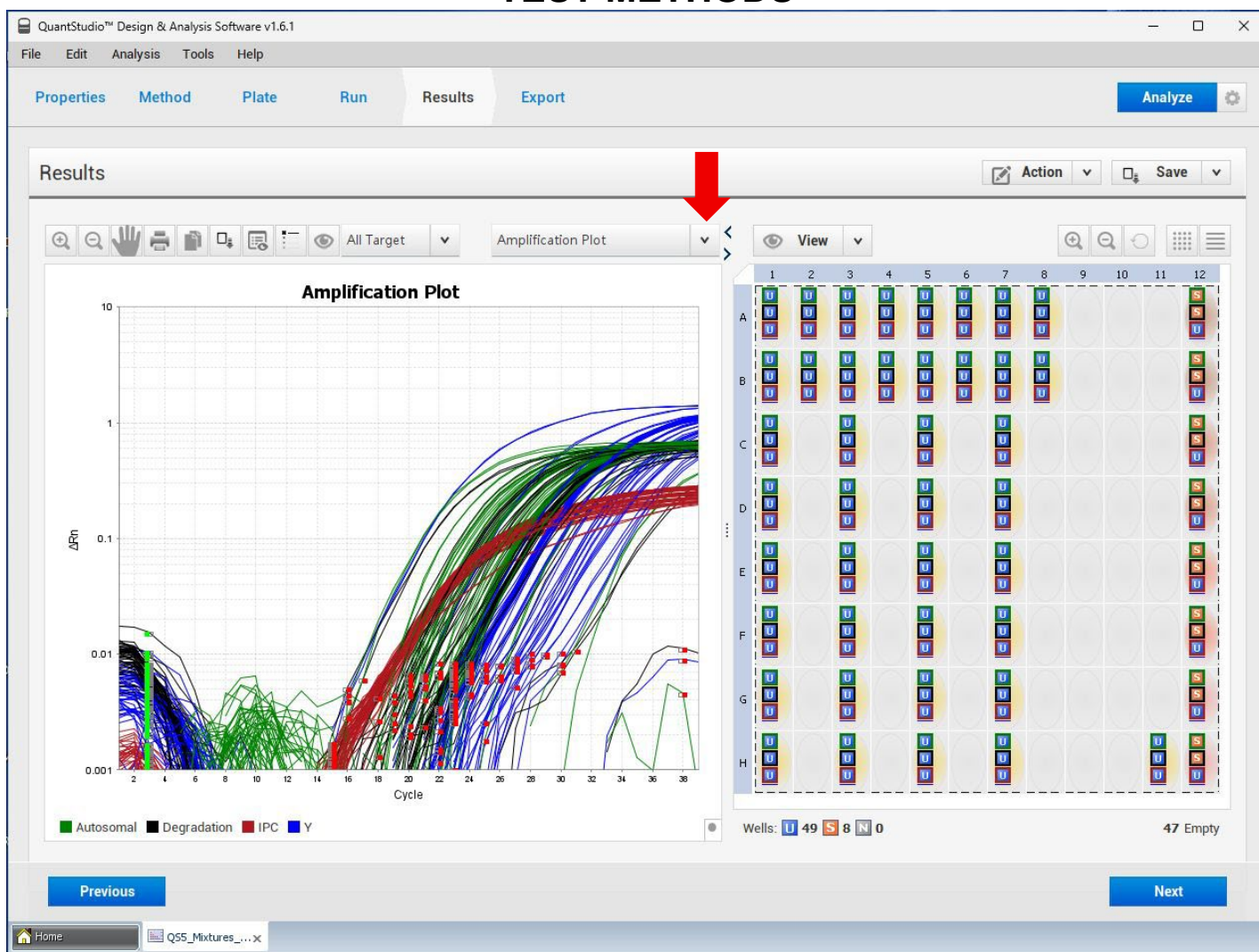


2.8.14.7.1.1 Up to 2 points from each curve may be deleted by right-clicking on the well, selecting **omit**, and selecting the appropriate target(s) or well. The results must be reanalyzed to incorporate the changes.

2.8.14.7.1.2 Omission of more than 2 points in any standard curve requires documented approval by a supervisor and notification to the Technical Leader.

2.8.14.7.2 The NTC, reagent blanks, and sample values can be evaluated in the **Results** tab by hovering the cursor over the respective sample well. The amplification plot can be viewed per sample by selecting the respective well in the plate map and selecting Amplification Plot as the graph view type.

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- 2.8.14.7.3** Select the **Export** tab from the top menu and export the .txt data file to the “CASEWORK” folder on the D: drive using the previously established export properties.
- 2.8.14.7.4** Import the .txt data file to the DNA Workbook **Quant** tab.
- 2.8.14.7.5** View and retain the **Quant Results** tab. The case notes shall contain the quantification values, IPC C_T values, degradation indices, and all three standard curve slope, R^2 , and y-intercept values.
- 2.8.14.7.6** The .eds run file and .txt data file shall be saved to each associated laboratory case number and request folder located in the analyst’s folders on the server.
- 2.8.14.7.7** Quantification values from the autosomal target shall be used for amplification of autosomal STRs.
- 2.8.14.7.8** Quantification values from the Y/male target shall be used for amplification of Y-STRs.
- 2.8.14.7.9** The quantification plate shall be discarded after the analysis is complete.

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2.8.14.8 Quantification Interpretation Guidelines

2.8.14.8.1 Standard Curve: Evaluate the Autosomal, Degradation, and Y standard curves individually or simultaneously.

2.8.14.8.1.1 Slope: Indicates the PCR amplification efficiency for the assay. A slope of -3.3 indicates 100% amplification efficiency. The analyst shall obtain documented Technical Leader approval to use data when a slope is outside the acceptable range. Scientific rounding rules to one decimal place apply.

Target	Acceptable slope range
Autosomal	-3.0 to -3.6
Degradation	-3.0 to -3.6
Y	-3.0 to -3.6

2.8.14.8.1.2 Y-intercept: The expected C_T value for a 1 ng/μl sample. Y-intercept averages may vary by instrument, kit lot, and pipetting differences. The analyst shall obtain documented Technical Leader approval to use data when a Y-intercept value is outside the acceptable range. Scientific rounding rules to one decimal place apply.

Target	Acceptable Y-intercept range (C_T)
Autosomal	25.0 to 28.0
Degradation	25.0 to 28.0
Y	25.0 to 28.0

2.8.14.8.1.3 R^2 value: A measure of the closeness of fit between the standard curve regression line and the individual C_T data points of the standard curve. A value of 1.00 indicates a perfect fit between the regression line and the data points. This value shall be ≥ 0.980 . If the R^2 value is <0.98 the experiment is inconclusive and shall be repeated with notification to the Technical Leader documented in the case record. Scientific rounding rules to two decimal places apply.

2.8.14.8.2 Internal PCR Control (IPC): Differentiates between a sample containing no human DNA and reactions affected by the presence of PCR inhibitors, assay setup, and chemistry or instrument failure.

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- 2.8.14.8.2.1 An elevated IPC C_T value (relative to quantitation standards) is an indication of the presence of an inhibitor or high concentration of human template DNA.
- 2.8.14.8.2.2 If inhibition is suspected, the analyst may re-Microcon® and/or Centri-sep the sample prior to amplification and typing of the sample.
- 2.8.14.8.2.3 If high concentration of human DNA is present, the analyst may make dilutions of the sample and re-quantify.
- 2.8.14.8.2.4 The analyst shall make reasonable efforts to rectify samples in which the IPC is undetected. If a DNA profile is not developed from a sample with an undetected IPC, it shall be reported as inconclusive.

Autosomal or Y targets	IPC	Interpretation	Suggestions / Options
Undetected	Detected	Negative result; No human DNA	No further analysis of sample
Undetected	Undetected	Invalid result; possible inhibition	Re-Microcon® and/or Centri-sep; Re-extract; or dilute and re-quantify
Detected (Low CT)	Undetected or Elevated	Inconclusive IPC result	Dilute and re-quantify (optional)
Detected (High CT)	Undetected or Elevated	Partial PCR Inhibition	Re-Microcon® and/or Centri-sep; Re-extract; or dilute and re-quantify

2.8.14.8.3 Non-Template Control (NTC): Contains PCR reagents with dilution buffer in place of template DNA and is therefore expected to be undetected (i.e., value of 0.00) for all three quantification values. However, background fluorescence or contamination may occur resulting in quantification values with one or more targets.

- 2.8.14.8.3.1 A NTC demonstrating quantification value(s) ≥ 0.001 ng/μl requires documented notification to the Technical Leader and shall be considered a failed control. The quantification shall be repeated.
- 2.8.14.8.3.2 A NTC demonstrating quantification value(s) < 0.001 ng/μl may be due to background fluorescence. The analyst may proceed with amplification and typing of the samples using caution with interpretation of any profiles obtained from the samples.

2.8.14.8.4 Dynamic range: The quantitation standard curve ranges from 50 to 0.0032 ng/μl. Quantitation values are most reliable when they fall near the middle of the standard curve and less reliable as they approach the ends and beyond.

- 2.8.14.8.4.1 If the quantity of Autosomal DNA in a sample is < 0.01 ng/μl, no additional autosomal STR analysis of that sample shall be performed.
- 2.8.14.8.4.2 If the quantity of male DNA in a sample is < 0.005 ng/μl, no additional Y-STR analysis of that sample shall be performed.

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2.8.14.8.4.3 The analyst should make a reasonable effort to target ≤ 50 ng/ μ l during extraction. If a sample quantifies > 50 ng/ μ l and is suitable for amplification, a dilution shall be made and quantified.

2.8.14.8.5 Female To Male Ratio (FTMR): In instances where it is valuable to identify a male contributor from a female contributor (i.e., sexual assault cases), the FTMR can assist in prioritizing samples and determining which are suitable for autosomal STR analysis and which are suitable for Y-STR analysis. The female DNA contribution is determined by subtracting the quantity of male DNA from the quantity of Autosomal DNA. The FTMR is determined by dividing the female contribution by the quantity of male DNA (equation: $[(\text{Auto}-Y)/Y]$). The selection of samples to continue DNA analysis may be prioritized at analyst discretion by using the FTMR and quantity of male DNA input as described below. Priority 1 samples and Priority 2 and 3 samples with male DNA concentrations ≥ 0.005 ng/ μ l should be amplified if no other samples have provided informative results.

2.8.14.8.5.1 Priority 1: Samples with FTMR $\leq 10:1$ are generally expected to yield interpretable information from male contributor with autosomal STR analysis.

2.8.14.8.5.2 Priority 2: Samples with FTMR between 10:1 and 25:1 may yield interpretable information from the minor male contributor with autosomal STR analysis if the concentration of male DNA is ≥ 0.005 ng/ μ l. If the male DNA concentration is less than 0.005 ng/ μ l, no further analysis should be performed.

2.8.14.8.5.3 Priority 3: Samples with FTMR between 25:1 and 50:1 are generally expected to yield minimal information from the minor male contributor with autosomal STR analysis if the concentration of male DNA is greater than 0.005 ng/ μ l. If the male DNA concentration is less than 0.005 ng/ μ l, no further analysis should be performed.

2.8.14.8.5.4 Priority 4: Samples with FTMR $> 50:1$ are generally not expected to yield interpretable information from the minor male contributor with autosomal STR analysis, therefore no further autosomal analysis should be performed. Y-STR analysis may be recommended if the concentration of male DNA is ≥ 0.005 ng/ μ l.

2.8.14.8.6 Degradation Ratio ([Auto]/[Deg]): A value to assist in the identification of degradation prior to amplification. The degradation ratio is calculated by dividing the quant values of the autosomal DNA target by the degradation target. An index > 2 may indicate the sample contains degraded human DNA, and the analyst should consider increasing the template DNA amount for autosomal STR amplification to generate interpretable data from the larger molecular weight loci.

2.8.14.8.7 Reagent blank: Should not have associated DNA and is therefore expected to be undetected (i.e., value of 0.00) for all three quantification values. However, background fluorescence or contamination may occur resulting in quantification values with one or more targets.

2.8.14.8.7.1 A reagent blank that demonstrates quantification value(s) ≥ 0.001 ng/ μ l requires documented notification to the Technical Leader. It shall be considered a failed control unless re-quantification and/or amplification

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demonstrate no contamination. It is recommended that the analyst evaluate the results for possible sample switching and requantify if that is suspected.

- 2.8.14.8.7.1.1 If the reagent blank is amplified and no peaks above analytical threshold are observed in the electropherogram, the associated samples may be interpreted with caution.
- 2.8.14.8.7.2 A reagent blank that demonstrates quantification value(s) <0.001 ng/ μ l may be due to background fluorescence or low-level contamination.
 - 2.8.14.8.7.2.1 If all associated samples fail to demonstrate a sufficient quantity of DNA for amplification (i.e., <0.01 ng/ μ l for autosomal STRs; <0.005 ng/ μ l for Y-STRs), they shall be reported as such.
 - 2.8.14.8.7.2.2 If the reagent blank is amplified and no peaks above analytical threshold are observed in the electropherogram, the associated samples may be interpreted normally.
- 2.8.14.8.7.3 A reagent blank that demonstrates peaks in the electropherogram shall be considered a failed control.
- 2.8.14.8.7.4 Any sample associated with a failed reagent blank but not amplified due to an insufficient quantity of DNA (i.e., <0.01 ng/ μ l for autosomal STRs; <0.005 ng/ μ l for Y-STRs) shall be reported as failed to demonstrate a sufficient quantity of (male) DNA for further/autosomal STR analysis.
- 2.8.14.8.7.5 Samples associated with a failed reagent blank shall not be submitted for Y-STR analysis.

2.8.15 PowerPlex® Fusion 6C System (Applied Biosystems® 3500 Series Genetic Analyzer)

2.8.15.1 Introduction

- 2.8.15.1.1 The Promega PowerPlex® Fusion 6C System is a 27-locus multiplex that amplifies 23 autosomal short tandem repeat (STR) loci (CSF1PO, FGA, Penta D, Penta E, SE33, TH01, TPOX, vWA, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, and D22S1045), two gender determining loci (Amelogenin and DYS391), and two additional rapidly mutating Y-STR loci (DYS570 and DYS576) in a single reaction. Separation and detection occurs using capillary electrophoresis with overlapping loci visualized simultaneously by using PCR primers labeled with six different fluorescent tags (FL-6C, JOE-6C, TMR-6C, CXR-6C, TOM-6C, and WEN-6C).
- 2.8.15.1.2 The Applied Biosystems® 3500 Series Genetic Analyzer utilizes electrokinetic injection of DNA molecules into polymer-filled capillaries which separates the DNA fragments by size. The fluorescent tag labeled primers incorporated into amplification products are responsive to the frequency of the 20mW solid state laser. Upon excitation, the fluorophores are raised to a higher energy level and when they return to their normal energy level a fluorescent signal is emitted. This signal is detected by a CCD (charge-coupled device) camera within the 3500 Genetic Analyzer which converts the signal to a computer image where it is visualized as a peak on the electropherogram.

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- 2.8.15.1.3** The data produced by the 3500 Genetic Analyzer is analyzed with GeneMapper™ ID-X Software which results in peaks labeled with their allele designation. The allele designation for each sample is accomplished through the use of an internal lane standard (ILS). The ILS contains 21 fragments of known length and is injected with each sample. The sample DNA fragments are compared to the ILS to determine the base pair size and the software assigns the allele designation by comparing the sizes to an allelic ladder.
- 2.8.15.1.4** The PowerPlex® Fusion 6C System locus-specific and allelic ladder information is available at www.promega.com/protocols/ in the PowerPlex® Fusion 6C System Technical Manual TMD045.
- 2.8.15.1.5** The PowerPlex® Fusion 6C 5X Primer Pair Mix, PowerPlex® Fusion 6C Allelic Ladder Mix, and WEN ILS 500 shall be protected from excessive exposure to light.
- 2.8.15.1.6** The preparation of the amplification reactions shall be performed in the PCR amplification set-up area.

2.8.15.2 PowerPlex® Fusion 6C Amplification Set-Up

- 2.8.15.2.1** For initial use, thaw the Amplification Grade Water, PowerPlex® Fusion 6C 5X Master Mix, and PowerPlex® Fusion 6C 5X Primer Pair Mix. Centrifuge briefly to bring contents to the bottom, then vortex to mix. Do not centrifuge after vortexing as this may cause the primers/dNTPs to be concentrated at the bottom of the tube.
- 2.8.15.2.2** Determine the number of samples to be amplified including controls. Add ~3 reactions to this number to compensate for the loss that occurs during reagent transfer.
- 2.8.15.2.3** Place one 0.2 ml PCR reaction tube for each sample into a rack and label appropriately. A 96-well plate may be used.
- 2.8.15.2.3.1** If utilizing a 96-well plate for amplification, well locations where allelic ladder will be utilized for capillary electrophoresis shall be left empty during amplification.
- 2.8.15.2.4** The recommended amplification target range is 1.0 to 2.0 ng of template DNA. Amplification of greater than 2.0 ng of template DNA may result in increased artifacts and interfere with interpretation. Only samples with concentration less than 0.066 ng/μl may target less than 1.0 ng template DNA and shall be interpreted with caution.
- 2.8.15.2.4.1** A higher template DNA amount (ex. 2.0 ng) should be considered for samples with an elevated degradation index (ex. >3).
- 2.8.15.2.5** For samples that will amplify 5 μl of template DNA, use the current version of the **DNA Analysis Workbook** to calculate the required amount of each component of the PCR master mix. Multiply the volume per reaction by the total number of reactions to obtain the final volume.

PCR Master Mix Component	Volume per Reaction (μl)
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Amplification Grade or Nuclease Free H ₂ O	10
PowerPlex® Fusion 6C 5X Master Mix	5
PowerPlex® Fusion 6C 5X Primer Pair Mix	5
Total Volume	20

- 2.8.15.2.6** Combine the calculated volume of each component into a 1.5 ml tube and vortex ~5 seconds.
- 2.8.15.2.7** Pipette 20 µl of the PCR master mix to each sample tube or well.
- 2.8.15.2.8** Add 5 µl of sample, reagent blank, positive amplification control, or negative amplification control to the appropriate tube or well.
- 2.8.15.2.8.1** For the positive amplification control, dilute the 2800M DNA standard supplied with the PowerPlex® Fusion 6C kit, or other approved DNA standard, to 0.2-0.4 ng/µl and record the final concentration on the amplification worksheet. A positive control shall be included in each thermal cyclor.
- 2.8.15.2.8.2** Amplification Grade H₂O or NF H₂O shall be used as a negative amplification control included with each thermal cyclor and will be the last sample in the batch.
- 2.8.15.2.9** For samples requiring more than 5 µl of template DNA, use the current version of the **DNA Analysis Workbook** worksheet to calculate the required amount of water and template DNA for individual samples. Prepare the PCR Master Mix without water. Multiply the volume per reaction by the total number of reactions to obtain the final volume.

PCR Master Mix Component	Volume per Reaction (µl)
PowerPlex® Fusion 6C 5X Master Mix	5
PowerPlex® Fusion 6C 5X Primer Pair Mix	5
Total Volume	10

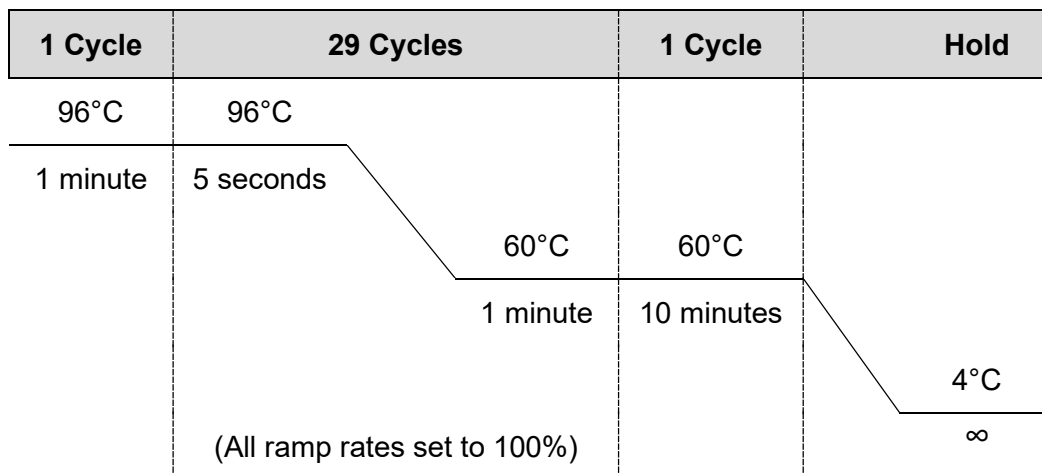
- 2.8.15.2.10** Combine the calculated volume of each component into a 1.5 ml tube and vortex ~5 seconds.
- 2.8.15.2.11** Pipette 10 µl of the PCR master mix to each sample tube or well.
- 2.8.15.2.12** Add the calculated volume of Amplification Grade H₂O or NF H₂O (0-10 µl) to each sample tube or well.
- 2.8.15.2.13** Add the calculated volume of sample, reagent blank, positive amplification control, or negative amplification control (5-15 µl) to each sample tube or well.
- 2.8.15.2.14** For samples amplified in a 96-well plate, seal the plate with adhesive foil. Press the foil well using a roller tool.
- 2.8.15.2.15** Centrifuge at 3000 rpm for approximately 10 seconds or spin using a salad spinner to remove any bubbles and force samples into the bottom of each well.

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2.8.15.3 PowerPlex® Fusion 6C Amplification

2.8.15.3.1 Place the tubes or 96-well plate in a Veriti™ Thermal Cycler.

2.8.15.3.2 Select the **Fusion 6C** protocol with the following amplification procedure. Approximately 1 hour cycling time.



2.8.15.3.3 Remove samples after the amplification process is completed and proceed with capillary electrophoresis or store the amplified samples frozen. Alternatively, amplified samples may be stored in the refrigerator if they are to be used within 2 days.

2.8.15.3.3.1 Any remaining amplification products shall be discarded after the case has been administratively reviewed.

2.8.15.4 Instrument setup for Applied Biosystems® 3500 Genetic Analyzer - Data Collection Software version 2.0 see [Appendix 3](#).

2.8.16 Applied Biosystems® 3500 Series Genetic Analyzer - Data Collection Software version 2.0 - PowerPlex® Fusion 6C Electrophoresis

2.8.16.1 Sample Preparation

2.8.16.1.1 Note: The quality of formamide is critical for the successful detection of a DNA profile. Deionized formamide shall be used that has a conductivity of less than 100µS/cm, such as Hi-Di™ Formamide. Multiple freeze-thaw cycles or long-term storage above 0°C may cause breakdown of the formamide which can create ions that compete with DNA during injection. This will cause lower peak heights and decreased sensitivity.

2.8.16.1.2 Caution: Formamide is an irritant and teratogen; therefore, universal precautions and a fume hood shall be utilized when manually working with formamide to avoid inhalation and contact with the skin.

2.8.16.1.3 For initial use, thaw the WEN ILS 500, PowerPlex® Fusion 6C allelic ladder, and an aliquot of Hi-Di™ Formamide. When thawed, centrifuge briefly to bring contents to the bottom, then vortex to mix.

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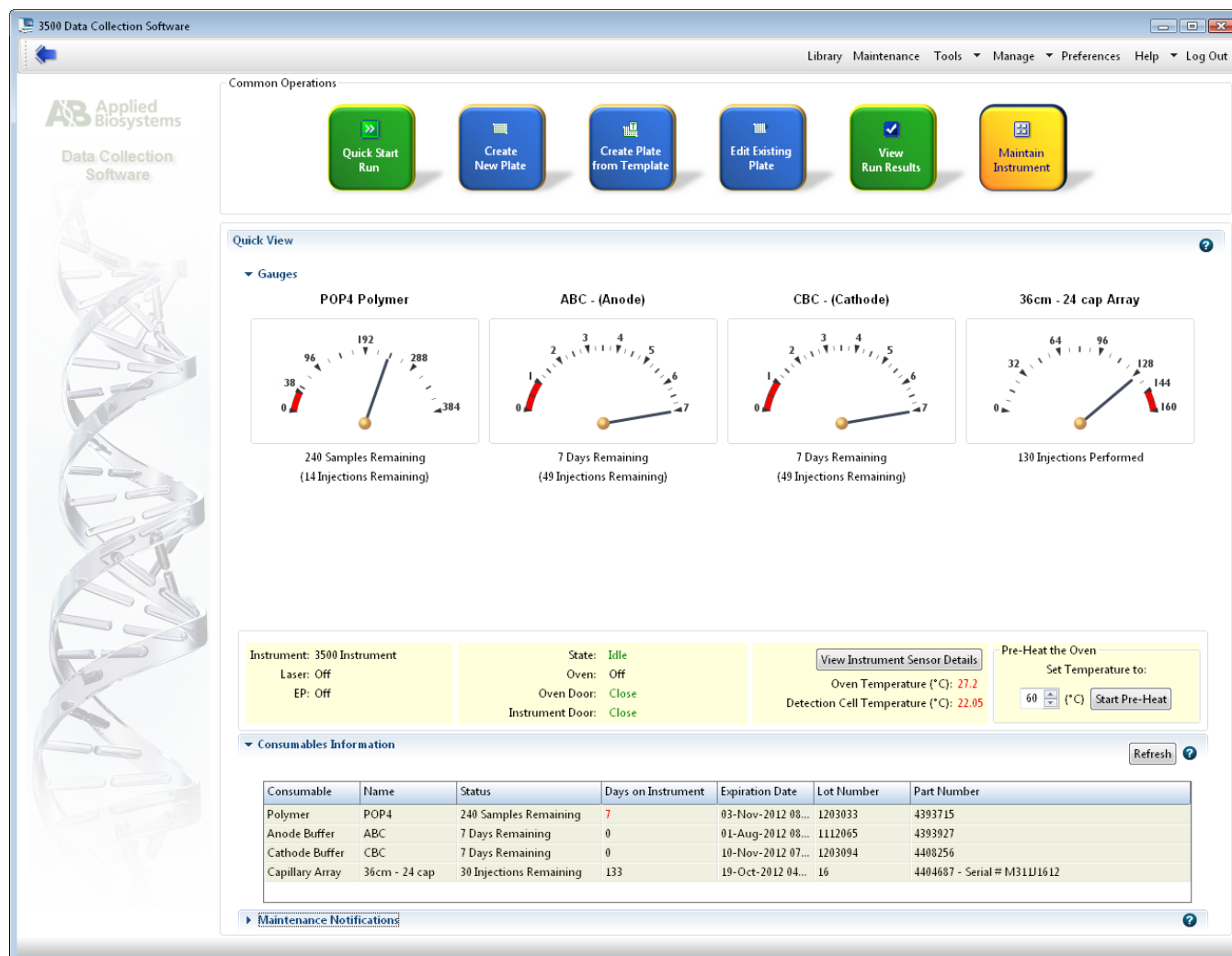
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- 2.8.16.1.4** Determine the number of samples to be injected, including allelic ladders and controls (reagent blanks, positive control, and amplification blank). Add ~3 reactions to compensate for the loss that occurs during pipetting.
- 2.8.16.1.5** Prepare a loading cocktail by combining 0.5-1.0 µl ILS with 9.5-9.0 µl formamide for a total volume of 10 µl per sample.
- 2.8.16.1.5.1 The volume of ILS used in the loading cocktail may be adjusted between 0.5 to 1.0 µl per sample to optimize size standard peaks. The amount of formamide shall be adjusted so that the total amount of loading cocktail for each well is 10 µl.
- 2.8.16.1.6** Vortex to mix.
- 2.8.16.1.7** Pipette 10 µl of the formamide/ILS mixture into each well containing sample, allelic ladder, or control. Add formamide or formamide/ILS mixture into empty wells to complete an injection set. Liquid must be present in every well in which an injection will occur.
- 2.8.16.1.8** Add 1 µl of amplified sample or allelic ladder to the appropriate wells. It is recommended that a minimum of one allelic ladder is present with every 24 samples injected (i.e., every three injections on the 8-capillary 3500 and within every injection on the 24-capillary 3500xL).
- 2.8.16.1.8.1 If amplified sample is in a 96-well plate, seal the amplification plate with a new aluminum foil seal for storage.
- 2.8.16.1.9** Cover the wells with the plate septa and briefly centrifuge to remove air bubbles.
- 2.8.16.1.10** Denature the samples at 95°C for 3 minutes, then immediately chill on crushed ice or frozen plate block for ~3 minutes. Denature the samples just prior to loading the instrument. Avoid denaturing the samples for longer than 3 minutes as extended heat denaturing can lead to the appearance of artifacts.
- 2.8.16.2 Create a Plate Record**
- 2.8.16.2.1** Open the 3500 Data Collection Software. The Dashboard screen will launch. Ensure that the **Consumable Information** and **Maintenance Notifications** are acceptable. The oven temperature should be set to 60°C.
- 2.8.16.2.2** Select **Start Pre-Heat**. This should be done at least 30 minutes prior to the first injection.

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Dashboard:



2.8.16.2.3 Select **Create New Plate** or **Create Plate from Template**.

2.8.16.2.3.1 Assign the plate name with the laboratory case number of the first case on the plate, the date, and the injection number of the plate (ex. 00A1234_01Jan16_01).

2.8.16.2.3.1.1 If the plate is re-injected the "01" number shall increase sequentially (00A1234_01Jan16_02).

2.8.16.2.3.1.2 If the plate is re-injected on a different day, the original date should still be used from when the plate was prepared.

2.8.16.2.3.1.3 If a second preparation of a plate is made on the same date as the first, it shall be designated with a "-2" after the date (00A1234_01Jan16-2_01).

2.8.16.2.4 Select "96" for Number of Wells.

2.8.16.2.5 Select "HID" for Plate Type.

2.8.16.2.6 Select "36"cm for Capillary Length.

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2.8.16.2.7 Select “POP4” for Polymer.

Create A Plate:

The screenshot displays the '3500 Data Collection Software' window. On the left is a sidebar with a 'Setup' section containing links for 'Define Plate Properties', 'Assign Plate Contents', 'Run Instrument', 'Review Results', and 'View Sequencing Results'. The main area is titled 'Plate Details' and contains several input fields: 'Name' (with a yellow highlight and an error message 'Plate Name is a required field. Provide a unique value.'), 'Number of Wells' (set to 96), 'Plate Type' (set to 'HID'), 'Capillary Length' (set to '36 cm'), and 'Polymer' (set to 'POP4'). There are also fields for 'Owner', 'Barcode', and 'Description'. At the bottom, there is a 'Secondary Analysis' section with a checkbox for 'Perform Auto-Analysis'.

2.8.16.2.8 Click “Assign Plate Contents”.

2.8.16.2.9 Enter sample information or import a plate record.

2.8.16.2.9.1 The sample name shall include the sample item/sub-item as well as the laboratory case number (ex. 1A1_00A1234) if more than one case is included on the plate. If only one case is present on the plate, then only the item number is required.

2.8.16.2.9.2 In order to aid in GeneMapper™ ID-X sample analysis, it is recommended to place a “z” in front of the sample name for known standards (ex. z1A1_00A1234”) here or in GeneMapper™ ID-X.

2.8.16.2.10 Under **Assays**, click “Add from Library” and select the appropriate Fusion6C assay. Double-click or click “Add to Plate” then “Close”.

2.8.16.2.11 Under **File Name Convention**, click “Add from Library” and select “Fusion6C_FNC”. Double-click or click “Add to Plate” then “Close”.

2.8.16.2.12 Under **Results Groups**, click “Add from Library” and select “Fusion6C_RG”. Double-click or click “Add to Plate” then “Close”.

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Assign Plate Contents:

	1	2	3	4	5	6	7	8	9	10	11	12
A	H Allelic Ladder	H 007A 00A-6789	H Allelic Ladder									
B	H 001A 00A-1234	H 008A 00A-6789	PC									
C	H 002A 00A-1234	H 009A 00A-6789	AB									
D	H 003A 00A-1234	H 010A 00A-6789										
E	H 004A 00A-1234	H 011A 00A-6789										
F	H 005A 00A-1234	H 012A 00A-6789										
G	H 006A 00A-1234	H 013A 00A-6789										
H	H RB 00A-1234	H RB 00A-6789										

Name: 00A-1234_01JANI6_01
Barcode:

Assays

☒ Fusion6C_1.2kv15s

File Name Conventions

☒ Fusion6C_FNC

Results Groups

☒ Fusion6C_RG

Customize Sample Info

Property	Value
1: Sample	
Sample Name	Allelic Ladder
Sample Type	Allelic Ladder
2: Custom	
User Defined Field 1	
User Defined Field 2	
User Defined Field 3	
User Defined Field 4	
User Defined Field 5	
3: Misc	

[Link Plate for Run](#)

2.8.16.2.13 On the bottom-right side of the screen expand the **Customize Sample Info** window so it appears as shown above.

2.8.16.2.13.1 Use the drop-down box to select a sample type for each sample (ex. Ladder, Sample, Positive Control and Negative Control). For imported plates, this may be done automatically.

2.8.16.2.14 Highlight the sample wells and then select the appropriate boxes under **Assays**, **File Name Conventions**, and **Results Groups**.

2.8.16.2.15 Place the plate on the instrument in position A. Select “Link Plate for Run”. Click “OK”. The instrument automatically senses the plate and assigns the information to the Plate A field. Click “OK”.

2.8.16.2.16 To add a second plate, follow steps 2.8.14.2.3 through 2.8.14.2.14. Place the plate in position B. Select the “Link Plate for Run” and click “OK”. Assign the information to the Plate B field. Click “OK”.

2.8.16.3 Start a Plate Run

2.8.16.3.1 A unique run name is automatically generated by the instrument for each plate.

2.8.16.3.2 To re-organize injections, click “Create Injection List” and use the arrows to arrange in the desired order.

2.8.16.3.3 Click “Start Run”.

2.8.16.3.4 After the run is complete, click “Unlink Plate” and remove it from the instrument. The plate may be discarded or labeled with the same title as the plate record and

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stored frozen. The plate shall be destroyed after the case has been administratively reviewed.

2.8.16.3.4.1 If the CE plate requires re-injection after frozen storage, thaw, and centrifuge to remove any air bubbles. Denature the plate at 95°C for 3 minutes, then immediately chill on crushed ice or frozen plate block for ~3 minutes prior to loading on the 3500 Genetic Analyzer.

2.8.16.3.5 The CE Plate Record Worksheet shall be maintained with each case record.

2.8.17 Setting Up GeneMapper™ ID-X Version 1.6 Software User Accounts and Security System
See [Appendix 4](#).

2.8.18 GeneMapper™ ID-X Version 1.6 Software - PowerPlex® Fusion 6C Data Analysis

2.8.18.1 Processing Sample Data

2.8.18.1.1 Import the sample files to be interpreted from a single run folder by selecting “Add Samples to Project”.

2.8.18.1.2 Navigate to the run folder that contains the sample files. If the entire run folder is to be imported, click on the folder to highlight it, then click the “Add to List” button at the bottom of the window. If only a portion of samples need to be selected, expand the folder to view the samples. Highlight the appropriate samples, ensuring that the allelic ladder and all desired samples are selected. Once all the samples are selected click the “Add to List” button at the bottom of the window.

2.8.18.1.2.1 A run folder shall not be created manually by manipulating sample files.

2.8.18.1.3 Ensure that the necessary files are now located in the “Samples to Add” window by double-clicking on the folder in the right pane, then click “Add”.

2.8.18.1.4 After the samples have been added to the project, first briefly scan the raw data to ensure that a bad injection did not occur.

2.8.18.1.4.1 To check the raw data, first expand the project folder in the left navigation pane, click on a sample file, then click on the “Raw Data” tab in the right GeneMapper™ ID-X window. To return to the “Samples” window, click on the project folder at the top of the left navigation pane.

2.8.18.1.5 The GeneMapper™ ID-X project shall contain at least one allelic ladder from each run folder included in the project for proper genotyping. Multiple allelic ladders within a run folder will be averaged by the software to calculate the allelic bins. If a ladder injection is of low quality, delete the ladder or change the sample type from “Allelic Ladder” to “Sample” to remove it from consideration in calculating the bins.

2.8.18.1.6 Set the **Table Setting** at the top of the screen to “STRmix_Fusion6C”.

2.8.18.1.7 In the **Sample Type** column, verify that the correct sample types are displayed (i.e., Allelic Ladder, Sample, Positive Control, and Negative Control). Should a sample type need altered, use the drop-down menu to select the appropriate type.

2.8.18.1.8 In the **Analysis Method** column,

2.8.18.1.8.1 For unknown profiles: select “STRmix_Fusion6C_v3” from the drop-down menu in row 1. Click the column header cell to highlight the entire column and press Ctrl+D or select Edit → Fill Down.

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2.8.18.1.8.2 For reference profiles: select “Fusion 6C_v3” from the drop-down menu in row 1. Click the column header cell to highlight the entire column and press Ctrl+D or select Edit → Fill Down.

2.8.18.1.8.2.1 If a reference profile is degraded and/or exhibits allelic dropout, either analysis method may be used. Stutter will need to be manually deleted. Notification of the Technical Leader documented in the case record is required if the STRmix analysis method is utilized.

2.8.18.1.8.2.2 A standard that will be utilized for kinship comparisons that exhibits degradation and allelic drop-out may utilize the STRmix analysis method and may be deconvoluted in STRmix to address potential allelic drop-out.

2.8.18.1.8.2.3 Reference profiles shall be analyzed in separate projects from unknown profiles and should be interpreted after unknown profiles.

2.8.18.1.8.2.3.1 Assumed profiles may be analyzed in the same project as unknowns.

2.8.18.1.8.2.4 When possible, reference profiles which are assumed prior to analysis of unknown profiles should be analyzed in separate projects from other reference profiles.

2.8.18.1.8.3 Positive controls can be analyzed with either analysis method; however, stutter will need to be manually deleted from positive control profiles.

2.8.18.1.8.4 Negative controls shall be analyzed with the lowest or lower analytical threshold of any associated samples.

2.8.18.1.8.5 A project may contain samples with different analysis methods. The project shall contain a separate ladder for each analysis method used.

2.8.18.1.8.6 A project does not need to be created for standards if no items of evidence in that case are amplified or demonstrate conclusive results.

2.8.18.1.9 In the **Panel** column,

2.8.18.1.9.1 For unknown profiles: select “STRmix_v3_Fusion_6C_ISP” from the drop-down menu in row 1. Click the column header cell to highlight the entire column and press Ctrl+D or select Edit → Fill Down.

2.8.18.1.9.2 For reference profiles: select “Fusion6C_v3_ISP” from the drop-down menu in row 1. Click the column header cell to highlight the entire column and press Ctrl+D or select Edit → Fill Down.

2.8.18.1.9.3 Ladders, positive and negative controls shall utilize the panel that corresponds to the analysis method used.

2.8.18.1.10 In the **Size Standard** column, select “WEN ILS 500” from the drop-down menu in row 1. Click the column header cell to highlight the entire column and press Ctrl+D or select Edit → Fill Down.

2.8.18.1.11 The Analysis Method, Size Standard, and Panel can be set as defaults when a GeneMapper™ ID-X project is opened. Select **File** → **Project Options**. Under

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the **Add Samples** tab select appropriate settings as the default in the drop-down menus for Analysis Method, Size Standard, and Panel. Click “OK”.

- 2.8.18.1.12** Select the green **Analyze** arrow button to start data analysis. A pop-up window will ask for a project name to save. The project name shall contain the date the 3500 plate was prepared so that it matches the run folder name. See the DNA Server Instructions [Appendix 9](#) for additional clarification. The case number is recommended but not required. Select the “ISP Casework Security Group”. Click “OK”.

2.8.18.2 Evaluating Sample Data

- 2.8.18.2.1** The **Sizing Quality** for unknown samples and allelic ladders shall be at least 0.75 for a sample to pass. The **Sizing Quality** for reference samples, negative controls and positive controls shall be at least 0.25 for a sample to pass. All appropriate ILS peaks shall be present and labeled correctly.

2.8.18.2.2 Allelic Ladders and Samples

- 2.8.18.2.2.1 Highlight the rows containing ladders or samples and click “Display Plots”. In the Samples Plot screen, change the “Plot Setting” drop-down box to “Fusion6C”. Magnify the area from about 50 to 500 bp ensuring that all ILS peaks are visible in the retained electropherogram.
- 2.8.18.2.2.2 Verify that the correct allele calls are made for each ladder peak and evaluate all sample calls as described in 2.8.21.3.
- 2.8.18.2.2.3 Retain the electropherograms.

2.8.18.2.3 Negative Controls

- 2.8.18.2.3.1 Highlight all sample rows containing negative controls and click “Display Plots”. Verify the Plot Setting is set to “Fusion6C”.
- 2.8.18.2.3.2 Ensure that no peaks above analytical threshold are present.
- 2.8.18.2.3.3 Retain the electropherograms ensuring that the primer peak is visible.

- 2.8.18.2.4** After all analysis is complete, select the **Genotypes** tab. Click “File”, then select “Export Table”. Export the genotypes table as a .txt file to be utilized for STRmix™ analysis and the generation of allele summary tables in the STRlite Excel program. The .txt file name shall be the same as the project name with the automatic extension “Genotypes Table” added during export.

- 2.8.18.2.5** The 3500 Data Collection run folder containing the sample files, associated GeneMapper™ ID-X projects, and exported genotypes table .txt file shall be saved under each associated laboratory case number and request folder located in the analyst’s folders on the server. Batch data generated by the Automation team is an exception. Automation batch data shall be stored only in the zAutomation folder and not duplicated per individual case. Projects should be deleted monthly from the “GeneMapper Manager” to maintain database space.

- 2.8.18.2.6** When exporting the GeneMapper™ ID-X project, ensure that the “Export with analysis settings” box is checked.

2.8.19 General Rules for PowerPlex® Fusion 6C Analysis On The Applied Biosystems® 3500 Genetic Analyzer

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- 2.8.19.1** At least one allelic ladder shall be present within each run folder. It is recommended that an allelic ladder be run within each set of 3 columns. This will help account for possible migration shifts due to external environmental factors. If a single injection contains both unknown and reference samples, it is recommended that two allelic ladders are run for GeneMapper™ ID-X analysis.
- 2.8.19.2** Samples in the 96-well plate should be oriented to allow for the injection of unknown samples prior to the injection of any reference samples for that case.
- 2.8.19.3** A signal range of 5,000 to 20,000 RFU will produce optimal results.
- 2.8.19.4** If multiple amplifications are analyzed for a sample, the analyst shall use the amplification which he or she determines sufficiently represents the sample based on peak heights, artifacts, and noise levels. Analysts shall interpret data that most clearly represents the sample and try to achieve better resolution if a sample has peak heights that are too high (resulting in artifacts, increased instrument noise, and pull-up) or too low (resulting in allelic drop out and loss of data).
- 2.8.19.5** Only the data used for interpretation needs to be published in the case record. However, other runs shall be noted in the case record and all data shall be saved under each associated laboratory case number and request folder located in the analyst's folder on the server.

2.8.20 Archiving Applied Biosystems® 3500 and GeneMapper™ ID-X Projects

- 2.8.20.1** The 3500 run folders containing all sample files for a case as well as the GeneMapper™ ID-X project and export files shall be saved under each associated laboratory case number and request folder located in the analyst's folder on the server and deleted from the hard drive of the instrument and/or analysis computer. The data stored on the server shall be routinely backed up to ensure security of data.
- 2.8.20.2** All electropherograms used in interpretation as well as the plate record shall be placed in the case record.

2.8.21 STRmix™ Installation and Set-up: See [Appendix 5](#)

2.8.22 Interpretation Guidelines for PowerPlex® Fusion 6C and STRmix™ Deconvolutions

2.8.22.1 Scope

- 2.8.22.1.1** The following interpretation guidelines shall aid in peak/true allele determinations and profile interpretations.
- 2.8.22.1.2** It shall be at the analyst's discretion, based on experience and training, as to which peaks are suitable for interpretation and input into STRmix™.
- 2.8.22.1.3** If an analyst has determined that a peak that has been labeled by the GeneMapper™ ID-X software is not a true allele peak, the analyst shall rename or delete the allele call label in GeneMapper™ ID-X.
- 2.8.22.1.4** Final allele calls shall be documented on the STRlite pages retained in the case record.
- 2.8.22.1.4.1** For unknown profiles, the STRlite Excel program utilizes a filter to display peaks in the allele table that fall below the mean + 3SD stutter value from the PowerPlex® Fusion 6C validation for backward (n-3/n-4/n-5) and forward (n+3/n+4/n+5) stutter and for n-2 stutter at SE33. This is only to aid in the

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analyst's ability to determine what are possibly true alleles for the determination of the number of contributors in the sample. STRmix™ analysis will make the official interpretative evaluations of these peaks.

2.8.22.1.5 STRmix™ is a probabilistic genotyping software program for the interpretation of autosomal STR DNA profiling results. STRmix™ can deconvolute (break down into individual contributors) a DNA mixture and attach a statistical weighting to comparisons of evidence sample profiles to reference profiles.

2.8.22.1.5.1 The ISP Forensic Services Division has validated STRmix™ v2.7.0 for casework.

2.8.22.2 DNA Profile Interpretation

2.8.22.2.1 Assumptions used in the interpretation of mixtures and interpretation decisions shall be documented in the case record.

2.8.22.2.2 Analysts shall consider the additive effects of allele sharing during interpretation.

2.8.22.2.3 The flow of interpretation generally follows the steps listed below (Clayton, et al., 1998):

- Identify the presence of a mixture.
- Designate the allele peaks.
- Identify the number of contributors.
- Estimate the relative ratio of the individual contributors to the mixture.
- Consider all genotype combinations.
- Compare reference samples.
- Statistical analysis (if necessary).

2.8.22.2.4 STRmix™ is utilized to perform the interpretation steps following the identification of the number of contributors. The general approach to profile interpretation with STRmix™ is outlined here:

A DNA profile or mixture previously analyzed in GeneMapper™ ID-X is assessed for suitability for STRmix™ analysis.

If suitable, the profile/mixture is interpreted/deconvoluted within STRmix™.

Reference samples are compared to the completed interpretation/deconvolution, and statistical calculations are performed reflecting that comparison (if necessary).

STRmix™ outputs are reviewed for quality and all appropriate results are reported.

2.8.22.2.5 Identify If the Profile Is A Mixture

2.8.22.2.5.1 Single Contributor DNA Profile Determination

2.8.22.2.5.1.1 Generally, a single source profile should contain no more than two alleles at each autosomal locus and no more than one allele at each Y-STR locus. However, three-peak allele patterns have been reported at autosomal loci for single-source stains and two-peak patterns have been reported at Y-STR loci, but these instances are rare. A profile where only one autosomal locus demonstrates three alleles or one Y-STR locus demonstrates two alleles could be an indication of:

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2.8.22.2.5.1.1.1 A tri-allelic pattern for an autosomal locus. If a tri-allelic pattern is indicated, it should be confirmed by concordance to at least one additional sample contained within the case and/or re-amplification.

2.8.22.2.5.1.1.1.1 Any locus with a confirmed tri-allelic pattern shall not be used for statistical evaluation.

2.8.22.2.5.1.1.2 A duplication event for a Y-STR locus. If a duplication is indicated, the profile should be confirmed by concordance to at least one additional sample contained within the case and/or re-amplification and documented notification to the Technical Leader. Alternatively, the sample may be amplified with PowerPlex® Y23 to confirm the profile with documented approval from a Biology Unit supervisor and notification to the Technical Leader.

2.8.22.2.5.1.2 For single source profiles, the peak height ratio (the smallest peak divided by the largest peak) of heterozygous individuals at a locus should be within 0.65.

2.8.22.2.5.1.2.1 Samples may display peak height ratios less than expected when the sample is of poor quality or the amplification target is below the ideal input of template DNA.

2.8.22.2.5.1.3 Conclusions shall be reported for all results in which the assumption of a single contributor is made.

2.8.22.2.5.2 Mixed DNA Profile Determination

2.8.22.2.5.2.1 A profile is defined as a mixture of two or more individuals when a locus demonstrates an additional allele, with the exception of a tri-allelic pattern at an autosomal locus or duplication event at a Y-STR locus.

2.8.22.2.5.2.2 A peak height ratio less than 0.65 at a locus may indicate a mixed profile and that allele sharing may be occurring.

2.8.22.2.5.2.2.1 Samples may display peak height ratios less than expected when the sample is of poor quality or the amplification target is below the ideal input of template DNA.

2.8.22.2.5.2.3 Peaks in stutter position that exceed the mean +3 SD stutter percentage may indicate the presence of a mixture.

2.8.22.2.5.2.4 All loci shall be considered when determining the presence of a mixture.

2.8.22.2.5.3 Single Contributor Profiles in Which A Single Additional Allele is Detected (not tri-allelic)

2.8.22.2.5.3.1 If the additional allele is present at Amelogenin or a Y-STR locus:

2.8.22.2.5.3.1.1 If it is possible drop-in (≤ 200 RFU), the analyst shall document that they interpreted the peak as possible drop-in and shall utilize the single source report wording.

2.8.22.2.5.3.1.2 If it is >200 RFU, this shall be considered an indication of an additional contributor. The profile shall be deconvoluted in STRmix™ with the assumed number of contributors increased to two contributors.

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2.8.22.2.5.3.1.2.1 If the peak is in stutter position, the expected stutter RFU (mean) does not count toward the 200 RFU cut-off to manually allow for the additive effect of stutter and drop-in.

2.8.22.2.5.3.2 If the additional allele is present at an autosomal locus:

2.8.22.2.5.3.2.1 If it is possible drop-in (≤ 200 RFU), the assumed number of contributors should not be increased to two contributors and STRmix™ shall be allowed to model the peak.

2.8.22.2.5.3.2.1.1 If the sample is not being modeled in STRmix™ due to it being consistent with an assumed standard, the analyst shall document that they interpreted the peak as drop-in and shall utilize the single source report wording. If the peak is in stutter position, the expected stutter RFU (mean) does not count toward the 200 RFU cut-off to manually allow for the additive effect of stutter and drop-in.

2.8.22.2.5.3.2.2 If it is >200 RFU, this shall be considered an indication of an additional contributor. The profile shall be deconvoluted in STRmix™ with the assumed number of contributors increased to two contributors.

2.8.22.3 Preliminary Evaluation of Allele Peaks

2.8.22.3.1 An analyst is required to visually confirm that all allelic ladders used for allele designation performed correctly.

2.8.22.3.2 The internally validated analytical threshold for Fusion 6C is 100 RFU for the Blue, Green, and Yellow dye channels, 120 RFU for the Red dye channel, and 60 RFU for the Purple and Orange dye channels. These values shall be used for interpretation of all unknown samples.

2.8.22.3.2.1 Peaks below analytical threshold shall not be interpreted, but may be considered when deciding if the possible number of contributors can be reasonably assumed.

2.8.22.3.2.2 For reference standards, 150 RFU shall be used as the analytical threshold for all Fusion 6C dye channels.

2.8.22.3.3 There is no static stochastic threshold used for STRmix™ interpretation. However, a stochastic threshold value of 950 RFU will be utilized to evaluate reference samples for completeness when a single allele is detected at a locus.

2.8.22.3.3.1 If a reference sample has a locus with a single peak present ≤ 950 RFU, the locus shall be ignored in the STRmix™ deconvolution (if the reference sample is being utilized for conditioning) or likelihood ratio calculation (if the reference sample is being utilized for comparison). Technical Leader approval is not necessary for ignoring more than one locus due to possible allelic drop-out of reference sample.

2.8.22.3.4 All interpretable autosomal peaks in unknown samples shall not exceed 28,000 RFU. Exceptions may be allowed with Technical Leader approval documented in the case record.

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- 2.8.22.3.4.1 Unknown profiles with autosomal peaks over 28,000 RFU should be interpreted with caution, as modeling parameters utilized in STRmix™ may not model profiles with saturated peaks optimally.
- 2.8.22.3.4.2 Reference samples and positive control profiles may have up to two autosomal loci with peaks over 28,000 RFU. These profiles should be interpreted with caution.
- 2.8.22.3.5** An initial interpretation of the data shall be made to distinguish non-allelic artifact peaks (e.g., pull-up, spikes, dye blobs, etc.) from true allelic data.
- 2.8.22.3.5.1 Artifacts have been observed and documented utilizing the PowerPlex® Fusion 6C amplification kit. Any artifact peaks called as alleles by the GeneMapper™ ID-X software shall be deleted or renamed in the software as an artifact. The case record electropherogram should indicate the reason for deletion, either manually or labeled in the software. Examples of documented artifacts are listed below. A complete list of artifacts, including nonhuman cross-reactivity, can be found in the PowerPlex® Fusion 6C Technical Manual. Some examples of artifacts that may be observed are:
- Amelogenin, D1S1656, DYS391, FGA – n-1
D13S317, D18S51, vWA, D7S820, D5S818, D19S433, FGA – n-2
Other DNA-independent artifacts have been documented in the following dyes:
- FL-6C (Blue) - ~65-75 bp, ~113 – 120 bp, ~137 – 145 bp
JOE-6C (Green) - ~60 – 66 bp
TMR-5C (Yellow) - ~57-62 bp
- 2.8.22.3.5.2 Pull-up or bleed through peaks can occur if signal intensity of sample or ILS peaks are too high or if a new spectral calibration needs to be run. Any pull-up peaks called as alleles by the GeneMapper™ ID-X software shall be deleted or renamed in the software as pull-up or artifact. The case record electropherogram should indicate the reason for deletion, either manually or labeled in the software. The sample should be re-run if a pull-up peak interferes with the analyst's ability to evaluate the profile based on their experience and training.
- 2.8.22.3.5.3 Spikes are peaks that generally appear in all colors and are sharper than regular peaks; however, they can occur predominantly in one color. Spikes are a natural consequence of capillary electrophoresis and can be caused by dust present in the system as well as urea crystals in the system. It is essential that the instrumentation be maintained and cleaned regularly to minimize the appearance of spikes. All spikes called as alleles by the GeneMapper™ ID-X software shall be deleted or renamed in the software as spikes or artifacts. The case record electropherogram should indicate the reason for deletion, either manually or labeled in the software. A sample should be re-injected when a spike interferes with the analyst's ability to evaluate the profile based on their experience and training.
- 2.8.22.3.6** Stutter peaks are artifacts of the amplification process and display less intensity than the true parent peak. Stutter peaks will typically be observed one repeat unit shorter than the parent peak (ex. n-4 at a tetranucleotide STR), but may also occur two repeats less (ex. n-8 at a tetranucleotide STR), or one repeat more (n+4 at a tetranucleotide STR). Additionally, n-2 artifact peaks have been

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reported at several loci amplified with PowerPlex® Fusion 6C. Mean and mean + 3SD backward, forward, and double-backward stutter values can be found in [Appendix 6](#).

- 2.8.22.3.6.1 Stutter peaks in the backward (n-3/n-4/n-5) and forward (n+3/n+4/n+5) positions at the autosomal loci, as well as in the n-2 position for locus SE33, are modeled by STRmix™ and **shall not be removed** from GeneMapper™ ID-X data of unknown profiles destined for STRmix™ analysis.
- 2.8.22.3.6.2 The stutter peaks listed below are not modeled by STRmix™ and **shall be removed** from GeneMapper™ ID-X data of unknown profiles destined for STRmix™ analysis. GMID-X stutter filters can aid in the removal of these stutter peaks below the mean + 3SD values. Peaks above the stutter filter, but determined to be stutter shall be deleted or renamed in the software as stutter or artifact. The case record electropherogram should indicate the reason for deletion, either manually or labeled in the software. Examples include:
 - 2.8.22.3.6.2.1 Stutter peaks in the double-backward position (n-6/n-8/n-10).
 - 2.8.22.3.6.2.2 Partial repeat (n-2/n+2) stutter peaks not included above (most common for D1S1656, D19S433, SE33 n+2 only and FGA).
 - 2.8.22.3.6.2.3 All stutter peaks at the Y-STR loci.
- 2.8.22.3.6.3 For reference profiles, all stutter peaks shall be deleted.
- 2.8.22.3.7** Rare variants (microvariants) have been described in the literature. These peaks will have a similar intensity to the other allelic peaks for that locus but will not line up with the allelic ladder and will fall outside of established bins.
 - 2.8.22.3.7.1 Any allele peak that is not present in the allelic ladder and does not have an associated “bin” in the GeneMapper™ ID-X analysis software is labeled “OL” (Off-Ladder) and shall be renamed by the analyst in the software.
 - 2.8.22.3.7.1.1 Alleles which are located between two alleles on the ladder shall be described as the short repeat followed by the number of base pairs it is larger (e.g., 0.1, 0.2, 0.3, or 0.4). Therefore, if a peak is 1 base pair larger than the 5 allele it shall be designated as 5.1. A microvariant 3 base pairs larger for than an allele on the ladder for a tri-nucleotide, 4 base pairs larger for a tetranucleotide, or 5 base pairs for a pentanucleotide may be designated with the full repeat number (e.g., A peak 4 base pairs larger than the 5 allele could be designated a 6; 5 base pairs larger a 6.1).
 - 2.8.22.3.7.1.2 Alleles which are located outside the range of the ladder or bin set but within the designated marker range of the locus shall be evaluated to determine the appropriate repeat number that should be assigned to the allele.
 - 2.8.22.3.7.1.2.1 Any allele recognized by the CODIS software to fall outside of the allelic ladder shall be renamed during CODIS entry as “<” the smallest allele or “>” the largest allele for the locus.
 - 2.8.22.3.7.1.3 Alleles which are labeled “OMR” (Outside Marker Range) by the GeneMapper™ ID-X analysis software shall be evaluated to determine

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which locus the allele likely belongs to. If the analyst can reasonably determine which locus the allele belongs to, the allele can be assigned to that locus in GeneMapper™ ID-X and the allele call can be made. If the analyst cannot reasonably determine which locus the allele belongs to, both loci surrounding the allele shall be ignored from the STRmix™ deconvolution.

2.8.22.3.7.1.4 In instances where non-numeric alleles (e.g., OL and/or OMR) are marked and retained in the GeneMapper™ ID-X data, the resulting STRmix™ run shall be analyzed with that locus ignored from analysis.

2.8.22.3.7.2 Unresolved peaks may be observed with alleles and/or stutter peaks that are one bp apart. Most often this is observed at loci that have partial repeat alleles (e.g., D1, D12) and it occurs if the allele peak is very large and the allele or stutter peak one bp away is small. Sometimes in this situation, the GeneMapper™ ID-X software does not recognize and call the smaller peak. It can typically be observed as a shoulder on the larger peak. Unresolved peaks shall be closely evaluated using the following guidelines:

- If the unresolved peak is estimated to be below the analytical threshold, no action is required, proceed with analysis.
- If the unresolved peak is above the analytical threshold, determine if the peak is potentially allelic or likely stutter. If the peak is potentially allelic, the locus shall be ignored in deconvolution.
- If the unresolved peak is likely stutter in a single source sample, the unlabeled stutter shall be documented in the notes and analysis can proceed as normal. Careful evaluation of the STRmix™ deconvolution shall be done as the stutter variance will be affected.
- If the unresolved peak is in a stutter position in a mixture, the estimated peak height shall be evaluated. If the estimated peak height is roughly equal or greater than the RFU height of minor alleles, the locus shall be omitted in the STRmix™ deconvolution. The Technical Leader shall be notified.

2.8.22.4 Evaluation of Controls

2.8.22.4.1 Failed controls require documented notification to the Technical Leader with appropriate documentation in the case record.

2.8.22.4.2 The appearance of pull-up or known artifact peaks does not render the following controls inconclusive.

2.8.22.4.3 Reagent Blank:

2.8.22.4.3.1 The purpose of the reagent blank is to determine if the reagents used to extract the associated samples were contaminated by human DNA. Therefore, no signal should be detected in this sample well other than the internal lane standard. If a signal is detected in the reagent blank, all results of samples associated with that reagent blank shall be considered inconclusive.

2.8.22.4.3.2 A reagent blank with peaks below the analytical threshold shall not prevent associated samples from being interpreted.

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2.8.22.4.3.3 A reagent blank with peaks at or above the analytical threshold shall be considered a failed negative control.

2.8.22.4.4 Positive Control:

2.8.22.4.4.1 The 2800M DNA is used as a positive control to demonstrate that the kit is performing properly. A positive control is considered a failed positive control if expected alleles are not detected, a single autosomal locus peak is ≤ 950 RFU, or if extra alleles are detected.

Positive Control 2800M (PowerPlex® Fusion 6C):

X, Y	17, 18	12, 13	10, 14	13, 15	9, 11	7, 14
Amelogenin	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E

9, 13	16, 18	22, 25	12	12, 13
D16S539	D18S51	D2S1338	CSF1PO	Penta D

6, 9.3	16, 19	29, 31.2	8, 11	12	11
TH01	vWA	D21S11	D7S820	D5S818	TPOX

14, 15	18, 23	13, 14	15, 16	16
D8S1179	D12S391	D19S433	SE33	D22S1045

10	20, 23	18	17
DYS391	FGA	DYS576	DYS570

2.8.22.4.5 Amplification Blank:

2.8.22.4.5.1 The purpose of the amplification blank is to determine if human DNA contaminated the samples at the amplification step. Because no template DNA was placed in the reaction, the sample should be blank except for the internal lane standard peaks.

2.8.22.4.5.2 An amplification blank with peaks below the analytical threshold shall not prevent associated samples from being interpreted.

2.8.22.4.5.3 An amplification blank with peaks at or above the analytical threshold shall be considered a failed negative control.

2.8.22.4.6 Interpreting, Comparing and Reporting DNA results associated with Failed Controls and Contamination Events

2.8.22.4.6.1 The Technical Leader shall be notified if a positive or negative control fails and/or if sample contamination occurs. Sample contamination is defined as additional peaks present in an unknown or reference sample that can be conclusively identified to not have originated in the sample (e.g., case to case contamination or a mixture in a reference sample).

2.8.22.4.6.2 Assessment of the integrity of the associated DNA results shall be done in consultation with the Technical Leader. This shall include the possible cause and effect of the failed control or contamination. Additionally, an assessment of the risk associated with moving forward with interpretation shall be documented, as well as the risks associated with retesting (e.g., unnecessary consumption of evidence).

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2.8.22.4.6.2.1 If retesting is performed, the reasons for doing so shall be documented.

2.8.22.4.6.2.2 If results are determined to be suitable for interpretation without retesting, the appropriate interpretations, comparisons, and statistical results and a description of the failed control or contamination event shall be reported per the Report Writing Guidelines.

2.8.22.4.6.2.3 If results are determined to not be suitable for interpretation and retesting cannot be performed, the results shall be reported as not suitable for comparison per the Report Writing Guidelines.

2.8.22.4.6.3 Documentation in the case record shall include at a minimum: the forensic sample, reference, or control test result that failed or was contaminated, the likely or known cause of the failed control or contamination, the likely or known source of the contamination, the impact on the integrity of the DNA results, and the determination if the test result is suitable or unsuitable for interpretation.

2.8.22.5 Determination of the Assumed Number of Contributors

2.8.22.5.1 The assignment of the number of contributors to a profile should be made by the analyst prior to the comparison of the profile to any reference samples. Reference sample profiles from individuals who are assumed to be present in a sample and will be used to condition on in the STRmix™ deconvolution shall be used to aid in the determination of number of contributors for that sample.

2.8.22.5.1.1 The profile from the corresponding fraction of a differential extraction, if amplified, should also be used to aid in the determination of the number of contributors.

2.8.22.5.2 Determination of the number of contributors shall be done at the analyst's discretion, based on experience and training, and should take into account peak height ratios and possible genotype pairings, as well as sub-analytical threshold and high stutter peaks.

2.8.22.5.3 Depending on sample data, an analyst may assign the number of contributors to a sample as one, two, three, four, or at least five.

2.8.22.5.4 In circumstances when the number of contributors cannot be determined, the profile is reported as too complex to interpret (inconclusive). Such profiles include:

- 5 person or greater mixtures
- Sample results with data at less than two autosomal loci
- Sample results with poor quality data in which peak number, peaks below analytical threshold, peak height ratios and other qualitative factors prevent the reasonable determination of the number of contributors

2.8.22.5.5 The following steps should be followed in assigning the number of contributors to a profile:

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- 2.8.22.5.5.1 Review the profile as a whole, assessing the level of degradation, presence of low level peaks, noisy or clean baseline and the general quality of the profile.
- 2.8.22.5.5.2 If replicate amplifications have been undertaken, all qualifying replicate profiles should be reviewed when determining the number of contributors.
- 2.8.22.5.5.3 Identify possible stutter peaks (both forward and backward) by reference to stutter table (see [Appendix 6](#)).
- 2.8.22.5.5.4 Find the locus with the highest number of unambiguous allelic peaks to determine the minimum number of contributors. Peak height imbalance between alleles may mean the presence of an additional contributor(s) above that indicated by allele count alone.
- 2.8.22.5.5.5 If one or more contributors at this locus is a clear major, check that this pattern is represented at other loci.
- 2.8.22.5.5.6 Apply the general pattern of contributors (e.g., number of alleles, peak height ratios) to other loci in the profile.
- 2.8.22.5.5.7 Discriminating loci such as FGA and SE33 are likely to be the most informative when determining the number of likely contributors.
- 2.8.22.5.5.8 The presence of a single minor peak or elevated stutter may indicate the presence of drop-in and not an additional contributor.
 - 2.8.22.5.5.8.1 If a single additional allele at a Y-STR locus that is ≤ 200 RFU is the only indication of an additional contributor, the analyst shall document that they interpreted the peak as possible drop-in. The number of contributors shall not be increased.
 - 2.8.22.5.5.8.2 If the peak is in stutter position the expected stutter RFU (mean) does not count toward the 200 RFU cut-off to account for the potential for drop-in and stutter being additive.
- 2.8.22.5.5.9 After initial assignment of the number of contributors and subsequent STRmix™ analysis, and prior to comparison with the person of interest reference profiles, the analyst may determine that the deconvolution did not conform to scientific expectation and may be re-run under a different number of contributors or with increased MCMC iteration accepts. In this case, the course of action shall be detailed in the case notes and the electronic file of the first deconvolution shall be maintained in the case record.
- 2.8.22.5.5.10 The Variable Number of Contributors (varNOC) function in STRmix™ may be utilized if the analyst is unable to determine a single number of contributors estimate but feels the data quality is suitable for STRmix™ analysis.
 - 2.8.22.5.5.10.1 It is recommended that the analyst utilize other troubleshooting methods to resolve the number of contributors (such a reamplification) prior to utilization of varNOC.
 - 2.8.22.5.5.10.2 Technical Leader permission is required if varNOC is utilized. DBLR software shall not be used with varNOC.

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2.8.22.5.5.10.3 The range of contributors evaluated in varNOC shall not exceed a difference of one contributor.

2.8.22.6 General Interpretation Considerations for STRmix™

2.8.22.6.1 STRmix™ should be used to deconvolute all qualifying samples, where a qualifying sample is defined as:

2.8.22.6.1.1 All evidence DNA samples where the number of contributors can be assumed (up to four contributors) unless the profile is completely accounted for by conditioning.

2.8.22.6.1.1.1 A sample demonstrating a partial profile shall be interpreted if the number of contributors can be reasonably assumed. STRmix™ requires a minimum of two autosomal loci for interpretation.

2.8.22.6.2 Evidence samples with identical profiles shall be analyzed independently with STRmix™.

2.8.22.6.3 Y-STR loci present in PowerPlex® Fusion 6C shall only be used for interpretation purposes and not for statistical evaluations. The Y-STR loci are not evaluated within the STRmix™ software.

2.8.22.6.4 The basis of STRmix™ is that, given a set of mass parameters (e.g., DNA amount, degradation, and amplification efficiency), the software can determine the expected height of a peak of a certain size at a given locus. If there are reasons for the expected peak height to be incorrect or for issues with allele designation, STRmix™ is unable to handle loci with these effects. Examples include:

2.8.22.6.4.1 Tri-allelic loci

2.8.22.6.4.2 Somatic and primer binding site mutations

2.8.22.6.4.3 Technical issues (saturated or poorly resolved GeneMapper™ ID-X data)

2.8.22.6.5 If these effects are discovered after the original deconvolution (e.g. upon inclusion of a tri-allelic standard), a second deconvolution should be performed ignoring the affected locus.

2.8.22.6.5.1 If the effect causes an analyst to change their original number of contributors decision, Technical Leader approval is required.

2.8.22.6.6 Non-numeric values such as OL, <, or > are not permitted within the STRmix™ input files.

2.8.22.6.7 The Ignore Locus function within STRmix™ may be used to ignore an affected locus within an interpretation. A locus with this option selected will be removed from the subsequent deconvolution or LR from Previous calculation.

2.8.22.6.7.1 Analyst discretion may be used to ignore a single locus within the STRmix™ deconvolution or LR from Previous calculation with reasoning documented in the case record. Ignoring more than one locus requires Technical Leader approval with documentation in the case record, except in the situation where ignoring multiple loci is necessary due to possible allele drop-out in a reference sample.

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2.8.22.6.7.2 If a locus has been ignored due to the inability of STRmix™ to handle a biological effect (e.g., a tri-allelic locus), an analyst may document their manual interpretation for CODIS entry purposes only.

2.8.22.6.8 A replicate is defined as a repeat amplification of the same extract and may be analyzed together in STRmix™ to aid sample interpretation, including determination of the number of contributors.

2.8.22.6.8.1 Replicates may only be analyzed together through STRmix™ with Technical Leader approval documented in the case record.

2.8.22.6.9 If it has been determined that a sample is unsuitable for interpretation, an explanation as to why the sample is un-interpretable should be given. Examples include:

2.8.22.6.9.1 No results: No peaks were detected in the electropherogram.

2.8.22.6.9.2 Inconclusive: Peaks were observed at one or more loci; however, no conclusive results could be drawn due to an inability to reasonably determine the number of contributors.

2.8.22.6.10 Test results from an unknown sample that are suitable for comparison to a reference sample may have the following conclusions reported:

2.8.22.6.10.1 Included: The STRmix™ calculated HPD (Highest Posterior Density) Likelihood Ratio favors inclusion.

2.8.22.6.10.2 Excluded: The profile obtained from the unknown stain had discrepant genotypes as compared to the profile of the reference sample (i.e., was not the same) and can be visually excluded or the STRmix™ calculated HPD Likelihood Ratio favors exclusion.

2.8.22.6.10.3 Uninformative: The profile obtained from the unknown stain has sufficient ambiguity when compared to the profile of the reference sample and the HPD Likelihood Ratio falls in the uninformative range.

2.8.22.6.11 If conclusive results are obtained from a sample, appropriate CODIS eligible profiles which do not match the victim shall be entered into CODIS for searching. The analyst and technical reviewer shall evaluate CODIS eligibility before submitting profiles for entry.

2.8.22.7 Setting Up Propositions for Calculating the Likelihood Ratio (LR)

2.8.22.7.1 The Likelihood Ratio (LR) assesses the probability of the evidence given two alternate propositions or hypotheses; one that aligns with the prosecution (H_1 or H_p) and one that aligns with the defense (H_2 or H_d). H_1 is typically inclusionary of the person(s) of interest. The defense proposition aligns with exclusion of the person(s) of interest.

2.8.22.7.2 Analysts will need to review all case information in order to determine which likelihood ratio calculations should be calculated and reported. Multiple propositions may need to be considered.

2.8.22.7.2.1 Examples:

2.8.22.7.2.1.1 Two-person mixture from intimate swab collected from victim. The victim and the Person of Interest (POI) cannot be excluded as possible contributors to the sample.

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H_1 : DNA originates from victim and POI

H_2 : DNA originates from victim and unknown contributor

2.8.22.7.2.1.2 Three-person mixture from firearm located at scene. The POI cannot be excluded as a possible contributor to the sample.

H_1 : DNA originates from POI and two unknown contributors

H_2 : DNA originates from three unknown contributors

2.8.22.7.2.1.3 Three-person mixture from firearm located at scene. POI1 and POI2 both cannot be excluded as possible contributors to the sample. Three separate LRs may be calculated:

H_{1-1} : DNA originates from POI1 and two unknown contributors

-Or-

H_{1-2} : DNA originates from POI2 and two unknown contributors

-Or-

H_{1-3} : DNA originates from POI1, POI2 and one unknown contributor

H_2 : DNA originates from three unknown contributors

2.8.22.7.3 Conditioning is defined as a DNA profile that is present in both propositions (H_1 and H_2) because it is reasonable to concede the presence of the individual's DNA. Conditioning where appropriate provides a more accurate likelihood ratio for the person of interest's possible contribution to that profile.

2.8.22.7.3.1 At this time, only DNA reference samples may be used for conditioning, except as indicated for CODIS purposes only.

2.8.22.7.3.2 The reference profiles shall have at least 20 complete autosomal loci (loci with no possible allelic drop-out). In cases where degradation of the reference sample results in less than 20 complete autosomal loci and conditioning may be beneficial to the sample, consultation with the Technical Leader is required to determine the best course of action.

2.8.22.7.3.3 If a reference profile being used for conditioning is a partial profile or has loci with possible allelic drop-out (a single allele $\leq$ 950 RFU), the loci with incomplete allelic information shall be omitted from the STRmix™ deconvolution.

2.8.22.7.3.4 The determination to use a reference profile for conditioning should be based on the analyst's visual determination that the individual is included in the evidence profile. A likelihood ratio shall not be performed to determine if a reference is included or excluded for this purpose.

2.8.22.7.3.5 Intimate samples shall be conditioned on the owner, unless a single source foreign profile is obtained (e.g., sperm fractions or blood of another on one's person), or the owner is visually excluded from a mixture.

2.8.22.7.3.5.1 Intimate samples shall also be conditioned on an elimination standard from a consensual partner, unless that individual is visually excluded.

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- 2.8.22.7.3.6 Non-intimate samples should be conditioned on elimination standards, depending on the profile and case information (e.g., steering wheel profile conditioned on owner or clothing profile conditioned on wearer), unless the elimination profile is visually excluded from the evidence profile.

2.8.22.8 Initial STRmix™ Analysis for Evidentiary Sample Data (Deconvolution)

- 2.8.22.8.1** To start a new STRmix™ deconvolution, from the STRmix™ main menu select **Interpretation**.
- 2.8.22.8.1.1 Alternately, **Batch Mode** for deconvolutions may be utilized for multiple samples, which is accessed from the main menu and functions as below.
- 2.8.22.8.2** In the **Interpretation** window, enter the case number in the “Case Number” box. The deconvolution number should be entered after the item number in the “Sample ID” box. For subsequent deconvolutions of the same item number, increase “Decon#” sequentially (e.g., 001A_Decon1, 001A_Decon2). If additional characters are added to the “Sample ID” designation, those characters shall be added after the specified naming convention.
- 2.8.22.8.3** Enter the proposed number of contributors for the sample (1, 2, 3, or 4), and select **Confirm**.
- 2.8.22.8.4** Ensure the **Contributor Range** box is not checked if varNOC is not being utilized.
- 2.8.22.8.4.1 If varNOC is being utilized, check the **Contributor Range** box. Enter the minimum and maximum number of contributors. The range of contributor number shall be +/- 1. Select **NIST_2017_Combined** as the population for the range.
- 2.8.22.8.5** Select **Run Settings** and ensure that the number of burn-in accepts per chain is set to **10,000**, and the number of post burn-in accepts per chain is set to **50,000**, and click **Apply**, then **Next**.
- 2.8.22.8.5.1 For troubleshooting purposes, an analyst may increase the number of burn-in accepts per chain to **100,000**, and the number of post burn-in accepts per chain to **500,000** with notification to the Technical Leader documented in the case record.
- 2.8.22.8.5.2 Aside from number of accepts, no other changes should be made in **Run Settings** without Technical Leader approval.
- 2.8.22.8.6** Verify that **ISP_Fusion6C_v3** is the selected DNA kit used.
- 2.8.22.8.7** Add a new profile to the box marked **Evidence Profile Data**. This may be done by selecting **+** and navigating the file tree, or by dragging and dropping the appropriate GeneMapper™ ID-X export file into the box. If multiple samples are present in the file, select the desired sample for analysis.
- 2.8.22.8.8** If conditioning on a reference sample, add a new profile to the box marked **Reference Profile Data**. This may be done by selecting **+** and navigating the file tree, or by dragging and dropping the appropriate GeneMapper™ ID-X export file or previous STRmix Evidence file into the box. Multiple reference profiles may be added if you are conditioning on multiple individuals.
- 2.8.22.8.9** After the reference profile is added, check the **Contributor to: Hd** box to reflect that it is a conditioned sample.

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- 2.8.22.8.10 Ensure that the **Perform Database Search** box is checked.
- 2.8.22.8.11 To ignore loci from deconvolution, select **Kit Settings**, then select **Loci**, then select **Ignore?** for appropriate loci, then select **Apply**.
- 2.8.22.8.12 Select **Start**. This will begin a deconvolution analysis of the chosen sample and search the results against the ISP QA DNA Database.
- 2.8.22.8.13 When the deconvolution is complete, STRmix™ will generate several data files and save them to the STRmix™ Results folder on the C: drive.
- 2.8.22.8.14 Save each deconvolution folder to the DNA server for the appropriate case.

2.8.22.9 Review of Run Diagnostics

- 2.8.22.9.1 Upon completion, the Deconvolution Report shall be reviewed for quality by assessing the deconvolution diagnostics. Skewed diagnostics may indicate that the sample is in need of further scrutiny (e.g., inclusion of inappropriate artifacts in import file or incorrect contributor number), that the deconvolution has not reached the appropriate sample space and additional accepts may be needed, or that the sample is of poor quality.
 - 2.8.22.9.1.1 The weights of the genotype combinations generated by STRmix™ should be assessed at each locus for their intuitiveness when compared visually to the accompanying sample electropherogram. These may be found under the **Weights** section of the Deconvolution report (example below).
 - 2.8.22.9.1.1.1 Rarely, due to limitations in stutter modeling for loci with complex repeat structures, STRmix™ may not model stutter peaks as expected (ex. 13 stutter peak from 14 allele at vWA). If an analyst finds that a peak has not modeled as expected, they may refer to the STRbase website ([NIST STRBase](#)) to investigate if the allele is one that may contain variations in the Longest Uninterrupted Sequence (LUS) value. This would provide weight to the decision to ignore the locus in the STRmix™ deconvolution.

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WEIGHTS

LOCUS	CONTRIBUTORS		WEIGHT (HIGHLIGHT ≥ 0.99)
	1 (52%)	2 (48%)	
D3S1358	15, 18	16, 16	2.7918E-1
	15, 16	16, 18	2.7166E-1
	16, 18	15, 16	2.3937E-1
	16, 16	15, 18	2.0979E-1
DIS1656	15.3, 17	15.3, 16	2.9155E-1
	15.3, 16	15.3, 17	2.5167E-1
	15.3, 15.3	16, 17	2.3808E-1
	16, 17	15.3, 15.3	2.1871E-1
D2S441	14, 14	11.3, 13	4.6170E-1
	13, 14	11.3, 14	2.9570E-1
	11.3, 13	14, 14	1.2257E-1
	11.3, 14	13, 14	1.1996E-1
	13, 14	11.3, 11.3	5.0760E-5
	11.3, 11.3	13, 14	1.6656E-5

2.8.22.9.1.2 Template (RFU) and Mixture Proportions

2.8.22.9.1.2.1 Mixture proportions are calculated from the template mass parameter per contributor. The template amount in RFU should approximate the lowest molecular weight alleles/loci for a heterozygous peak (the value would need to be doubled for a homozygous genotype). The template and mixture proportions should be intuitive to the analyst's interpretation of the profile. Significant deviations from the expected template and/or mixture proportions may require further scrutiny and additional rework.

2.8.22.9.1.3 Degradation

2.8.22.9.1.3.1 The degradation mass parameter per contributor is displayed in the STRmix™ report as two values. The first value is the Linear approximation (RFU/bp) which demonstrates the average decrease in RFU per base pair. The second value is the Degradation exponential curve which is the actual mass parameter utilized by STRmix™ for the calculation of Total Allelic Product. This value has a maximum threshold of 0.01 in the standard kit settings. For either value a larger value indicates a steeper curve and more degradation; however, a sample with significant degradation cannot exceed the 0.01 exponential curve threshold and may result in additional skewed diagnostics and/or unintuitive genotype weightings. If an elevated degradation value is observed (likely greater than 0.009) and potentially hindering the interpretation of the sample, consultation with the Technical Leader is recommended to consider increasing the threshold up to 0.02.

2.8.22.9.1.4 Total Iterations and Acceptance Rate

2.8.22.9.1.4.1 The Total Iterations value displayed in the **Post Burn-In Summary** indicates the total number of post burn-in MCMC iterations. This value, along with the number of accepts chosen for the analysis can inform the

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user as to how often a new proposed set of parameters was accepted. This is referred to as the acceptance rate.

2.8.22.9.1.4.2 A very low acceptance rate (e.g., 1 in thousands to millions) may, in combination with the other diagnostics, indicate that the analysis may benefit from a run with additional iterations. On its own (and without any other indication of sub-optimal results) a low acceptance rate is not an indication that rework is required.

POST BURN-IN SUMMARY

Total iterations	532,424	Acceptance rate	1 in 1.33
Effective sample size	5,141.61	log(likelihood)	49.66
Gelman-Rubin convergence diagnostic	1.07		
Allele variance (mode = 8.769)	10.884	Back Stutter variance (mode = 5.992)	10.385
Forward Stutter variance (mode = 3.186)	4.165	SE33 -2 Stutter variance (mode = 0.694)	1.732

2.8.22.9.1.5 The Effective Sample Size (ESS) value displayed in the **Post Burn-In Summary** is the number of independent samples the MCMC has taken from the posterior distribution of all parameters. A low ESS in relation to the total number of iterations suggests that the MCMC has not moved very far with each step or has had a low acceptance rate. A low absolute value of ESS (e.g., 10s or 100s) will mean that there is potential for a large difference in weights if the analysis was run again. On its own (and without any other indication of sub-optimal results) a small ESS is not an indication that rework is required.

2.8.22.9.1.6 The log(likelihood) value displayed in the **Post Burn-In Summary** is the log of the average likelihood (or probability) value created at each of the post burn-in MCMC iterations. The larger this value the better STRmix™ has been able to describe the observed data. A negative value suggests that STRmix™ has not been able to describe the data very well given the information it has been provided. Reasons why this value may be low or negative are:

- The profile is simply very low level and there is very little data making up the likelihood.
- The proposed number of contributors does not model well (and may be incorrect), forcing an interpretation of large stochastic events (e.g., large heterozygote peak imbalances or variation in mixture proportions across the profile) in the STRmix™ run.
- Data has been removed that was real, (e.g., stutter peaks), forcing an interpretation of dropout by STRmix™.
- Artifacts have been left labelled (not removed) in the imported data, forcing an interpretation of drop-in by STRmix™.

2.8.22.9.1.6.1 A low or negative value for the average log(likelihood) may indicate to users that the analysis requires additional scrutiny.

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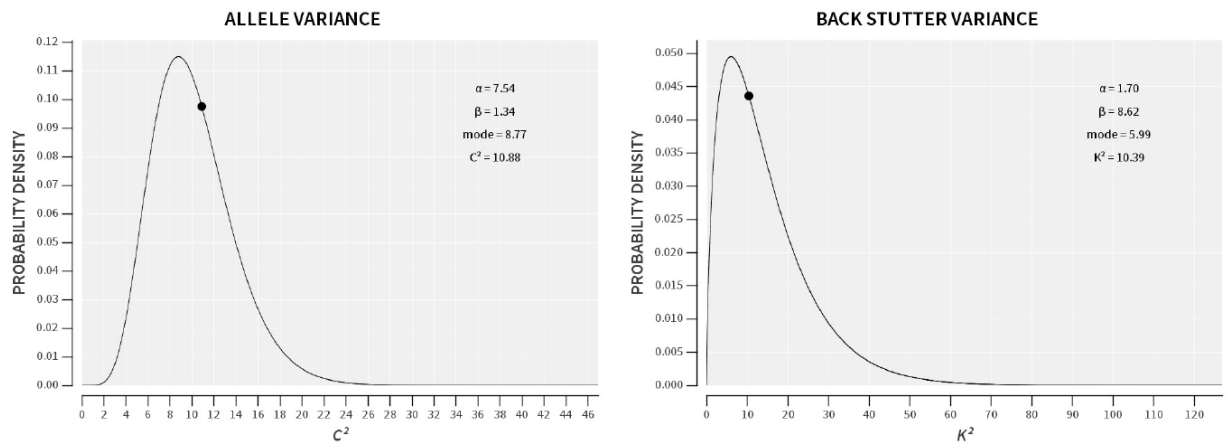
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- 2.8.22.9.1.6.2 Good quality mixed DNA profiles are likely to give higher average log(likelihood) values than good quality single source profiles.
- 2.8.22.9.1.6.3 Low average log(likelihood) values alone are not necessarily an indicator of an issue, especially if the profile is single source.
- 2.8.22.9.1.7 The Gelman-Rubin Convergence Diagnostic (GR) value displayed in the **Post Burn-In Summary** informs the user whether the MCMC analysis has likely converged. STRmix™ uses multiple MCMC chains to carry out the deconvolution, and ideally each chain will be sampling in the same space after burn-in. Too few accepts may result in a failure to converge. If the chains spend their time in different spaces, then it is likely that the analysis has not run for sufficient iterations. Whether or not the chains have spent time in the same space can be gauged by the within-chain and between-chain variances. These two variances are used to calculate the variance of what is called the 'stationary distribution'. If all chains have sampled the same space, then the within chain variance and the variance of the stationary distribution will be approximately equal. If chains have spent time in different spaces, then the variation between the chains is likely to be larger than the variation within the chains. The GR is the ratio of the stationary distribution and within-chain variances. For a perfectly converged analysis, GR = 1.
- 2.8.22.9.1.7.1 If the GR is above 1.2 then there exists the possibility that the analysis hasn't converged. If the GR value is above 1.2, STRmix™ will automatically run an additional 50,000 post burn-in accepts. This may decrease the GR to below 1.2, yielding a clearer deconvolution.
- 2.8.22.9.1.7.2 If the GR continues to remain above 1.2 after the additional post burn-in accepts, this may be an indicator that the sample requires rework.
- 2.8.22.9.1.8 The allele, stutter, and Locus Specific Amplification Efficiency (LSAE) variance data depicted in the **Variance Charts** (see below for allele and back stutter variance only) can be used as a guide as to the level of stochastic variation in peak heights that is present in the profile. The values listed in the **Post Burn-In Summary** of the report include the average value for allele, stutter, and LSAE variance constants across the entire post burn-in MCMC analysis.

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2.8.22.9.1.8.1 If the variance constant has increased markedly from the mode of the prior distribution, then this may indicate that the DNA profile is sub-optimal or that the number of contributors is incorrect.

2.8.22.9.1.8.2 Used in conjunction with the average log(likelihood), a large allele variance or stutter variance constant can indicate poor PCR.

2.8.22.9.1.8.2.1 A low-level sample may result in a low average log(likelihood) and an average variance constant.

2.8.22.9.1.8.2.2 Data with missing information, extra information (e.g., artifact peaks), or misinterpreted data may result in a low average log(likelihood) and high variances.

2.8.22.10 Indiana State Police Forensic Services Division Quality Assurance DNA Database Search Review

2.8.22.10.1 In the Database Search Report, verify that no positive associations with $LR \geq 10,000$ have occurred.

2.8.22.10.2 Any database matches with a $LR \geq 10,000$ should be evaluated for the likelihood of potential contamination. The Technical Leader shall be consulted for evaluation of the match. If the match is determined to be a suspected contamination event a root cause analysis shall be performed to determine the possible cause. Appropriate report wording shall be utilized if contamination is suspected.

2.8.22.10.2.1 Database matches ($LR \geq 10,000$) shall be interpreted with caution and acknowledged in the report wording.

2.8.22.10.2.1.1 The database profile shall be utilized and conditioned upon in an additional STRmix™ deconvolution.

2.8.22.10.2.1.2 The database Excel tool shall be used to convert the database profile into the correct STRmix™ reference profile format.

2.8.22.10.2.1.3 Any LRs calculated for the samples shall be performed on the deconvolution conditioned on the database profile.

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2.8.22.11 Evaluating Deconvolution Components for Comparison Suitability – applicable only to deconvolutions performed after June 17, 2024.

- 2.8.22.11.1** Any component of a mixture with STRmix™ determined template <50 RFU shall be considered unsuitable for comparisons. This does not apply to assumed individuals.
- 2.8.22.11.2** All single-source profiles and STRmix™ determined template amounts for 2-person mixture components >150 RFU, 3-Person mixture components >250 RFU, and 4-Person mixture components >350 RFU shall be considered suitable for comparisons.
- 2.8.22.11.3** DBLR™ v1.5 or currently approved version shall be used to evaluate individual contributor positions for the following components prior to comparison of reference standards, except assumed contributor(s), and calculation of LR:
- 2-Person mixture components with STRmix™ determined template amounts from 50-150 RFU.
 - 3-Person mixture components with STRmix™ determined template amounts from 50-250 RFU.
 - 4-Person mixture components with STRmix™ determined template amounts from 50-350 RFU.
- 2.8.22.11.3.1** Monte Carlo estimates <0.01 for $P(\text{Log}_{10}\text{LR} \geq 0 \mid H_2)$ shall be considered suitable for comparison to reference profiles and LR shall be calculated/reported.
- 2.8.22.11.3.2** Contributor positions which demonstrate a Monte Carlo estimate ≥ 0.01 for $P(\text{Log}_{10}\text{LR} \geq 0 \mid H_2)$ shall be considered unsuitable for comparison.
- 2.8.22.11.3.2.1** Once a higher contributor has been identified as unsuitable for comparison, the lower contributors are automatically deemed unsuitable for comparison and DBLR™ shall not be run. For example, in a 4 person mixture, if contributor 2 is determined to be unsuitable for comparison utilizing DBLR™, contributors 3 and 4 are also determined to be unsuitable without DBLR™ evaluation.
- 2.8.22.11.3.2.2** Any reference standard in which an LR is calculated and the contributor order giving the highest LR aligns with a contributor position determined to be unsuitable for comparison shall be reported as an exclusion. The LR shall be marked as not used or applicable in the notes. The reference standard shall not be utilized for a combined LR proposition set.
- 2.8.22.11.3.2.2.1** When running a combined LR with reference standards that have support for inclusion into the comparable portion of a mixture individually, and the combined LR statistic aligns one of the references into a contributor position determined to be unsuitable for comparison, the individual statistics shall be reported and the

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reporting note indicating that they cannot be together in the interpretable portion of the mixture shall be utilized.

- 2.8.22.11.3.2.3 An LR need not be calculated for a reference standard that can be unambiguously excluded from the interpretable portion(s) of the mixture.

2.8.22.11.4 To start a new DBLR™ evaluation, from the DBLR™ main menu select **Explore Deconvolution**.

- 2.8.22.11.4.1 **Drag and drop** the sample deconvolution file folder or select **Browse** to navigate to the sample deconvolution and open the config file.
- 2.8.22.11.4.2 Expand the **Simulation** menu and mark an appropriate contributor position for evaluation. If more than one contributor position requires evaluation, they shall be simulated independently.
- 2.8.22.11.4.3 Open the **Population** menu and select *ISP_NIST_Combined w iSNP: ISP_NIST_combined w iSNP (100%, Fst=0.01)*.
- 2.8.22.11.4.4 Ensure the remaining search configuration settings match below:
- 2.8.22.11.4.4.1 H1 Relationship: Contributor; H2 Relationship: Unrelated
- 2.8.22.11.4.4.2 Include loci: All loci marked
- 2.8.22.11.4.4.3 Log10(LR) threshold: 0 (corresponds to LR threshold of 1)
- 2.8.22.11.4.4.4 Extended Output: Unmarked
- 2.8.22.11.4.4.5 Sample: H1 true and H2 true marked; Additional hypothesis (H3) unmarked.
- 2.8.22.11.4.4.6 Seed: Random
- 2.8.22.11.4.4.7 Number of samples: 100,000
- 2.8.22.11.4.5 Select **Sample Likelihood Ratios**
- 2.8.22.11.4.6 Select **Save Simulation Results**. Save the DBLR results folder to the DNA data drive and add the PDF report to the case file.

2.8.22.12 Comparing Sample Deconvolutions to Reference Standards (LR from Previous)

- 2.8.22.12.1 A reference standard may be visually excluded without STRmix™ analysis.
- 2.8.22.12.2 To compare reference STR profiles to a previously run STRmix™ deconvolution, from the STRmix™ main menu, select **Investigation**, then **LR from Previous**.
- 2.8.22.12.2.1 Alternately, a **Batch Mode** for LR from Previous may be utilized for multiple samples, which is accessed from the main menu. In the **Add to Batch** drop down menu, select **add LR from Previous** and then follow the instructions below.
- 2.8.22.12.3 In the **Previous Interpretation** box, select **Browse** and navigate to the config.xml file for the appropriate STRmix™ deconvolution or drag and drop the deconvolution folder into the window.
- 2.8.22.12.4 Leave the Case Number as it is carried over. In the “Sample ID” box the appropriate LR number should be added behind LRPrev (e.g., 001A_Decon1_LRPrev1). Each LR calculated from a deconvolution shall have a unique identifier. If additional characters are added to the “Sample ID”

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designation at this step, those characters shall be added after the specified naming convention. Select **Next**.

- 2.8.22.12.4.1 The number of contributors, number of burn-in accepts per chain, and number of post burn-in accepts per chain will be grayed out and cannot be changed. No changes should be made to **Run Settings**.
- 2.8.22.12.5** Add a new profile to the box marked **Reference Profile Data**. This may be done by selecting **+** and navigating the file tree, or by dragging and dropping the appropriate GeneMapper® ID-X export file or previous STRmix™ evidence file into the box. Multiple reference profiles may be added, one at a time or highlight and add multiple profiles at one time.
- 2.8.22.12.6** To ignore loci from deconvolution, select **Kit Settings**, then select **Loci**, then select **Ignore?** for appropriate loci, then select **Apply**.
- 2.8.22.12.6.1 If a locus in the reference profile has potential allelic drop-out (a single allele ≤ 950 RFU), the locus shall be omitted from the LR from Previous calculation.
- 2.8.22.12.7** If a varNOC deconvolution was performed, select **LR Settings**. Under **Contributor Range**, select **MLE** for the priors method, then select **Apply**.
- 2.8.22.12.7.1 Upon request by the submitting agency, Prosecuting or Defense attorney or with Technical Leader permission documented in the casefile, a User Defined LR may be performed.
- 2.8.22.12.7.1.1 Select **LR Settings**. Under **Contributor Range**, select **User Defined** for the priors method, enter the selected number of contributors for Hp and Hd, then select **Apply**.
- 2.8.22.12.8** Select **Start**. This will begin the LR calculation of the chosen sample.
- 2.8.22.12.9** When the analysis is complete, STRmix™ will generate several data files and save them to the Results folder on the C: drive.
- 2.8.22.12.10** Save the results folder for the LR calculation containing all appropriate STRmix™ files to the corresponding deconvolution master folder located on the DNA server for the appropriate case.

2.8.22.13 LR Calculations

- 2.8.22.13.1** Each analyzed proposition (and therefore each LR From Previous report) will contain a generated set of calculations including a Likelihood Ratio (LR) Total (sub-sub-source), Factor of N! (sub-source) LR, and a 99% 1-Sided Lower HPD Interval.
- 2.8.22.13.2** The (sub-sub-source) LR Total is a ratio between the probability of the evidence given two competing propositions or hypotheses and forms the basis for subsequent statistical calculations.
- 2.8.22.13.2.1 Calculations for (sub-sub-source) LR Total utilize the National Research Council recommendation 4.2, equations 4.10, more commonly referred to as the Balding & Nichols formulae.
- 2.8.22.13.2.1.1 Homozygous
$$\frac{[2\theta + (1-\theta)p][3\theta + (1-\theta)p]}{[1+\theta][1+2\theta]}$$
- 2.8.22.13.2.1.2 Heterozygous
$$2[\theta + (1-\theta)p][\theta + (1-\theta)q]$$

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$$[1+\theta][1+2\theta]$$

2.8.22.13.2.1.3 Each locus LR shall be multiplied together to form the multi-locus LR for the profile.

2.8.22.13.3 The Factor of N! (sub-source) LR is a calculation that uses the (sub-sub-source) LR Total with additional consideration for the number of ways to combine the different contributor probabilities in a mixture.

2.8.22.13.4 The HPD calculation uses the Factor of N! (sub-source) LR with additional consideration for the uncertainty in the allele frequencies and deconvolution process. HPD is the most conservative of the three calculations and will be used for reporting the significance of a comparison.

2.8.22.13.5 Additionally, a Unified LR may be calculated under propositions that consider only a single person of interest. The unified LR takes into account that the unknown contributor(s) are made up of both unrelated people and relatives of the person of interest. These calculations may be most useful when the alternate proposition is that the true donor of the evidence sample is a relative of the person tested.

2.8.22.13.6 The varNOC Maximum Likelihood Estimate LR (MLE) assigns an LR using the most probable number of contributors under H_1 and H_2 . This means the number of contributors in the numerator and denominator may be the same or they may be different. The number of contributors will depend on which number of contributor analysis has the highest likelihood given the proposition for each.

2.8.22.14 Troubleshooting STRmix™ Results

2.8.22.14.1 After a STRmix™ analysis has completed, the results are interpreted by examining the weightings of various genotypes and the DNA results observed. There are instances when the results obtained do not intuitively seem correct. Examples of this are:

- Large LR's (greater than 1) are obtained for each locus, except one where the LR is 0.
- The mixture proportions do not reflect what is observed.
- The interpreted contributor genotypes are not intuitively correct.

2.8.22.14.2 Possible causes for these issues include:

- The MCMC has not run for enough iterations.
- The number of contributors has not been correctly chosen.
- An artifact was left in the GeneMapper™ ID-X interpretation.

2.8.22.14.3 Should the weights and/or the diagnostics highlight to the user that further scrutiny is necessary then a number of rework options are available, if required. For example, a review of the proposed number of contributors could be considered. Further analytical work such as a re-electrophoresis, a re-amplification, or a clean up to strengthen the number of contributors assumption or assist with allele designation/sub optimal PCR performance. Extended total iterations (x10) may be considered, if the acceptance rate is low, the ESS is low and/or the GR value is significantly above 1.2. If extended iterations are

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manually utilized, the Technical Leader shall be notified with documentation included in the case record.

2.8.22.14.3.1 Additional troubleshooting options may be available after consultation with the Technical Leader (documented in the case record). This may include utilizing informed priors to designate the mixture proportions utilized in the deconvolution.

2.8.22.14.4 If after comparison to a reference sample from a person of interest, an analyst suspects an error was made during the interpretation of the sample (e.g., determining the wrong number of contributors or an artifact was not deleted), independent analysis and interpretation of the profile by the Technical Leader or their designee shall be performed. A referee analysis is not required if the error determined after comparison to a reference profile of an individual who may be assumed present in the sample. If the error is caught during technical review of the case, and the reviewer has not examined the profiles of the person(s) of interest, the referee analysis is not required.

2.8.22.14.4.1 The analyst shall only provide the .hid files for the unknown sample of concern and the allelic ladder to the Technical Leader. If a reference standard may be assumed, the .hid file for that reference may also be included. If the analysis method and panels and bins in GMID-X are not current methods, the analyst shall notify the Technical Leader of the appropriate analysis methods and settings to utilize. No additional case information or information regarding the interpretation issue shall be given. The Technical Leader may request limited additional information only if it is needed for analysis.

2.8.22.14.5 In a single source sample, if an error is discovered that did not affect the STRmix deconvolution of the sample (ex. a pull-up peak was not deleted, but it was modeled as drop-in), a second deconvolution does not need to be performed. The error shall be documented in the case record with an explanation why it did not affect the interpretation.

2.8.22.14.5.1 An error of this type requires a new deconvolution in a mixed sample.

2.8.22.15 CODIS Eligibility and Entry

2.8.22.15.1 Casework profiles shall be carefully evaluated for CODIS eligibility in accordance with National DNA Index System (NDIS) Operational Procedures "DNA Records Accepted at NDIS" and the flowchart "A Guide to Determining What is Allowable in the Forensic Index at NDIS," both available on the CODISPortal. Refer to the CODIS Test Method for details.

2.8.22.15.2 Each year, every qualified analyst shall complete the 'Annual Review of DNA Data Accepted as NDIS'. The timing of this review is specified by NDIS and completion is documented within the CODIS learning management system. Appropriate extensions, if needed, will be maintained by the CODIS Administrator.

2.8.22.15.3 If a casework profile is determined to be ineligible after CODIS entry and upload, that profile shall be deleted by a CODIS administrator. The case analyst shall issue a supplemental report stating that the profile was removed.

2.8.22.16 Use of STRmix™ to aid in deconvolution of mixtures for CODIS entry

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2.8.22.16.1 Additional STRmix™ deconvolutions utilizing conditioning may be performed to aid in the deconvolution of a mixture for improved CODIS entry.

2.8.22.16.1.1 If a mixture includes support for inclusion of a reference profile (an LR has been calculated in support for H_1), the mixture may be deconvoluted conditioning on that reference profile to provide improved genotype weightings of an additional contributor to be entered into the database.

2.8.22.16.1.1.1 The case documentation of this deconvolution shall be clearly marked as for CODIS purposes only.

2.8.22.16.1.1.2 The analyst shall ensure all alleles to be entered in CODIS are present in the original mixture.

2.8.22.16.1.1.3 The original deconvolution should generally be utilized for comparison if an evidentiary reference standard is submitted at a later date.

2.8.22.16.1.2 If a mixture indicates that an additional single source unknown profile in the case cannot be excluded as a contributor, that unknown profile may be utilized to improve genotype weightings of an additional contributor for CODIS entry.

2.8.22.16.1.2.1 The single source unknown profile may be reanalyzed in GeneMapper™ ID-X utilizing reference profile settings.

2.8.22.16.1.2.2 The mixture may be deconvoluted conditioning on the reanalyzed single source unknown profile.

2.8.22.16.1.2.3 The analyst shall ensure all alleles to be entered in CODIS are present in the original mixture.

2.8.22.16.1.2.4 The case documentation of the reanalysis and the deconvolution shall be clearly marked as for CODIS purposes only.

2.8.22.16.1.2.5 The original deconvolution with no conditioning on the unknown profile shall be utilized for all LR calculations.

2.8.22.17 Use of the STRlite Excel program

2.8.22.17.1 The ISP STRlite Excel program shall be utilized to provide a summary of GeneMapper™ ID-X allele calls for reference and unknown samples; as well as information collated from the STRmix™ deconvolution, database search and LR from Previous reports for sample analysis utilized in the interpretation of the profile.

2.8.22.17.1.1 STRlite v2.0 (or most current version as approved by the Technical Leader) shall be utilized.

2.8.22.17.1.2 Worksheets, as appropriate, from the STRlite program shall be maintained in the case record. These worksheets may include Pre-STRmix, Deconvolution, Likelihood Ratios, Standards, CODIS, 1P Combo, and 2P Combo. An electronic copy of STRlite containing results shall not be maintained on the DNA server to avoid duplication of items between LIMS and the server.

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- 2.8.22.17.1.2.1 The STRlite Deconvolution page or a 1P/2P Combo worksheet shall be maintained for all deconvolutions that represent a valid interpretation of the profile.
- 2.8.22.17.1.2.1.1 If an analyst error is made during a deconvolution run (e.g., An unintended number of contributors was entered, a pull-up peak was not deleted etc.), a deconvolution worksheet does not need to be generated for that run, but the STRmix™ deconvolution files shall be maintained electronically and the case record shall document why the run was not used.
- 2.8.22.17.1.2.2 On STRlite Deconvolution pages, Combo worksheets, and CODIS pages, the use of “{ }” at a locus with one or two alleles clarifies that the 99% cumulative weighting contains more than one possible genotype (mixture or partial) and would be searched with moderate stringency in CODIS.
- 2.8.22.17.1.2.3 For single source profiles, the analyst shall manually add results for Amelogenin to the CODIS worksheet.
- 2.8.22.17.1.2.4 For mixed profiles, the analyst may manually add results for Amelogenin to the CODIS worksheet if they feel the data supports the determination.
- 2.8.22.17.1.3 The electronic STRmix™ reports saved to the case folder on the DNA server shall be referenced for further troubleshooting as needed.

2.9 Records:

- 2.9.1 The appropriate worksheets as contained in the Worksheet Manual, STRlite, or the equivalent workbooks shall be used to record all procedures.
- 2.9.2 All data sheets, notes, photographs, and other information generated from the laboratory examination shall be kept in the case record.
- 2.9.3 The technical review of the case record shall be recorded on the technical review worksheet.
- 2.9.4 Electronic records shall be retained as indicated in [Appendix 9](#).

2.10 Interpretations of Results:

- 2.10.1 Interpretation guidelines are located within the **Procedures Section 2.8**.

2.11 References:

- 2.11.1 Federal Bureau of Investigation. Procedures for the Detection of Restriction Fragment Length Polymorphisms in Human DNA. FBI Laboratory. 1990.
- 2.11.2 Federal Bureau of Investigation. PCR-Based Typing Protocols. FBI Laboratory. 1994.
- 2.11.3 Applied Biosystems. 3500/3500xL Genetic Analyzer 3500 Series Software 2 User's Manual. P/N 4476988, rev. A. 5/2012.
- 2.11.4 Applied Biosystems. Veriti™ Thermal Cycler User Guide. P/N 4375799, Rev. E. 2010
- 2.11.5 Promega Corporation. PowerPlex® Fusion 6C System Technical Manual. Part No. TMD045. Most current issue.
- 2.11.6 Promega Corporation. PowerPlex® 6C Matrix Standard Technical Manual. Part No. DG4900. 10/15 or most current issue.

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- 2.11.7 Promega Corporation. Maxwell® FSC Instrument Operation Manual. Part No. TM462. 2024 or most current issue
- 2.11.8 Promega Corporation. Maxwell® RSC 48 Instrument Operation Manual. Part No. TM510. 2024 or most current issue
- 2.11.9 Promega Corporation. Maxwell® FSC DNA IQ™ Casework Kit Technical Manual. Part No. TM499. 2024 or most current issue.
- 2.11.10 Promega Corporation. Tissue and Hair Extraction Kit (for use with the DNA IQ™). Part No. TB307. 2016 or most current issue.
- 2.11.11 Applied Biosystems. GeneMapper™ ID-X Software Version 1.0 Getting Started Guide. Part No. 4375574 Rev. A. 10/2007
- 2.11.12 Applied Biosystems. GeneMapper™ ID-X Software Version 1.2 Reference Guide. Part No. 4426481 Rev. A. 10/2009
- 2.11.13 Applied Biosystems. GeneMapper™ ID-X Software Version 1.0 Administrator's Guide. Part No. 4376327 Rev A. 09/2007.
- 2.11.14 Applied Biosystems. GeneMapper™ ID-X Software Version 1.4 User Bulletin. Part No. 77684 Rev. A. 08/2012
- 2.11.15 Applied Biosystems. GeneMapper™ ID-X Software Version 1.6 User Bulletin. Pub. No. 100073905 Rev. B. 02/2019.
- 2.11.16 Smith, J., Budowle, B. Source Identification of Body Fluid Stains Using DNA Profiling. Proceedings of the Second European Symposium, Innsbruck, Austria. 6/98
- 2.11.17 Applied Biosystems. Quantifiler® HP and Trio DNA Quantification Kit User Guide. P/N 4485354 most current version.
- 2.11.18 Applied Biosystems. QuantStudio 3 and 5 Real-Time PCR Systems. Installation, Use, and Maintenance. Firmware v1.6. Pub No. MAN0010407. Rev G
- 2.11.19 Promega Corporation. PowerQuant Technical Manual. TMD047 or most current issue
- 2.11.20 Bär, W. et al. DNA recommendations: Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. *Int. J. Legal Med.* (1997) 110, 175-176.
- 2.11.21 Gill, P. et al. Considerations from the European DNA profiling group (EDNAP) concerning STR nomenclature. *Forensic Science International* (1997) 87, 185-192.
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3. Forensic Relationship Comparison Methods:

3.1. Scope:

- 3.1.1. Forensic relationship comparisons may be requested as part of some criminal investigation cases. Types of relationship comparisons that can be performed include paternity, maternity, reverse paternity, sibling relationship (sibship), etc. These comparisons may establish potential relationships among individuals and/or aid in the identification of human remains and missing persons. Samples for forensic relationship testing shall be processed in accordance with the test methods outlined in the DNA methods section of this document.

3.2. Precautions/Limitations:

- 3.2.1. Cases submitted for relationship comparisons should be evaluated by a member of the relationship comparison team before any testing is performed. It may be necessary to outsource some types of cases to a vendor laboratory for analysis. Cases shall be refused when they do not meet the requirements of forensic relationship comparison analysis.

3.2.1.1. Cousin comparisons should not be performed unless it is part of a pedigree.

3.3. Related Information:

- 3.3.1. See Relationship Comparison Statistical Reference Sheet ([Appendix 10](#))

3.4. Instruments:

- 3.4.1. See DNA Test Methods Section 2.4

3.5. Reagents/Materials:

- 3.5.1. See DNA Test Methods Section 2.5

3.6. Hazards/Safety:

- 3.6.1. See DNA Test Methods Section 2.6

3.7. Reference Materials/Controls/Calibration Checks:

- 3.7.1. See DNA Test Methods Section 2.7

3.8. Procedures/Instructions:

- 3.8.1. See DNA Test Methods Section 2.8

3.9. Interpretation of Relationship Testing Results:

3.9.1. Possible Outcomes of Relationship Comparisons

- 3.9.1.1. The genetic profiles from standards and samples are compared to evaluate relationship. The following conclusions may be reported:

3.9.1.2. For relationship cases using DBLR™:

- [Limited/moderate/strong/very strong] support for the alleged relationship: $CRI > 1$.
- Uninformative: $CRI = 1$
- Limited support for unrelatedness: $2 \leq 1/CRI < 100$
- Unrelated: $1/CRI \geq 100$ or visual exclusion for parentage cases

3.9.2. Inconsistent Profiles in Parentage Relationship Cases

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- 3.9.2.1. If the alleged father's profile is inconsistent with the child's obligate paternal alleles at more than three loci with PowerPlex® Fusion 6C, then no relationship/exclusion shall be concluded.
- 3.9.2.2. If the alleged father's profile is inconsistent with the child's obligate paternal alleles at fewer than four loci with PowerPlex® Fusion 6C, then other alternatives may be evaluated for these inconsistencies. Other alternatives may include possible mutation, null allele, first degree relative, or true exclusion. If it is determined that a possible mutation or a null/silent allele could account for the inconsistencies, then the mutation or null allele shall be included into the statistical calculations.
 - 3.9.2.2.1 A mutation is typically observed to be a 1-step (repeat unit) smaller or larger from the parental allele (family study estimates ~90%). 2-step (~9%) or 3-step (~1%) mutations are possible, but very uncommon.
 - 3.9.2.2.2 A null/silent allele may be observed when both the child and either parent appear homozygous at a locus without a concordant allele and the overall peak height of the homozygous allele is approximately half expectation based on the peak heights of other loci in the same dye channel. Additional indicators may include a possible peak below AT that would be concordant between the parent and child and/or requiring more than a 1-step mutation to explain the discordant locus. The Technical Leader shall be consulted for incorporation of a null allele into the statistical calculation.

3.9.3. Mixtures

- 3.9.3.1. With product of conception/fetal samples, it may be necessary to have a standard from the mother. Using the biological mother's standard, a deconvolution may be performed to determine the genotype of the child.
- 3.9.3.2. Secondary standards, including personal effects believed to belong to a missing person, may be suitable for comparison. Additional standards may be requested to aid in interpretation or to be conditioned upon, if appropriate.

3.10. Guidelines for Additional Testing

3.10.1. Y-STR testing for kinship comparisons should only be done in the following circumstances:

- 3.10.1.1. Kinship analysis involving male individuals proposed to be paternally related that demonstrate limited support for H1 or H2 or an uninformative result shall have additional Y-STR analysis performed to provide further information regarding a potential relationship.
- 3.10.1.2. Kinship analysis involving male individuals proposed to be paternally related that demonstrate moderate support for H1 may have additional Y-STR analysis performed to provide further information regarding a potential relationship.
- 3.10.1.3. If a mutation is suspected at one or two Y-STR loci with PowerPlex® Fusion 6C, additional genetic testing should be performed to evaluate additional Y-STR loci.

3.10.2. If there are additional Y-STR discordances that do not support an alleged relationship (such as full siblings), the analyst shall attempt to confirm with the contributing agency if there is potential for a different relationship (such as half-siblings). If the agency is unable to confirm a different relationship is possible, the results shall be reported as no support for the originally designated relationship and additional family standards should be requested. If confirmation of a potential relationship in which Y-STR profiles are not expected to match is obtained, the new relationship statistic should be performed along with a report statement indicating that the Y-STR results do not demonstrate support for the original relationship.

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3.11. Rules for Relationship Statistical Calculations using DBLR™:

- 3.11.1. Calculations shall be performed using DBLR™ version 1.5 or currently approved version.
- 3.11.2. Statistical calculations shall be performed and reported in forensic relationship comparison cases where Results/Opinions/Interpretations are being given in regards to relationship.
- 3.11.3. A minimum of eight autosomal loci with no indication of allelic drop-out are required for comparison and statistical calculations. STRmix™ deconvolutions may be utilized for degraded standards or for standards demonstrating mixtures as allowed in 3.9.3. For STRmix™ deconvolutions, the 99% contributor designation shall be utilized for this purpose.
- 3.11.4. Statistical calculations shall not be performed in parentage cases with more than three genetic inconsistencies.
- 3.11.5. Linkage shall be enabled for all calculations, regardless of the type of relationship under evaluation.
- 3.11.6. The addition of Y-STR loci (DYS391, DYS576, and DYS570) into the PowerPlex® Fusion 6C system is intended to assist with interpretation of the number of male contributors in a sample and with samples demonstrating a possible null Y allele at Amelogenin. Therefore, statistical evaluation of the Y-STR loci shall not be performed or incorporated in the Combined Relationship Index unless a mutation is suspected.
- 3.11.7. See Relationship Comparison Statistical Reference Sheet ([Appendix 10](#)) for a list of formulas for calculations.
- 3.11.8. If more than one standard/sample is available for comparison, the most representative standard/sample or the standard/sample where the most obligate paternal alleles can be determined shall be used for statistical calculations.
- 3.11.9. Relationship statistical calculations utilize the race of the alleged individual. The calculations shall be performed with the appropriate NIST 2017 population data using the racial information provided by the contributing agency. If racial information is unknown or reported as multi-racial, calculations shall be performed using the Combined population data.
- 3.11.10. Manual calculations performed by an analyst shall be verified by a qualified technical reviewer and/or a qualified unit supervisor.
- 3.11.11. Mutations shall only be enabled in DBLR™ when necessary.
 - 3.11.11.1. The appropriate Maternal or Paternal mutation rate file shall be selected in DBLR™ based on the suspected mutation source.
 - 3.11.11.2. In the event that a mutation is suspected at one Y-STR locus other than DYS570, DYS576, DYS612, and DYF387S1, a mutation factor of 0.001 shall be used as a haplotype Relationship Index and incorporated through manual calculation into the Combined Relationship Index derived from DBLR™. DYS570, DYS576, DYS612, and DYF387S1 are generally regarded as rapidly mutating Y-STR loci. The mutation factor for a single discordant Y-STR locus defined as rapidly mutating will be incorporated as a haplotype Relationship Index and incorporated manually into the CRI by multiplying by 0.01. TL consultation is required for two suspected Y-STR mutations.
- 3.11.12. Mutation rates for historical loci (PowerPlex® 16 HS and SE33) have been reported in the AABB Annual Report Summary. For any locus without a published mutation rate, the rate

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shall be calculated by averaging the available tetranucleotide STR mutation rates (excluding SE33).

- 3.11.13. For parentage calculations utilizing a mother's standard and an alleged father's standard in which a null allele from the alleged father is suspected, the Paternity Index (PI) for a locus demonstrating a possible null allele should be manually calculated using the formula described in [Appendix 10](#). The frequency of a silent allele(s) is 0.001 based on the typical observation of a null allele in modern STR typing kit loci is less than 1 in 1,000. The Technical Leader shall be consulted if a null allele is suspected.
- 3.11.14. The F_{ST} Coancestry Coefficient shall be enabled for all calculations. An F_{ST} of 0.01 shall be utilized for all populations.

3.12. Relationship Calculations using DBLR™

- 3.12.1. To start a new DBLR™ relationship calculation, from the DBLR™ main menu select **Kinship**.
- 3.12.2. In the H1 screen, construct the pedigree.
 - 3.12.2.1. The symbols used should reflect the sex of the corresponding sample, with squares for males and circles for females.
 - 3.12.2.2. A template may be chosen from the drop-down menu.
 - 3.12.2.3. A custom pedigree may be constructed by right clicking on the symbol for Individual A and adding parents or offspring as required.
 - 3.12.2.4. The default sample names may be edited to reflect case information by highlighting the assigned letter and typing the desired label.
- 3.12.3. Add H2 by clicking the "+" symbol next to H1.
 - 3.12.3.1. Add the unknown, unrelated individual by choosing the male or female symbol from the toolbar, as appropriate.
- 3.12.4. Verify that all symbols and labels are visible and adjust spacing as needed.
 - 3.12.4.1. The pedigrees can be rearranged by clicking and dragging symbols.
- 3.12.5. Choose **Numeric Kinship** in the bottom, right corner.
- 3.12.6. Add the profiles to the box marked **Load Samples**. This may be done by dragging and dropping the appropriate files, or by selecting **Browse** and navigating the file tree.
 - 3.12.6.1. If a Genotypes Table file is used, ensure that all samples in the project have unique names.
 - 3.12.6.2. If necessary, a locus may be omitted in DBLR™ in instances of tri-allele, null allele, or a single autosomal locus with only one allele detected below the Analytical Threshold (i.e., 950 RFU). Alternatively, when multiple loci demonstrate one allele below the AT a STRmix™ deconvolution should be performed.
 - 3.12.6.3. When loading samples, select the pencil icon next to **Ignored loci** for the appropriate sample and mark the necessary loci to be omitted.
- 3.12.7. Under **Configuration**, add the appropriate Analysis Name. At a minimum, the name shall include the case number, followed by an underscore and the DBLR run number, increasing sequentially (e.g., 23L1234_DBLR1, 23L1234_DBLR2). If additional characters are added to the "Analysis Name" designation, those characters shall be added after the specified naming convention.

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- 3.12.8.** Under **Parameters**, Verify the Population Stratification is set to “No stratification” and choose the appropriate Allele Frequencies file. Verify that “Model mutations” is unchecked unless a mutation is suspected. Verify that “Model Fst” is checked and set to 0.01. Verify that “Model linkage” is checked and the appropriate Linkage map is selected.
- 3.12.9.** Select **Confirm Inputs** once all profiles are loaded and settings are verified.
- 3.12.10.** Link all loaded profiles to the appropriate positions in H1 and H2. This may be accomplished by dragging the sample name to the appropriate position in the pedigree, or by clicking **Add Relationship** and selecting the person in the pedigree and its corresponding sample from the drop-down menus.
- 3.12.10.1. Verify that the correct Contributor Position is selected if a STRmix deconvolution is used as a sample input.
- 3.12.10.2. When F_{ST} is enabled, a warning message will show that there are unexplained contributor positions until all loaded samples are linked to the pedigree. This must be resolved before a likelihood ratio can be calculated.
- 3.12.10.3. The Locus Exclusion Precheck Warning shows either an information or warning symbol under H1 and H2 based on how the genotypes fit the pedigree. A warning symbol occurs when at least one genotype cannot occur with the chosen relationship. Clicking on the symbol opens a pop-up window to review the inputs and pedigree for troubleshooting purposes. This does not need to be resolved for a calculation to progress.
- 3.12.11.** Click **Compute Likelihood Ratios** and review the data.
- 3.12.12.** Save a copy of the Kinship Report in the casefile and the run folder on the DNA server (See [Appendix 9](#)).
- 3.12.12.1. The report will list linked loci together whenever Linkage is enabled. However, the model is only applied when needed based on the pedigree and sample profiles.

3.13. Report Writing for Relationship Comparisons Using DBLR™ General Guidelines:

- 3.13.1.** The Combined Paternity Index shall be reported in the Results/Opinions/Interpretations area of the Certificate of Analysis when applicable as detailed in the report wording section ([Appendix 7](#)).
- 3.13.1.1. No statistical calculations shall be reported for exclusions determined from greater than three inconsistencies in parentage comparisons.
- 3.13.2.** All reports associated with a Paternity/Relationship Comparison Request shall include an Appendix ([Appendix 7](#)). Each profile development statement in the report shall contain the citation for the appropriate kit utilized statement in the Appendix.
- 3.13.3.** The items used for comparison will be reported in the format listed in the report wording section ([Appendix 7](#)).
- 3.13.4.** Alternate report wording may be used depending on the type of relationship and calculation performed.
- 3.13.5.** Terminology of Parentage, Paternity, Maternity, Relationship, Kinship, and Sibling Relationship (Sibship) may be used interchangeably where applicable.
- 3.13.6.** The Combined Paternity Index shall be reported to two significant figures.
- 3.13.6.1. Combined Relationship/Paternity Index values supporting the relationship (H1) shall be rounded down.

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3.13.6.2. Combined Relationship/Paternity Index values supporting exclusion (H2) shall be inverted (1/CRI) and rounded up.

3.13.6.3. Combined Relationship/Paternity Index values less than 2 shall be rounded down to 1.0 (uninformative).

3.13.7. Wording of Relationship Comparisons for Results/Opinions/Interpretations are located in [Appendix 7](#).

3.14. Support for Investigative Genetic Genealogy

3.14.1. Y-STR testing may be utilized to aid in paternal lineage identification.

3.14.2. At a minimum, 12 Y-STR loci are required to draw a conclusion on paternal lineage.

3.14.3. Interpretations will be based on the number of loci evaluated and mismatches observed.

3.14.3.1. When at least 20 Y-STR loci are available for comparison the following interpretation guidelines shall apply:

- 0-1 mismatches: support the donors of the Y-STR profiles are related
- 2 mismatches: support the donors of the Y-STR profiles are probably related
- 3 mismatches: support the donors of the Y-STR profiles are possibly related
- 4+ mismatches: support the donors of the Y-STR profiles are not related within four generational steps

3.14.3.2. When 12-19 Y-STR loci are available for comparison the following interpretation guidelines shall apply:

- 0-1 mismatches: support the donors of the Y-STR profiles are probably related
- 2 mismatches: support the donors of the Y-STR profiles are possibly related
- 3+ mismatches: support the donors of the Y-STR profiles are not related within four generational steps

3.14.4. Interpretations of Y-STR testing for paternal lineage identification shall be issued to the interested parties via a memo as shown in [Appendix 7](#).

3.15. Definitions:

3.15.1. **Random Man Not Excluded (RMNE)** – The frequency of selecting a random man from the population that could not be excluded as the biological father.

3.15.2. **Probability of Exclusion/Power of Exclusion (PE)** – The probability of excluding a random man from the population as being the biological father; is dependent on the genotypes of the child and mother and the race of the alleged father.

3.15.3. **Parentage Index (PI)/Relationship Index (RI)** – A likelihood ratio based on two different conditional probabilities; PI/RI is calculated for each locus in a system.

3.15.4. **Combined Parentage Index (CPI)/Combined Relationship Index (CRI)** – CPI/CRI is the product of all PIs/RIs for the loci in a system; a measure of the strength of the genetic evidence.

3.15.5. **Probability of Parentage (W)** – Also known as the probability of relatedness. It is the measure of the strength of the genetic evidence and the non-genetic evidence that an individual is a biological relative as compared to an unrelated, random individual in the same population.

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- 3.15.6. Prior Probability (Pr)** – The strength of the non-genetic evidence that the alleged individual is a biological relative.

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4. Y-STR Test Methods:

- 4.1. Scope:** Y-STR analysis may be performed in situations where male DNA needs to be differentiated from female DNA. DNA analysis performed prior to Y-STR analysis shall be in accordance with the test methods outlined in the DNA Methods section of this document.
- 4.2. Precautions/Limitations:** Samples that may be suitable for Y-STR analysis should be evaluated by a member of the Y-STR team to determine the most appropriate sample(s) to analyze in a case. The following should be considered:
- 4.2.1. Cases from sexual assault/misconduct should be considered for analysis.
 - 4.2.1.1. Due to the limitations of the technology, only body swabs or body fluid stains from crimes against a person shall be considered for Y-STR analysis. In general, suspected epithelial cell samples shall not be tested from areas other than body area samples. Exceptions require supervisor approval.
 - 4.2.1.2. Special consideration shall be given for cold case sexual assaults and homicides where Forensic Investigative Genetic Genealogy or additional investigative techniques may be pursued. Supervisor notification of Y-STR analysis in these cases is required.
 - 4.2.2. Cases that have not provided meaningful autosomal results shall be submitted for Y-STR analysis considering the following:
 - 4.2.2.1. If a sample or samples from more intimate/informative areas qualify for Y-STR analysis, those samples shall be submitted.
 - 4.2.2.1.1. i.e., if the autosomal result is from a non-genital swab, all genital swabs that qualify for Y-STR analysis shall be submitted.
 - 4.2.2.2. If the LR associated with the autosomal result is in the lower moderate to limited verbal scale, samples shall be submitted for Y-STR analysis.
 - 4.2.2.3. Due to the high probability of extraneous transfer and non-probative DNA results, clothing, hand swabs and fingernail scrapings shall not be submitted for Y-STR analysis if the victim and suspect live in the same household. Exceptions require supervisor approval.
 - 4.2.2.4. In general, male-on-male sexual assaults shall not be submitted for Y-STR analysis. Exceptions require supervisor approval.
- 4.3. Related Information:**
- 4.3.1. Worksheet Manual
 - 4.3.2. [Appendix 13](#) PowerPlex® Y23 Stutter Table
- 4.4. Instruments:**
- 4.4.1. See DNA Test Methods Section 2.4
- 4.5. Reagents/Materials:**
- 4.5.1. See DNA Test Methods Section 2.5
- 4.6. Hazards/Safety:**
- 4.6.1. See DNA Test Methods Section 2.6
- 4.7. Reference Materials/Controls/Calibration Checks:**
- 4.7.1. See DNA Test Methods Section 2.7

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4.8. Procedures/Instructions:

4.8.1. See DNA Test Methods Section 2.8 for extraction procedures

- 4.8.1.1. Performing Y-STR analysis before or after autosomal STR analysis will be evaluated on a case by case basis.
- 4.8.1.2. Y-STR analysis should be performed on extracts previously used for autosomal STR testing. Some instances may require re-extraction of a sample.
- 4.8.1.3. The male quantification value using Quantifiler® Trio shall be used for amplification of the sample.

4.8.2. PowerPlex® Y23 (Applied Biosystems® 3500 Genetic Analyzer)

4.8.2.1. Introduction

- 4.8.2.1.1. Short tandem repeat (STR) markers on the Y chromosome (Y-STR) have qualities that are distinct from autosomal markers and are useful for human male identification. The Promega PowerPlex® Y23 System allows co-amplification of twenty-three loci (DYS576, DYS389 I, DYS448, DYS389 II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385 a/b, DYS456, and YGATAH4) in a single reaction. Separation and detection occur using capillary electrophoresis with overlapping loci visualized simultaneously by using PCR primers labeled with four different fluorescent tags (Fluorescein, JOE, TMR-ET, and CXR-ET).
- 4.8.2.1.2. The Applied Biosystems® 3500 Genetic Analyzer utilizes electrokinetic injection of DNA molecules into polymer-filled capillaries which separates the DNA fragments by size. The fluorescent tag labeled primers incorporated into the PowerPlex® Y23 amplification products are responsive to the frequency of the 20 mW solid-state laser. Upon excitation, the fluorophores are raised to a higher energy level. When the fluorophores return to their normal energy level, a fluorescent signal is emitted. This signal is then detected by a camera within the 3500 capillary electrophoresis instrument which converts the signal to a computer image where it is visualized in an electropherogram as a peak.
- 4.8.2.1.3. The data produced by the Applied Biosystems® 3500 Genetic Analyzer is analyzed with GeneMapper™ ID-X Software which results in peaks labeled with their allele designation. The Internal Lane Standard (WEN ILS 500) is injected with each sample and it contains 21 fragments of 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, and 500 bases in length. Each fragment is labeled with WEN dye and can be detected separately (as a fifth color) in the presence of PowerPlex® Y23 amplified material. The Internal Lane Standard determines the base pair size of the fragments in the sample and the software compares the sizes to an allelic ladder to determine the allele designation.
- 4.8.2.1.4. The PowerPlex® Y23 locus-specific and allelic ladder information is available at www.promega.com/protocols/ in the PowerPlex® Y23 System Technical Manual TMD035.
- 4.8.2.1.5. The PowerPlex® Y23 Primer Pair Mix, PowerPlex® Y23 Allelic Ladder Mix, and the WEN ILS 500 Y23 shall be protected from excessive exposure to light.
- 4.8.2.1.6. The preparation of the amplification reactions shall be performed in the PCR amplification set-up area.

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4.8.2.2. PowerPlex® Y23 Amplification Set-Up

- 4.8.2.2.1. Thaw the Amplification Grade Water, PowerPlex® Y23 5X Master Mix, and PowerPlex® Y23 10X Primer Pair Mix. Centrifuge briefly to bring contents to the bottom, then vortex to mix. Do not centrifuge after vortexing as this may cause the primers/dNTPs to be concentrated at the bottom of the tube.
- 4.8.2.2.2. Determine the number of samples to be amplified, including controls. Add 2 to 4 reactions to this number to compensate for the loss that occurs during reagent transfers.
- 4.8.2.2.3. Place one 0.2 ml PCR reaction tube for each sample into a rack and label appropriately. A 96-well plate may be used.
 - 4.8.2.2.3.1. If utilizing a 96-well plate for amplification, well locations where allelic ladder will be utilized for capillary electrophoresis shall be left empty during amplification.
- 4.8.2.2.4. 0.1 ng to 0.5 ng of template DNA is recommended. Amplification of greater than 0.5 ng of template DNA should not be used due to off-scale peak heights and may result in increased artifacts and interfere with interpretation. Targeting less than 0.5 ng of template DNA should be used only with samples whose concentration falls between 0.006 ng/μl and 0.029 ng/μl. Such samples shall be interpreted with caution. Samples with concentrations greater than 0.029 ng/μl should target 0.5 ng but shall not exceed 1.0 ng for amplification.
 - 4.8.2.2.4.1. A higher template DNA amount (ex. 1.0 ng) should be considered for samples with an elevated degradation index (ex. >3).
- 4.8.2.2.5. For samples that will amplify 5 μl of template DNA, use the current version of the **DNA Analysis Workbook** to calculate the required amount of each component of the PCR master mix. Multiply the volume per reaction by the total number of reactions to obtain the final volume.

PCR Master Mix Component	Volume per Reaction (μl)
Amplification Grade or Nuclease Free H ₂ O	12.5
PowerPlex® Y23 5X Master Mix	5
PowerPlex® Y23 10X Primer Pair Mix	2.5
Total Volume	20

- 4.8.2.2.6. Add the calculated volume of each component to a 1.5 ml tube. Mix gently.
- 4.8.2.2.7. Add 20 μl of PCR master mix to each sample tube or well.
- 4.8.2.2.8. Pipette 5 μl of sample, reagent blank, positive amplification control, or negative amplification control into the respective tube or well.
 - 4.8.2.2.8.1. For the positive amplification control, dilute the 2800M or other approved positive DNA standard supplied with the PowerPlex® Y23 kit to 0.02 ng/μl to 0.1 ng/μl and record the final concentration on the amplification

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worksheet. A positive control shall be included in each thermal cycler. The diluted positive control should be stored at 2-10°C.

4.8.2.2.8.2. Amplification grade H₂O or NF H₂O shall be used as a negative amplification control included with each thermal cycler as the last sample in the batch.

4.8.2.2.9. For samples requiring more than 5 µl of sample volume, use the current version of the **DNA Analysis Workbook** to calculate the required amount of water and template DNA for individual samples. Prepare the PCR Master Mix without water. Multiply the volume per reaction by the total number of reactions to obtain the final volume.

PCR Master Mix Component	Volume per Reaction (µl)
PowerPlex® Y23 5X Master Mix	5
PowerPlex® Y23 10X Primer Pair Mix	2.5
Total Volume	7.5

4.8.2.2.10. Add the calculated volume of each component to a 1.5 ml tube. Mix gently.

4.8.2.2.11. Add 7.5 µl of PCR master mix to each sample tube or well.

4.8.2.2.12. Add the calculated volume of Amplification Grade H₂O or NF H₂O to each sample tube or well.

4.8.2.2.13. Pipette the appropriate amount of each sample, reagent blank, positive amplification control into the respective tube or well containing master mix.

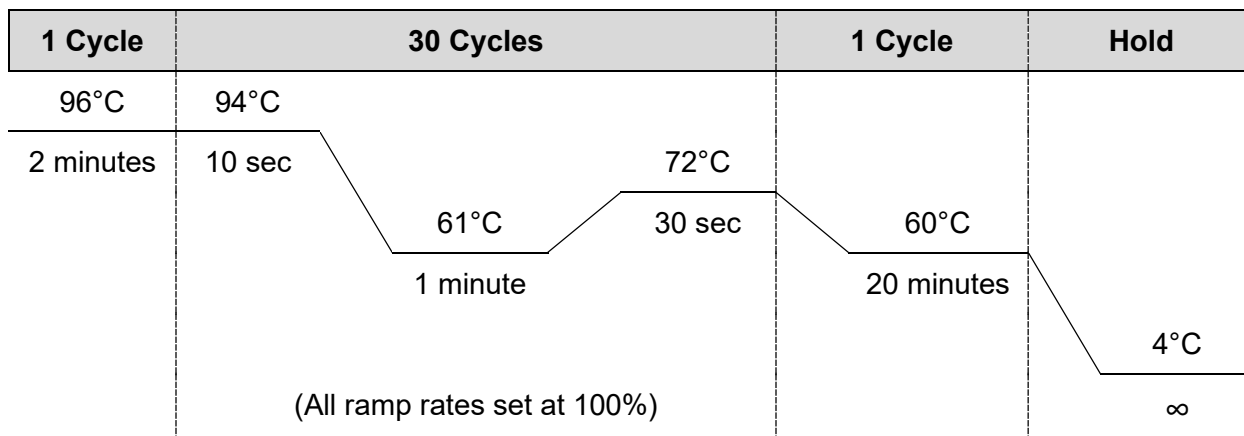
4.8.2.2.14. For samples amplified in a 96-well plate, seal the plate with adhesive foil. Press the foil well using a roller tool.

4.8.2.3. PowerPlex® Y23 Amplification

4.8.2.3.1. Place the tubes or 96-well plate in a Veriti™ thermal cycler.

4.8.2.3.2. Select and run the thermal cycling protocol below:

4.8.2.3.3. Y23 Veriti™ Thermal Cycler Protocol



4.8.2.3.3.1. Select 25 µl for the volume in the amp tubes.

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4.8.2.3.4. Remove samples after the amplification process is completed and proceed with capillary electrophoresis or store the amplified samples frozen. Alternatively, amplified samples may be stored in the refrigerator if they are to be used within 2 days.

4.8.2.3.4.1. Any remaining amplified products shall be discarded after the case is administratively reviewed.

4.8.2.4. **PowerPlex® Y23 Direct Amplification Set-Up for Databasing Lysates**

4.8.2.4.1. Thaw the Amplification Grade Water, PowerPlex® Y23 5X Master Mix, and PowerPlex® Y23 10X Primer Pair Mix. Centrifuge briefly to bring contents to the bottom, then vortex to mix. Do not centrifuge after vortexing as this may cause the primers/dNTPs to be concentrated at the bottom of the tube. Once thawed, the PowerPlex® Y23 kit may be stored in the refrigerator for up to one year or refrozen.

4.8.2.4.2. Determine the number of samples to be amplified, including controls. Add 2 to 4 reactions to this number to compensate for the loss that occurs during reagent transfers.

4.8.2.4.3. Place one 0.2 ml PCR reaction tube for each sample into a rack and label appropriately. A 96-well plate may be used.

4.8.2.4.3.1. If utilizing a 96-well plate for amplification, well locations where allelic ladder will be utilized for capillary electrophoresis shall be left empty during amplification.

4.8.2.4.4. For samples and controls, use the current version of the **DNA Analysis Workbook** to calculate the required amount of each component of the PCR master mix. Multiply the volume per reaction by the total number of reactions to obtain the final volume.

PCR Master Mix Component	Volume per Reaction (µl)
Amplification Grade or Nuclease Free H ₂ O	15.5
PowerPlex® Y23 5X Master Mix	5
PowerPlex® Y23 10X Primer Pair Mix	2.5
Total Volume	23

4.8.2.4.5. Add the calculated volume of each component to a 1.5 ml tube. Mix gently.

4.8.2.4.6. Add 23 µl of PCR master mix to each sample tube/well.

4.8.2.4.7. Pipette 2 µl of sample, reagent blank, positive amplification control, or negative amplification control into the respective tube or well containing Master Mix.

4.8.2.4.7.1. For the positive amplification control, dilute the 2800M or other approved positive DNA standard supplied with the PowerPlex® Y23 kit to 1 ng/µl to 1.5 ng/µl and record the final concentration on the amplification worksheet. A positive control shall be included in each thermal cycler.

4.8.2.4.7.2. Amplification grade H₂O or NF H₂O shall be used as a negative amplification control included with each thermal cycler as the last sample in the batch.

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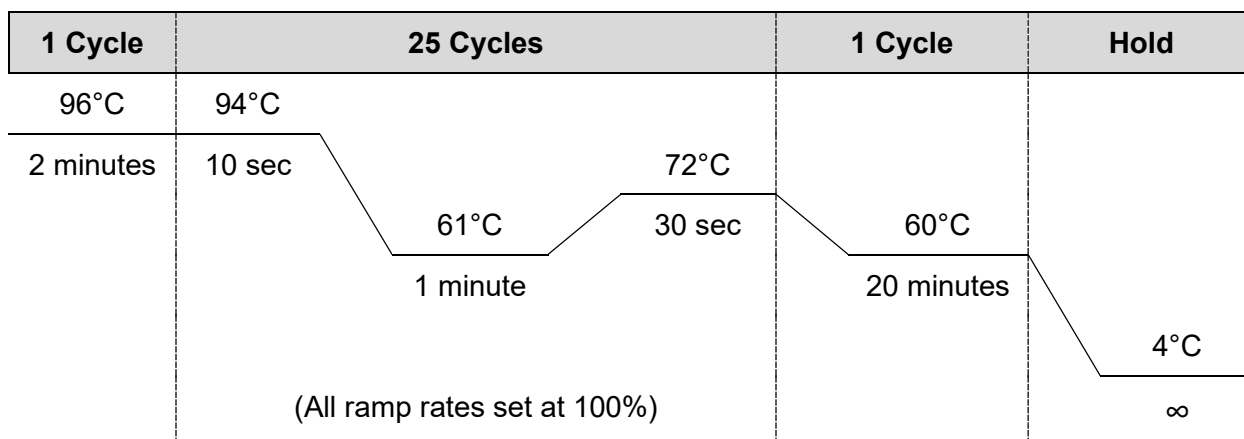
4.8.2.4.8. For samples amplified in a 96-well plate, seal the plate with adhesive foil. Press the foil well using a roller tool.

4.8.2.5. PowerPlex® Y23 Direct Amplification for Standards

4.8.2.5.1. Place the tubes or 96-well plate in a Veriti™ thermal cycler.

4.8.2.5.2. Select and run the thermal cycling protocol below:

4.8.2.5.3. Y23direct Veriti™ Thermal Cycler Protocol



4.8.2.5.3.1. Select 25 µl for the volume in the amp tubes.

4.8.2.5.4. Remove samples after the amplification process is completed and proceed with capillary electrophoresis or store the amplified samples frozen. Alternatively, amplified samples may be stored in the refrigerator if they are to be used within 2 days.

4.8.2.5.4.1. Any remaining amplified products shall be discarded after the case is administratively reviewed.

4.8.3. **Applied Biosystems® 3500 Genetic Analyzer - Data Collection Software version 2.0 – Instrument Set-up.** See [Appendix 11](#)

4.8.4. **Applied Biosystems® 3500 Genetic Analyzer - Data Collection Software version 2.0 - PowerPlex® Y23 Electrophoresis**

4.8.4.1. Sample Preparation

4.8.4.1.1. **Note:** The quality of formamide is critical for the successful detection of a DNA profile. Deionized formamide shall be used that has a conductivity of less than 100µS/cm, such as Hi-Di™ Formamide. Multiple freeze-thaw cycles or long-term storage above 0°C may cause breakdown of the formamide which can create ions that compete with DNA during injection. This will cause lower peak heights and decreased sensitivity.

4.8.4.1.2. **Caution:** Formamide is an irritant and teratogen; therefore, universal precautions and a fume hood shall be utilized when manually working with formamide to avoid inhalation and contact with the skin.

4.8.4.1.3. Thaw the ILS (WEN ILS 500 Y23), the allelic ladder, and an aliquot of Hi-Di™ Formamide. Once thawed initially, the ILS shall not be refrozen. When all components are thawed, centrifuge briefly then vortex to mix. Do not centrifuge the ILS after vortexing.

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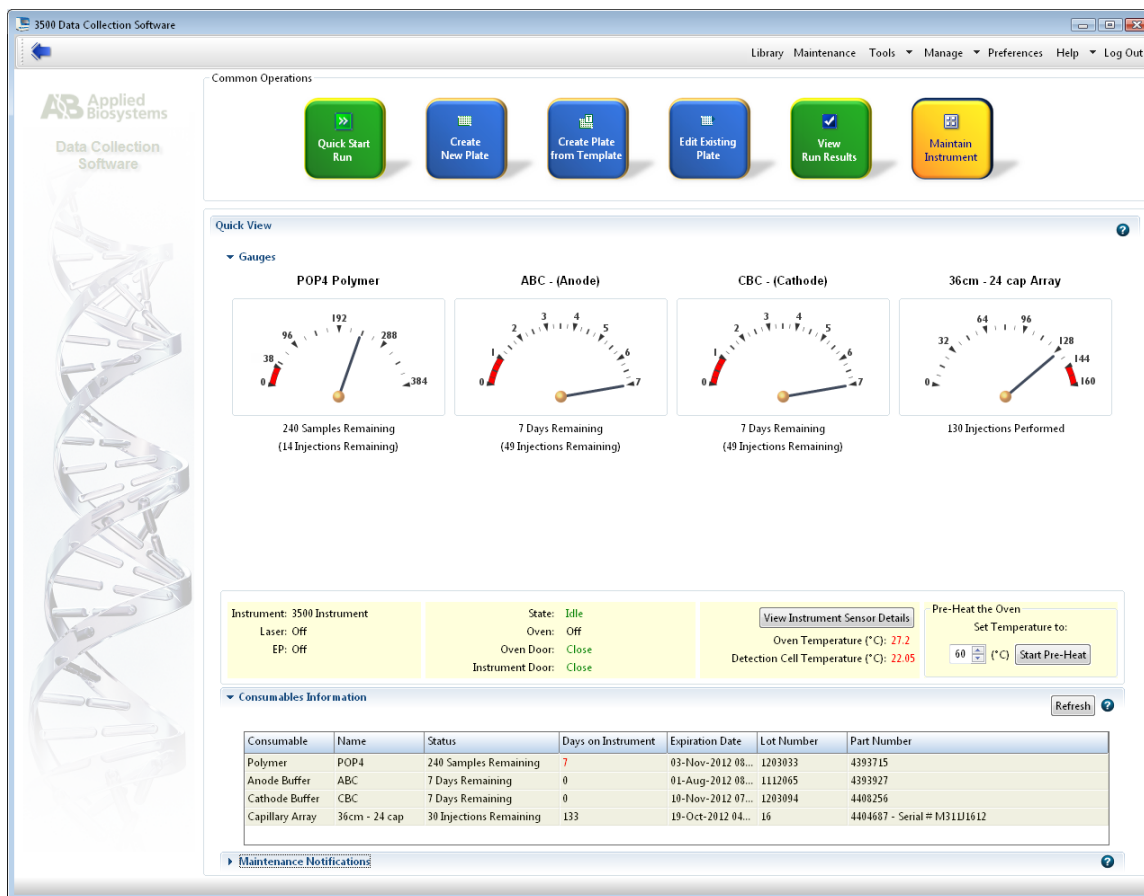
- 4.8.4.1.4. Determine the number of samples to be injected, including controls and allelic ladders. Add 2 to 4 reactions to this number to compensate for the loss that occurs during reagent transfers.
- 4.8.4.1.5. Prepare a loading cocktail by combining 0.5-1.0 µl ILS with 9.5-9.0 µl Hi-Di™ Formamide for a total volume of 10 µl.
 - 4.8.4.1.5.1. The volume of ILS used in the loading cocktail may be adjusted between 0.5 to 1.0 µl per sample to optimize size standard peaks. The amount of formamide shall be adjusted so that the total amount of loading cocktail for each well is 10 µl.
- 4.8.4.1.6. Vortex to mix.
- 4.8.4.1.7. Pipette 10 µl of the formamide/ILS mixture into each well. Add formamide or formamide/ILS mixture into empty wells to complete an injection set of eight. Every well in which an injection is occurring must contain liquid.
- 4.8.4.1.8. Add 1 µl of amplified sample or allelic ladder to the appropriate wells. It is recommended that a minimum of one allelic ladder is present with every 24 samples injected (i.e., every three injections on the 8-capillary 3500). At least one allelic ladder is required within each run folder.
 - 4.8.4.1.8.1. If the amplified sample is in a 96-well plate, seal the amplification plate with a new aluminum foil seal for storage.
- 4.8.4.1.9. Cover the wells with the plate septa and briefly spin down to remove air bubbles.
- 4.8.4.1.10. Denature the samples at 95°C for ~3 minutes, then immediately chill on crushed ice or a frozen plate block for ~3 minutes. Denature the samples just prior to loading the instrument. Avoid denaturing the samples for longer than 3 minutes as extended heat denaturing can lead to the appearance of artifacts.

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4.8.4.2. Creating a Plate Record

- 4.8.4.2.1. Open the 3500 Data Collection Software. The Dashboard screen will launch. Ensure that the **Consumable Information** and **Maintenance Notifications** are acceptable. The oven temperature should be set to 60°C.
- 4.8.4.2.2. Select **Start Pre-Heat**. This should be done at least 30 minutes prior to the first injection.

Dashboard:



- 4.8.4.2.3. Select **Create New Plate**. Alternatively, **Create Plate from Template** may be used.

- 4.8.4.2.3.1. Assign the plate name with the laboratory case number, the date and the injection number of the plate (ex. 00A1234_01Jan13_01).

4.8.4.2.3.1.1. If the plate is re-injected the "01" number shall increase sequentially (00A1234_01Jan13_02).

4.8.4.2.3.1.2. If the plate is re-injected on a different day, the original date should still be used from when the plate was prepared.

4.8.4.2.3.1.3. If a second preparation of a plate is made on the same date as the first, it shall be designated with a "-2" after the date (00A1234_01Jan13-2_01).

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4.8.4.2.4. Select “96” for Number of Wells.

4.8.4.2.5. Select “HID” for Plate Type.

4.8.4.2.6. Select “36” for Capillary Length.

4.8.4.2.7. Select “POP4” for Polymer.

Create A Plate:

The screenshot displays the '3500 Data Collection Software' window. On the left is a sidebar with a DNA double helix graphic and a 'Setup' section containing links: 'Define Plate Properties', 'Assign Plate Contents', 'Run Instrument', 'Load Plates for Run', 'Preview Run', 'Monitor Run', 'Review Results', 'View Sequencing Results', and 'View Fragment/HID Results'. The main window has a top menu bar with 'Dashboard', 'Edit', 'Library', 'Maintenance', 'Tools', 'Manage', 'Preferences', 'Help', and 'Log Out'. Below the menu is a 'Plate Name:' section with buttons: 'New Plate', 'Open Plate', 'Save Plate', 'Close Plate', and 'Start Run'. The 'Plate Details' form contains the following fields: 'Name' (text input with a validation error: 'Plate Name is a required field. Provide a unique value.'), 'Number of Wells' (text input), 'Plate Type' (dropdown menu set to 'HID'), 'Capillary Length' (dropdown menu set to '36' with 'cm' unit), 'Polymer' (dropdown menu set to 'POP4'), 'Owner' (text input), 'Barcode' (text input), and 'Description' (text area). At the bottom of the form is a 'Secondary Analysis' section with a 'Perform Auto-Analysis' checkbox. A yellow bar at the very bottom of the window contains the 'Assign Plate Contents' button.

4.8.4.2.8. Click on “Assign Plate Contents”.

4.8.4.2.9. Enter sample information or import a plate record.

4.8.4.2.9.1. The sample name shall include the sample item/subitem as well as the laboratory case number (ex. 1A1_00A1234) if more than one case is included on the plate. If only one case is present on the plate, then only the item/subitem number is required.

4.8.4.2.9.2. In order to aid in GeneMapper™ ID-X sample analysis, it is recommended to place a “z” in front of the sample name for reference standards (ex. z1A1_00A1234”) here or in GeneMapper™ ID-X under sample name.

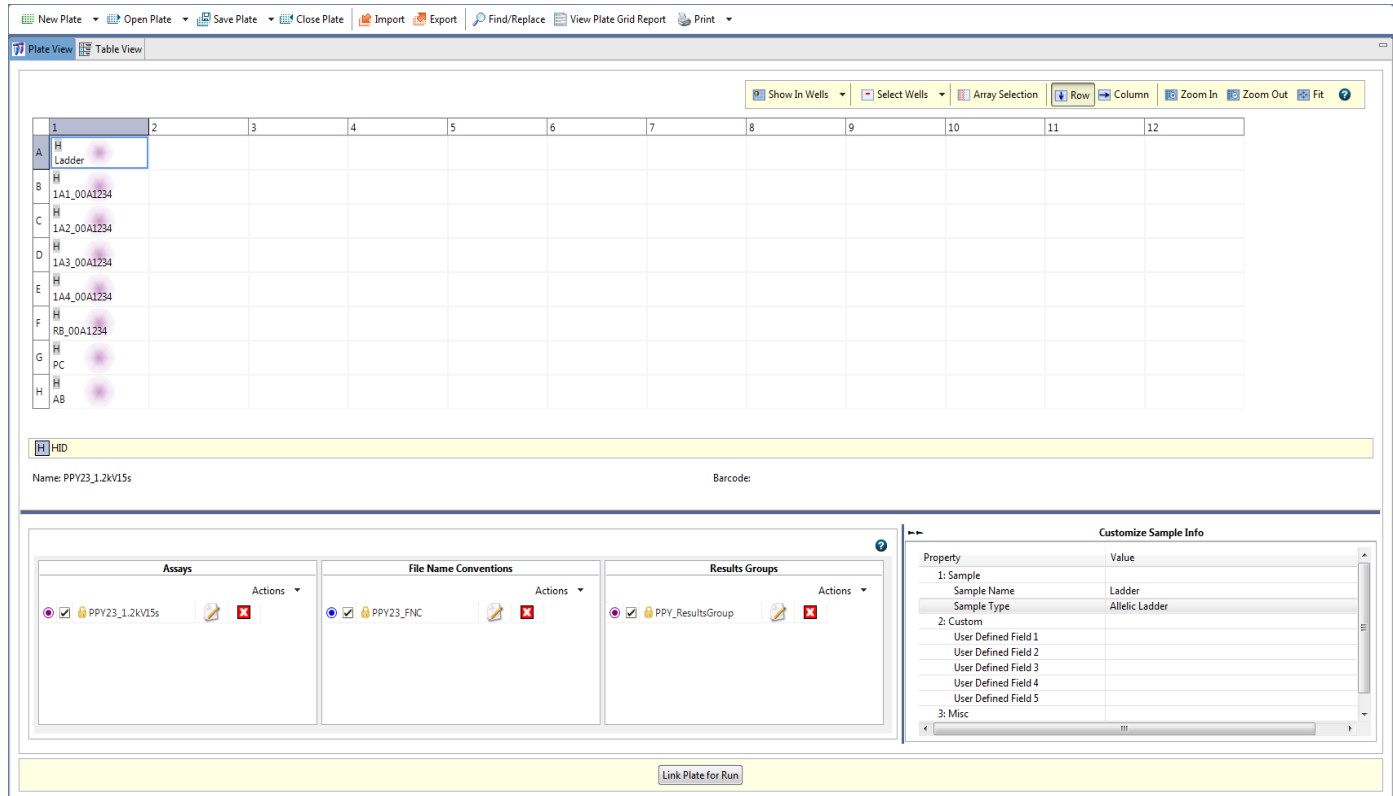
4.8.4.2.10. Under **Assays** click “Add from Library” and select “PPY23_1.2kV15s”. Click “Add to Plate” then “Close”.

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4.8.4.2.11. Under **File Name Convention**, click “Add from Library” and select “PPY23_FNC”. Click “Add to Plate” then “Close”.

4.8.4.2.12. Under **Results Groups**, click “Add from Library” and select “PPY_ResultsGroup”. Click “Add to Plate” then “Close”.

Assign Plate Contents:



4.8.4.2.13. On the bottom-right side of the screen expand the **Customize Sample Info** window so it appears as shown above.

4.8.4.2.13.1. Use the drop-down box to select a sample type for each sample (e.g., Ladder, Sample, Positive Control and Negative Control). For imported plates, this will be done automatically.

4.8.4.2.13.2. Minimize the “Customize Sample Info” window.

4.8.4.2.14. Highlight the sample wells and then select the boxes in the **Assays**, **File Name Conventions**, and **Results Groups** that pertain to those samples.

4.8.4.2.15. Place plate on instrument in position A. Select “Link Plate for Run”. Click “OK”. The instrument automatically senses the plate and puts the information in the Plate A field. Click “OK”.

4.8.4.2.16. To add a second plate, follow steps 4.8.4.2.3 through 4.8.4.2.15. Place the plate in position B. Select the “Link Plate for Run” and click “OK”. Assign the information to the Plate B field. Click “OK”.

4.8.4.3. Start a Plate Run

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- 4.8.4.3.1. A unique run name is automatically generated by the instrument for each plate.
- 4.8.4.3.2. To re-organize injections, click “Create Injection List” and use the arrows to re-order the injections.
- 4.8.4.3.3. Click “Start Run”.
- 4.8.4.3.4. After the run is complete, click “Unlink Plate” and remove it from the instrument. The plate may be discarded or labeled with the same title as the plate record and stored frozen. The plate shall be destroyed after the case has been administratively reviewed.
 - 4.8.4.3.4.1. If the CE plate requires re-injection after frozen storage, thaw and centrifuge to remove any air bubbles. Denature the plate at 95°C for 3 minutes, then immediately chill on crushed ice or frozen plate block for ~3 minutes prior to loading on the 3500 Genetic Analyzer.
- 4.8.4.3.5. The CE Plate Record Worksheet shall be maintained with each case record.
- 4.8.5. **See Casework Test Methods [Appendix 4](#) for GeneMapper™ ID-X Version 1.6 Software User Accounts and Security Setting Procedures. GeneMapper™ ID-X Version 1.6 Software - PowerPlex® Y23 Software Settings. See [Appendix 12](#)**
- 4.8.7. **GeneMapper™ ID-X Version 1.6 Software - PowerPlex® Y23 Data Analysis**
 - 4.8.7.1. **Processing Sample Data**
 - 4.8.7.1.1. Import the sample files from a single run folder by “Edit”, then selecting “Add Samples to Project”.
 - 4.8.7.1.2. In the “Add Samples to Project” screen, navigate to the run folder that contains the sample files. If the entire run folder is to be imported, click on the folder to highlight it; then click the “Add to List” button at the bottom of the window. If the run folder was shared between multiple cases, expand the folder to view the samples. Highlight the appropriate samples, ensuring that the allelic ladder and all the desired samples are selected. Once all the samples are selected click the “Add to List” button at the bottom of the window.
 - 4.8.7.1.2.1. A run folder shall not be created manually by manipulating sample files.
 - 4.8.7.1.3. Ensure that the necessary files are now located in the “Samples to Add” window by double-clicking on the folder in the right pane, then click “Add”.
 - 4.8.7.1.4. After the samples have been added to the project, first briefly scan the raw data to ensure that a bad injection did not occur. To check the raw data, first expand the project folder in the left navigation pane, then click on a sample file, then click on the “Raw Data” tab in the right GeneMapper™ ID-X window. To return to the “Samples” window, click on the project folder at the top of the left navigation pane.
 - 4.8.7.1.5. The GeneMapper™ ID-X project shall contain at least one allelic ladder from each run folder included in the project for proper genotyping. Multiple allelic ladders within a run folder will be averaged by the software to calculate the allelic bins. If a ladder injection is of low quality, delete the ladder or change the sample type from “Allelic Ladder” to “Sample” to remove it from consideration in calculating the bins.
 - 4.8.7.1.6. Ensure that the **Table Setting** at the top of the screen is set to “PPY23”.

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- 4.8.7.1.7. In the **Sample Type** column, verify that the correct sample types are displayed (e.g., Allelic Ladder, Sample, Positive Control, and Negative Control). Should a sample type need altered, use the drop-down menu to select the appropriate type.
 - 4.8.7.1.8. In the **Analysis Method** column, select the appropriate method from the drop-down menu: "PowerPlex Y23 WEN". Click the column header cell to highlight the entire column, then select "Edit", then "Fill Down" (or the shortcut Ctrl + D).
 - 4.8.7.1.9. In the **Panel** column, select the appropriate panel file: Open the "PowerPlexY23_Panels_IDX_v2.0" folder then select "PowerPlexY23_IDX_v2.0". Click the column header cell to highlight the entire column, then select "Edit", then "Fill Down" (or the shortcut Ctrl + D).
 - 4.8.7.1.10. In the **Size Standard** column, select the appropriate ILS from the drop-down menu: "WEN ILS 500". Click the column header cell to highlight the entire column, then select "Edit", then "Fill Down" (or the shortcut Ctrl + D).
 - 4.8.7.1.11. The Analysis Method, Size Standard, and Panel can be set as defaults when a GeneMapper™ ID-X project is opened. Select **File** → **Project Options**. Under the "Add Samples" tab select the above settings as the default in the drop-down menus for Analysis Method, Size Standard and Panel. Click "OK".
 - 4.8.7.1.12. Select the green **Analyze** arrow button to start the data analysis. At the Project name prompt, save the project. The project name shall contain the date the 3500 plate was prepared so that it matches the run folder name. See the DNA Server Instructions [Appendix 9](#) for additional clarification. The case number is recommended but not required. Select the "ISP Casework Security Group".
- 4.8.7.2. **Evaluating Sample Data**
- 4.8.7.2.1. The **Sizing Quality** for unknown samples and allelic ladders shall be at least 0.75 for a sample to pass. The **Sizing Quality** for reference samples, negative controls, and positive controls shall be at least 0.25 for a sample to pass. All appropriate ILS peaks shall be present and labeled correctly.
 - 4.8.7.2.2. **Allelic Ladders and Samples**
 - 4.8.7.2.2.1. Highlight all sample rows containing ladders or samples and click "View", then "Display Plots". In the Samples Plot screen, change the "Plot Setting" drop-down box to "PPY23". Magnify the area from approximately 50 bp to 500 bp ensuring that all ILS peaks are visible in the retained electropherogram.
 - 4.8.7.2.2.2. Verify the correct allele calls are made for each ladder peak and evaluate all allele calls as described in 4.8.10.
 - 4.8.7.2.2.3. Retain the electropherograms.
 - 4.8.7.2.3. **Negative Controls** (e.g., amplification blanks and reagent blanks).
 - 4.8.7.2.3.1. Highlight all sample rows containing negative controls and click "View", then "Display Plots". In the Samples Plot screen, change the "Plot Setting" drop-down box to "PPY23".
 - 4.8.7.2.3.2. Ensure that no peaks above analytical threshold are present.
 - 4.8.7.2.3.3. Retain the electropherograms ensuring that the primer peak is visible.

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- 4.8.7.2.4. After all analysis is complete, select the **Genotypes** tab. Click “File”, then select “Export Table”. Export the genotypes table as a .txt file to be utilized for the generation of allele summary tables in the Y-IndySTR v2.0 Excel program. The .txt file name should be the same as the project name with the automatic extension “Genotypes Table” added during export.
- 4.8.7.2.5. The 3500 Data Collection run folder containing the sample files, associated GeneMapper™ ID-X projects, and exported genotypes table .txt file shall be saved under each associated laboratory case number and request folder located in the analyst’s folders on the server. Projects should be deleted monthly from the “GeneMapper Manager” to maintain database space.
- 4.8.7.2.6. When exporting the GeneMapper™ ID-X project, ensure that the “Export with analysis settings” box is checked.
- 4.8.8. **General Rules for PowerPlex® Y23 Analysis On The Applied Biosystems® 3500 Genetic Analyzer**
- 4.8.8.1. It is required that at least one allelic ladder is present within a run folder. However, it is recommended that an allelic ladder is run within each set of 3 injections on the 8 capillary 3500 (one ladder per 24 capillaries). External environmental factors during a plate run can cause a shift in the migration of DNA fragments within the capillary which may cause a small difference in the base pair length determined for an allele. If an allelic ladder sample was not run within a reasonable time as the sample, this may cause an allele to be called off ladder.
- 4.8.8.2. The placement of unknown samples in the 96-well plate should be done so that the orientation allows for the injection of unknown samples prior to the injection of any reference samples for that case.
- 4.8.8.3. Peak heights of analyzed samples should not exceed 28,000 RFU. This level of peak height is approaching saturation and will lead to the appearance of artifacts. Use of data with more than two peaks >28,000 RFU may be allowed with the approval of a supervisor and notification to the Technical Leader documented in the case record.
- 4.8.8.4. If multiple amplifications are analyzed for a sample, the analyst shall use the amplification which he or she determines sufficiently represents the sample based on peak heights, artifacts, and noise levels. Analysts shall interpret data that most clearly represents the sample and try to achieve better resolution if a sample has peak heights that are too high (resulting in artifacts, increased instrument noise, and pull-up) or too low (resulting in allelic drop out and loss of data).
- 4.8.8.5. Only the data used for interpretation needs to be published in the case record. However, other runs shall be noted in the case record and all data shall be saved under each associated laboratory case number and request folder located in the analysts’ folders on the server.
- 4.8.9. **Archiving Applied Biosystems® 3500 and GeneMapper™ ID-X Projects**
- 4.8.9.1. The 3500 run folders containing all sample files for a case as well as the GeneMapper™ ID-X project file shall be saved under each associated laboratory case number and request folder located in the analysts’ folders on the server and deleted from the hard drive of the instrument and/or analysis computer. The data stored on the server shall be routinely backed up to ensure security of data.

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4.8.9.2. All electropherograms used in interpretation as well as the plate record shall be placed in the case record.

4.8.10. Interpretation Guidelines for PowerPlex® Y23

- 4.8.10.1.1. An analyst is required to visually confirm all allelic ladders used for allele designation.
- 4.8.10.1.2. The internally validated analytical threshold for data interpretation is 100 RFU for all dye channels.
 - 4.8.10.1.2.1. Peaks below 100 RFU shall not be interpreted or marked on the STR summary sheet but may be considered when determining suitability for interpretation and/or number of contributors for a sample.
- 4.8.10.1.3. The stochastic threshold for data interpretation of the DYS385 a/b locus is 225 RFU. There is no stochastic threshold for the remaining loci.
 - 4.8.10.1.3.1. Peaks less than 225 RFU at the DYS385 a/b locus shall be reported with a bracket (e.g., [13]) on the Y-STR summary sheet.
 - 4.8.10.1.3.2. Interpretation of peaks less than 225 RFU at the DYS385 a/b locus is a qualitative assessment. If the DYS385 a/b locus demonstrates a single allele that is above 100 RFU and below 225 RFU, true homozygosity cannot be determined due to possible stochastic effects.
- 4.8.10.1.4. An initial interpretation of the data shall be made to distinguish non-allelic artifact peaks (e.g., pull-up, spikes, dye blobs, etc.) from true allelic data.
 - 4.8.10.1.4.1. Artifacts have been observed and documented utilizing the PowerPlex® Y23 amplification kit. Any artifact peaks called alleles by the GeneMapper™ ID-X software shall be deleted or renamed on the electropherogram as an artifact. Many DNA-independent and DNA-dependent artifacts have been observed. A complete list of artifacts can be found in the PowerPlex® Y23 Technical Manual.
 - 4.8.10.1.4.2. Pull-up or bleed through peaks can occur if signal intensity of sample or ILS peaks is too high or if a new spectral calibration needs to be run. Any pull-up peaks called as alleles by the GeneMapper™ ID-X software shall be labeled on the electropherogram as pull-up or artifact. The sample should be re-run if a pull-up peak interferes with the analyst's ability to evaluate the profile based on their experience and training.
 - 4.8.10.1.4.3. Spikes are peaks that generally appear in all colors and are sharper than regular peaks; however, they can occur predominantly in one color. Spikes are a natural consequence of capillary electrophoresis and can be caused by dust present in the system as well as urea crystals in the system. It is essential that the instrumentation be maintained and cleaned regularly to minimize the appearance of spikes. All spikes called as alleles by the GeneMapper™ ID-X software shall be clearly labeled as spikes or artifacts on the electropherogram. A sample should be re-injected when a spike interferes with the analyst's ability to evaluate the profile based on their experience and training.
- 4.8.10.1.5. Stutter peaks are artifacts of the amplification process. The peak heights of stutter peaks will be less intense than that of the major peak. Stutter peaks will typically be observed one repeat unit shorter than the parent peak (e.g., n-4 position at a

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tetranucleotide repeat locus) but may also occur two repeat units less (e.g., n-8 at a tetranucleotide repeat locus) or one repeat unit more (e.g., n+4 at a tetranucleotide repeat locus). Additionally, n-2 and n+2 artifact peaks have been observed. Any peaks determined to be stutter that were not filtered out by the GeneMapper™ ID-X software shall be labeled as stutter or artifact on the electropherogram. Mean and mean + 3SD stutter values can be found in [Appendix 13](#).

- 4.8.10.1.6. Rare variants have been described in the literature. The causes of these rare variants are microvariants or chromosomal mutations (duplication or deletion).
- 4.8.10.1.6.1. Microvariants represent a partial repeat for a given allele. These peaks have a similar intensity to other allelic peaks but will not line up with the allelic ladder and will fall outside established bins.
 - 4.8.10.1.6.1.1. Any allele peak that is not present in the allelic ladder and does not have an associated “bin” in the GeneMapper™ ID-X analysis software is labeled “OL” (Off-Ladder) and shall be renamed by the analyst on the electropherogram.
 - 4.8.10.1.6.1.2. Alleles which are located between two alleles on the ladder shall be described as the short repeat followed by the number of base pairs it is larger (e.g., 0.1, 0.2, 0.3, 0.4, or 0.5). Therefore, if a peak is 1 base pair larger than the 5 allele it shall be designated as 5.1. A microvariant 3 base pairs larger than an allele on the ladder for a trinucleotide, 4 base pairs larger for a tetranucleotide, 5 base pairs larger for a pentanucleotide, or 6 base pairs larger for a hexanucleotide may be designated with the full repeat number (A peak 4 base pairs larger than the 5 allele could be designated a 6; 5 base pairs larger a 6.1).
 - 4.8.10.1.6.1.3. Alleles which are located outside the range of the ladder or bin set but within the designated marker range of the locus shall be evaluated to determine the appropriate repeat number that should be assigned to the allele. The allele may also be described as “<” or “>” the largest or smallest allele for that locus with a set of () placed around the off ladder allele. For example, if a peak is located above the largest allele for the DYS19 locus, it would be designated as “(>19)”.
 - 4.8.10.1.6.1.4. Alleles which are labeled “OMR” (Outside Marker Range) by the GeneMapper™ ID-X analysis software shall be evaluated to determine which locus the allele likely belongs to. If the analyst can reasonably determine which locus the allele belongs to, the allele can be assigned to that locus in GeneMapper™ ID-X and the allele call can be made. If the analyst cannot reasonably determine which locus the allele belongs to, both loci surrounding the allele shall be marked inconclusive.
- 4.8.10.1.6.2. A duplication occurs when a DNA sequence on the Y-chromosome is duplicated and results in more than one allele at a locus. Duplications have been documented with the PowerPlex® Y23 amplification kit. Instances of multiple duplications within a sample have been documented as well. Most duplications have a one repeat difference; however, two, three, and four repeat unit differences have been observed.
 - 4.8.10.1.6.2.1. If a locus other than DYS385 a/b demonstrates more than one allele, evaluate the sample to distinguish between a mixture and a duplication.

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4.8.10.1.6.2.1.1. Determine the number of loci containing more than one peak and consider the position on the Y-chromosome if multiple loci have two peaks.

4.8.10.1.6.2.1.2. Determine if the repeat spread is >1 repeat unit.

4.8.10.1.6.2.1.3. Evaluate the peak height ratio of the two peaks present at the locus.

4.8.10.1.6.2.1.4. Examine DYS385 a/b for the presence of >2 peaks.

4.8.10.1.6.2.2. If a two-peak pattern is observed for a single-source unknown sample and is believed to be a duplication, the profile should be confirmed by concordance to at least one additional sample contained within the case and/or re-amplification. Documented notification of the duplication event to the Technical Leader is required.

4.8.10.1.6.3. Null alleles can occur due to a deletion of a portion of the Y chromosome or a mutation to the primer binding site for a particular locus. The result is a lack of a peak at a single locus or multiple loci.

4.8.10.1.6.3.1. The lack of an allele at a locus that is believed to be a null allele may be distinguished from an undetected allele due to allelic drop out by evaluating the overall quality of the sample, including peak heights and degradation. The location of the missing allele may also be considered. Documented notification of the null allele to the Technical Leader is required.

4.8.10.1.6.3.1.1. A deletion has been reported at the DYS448 locus. Samples with this deletion will show two peaks (duplication) in DYS576 and a null allele in DYS448.

4.8.10.2. Evaluation of Controls

4.8.10.2.1. Failed controls require notification to the Technical Leader, documented in the case record.

4.8.10.2.2. The appearance of pull-up or known artifact peaks does not render the following controls inconclusive.

4.8.10.2.3. Reagent Blank:

4.8.10.2.3.1. The purpose of the reagent blank is to determine if the reagents used to extract the associated samples were contaminated by male DNA. Therefore, no signal should be detected in this sample well other than the internal lane standard. If a signal is detected in the reagent blank, all results of samples associated with that reagent blank shall be considered inconclusive.

4.8.10.2.3.2. A reagent blank with peaks below analytical threshold shall not prevent associated samples from being interpreted.

4.8.10.2.3.3. A reagent blank with peaks at or above the analytical threshold shall be considered a failed negative control.

4.8.10.2.4. Positive Control:

4.8.10.2.4.1. 2800M (or other male standard provided with the PowerPlex® Y23 kit) is used as a positive control to demonstrate that the kit is performing properly. A

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positive control is considered a failed positive control if expected alleles are not detected or if additional alleles are detected.

Positive Control 2800M (PowerPlex® Y23):

18	14	19	31	14	
DYS576	DYS389 I	DYS448	DYS389 II	DYS19	

10	22	13	12	9	14
DYS391	DYS481	DYS549	DYS533	DYS438	DYS437

17	21	24	12	13	10
DYS570	DYS635	DYS390	DYS439	DYS392	DYS643

13	17	13, 16	17	11	
DYS393	DYS458	DYS385 a/b	DYS456	Y-GATA-H4	

4.8.10.2.5. Amplification Blank:

- 4.8.10.2.5.1. The purpose of the amplification blank is to determine if male DNA contaminated the samples at the amplification step. Because no template DNA was placed in the reaction, the sample well should be blank except for the internal lane standard peaks.
- 4.8.10.2.5.2. An amplification blank with peaks below analytical threshold shall not prevent associated samples from being interpreted.
- 4.8.10.2.5.3. An amplification blank with peaks at or above analytical threshold shall be considered a failed negative control.

4.8.10.2.6. Interpreting, Comparing and Reporting DNA Results Associated with Failed Controls and Contamination Events

- 4.8.10.2.6.1. The Technical Leader shall be notified if a positive or negative control fails and/or if sample contamination occurs. Sample contamination is defined as additional peaks present in an unknown or reference sample that can be conclusively identified to not have originated in the sample (e.g., case to case contamination or a mixture in a reference sample).
- 4.8.10.2.6.2. Assessment of the integrity of the associated DNA results shall be done in consultation with the Technical Leader. This shall include the possible cause and effect of the failed control or contamination. Additionally, an assessment of the risk associated with moving forward with interpretation shall be documented, as well as the risks associated with retesting (e.g., unnecessary consumption of evidence).
 - 4.8.10.2.6.2.1. If retesting is performed, the reasons for doing so shall be documented.
 - 4.8.10.2.6.2.2. If results are determined to be suitable for interpretation without retesting, the appropriate interpretations, comparisons, and statistical results and a description of the failed control or

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contamination event shall be reported per the Report Writing Guidelines.

4.8.10.2.6.2.3. If results are determined to be not suitable for interpretation and retesting cannot be performed, the results shall be reported as not suitable for comparison per the Report Writing Guidelines.

4.8.10.2.6.3. Documentation in the case record shall include at a minimum: the forensic sample, reference or control test results that failed or was contaminated, the likely or known cause of the failed control or contamination, the likely or known source of the contamination, the impact on the integrity of the DNA results, and the determination if the test result is suitable or unsuitable for interpretation.

4.8.10.3. **Y-STR DNA Profile Interpretation**

4.8.10.3.1. All interpretation decisions shall be documented prior to comparison of reference profiles. If an interpretation error is discovered after comparison to a reference profile, the Technical Leader shall be consulted to determine the appropriate action. The error and Technical Leader consultation shall be documented in the case record.

4.8.10.3.2. If available, a reference profile from a male victim who is not paternally related to the person of interest may be assumed for single source and major Y-STR profiles and no statistical analysis is required.

4.8.10.3.3. The Indiana State Police Forensic Services Division Quality Assurance DNA Database is searched automatically by the Y-IndySTR v2.0 excel program. If there are any loci missing allele calls in the QA database sample in a database hit, those hits shall be investigated manually using the original data on the QA drive. The Technical Leader shall be consulted for evaluation of the match. If the match is determined to be a suspected contamination event, a root cause analysis shall be performed to determine the possible cause. Appropriate report wording shall be utilized if contamination is suspected.

4.8.10.3.3.1. QA database hits from single source profiles or major alleles in a mixture sample with fewer than 12 loci eligible for interpretation can be considered adventitious hits and require no further investigation. However, if TL permission is granted to use profile with fewer than 12 loci for comparison, then any QA database hits shall be investigated and reported to the TL.

4.8.10.3.4. There are four possible outcomes of Y-STR analysis:

4.8.10.3.4.1. No results: No peaks were detected on the electropherogram.

4.8.10.3.4.2. Inconclusive: Peaks were observed at one or more loci; however, no conclusive results can be drawn from them.

4.8.10.3.4.3. Inclusion: The profile obtained from the unknown sample had no discrepant alleles as compared to the profile of the reference standard. Allelic drop-out may occur in low concentrations or mixtures.

4.8.10.3.4.4. Exclusion: The profile obtained from the unknown sample was not the same as the reference sample.

4.8.10.3.5. **Single Source Profile Interpretation**

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4.8.10.3.5.1. Generally, a single source profile should contain no more than one allele at each locus except for DYS385 a/b, which may demonstrate up to two alleles. A profile where only one locus demonstrates two alleles could be an indication of:

4.8.10.3.5.1.1. A mixture where only one allele from a minor contributor is detected above the analytical threshold.

4.8.10.3.5.1.2. Extraneous DNA.

4.8.10.3.5.1.3. A duplication.

4.8.10.3.5.1.4. In situations 1 and 2, the sample shall be reported with the additional allele wording outlined in the Report Wording Guidelines ([Appendix 7](#)). In situation 3, no mention is required in the Certificate of Analysis.

4.8.10.3.5.2. At least twelve loci eligible for statistical calculations are required for interpretation of a single source profile. Profiles demonstrating fewer than twelve loci shall be reported as inconclusive. Interpretation of single source profiles with fewer than twelve loci shall require consultation and documented approval from the technical leader.

4.8.10.3.5.3. If DYS385 a/b demonstrates two alleles, the two peaks should have a peak height ratio of 65%. If the peak height ratio is less than 65%, evaluate the sample quality to determine if the locus should be used in statistical analysis.

4.8.10.3.5.4. If DYS385 a/b demonstrates two alleles in which one or both alleles are above 100 RFU and below 225 RFU, both alleles are interpretable and may be used for statistical analysis.

4.8.10.3.5.5. If only one allele is present at DYS385 a/b and is below 225 RFU, the locus shall not be used for statistical analysis.

4.8.10.3.6. **Mixture Interpretation**

4.8.10.3.6.1. A profile is defined as a mixture between two or more males when two or more loci demonstrate two or more alleles, with the exception of two alleles at DYS385 a/b or a duplication event.

4.8.10.3.6.2. If the profile is determined to be a mixture, each locus should be evaluated and classified as below:

4.8.10.3.6.2.1. Indistinguishable mixture between two or more males

4.8.10.3.6.2.2. Mixture demonstrating a major contributor.

4.8.10.3.6.2.3. Demonstrates the potential for allelic drop-out.

4.8.10.3.6.3. For mixtures of two male individuals, a major single source profile shall be calculated based on the following criteria:

4.8.10.3.6.3.1. If the peak height ratio between the peaks is less than 20%, then the highest peak can be called a major at that locus.

4.8.10.3.6.3.2. If there is only one peak at a particular locus (and a major can be determined at one or more loci with more than one peak), this peak can be called a major. All loci must be considered in making this determination.

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- 4.8.10.3.6.3.3. A mixture threshold of 500 RFU shall be used to interpret single peaks and major peaks at a locus. Peaks below 500 RFU shall not be used for interpretation of the mixture.
- 4.8.10.3.6.3.4. When evaluating DYS385 a/b, a major consisting of two peaks should have a peak height ratio of 65% and the tallest of the minor peak shall have a peak height ratio of less than 20% of the shortest major peak.
- 4.8.10.3.6.3.5. If the sample does not meet these criteria at 12 or more loci, a major profile should not be determined for the sample. Determination of a major profile with fewer than 12 loci requires documented TL approval.
- 4.8.10.3.6.4. Mixtures of three males which meet the above criteria should be interpreted with caution.
- 4.8.10.3.6.5. Mixtures of four or more males shall be evaluated based on the following criteria. These samples should be interpreted with caution and only with approval of the Technical Leader.
 - 4.8.10.3.6.5.1. If the peak height ratio between the highest peak and tallest minor peak is less than 15%, the highest peak can be called a major at that locus.
 - 4.8.10.3.6.5.2. A mixture threshold of 1000 RFU shall be used to interpret single peaks and major peaks at a locus.
 - 4.8.10.3.6.5.3. When evaluating DYS385 a/b, a major consisting of two peaks should have a peak height ratio of 65% and the tallest of the minor peaks shall have a peak height ratio of less than 15% of the shortest major peak.
 - 4.8.10.3.6.5.4. If the sample does not meet these criteria at 12 or more loci, a major profile should not be determined for the sample. Determination of a major profile with fewer than 12 loci requires documented TL approval.
- 4.8.10.3.6.6. If a major profile cannot be determined in a mixture of two or more males, the mixture is considered indistinguishable. In this case, since no statistical interpretations can be made, the sample shall be reported as inconclusive.
- 4.8.10.4. **Y-STR Statistical Analysis and Database Searching**
 - 4.8.10.4.1. A Y-STR profile is treated as a single locus because it is a haplotype.
 - 4.8.10.4.2. Once a comparison between a reference sample(s) and an unknown sample is completed and an inclusion for a single source or major Y-STR profile is reported, the significance of that inclusion shall be estimated. It shall be reported that all paternally related males will share the same Y-STR profile.
 - 4.8.10.4.3. The unknown haplotype developed for the evidence profile shall be searched against the Y-Chromosome Haplotype Reference Database (YHRD), found online at <http://yhrd.org>.
 - 4.8.10.4.4. The unknown haplotype shall be searched by selecting the PowerPlex® Y23 Kit and the Y17 Dataset. Loci from the PowerPlex® Y23 kit include DYS576,

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DYS389 I, DYS448, DYS389 II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385 a/b, DYS456, and Y-GATA-H4.

4.8.10.4.4.1. If the Y-STR profile contains a locus with a duplication, the duplication can be entered in the format of "X, Y".

4.8.10.4.4.2. If the Y-STR profile contains a locus with a null allele, a "0" will be entered.

4.8.10.4.5. The counting method with the application of a 95% upper confidence interval using the Clopper and Pearson method along with a theta-corrected match probability are used for statistical calculations.

4.8.10.4.5.1. The feature National Database (with Subpopulations, 2014 SWGDAM-compliant) shall be added to the statistical report after the Y-STR profile has been searched.

4.8.10.4.6. A statistical result from YHRD shall be generated for every unique Y-STR haplotype/profile searched. The applicable sample ID(s) shall be clearly documented on the sheet.

4.8.10.4.7. A likelihood ratio that incorporates the Theta-corrected Match Probability (without Native American) shall be included in the Certificate of Analysis. The likelihood ratio is the inverse of the match probability. A verbal scale shall accompany the likelihood ratio result to provide additional explanation of the weight of the evidence.

<u>Likelihood Ratio</u>	<u>Support for Inclusion</u>
1	Uninformative
$2 \leq LR < 100$	Provides limited support
$100 \leq LR < 10,000$	Provides moderate support
$10,000 \leq LR < 1,000,000$	Provides strong support
$1,000,000 \leq LR$	Provides very strong support

4.8.10.4.8. The Certificate of Analysis shall acknowledge the date of the search and the release number of YHRD at the time of the search.

4.8.10.4.8.1. Statistics from a previous database search will not be recalculated. Exceptions require permission from the Technical Leader.

4.9. Records:

- 4.9.1. The appropriate worksheets or the equivalent workbooks shall be used to record all procedures.
- 4.9.2. All data sheets, notes and other information generated from the laboratory examination shall be kept in the case record.
- 4.9.3. The technical review of the case record shall be recorded on the technical review worksheet.
- 4.9.4. Electronic records shall be retained as indicated in [Appendix 9](#).

4.10. Interpretation of Results:

- 4.10.1. Interpretation guidelines are located with **Procedures Section 4.8**.

4.11. Reporting of Y-STR Analysis:

- 4.11.1. See [Appendix 7](#) for formatting and Y-STR reporting for specific examples

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4.12. References:

- 4.12.1. Promega Corporation, (2014 or most current version). PowerPlex® Y23 System Technical Manual. Part No. TMD035. Madison, WI.
- 4.12.2. Promega Corporation. PowerPlex® 5C Matrix Standard Technical Manual. Part No. DG4850. 10/23 or most current issue.
- 4.12.3. Butler, J.M. 2011. Y Chromosome DNA Testing. Advanced Topics in Forensic DNA Typing: Methodology. pp 371-403. Elsevier Academic Press. Burlington, MA.
- 4.12.4. Butler, J.M. 2010. Lineage Markers: Y Chromosome and mtDNA testing. Fundamentals of Forensic DNA Typing. pp 363-374. Academic Press. Burlington, MA.
- 4.12.5. Butler, J.M. 2005. Y Chromosome DNA Testing. Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers. Second Edition. pp 201-239. Elsevier Academic Press. Burlington, MA.
- 4.12.6. SWGDAM (2022). SWGDAM Interpretation Guidelines for Y-Chromosome STR Testing. Available at <http://www.swgdam.org/publications> SWGDAM (2022). Supplemental Information for the SWGDAM Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Laboratories. Available at <http://www.swgdam.org/publications>
- 4.12.7. Ballantyne, J. and Fatolitis, L. The U.S. Y-STR database. Profiles in DNA. 2008. 11(1): 13 – 14.
- 4.12.8. Y-Chromosome Haplotype Reference Database (YHRD). <http://yhrd.org>

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5. DNA Sample Processing using the Biomek i5 Automated Workstations:

5.1. Scope: This test method is designed for the direction of Biology Section personnel who will operate the and Biomek i5 automation workstations to process DNA samples from extraction/purification through CE (capillary electrophoresis) setup. The Biomek i5 workstation is capable of extracting up to 87 samples and blanks in two hours, setting up quantification plates, normalizing samples and setting up amplification. This test method may be expanded or altered as techniques and/or new genetic analyses are found applicable and validated.

5.2. Precautions/Limitations:

5.2.1. Due to the hands-off nature of automated processing, it is vital that submitted samples be prepared following procedures determined by the automation validation and that the ordered flow of data throughout the process be maintained.

5.2.2. Customization or modification of sample processing procedures will not be allowed without consultation with the automation team and documented approval of the Technical Leader.

5.3. Related Information:

5.3.1. N/A

5.4. Instruments:

5.4.1. Biomek i5 Automated Laboratory Workstation— robotic DNA processing system.

5.5. Reagents/Materials:

- 5.5.1.** Digest/Wash Buffer
- 5.5.2.** Isopropyl Alcohol
- 5.5.3.** Nuclease Free Water (NFW₂O)
- 5.5.4.** DTT 1M
- 5.5.5.** Proteinase K (10 mg/ml)
- 5.5.6.** Proteinase K (18 mg/ml)
- 5.5.7.** Ethanol
- 5.5.8.** Sarkosyl 20% w/v
- 5.5.9.** Stain Extraction Buffer for Automation
- 5.5.10.** TRIS/EDTA/NaCl Solution
- 5.5.11.** DNA IQ™ Extraction Kit
 - 5.5.11.1.** Lysis Buffer
 - 5.5.11.2.** Elution Buffer
 - 5.5.11.3.** Magnetic Resin
 - 5.5.11.4.** Wash Buffer

5.6. Hazards/Safety:

5.6.1. All chemicals shall be handled in a safe method as referenced in the specific Safety Data Sheets (SDS).

5.6.2. Universal Precautions shall be observed whenever biological materials are being handled.

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5.6.3. Biological waste shall be disposed of in the appropriate waste receptacle.

5.6.4. On the Biomek i5, a light curtain prevents interaction with the deck or the robotic arm while a procedure is running.

5.7. Reference Materials/Calibration Checks:

5.7.1. DNA processing using the automated workstation does not require any additional controls to those used in manual DNA processing.

5.7.2. Annual calibration of the pipetting accuracy will be performed by Beckman Coulter Operational Qualification 3 (OQ3).

5.8. Procedures/Instructions:

5.8.1. General Rules

5.8.1.1. Batch ID numbers shall be named sequentially “year-run number” (ex. 2011-001).

5.8.1.2. Each case shall contain at least one reagent blank for unknowns when processed on the automated workstation.

5.8.1.3. Each group of reference samples (per analyst) processed on the automated workstation shall contain at least one reagent blank. On the pre-extraction worksheet (submission form), the case number for the grouped reagent blank shall be the first case number in the batch it is associated with.

5.8.1.4. A submitting analyst may request that an assumed reference standard (for any type of case) be included within the same extraction batch as the associated unknowns as long as:

5.8.1.4.1. All pre-robot processing steps were performed separately (separated by time and/or space); AND

5.8.1.4.2. There is no additional evidence within the batch where the reference cannot be assumed. (For example: If sexual assault kit samples are submitted to the robot along with a penile swab from the suspect, the assumed victim standard cannot be included on that robot batch plate.)

5.8.1.5. Reagent blanks shall contain “RB” in the name on the pre-extraction worksheet.

5.8.1.6. At the Indianapolis Lab: Within 24 hours of incubation, analysts shall deliver samples to be processed to the automation room. An e-mail shall be sent to the automation team at DAutomation@ISP.IN.gov with the completed pre-extraction worksheet attached. The worksheet filename shall include the analyst’s initials, PE number, and the date the samples were submitted (ex. AB1234_01Jan11). The tube rack containing the samples shall be labeled to include the same information. See Section 5.9 for more information regarding retention of records for chain of custody.

5.8.1.6.1. Samples from Evansville, Fort Wayne, and Lowell shall be delivered to their designated transfer location within 72 hours of incubation. Tubes shall be placed in a rack with a lid, and the lid shall be secured with tape. The covered rack shall be placed in a convenience package and labeled

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with the worksheet filename. An email with the completed pre-extraction worksheet attached shall be sent on the day of incubation to DAutomation@ISP.IN.gov.

5.8.1.7. To maximize efficiency of each run, the automation team may process samples up to 14 days from their submission date.

5.8.1.8. In addition to the documentation of data as described in the Forensic Biology Section Test Method (DNA Test Methods, section 2), the associated automation documents to be retained in the case record are: emails documenting submission of samples and batch completion, the pre-extraction worksheet, the extraction/quantification plate record, the Samples Not Amplified summary and/or the Normalization/Amplification summary sheet as appropriate, and the amplification/capillary electrophoresis plate record if case samples are amplified.

5.8.2. Sample Triage with Automated Sample Processing

5.8.2.1. General Triage Rules

- 5.8.2.1.1. The Automation Workbook will triage samples based on their quantification data, their letter code(s) as assigned on the “Samples” tab (initially provided by the analyst on the Pre-Extraction Worksheet), and their context within a case as defined by their case number.
 - 5.8.2.1.1.1. If a single case contains multiple sexual assault evidence collection kits that each require their own individual triage, each kit’s samples should be associated to the case number written a unique way. There are four ways to write a case number that the triage will consider unique, with/without a dash and with/without leading zeroes (e.g., 26L1234, 26L-1234, 26L01234, 26L-01234).
 - 5.8.2.1.2. No samples containing less than 0.01 ng/uL small autosomal DNA will be amplified, regardless of sample code or “locked” status.
 - 5.8.2.1.3. Whenever comparisons between code M samples are made (Differential Triage or Priority System Triage), the “better” or “winning” sample is defined as the one with more actual male DNA input into the amplification reaction.
 - 5.8.2.1.3.1. This calculation prioritizes maximum male DNA input rather than only the F:M ratio or only the Y quantification value.
 - 5.8.2.1.3.2. The calculation takes into account the normalization of the small autosomal DNA amount (i.e., normalizing a high autosomal value requires dilution, which also lowers the amount of total male DNA input).
 - 5.8.2.1.3.3. *Example: SampleA has 0.018 ng/uL of autosomal DNA and 0.008 ng/uL of male DNA, for an F:M ratio of 1.25 and 0.12 ng of total male input in a 15 uL reaction. SampleB has 0.072 ng/uL of autosomal DNA and 0.02 ng/uL of male DNA, for an F:M ratio of 2.6 and 0.3 ng of total male input in a 15 uL reaction. SampleB “wins” despite having a higher F:M ratio than sample A.*

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- 5.8.2.1.3.4. Case numbers, item numbers, and sample codes will be read as-is. Typos in any of these may result in extra samples being amplified beyond the triage process.

5.8.2.2. Sample Codes

- 5.8.2.2.1. Sample codes are specific letters or combinations of letters entered by the analyst in the "Code" column of the Pre-Extraction Worksheet. These codes determine certain properties of the sample and how it will be processed on the Automation platform. Code letters may be entered in any order and are case-insensitive.
- 5.8.2.2.2. **Code T (Tube):** Code T merely indicates that the extract will be returned to a tube after processing; it does not affect any other analysis. It can be combined with other codes.
- 5.8.2.2.3. **Code S (Standard):** Code S indicates that the sample is a standard, or a reagent blank associated with a standard. Standards are "freed" from their case numbers; they do not undergo case-level triage and are assigned to extraction sets differently. If a sample is marked S, an M or L code will not affect it.
- 5.8.2.2.3.1. This allows one reagent blank to be associated with standards from multiple cases.
- 5.8.2.2.4. **Code M (Male DNA):** Code M is the equivalent of "stop if low male," used formerly. It indicates that the sample must be Priority 3 or greater to amplify. The sample will also undergo case-level triage, which includes the Priority system ratings, comparison between differential fractions (Differential Triage), and selection of the five best samples to amplify based on total male DNA input (Priority System Triage). The function of code M is not affected by code T, but code S will cancel the functionality of code M.
- 5.8.2.2.4.1. Code M samples will not be amplified if the F:M ratio is greater than 50, regardless of whether they are locked.
- 5.8.2.2.5. **Code L (Locked):** Code L is combined with code M to "lock" a sample for amplification. It is a way of bypassing the case-level triage while still requiring sufficient male DNA for amplification (i.e., Priority 3 or greater, and sufficient autosomal DNA). Code L does not affect any other code except M.
- 5.8.2.2.5.1. Locked samples will not affect the triage of other samples within the same case. In other words, locking samples that turn out to be poorer quality will *not* prevent better quality, "unlocked" samples from being amplified. Locked samples will be amplified in addition to better quality unlocked samples.

5.8.2.3. Differential Triage

- 5.8.2.3.1. This section only applies to code M samples designated as sperm or non-sperm fractions as described below.

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- 5.8.2.3.1.1. Non-sperm fraction samples are designated by the presence of “ns” in the item name. Note that this includes either “ns” or “nsf” (e.g., “1C1_nsf” or “1C1_ns”).
- 5.8.2.3.1.2. Sperm fraction samples are designated by the presence of “sf” or “sp” and not “ns” in the item name (e.g., “1C1-sf” or “1F2sf_2”).
- 5.8.2.3.2. A pair of samples with *identical* case numbers and item numbers, except for the “ns/sf” designations, will be detected as a differential set.
 - 5.8.2.3.2.1. *Example: “99F9999_1Cnsf” and “99F9999_1Csf” will be detected as a differential set.*
 - 5.8.2.3.2.2. *Example: “99F9999_1C-ns” and “99F9999_1Csf” will not be detected as a differential set because of the dash in the non-sperm sample name.*
- 5.8.2.3.3. If a differential set is detected, the two fractions will be compared to each other and only the better fraction (see General Triage Rules, above) will proceed to Priority System Triage.
 - 5.8.2.3.3.1. The “losing” fraction will not be amplified unless it is locked.

5.8.2.4. Priority System Triage

- 5.8.2.4.1. This section only applies to code M samples.
- 5.8.2.4.2. Non-differential samples and the “winning” fractions of differential sets (see Differential Triage section) are sorted by their F:M ratio into Priority 1, 2, or 3 categories (P1, P2, or P3).
- 5.8.2.4.3. The best five samples within a case will be amplified.
 - 5.8.2.4.3.1. These samples are chosen by Priority level (P1>P2>P3) and then by total male DNA amplified (see General Triage Rules, above).
 - 5.8.2.4.3.2. *Example: A case contains seven samples marked code M. Of these, one is P1, five are P2, and one is P3. The P1 sample is amplified, and the P2 samples are compared to find the best four. The remaining P2 sample and the P3 sample are not amplified.*
- 5.8.2.4.4. After the best five samples are chosen, any remaining locked (code L) samples will also be amplified if eligible.

5.8.3. Sample Preparation

5.8.3.1. Preparation of Samples for Blood, Hair, Saliva, Epithelial Cell Samples And Blood Or Buccal Swab Standards

- 5.8.3.1.1. For each sample, combine 350 µl Stain Extraction Buffer for Automation (SEBA) with 10 µl Pro K (18 mg/ml) and 40 µl DTT (1M). *Example: for 16 samples, combine 5,600 µl Incubation Buffer (or SEBA) with 160 µl Pro K and 640 µl DTT.*

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- 5.8.3.1.2. Place sample at the bottom of a Qiagen® Investigator® Lyse&Spin basket with 2 ml collection tube. Do not use “dolphin-style” or other microcentrifuge tubes. Each group of samples being extracted shall include a reagent blank as the last sample in the batch.
- 5.8.3.1.3. To the sample add 400 µl Buffer/Pro K/DTT Master Mix. Vortex and spin briefly in a microcentrifuge or tap the tube to force the cutting into the extraction fluid.
 - 5.8.3.1.3.1. Do NOT add more than 400 µl of liquid per sample.
- 5.8.3.1.4. Incubate the sample at 56°C for at least one hour. Alternatively, samples may be incubated overnight.
- 5.8.3.1.5. Spin in a microcentrifuge at 10,000 – 14,000 rcf for at least 1 minute. Remove and discard the basket insert.
- 5.8.3.1.6. Close the lid of the sample tube and cut the hinge.
- 5.8.3.1.7. Save the extract until ready for automated DNA extraction. Do not refrigerate or freeze sample. Prior to submission to the automation team, processed samples may be left at room temperature (22-25°C) up to 24 hours, if necessary.

5.8.3.2. Preparation of Samples for Differential Extraction

- 5.8.3.2.1. For each sample, combine 240 µl Tris/EDTA/NaCl, 6 µl 20% Sarkosyl, 54 µl NFH₂O and 3 µl Proteinase K (10 mg/ml). *Example: for 16 samples, combine 3840 µl Tris/EDTA/NaCl, 96 µl 20% Sarkosyl, 864 µl NFH₂O, and 48 µl Pro K.*
- 5.8.3.2.2. Place the sample in 1.5 ml microcentrifuge tube or 2 ml Qiagen® Investigator® Lyse&Spin collection tube. Qiagen® Investigator® Lyse&Spin baskets shall not be used for differential extractions. Each group of samples being extracted shall include reagent blanks (sperm and non-sperm fraction) as the last samples in the batch.
 - 5.8.3.2.2.1. For extraction on the Biomek i5 workstation, samples shall be loaded specifically into Qiagen® Investigator® Lyse&Spin 2 ml collection tubes. Do not use “dolphin-style” or other microcentrifuge tubes. If the differential steps are performed with an alternate 1.5 ml tube the sample extract shall be transferred to a Lyse&Spin 2 ml collection tube prior to submission.
 - 5.8.3.2.2.2. A differential set shall be named as “sp” or “sf” for sperm fractions and “ns” or “nsf” for non-sperm fractions.
- 5.8.3.2.3. To the sample add 300 µl of the Master Mix. Vortex and spin briefly in a microcentrifuge or tap the tube to force the cutting into the extraction fluid.
- 5.8.3.2.4. Incubate the sample at 37°C for 2 hours.
- 5.8.3.2.5. Spin briefly in a microcentrifuge to force the condensate into the bottom of the tube.

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- 5.8.3.2.6. Using a wooden applicator stick, remove the cutting and spin in a microcentrifuge for about 5 minutes at approximately 14,000 rcf.
- 5.8.3.2.7. Alternately transfer the cutting into a spin basket insert. Place the basket insert into the tube containing the stain extract. Spin in a microcentrifuge at approximately 14,000 rcf for about 5 minutes. Remove and discard the basket insert.
- 5.8.3.2.8. While being very careful to not disturb any pelleted material, remove the supernatant to a clean, labeled Qiagen® Investigator® Lyse&Spin 2 ml collection tube. This is the non-sperm fraction. Analysis of the non-sperm fraction resumes at 5.8.2.2.11. The pellet remaining in the tube is the sperm fraction.
- 5.8.3.2.9. Wash the sperm fraction by resuspending it in 500 µl digest/wash buffer, vortexing the suspension briefly, and spinning the tube in a microcentrifuge at approximately 14,000 rcf for about 5 minutes. Remove and discard the supernatant fluid, being careful not to disturb the cell pellet.
- 5.8.3.2.10. Repeat step 5.8.3.2.9 two times for a total of three washes.
- 5.8.3.2.11. Lysis buffer step:
 - 5.8.3.2.11.1. For each sample pair (sperm and non-sperm fraction) combine 600 µl of Lysis Buffer with 6 µl of 1M DTT.
 - 5.8.3.2.11.2. Add 400 µl of the Lysis Buffer master mix to each sperm fraction sample and 200 µl to each non-sperm fraction sample.
- 5.8.3.2.12. Vortex sample and spin down briefly. Cut the hinge of the sample tube.
- 5.8.3.2.13. Save until ready for automated DNA extraction. Do not refrigerate or freeze sample. Prior to submission to the automation team, processed samples may be left at room temperature (22–25°C) overnight, if necessary.

5.8.4. Biomek i5 Preparation

- 5.8.4.1. Wipe down the deck with 70% ethanol, 3-6% hydrogen peroxide (with 70% ethanol rinse) or 10% bleach.
- 5.8.4.2. Ensure that the workstation trash is not full.
- 5.8.4.3. Ensure that the waste carboy is not full.
- 5.8.4.4. Ensure that there is enough distilled water in the intake carboy (the valve must be underwater).
- 5.8.4.5. Ensure that the software is connected to the instrument and not in Simulate mode:
 - 5.8.4.5.1. “Utilities” tab → “Hardware Setup”.
 - 5.8.4.5.2. Set “Name” to “Biomek”.

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5.8.4.5.3. Click “Accept”.

5.8.4.6. Run “Home All Axes” under the “Method” tab.

5.8.4.6.1. Let the tips flush until there are no bubbles created when the pistons move.

5.8.4.6.2. There may be a large bubble stationary at the tops of the syringes; this is normal.

5.8.4.7. Ensure that ethanol and isopropyl alcohol have been added to the alcohol wash buffer in the DNA IQ™ kit (35 mL of each).

5.8.5. Tube to Plate Transfer

5.8.5.1. Place sample tubes in the tube racks (4x6 with white adapters), arranging the tubes according to the order shown in the Automation Workbook. Do not skip columns or spaces between samples.

5.8.5.2. Ensure that the sample tube lid hinges have been cut.

5.8.5.3. Spin down the tube racks (containing the sample tubes) in the centrifuge. Carefully remove the sample tube lids and discard.

5.8.5.3.1. CAUTION: Once the lids are removed, the Automation Workbook is the only record of the order and identity of each sample.

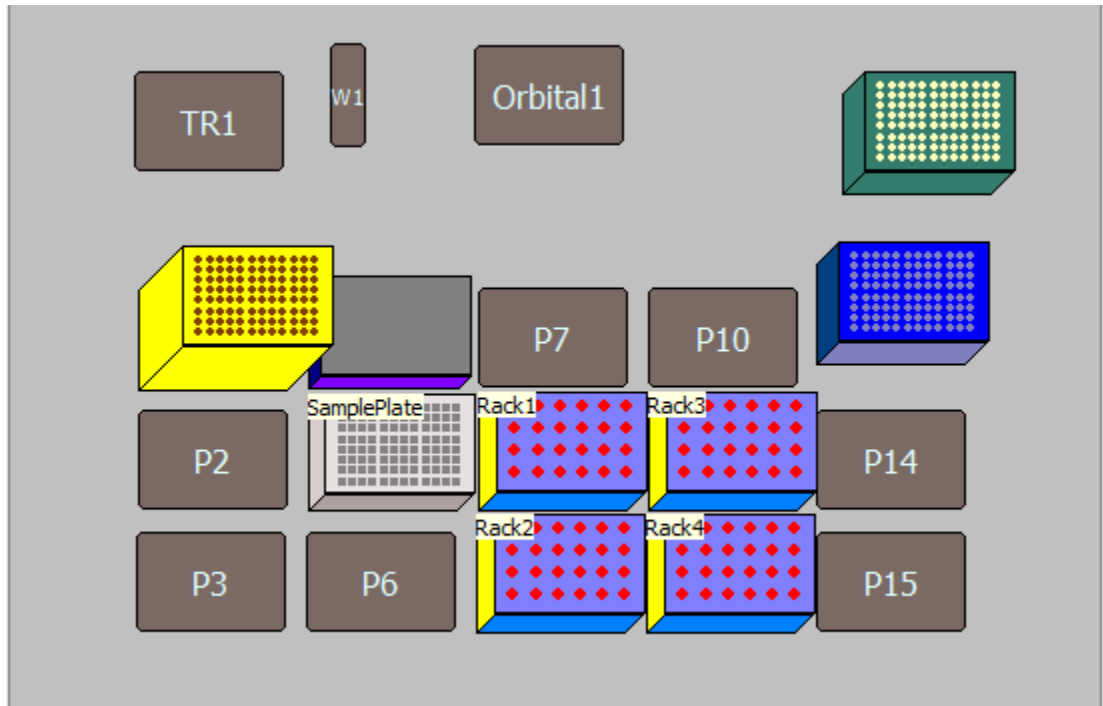
5.8.5.4. Arrange the deck layout as shown in the figure below:

5.8.5.4.1. Place sample tube racks on positions P8, P9, P11, and P12, again paying attention to sample order according to the Automation Workbook.

5.8.5.4.2. Place a new Promega (Madison, WI) 2.2 ml Square-Well Deep Well Plate (Cat. no. V6781) on position P5. Orient the plate so that the clipped corner is on the bottom left.

5.8.5.4.3. Place a new box of Beckman Coulter (Fullerton, CA) Biomek i-Series 190 µl Barrier tips (Cat. no. B85911) on the Rapid-Shuck module (top right).

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5.8.5.5. Open the “2_TP_Tube to Plate Transfer” method contained in the Active project folder.

5.8.5.6. Click the green “Run” button at the top of the screen.

5.8.5.7. The method will prompt for two values:

5.8.5.7.1. SampleColumns = the number of full or partial columns of sample on the extraction plate.

5.8.5.7.1.1. *Example: 20 samples/blanks will use two full columns and half of a third. Enter “SampleColumns” as 3.*

5.8.5.7.2. SampleVolume = the approximate volume of liquid in the sample tubes, in microliters. Round to the next highest hundred microliters, to a maximum of 400 μ l.

5.8.5.7.2.1. *Example: Most of the sample tubes contain 300 μ l, but one tube contains 340 μ l. Enter “SampleVolume” as 400.*

5.8.5.8. When the instrument has finished transferring sample from the tubes to the sample plate, remove the tube racks from the deck and discard the empty sample tubes.

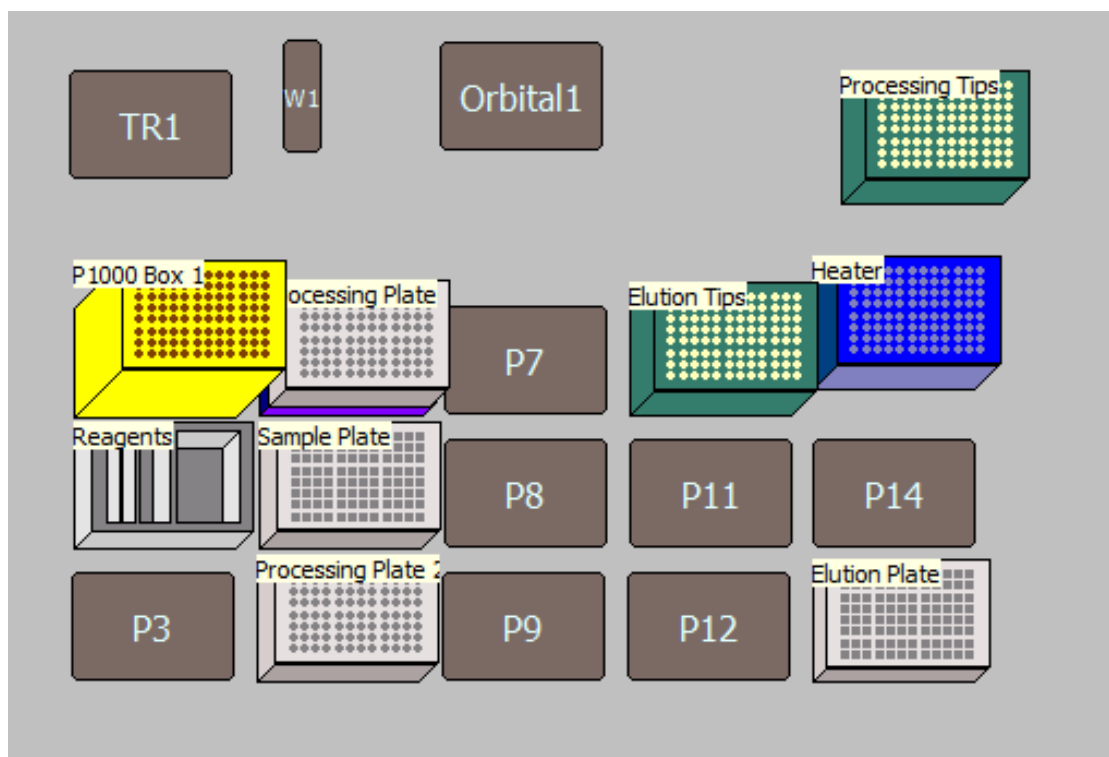
5.8.5.9. Leave the used Biomek i-Series 190 μ l Barrier Tips on the Rapid-Shuck module for subsequent use in DNA IQ™ automated extraction. Each tip is now associated with a single sample and will only be used to pipet that sample.

5.8.6. DNA IQ™ Automated Extraction Procedure

5.8.6.1. Instrument Deck Setup

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- 5.8.6.1.1. Turn on the heating block and ensure that temperature is set to 85°C.
- 5.8.6.1.2. Place the 2.2 ml Square-Well Deep Well Plate sample plate [containing sample transferred during Initial Tube Transfer (5.8.4)] on position P5.
- 5.8.6.1.3. The box of Biomek 190 µl Barrier Tips used in the Tube to Plate Transfer (5.8.4) should be unchanged on the Rapid-Shuck module. DO NOT change the orientation of this box.
- 5.8.6.1.4. Place the following labware on the deck as shown in the figure below:
 - 5.8.6.1.4.1. Box of Beckman Coulter Biomek i-Series 1025 µl Barrier tips (Cat. no. B85955) on position P1. If there are fewer than five columns of tips available, the program will prompt for a full box of 1025 µL tips on position P3, otherwise, a partial box may be used.
 - 5.8.6.1.4.2. Box of new i-Series 190 µl Barrier tips on position P10.
 - 5.8.6.1.4.3. Promega 1.2 ml Round-Bottom Deep Well Plates (Cat. no. V6771) on positions P6 and P4 (aligned on top of the MagnaBot module in P4). Orient each plate so that the clipped corner is on the bottom left.
 - 5.8.6.1.4.4. Promega 1.1 ml Square-Well V-Bottom Deep Well Plate (Cat. no. V6821) on position P15. Orient the plate so that the clipped corner is on the bottom left.



5.8.6.2. Reagent Setup

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- 5.8.6.2.1. Open the Automation Workbook from the “Robot Team” network folder.
- 5.8.6.2.2. The “Samples” tab has a description of the reagents and supplies needed to run automated DNA IQ™ extraction.
 - 5.8.6.2.2.1. Enter the number of samples on the plate, including reagent blanks.
 - 5.8.6.2.2.2. Enter the desired elution volume (between 50-100 µl per sample).
 - 5.8.6.2.2.3. Do not count the No Template Control (NTC) or quantification standards.
 - 5.8.6.2.2.4. Do not add extra samples for excess volume; it is already calculated into the program.
- 5.8.6.2.3. In a reservoir holder, place the following clean reservoirs in order from left to right: one Quarter Module reservoir (Beckman Coulter, Fullerton CA, cat. no. 372790) divided by length, one additional Quarter Module reservoir, and one Half Module reservoir (Beckman Coulter, cat. no 372786).
- 5.8.6.2.4. Load reagents in each reservoir as indicated in the “Samples” tab, adding elution buffer last.
- 5.8.6.2.5. Place the reservoir holder on deck position P2.

5.8.6.3. Running the DNA IQ™ Method

- 5.8.6.3.1. Open the “3_X_Extraction (v2.1.0.ISP)” method, contained in the Active project folder.
- 5.8.6.3.2. Click the “Run” button at the top of the screen.
- 5.8.6.3.3. The method will prompt for three values:
 - 5.8.6.3.3.1. UsedP1000TipCols = the number of **used** full or partial columns in the 1000 µl tip box on position P1. For a new box, enter 0.
 - 5.8.6.3.3.1.1. *Example: If two and a half columns of P1000 tips have been used, enter “UsedP1000TipCols” as 3.*
 - 5.8.6.3.3.2. UsedP200TipCols = the number of **used** full or partial columns in the 190 µl tip box on the Rapid-Shuck module. Assuming a new box was used during the Tube to Plate Transfer (5.8.4), the value should be 0.
 - 5.8.6.3.3.3. SampleCount = the number of total samples to be extracted, up to 87. Wells may not be skipped; this number corresponds to the last well of the plate containing a sample, counting down columns then across rows (e.g., well G11 = 87th well)
 - 5.8.6.3.3.3.1. For a default (aqueous) extraction, simply enter the sample count number (e.g., “87”). This extraction type accommodates a 400 µL starting sample volume and 75 µL elution volume.

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5.8.6.3.3.2. To re-extract/purify a previously extracted lysate plate (lysis extraction), enter the sample count number followed by the word "Lysate" (e.g., "87Lysate"). This extraction type accommodates an 800 µL starting sample volume and 75 µL elution volume.

5.8.6.3.4. The protocol will run for approximately 1 hour 45 minutes, depending on how many samples are being processed.

5.8.6.3.5. When the run is finished, reservoirs may be carefully rinsed, labeled, and reused. Discard partial tip box from position P10. Remaining partial tip boxes may be used again. Discard processing plates (both 1.2 ml Round-Bottom Deep Well Plates). Turn off the heating block.

5.8.6.3.6. Label and retain the original sample plate (2.2 ml Square-Well Deep Well Plate) containing the lysate, as back-up until the samples have been completely processed. Cover and store at room temperature.

5.8.6.3.7. Cover the elution plate (1.1 ml Square-Well V-Bottom Deep Well Plate) containing the extracted samples with a temporary seal and refrigerate if the plate is not to be quantified immediately.

5.8.7. Combined Initial Tube Transfer/DNA IQ™ Extraction

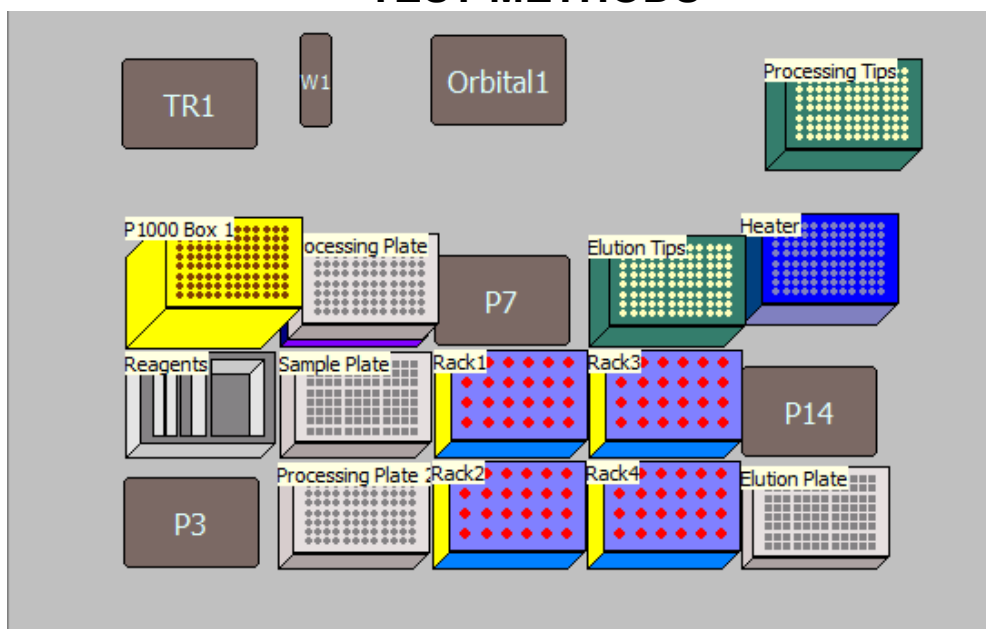
5.8.7.1. If desired, the Tube to Plate Transfer and Extraction steps may be completed in a single method.

5.8.7.2. Instrument Deck Setup

5.8.7.2.1. In the Biomek Software, open the "1_C_Combined TP & X" method contained in the Active project folder.

5.8.7.2.2. Follow the deck setup for both the Tube to Plate Transfer and Extraction methods described above. Both will fit concurrently on the deck.

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5.8.7.2.3. Click the “Run” button at the top of the screen.

5.8.8. REAL-TIME PCR QUANTIFICATION USING QUANTIFILER® TRIO Prepared on the Biomek i5 Laboratory Automation Workstation

5.8.8.1. Instrument Deck Setup

5.8.8.1.1. In the Biomek Software, open the “4_Q_Trio Quant” method contained in the Active project folder.

5.8.8.1.2. Click on the “User Interface” line within the method.

5.8.8.1.2.1. To the right, check that “Quantifiler Trio” is selected.

5.8.8.1.2.2. Enter the number of samples to process (equal to the number of elution plate wells that contain extract).

5.8.8.1.2.3. Do not count the NTC or quantification standards.

5.8.8.1.2.4. Do not add extra samples for excess volume; it is already calculated into the program.

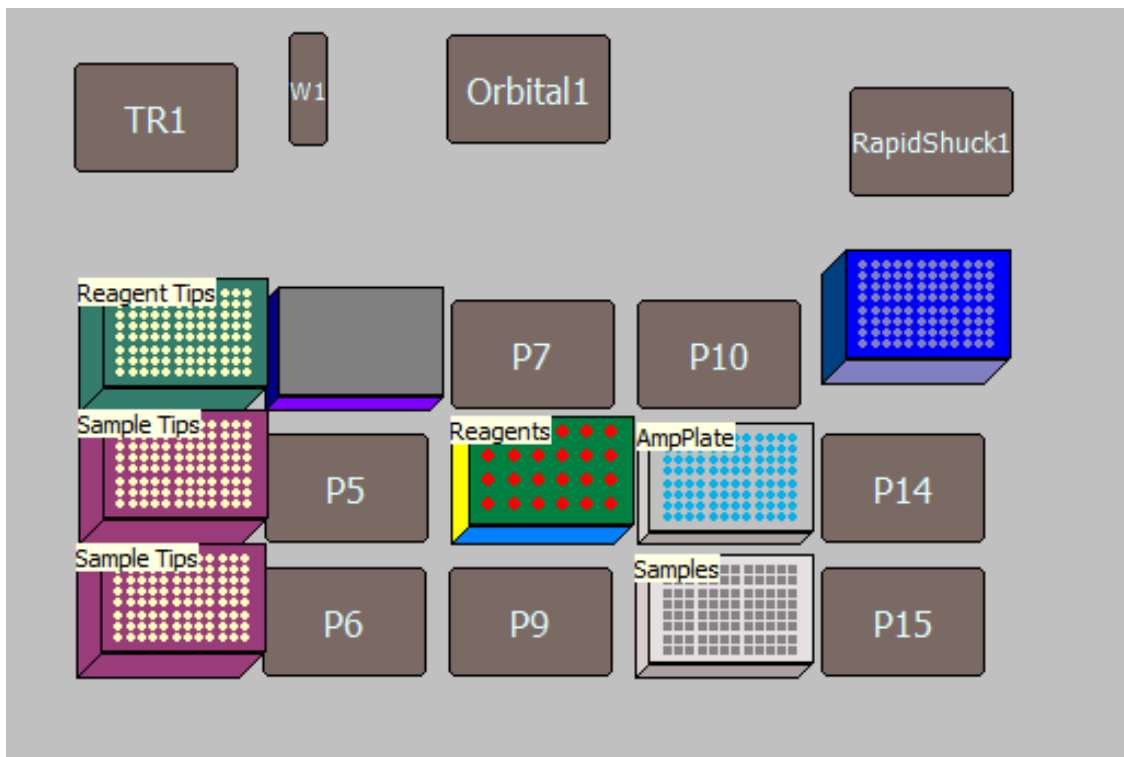
5.8.8.1.3. Place the following labware on the deck as shown in the figure below:

5.8.8.1.3.1. New box of Beckman Coulter Biomek i-Series 50 µl Barrier tips (Cat. no. B85888) on position P2. Alternatively, a partial box may be used by selecting

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the number and position of remaining tips in the box under the “Properties” tab of the “Instrument Setup” step.

- 5.8.8.1.3.2. Box of i-Series 190 µl Barrier tips on position P1. A partial box may be used (as above) if at least one column is available.
- 5.8.8.1.3.3. New Applied Biosystems (Foster City, CA) MicroAmp® Optical 96-Well reaction plate (Cat. no. N801-0560) on position P11.
- 5.8.8.1.3.4. The elution plate containing extracted samples on position P12. This plate must be centrifuged prior to quantification to prevent bubbles in the sample wells.

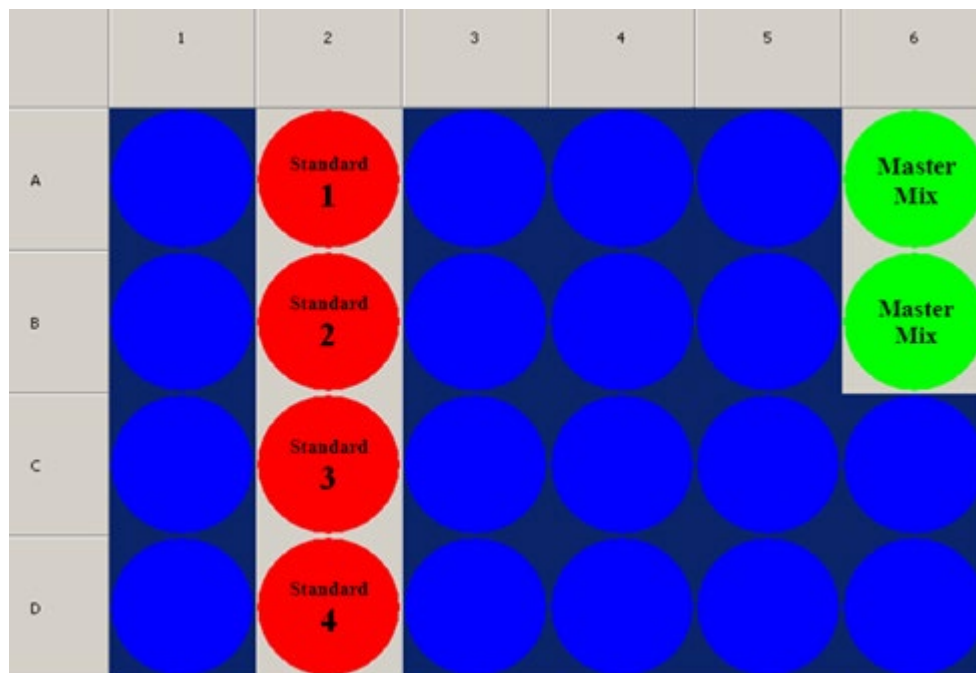


5.8.8.2. Reagent Preparation

- 5.8.8.2.1. Open the Automation Workbook from the “Robot Team” network folder.
- 5.8.8.2.2. The “Samples” tab has a description of the reagents and supplies needed to run automated quantification setup.
 - 5.8.8.2.2.1. Enter the number of samples on the plate, the same as entered into the “User Interface” above.
- 5.8.8.2.3. When a new Quantifiler® Trio kit is opened, Quantification Enhancer may be added to the reaction mix as follows:

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- 5.8.8.2.3.1. Using a 2.5 µl pipettor, carefully add 1 µl of Quantification Enhancer to each fresh tube of reaction mix. Vortex each reaction mix tube briefly.
- 5.8.8.2.3.2. Mark each tube of reaction mix with “QE” to indicate that Quantification Enhancer has been added.
- 5.8.8.2.3.3. Record the lot number of the Quantification Enhancer on the Automated Extraction/Quantification Worksheet.
- 5.8.8.2.3.4. Quantification Enhancer shall be stored at room temperature.
- 5.8.8.2.4. Prepare the master mix as described in section 2 of the Forensic Biology Section Casework Test Method, using the volumes indicated on the Reagents tab. Note that for 40 or more samples, the master mix must be divided evenly into two tubes. Vortex the Master Mix 3 to 5 seconds, then centrifuge briefly.
- 5.8.8.2.5. Place the master mix tube(s) in wells A6 (and B6) of the Reagent Tube Rack as indicated below.
- 5.8.8.2.6. Prepare the standard dilution series as described in Quantifiler® Trio section of the Biology Section Casework Test Method or use a previously prepared dilution series. Each tube must contain at least 20 µl of standard.
- 5.8.8.2.7. Place the standard tubes in column 2 of the Reagent Tube Rack as indicated below.
- 5.8.8.2.8. Open and fold back all tube caps and place the Reagent Tube Rack on deck position P8.



5.8.8.3. Running the Automated Quant Trio Setup Method

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- 5.8.8.3.1. With the “4_Q_Trio Quant” method open and the number of samples specified, click the “Run” button at the top of the screen.
- 5.8.8.3.2. A window will appear to confirm the amount and placement of the master mix tubes. Click “OK”.
- 5.8.8.3.3. A window will appear to confirm the deck layout as shown by the software. Click “OK”.
- 5.8.8.3.4. A window will appear to confirm the total amount of master mix, the amount of standard in each of the standard tubes, the sample count, and the quantification kit. Click “OK”.
- 5.8.8.3.5. The protocol will begin. When the run is finished, remove the elution plate with extracts and cover with a temporary seal until ready to proceed with amplification.
- 5.8.8.3.6. Seal the optical plate with an optical adhesive cover. Run the edge of the cover applicator between the rows and columns of the wells to ensure that all wells are sealed properly.
- 5.8.8.3.7. Spin the plate using either a centrifuge or a salad spinner to remove any bubbles and force samples into the bottom of each well.
- 5.8.8.3.8. Place the plate in the real-time PCR instrument, turn on the instrument, and open the 7500 System software.

5.8.8.4. Create A Plate Document

- 5.8.8.4.1. Open the Automation Workbook file containing the batch of samples being run, and click on the “Extraction-Quant” tab. Ensure that the corresponding plate document contains sample information, otherwise, add and save that information now. Also ensure that the batch number has been entered into the yellow box on the “Samples” tab.
- 5.8.8.4.2. Press the “Create Quant Setup File” button. A new text file named “Batch#_QuantSetup_Date” will appear in the same folder that contains the Automation Workbook. Click “OK” to proceed.
 - 5.8.8.4.2.1. If there are duplicate sample names in the batch, an error message will list them and the Quant Setup file will not be created until the duplicate names are fixed.
- 5.8.8.4.3. On the computer connected to the real-time PCR instrument, create a new plate document as described in the Quantifiler® Trio section of the Biology Section Casework Test Method.
- 5.8.8.4.4. Import the text file you just created. The wells should populate with the names of the samples and standards.

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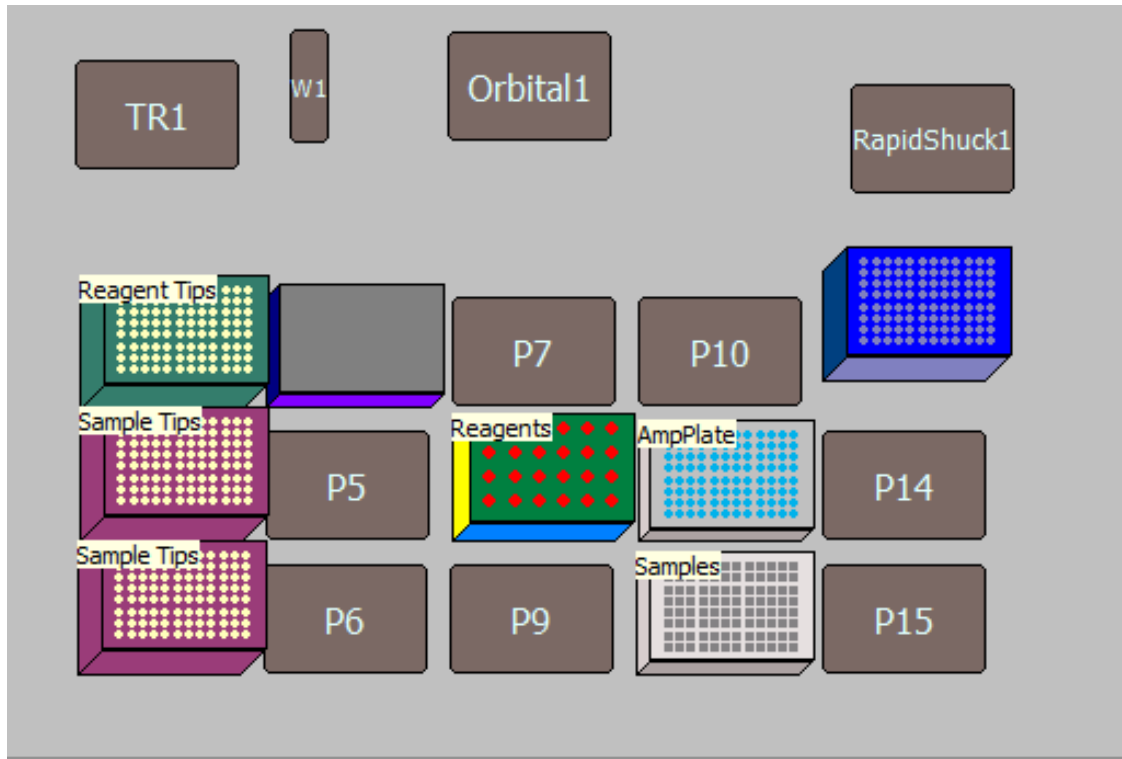
- 5.8.8.4.5. Enter the plate name under “Experiment”. Save the plate document and proceed with real-time PCR analysis as described in the Quantifiler® Trio section of the Biology Section Casework Test Method.

5.8.9. Real-Time PCR Quantification Using Promega PowerQuant® System Prepared on the Biomek i5 Laboratory Automation Workstation

5.8.9.1. Instrument Deck Setup

- 5.8.9.1.1. In the Biomek Software, open the “4_Q_PowerQuant” method contained in the Active project folder.
- 5.8.9.1.2. Click on the “User Interface” line within the method.
- 5.8.9.1.2.1. To the right, check that “PowerQuant” is selected.
 - 5.8.9.1.2.2. Enter the number of samples to process (equal to the number of elution plate wells that contain extract).
 - 5.8.9.1.2.3. Do not count the NTC or quantification standards.
 - 5.8.9.1.2.4. Do not add extra samples for excess volume; it is already calculated into the program.
- 5.8.9.1.3. Place the following labware on the deck as shown in the figure below:
- 5.8.9.1.3.1. New box of Beckman Coulter Biomek i-Series 50 µl Barrier tips (Cat. no. B85888) on position P2. Alternatively, a partial box may be used by selecting the number and position of remaining tips in the box under the “Properties” tab of the “Instrument Setup” step.
 - 5.8.9.1.3.2. Box of i-Series 190 µl Barrier tips on position P1. A partial box may be used (as above) if at least one column is available.
 - 5.8.9.1.3.3. New Applied Biosystems (Foster City, CA) MicroAmp® Optical 96-Well reaction plate (Cat. no. N801-0560) on position P11.
 - 5.8.9.1.3.4. The elution plate containing extracted samples on position P12. This plate must be centrifuged prior to quantification to prevent bubbles in the sample wells.

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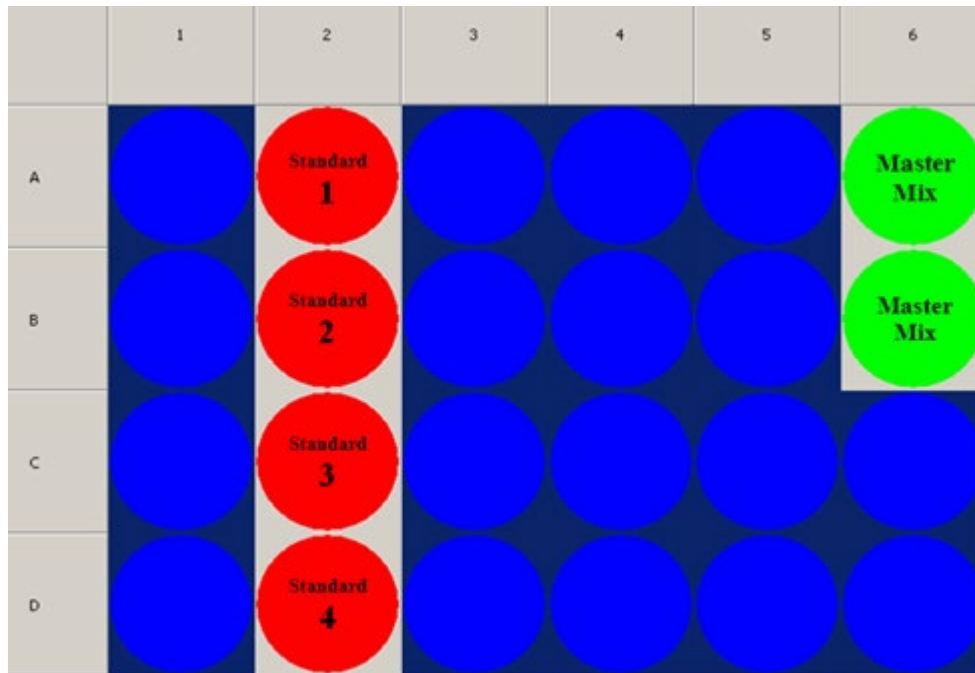


5.8.9.2. Reagent Preparation

- 5.8.9.2.1. Open the Automation Workbook from the “Robot Team” network folder.
- 5.8.9.2.2. The “Samples” tab has a description of the reagents and supplies needed to run automated quantification setup.
 - 5.8.9.2.2.1. Enter the number of samples on the plate, the same as entered into the “User Interface” above.
- 5.8.9.2.3. Prepare the master mix as described in section 2 of the Forensic Biology Section Casework Test Method, using the volumes indicated on the Reagents tab. Note that for 40 or more samples, the master mix must be divided evenly into two tubes. Vortex the Master Mix 3 to 5 seconds, then centrifuge briefly.
- 5.8.9.2.4. Place the master mix tube(s) in wells A6 (and B6) of the Reagent Tube Rack as indicated below.
- 5.8.9.2.5. Prepare the standard dilution series as described in PowerQuant® section of the Biology Section Casework Test Method or use a previously prepared dilution series. Each tube must contain at least 20 µl of standard.
- 5.8.9.2.6. Place the standard tubes in column 2 of the Reagent Tube Rack as indicated below.

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- 5.8.9.2.7. Open and fold back all tube caps and place the Reagent Tube Rack on deck position P8.



5.8.9.3. Running the Automated PowerQuant® Setup Method

- 5.8.9.3.1. With the "4_Q_PowerQuant" method open and the number of samples specified, click the "Run" button at the top of the screen.
- 5.8.9.3.2. A window will appear to confirm the amount and placement of the master mix tubes. Click "OK".
- 5.8.9.3.3. A window will appear to confirm the deck layout as shown by the software. Click "OK".
- 5.8.9.3.4. A window will appear to confirm the total amount of master mix, the amount of standard in each of the standard tubes, the sample count, and the quantification kit. Click "OK".
- 5.8.9.3.5. The protocol will begin. When the run is finished, remove the elution plate with extracts and cover with a temporary seal until ready to proceed with amplification.
- 5.8.9.3.6. Seal the optical plate with an optical adhesive cover. Run the edge of the cover applicator between the rows and columns of the wells to ensure that all wells are sealed properly.
- 5.8.9.3.7. Spin the plate using either a centrifuge or a salad spinner to remove any bubbles and force samples into the bottom of each well.

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- 5.8.9.3.8. Place the plate in the QuantStudio™ 5 Real-Time instrument, turn on the instrument, and open the template in the Design & Analysis software saved to the desktop.

5.8.9.4. Create A Plate Document

- 5.8.9.4.1. Open the Automation Workbook file containing the batch of samples being run, and click on the “Extraction-Quant” tab. Ensure that the corresponding plate document contains sample information, otherwise, add and save that information now. Also ensure that the batch number has been entered into the yellow box on the “Samples” tab.
- 5.8.9.4.2. Press the “Create Quant Setup File” button. A new text file named “Batch#_QuantSetup_Date” will appear in the same folder that contains the Automation Workbook. Click “OK” to proceed.
- 5.8.9.4.2.1. If there are duplicate sample names in the batch, an error message will list them and the Quant Setup file will not be created until the duplicate names are fixed
- 5.8.9.4.3. On the computer connected to the real-time PCR instrument, create a new plate document as described in the PowerQuant® section of the Biology Section Casework Test Method.
- 5.8.9.4.4. Enter the plate name under “Experiment” tab in the Name field under Experiment Properties.
- 5.8.9.4.5. Import the text file you just created. The wells should populate with the names of the samples and standards.
- 5.8.9.4.6. Save the plate by clicking Start Run and proceed with real-time PCR analysis as described in the PowerQuant® section of the Biology Section Casework Test Method

5.8.10. PowerPlex® Fusion 6C Amplification Prepared on the Biomek i5 Laboratory Automation Workstation

5.8.10.1. Preparing Extracts

- 5.8.10.1.1. Spin down the extract plate again before proceeding to amplification set-up. (CAUTION: Ensure that there are no bubbles in the wells.) Remove temporary seal.

5.8.10.2. Normalization Data Setup

- 5.8.10.2.1. Open the Automation Workbook file containing the batch to be amplified.
- 5.8.10.2.2. On the “Extraction-Quant” tab, click the “Import & Analyze Quant Results” button.
- 5.8.10.2.3. Browse for the appropriate text import file containing quantification data for this batch. Click “Open”.

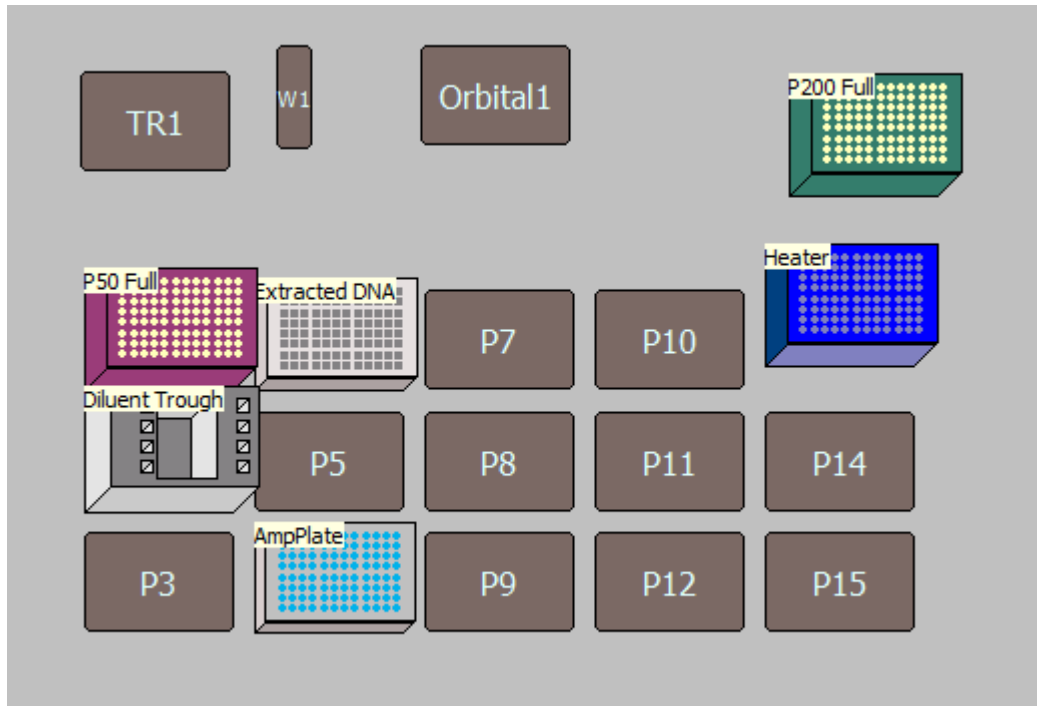
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- 5.8.10.2.4. The Automation Workbook will analyze the batch, create the Quantification Results and Standard Curve worksheets, create the Amplified Samples summary, create the Samples Not Amplified summary, and fill the samples on the Amplification Setup/CE Plate Record.
 - 5.8.10.2.4.1. If any standard curve parameter fails, or if the NTC contains a value, or if a standard curve well has an undetected IPC, an error message will appear and the batch will not be analyzed until the standard curve is fixed. Points should be omitted in the Real-Time PCR software and a new results file exported.
- 5.8.10.2.5. A new .csv file named "Batch#_AmpSetup" (the AmpSetup file) will appear in the same folder that contains the Automation Workbook. Click "OK" to proceed.
- 5.8.10.2.6. Move or copy this AmpSetup file to the folder //IN PROGRESS/Amp Setups on the "Robot Team" drive.

5.8.10.3. Instrument Deck Setup

- 5.8.10.3.1. In the Biomek Software, open the "5a_A_Fusion 6C Normalize-Amp" method contained in the Promega project.
- 5.8.10.3.2. Place the following labware on the deck as shown in the figure below:
 - 5.8.10.3.2.1. Box of i-Series 190 µl Barrier tips on the Rapid-Shuck module.
 - 5.8.10.3.2.2. Box of i-Series 50 µl Barrier tips on position P1. A second box of P50 Barrier tips may be required on position P3.
 - 5.8.10.3.2.3. 1.1 ml square-bottom deepwell plate on position P5. A second 1.1 ml square-bottom deepwell plate may be required on position P8.
 - 5.8.10.3.2.4. New 96-well optical amplification plate on position P6.
 - 5.8.10.3.2.5. Extract plate containing samples on position P4.
 - 5.8.10.3.2.5.1. CAUTION: ensure that the MagnaBot module has been removed.

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5.8.10.3.3. Click “Run”.

5.8.10.3.4. The program will prompt for three values:

5.8.10.3.4.1. BatchNumber = The batch number being amplified, in the format Year-XXX. This directs the Biomek software to import the correct Amp Setup file from the //IN PROGRESS/Amp Setups folder.

5.8.10.3.4.2. First_P250_Tip = the number of the first unused tip in the 190 µL tip box on the Rapid-Shuck module, numbering down columns and then across. For a new box, enter 1.

5.8.10.3.4.2.1. *Example: If two and a half columns (twenty tips) of P250 tips have been used, enter “First_P250_Tip” as 21.*

5.8.10.3.4.3. First_P50_Tip = the number of the first unused tip in the 50 µl tip box on position P1, numbering down columns and then across. For a new box, enter 1.

5.8.10.4. Reagent Preparation

5.8.10.4.1. Follow the prompts within the Biomek Software. Note that depending on the dilution of samples within the batch, the protocol may require an additional 1.1 ml square-bottom deepwell plate on position P8.

5.8.10.4.2. Place the STR Master Mix tube in position 4 (front-most) of the Left Tube Holder on the reagent rack.

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5.8.10.4.3. Place the tube containing diluted positive control (at least 20 µl) in position 1 (rear-most) of the Right Tube Holder on the reagent rack. Click “OK”.

5.8.10.4.4. Pipet the indicated amount of amplification-grade water or nuclease-free water into the center trough of the reagent rack. Place the reagent rack on deck position P2.

5.8.10.5. Running the Normalization/Amp Set-up Method

5.8.10.5.1. Check that the deck layout matches the layout indicated in the next prompt. Click “OK”.

5.8.10.5.2. The run will begin, performing normalization and amplification setup in one step.

5.8.10.5.3. When the run is finished, remove the amplification plate and cover with adhesive foil. Press the foil well using a roller tool.

5.8.10.5.4. Remove the elution plate containing extracted samples and cover with a temporary seal and refrigerate.

5.8.10.6. Create CE Plate Record

5.8.10.6.1. The Amp-CE Plate Record in the Automation Workbook is automatically populated with the amplified samples when the batch is analyzed with quantification results.

5.8.10.6.2. Open the Automation Workbook containing the batch of interest and click on the “Amp-CE” tab.

5.8.10.6.3. Click the “Export CE Setup File” button. A new text file named “Batch#_CESetup_Date” will appear in the same folder that contains the Automation Workbook. Click “OK” to proceed.

5.8.10.6.3.1. If no plate name has been filled in, the file will contain a default plate name in the format “Batch#_Date_01”. This can be changed on the “Amp-CE” tab or in the genetic analyzer software.

5.8.10.6.4. Samples may be added manually to the CE plate record and “CE Setup” tab, as needed, before clicking the “Export CE Setup File” button. Only samples listed on the “CE Setup” tab will be included in the exported CE Setup file.

5.8.10.6.5. Fill out the rest of the worksheet as designated, including dates, reagent lot numbers, amplification reaction type, and injection protocols.

5.8.11. PowerPlex® Y23 Amplification

Prepared on the Biomek i5 Laboratory Automation Workstation

5.8.11.1. NOTE: PowerPlex® Y23 amplification via Biomek automation is not validated for casework unknowns and shall only be performed on known samples.

5.8.11.2. Normalization Data Setup

5.8.11.2.1. Open the Automation Workbook file containing the batch to be amplified.

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- 5.8.11.2.2. Assuming the quantitation data for the batch has already been imported and processed (see preceding section for Fusion 6C amplification), go to the Data Extract tab.
- 5.8.11.2.2.1. The “Amp YSTR” column on the right contains TRUE/FALSE designations for each sample. This controls whether a sample will be included in a Y-STR amplification. Samples are automatically marked “TRUE” for Y-STR amplification if they both:
- 1) Have a F:M ratio ≤ 5
 - 2) Are marked as a standard (Code S)
- 5.8.11.2.2.2. Y-STR amplification may be manually turned on/off per sample by editing the Amp YSTR column values.
- 5.8.11.2.2.3. If the Amp YSTR column reflects the samples that should be amplified, click the “Amp Y-STR” button above it.
- 5.8.11.2.3. The Automation Workbook will analyze the batch according to the Y quant values and create a Y-STR version of the following worksheets: Amplified Samples Summary, Amplification Setup/CE Plate Record, and CE Setup. These worksheets are normally hidden in the Workbook but will appear as blue tabs.
- 5.8.11.2.4. A new .csv file named “Batch#_YSTR_AmpSetup” (the AmpSetup file) will appear in the same folder that contains the Automation Workbook. Click “OK” to proceed.
- 5.8.11.2.5. Move or copy this YSTR_AmpSetup file to the folder //IN PROGRESS/Amp Setups on the “Robot Team” drive.
- 5.8.11.2.6. Proceed with Instrument Deck Setup, Reagent Preparation, Running the Normalization/Amp Setup Method, and Create CE Plate Record as described in the previous section, *except*:
- 5.8.11.2.6.1. For Instrument Deck Setup, open the “5b_A_PPY23 Normalize-Amp” method contained in the Active project folder.
- 5.8.11.2.6.2. For Reagent Preparation, be sure to use the PowerPlex® Y23 amplification kit. Follow the master mix preparation recipe as prompted in the Biomek software.
- 5.8.11.2.6.3. For Create CE Plate Record, click on the “Export CE Setup File” on the “Y-Amp-CE” worksheet.

5.8.12. Final Tube Transfer

- 5.8.12.1. The Automation Workbook consolidates samples that are to be returned to tubes and creates an output for the robot to cherry-pick those samples. The remaining samples will remain in the elution plate. Samples returned to tubes will include those given code “T” by the case analyst, samples with more than 50 ng/uL of small autosomal DNA, and any samples that meet ALL of the following criteria:

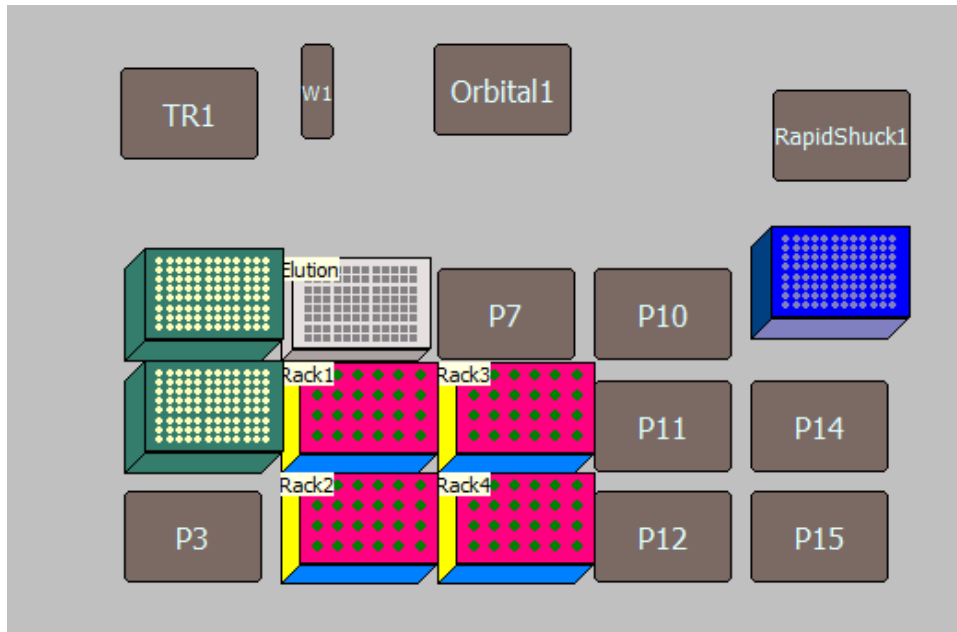
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- 5.8.12.1.1. The sample is code "M".
- 5.8.12.1.2. The sample is not a standard.
- 5.8.12.1.3. The sample contains male DNA ≥ 0.005 ng/uL.
- 5.8.12.1.4. The sample contains < 0.01 ng/uL of small autosomal DNA or has a female:male ratio > 50 .
- 5.8.12.2.** Open the Automation Workbook containing the set of samples that have been run, and click on the "FTT Layout" tab. Click the "Consolidate Samples" button on the upper right.
 - 5.8.12.2.1. The layout worksheet will tell you how many tubes/labels you will need. If you wish to print labels on a partially used sheet, you may designate how many labels are missing from the sheet in the "# of used labels" box. A map of a label sheet is included on the right for reference.
 - 5.8.12.2.1.1. You cannot use a partial sheet if the # of used labels + # of tubes required is greater than 80.
- 5.8.12.3.** Click the "Export FTT File" button. A new .csv file named "<batch ID>_FTT Setup" will appear in the same folder that contains the Automation Workbook. Click "OK" to proceed.
- 5.8.12.4.** Move or copy the .csv file to the folder //IN PROGRESS/FTT Setups on the "Robot Team" drive.
- 5.8.12.5.** Place new, empty, labeled Molecular BioProducts (San Diego, CA) 1.5 ml screw-top tubes (Cat. no. 3474) in the tube racks (4x6 with white adapters), arranging the tubes according to the order shown in the "Consolidated Extracts in Tubes" layout on the "FTT Layout" tab of the Automation Workbook. Do not skip columns or spaces between samples.
- 5.8.12.6.** Arrange the deck layout as shown in the figure below:
 - 5.8.12.6.1. Place sample tube racks on positions P5, P6, P8, and P9, again paying attention to sample order according to the FTT Layout.
 - 5.8.12.6.2. Place the plate containing extracted samples on position P4.
 - 5.8.12.6.3. Place a new box of Biomek i-series 190 μ l Barrier Tips on position P1.

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Biomek i5

5.8.12.7. Open the “6_PT_Plate to Tube Transfer” method contained in the Promega project.

5.8.12.8. Click the green “Run” button at the top of the screen.

5.8.12.8.1. The method will prompt for the Batch ID. Type the Batch ID in the format “Year-000” (e.g., “2017-001”). This tells the software which .csv file to use in the folder “FTT Setups” mentioned above.

5.8.12.9. When the instrument has finished transferring sample from the extraction plate to the tubes, remove the tube racks from the deck and cap the screw-top tubes.

5.8.12.9.1. The extraction plate, which now contains only extracts of reference samples and property crime samples, shall be covered with a temporary seal, refrigerated, and saved for a minimum of 60 days, after which it may be discarded.

5.8.13. When processing is completed, the submitting analyst will be notified by e-mail that the data is available on the DNA server and the extracted DNA samples can be retrieved from a designated location.

5.9. Records:

5.9.1. Sample Tracking for Automated Processing of Casework Samples

5.9.1.1. The below documentation steps are unnecessary if an automation analyst is processing their own case samples throughout the entire automation process.

5.9.1.2. As part of the process of preparing samples for automated processing, the analyst shall complete the most current version of the Robot Submission Form, then email the form to DAutomation@isp.in.gov.

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- 5.9.1.3.** This form, as well as the email that included it, shall be retained by the analyst in the case record.
- 5.9.1.4.** This documents a step in the chain of custody and denotes the point of divergence of extracts from their associated parent samples' chain of custody if the extract is later requested to be retained as a subitem. The extracts leave the analyst's custody and are transferred to a storage area where they will be retrieved for automated processing.
- 5.9.1.5.** At IRL - Within the same workday that the Robot Submission Form is submitted, the analyst shall deliver all associated samples to the IRL S201A robot room storage area. At ERL, LRL, and FWRL - Samples will be delivered by an FSD employee at the earliest available transfer.
- 5.9.1.6.** The robot operator shall collect samples from the storage area for automated processing. The transfer shall be documented for the case record on the Biomek Automated Extraction/Quantitation Worksheet.
- 5.9.1.7.** The name (or initials) of robot operator receiving custody of samples, as well as the date of transfer, shall be marked on the worksheet.
- 5.9.1.8.** Following extraction, the lysis portion of the samples shall be retained (sealed in plate) in the IRL S201 robot room storage area. The lysis plate shall be retained for approximately 60 days.
- 5.9.1.9.** If the lysis plate is required for reprocessing, the robot operator shall assume custody of the lysis plate and associated samples. This transfer shall be documented in the case record to illustrate a clear, linear chain of custody and shall include, at a minimum, the name (or initials) of robot operator and date of transfer.
- 5.9.1.10.** After approximately 60 days post-processing, the lysis plate shall be discarded.
- 5.9.1.11.** Each subsequent step of automated processing (quantitation, amplification, and CE Injection) may be performed by the initial operator, or by another robot operator. For each step, name (or initials) of the robot operator, as well as the date of the step performed, shall be documented in the case record on the corresponding automation worksheets.
- 5.9.1.12.** When different operators perform downstream steps of automated processing, the worksheets serve to document the dates of transfer from operator to operator at each point of automated processing.
- 5.9.1.13.** If processing requires repeating steps or changes that result in additional transfers between operators and/or analysts, this shall be documented in the case record to illustrate a clear, linear chain of custody and shall include, at a minimum, the name (or initials) of individuals and date of transfer.
- 5.9.1.14.** Following CE data generation, the robot operator shall send an email to the analyst informing them that data (and, if applicable, extracts) are available for collection.
- 5.9.1.15.** If any associated extracts are designated to be retained for preservation or additional manual processing (e.g., Y-STR), a robot operator shall transfer those extracts into

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individual tubes and return these extracts to the IRL S201A robot room storage area within the same workday that the notification email is delivered.

- 5.9.1.16.** This email shall be retained by the analyst in the case record. This documents a step in the chain of custody and denotes the point where the extract is transferred from the operator's custody to the storage location.
- 5.9.1.17.** An analyst may transfer extracts destined for additional manual processing from the plate into individual tubes. This shall be clearly documented by the analyst in the case record. This documents a step in the chain of custody from robot operator to analyst, and shall include, at a minimum, the name (or initials) of analyst and the date of transfer.
- 5.9.1.18.** If an extract designated to be retained is completely consumed (or if the extract has completely evaporated prior to tube transfer), this shall be clearly documented by the robot operator in the automation case worksheets, and no tube shall be generated.
- 5.9.1.19.** The extracts not designated to be retained (i.e., remaining in the sample plate) shall be transferred (sealed in plate) to the IRL S201A robot room storage area. These samples will be retained for approximately 60 days, in the event that the samples are required for retention or retesting (see above).
- 5.9.1.20.** After approximately 60 days post-processing, any remaining extracts not designated for retention shall be discarded.
- 5.9.1.21.** The analyst shall retrieve any retained extracts from the robot room storage area to return them to their custody. The date of the transfer shall be recorded by the analyst in the case record.
- 5.9.1.22.** This documents a step in the chain of custody and denotes the point where the extract re-enters the analysts' custody and adopts the associated parent samples' chain of custody.

5.10. Interpretation of Results:

5.10.1. N/A

5.11. Report Wording:

5.11.1. N/A

5.12. References:

5.12.1. N/A

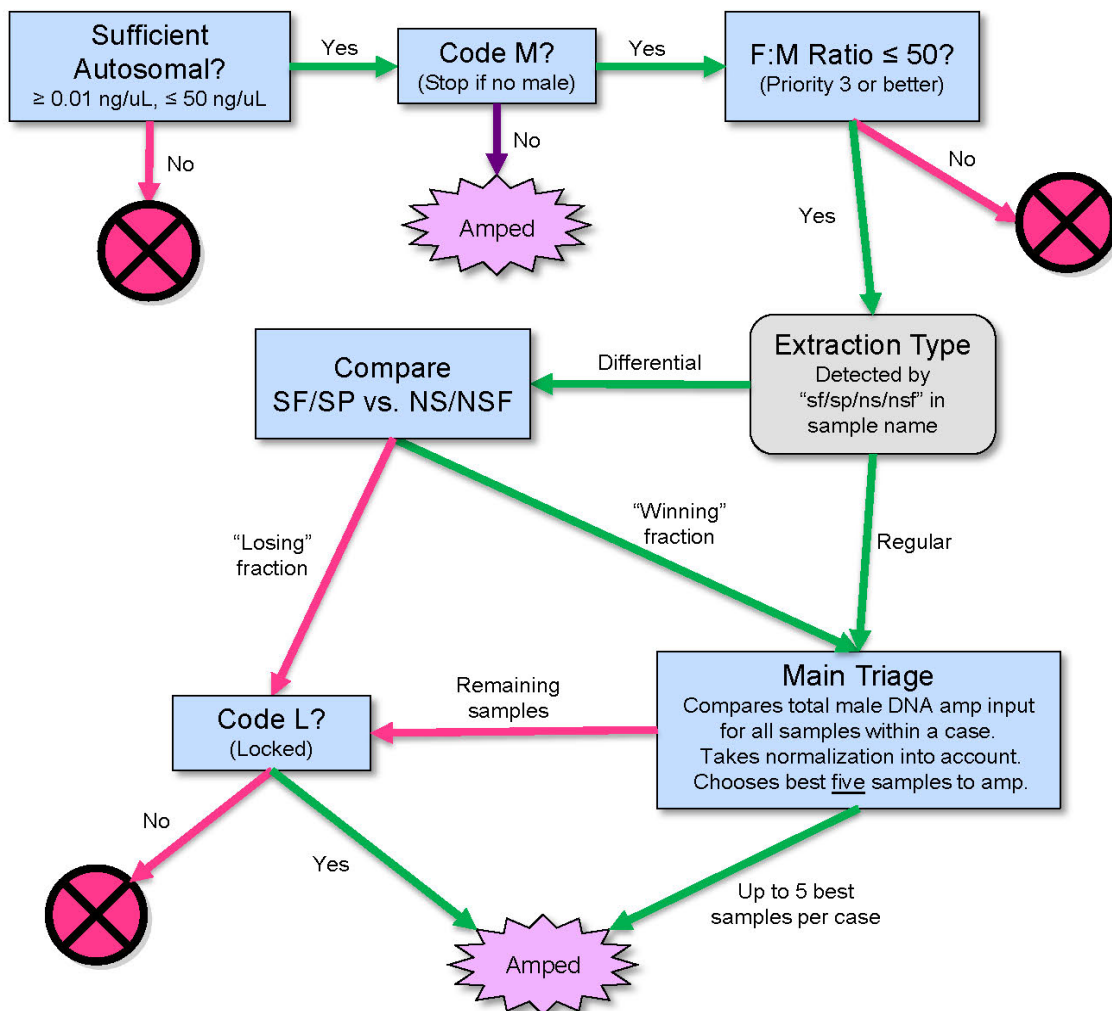
APPENDIX 1

Automation Sample Triage

Sample Property Codes:

S	Standard	Allows standards from different cases to share a reagent blank (RB must also be marked S). As usual, standards will be placed last in a robot plate.
M	Stop if no Male	Marks a sample for triage based on total amplifiable male DNA (see below). Has no effect on reagent blanks or Standards (S).
L	Locked	Combined with code M, causes a sample to be amplified if it has sufficient male DNA (Priority 3 or better) regardless of triage. Will not prevent better samples from being amplified. No effect unless combined with code M, and no effect on reagent blanks.
T	Return to Tube	Marks any sample or reagent blank to be returned to a tube. No effect on other codes.

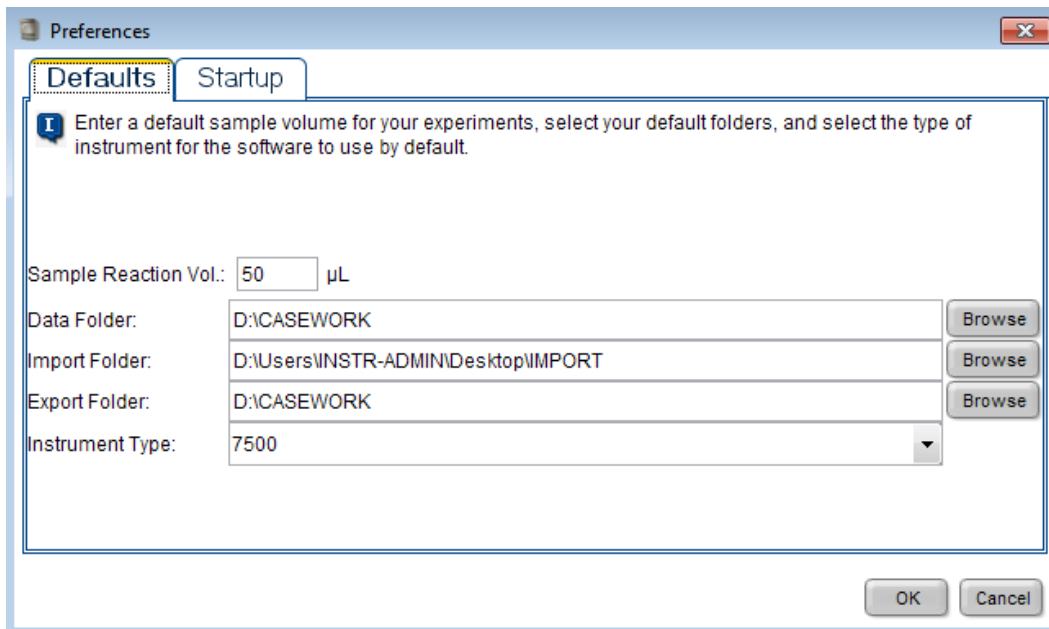
Triage Process:



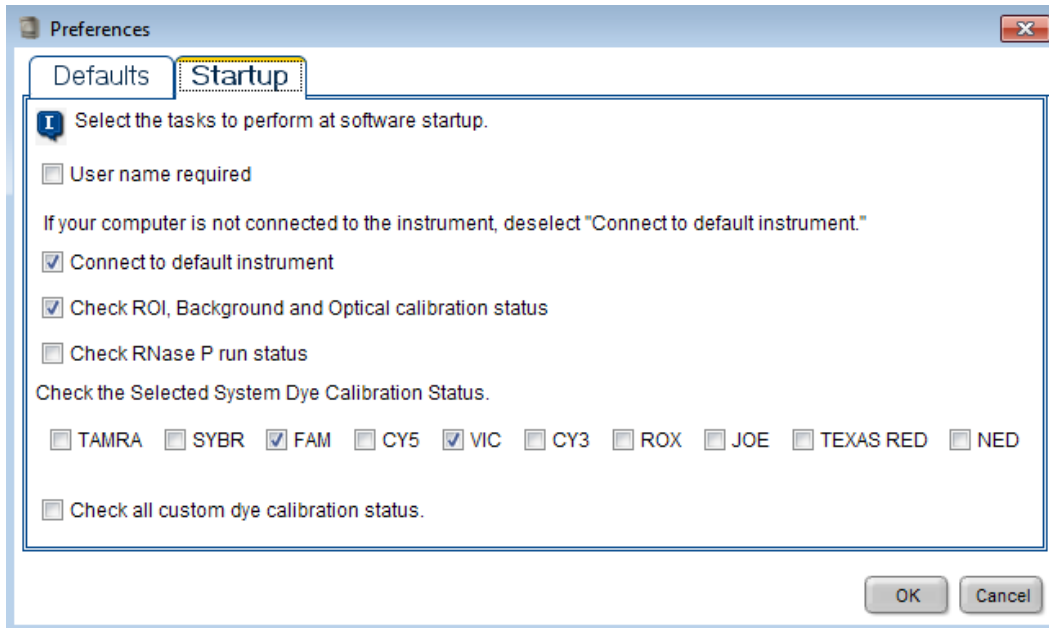
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1. HID Real-Time PCR Analysis Software v1.2 and Quantifiler® Trio template set-up

- 1.1 Create a “CASEWORK” folder on the AB_SW&DATA D: drive and a shortcut on the desktop.
- 1.2 Create an “IMPORT” folder on the desktop.
- 1.3 Open the HID Real-Time PCR Analysis Software v1.2.
- 1.4 Select **Tools** → **Preferences** and select the “Defaults” tab.
 - 1.4.1 Change the default Data Folder and Export Folder destinations to the “CASEWORK” folder found on the D: drive. Change the default Import Folder destination to the “IMPORT” folder on the desktop.
 - 1.4.2 Select the “Startup” tab and deselect “User name required”. Mark only “FAM” and “VIC” for calibration status.

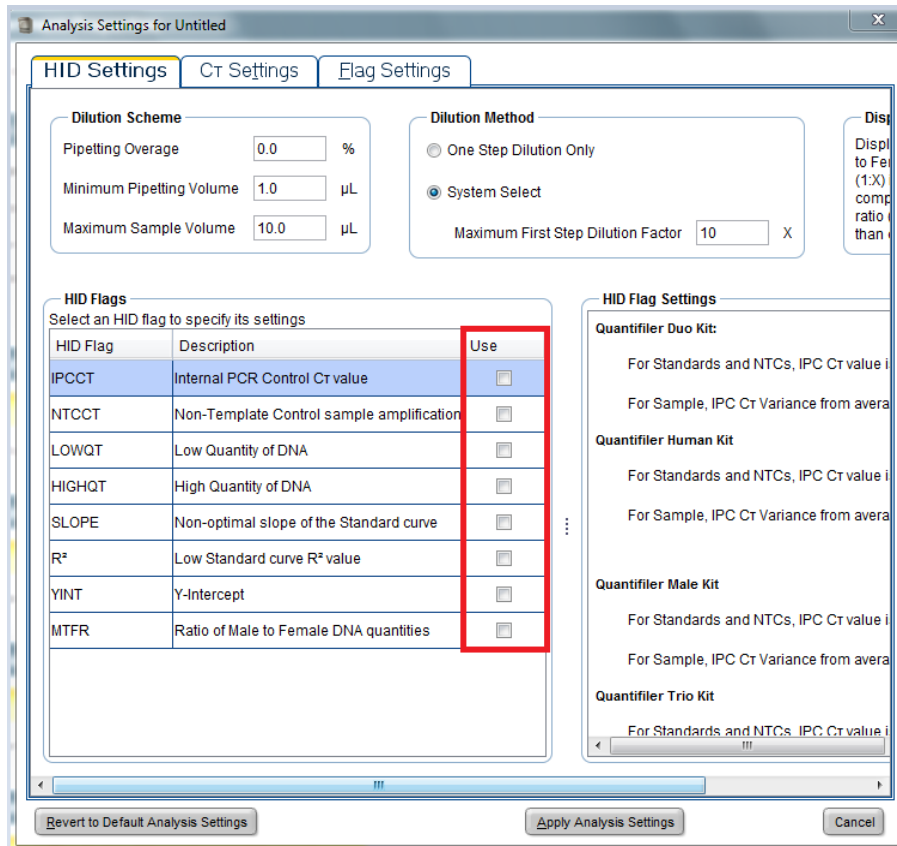


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1.5 Select the **Quantifiler® Trio** icon on the Home screen.

1.6 Select **Analysis** → **Analysis Settings** to remove all flags from the "HID Settings" and "Flag Settings" tabs.



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Flag	Description	Use	Attribute	Condition	Value	Reject Well
AMPNC	Amplification in ...	☐	Cr	<	35	☐
BADROX	Bad passive ref...	☐	Bad passive ref...	>	0.6	☐
BLFAIL	Baseline algorit...	☐				☐
CTFAIL	Cr algorithm fail...	☐				☐
EXPFAIL	Exponential alg...	☐				☐
OFFSCALE	Fluorescence is...	☐				☐
HIGHSD	High standard d...	☐	Cr standard dev...	>	0.5	☐
NOAMP	No amplification	☐	Amplification al...	<	0.1	☐
NOISE	Noise higher th...	☐	Relative noise	>	4	☐
SPIKE	Noise spikes	☐	Spike algorithm...	>	1	☐
NOSIGNAL	No signal in well	☐				☐
OUTLIERRG	Outlier in replic...	☐				☐
THOLDFAIL	Thresholding al...	☐				☐

Buttons: Revert to Default Analysis Settings, Apply Analysis Settings, Cancel

1.7 Verify the CT Settings in the image below.

Default Cr Settings
Default Cr settings are used to calculate the Cr for targets without custom settings. To edit the default settings, click "Edit Default Settings."
Threshold: 0.2 Baseline Start Cycle: 3 Baseline End Cycle: 15 [Edit Default Settings](#)

Target	Threshold	Baseline Start	Baseline End
T.IPC	0.1	3	15
T.Large Autosomal	0.2	3	15
T.Small Autosomal	0.2	3	15
T.Y	0.2	3	15

Cr Settings for T.IPC
Cr Settings to Use: ☐ Use Default Settings
☐ Automatic Threshold
Threshold: 0.1
☐ Automatic Baseline
Baseline Start Cycle: 3 End Cycle: 15

Buttons: Revert to Default Analysis Settings, Apply Analysis Settings, Cancel

1.8 Select **Plate Setup** from the left navigation pane and assign the 4 standard curve points to column 12 in duplicate.

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- 1.8.1 The task for the Lg. Auto, Sm. Auto, and Y targets for each standard curve point shall be designated as “Standard”. The task for the IPC target shall be designated as “Unknown”.
- 1.8.2 Enter the appropriate quantity for each Lg. Auto, Sm. Auto, and Y targets. (See the [Standard Curve Prep Table](#) above)
- 1.9 Assign the NTC to well H11 and mark the task for the Lg. Auto, Sm. Auto, and Y targets as “Negative control”. The task for the IPC target shall be designated as “Unknown”.
- 1.10 All remaining wells shall be left blank.

Experiment: **Untitled** Type: **HID Standard Curve** Kit Name: **Quantifiler Trio** **START RUN** ?

Define Targets and Samples **Assign Targets and Samples**

Instructions: Standards and NTC are set by default. Select wells, then assign targets if applicable.

Assign sample(s) to the selected wells.

Assign	Sample
☒	Trio Standard 1
☐	Trio Standard 2
☐	Trio Standard 3
☐	Trio Standard 4
☐	NTC

Assign target(s) to the selected wells.

Assign	Target	Task	Quantity
☒	T.Large A...	U S N	50
☒	T.Small A...	U S N	50
☒	T.IPC	U S N	
☒	T.Y	U S N	50

☒ Mixed
 ☒ Unknown
 ☒ Standard
 ☒ Negative Control

Select the dye to use as the passive reference.

MP

View Plate Layout View Well Table

Select Wells With: - Select Item - - Select Item -

Show in Wells
 View Legend
 Set as Default
 Print
 Refresh
 Close

	1	2	3	4	5	6	7	8	9	10	11	12
A												T.Large A T.Small A
B												T.Large A T.Small A
C												T.Large A T.Small A
D												T.Large A T.Small A
E												T.Large A T.Small A
F												T.Large A T.Small A
G												T.Large A T.Small A
H										T.Large A T.Small A	T.Large A T.Small A	

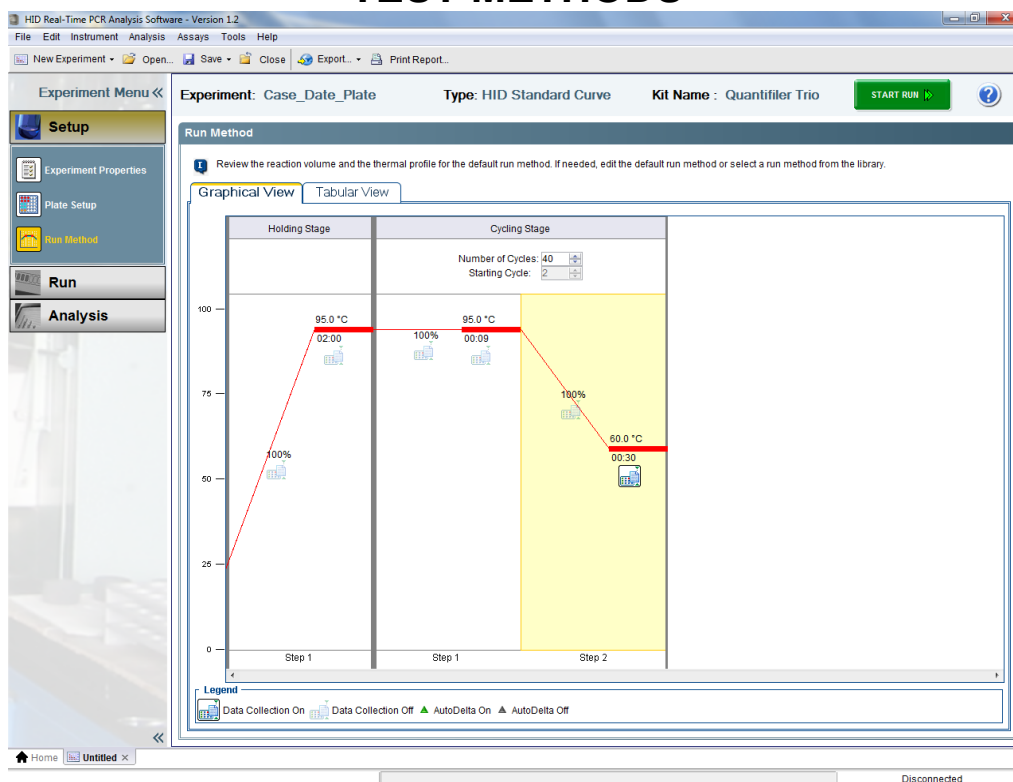
Wells: U 9 Unknown S 8 Standard N 1 Negative Control 87 Empty

1.11 Verify the **Run Method**.

Holding Stage: 95.0°C for 2.00 minutes.

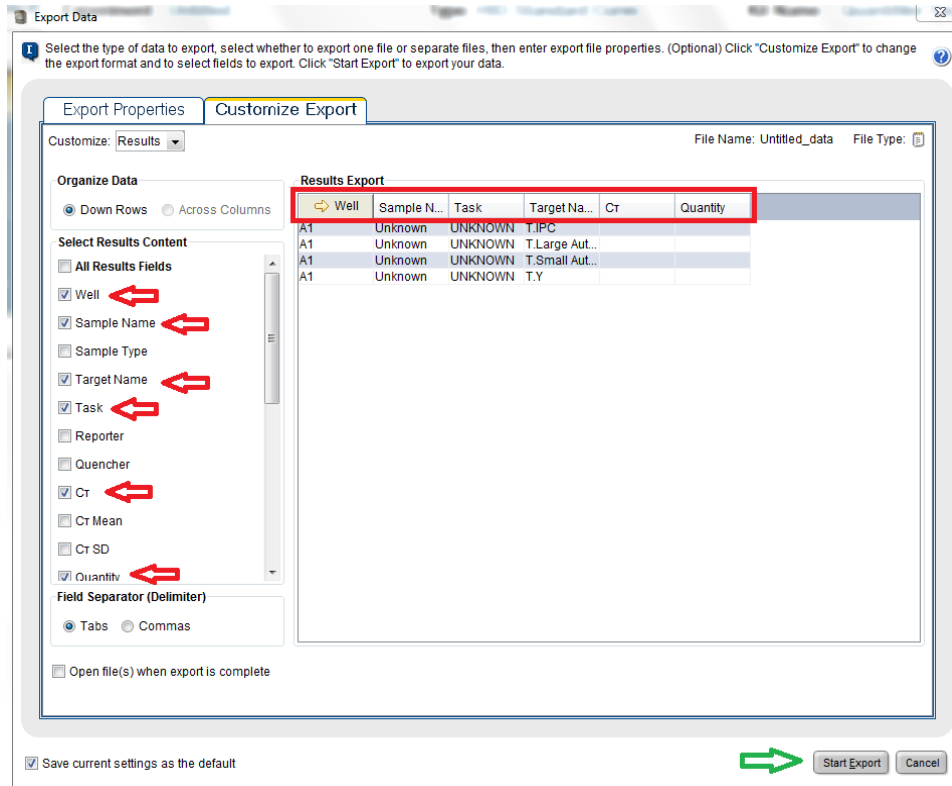
Cycling stage: 40 cycles of 95°C for 9 seconds, 60.0°C for 30 seconds.

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- 1.12 Select **Export** and change the options to match the following two images. Ensure the Results Export are in the following order: Well, Sample Name, Task, Target Name, C_T , and Quantity. Select **Save as default** at the bottom and **Start Export**.

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- 1.13 Overwrite the QuantifilerTrio.edt template by selecting **Save** → **Save changes as template**. Navigate to **AB_SW&DATA (D:) → Applied Biosystems** folder → **7500** folder → **config** folder → **templates** folder and select **QuantifilerTrio.edt**.

2. QuantStudio™ Design and Analysis Desktop Software v1.6.1 and Promega PowerQuant® run template set-up

- 2.1. On the instrument computer, download the Design and Analysis Software 1.6.1 from the PowerQuant and Quant Studio validation folder on the BiologyQA drive.
 - 2.1.1. Click on the “setup” file within the DA 1.6.1 Installer (Win 10) folder.
 - 2.1.2. Follow the Wizard instructions.
- 2.2. Create a “CASEWORK” folder and an “IMPORT” folder on the desktop.
- 2.3. Open the Design and Analysis Desktop Software on the desktop.
- 2.4. To add the PowerQuant® dyes,
 - 2.4.1. Select **Tools** from the toolbar.
 - 2.4.2. Select **Dye Library** from the drop-down menu.
 - 2.4.3. Add the five PowerQuant® custom dyes names by selecting the **New** button.
 - 2.4.4. Enter and save the following dye names: “PQ_FAM”, “PQ_CFG540”, “PQ_TMR”, “PQ_Q670”, and “PQ_CXR”.

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- 2.4.5. Confirm that “Reporter” is selected as the “Type” for each dye. **Note:** The dye names must match those entered in the Custom Dye selection when the dye calibrations were performed.
- 2.5. To create a run template,
- 2.5.1. Select the **Create New Experiment** icon on the home screen.
 - 2.5.2. Specify the following parameters in the Experiment Properties form (Figure 1).
 - 2.5.2.1. Enter PowerQuant_v#_Date in the Name field.
 - 2.5.2.2. Select **QuantStudio™ 5 System** for the instrument type.
 - 2.5.2.3. Select **96-Well 0.2-mL Block** for the block Type.
 - 2.5.2.4. Select **Standard Curve** for the experiment type.
 - 2.5.2.5. Select **TaqMan® Reagents** for the chemistry.
 - 2.5.2.6. Select **Standard** as the run mode.

QuantStudio™ Design & Analysis Software v1.6.1

File Edit Analysis Tools Help

Properties Method Plate Run Results Export

Experiment Properties

Save

Name: PowerQuant

Barcode: NA

User name: NA

Instrument type: QuantStudio™ 5 System

Block type: 96-Well 0.2-mL Block

Experiment type: Standard Curve

Chemistry: TaqMan® Reagents

Run mode: Standard

Manage chemistry details

Type in plate name!

Next

Home PowerQuant_v... x

Figure 1. The “Experiment Properties” screen.

- 2.5.3. Click **Next**.
- 2.5.4. Select the “Experiment Method” tab and modify the default run method as below. The completed PowerQuant® System run method is shown in Figure 2.

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- 2.5.4.1. Enter “20” in the “Volume” box.
- 2.5.4.2. Select the first Holding Stage and select the [-] icon to delete it.
- 2.5.4.3. Change the remaining Holding Stage to 98°C for 2 minutes.
- 2.5.4.4. Change Step 1 of the PCR Stage to 98°C for 15 seconds.
- 2.5.4.5. Change Step 2 of the PCR Stage to 62°C for 35 seconds.
- 2.5.4.6. Ensure the **Data Collection On** icon is active for Step 2 of the PCR Stage. **Note:** The icon legends are shown at the bottom of the screen.
- 2.5.4.7. Enter “39” for the number of cycles in the box below the PCR Stage.
- 2.5.4.8. Change the ramp rates for all three steps to “2.44°C/s”.

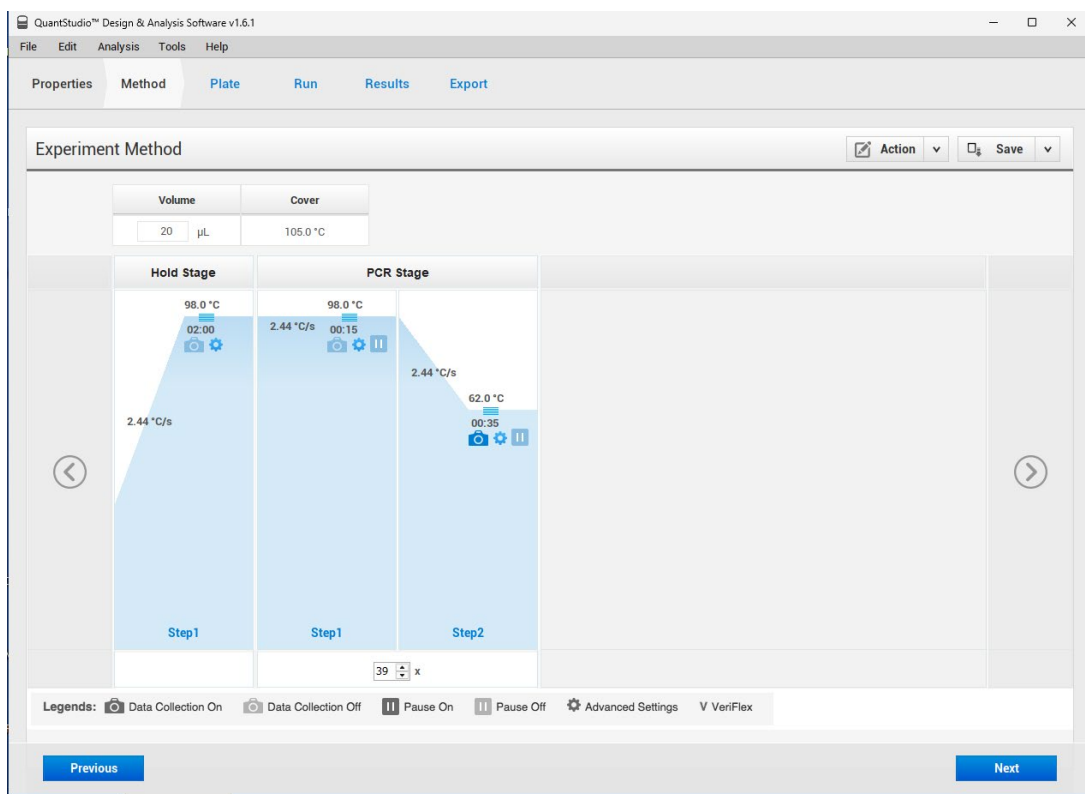


Figure 2. The completed PowerQuant® System run method.

- 2.5.5. Click **Next**.
- 2.5.6. Under the ‘Plate’ tab, enter the PowerQuant® targets in the Targets section of the “Advanced Setup” tab in the Assign Targets and Samples section. Select **[+] Add** three additional times and enter the following target-specific information.

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Target Name	Reporter	Quencher
Autosomal	PQ_FAM	NFQ-MGB
Degradation	PQ_Q670	NFQ-MGB
Y	PQ_CFG540	NFQ-MGB
IPC	PQ_TMR	NFQ-MGB

- 2.5.7. Highlight all wells (A12 – H12) that contain the DNA standards and select **S** as the Task for the autosomal, degradation, and Y targets. The Task for the IPC target should be **U**.
- 2.5.8. Enter the concentration of each DNA standard in the Quantity field without a unit of measure for all three targets. Highlight wells with DNA standards of the same concentration simultaneously then enter the value. The IPC Quantity will be blank.
 - 2.5.8.1. Enter “50” for wells A12 and B12.
 - 2.5.8.2. Enter “2” for wells C12 and D12.
 - 2.5.8.3. Enter “0.08” for wells E12 and F12.
 - 2.5.8.4. Enter “0.032” for wells G12 and H12.
- 2.5.9. In the Samples section of the Advanced Setup’ tab, select **[+] Add** three times and enter a sample name for each concentration of the DNA standards (e.g., Standard 1, Standard 2, Standard 3, and Standard 4).
- 2.5.10. Highlight all wells with DNA standards of the same name. Assign the DNA standard name to the selected wells by selecting the box adjacent to the corresponding DNA standard name under the Samples section. Repeat for each DNA standard.
- 2.5.11. Select the ‘Quick Setup’ tab. In the Plate Attributes section, select **PQ_CXR** as the Passive Reference.
- 2.5.12. Select **Analysis Settings** from the ‘Analysis’ drop-down menu.
- 2.5.13. On the ‘CT Settings’ tab, select the Autosomal Target.
- 2.5.14. In the CT Settings for the Autosomal section, uncheck the “Default Settings” box and uncheck the “Automatic Threshold” box. Enter “0.08” for the threshold. The “Automatic Baseline” box should be selected.
- 2.5.15. Repeat the process for the Degradation and Y targets using a threshold of “0.08” for each.
- 2.5.16. Repeat the process for the IPC target; however, enter “0.015” for the threshold. Uncheck the “Automatic Baseline” box and manually enter “3” for the ‘Baseline Start Cycle’ and “15” for the ‘End Cycle’.
- 2.5.17. Select **Apply**.
- 2.5.18. Select the ‘Export’ tab.

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- 2.5.19. Set the File Type to "QuantStudio" and ".txt".
- 2.5.20. Uncheck the "Open exported files when complete" box.
- 2.5.21. The "Results" box should be selected in the Content section. Deselect the "Sample Setup", "Amplification Data", "Raw Data", "Multicomponent Data", and "Reagent Information" boxes.
- 2.5.22. Under the Options selection, "Unify the above content into one file" should be selected.
- 2.5.23. Select the **Customize** button and confirm the following options in the Select Content list are selected: Well Position, Sample Name, Target Name, Task, CT, and Quantity. All other options should be unselected.
- 2.5.24. The boxes for "Skip Empty Wells" and "Skip Omitted Wells" at the top of the 'Customize' section should be unselected.
- 2.5.25. Click "Browse" to select a default location for files to be exported. This should be the CASEWORK folder on the desktop.
- 2.6. To save the run template with the above settings,
 - 2.6.1. Select **File** from the toolbar at the top of the screen, select **Save As**.
 - 2.6.2. Choose the desktop or another location to save the template file as an .edt file. Name the file the same name as chosen in Experiment Properties.
 - 2.6.3. From the desktop, click on the .edt file.
 - 2.6.4. Click on Properties.
 - 2.6.5. Make this template file "Read Only" by checking the appropriate box in the Attributes section. Select Apply and then OK.

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Instrument setup for Applied Biosystems® 3500 Genetic Analyzer - Data Collection Software version 2.0

(only necessary the first time PowerPlex® Fusion 6C samples are run or if the parameters change)

1.0 Configure the Security Settings

- 1.1 Navigate to **Tools** and select “Security”.
- 1.2 Change Screen settings to those depicted below.
- 1.3 Click “Save Settings”.

Security Screen:

Dashboard Edit ▾ Library Maintenance Tools ▾ Manage ▾ Preferences Help ▾ Log Out

Settings Resources

Applied Biosystems

Manage Reports
Audit Reports
E-Signature Reports

Manage Users
Users

Manage Settings
Security
Audit
E-Signature

Import
Export
Main Workflow

Enable System Security Disable System Security ⓘ

Account Setup ⓘ

User Name
The length of user names must be between 8 and 32 characters.

Define name spacing
☐ Leading ☐ Trailing ☒ Consecutive

Define name characteristics
☒ Alpha ☒ Numeric ☒ Uppercase ☒ Lowercase ☒ Special

User Password
The length of user passwords must be between 8 and 32 characters.

Define password spacing
☐ Leading ☐ Trailing ☒ Consecutive

Define password characteristics
0 Alpha 0 Numeric 0 Uppercase 0 Lowercase 0 Special

User may not reuse the previous 3 passwords.

Security Policies ⓘ

Password Expiration
Passwords will expire ☐ Yes ☒ No
every 60 days.
Notify the user 3 days before expiration.

Account Suspension
Login attempts with an incorrect password will suspend the user account ☐ Yes ☒ No
for the next 24 Hours
if consecutively failing 3 time(s)
within any 60 minute span.

Session Timeout
User sessions will be timed out if there is no user activity ☒ Yes ☐ No
for 60 minutes.
(An instrument run is not considered user activity.)

Setup Messaging Notifications Set Defaults Save Settings

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2.0 Edit User Roles

- 2.1 Click the "Users" button in the left navigation pane.
- 2.2 Open the "Roles" tab.
- 2.3 Set preferences to those depicted below.
- 2.4 Select "Scientist" and click "Edit".
- 2.5 Click "Save Role".

Scientist User Role:

The screenshot shows the 'Edit Role' window with the following content:

- Instruction:** Enter the name and description for your role, then select the permission(s) to assign to this role. When you are done select the "Save Role" button. * = Required
- 1. Define New Role:**
 - * Role Name: Scientist
 - Description:
- 2. Select Permission(s):**
 - ☒ Select All Permissions
 - ☒ Setup
 - ☒ Create Plate/Plate Template
 - ☒ Run
 - ☐ Edit Default Instrument Run Name
 - ☒ Manage Injection List
 - ☒ Duplicate Injection
 - ☒ Re-Inject
 - ☒ Primary Analysis
 - ☒ Edit Samples
 - ☐ Export Sequencing Results
 - ☐ Assays
 - ☐ Filename Conventions
 - ☐ Results Group
 - ☐ Instrument Protocol
 - ☐ PA Protocol
 - ☐ SA Protocol
 - ☐ QC Protocol
 - ☐ Size Standard
 - ☒ Plates and Plate Templates
 - ☒ Edit Plate and Plate Template
 - ☒ Delete Plate and Plate Template
 - ☒ Import Plate and Plate Template
 - ☒ Export Plate and Plate Template
 - ☐ Dye Sets
 - ☐ Locking

Buttons at the bottom: Close, Save Role

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

Edit Role

Instruction
Enter the name and description for your role, then select the permission(s) to assign to this role. When you are done select the "Save Role" button.
* = Required

1. Define New Role

* Role Name:

Description:

2. Select Permission(s)

- ☐ Locking
- ☒ Preferences
 - ☒ Edit System Preferences
 - ☐ Export System Preferences
 - ☐ Import System Preferences
 - ☐ Export User Preferences(All)
- ☒ Calibrations
 - ☒ Perform Spatial Calibration Run
 - ☒ Perform Spectral Calibration
- ☒ Performance Check
 - ☒ Running Performance Check Install Standards
- ☒ Archiving
 - ☒ Archive Datastore Objects
 - ☒ Purge Datastore Objects
 - ☒ Restore Datastore Objects
- ☐ SAE Configuration

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3.0 Create User Accounts

- 3.1 Click on the “Users” tab.
- 3.2 Click “Create” to access a New User window.
- 3.3 Enter a unique User Name (ex. jdoe8251), set “Password” to lowercase “password” and re-enter. Enter user’s first and last name and change settings to those depicted below. Ensure the “User Role” is either set as an “Administrator” or “Scientist”.
- 3.4 Click Save.

New User Window:

New User:

Setup a User

* User Name: first initial, last name, PE#

* Password: ••••••••

* Re-Enter Password: ••••••••

* First Name: Jane

* User Role: Scientist

* Dx User: ☐

Email:

Comments:

Created By Admin On:

☒ Pre-expired

Password Expires On:

MI:

* Last Name: Doe

* Status: Active

Electronic Signature ☐ Enable ☒ Disable

Phone:

Close Save

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

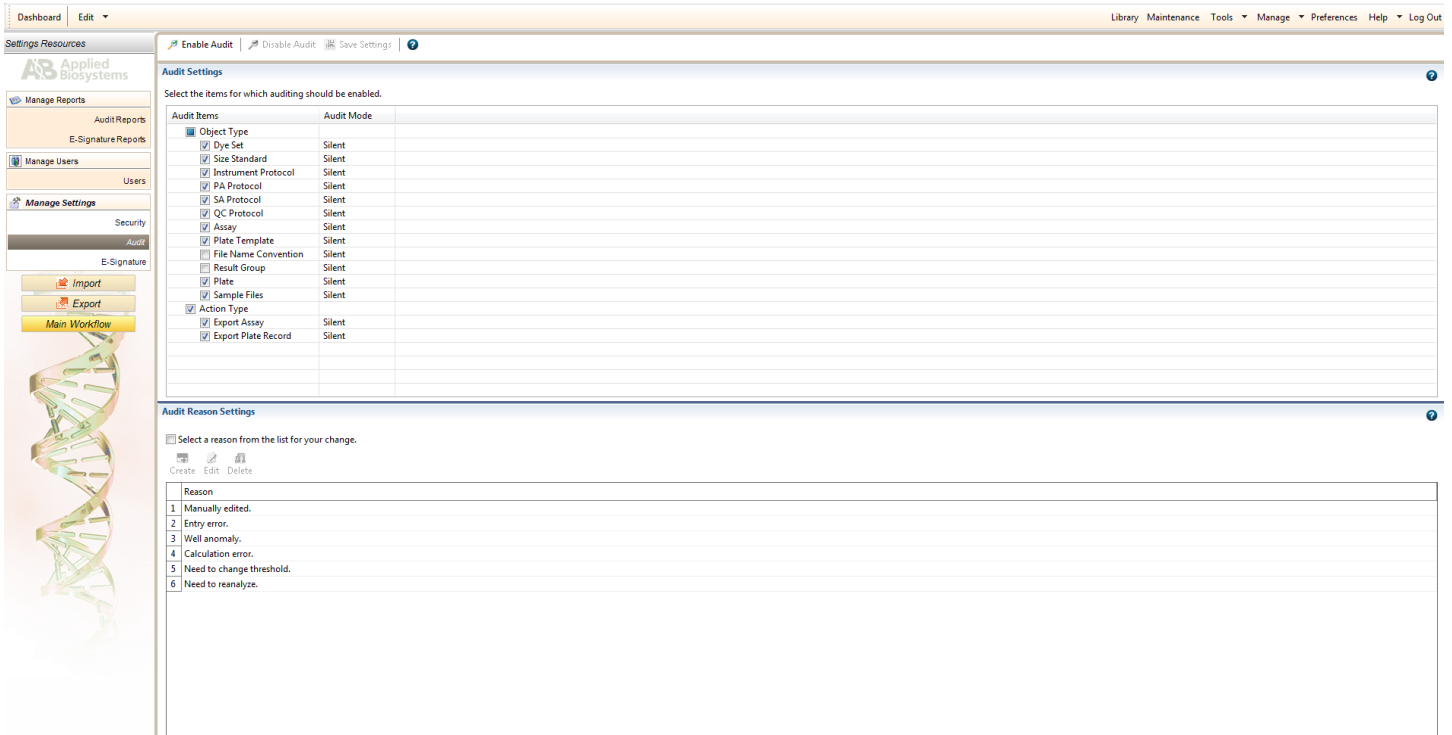
4.0 Manage Audit Settings

4.1 Navigate to **Tools** and Select “Audit”.

4.2 Turn Auditing off by ensuring the “Disable Audit” is grayed out and the “Enable Audit” appears black as depicted below.

4.3 Audit settings may be altered depending on current need.

Disable Audit Settings:



Dashboard | Edit

Library | Maintenance | Tools | Manage | Preferences | Help | Log Out

Settings Resources

AS Applied Biosystems

Manage Reports

Audit Reports

E-Signature Reports

Manage Users

Users

Manage Settings

Security

Audit

E-Signature

Import

Export

Main Workflow

Enable Audit | Disable Audit | Save Settings

Audit Settings

Select the items for which auditing should be enabled.

Audit Items	Audit Mode
☒ Object Type	
☒ Dye Set	Silent
☒ Size Standard	Silent
☒ Instrument Protocol	Silent
☒ PA Protocol	Silent
☒ SA Protocol	Silent
☒ QC Protocol	Silent
☒ Assay	Silent
☒ Plate Template	Silent
☐ File Name Convention	Silent
☐ Result Group	Silent
☒ Plate	Silent
☒ Sample Files	Silent
☒ Action Type	
☒ Export Assay	Silent
☒ Export Plate Record	Silent

Audit Reason Settings

Select a reason from the list for your change.

Create Edit Delete

Reason
1 Manually edited.
2 Entry error.
3 Well anomaly.
4 Calculation error.
5 Need to change threshold.
6 Need to reanalyze.

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5.0 Manage Electronic Signature Settings

5.1 Navigate to **Tools** and Select “E-Signature”.

5.2 Turn E-Signature off by ensuring that the “Disable E-Sig” is grayed out and the “Enable E-sig” is black as depicted below.

5.3 E-Signature settings may be altered depending on need.

Disable E-Signature Settings:

Dashboard | Edit | Library | Maintenance | Tools | Manage | Preferences | Help | Log Out

Settings Resources

AS Applied Biosystems

Manage Reports
Audit Reports
E-Signature Reports
Manage Users
Users
Manage Settings
Security
Audit
E-Signature
Import
Export
Main Workflow

Enable E-Sig | Disable E-Sig | Save Settings | ?

E-Signature Settings

Select the electronic signature types that should be allowed.

E-Signature Type
☐ Approve Dye Set
☐ Approve Size Standard
☐ Approve Spatial Calibration
☐ Approve Spectral Calibration
☐ Approve Instrument Protocol
☐ Approve Sizecall Protocol
☐ Approve Basecall Protocol
☐ Approve QC Protocol
☐ Approve Gene Mapper Protocol
☐ Approve Gene Mapper IDx Protocol
☐ Approve SeqScape Protocol
☐ Approve MicroSeq ID Protocol
☐ Approve Assay
☐ Approve Plate Template
☐ Approve Plate
☐ Approve Sample
☐ Approve Sequencing Install Standard Results
☐ Approve MicroSeqID Install Standard Results
☐ Approve BDTv1.1POP6 Install Standard Results
☐ Approve Fragment Install Standard Results
☐ Approve HID Install Standard Results

Select the functions after which the system will prompt for an electronic signature of the selected type.

Function

Select the functions before which the system will check for required electronic signatures.

Function	# Signatures Required	Authorities Required

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6.0 Create an Instrument Protocol

- 6.1 Navigate to the **Library** and select “Instrument Protocols”.
- 6.2 Select “Create”.
- 6.3 Select “HID” for the Application Type.
- 6.4 Select “36” for the Capillary Length.
- 6.5 Select “POP4” for the Polymer.
- 6.6 Select “Promega_J6” for the Dye Set.
- 6.7 Select “HID36_POP4” for the Run Module on the 8-capillary 3500. Select “HID36_POP4xl” on the 24-capillary 3500xl.
- 6.8 Enter “13.0” for the Run Voltage (kVolts).
- 6.9 Enter “1.2” for Injection Voltage (kVolts).
- 6.10 Enter “15” seconds for the Injection Time on the 8-capillary 3500. Enter “20” seconds on the 24-capillary 3500xl.
- 6.11 Change the Run Time (sec) to “1500”.
- 6.12 Name the protocol with the kit name and the injection voltage and time (i.e., Fusion6C_1.2kv15s).
- 6.13 Mark “Locked” and click “Save”.

Instrument Protocol:

Setup an Instrument Protocol

Application Type: **HID** Capillary Length: **36** cm Polymer: **POP4**

Dye Set: **Promega_J6**

Instrument Protocol Properties

* Run Module: **HID36_POP4**

* Protocol Name: **Fusion6C_1.2kv15s** ☒ Locked

Description: **Fusion6C for 8-capillary**

Oven Temperature (°C): **60** Run Voltage (kVolts): **13.0** PreRun Voltage (kVolts): **15** Injection Voltage (kVolts): **1.2**

Run Time (sec.): **1500** PreRun Time (sec.): **180** Injection Time (sec.): **15** Data Delay (sec.): **1**

▶ Advanced Options

Close Save

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7.0 Create a Size Standard

7.1 Navigate to the **Library** and select “Size Standard”.

7.2 Select “Create”.

7.3 Assign the name “WEN ILS 500”.

7.4 Select “Orange” for the Dye Color.

7.5 Type the following into the left column: 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, and 500. Click “Add Size(s) >>”.

7.6 Mark “Locked” and click “Save”.

Size Standard:

Edit Size Standard WEN ILS 500

Setup a Size Standard

* Size Standard: WEN ILS 500 ☒ Locked

Description: Fusion6C (60-500)

* Dye Color: Orange

Enter sizes in the field below separated by a comma, space, or return then click the "Add Size(s) >>" button to add them to the current size standard definition.

Enter new Size Standard definition: (e.g. 11.0, 34.2, 55)

Add Size(s) >>

* Current Size Standard definition: Delete Selected Sizes

60.0
65.0
80.0
100.0
120.0
140.0
160.0
180.0
200.0
225.0
250.0
275.0
300.0
325.0
350.0
375.0
400.0
425.0
450.0
475.0
500.0

Close Save

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8.0 Create a QC Protocol

- 8.1 Navigate to the **Library** and select “QC Protocols”.
- 8.2 Select “Create”.
- 8.3 Assign the name “WEN ILS 500”.
- 8.4 Change settings to match the figure below.
- 8.5 Mark “Locked” and click “Save”.

QC Protocol:

Edit QC Protocol Fusion6C (WEN ILS 500)

Setup a QC Protocol

* **Protocol Name:** Fusion6C (WEN ILS 500) ☐ Locked

Description:

Size Standard: WEN ILS 500

Sizecaller: SizeCaller v1.1.0

Analysis Settings **QC Settings**

Analysis Range: Full **Analysis Start Point:** 0 **Analysis Stop Point:** 1000000

Sizing Range: Partial **Sizing Start Size:** 60 **Sizing Stop Size:** 500

Size Calling Method: Local Southern

	Blue	Green	Yellow	Red	Purple	Orange
Peak Amplitude Threshold	150	150	150	150	150	150

Common Settings

Use Smoothing: Light

Use Baseline (Baseline Window (Pts)): 51

Minimum Peak Half Width: 2

Peak Window Size: 15

Polynomial Degree: 3

Slope Threshold Peak Start: 0.0

Slope Threshold Peak End: 0.0

Close **Save**

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9.0 Create an Assay

- 9.1 Navigate to the **Library** and Select “Assays”.
- 9.2 Select “Create”.
- 9.3 Title the assay name “Fusion6C_1.2kv15s” on the 8-capillary 3500, and “Fusion6C_1.2kv20s” on the 24-capillary 3500xl.
- 9.4 Select “HID” for the Application Type.
- 9.5 Select “Fusion6C (WEN ILS 500)” as the QC Protocol.
- 9.6 Select the appropriate instrument protocol.
- 9.7 Mark “Locked” and click “Save”.

Assay:

Edit Assay Fusion6C_1.2kv15s

Setup an Assay

* Assay Name: Fusion6C_1.2kv15s ☐ Locked Color: Cyan

Application Type: HID

Protocols

Do you wish to assign multiple instrument protocols to this assay? ☒ No ☐ Yes

* Instrument Protocol: Fusion6C_1.2kv15s Edit Create New

* QC Protocol: Fusion6C (WEN ILS 500) Edit Create New

GeneMapper IDX Protocol: Edit Create New

Close Save

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10.0 Create a Naming Convention

- 10.1 Navigate to the **Library** and Select “File Name Convention”.
- 10.2 Select “Create”.
- 10.3 Title the name convention “Fusion6C_FNC”.
- 10.4 Select the attributes and delimiters in the image below, click “Add”. The order of the attributes and delimiters can be changed using the “Move Up” and “Move Down” buttons.
- 10.5 Leave file location as the default.
- 10.6 Look at the “Preview of File Name” field for accuracy.
- 10.7 Mark “Locked”, and Click “Save”.

File Name Convention:

The screenshot shows the 'Edit File Name Convention' window for 'Fusion6C_FNC'. The window has a title bar with the text 'Edit File Name Convention Fusion6C_FNC'. Below the title bar is a header area with the text 'Setup a File Name Convention'. The main area contains several sections:

- Name:** A text field containing 'Fusion6C_FNC'.
- Locked:** A checkbox that is currently checked.
- Color:** A dropdown menu set to 'Cyan'.
- Select File Name Attributes:** A section with a 'Preview of File Name' field showing '<Sample Name>_<Instrument Protocol>_<Date of Run>_<Well Position>'. Below this are two lists: 'Available Attributes' (Capillary Number, Custom Text1, Custom Text2, Custom Text3, Injection Number, Instrument Name, Owner Name) and 'Selected Attributes' (Sample Name, Underscore (_), Instrument Protocol, Underscore (_), Date of Run, Underscore (_), Well Position). Between these lists are buttons for 'Add >>', '<< Remove', 'Move Up', and 'Move Down'. Below the 'Available Attributes' list is a 'Delimiters' section with a 'Select a delimiter' dropdown set to 'Underscore (_)' and an 'Add >>' button. There is also a checkbox for 'Add between attributes'.
- Add a custom value to available attributes (optional):** Three text fields labeled 'Custom Text 1:', 'Custom Text 2:', and 'Custom Text 3:'.
- Select File Location:** Two radio buttons: 'Default File Location' (selected) and 'Custom File Location'. The 'Default File Location' is set to 'D:\Applied Biosystems\3500\Data'. The 'Custom File Location' has a 'Browse...' button next to it.

At the bottom of the window are two buttons: 'Close' and 'Save'.

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11.0 Create a Results Group

- 11.1 Navigate to the **Library** and Select “Results Group”.
- 11.2 Select “Create”.
- 11.3 Title the results group “Fusion6C_RG”.
- 11.4 Select the attributes and delimiters in the image below, click “Add”. The order of the attributes and delimiters can be changed using the “Move Up” and “Move Down” buttons.
- 11.5 Ensure that “Store reinjection sample file with original sample files (same level)” is selected.
- 11.6 Leave file location as the default.
- 11.7 Click so the “Include a Result Group Name folder” box is selected.
- 11.8 Look at the “Preview of Results Group Name” field for accuracy.
- 11.9 Mark “Locked” and click “Save”.

Results Group:

Edit Results Group Fusion6C_RG

Setup a Results Group

* Name: Fusion6C_RG ☐ Locked Color: Cyan

Select Results Group Attributes

Preview of Results Group Name: <Plate Name>_<IP Name>

Available Attributes

Assay Name

Injection Number

Delimiters

Select a delimiter Underscore (_)

☒ Add between attributes

Add >>

Selected Attributes

Plate Name

Underscore (_)

IP Name

Add >>

<< Remove

Move Up

Move Down

Enter a custom value as either the Prefix or Suffix (optional)

Prefix: Suffix:

Select Reinjection Folder Option

☐ Store reinjection sample files in a separate Reinjection folder (same level as Injection folders)

☒ Store reinjection sample files with original sample files (same level)

Select Folder Option

☒ Default file location D:\Applied Biosystems\3500\Data\<Plate Name>_<IP Name>\

☐ Custom file location Browse...

☐ Include an Instrument Run Name folder

☒ Include a Result Group Name folder

☐ Include an Injection folder

Close Save

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS APPENDIX 4 GeneMapper™ ID-X

1. Setting Up GeneMapper™ ID-X Version 1.6 Software User Accounts and Security System

1.1 Setting Up Password Policies

- 1.1.1 Open the GeneMapper™ ID-X version 1.6 software.
- 1.1.2 Login using an administrator user account.
- 1.1.3 Select “Admin” then “Security Manager”.
- 1.1.4 Login with the same administrator user account.
- 1.1.5 Select “Settings” then “Password Policies”.
- 1.1.6 Change the settings to match the figure below.

Password Policies:

The screenshot shows the 'Password Policies' dialog box with the following settings:

- Attempts:**
 - Max Login Attempts: 5 count
 - Upon Failure:**
 - ☒ Send log message
 - Set User State:**
 - ☐ Remain active
 - ☒ Suspend for 10 min(s)
- Password:**
 - ☐ Password Lifetime: 90 days
 - Password Grace Logins: 6 count
- Password Reusability:**
 - ☒ Password Reuse Period: 30 days
 - Passwords kept per user: 10 count
- Password Format:**
 - Minimum Password Length: 6 characters

Buttons at the bottom: Save Changes, Cancel.

- 1.1.7 Save changes.

1.2 Creating a User Group

- 1.2.1 Open the Security Manager.
- 1.2.2 Select the appropriate default user group in the left navigation pane (e.g., “Casework User Group”).
- 1.2.3 Select “Edit” then “Duplicate”.

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- 1.2.4 In the “General” section, enter a name for the user group (e.g., “ISP Casework User Group”).
- 1.2.5 In the “Default Rights” section, make sure the “Read” and “Update” options are checked.
- 1.2.6 Select the “Security Groups” tab. Ensure all associated security groups are checked, including the “GeneMapper ID-X” security group.

1.3 Creating a Security Group

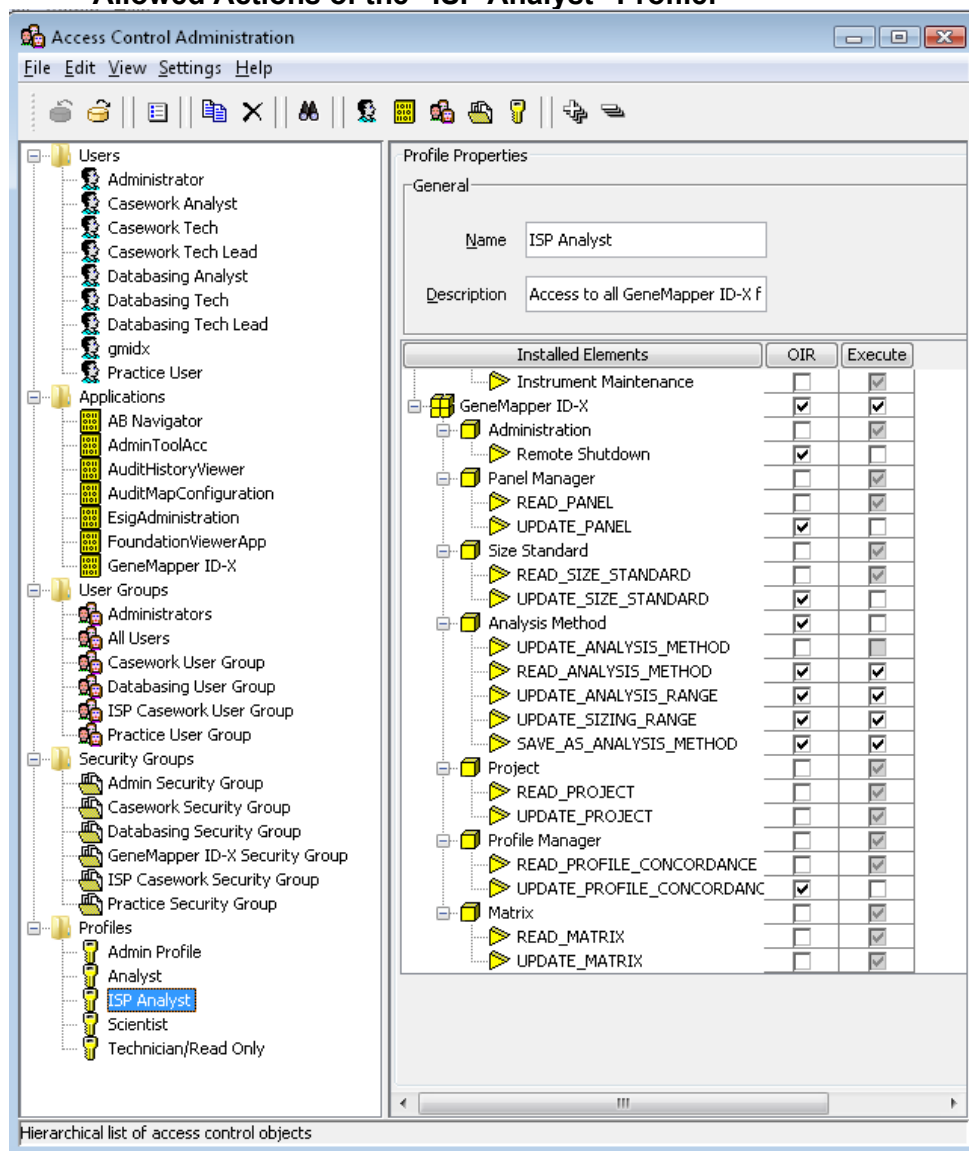
- 1.3.1 Open the Security Manager.
- 1.3.2 Select the appropriate default security group in the left navigation pane (e.g., “Casework Security Group”).
- 1.3.3 Select “Edit” then “Duplicate”.
- 1.3.4 In the “General” section, enter a name for the security group (e.g., “ISP Casework Security Group”).
- 1.3.5 In the “Associate” column, select the appropriate user groups to associate with that security group (e.g., “ISP Casework User Group”). Make sure the “Read” and “Update” options are checked for the selected user groups.

1.4 Creating a Profile

- 1.4.1 Open the Security Manager.
- 1.4.2 Select the appropriate default profile in the left navigation pane (e.g., “Analyst”).
- 1.4.3 Select “Edit” then “Duplicate”.
- 1.4.4 In the “General” section, enter a name for the profile (e.g., “ISP Analyst”).
- 1.4.5 In the “Installed Elements” table, select allowed actions for that particular profile. Allowed actions are not automatically inherited from the default profile and have to be manually entered. For the “ISP Analyst” profile, check the same allowed actions as those for the default GeneMapper™ ID-X “Analyst” profile, with the exception of the “Update Panel” and “Update Size Standard” actions. Change those actions to match the selections in the image below.

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Allowed Actions of the “ISP Analyst” Profile:



1.5 Setting Up a User Account

- 1.5.1 Open the Security Manager.
- 1.5.2 Select the appropriate default user type in the left navigation pane for that particular user (e.g., “Casework Analyst”).
- 1.5.3 Select “Edit” then “Duplicate”.
- 1.5.4 In the “General” section, enter a name. This will be the login name.
- 1.5.5 In the “User Details” section, enter a full name. Leave the status set to “Active.” Uncheck “Show EULA”.
- 1.5.6 Select a profile (e.g., “ISP Analyst”).
- 1.5.7 Select one or more user groups (e.g., “ISP Casework User Group”).

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- 1.5.8 In the “Password” section, select “Set Password”. Enter the same password twice and click “OK”. Check “Pre-Expire”.

2.0 GeneMapper™ ID-X Version 1.6 Software Settings - PowerPlex® Fusion 6C

2.1 Import Panel and Bin Files

- 2.1.1 Open the GeneMapper™ ID-X version 1.6 software.
- 2.1.2 Select **Tools** → **Panel Manager**.
- 2.1.3 Highlight the “Panel Manager” icon in the navigation pane.
- 2.1.4 Select **File** → **Import Panels**.
- 2.1.5 Navigate to the saved panel, bin, and stutter files. Select “Fusion6C_v3_ISP_Panels” and click “Import”.
- 2.1.6 Select the “ISP Casework Security Group”. Click “OK”.
- 2.1.7 In the navigation pane, highlight the “Fusion6C_v3_ISP” folder.
- 2.1.8 Select **File** → **Import Bin Set**.
- 2.1.9 Select “Fusion6C_v3_ISP_Bins” and click “Import”.
- 2.1.10 In the navigation pane, highlight the “Fusion6C_v3_ISP” folder.
- 2.1.11 Select **File** → **Import Marker Stutter**. A warning box will appear asking to overwrite the current values. Select “Yes”.
- 2.1.12 Select “Fusion6C_v3_ISP_stutter” and click “Import”.
- 2.1.13 In the Panel Manager window, select “Apply”, then “OK”.
- 2.1.14 Repeat this process with the panels, bins and marker stutter files for analysis of unknowns to be run in STRmix™.
 - 2.1.14.1 Panels: “STRmix_v3_Fusion_6C_ISP_Panels”.
 - 2.1.14.2 Bins: “STRmix_v3_Fusion6C_ISP_Bins”.
 - 2.1.14.3 Marker Stutter: “STRmix_v3_Fusion6C_ISP_stutter”.

2.2 Create a Casework Analysis Method

- 2.2.1 Select **Tools** → **GeneMapper Manager**.
- 2.2.2 Select the **Analysis Methods** tab.
- 2.2.3 Enter the name “Fusion6C_v3”.
- 2.2.4 Select the “ISP Casework Security Group”.
- 2.2.5 Enter “3500” as the instrument.
- 2.2.6 Select the **Allele** tab. In the “Bin Set” drop-down menu select “Fusion6C_ISP_Bins”. Verify that the “Use marker-specific stutter ratio and distance if available” box is checked. All values shall be “0.0”.
- 2.2.7 Select the Peak Detector tab. Change the settings to match those shown in the following figure.

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Peak Detector Tab:

Analysis Method Editor

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

Peak Detection Algorithm: Advanced

Ranges

Analysis	Sizing
Full Range	All Sizes
Start Pt: 0	Start Size: 0
Stop Pt: 10000	Stop Size: 1000

Smoothing and Baseline

Smoothing ☐ None ☒ Light ☐ Heavy

Baseline Window: 51 pts

Size Calling Method

☐ 2nd Order Least Squares
☐ 3rd Order Least Squares
☐ Cubic Spline Interpolation
☒ Local Southern Method
☐ Global Southern Method

Peak Detection

Peak Amplitude Thresholds:

B:	150	R:	150
G:	150	P:	150
Y:	150	O:	60

Min. Peak Half Width: 2 pts

Polynomial Degree: 7

Peak Window Size: 11 pts

Slope Threshold

Peak Start: 0.0

Peak End: 0.0

Normalization

☐ Use Normalization, if applicable

Factory Defaults

Save As Save Cancel Help

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2.2.8 Select the **Peak Quality** tab. Change the settings to match the following figure.

Peak Quality Tab:

The screenshot shows the 'Analysis Method Editor' window with the 'Peak Quality' tab selected. The window has a title bar with a close button. Below the title bar are five tabs: 'General', 'Allele', 'Peak Detector', 'Peak Quality' (selected), and 'SQ & GQ Settings'. The 'Peak Quality' tab contains several settings groups:

- Min/Max Peak Height (LPH/MPH):**
 - Homozygous min peak height: 950.0
 - Heterozygous min peak height: 150.0
 - Max Peak Height (MPH): 28000.0
- Peak Height Ratio (PHR):**
 - Min peak height ratio: 0.65
- Broad Peak (BD):**
 - Max peak width (basepairs): 1.5
- Allele Number (AN):**
 - Max expected alleles:
 - For autosomal markers & AMEL: 2
 - For Y markers: 1
- Allelic Ladder Spike:**
 - Spike Detection: Enable (dropdown menu)
 - Cut-off value: 0.2
- Sample Spike Detection:**
 - Spike Detection: Enable (dropdown menu)

At the bottom right of the settings area is a 'Factory Defaults' button. At the bottom of the window are four buttons: 'Save As', 'Save', 'Cancel', and 'Help'.

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2.2.9 Select the **SQ and GQ Settings** tab. Change the settings to match the following figure.

SQ and GQ Settings:

The screenshot shows the 'Analysis Method Editor' dialog box with the 'SQ & GQ Settings' tab selected. The dialog contains several sections for configuring quality weights and ranges.

Quality weights are between 0 and 1.

Sample and Control GQ Weighting

Broad Peak (BD)	0.8	Allele Number (AN)	1.0
Out of Bin Allele (BIN)	0.8	Low Peak Height (LPH)	0.3
Overlap (OVL)	0.8	Max Peak Height (MPH)	0.3
Marker Spike (SPK)	0.3	Off-scale (OS)	0.8
AMEL Cross Check (ACC)	0.0	Peak Height Ratio (PHR)	0.3

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

SQ Weighting

Broad Peak (BD) 0.5

Allelic Ladder GQ Weighting

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

SQ & GQ Ranges

	Pass Range:	Low Quality Range:
Sizing Quality:	From 0.75 to 1.0	From 0.0 to 0.25
Genotype Quality:	From 0.75 to 1.0	From 0.0 to 0.25

Reset Defaults

Save As Save Cancel Help

2.2.10 Select "Save".

2.2.11 Repeat these steps to create the "STRmix_Fusion6C_v3" analysis method used to analyze unknown profiles for STRmix™ interpretation. All steps are identical except the following:

2.2.11.1 Enter the name "STRmix_Fusion6C_v3".

2.2.11.2 Select the **Allele** tab. In the "Bin Set" drop-down menu select "STRmix_Fusion6C_ISP_Bins".

2.2.11.3 Select the **Peak Detector** tab. Ensure that the dye channel peak amplitude thresholds are set to the values in the following figure (100 for Blue, Green, and Yellow, 120 for Red, and 60 for Purple and Orange).

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Peak Detector Tab:

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Detector' tab selected. The 'Peak Detection Algorithm' is set to 'Advanced'. The 'Ranges' section has 'Analysis' set to 'Full Range' and 'Sizing' set to 'All Sizes'. The 'Start Pt' is 0 and 'Stop Pt' is 10000. The 'Start Size' is 0 and 'Stop Size' is 1000. The 'Smoothing and Baseline' section has 'Smoothing' set to 'Light' and 'Baseline Window' set to 51 pts. The 'Size Calling Method' section has 'Local Southern Method' selected. The 'Peak Detection' section has 'Peak Amplitude Thresholds' set to B: 100, R: 120, G: 100, P: 60, Y: 100, O: 60. The 'Min. Peak Half Width' is 2 pts, 'Polynomial Degree' is 7, and 'Peak Window Size' is 11 pts. The 'Slope Threshold' section has 'Peak Start' and 'Peak End' both set to 0.0. The 'Normalization' section has 'Use Normalization, if applicable' checked. A 'Factory Defaults' button is at the bottom right. At the very bottom of the dialog are 'Save As', 'Save', 'Cancel', and 'Help' buttons.

Analysis Method Editor

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

Peak Detection Algorithm: Advanced

Ranges

Analysis: Full Range Sizing: All Sizes

Start Pt: 0 Start Size: 0

Stop Pt: 10000 Stop Size: 1000

Smoothing and Baseline

Smoothing: ☐ None ☒ Light ☐ Heavy

Baseline Window: 51 pts

Size Calling Method

☐ 2nd Order Least Squares

☐ 3rd Order Least Squares

☐ Cubic Spline Interpolation

☒ Local Southern Method

☐ Global Southern Method

Peak Detection

Peak Amplitude Thresholds:

B: 100 R: 120

G: 100 P: 60

Y: 100 O: 60

Min. Peak Half Width: 2 pts

Polynomial Degree: 7

Peak Window Size: 11 pts

Slope Threshold

Peak Start: 0.0

Peak End: 0.0

Normalization

☒ Use Normalization, if applicable

Factory Defaults

Save As Save Cancel Help

2.2.11.4 Select the **Peak Quality** tab. Change the settings to match the following figure.

Peak Quality Tab:

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The screenshot shows the 'Analysis Method Editor' dialog box with the 'SQ & GQ Settings' tab selected. The dialog has several sections with input fields and dropdown menus. At the bottom are buttons for 'Save As', 'Save', 'Cancel', 'Help', and a 'Factory Defaults' button.

Section	Parameter	Value
Min/Max Peak Height (LPH/MPH)	Homozygous min peak height	60.0
	Heterozygous min peak height	60.0
	Max Peak Height (MPH)	28000.0
Peak Height Ratio (PHR)	Min peak height ratio	0.01
Broad Peak (BD)	Max peak width (basepairs)	1.5
Allele Number (AN)	Max expected alleles:	
	For autosomal markers & AMEL	20
	For Y markers	20
Allelic Ladder Spike	Spike Detection	Enable
	Cut-off value	0.2
Sample Spike Detection	Spike Detection	Enable

2.3 Create a Size Standard

- 2.3.1 Select **Tools** → **GeneMapper Manager**.
- 2.3.2 Select the **Size Standard** tab and click "New".
- 2.3.3 Name the size standard "WEN ILS 500" in the "Size Standard Editor" screen, choose the "ISP Casework Security Group", and choose "Orange" as the color for the size standard dye. If "WEN ILS 500" is being utilized for both casework and databasing analysis methods on the same GMID-X database, the "GeneMapper ID-X Security Group" should be chosen to allow both the ISP Casework and Database security groups access.
- 2.3.4 Enter the sizes of the 21 allelic ladder fragments: 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, and 500.
- 2.3.5 Select "OK".

2.4 Create a Table Setting

- 2.4.1 Select **Tools** → **GeneMapper Manager**.
- 2.4.2 Select the **Table Setting** tab and click "New".

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TEST METHODS

- 2.4.3 Under the **General** tab name the Table Setting “STRmix_Fusion6C” and select the “ISP Casework Security Group”.
- 2.4.4 Under the **Samples** tab ensure that check marks are located next to only the following: Status, Sample File, Sample Name, Sample Type, Analysis Method, Panel, Size Standard, Sizing Quality Overridden, Sample File Not Found, Sample Off-Scale, and Sizing Quality.
 - 2.4.4.1 Sort by “Sample Type” and then by “Sample Name”. Select the “Ascending” option for both, and leave the final sort option to “None”.
 - 2.4.4.2 Leave the font “Arial” and the size “11”.
- 2.4.5 Under the **Genotypes** tab ensure that check marks are located next to only the following: Sample Name, Marker, Allele, Size, and Height.
 - 2.4.5.1 Sort by “Sample File”, then by “Sample Name”, and then by “Marker”. Select the “Ascending” option for all three.
 - 2.4.5.2 Change the “Show number of alleles” to 20 and ensure that “Keep Allele, Size, Height, Area, Data Point, Mutation and Comment together” is NOT checked. (If the option to “Keep Allele, Size, Height...” is not visible in the window, expand the window size until the option appears.)
 - 2.4.5.3 Leave the font “Arial” and the size “11”.
- 2.4.6 Select “OK”.
- 2.5 **Create a Plot Setting – Samples, Controls, and Ladders**
 - 2.5.1 Select **Tools** → **GeneMapper Manager**.
 - 2.5.2 Select the **Plot Settings** tab and click “New”.
 - 2.5.3 Under the **General** tab name the Plot Setting “Fusion6C” and select the “ISP Casework Security Group”.
 - 2.5.4 Under the **Sample Header** tab ensure that check marks are located next to the following: Sample File, Sample Name, Panel, Sizing Quality Overridden, Sample Off-Scale, and Sizing Quality.
 - 2.5.5 Under the **Genotype Header** tab ensure that check marks are located next to the following: Sample File, Sample Name, Panel, Marker, Sample Off-Scale, Out of Bin Allele, Peak Height Ratio, Control Concordance, and Genotype Quality.
 - 2.5.6 Under the **Sizing Table** tab ensure that check marks are located next to the following: Dye/Sample Peak, Sample File Name, Marker, Allele, Size, Height, Area, and Data Point. Leave the font “Arial” and the size “11”.
 - 2.5.7 Select the **Labels** tab. Change the settings to match the following figure. Alternatively, if the “allele edit comment” function is not being used, “Label 4” for “Assigned Allele”, “Custom Allele” and “Artifact” can be changed to “NONE”.

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Labels Tab:

Plot Settings Editor

General Sample Header Genotype Header Sizing Table **Labels** Display Settings

Show Labels on Samples and Genotypes Plot:

Labels

	Assigned Allele	Custom Allele	Allelic Ladder	Artifact
Label 1:	Allele Call	Allele Call	Allele Call	Artifact Label
Label 2:	Height	Height	NONE	Height
Label 3:	Size	Size	NONE	Size
Label 4:	AE Reason for Cha...	NONE	NONE	NONE

Font

Font: Times New Roman

Size: 9

When opening the Plot Window:

- ☐ Show PQV trigger peak (LPH,MPH,BD,OS)
- ☐ Show data type prefixes
- ☒ Display virtual allele label in black
- ☐ Show type of edit

Label Color: Dye Color-Border

OK Cancel Help

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2.5.8 Under the **Display Settings** tab, change the display settings so that they match the following figure and select "OK".

Display Settings Tab:

Plot Settings Editor

General | Sample Header | Genotype Header | Sizing Table | Labels | **Display Settings**

When opening the Plot Window:

☐ Use the display settings last used for this plot

☒ Use these display settings:

For both Sample and Genotype plots:

Panes: 2

Labels:

☐ No Labels

☐ Horizontal Labels

☒ Vertical Labels

Show:

☒ Plot Header

☒ Marker Range

☐ Marker Indicators

☒ Bins

☒ Toolbar

☐ Peak Positions

☐ Bring Ctrl's to Top

☐ Bring Ladders to Top

☒ Allele Changes

☒ Off-scale

Axes:

Y-Axis: Scale individu...

X-Axis: Basepairs

For Sample plot only:

Select Dyes:

☒ Blue

☒ Green

☒ Yellow

☒ Red

☒ Purple

☒ Orange

☒ All Dyes

All-Dye Range (bp): *

Start Range: 0.0

End Range: 600.0

Labels:

☒ Size Std Labels

Print:

☐ Quality Value Details

Tables:

☒ No Table

☐ Sizing Table

☐ Genotypes Table

☐ Label Edit Viewer

Dye Layout:

☐ Combine Dyes

☒ Separate Dyes

☐ Overlay All

Custom Colors

For Genotype plot only:

Marker Margin: 5 bp

* Will be overridden if Retain X-axis Zoom Range is enabled on Plots ->Zoom menu

OK Cancel Help

2.6 GeneMapper™ ID-X Maintenance

2.6.1 The number of audit records on the GeneMapper™ ID-X database should be routinely checked. Audit records should be backed-up, saved to the DNA server, and then deleted from the GeneMapper™ ID-X database routinely or if the number of records exceeds 40,000. If the number of audit records exceeds 60,000, the performance of the software may be affected.

2.6.2 The amount of database space in the GeneMapper™ ID-X software should be routinely checked. If the occupied space exceeds 80%, additional disk space should be allocated.

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS APPENDIX 5 STRmix™ SETTINGS

1.0 STRmix™ Installation and Set-up

- 1.1 Install the current version of STRmix™ (v2.7.0).
- 1.2 Copy and Replace the following files from the BiologyQA drive to the STRmix™ Program files located on the (C:) drive:
 - 1.2.1 AlleleFreq folder, config folder, Databases folder, jre folder, Kits folder, logs folder, Populations folder, Results folder, stutters folder, Default Configuration settings, ISP Lab Logo, and Report Defaults XML document.
- 1.3 Update to the floating license:
 - 1.3.1 After opening STRmix™, if no prompt comes up with the ability to install a license/validate license server, select **About**, and then select **Remove License**. It will tell you that you won't be able to use STRmix™ again until a license is installed and will close the program.
 - 1.3.2 Reopen the program and follow the prompt to validate the license server.
 - 1.3.3 In the Host name, put the following IP address – 10.16.105.102 and the port# 16090.
 - 1.3.4 Select **Validate**. It should then connect via the floating license server and access a license.
- 1.4 Kit Parameters
 - 1.4.1 To access the run parameter settings from the STRmix™ main menu, select **Administration** (an administrator password shall be required), then select **Profiling Kits**.
 - 1.4.2 Navigating the **General**, **Stutters**, and **Loci** tabs, ensure that the correct kit settings are established. The following settings shall be used for the interpretation of profiles:

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STRmix

STRmix.

< MANAGE KITS

Profiling Kits

ISP_Fusion6C

ISP_Fusion6C_v2

ISP_Fusion6C_v3

GENERAL

STUTTERS

LOCI

IMPORT

Kit Type

Fusion6C

Size Regression File

Fusion6C_SizeRegression_ISP.csv

Edit

VARIANCE

Allelic Variance

8.249, 1.296

Locus Amplification Variance

0.008

Minimum Variance Factor

0.5

Variance Minimization Parameter

1,000

Cancel

Save

Profiling Kits

ISP_Fusion6C

ISP_Fusion6C_v2

ISP_Fusion6C_v3

GENERAL

STUTTERS

LOCI

IMPORT

DROP-IN

Drop-in Cap

200

Drop-in Frequency

0.000371

Drop-in Distribution Parameters

☐ Uniform 2.4, 28.93

ADDITIONAL THRESHOLDS

Maximum Degradation

0.01

Saturation Threshold

28,000

Degradation Start Point

☒ Use Smallest Peak

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

GENERAL	STUTTERS	LOCI	IMPORT
BACK STUTTER			
☒ Stutter Enabled		Position Relative to Parent -1, 0	
Inversely Proportional To Observed Height of Parent Allele ▼			
Maximum Stutter Ratio ☐ No Maximum 0.3		Variance 1.825, 9.576	
Stutter Regression File Indiana_Fusion_6C_backward_stutter_v2.csv ▼			Edit
Stutter Exceptions File Indiana_stutter_exception_table_v2.csv ▼			Edit

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

FORWARD STUTTER

☒ Stutter Enabled

Position Relative to Parent

1, 0

Inversely Proportional To

Expected Height of Stutter Peak ▼

Maximum Stutter Ratio

☐ No Maximum 0.15

Variance

2.208, 2.915

Stutter Regression File

Indiana_Fusion_6C_forward stutter_v2.csv ▼

Edit

Stutter Exceptions File

Select a value ▼

Edit

SE33 -2 STUTTER

☒ Stutter Enabled

Position Relative to Parent

0, -2

Inversely Proportional To

Expected Height of Stutter Peak ▼

Maximum Stutter Ratio

☐ No Maximum 0.15

Variance

1.867, 0.767

Stutter Regression File

Indiana_Fusion_6C_half-backward stutter_v2.csv ▼

Edit

Stutter Exceptions File

Select a value ▼

Edit

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

GENERAL		STUTTERS	LOCI	IMPORT			
LOCUS NAME	GENDER?	REPEAT LENGTH	IGNORE?	DETECTION THRESHOLD	BACK STUTTER	FORWARD STUTTER	SE33 -2 STUTTER
AMEL	☒						
D3S1358	☐	4	☐	100	☒	☒	☐
D1S1656	☐	4	☐	100	☒	☒	☐
D2S441	☐	4	☐	100	☒	☒	☐
D10S1248	☐	4	☐	100	☒	☒	☐
D13S317	☐	4	☐	100	☒	☒	☐
Penta E	☐	5	☐	100	☒	☒	☐
D16S539	☐	4	☐	100	☒	☒	☐
LOCUS NAME	GENDER?	REPEAT LENGTH	IGNORE?	DETECTION THRESHOLD	BACK STUTTER	FORWARD STUTTER	SE33 -2 STUTTER
D18S51	☐	4	☐	100	☒	☒	☐
D2S1338	☐	4	☐	100	☒	☒	☐
CSF1PO	☐	4	☐	100	☒	☒	☐
Penta D	☐	5	☐	100	☒	☒	☐
TH01	☐	4	☐	100	☒	☒	☐
vWA	☐	4	☐	100	☒	☒	☐
D21S11	☐	4	☐	100	☒	☒	☐
D7S820	☐	4	☐	100	☒	☒	☐

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

LOCUS NAME	GENDER?	REPEAT LENGTH	IGNORE?	DETECTION THRESHOLD	BACK STUTTER	FORWARD STUTTER	SE33 -2 STUTTER
D5S818	☐	4	☐	100	☒	☒	☐
TPOX	☐	4	☐	100	☒	☒	☐
D8S1179	☐	4	☐	120	☒	☒	☐
D12S391	☐	4	☐	120	☒	☒	☐
D19S433	☐	4	☐	120	☒	☒	☐
SE33	☐	4	☐	120	☒	☒	☒
D22S1045	☐	3	☐	120	☒	☒	☐
DYS391	☒						
FGA	☐	4	☐	60	☒	☒	☐
DYS576	☒						
DYS570	☒						

1.4.3 In the “Gender?” column, AMEL, DYS391, DYS576, and DYS570 shall be checked.

1.4.5 The Detection Threshold for shall be set at 100, 120, or 60, depending on the associated dye.

1.4.6 In the “Back Stutter” and “Forward Stutter” columns, all autosomal loci shall be checked.

1.4.7 In the “SE33 -2” column, SE33 shall be checked.

1.5 Default Settings

1.5.1 To access the default settings, from the STRmix™ main menu, select **Administration** (an administrator password shall be required), then select **STRmix Defaults**.

1.5.1.1 Navigating the **General**, **Likelihood Ratio**, and **User Interface** tabs, ensure that the correct kit settings are established. The following default analysis settings shall be used for the interpretation of profiles:

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

< DEFAULT SETTINGS

GENERAL

LIKELIHOOD RATIO

DATABASE SEARCH

USER INTERFACE

Default Profiling Kit

ISP_Fusion6C_v3

Results Directory

Results

Browse

MCMC

Number of Chains

8

Burn-in Accepts (per chain)

10,000

Post Burn-in Accepts (per chain)

50,000

Random Walk SD

0.005

Post Burn-in Shortlist

9

☐ Extended Output

GELMAN-RUBIN

☒ Auto-Continue on GR

Gelman-Rubin Threshold

1.2

Extra Accepts

50,000

CONTRIBUTOR RANGE

Hyper-Rectangle Percent Accepts

2.5

PERFORMANCE

Number of Threads

8

☐ Low Memory Mode

GENERAL

LIKELIHOOD RATIO

DATABASE SEARCH

USER INTERFACE

DEFAULT POPULATIONS

NIST_2017_Combined

SUB-SOURCE LR

☒ Assign Sub-Source LR

CONTRIBUTOR RANGE

Priors Method

MLE

SAMPLING VARIATION

☒ Calculate HPD

☒ MCMC Uncertainty

☒ Allele Frequency Uncertainty

Number of HPD Iterations

1000

Probability Interval Quantile

99

Probability Interval Sides

1

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GENERAL	LIKELIHOOD RATIO	DATABASE SEARCH	USER INTERFACE
Database File Q:\STRmix Database\STRmix Employee Database.csv Browse			
Minimum LR 10,000	Population For Search NIST_2017_Combined		
Type of Search Standard	FST For Search 0.01b(1.0, 1.0)	☒ Assign Sub-Source LR	Priors Method MLE
☒ Auto Database Search			

GENERAL	LIKELIHOOD RATIO	DATABASE SEARCH	USER INTERFACE
Default Text File Directory C:\ProgramData\STRmix\Results Browse			
☐ Allow Text Sample Renaming			

1.6 Population Settings

- 1.6.1 The NIST_2017_Combined population database shall be used to perform statistical calculations.
- 1.6.2 To access the population parameter settings, from the STRmix™ main menu, select **Administration**, then select **Populations**.
- 1.6.3 The following population settings shall be used for the interpretation of profiles.
 - 1.6.3.1 F_{ST} Coancestry Coefficient, Theta (θ): corrects for two alleles identical by descent in different individuals. An F_{ST} of 0.01 was selected for the four populations.

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

Allele Frequency File NIST_2017_Combined.csv ▼		
Edit		
Population Proportion 1	Default FST 0.01b(1.0, 1.0)	
Population Size 6,500,000	Children Per Family 4	
Sibling Proportion 4.615384615384616E-7	Child Proportion 4.102564102564102E-7	Parent Proportion 2.051282051282051E-7
Uncle/Aunt Proportion 6.153846153846154E-7	Niece/Nephew Proportion 1.230769230769231E-6	Grandparent Proportion 2.051282051282051E-7
Grandchild Proportion 8.205128205128205E-7	Cousin Proportion 3.692307692307692E-6	Unrelated Proportion 0.9999923589743589

1.7 Report Settings

- 1.7.1 To access the Summary Report settings, from the STRmix™ main menu, select **Administration**, then select **Report Defaults**, and then select **Summary Report**.
- 1.7.2 Navigating the **Per Report Settings** and **General Settings** tabs, ensure that the correct settings are established. The following report settings shall be used. Drag the individual **Included Components** to arrange their reporting order.

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

PER REPORT SETTINGS
GENERAL SETTINGS

Calculation Type
Interpretation/LR From Previous

REPORTS TO RUN

☒ Summary report
☐ CODIS report

SUMMARY REPORT SETTINGS

COMPONENT	INCLUDE?	NEW PAGE?
Contributor Summary	☒	☐
Post Burn-In Summary	☒	☐
Evidence Peak Issues	☒	☐
Variance Charts	☒	☐
Contributor Degradation Curves	☒	☐
Locus Efficiencies	☒	☐
Evidence Files	☒	☐
Reference Files	☒	☐
Settings	☒	☐
Kit Files	☒	☐
Interpretation Details	☒	☐
Weights	☒	☐
Component Interpretation	☒	☐
LR Summary	☒	☐

<
DEFAULT REPORT SETTINGS

PER REPORT SETTINGS
GENERAL SETTINGS

Lab Logo
C:\Users\Johnson7457\Desktop\STRmix 2.7 Trial\ISP Lab Logo.jpg

Browse

Page Size
Letter

Bottom Margin
0

Date Format
dd MMM yyyy HH:mm:ss

☒ Include Audit Info

☐ Auto-Open Report

1.7.5 You may need to browse to your local STRmix™ folder to correctly path to the lab logo.

1.7.6 Select **Save**.

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Appendix 6

PowerPlex® Fusion 6C Mean and Mean + 3SD Stutter

The average observed percent stutter for each locus and mean +3SD (99.7% confidence level) of stutter observed in the PowerPlex® Fusion 6C validation are listed in the table below. Stutter exception files utilized in STRmix™ analysis are determined through different methods outlined and documented in the STRmix™ validation.

	Backward n-3/n-4/n-5 bp		Double Backward n-6/n-8/n-10 bp		Forward n+3/n+4/n+5 bp	
	Mean %	Mean + 3SD %	Mean %	Mean + 3SD %	Mean %	Mean + 3SD %
D3S1358	9.13	14.1	0.74	1.43	0.64	1.39
D1S1656	8.51	14.3	0.91	1.87	0.97	1.84
D2S441	5.03	8.55	0.38	0.69	0.78	1.49
D10S1248	8.31	13.8	0.73	1.46	0.35	1.04
D13S317	5.05	10.9	0.39	0.65	0.87	2.01
Penta E	2.75	6.99	0.41	0.92	0.37	0.84
D16S539	7.04	12.6	0.61	1.27	1.07	2.37
D18S51	8.08	14.9	0.80	1.59	1.07	3.11
D2S1338	8.23	13.8	0.78	1.49	0.80	1.54
CSF1PO	6.50	12.1	0.51	0.81	1.08	2.49
Penta D	1.90	3.91	0.43	1.10	0.56	1.37
TH01	2.07	4.60	0.34	0.82	0.38	0.79
vWA	7.43	16.8	0.75	1.57	0.66	1.74
D21S11	8.51	12.3	0.72	1.42	1.07	1.90
D7S820	5.38	11.1	0.53	1.12	0.76	1.76
D5S818	6.53	11.1	0.55	1.12	1.00	1.88
TPOX	3.01	6.42	0.31	0.50	0.23	0.31
D8S1179	6.96	10.7	0.60	1.08	0.70	1.28
D12S391	9.68	17.9	1.01	2.40	0.81	2.38
D19S433	7.85	12.5	0.81	2.37	1.06	2.58
SE33	10.5	18.1	1.05	2.02	1.04	2.25
D22S1045	7.55	18.1	1.01	1.86	4.50	9.48
DYS391	6.79	8.71	0.77	1.64	0.73	2.02
FGA	7.21	12.4	0.81	1.86	0.77	2.21
DYS576	8.89	12.1	0.89	2.27	1.28	2.83
DYS570	8.96	12.9	0.97	1.62	1.07	1.82

	Half Backward n-2 bp	
	Mean %	Mean + 3SD %
D1S1656	1.68	2.97
SE33	4.46	5.53
FGA	0.47	0.97

	Half Forward n+2 bp	
	Mean %	Mean + 3SD %
SE33	1.26	2.25

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REPORT WORDING RULES

These are general wording guidelines for commonly occurring test results. Alterations must be approved by a supervisor. Many of the words in these guidelines that are in uppercase should be changed to reflect your results (e.g. PERSON, ITEM X, etc.). Words or phrases in [] are optional.

In this appendix, the word “subitem” refers to a part of a submitted item of evidence that is considered distinctly separate and would not be combined with other subitems for DNA testing (due to case circumstances, sample purpose, serological results, etc.), regardless of whether or not a sample is retained. (E.g., vaginal/cervical swabs and external genital swabs are subitems within a kit item; a belt and a shirt may be subitems within a whole item; two submitted swabs with different serology results are considered separate subitems.)

REPORT FORMATTING

1. Reports shall be typed into a Word document with specific formatting.
2. The Word document shall have no indents, tabs, special formatting, or font/size changes. The report shall be written in default Normal style.
3. **Original items** and their descriptions shall be bolded and underlined. Multiple original items shall be separated with a horizontal line of underscores.
 - If related, original items shall be addressed in additional reports such as additional standard comparisons, Y-STR and paternity requests (see example [10](#))
4. **Subitems** and their descriptions (if applicable) may be bolded (see examples [2,3](#) and [4](#)).
 - Subitem headers and required formatting (bold, underlined, or italicized) are required when you have different tiers in the same report (e.g. in SAK's). Headers are not required for simple items, especially if nothing is retained or amplified (see example [9](#)).
 - When a subitem is reported it shall be bolded and underlined if the original item is not present and was previously reported (as in additional submissions).
5. Sub-subitems may be grouped by results under underlined headings (see example [6](#)).

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6. *Sperm and Non-sperm fractions*, where DNA results are different, shall be separated under italicized headings (see item 001C in [example 1](#)).
7. *Sub-sub-subitems*, if needed, are listed under italicized headings.
8. Line breaks: At least one blank line shall separate all items, subitems, and other individual results. Multiple blank lines may be used to enhance clarity or readability.
9. Exclusions and comparisons without LR (inclusions of unknown individuals) shall be listed with the contributor assumption before the bullets (see item 002A in [example 6](#)). The word excluded shall be bolded, except when *including* an individual (i.e., cannot be excluded).
10. Likelihood ratios:
 - Each likelihood ratio paragraph shall be preceded by a triangle text bullet: ►
 - The word inclusion/exclusion/uninformative in the LR paragraph shall be bolded.

GENERAL REPORT RULES

1. Report wording may be altered with the approval of a Biology Unit Supervisor.
2. The appropriate appendix with appropriate definitions and explanations shall be attached to the end of each report. Reports in which the entire request has been withdrawn do not require an appendix.
3. Serology results shall be reported prior to any DNA results for each item.
4. No serology statement is needed if no serological testing is performed.
5. Item numbers shall include all leading zeroes.
6. Items shall be reported by item in numerical order.
7. Item descriptions in the results section:
 - The description of each item shall be similar to but need not be identical to the item description in the submission section.
 - The description shall not include the packaging.
 - The description shall include the number of swabs/samples in parentheses when it is not otherwise apparent.
8. If subitems created for DNA sampling are retained by the Forensic Services Division, this shall be reported (see [examples](#)). When multiple swabs/samples within an item are being tested, the number of items tested shall be reported (see [examples](#)).
9. Retention statements generally will be in the opening paragraph for an item.

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10. The significant contents of an item shall be listed in the results section.
11. At a minimum, the first and last name of any individuals being compared shall be used throughout the entire report. The item number of the standard shall not follow the name in the results unless individuals cannot otherwise be distinguished.
12. Individual items from another case should not be listed in the item descriptions or as separate items in the body of the report.
13. Similar results within an item may be grouped together.
14. Swabs utilized for DNA sampling, shall be reported as a "sample" of the item being tested or the sample itself. When multiple samples of an item are extracted separately, they are considered separate samples and the number of samples shall be reported.
15. All submitted samples in an item (excluding standards) not tested shall be described as not tested in the report using the appropriate wording.
16. When two or more samples are combined at any point in the analysis process, the report shall refer to the samples as "combined". The analyst's notes shall clearly describe which samples and at which stage of the process the samples were combined.
 - If all subitems or samples within the item (not collected by the analyst) are not combined into one extract, the report shall indicate how many subitems/samples were combined and how many were not tested.
 - If multiple swabs are collected by the analyst and treated as one sample, the use of "combined" is not required.
17. In a sample with insufficient (male) DNA for amplification, where it is valuable to identify a male contributor from a female contributor (i.e., sexual assault cases), results shall be reported based on the Quantifiler™ Trio and/or PowerQuant® Y target.
18. When differential extractions are employed, the terminology "sperm [cell] fraction" and "non-sperm [cell] fraction" shall be used in the report any time a portion of the sample is amplified or qualifies for autosomal amplification. The results shall be reported as single result not referring to the fractions in the following situations:
 - When both fractions of a differential extraction exhibit the same quantitation testing result and are not amplified, the results shall be reported as a single result referring to the item only and not the individual fractions.
 - If fractions of the same extract have different results regarding insufficient male DNA for autosomal analysis, insufficient male DNA for further analysis, and/or no male DNA, the results shall be reported as a single result using

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the appropriate insufficient male DNA wording that indicates the largest amount of male DNA present between the two fractions.

DNA PROFILE COMPARISONS

1. When reporting a mixture, the assumed number of contributors shall be stated.
2. When a person or a group of people are visually excluded from a sample, their exclusion shall be reported. Exceptions for complex cases require supervisor/technical leader consultation.
3. A unique unknown individual may be determined and reported if there are twelve or more complete autosomal loci with > 99% weighting in a single source sample or a single profile determined from a mixture.
 - A unique individual shall be identified in the report as male or female if an unambiguous determination can be made from Amelogenin and the Y-STR loci results.
 - A unique unknown individual can be included/excluded from any additional single source or mixed sample (including samples with fewer than 12 loci) at the analyst's discretion. Care should be taken to ensure that this conclusion is unambiguous as the comparison within STRmix™ will not take place until a standard is submitted.
4. For each report detailing a new evidentiary DNA profile, the appropriate CODIS statement shall be included. The CODIS statement shall be with the closing statements at the end of the report.
5. All positive associations shall utilize a STRmix™ deconvolution and have a statistical evaluation applied and reported. The only exceptions being the following:
 - The owner on an intimate sample or an elimination standard reasonably assumed to be present on an item. Report wording shall include a statement indicating that the assumption was made.
 - A profile may be reported as being consistent with an assumed individual with no conclusions drawn on the remainder of the sample due to the inability to determine a number of contributors.
 - A profile from one fraction of a differential extraction that is consistent with a conditioned individual and a profile from the corresponding fraction.
 - The profile from the corresponding fraction may be utilized if it is single source or a single source profile determined by conditioning.

STATISTICAL EVALUATIONS

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1. When reporting out the Likelihood Ratio, it can be reported numerically or in word form, (i.e., 1,000,000 versus 1 million).
2. A reporting ceiling of 1 trillion will be utilized. When a calculated LR is more than 1 trillion times the phrase "at least 1 trillion" shall be utilized in place of the calculated LR number.
 - A verbal scale shall accompany the LR result in an effort to provide additional explanation of the weight of the evidence.

Likelihood Ratio	Support Level	Support for:
$LR \geq 1,000,000$	Very strong	Inclusion
$10,000 \leq LR < 1,000,000$	Strong	Inclusion
$100 \leq LR < 10,000$	Moderate	Inclusion
$2 \leq LR < 100$	Limited	Inclusion
1	Uninformative	Uninformative
$2 \leq (1/LR) < 100$	Limited	Exclusion
$100 \leq (1/LR)$	--	Exclusion

3. Analysts have the discretion to report unambiguous exclusions without using STRmix™ to calculate a LR.
4. DNA mixtures in which multiple reference profiles indicate inclusion (LR support for H1) shall have multiple LR propositions calculated. The reference profile LRs shall be run individually to indicate individual weight in the mixture. Additionally, LRs shall be calculated to indicate the weight of all included individuals combined together in the mixture.
 - There may be scenarios when multiple individuals (possibly even more individuals than present in the mixture) have a LR supporting H1 when compared to an evidence profile in isolation; however, the LR could favor exclusion when the individuals are compared to the evidence profile in combination with each other. In this scenario, the evidence profile could not have come from that combination of individuals. Such groups are termed 'incompatible' as, combined, they are incompatible with the evidence profile. This scenario is not unexpected when related individuals are compared to minor or low level DNA profiles. When appropriate, an analyst can attempt to test different combinations to provide further information. However, it is not always possible to test or list all incompatible groups.
5. When multiple LR propositions are calculated, it is recommended that the analyst report the single proposition that most reasonably explains the evidence based on the case information available, if possible. Case circumstances will dictate which of these calculations are most appropriate for the report and multiple propositions may need to be reported.
 - Reporting individual LRs is required when the LR favoring inclusion of one individual falls in a different level of the verbal scale than the other individual. However, if each individual LR falls in the same level of the

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verbal scale (i.e., all individuals indicate very strong support for H1), it is appropriate to only report the combined LR.

- The combined LR shall be reported when the calculated LR of all individuals combined equals 0 (no support for the individuals making up the mixture together).
 - The appropriate inclusionary individual LRs shall be reported along with the additional report wording statement indicating the individuals cannot make up the mixture together.
- 6. If the analyst cannot clearly exclude an individual, STRmix™ may be used to assist in the comparison. If STRmix™ is used to determine an exclusion, the appropriate exclusion statement shall be included in the report for that individual.
 - LR values less than 1 favor the defense proposition (H2). LR values less than 1 will be evaluated for the verbal equivalent as 1/LR.
 - 1/LR values between 2 and 99 will be reported as limited support for exclusion and will include the hypotheses and calculated 1/LR value in the report as stated in the report wording guidelines.
 - 1/LR values above 99 shall use the general exclusion statement as stated in the report wording guidelines.
 - An LR value of zero indicates no scientific support for the prosecution proposition (H1) and shall use the general exclusion statement as stated in the report wording guidelines.
- 7. The 99% 1-sided lower HPD LR values shall be reported using the ISP_NIST_Combined allele frequencies.
 - LR values in the support of H1 will be rounded down to two significant figures for reporting.
 - If the LR in support of H1 is less than 2, it shall be rounded down to 1.0 (uninformative).
 - If the LR value is in support of H2, the 1/LR value will be rounded up to two significant figures for reporting.
 - If the LR in support of H2 is less than 2, it shall be rounded up to 2.0 (limited support for exclusion).
- 8. Additional LR calculations performed by request after the initial report has been released shall be reported in a Supplemental Certificate of Analysis.
- 9. Reports issued with a verbal equivalent statement prior to September 24, 2018 shall not be reissued unless new statistical comparisons are made. If new comparisons are made, the verbal equivalents shall be updated as appropriate and appropriate wording shall be added to the report to indicate the change.

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10. Any statistical result for analysis reported prior to July 13, 2015 shall be recalculated with the original statistical method used and appropriate updated allele frequencies utilizing IndySTR v2.2_04-01-2016 if:
 - Additional evidence is submitted in the case;
 - The case is going to court; or
 - Upon request;
 - If the reported statistical results change, a new (additional evidence submitted) or amended (for court or upon request) Certificate of Analysis shall be issued with notification included in the report and the statistical worksheets uploaded under the associated request number. If the reported statistical results do not change and there is an active new request, the new statistical worksheets shall be uploaded under the active request number. If the reported statistical results do not change and there is no active request, the new statistical worksheets shall be uploaded under the original request as V2.
11. Previously calculated statistics and comparisons should utilize the appropriate report writing statements approved at the date the calculations were originally performed. Supervisor approval shall be obtained.

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SEROLOGY STATEMENTS

Citations shall refer to the Report Appendix to reflect the test(s) supporting the result.

NEGATIVE RESULT

No blood was detected.¹

No seminal material was detected.²

POSITIVE CONFIRMATORY RESULT

Human blood was confirmed.³ [DNA analysis was performed on X samples.]

Seminal material was confirmed.⁴ [DNA analysis was performed on X samples.]

POSITIVE PRESUMPTIVE RESULT

Blood was indicated by presumptive testing.¹ [DNA analysis was performed on X samples.]

Seminal material was indicated by presumptive testing.² [DNA analysis was performed on X samples.]

NEGATIVE CONFIRMATORY RESULT

In addition to positive presumptive result.

Confirmatory testing was negative [for human blood/seminal material].^{3/4}

SEXUAL ASSAULT KIT

Could apply to any item containing multiple subitems which need to be addressed.

Item 00X: Evidence Collection Kit – PERSON

Items 00XA through 00XX were present [or prepared from items] in the kit.

NO SEROLOGICAL TESTING PERFORMED

For cases where it is important to note a lack of staining to test; not for routine use.

No stains were observed for serological testing.

SEROLOGY ONLY REPORTS

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For use in reports where one DNA analyst has performed serological testing and performed some or all of the DNA lab work, but DNA analysis and interpretation will be reported by another analyst.

DNA processing was performed on[# samples/the combined swabs].

TRACE EVIDENCE

Potential trace evidence was observed.⁶

HAIR STATEMENTS

Hair demonstrating characteristics of human hair with the potential for nuclear DNA analysis was observed/tested as item XA.⁵

When no microscopic analysis is performed. Ends sampled for DNA.

DNA analysis was performed on # hair-like fiber(s).

Human hair without roots, or other hairs may be considered trace evidence when not significant. The wording below is to be used when hair is of evidentiary value.

Hair demonstrating characteristics of human hair but without the potential for nuclear DNA analysis was observed.⁵

Hair demonstrating characteristics not consistent with human hair was observed.⁵

ITEMS OR SUBITEMS NOT EXAMINED

For subitems not examined, the statement is placed at the end of the item.

The ITEMDESCRIPTION, ITEMDESCRIPTION, and ITEMDESCRIPTION (items XA-XZ) were not examined.

AUTOSOMAL DNA STATEMENTS

THE BEGINNING OF RESULTS SECTION (if applicable)

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IDENTICAL TWINS

The DNA profiles obtained from TWIN1 and TWIN2 demonstrated the same DNA profile. This is typically associated with identical twins. The conclusions reflect the comparisons with the standards provided.

SPERM/NON-SPERM FRACTION STATEMENT

To be placed at the beginning of the results section if sperm and non-sperm fractions are reported.

In the results detailed below, [a portion of the] samples were processed utilizing a differential extraction procedure. The terms "sperm fraction" and "non-sperm fraction" refer to the two portions of the extract generated during the differential extraction procedure. The presence of DNA in the "sperm fraction" does not confirm the presence of seminal material and is not directly correlated to the cell types from which the DNA originated.

EXCLUSION

Excluded visually or with an individual STRmix™ calculated LR=0, 1/LR ≥100, or falling into an unsuitable contributor position when there are available positions for inclusion. To be placed with the appropriate interpretation statement, before the LR bullet points.

PERSON is **excluded**.

SINGLE SOURCE STATEMENTS

The DNA profile [from ITEMDESCRIPTION] was interpreted as originating from a single individual.

PERSON was considered as a contributor:

- ▶ The DNA profile is at least 1 trillion/LR times more likely if it originated from PERSON than if it originated from an unknown, unrelated individual.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON is **included**.

SINGLE UNKNOWN PROFILE

STRmix™ deconvolution performed, but no LR calculated. [(Unknown #) Statement allowed if a designated unknown is determined at 12 or more loci in a single source]

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profile or in a two-person mixture in which the other individual is assumed (i.e. only one foreign individual). Utilized at analyst discretion.]

The DNA profile [from ITEMDESCRIPTION] was interpreted as originating from a single male/female/individual [(Unknown Male/Female/Individual #)].

The DNA profile from the combined swabs was interpreted as a mixture of two individuals. Assuming PERSON as a contributor, the mixture contains a single [male/female] individual [Unknown Male/Female/Individual #1].

CONSISTENT WITH ELIMINATION STANDARD

No deconvolution performed.

The DNA profile [from ITEMDESCRIPTION] was interpreted as originating from a single individual. Assuming PERSON as a contributor, no additional alleles were detected.

MIXTURE STATEMENTS

DESIGNATED UNKNOWN INDIVIDUAL IN A MIXTURE

Statement utilized any time a designated unknown is either determined at 12 or more loci OR not excluded from the mixture. Utilized at analyst discretion ('excluded' is not bolded). This is the only option if there is more than one foreign individual in a profile.

Unknown [Female/Male/Individual] #1 cannot be excluded.

UNKNOWN MIXTURE (NO LR)

Statement utilized when multiple unknown individuals are in the mixture.

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of N individuals, [including at least one male]. [PERSON was assumed as a contributor.] [Unknown Female/Male/Individual #1 cannot be excluded.]

GENERAL MIXTURE STATEMENT WITH LR

LR ≥ 2 , result supports H1, inclusion of one person of interest.

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of N individuals.

PERSON was considered as a contributor:

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- ▶ The DNA profile is at least 1 trillion/LR times more likely if it originated from PERSON and N-1 unknown individual[s] than if it originated from N unknown, unrelated individuals.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON is **included**.

GENERAL MIXTURE STATEMENT – COMBINED LR

LR ≥ 2 , result supports H1, inclusion of multiple persons of interest.

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of N individuals. [A portion of the profile was determined to be unsuitable for comparison. All conclusions are based on the remaining portion.] [PERSON3 is **excluded**.]

PERSON1 and PERSON2 were considered as contributors:

- ▶ The DNA profile is at least 1 trillion/LR times more likely if it originated from PERSON1, PERSON2, and N-2 unknown individual[s] than if it originated from N unknown, unrelated individuals.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that [both] PERSON1 and PERSON2 are **included**.

GENERAL MIXTURE STATEMENT WITH UNSUITABLE PORTION(S)

LR ≥ 2 , result supports H1, inclusion of one person of interest, exclusion of another. DBLR or template determined portion that is unsuitable for comparison.

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of N individuals. A portion of the profile was determined to be unsuitable for comparison. All conclusions are based on the remaining portion. PERSON2 is **excluded**.

PERSON was considered as a contributor:

- ▶ The DNA profile is at least 1 trillion/LR times more likely if it originated from PERSON and N-1 unknown individual[s] than if it originated from N unknown, unrelated individuals.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON is **included**.

MIXTURE WITH CONDITIONING

LR ≥ 2 , result supports H1, inclusion of one person of interest.

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of two individuals. PERSON2 was assumed as a contributor.

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PERSON was considered as a contributor:

- ▶ The DNA profile is at least 1 trillion/LR times more likely if it originated from PERSON2 and PERSON1 than if it originated from PERSON2 and an unknown, unrelated individual.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON1 is **included**.

MIXTURE WITH CONDITIONING AND UNSUITABLE PORTION(S)

DBLR/template unsuitable for remaining portion of profile.

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of N individuals, [including at least one male]. PERSON2 was assumed as a contributor. The remaining portion of the profile was determined to be unsuitable for comparison.

MIXTURE WITH CONDITIONING

LR ≥ 2 , result supports H1, inclusion of one person of interest with DBLR/template unsuitable for portion of profile.

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of N individuals. PERSON2 was assumed as a contributor. A portion of the profile was determined to be unsuitable for comparison. All conclusions are based on the remaining portion. [Unknown Female/Male/Individual #1 cannot be excluded.]

PERSON1 was considered as a contributor:

- ▶ The DNA profile is at least 1 trillion/LR times more likely if it originated from PERSON2, PERSON1, and N-2 unknowns individual[s] than if it originated from PERSON2 and N-1 unknown, unrelated individual[s].
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON1 is **included**.

PROFILES ONLY WHEN VARNOC UTILIZED

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of N or N+1 individuals [, including at least one male]. [LR or exclusion wording, as needed]

CONSISTENT WITH MULTIPLE ELIMINATION STANDARDS

No deconvolution performed. No other inclusions or exclusions made from the profile.

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of two individuals. Assuming PERSON1 and PERSON2 as contributors, no additional alleles were detected.

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The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of three individuals. PERSON1 and PERSON2 were assumed as contributors. The remaining portion of the profile was determined to be unsuitable for comparison.

CONSISTENT WITH ELIMINATION STANDARD + DIFFERENTIAL FRACTION

In one differential fraction, may assume corresponding differential fraction. No deconvolution performed.

The DNA profile from the non-sperm/sperm fraction [of ITEMDESCRIPTION] was interpreted as a mixture of two individuals. Assuming PERSON and the corresponding sperm/non-sperm fraction as contributors, no additional alleles were detected.

LIMITED EXCLUSIONARY LR

STRmix™ LR calculated, $2 \leq 1/LR < 100$, result supports H2. [DBLR/template unsuitable for portion of profile.]

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of N individuals. [A portion of the profile was determined to be unsuitable for comparison. All conclusions are based on the remaining portion.]

PERSON was considered as a contributor:

- ▶ The DNA profile is LR times more likely if it originated from N unknown, unrelated individuals than if it originated from PERSON and N-1 unknown individual[s].
- ▶ This statistical analysis provides limited support for the proposition that PERSON is **excluded**.

INCONCLUSIVE LR

STRmix™ LR calculated, LR rounds to 1.0. [DBLR/template unsuitable for portion of profile.]

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of N individuals. [A portion of the profile was determined to be unsuitable for comparison. All conclusions are based on the remaining portion.]

PERSON was considered as a contributor:

- ▶ A likelihood ratio of 1.0 was obtained. The DNA profile is equally as likely if it originated from PERSON and N-1 unknown individual[s] or if it originated from N unknown, unrelated individuals.
- ▶ This result is considered **uninformative**.

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ALTERNATE PROPOSITIONS LISTED IN RESULT STATEMENT

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of N individuals. [A portion of the profile was determined to be unsuitable for comparison. All conclusions are based on the remaining portion.] [Exclusions, if needed] The probability of the evidence has been calculated by considering multiple propositions.

PERSON1 was considered as a contributor:

- ▶ The DNA profile is at least 1 trillion/LR times more likely if it originated from PERSON1 and N-1 unknown individual[s] than if it originated from N unknown, unrelated individuals.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON1 is **included**.

PERSON2 was considered as a contributor:

- ▶ The DNA profile is at least 1 trillion/LR times more likely if it originated from PERSON2 and N-1 unknown individual[s] than if it originated from N unknown, unrelated individuals.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON2 is **included**.

INDIVIDUAL INCLUSIONS, BUT EXCLUDED TOGETHER

To be placed after the appropriate individual inclusionary statements.

Note:

Given the interpretation that the mixture originated from N individuals, there is support for the propositions that the [comparable portion of the] DNA profile originated from PERSON1 or PERSON2 individually. However, there is no scientific support for the proposition that the [comparable portion of the] DNA profile originated from PERSON1 and PERSON2 together. Refer to the independent inclusionary statistics reported above.

VARIABLE NUMBER OF CONTRIBUTORS

The appropriate mixture wording should be adjusted to accurately reflect results. One example below.

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of N or N+1 individuals.

PERSON was considered as a contributor:

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- ▶ The DNA profile is at least 1 trillion/LR times more likely if it originated from PERSON and N unknown individual[s] than if it originated from N+1/N-1 unknown, unrelated individuals.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON is **included**.

INCONCLUSIVE SAMPLES

NO ALLELES DETECTED

ITEMDESCRIPTION failed to demonstrate a DNA profile.

INCONCLUSIVE NUMBER OF CONTRIBUTORS

The DNA profile [from ITEMDESCRIPTION] has limited data/is a mixture. No further conclusions can be drawn due to the uncertainty in the number of contributors.

CONSISTENT WITH ELIMINATION STANDARD + INCONCLUSIVE NOC

Consistent with elimination standard but no conclusion being drawn from additional alleles.

The DNA profile [from ITEMDESCRIPTION] is a mixture. Assuming PERSON as a contributor, no further conclusions can be drawn due to the uncertainty in the number of contributors.

ALL CONTRIBUTORS UNSUITABLE FOR COMPARISON

STRmix™ deconvolution performed, but no LR calculated. DBLR/template unsuitable for all portions of profile.

The DNA profile [from ITEMDESCRIPTION] was interpreted as a mixture of two/three/four individuals. The profile was determined to be unsuitable for comparison.

>4 CONTRIBUTORS

No conclusion can be drawn [from ITEMDESCRIPTION] because the DNA profile is a mixture of more than four individuals.

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FAILED CONTROL

Statement to be placed before listed results for an item. Not all possible combinations are given; consultation with the Technical Leader and/or Supervisor is required for situations not covered below.

During analysis [of ITEMDESCRIPTION], a positive/negative test control failed. Data was evaluated and determined to be suitable for interpretation.

During analysis [of ITEMDESCRIPTION], a positive/negative test control failed. Data was evaluated and determined to be unsuitable for interpretation; therefore, no conclusions can be drawn.

CONTAMINATION

When Database Search LR $\geq 10,000$. Not all possible combinations are given; consultation with the Technical Leader and/or Supervisor is required.

The DNA profile [from ITEMDESCRIPTION] was interpreted as originating from a single identified individual from the Indiana State Police Forensic Services Division Quality Assurance DNA Database. Assuming this individual as a contributor, no additional alleles were detected.

The DNA profile was interpreted as a mixture of N individuals. An identified individual from the Indiana State Police Forensic Services Division Quality Assurance DNA Database was present and assumed as a contributor.

PERSON was considered as a contributor:

- ▶ The DNA profile is at least 1 trillion/LR times more likely if it originated from the Database individual and PERSON than if it originated from the Database individual and an unknown, unrelated individual.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON is **included**.

SAMPLES NOT AMPLIFIED / QUANT-OUTS

In a sample with insufficient (male) DNA for amplification, where it is valuable to identify a male contributor from a female contributor (i.e. sexual assault cases), the reported results shall be based on the Quantifiler® Trio Y target.

INSUFFICIENT HUMAN DNA

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ITEMDESCRIPTION demonstrated an insufficient quantity of DNA for further analysis.

INSUFFICIENT MALE DNA for AUTOSOMAL ANALYSIS

Not eligible for autosomal, but male DNA is greater than 0.006 ng/μl

ITEMDESCRIPTION demonstrated an insufficient quantity of male DNA for autosomal STR analysis.

INSUFFICIENT MALE DNA OR UNDETECTED MALE DNA

Not eligible for either autosomal or Y-STR analysis, or undetected male DNA

No male DNA or an insufficient quantity of male DNA for further analysis was detected [on ITEMDESCRIPTION].

SAMPLE TRIAGED

Eligible for autosomal analysis, but not amplified

ITEMDESCRIPTION was/were extracted and quantified; however, no additional DNA analysis was performed [due to the development of DNA profiles on other items].

SAMPLE/SUBITEM NOT TESTED FOR DNA

May modify as shown if permission to consume is required.

No DNA analysis was performed. [If further DNA analysis is desired, permission to consume the sample is required. Please contact the reporting analyst.]

STANDARD ITEMS

Item X: DNA Standard – PERSON

STAIN CARD

A stain card was prepared [and returned to the original packaging].

PROFILE DEVELOPED/NOT DEVELOPED

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The DNA profile [of PERSON] was [previously] developed.

No DNA profile [of PERSON] was developed [at this time].

SEXUAL ASSAULT KIT ORAL SWAB DEVELOPED AS STANDARD

If female victim:

[Appropriate male quantitation statement]. The DNA profile was developed as a standard for PERSON.

If male victim:

The DNA profile [from ITEMDESCRIPTION] was interpreted as originating from a single individual. This profile will be used as a DNA standard for PERSON.

PROFILE DEVELOPED BUT NOT INTERPRETED

The sample was processed but not interpreted due to a lack of evidence for comparison.

STANDARD DEVELOPED WITH UPDATED CHEMISTRY

The DNA profile was updated with the current DNA chemistry.

MIXED PROFILE OBTAINED

To be used only with Technical Leader consultation and approval.

The DNA profile was interpreted as a mixture of N individuals. A distinct female/male profile was determined to be used as the assumed standard for PERSON.

MULTIPLE STANDARDS

The standard was not examined due to the presence of multiple standards.

STANDARD COMPARED TO EVIDENCE IN OTHER CASE

For the case that contains the standard

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The DNA profile of PERSON (item XXA) was compared to the evidence in Indiana State Police Forensic Services Division Case XXX-XXXXX, Other Police Department Case XXXXX.

EVIDENCE COMPARED TO STANDARD FROM OTHER CASE

For the case that contains the evidence but not the standard. Placed at the beginning of the report.

Evidence was compared to the DNA profile of PERSON (item XA) developed in Indiana State Police Forensic Services Division Case XXX-XXXXX, Other Police Department Case XXXXX.

CODIS STATEMENTS

All placed in the end section of the report.

[The] DNA profile[s] from [list item numbers] [was/were] entered into the Combined DNA Index System (CODIS) and will be searched on a routine basis. In the event of a database match, additional information will be provided separately.

PENDING STANDARD SUBMISSION

Upon submission of an appropriate DNA standard from PERSON, the DNA profile from item number will be re-evaluated for possible entry into the Combined DNA Index System (CODIS).

PENDING ADDITIONAL INFORMATION

Additional information is required [regarding ITEM] before it can be determined if any DNA profiles are eligible for the Combined DNA Index System (CODIS). Please contact the reporting analyst if a database search is desired.

NO CODIS ENTRIES

To be used when profiles are developed but none are suitable for entry.

No DNA profiles were entered into the Combined DNA Index System (CODIS).

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REMOVAL FROM CODIS

To be used in supplemental reports when profiles are deleted from the database.

[The] DNA profile[s] from [list item numbers] [was/were] removed from the Combined DNA Index System (CODIS).

OTHER STATEMENTS

All wording in this section placed at the end of the report unless otherwise indicated.

ALTERNATE CALCULATIONS

To be used when additional/alternate LR's were calculated but not included in the report.

Alternate calculations were performed for item(s) XA, XB and XC and are available upon request.

STATISTICS STATEMENT WHEN FREQUENCY REPORTED

To be placed prior to the appropriate SWGDAM statement.

The data in this report was interpreted and evaluated prior to the implementation of Likelihood Ratios at the Indiana State Police Forensic Services Division. The statistical estimates are presented in the form of a frequency.

POWERPLEX® FUSION 6C DATA WITH RMP/CPI

To be used when Fusion 6C data was reported with a frequency prior to November 1, 2017.

Statistical estimates were performed based on the recommended concepts endorsed by the Scientific Working Group on DNA Analysis Methods (1/12/17). Y-STR loci included with the PowerPlex® Fusion 6C System were used for interpretations but were not included in statistical estimates. Calculations for the Caucasian, African American, and Hispanic populations have been performed. The most common frequency has been reported unless all calculated frequencies exceed 1 in 8 trillion.

RE-EVALUATING DATA WITH NEW FBI ALLELE FREQUENCIES

Stats calculated prior to July 13, 2015 and re-calculated with updated allele frequencies.

The statistical calculations were adjusted to reflect the amended allele frequencies published by the FBI. *Moretti, T.R., Budowle, B. and Buckleton, J. S., Erratum, Journal of Forensic Sciences 2015; 60(4).*

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RE-EVALUATING DATA WITH NEW INTERPRETATION GUIDELINES

Requires Supervisor's approval and shall be placed at the beginning of the results section.

In the DNA analysis detailed below, sample data was re-evaluated using methods implemented after the original report was issued. Accordingly, the following conclusions may differ from the original report.

UPDATING STATISTICS WITH VERBAL EQUIVALENT

Shall be placed at the beginning of the results section. Applies to reports issued with a verbal equivalent statement prior to September 24, 2018.

In the DNA analysis detailed below, sample conclusions were updated utilizing a verbal qualifying statement recommended by the Scientific Working Group on DNA Analysis Methods. Accordingly, the following conclusions may differ from the original report.

RE-ANALYSIS OF SAMPLE FOR ADDITIONAL DATA

Requires Supervisor's approval and shall be placed at the beginning of the results section. Other situations may be listed as needed with supervisor approval.

Additional analysis of the ITEMDESCRIPTION was performed. The resulting profile was suitable/not suitable for familial searching/entry/updating in the Combined DNA Index System (CODIS). In the event of a database match, additional information will be provided separately.

RE-ANALYSIS OF SAMPLE TO UPDATE CHEMISTRY AND INTERPRET USING CURRENT SOFTWARE OPTIONS

The DNA profile was updated with the current DNA chemistry.

REQUEST FOR STANDARDS

If additional comparisons are desired, please submit an appropriate DNA standard (such as an oral/buccal swab standard) from [victim/consensual partner/named suspect or] any [additional] suspect(s).

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Autosomal DNA/Y-STR/SNP ANALYSIS PENDING

When completed, results from the [DNA/Y-STR/SNP] analysis will be provided separately.

PATERNITY/KINSHIP ANALYSIS PENDING

When completed, Paternity/Kinship analysis of the DNA profiles determined for PERSON1, PERSON2, and PERSON3 will be provided separately.

SAMPLES PROCESSED BY ANOTHER ANALYST

For use in reports where a forensic technician or DNA analyst has prepared or processed samples, but DNA results will be reported by another analyst.

Original items of evidence were processed by NAME at the [Evansville/Fort Wayne/Indianapolis/Lowell] Laboratory.

SAMPLES PROCESSED AT MULTIPLE LABORATORIES

Applies to robot samples sent from regional labs and to technician cases that were processed at one lab and sent to another for reporting.

Laboratory activities were performed at the [Evansville/Fort Wayne/Indianapolis/Lowell] and [Evansville/Fort Wayne/Indianapolis/Lowell] Laboratories.^{xv}

SAMPLES SENT TO AN OUTSOURCE LABORATORY

Item(s) XA [were/was] sent to XYZ Laboratory, city, state for [additional] analysis as per the authority of John Smith, Police Department.

WITHDRAWAL STATEMENTS

The request for DNA analysis was withdrawn by John Smith, Police Department, on Date. The items will be returned to the submitting agency.

The request for DNA analysis was withdrawn. The evidence is not eligible for entry into the Combined DNA Index System (CODIS). Submission of appropriate DNA standard(s) (such as an oral/buccal swab standard) is required before analysis can be completed. The evidence will be returned to the submitting agency.

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The request for DNA analysis was withdrawn in order to preserve the sampling area for Latent Print examination. The items will be returned to the submitting agency.

The request for laboratory examination was withdrawn per information found in MyCase, Cause Number: CAUSE NO, DATE. The items will be returned to the submitting agency. If analysis is needed, please re-submit the item(s) to the Forensic Services Division.

EXAMPLES

Example 1: Homicide with Kit

In the results detailed below, a portion of the samples were processed utilizing a differential extraction procedure. The terms "sperm fraction" and "non-sperm fraction" refer to the two portions of the extract generated during the differential extraction procedure. The presence of DNA in the "sperm fraction" does not confirm the presence of seminal material and is not directly correlated to the cell types from which the DNA originated.

Item 001: Evidence Collection Kit – Jane Smith

Items 001A through 001K were present or prepared from items in the kit.

Item 001A: Blood standard

A stain card was prepared. The DNA profile was developed.

Item 001B: Vaginal wash

The vaginal wash demonstrated an insufficient quantity of male DNA for autosomal STR analysis.

Item 001C: Vaginal/cervical swabs (2)

Sperm fraction

The DNA profile from the combined swabs was interpreted as a mixture of two individuals. Jane Smith was assumed as a contributor.

John Doe was considered as a contributor:

► The DNA profile is at least 1 trillion times more likely if it originated from Jane Smith and John Doe than if it originated from Jane Smith and an unknown, unrelated individual.

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► This statistical analysis provides very strong support for the proposition that John Doe is **included**.

Non-sperm fraction

The DNA profile from the combined swabs was interpreted as a mixture of two individuals. Assuming Jane Smith and the corresponding sperm fraction as contributors, no additional alleles were detected.

Item 001D: Neck swabs (2)

The DNA profile from the combined swabs was interpreted as a mixture of two individuals, including at least one male. Jane Smith was assumed as a contributor. Unknown Male #1 cannot be excluded.

Item 001E: External genital swab

The swab demonstrated an insufficient quantity of male DNA for autosomal STR analysis.

Item 001F: Anal swabs (6)

Four combined swabs demonstrated an insufficient quantity of male DNA for autosomal STR analysis. No DNA analysis was performed on two remaining swabs.

Item 001G: Underwear

DNA analysis was performed on one sample and one hair-like fiber.

Item 001G1

Sperm fraction

The DNA profile from the sample was interpreted as originating from a single individual.

John Doe was considered as a contributor:

► The DNA profile is at least 1 trillion times more likely if it originated from John Doe than if it originated from an unknown, unrelated individual.

► This statistical analysis provides very strong support for the proposition that John Doe is **included**.

Non-sperm fraction

The sample demonstrated an insufficient quantity of male DNA for further analysis.

Item 001G2

The hair-like fiber demonstrated an insufficient quantity of male DNA for further analysis.

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The pubic hair combings, head hair combings, foreign material collection, and underwear (items 001H through 001K) were not examined.

Item 002: DNA standard – John Doe

The DNA profile was developed.

Item 003: Knife

Human blood was confirmed.³ DNA analysis was performed on two samples.

Item 003A: Knife blade

The DNA profile from the knife blade was interpreted as a mixture of two individuals. John Doe is **excluded**. Unknown Male #1 cannot be excluded.

Jane Smith was considered as a contributor:

- ▶ The DNA profile is 200 times more likely if it originated from Jane Smith and one unknown individual than if it originated from two unknown, unrelated individuals.
- ▶ This statistical analysis provides moderate support for the proposition that Jane Smith is **included**.

Item 003B: Knife handle

The DNA profile from the knife handle was interpreted as originating from a single male.

Item 004: Shirt

Human blood was confirmed.³ Seminal material was indicated by presumptive testing.² DNA analysis was performed on four samples.

Item 004A

Sperm Fraction

The DNA profile was interpreted as originating from a single male.

Non-Sperm Fraction

The DNA profile was interpreted as originating from a single individual. Assuming John Doe as a contributor, no additional alleles were detected.

Items 004B and 004C

The DNA profiles were each interpreted as originating from a single individual.

Jane Smith was considered as a contributor:

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- ▶ Each DNA profile is at least 1 trillion times more likely if it originated from Jane Smith than if it originated from an unknown, unrelated individual.
- ▶ Each statistical analysis provides very strong support for the proposition that Jane Smith is **included**.

Item 004D

The sample demonstrated an insufficient quantity of DNA for further analysis.

DNA profiles from items 001D and 004A were entered into the Combined DNA Index System (CODIS) and will be searched on a routine basis. In the event of a database match, additional information will be provided separately.

Example 2: Crime against person – single item multiple components

Use the kit format for a collection of different objects within one evidence item.

In the results detailed below, a portion of the samples were processed utilizing a differential extraction procedure. The terms “sperm fraction” and “non-sperm fraction” refer to the two portions of the extract generated during the differential extraction procedure. The presence of DNA in the “sperm fraction” does not confirm the presence of seminal material and is not directly correlated to the cell types from which the DNA originated.

Item 005: Bag of trash

Items 005A-005D were present in the bag of trash.

Item 005A: Condom wrapper

The DNA profile was interpreted as originating from a single female individual. Bob Roberts is **excluded**.

Jane Smith was considered as a contributor:

- ▶ The DNA profile is 17 times more likely if it originated from Jane Smith than if it originated from an unknown, unrelated individual.
- ▶ This statistical analysis provides limited support for the proposition that Jane Smith is **included**.

Item 005B: Piece of gum

Seminal material was indicated by presumptive testing.²

Sperm Fraction

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The DNA profile was interpreted as originating from a single male individual.

Bob Roberts was considered as a contributor:

- ▶ The DNA profile is 17 times more likely if it originated from Bob Roberts than if it originated from an unknown, unrelated individual.
- ▶ This statistical analysis provides limited support for the proposition that Bob Roberts is **included**.

Non-Sperm Fraction

The DNA profile was interpreted as originating from a single female individual.

Jane Smith was considered as a contributor:

- ▶ The DNA profile is at least one trillion times more likely if it originated from Jane Smith than if it originated from an unknown, unrelated individual.
 - ▶ This statistical analysis provides very strong support for the proposition that Jane Smith is **included**.
-

The banana peel and apple core (items 005C and 005D) were not examined.

Example 3: Crime against person – single item multiple components - clothing
Use the kit format for a collection of different objects within one evidence item.

In the results detailed below, a portion of the samples were processed utilizing a differential extraction procedure. The terms “sperm fraction” and “non-sperm fraction” refer to the two portions of the extract generated during the differential extraction procedure. The presence of DNA in the “sperm fraction” does not confirm the presence of seminal material and is not directly correlated to the cell types from which the DNA originated.

Item 001: Bag of clothing

A shirt (item 001A), jeans (item 001B) and a pair of socks (item 001C) and a pair of shoes (item 001D) were present.

Item 001A: Shirt

Human blood was confirmed.³ DNA analysis was performed on two samples.

Item 001A1

The DNA profile from sample was interpreted as originating from a single individual (Unknown Male #1).

Item 001A2

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The DNA profile was interpreted as originating from a single individual. Assuming Jane Doe as a contributor, no additional alleles were detected.

Item 001B: Jeans

Seminal material was indicated by presumptive testing.²

Sperm Fraction

The DNA profile was interpreted as originating from a single individual (Unknown Male #2).

Non-Sperm Fraction

The DNA profile was interpreted as originating from a single individual. Assuming Jane Doe as a contributor, no additional alleles were detected.

Item 001C: Socks

Blood was indicated by presumptive testing on each sock.¹ DNA analysis was performed on three samples.

Item 001C1

Item 001C1a

The DNA profile from sample was interpreted as originating from a single individual (Unknown Male #1).

Item 001C1b

The sample demonstrated an insufficient quantity of DNA for further analysis.

Item 001C2

The DNA profile was interpreted as originating from a single individual. Assuming Jane Doe as a contributor, no additional alleles were detected.

Item 001D: Shoes

The DNA profile was interpreted as originating from a single individual. Assuming Jane Doe as a contributor, no additional alleles were detected.

Item 002: DNA standard – Jane Doe

The DNA profile was developed.

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DNA profiles from items 001A1 and the sperm fraction of 001B were entered into the Combined DNA Index System (CODIS) and will be searched on a routine basis. In the event of a database match, additional information will be provided separately.

Example 4: Property crime with serological testing

Submitted swabs that are considered distinctly separate (due to case circumstances, sample purpose, serology results, etc.) get separate (subitem) designations. Item 001 in the following example contains swabs that are distinct because of serological results and may have different DNA treatment. Item 002 contains swabs that have no reason to be considered separately.

Item 001: Swabs of window (2)

Item 001A

Blood was indicated by presumptive testing.¹ Confirmatory testing was negative.³

The DNA profile was interpreted as originating from a single female individual.

Item 001B

No blood was detected on the remaining swab.¹ No DNA analysis was performed.

Item 002: Swabs of floor (2)

Blood was indicated by presumptive testing on the two swabs.¹ Human blood was confirmed on one swab.³ No confirmatory testing was performed on the remaining swab.

The DNA profile from the combined swabs was interpreted as originating from a single individual (unknown male #1).

Item 003: Buccal swab standard – Robert Jones

The DNA profile was developed.

Example 5: Property crime – no serological testing

Item 001: Crowbar

A sample of the crowbar demonstrated an insufficient quantity of DNA for further analysis.

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Item 002: Swabs of dresser drawer (2)

The DNA profile from the combined swabs was interpreted as originating from a single individual. Assuming Sally Smith as a contributor, no additional alleles were detected.

Item 003: DNA standard – Sally Smith

The DNA profile was developed.

Example 6: Crime against person – many areas on one item

Item 001: Victim's Blanket

Human blood was confirmed.³ DNA analysis was performed on six samples.

Items 001A and 001B

Each sample demonstrated an insufficient quantity of DNA for further analysis.

Items 001C and 001D

Each DNA profile was interpreted as originating from a single individual. Assuming Mary Doe as a contributor, no additional alleles were detected.

Items 001E and 001F

The DNA profiles were each interpreted as mixtures of two individuals, including at least one male. Mary Doe and Bob Doe are **excluded**.

John Doe was considered as a contributor:

- ▶ Each DNA profile is at least 1 trillion times more likely if it originated from John Doe and an unknown individual than if it originated from two unknown, unrelated individuals.
 - ▶ Each statistical analysis provides very strong support for the proposition that John Doe is **included**.
-

Item 002: Shirt

Human blood was confirmed.³

The DNA profile was interpreted as a mixture of two individuals, including at least one male. The probability of the evidence has been calculated by considering multiple propositions. Bob Doe is **excluded**.

Mary Doe was considered as a contributor:

- ▶ The DNA profile is 40,000 times more likely if it originated from Mary Doe and one unknown individual than if it originated from two unknown, unrelated individuals.

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► This statistical analysis provides strong support for the proposition that Mary Doe is **included**.

John Doe was considered as a contributor:

► The DNA profile is 8,500 times more likely if it originated from John Doe and one unknown individual than if it originated from two unknown, unrelated individuals.

► This statistical analysis provides moderate support for the proposition that John Doe is **included**.

Note:

Given the interpretation that the mixture originated from two individuals, there is support for the propositions that the DNA profile originated from Mary Doe or John Doe individually. However, there is no scientific support for the proposition that the DNA profile originated from Mary Doe and John Doe together. Refer to the independent inclusionary statistics reported above.

Example 7: Possession of firearm (swabs submitted)

Item 001: Swabs of Firearm (3)

Items 001A1 and 001A3

The DNA profiles from the swabs were each interpreted as originating from a single individual (Unknown Male #1).

Item 001A2

The swab demonstrated an insufficient quantity of DNA for further analysis.

Example 8: Possession of firearm (firearm submitted)

Item 001: Firearm

DNA analysis was performed on three samples.

Items 001A1 and 001A3

The DNA profiles from two samples were each interpreted as originating from a single individual (Unknown Male #1).

Item 001A2

The swab demonstrated an insufficient quantity of DNA for further analysis.

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Example 9: Simple items (swabs distinguished by description, headers not required)

Item 001: Swabs from Chevy (2)

The swabs each demonstrated an insufficient quantity of DNA for further analysis.

Item 002: Swabs from Ford (2)

The DNA profile from the steering wheel swab was interpreted as originating from a single individual (Unknown Male #1).

The swab of the gear shift demonstrated an insufficient quantity of DNA for further analysis.

Example 10: Additional reports without subitems in which the original parent item is related in LIMS (new comparisons, Y-STR, paternity testing, etc.)

Item 001: Evidence Collection Kit – Jane Smith

Item 001A: DNA standard – Jane Smith

The DNA profile was previously developed.

Example 11: Serology only. If serology is required on any item in the initial request, all items must be addressed in the report. In this example, a firearm was swabbed, a shirt was tested for blood and sampled for transfer DNA, and swabs were combined and consumed for DNA with no serology.

Item 001: Firearm

DNA processing was performed on four samples.

Item 002: Shirt

Human blood was confirmed.³ DNA processing was performed on two samples.

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Item 003: DNA standard – Jane Doe

DNA processing was performed.

Item 004: Steering wheel swabs (2)

DNA processing was performed on the combined swabs.

When completed, results from the DNA analysis will be provided separately.

The Appendix below will be added to all Biology Requests in Justice Trax after the analyst's signature, formatted as a stand-alone page.

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Biology Report Appendix

The following includes supplemental information on biological analysis performed at the Indiana State Police Forensic Services Division (FSD) and definitions of some commonly used terms. Not all terms referenced in the appendix may apply to the testing in the associated case report. Please contact the reporting analyst with specific questions regarding the DNA analysis in this case.

Serological testing: Citations in the report refer to the test(s) listed below.

Presumptive testing indicates the possible presence of the substance in question.

1. Blood: Kastle-Meyer/phenolphthalein chemical test
2. Seminal material: Acid Phosphatase chemical test

Confirmatory testing identifies the substance in question.

3. Human blood: HemDirect™ immunological test + Kastle-Meyer/phenolphthalein test
4. Seminal material: microscopic sperm search + Acid Phosphatase test

Other examinations:

5. A macroscopic and microscopic examination of a potential hair is performed to determine if it is consistent with human hair and if root material may be present.
6. Trace evidence may include but is not limited to paint, glass, hair, and fibers.

- I. This report and evaluation only provide information that helps address who may be the source of the DNA - not how or when the DNA was deposited. This report cannot contribute any information regarding the mechanisms, timings, or actions that led to the deposition of the recovered biological material.
- II. Biological (DNA-based) sex information reported here is based solely on the detection of X and Y chromosome markers. Reference to male and female in the report should not be misinterpreted to mean that an individual must appear as male or female based upon this indication alone.
- III. **Insufficient DNA:** Following extraction and quantitation, DNA profiles are not developed from samples which do not yield enough DNA based on the FSD's established threshold.
- IV. **Insufficient Male DNA:** In cases where male DNA is of interest, autosomal DNA profiles are not developed from samples which did not yield enough male DNA based on the FSD's established threshold. These samples may still be eligible for Y-STR analysis. Male DNA quantitation at low levels can be unreliable and does not confirm the presence of male DNA.
- V. An **unknown individual** may be given a designation (e.g., "Unknown Male #1") to distinguish between multiple unknown profiles within a case or to indicate multiple samples consistent with the same unknown profile.
- VI. A **mixture** is a sample that contains DNA from more than one person. Samples in which the number of contributors cannot be determined, mixtures of more than four contributors, and portions of mixtures with an increased risk of false inclusions or exclusions are not suitable for comparison by the FSD's current methods.
- VII. An individual is **assumed** to be a contributor when their contribution to a DNA sample is reasonably expected based upon the context of the sample and the presence of their DNA is not in question. This may include samples recovered from the individual's own body or from an item they regularly use. Using the DNA profile of the assumed individual allows for a more accurate interpretation and representative statistic for the portion of the profile that is in question.
- VIII. Statistics are presented in the form of a **likelihood ratio** (LR), which is a comparison between two competing hypotheses to explain the evidence profile. If all parties agree that the DNA profile originated from the same individual(s), the LR would equal 1. An LR of 0 is an exclusion. Calculations are performed using a combined dataset of Caucasian, African American, Hispanic, and Asian populations. The maximum reported LR is 1 trillion. The LR is based upon the case information provided. If additional information should be considered, please contact the reporting analyst.
- IX. In some DNA mixtures, false inclusions of other closely related **family members** (such as parents, offspring, and siblings) can occur due to the elevated sharing of genetic information between relatives. When first order relatives share many alleles with a person of interest, an adventitious likelihood ratio can give strong support for inclusion. The rate of adventitious support with related individuals increases with increasing mixture complexity. If a case involves potential related individuals, the best course of action is to submit known DNA standards for direct comparison and statistical evaluation.
- X. Statistical calculations are reported with a **verbal qualifying statement** to express the strength of statistical support. These statements follow the verbal scale recommended by the Scientific Working Group on DNA Analysis Methods (7/12/18). The scale reflects stronger degrees of support as the likelihood ratio increases. Reported exclusion(s) may include statistical calculations to support the verbal qualifying statement.

Likelihood Ratio	Support Level	Support for:
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$LR \geq 1,000,000$	Very strong	Inclusion
$10,000 \leq LR < 1,000,000$	Strong	Inclusion
$100 \leq LR < 10,000$	Moderate	Inclusion
$2 \leq LR < 100$	Limited	Inclusion
1	Uninformative	Uninformative
$2 \leq (1/LR) < 100$	Limited	Exclusion
$100 \leq (1/LR)$	--	Exclusion

- XI. Evidence disposition:** Unless otherwise noted, all portions of the evidence not consumed during analysis will be returned to the submitting agency. Retained items will remain at the Indiana State Police Forensic Services Division. The remainder of the evidence or the original packaging will be returned.
- XII. DNA chemistry:** Unless otherwise noted, DNA profiles are developed using Polymerase Chain Reaction (PCR) with the PowerPlex® Fusion 6C System by Promega Corporation. Y-STR loci may be used for interpretations but are not included in statistical estimates.
- XIII. Laboratory Locations**
- a. Evansville: 19411 Highway 41 North, Evansville, IN 47725
 - b. Fort Wayne: 5811 Ellison Road, Fort Wayne, IN 46804
 - c. Indianapolis: 550 West 16th Street, Indianapolis, IN 46202
 - d. Lowell: 1550 East 181st Street, Lowell, IN 46356

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Relationship Testing Wording

INCONCLUSIVE

The DNA profile [from ITEMDESCRIPTION] has [limited data/is a mixture]. ^{1/2/3} No further conclusions can be drawn due to insufficient quality of the data.

DNA STANDARD RESULT STATEMENTS

Citations shall refer to the Report Appendix to reflect the technology and amplification kit used to generate the profile.

MIXTURE - PRODUCTS OF CONCEPTION

The DNA profile was interpreted as a mixture of two individuals. Assuming BIOMOTHER as a contributor, a distinct male/female profile was determined to be used as the standard for the product of conception. ^{1/2/3}

MIXTURE - SECONDARY STANDARDS

The DNA profile was interpreted as a mixture of two/three individuals. A distinct male/female profile was determined to be used as the secondary standard for PERSON. ^{1/2/3}

TRASH PULL – PROFILE TO BE USED FOR STATISTICAL CALCULATIONS

The DNA profile [from ITEMDESCRIPTION] was interpreted as [a mixture / originating from a single individual]. A [distinct] male/female profile was determined from the sample and was purported to belong to PERSON. ^{1/2/3}

MULTIPLE EVIDENTIARY OR STANDARD SAMPLES

For varied mixture results

DNA profiles were developed for # additional samples of ITEMDESCRIPTION; however, these profiles were not used for statistical purposes. ^{1/2/3}

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PARENTAGE RESULTS

ALLEGED INDIVIDUAL CANNOT BE EXCLUDED

STANDARD TRIO AND SINGLE PARENTAGE

PERSON1 and CHILD were evaluated as biological father and child by considering the following propositions:

- ▶ H1: [PERSON2 is the mother and] PERSON1 is the father of CHILD.
- ▶ H2: [PERSON2 is the mother and] A[a]n unknown, unrelated man is the father of CHILD.
- ▶ The genetic results are CPI times more likely if PERSON1 is the father of CHILD than if an unknown, unrelated man is the father of CHILD.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON1 and CHILD are related as biological father and child.

INCESTUOUS PARENTAGE

PERSON1 and CHILD were evaluated as biological father and child by considering the following propositions:

- ▶ H1: PERSON2 is the mother of CHILD; PERSON1 is the father of PERSON2 and CHILD.
- ▶ H2: PERSON2 is the mother of CHILD; PERSON 1 is the father of PERSON2; and an unknown, unrelated man is the father of CHILD.
- ▶ The genetic results are CPI times more likely if PERSON1 is the father of CHILD than if an unknown, unrelated man is the father of CHILD.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON1 and CHILD are related as biological father and child.

REVERSE PARENTAGE

PERSON1 and PERSON2 were evaluated as the biological parents of CHILD by considering the following propositions:

- ▶ H1: PERSON1 and PERSON2 are the parents of CHILD.
- ▶ H2: Two unknown, unrelated individuals are the parents of CHILD.

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- ▶ The genetic results are CPI times more likely if PERSON1 and PERSON2 are the parents of CHILD than if two unknown, unrelated individuals are the parents of CHILD.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON1 and PERSON2 are the biological parents of CHILD.

ALLEGED INDIVIDUAL CAN BE VISUALLY EXCLUDED

Given the lack of genetic markers that must be contributed to the child by the biological father in the genetic results, PERSON can be excluded as the biological father of CHILD.

OTHER RELATIONSHIPS

SIBSHIP (LR SUPPORT FOR RELATIONSHIP)

CHILD1 and CHILD2 were evaluated as full siblings by considering the following propositions:

- ▶ H1: CHILD1 and CHILD2 are related as full siblings.
 - ▶ H2: CHILD1 and CHILD2 are unrelated.
- ▶ The genetic results are CPI times more likely if CHILD1 and CHILD2 are related as full siblings than if CHILD1 and CHILD2 are unrelated.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that CHILD1 and CHILD2 are related as full siblings.

PEDIGREE OPTION 1

PERSON1, PERSON2, and PERSON3 were evaluated for relatedness by considering the following propositions:

- ▶ H1: PERSON1 is the X of PERSON2 and [and the Y of] PERSON3.
 - ▶ H2: PERSON1 is the X of PERSON2; PERSON3 is unrelated.
- ▶ The genetic results are CPI times more likely if PERSON1, PERSON2, and PERSON3 are related as alleged (H1) than if PERSON3 is unrelated to PERSON1 and PERSON2 (H2).
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that includes the alleged relationship (H1).

PEDIGREE OPTION 2

PERSON1, PERSON2, and PERSON3 were evaluated for relatedness by considering the following propositions:

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- ▶ H1: PERSON1 is the X of PERSON2 and [and the Y of] PERSON3.
- ▶ H2: PERSON1 is the X of PERSON2; PERSON3 is unrelated.

- ▶ The genetic results are CPI times more likely if PERSON1, PERSON2, and PERSON3 are related as X [and Y] than if PERSON3 is unrelated to PERSON1 and PERSON2.
- ▶ This statistical analysis provides limited/moderate/strong/very strong support for the proposition that PERSON3 is a X [and Y] of PERSON1 and PERSON2.

ADDITIONAL STATEMENT FOR LIMITED SUPPORT FOR H1

The statistical results for RELATIONSHIP are in a range where adventitious support with unrelated individuals can occur. These results should be considered in the context of other information known in the case when making determinations of the identity of the individual. Additional genetic testing is recommended if further information is needed. Please contact the reporting analyst if desired.

UNINFORMATIVE RESULTS

CHILD1 and CHILD2 were evaluated as full siblings by considering the following propositions:

- ▶ H1: CHILD1 and CHILD2 are related as full siblings.
- ▶ H2: CHILD1 and CHILD2 are unrelated.

- ▶ The genetic results are equally as likely if CHILD1 and CHILD2 are related as full siblings or if CHILD1 and CHILD2 are unrelated.
- ▶ This result is considered uninformative.

LR SUPPORT FOR LIMITED EXCLUSION

CHILD1 and CHILD2 were evaluated as full siblings by considering the following propositions:

- ▶ H1: CHILD1 and CHILD2 are related as full siblings.
- ▶ H2: CHILD1 and CHILD2 are unrelated.

- ▶ The genetic results are CPI times more likely if CHILD1 and CHILD2 are unrelated than if CHILD1 and CHILD2 are related as full siblings.
- ▶ This statistical analysis provides limited support for the proposition that CHILD1 and CHILD2 are unrelated.

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The statistical results for full siblings are in a range where the alleged relationship is not supported despite there being a potential biological relationship. These results should be considered in the context of other information known in the case when making determinations of the identity of the individual. Additional genetic testing is recommended if further information is needed. Please contact the reporting analyst if desired.

ALLEGED INDIVIDUAL IS EXCLUDED BY CPI ($1/CPI \geq 100$)

CHILD1 and CHILD2 were evaluated as full siblings by considering the following propositions:

- ▶ H1: CHILD1 and CHILD2 are related as full siblings.
- ▶ H2: CHILD1 and CHILD2 are unrelated.
- ▶ The genetic results are CPI times more likely if CHILD1 and CHILD2 are unrelated than if CHILD1 and CHILD2 are related as full siblings.
- ▶ This statistical analysis supports the proposition that CHILD1 and CHILD2 are unrelated individuals.

ALLEGED INDIVIDUAL IS EXCLUDED BY FULL Y-STR ANALYSIS

Given the inconsistencies between the Y-STR genetic markers, CHILD1 can be excluded as the biological full sibling of CHILD2.

ALLEGED INDIVIDUAL IS INCLUDED BY FULL Y-STR ANALYSIS (NO INCONSISTENCIES)

The Y-STR profile from PERSON 1 was compared to the Y-STR profile developed from PERSON 2. No mismatches were observed out of # evaluated Y-STR loci. Based on this comparison, it is likely that the donors of the Y-STR profiles are paternally related. These results are based only on the Y-STR data developed at the time of reporting and do not supersede any additional genetic testing that may be performed. They should be evaluated in context with the autosomal kinship statistical results reported above.

STATEMENTS LOCATED AT THE END OF THE REPORT

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OBSERVED MUTATION or NULL ALLELE

Incorporating a null allele into kinship calculations requires Technical Leader permission.

A single genetic inconsistency between CHILD and ALLEGED FATHER was observed at LOCUS. This has been incorporated into the calculation as a [mutation/null allele].

OBSERVED MUTATION AT A Y-STR LOCUS WITH FULL Y-STR ANALYSIS

A single genetic inconsistency between the decedent and JOHN DOE was observed at LOCUS. This has been incorporated into the calculation as a mutation. The remaining 22 Y-STR loci were not evaluated statistically; however, these Y-STR results support the proposition that John DOE and the decedent are paternally related.

BODY IDENTIFICATION WITH CODIS ENTRY

If a positive identification is made, notify the reporting analyst such that associated family reference standards may be removed from CODIS as required.

The Appendix below will be added to all Paternity and Missing Person Requests in Justice Trax after the analyst's signature, formatted as a stand-alone page.

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Relationship Comparison Report Appendix

The following includes supplemental information on relationship comparisons performed at the Indiana State Police Forensic Services Division (FSD) and definitions of some commonly used terms. Not all terms referenced in the appendix may apply to the testing in the associated case report. Please contact the reporting analyst with specific questions regarding the DNA analysis in this case.

DNA Kit Chemistry and Technology: Citations in the report refer to the test(s) listed below.

1. PowerPlex® Fusion 6C: DNA profiles are developed using Polymerase Chain Reaction (PCR) and capillary electrophoresis with the PowerPlex® Fusion 6C System by Promega Corporation. Y-STR loci may be used for interpretations but are not included in statistical estimates.
2. ForenSeq® DNA Signature Plus, Primer Set B: DNA profiles are developed using Polymerase Chain Reaction (PCR) and Next-Generation Sequencing (NGS) with the ForenSeq® DNA Signature Plus Kit, Primer Set B. Y-STR and identity informative SNP loci may be used for interpretations but may not be included in statistical estimates. X-STR loci may be used for interpretations but are not included in statistical estimates. Phenotype informative SNP loci and ancestry informative SNP loci may be used for investigative purposes only.
3. PowerPlex® Y23: Y-STR profiles were developed using Polymerase Chain Reaction (PCR) and capillary electrophoresis with the PowerPlex® Y23 System by Promega Corporation. Y-STR loci may be used for interpretations but are not included in statistical estimates for relationship comparisons unless a mutation is suspected.

- I. Insufficient DNA:** Following extraction and quantitation, DNA profiles are not developed from samples which do not yield enough DNA based on the FSD's established threshold.
- II. An unknown individual** may be given a designation (e.g., "Unknown Male #1") to distinguish between multiple unknown profiles within a case or to indicate multiple samples consistent with the same unknown profile.
- III. A mixture** is a sample that contains DNA from more than one person. Mixtures with limited or low quality data and mixtures of more than four contributors are not interpretable by the FSD's current methods.
- IV.** An individual is **assumed** to be a contributor when their contribution to a DNA sample is reasonably expected based upon the context of the sample and the presence of their DNA is not in question. This may include samples recovered from the individual's own body or from an item they regularly use. Using the DNA profile of the assumed individual allows for a more accurate interpretation and representative statistic for the portion of the profile that is in question.
- V.** Statistics are presented in the form of a **likelihood ratio** (LR), which is a comparison between two competing hypotheses to explain the profiles. If all parties agree that the alleged relationship is true, the LR would equal 1. An LR of 0 indicates no support for the relationship. Calculations are only reflective of the relationship(s) tested. If an alternate relationship should be considered, please contact the reporting analyst.
- VI.** Calculations are performed utilizing the population data associated with the racial information provided for the alleged individual and is documented in the case record. Population data from the Caucasian, African American, Hispanic, and Asian populations are available for calculation. If an individual is multi-racial or if racial information is unknown the Combined population data is utilized. If additional information should be considered for this case, please contact the reporting analyst.
- VII.** Closely related **family members** (such as parents, offspring, and siblings) share genetic information. If a case involves a related individual as an additional person of interest, the best course of action is to submit known DNA standards for direct comparison and statistical evaluation.
- VIII.** Statistical calculations are reported with a **verbal qualifying statement** to express the strength of statistical support. These statements follow the verbal scale recommended by the Scientific Working Group on DNA Analysis Methods (7/12/18). The scale reflects stronger degrees of support as the likelihood ratio increases.

Likelihood Ratio	Support Level	Support for:
$LR \geq 1,000,000$	Very strong	Alleged Relationship
$10,000 \leq LR < 1,000,000$	Strong	Alleged Relationship
$100 \leq LR < 10,000$	Moderate	Alleged Relationship
$2 \leq LR < 100$	Limited	Alleged Relationship
1	Uninformative	Uninformative
$2 \leq (1/LR) < 100$	Limited	Unrelated
$100 \leq (1/LR)$	--	Unrelated

- IX. Evidence disposition:** Unless otherwise noted, all portions of the evidence not consumed during analysis will be returned to the submitting agency. Retained items will remain at the Indiana State Police Forensic Services Division. The remainder of the evidence or the original packaging will be returned.

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RELATIONSHIP TESTING EXAMPLES

Example 1: Standard paternity trio

Item 001: DNA standard - John Smith

The DNA profile was developed.¹

Item 002: Product of conception

The DNA profile was interpreted as a mixture of two individuals. Assuming Sally Smith as a contributor, a distinct male profile was determined to be used as the standard for the product of conception.¹

Item 003: DNA standard - Sally Smith

The DNA profile was developed.¹

John Smith and the product of conception were evaluated as biological father and child by considering the following propositions:

- ▶ H1: Sally Smith is the mother and John Smith is the father of the product of conception.
- ▶ H2: Sally Smith is the mother and an unknown, unrelated man is the father of the product of conception.
- ▶ The genetic results are 1,000,000 times more likely if John Smith is the father of the product of conception than if an unknown, unrelated man is the father of the product of conception.
- ▶ This statistical analysis provides very strong support for the proposition that John Smith and the product of conception are related as biological father and child.

Example 2: Direct comparison of remains to secondary standard

Item 001: DNA standard - Decedent

The DNA profile was interpreted as originating from a single individual.¹

- ▶ The DNA profile is at least 1 trillion times more likely if it originated from Ron Smith than if it originated from an unknown, unrelated individual.

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► This statistical analysis provides very strong support for the proposition that Ron Smith is **included**.

Item 002: Toothbrush (secondary standard) - Ron Smith

The DNA profile was interpreted as a mixture of two individuals. A distinct male profile was determined to be used as the secondary standard for Ron Smith.¹

Example 3: Sibship with limited support for exclusion

Item 001: DNA standard – John Smith

The DNA profile was developed.¹

Item 002: DNA standard – Sally Smith

The DNA profile was developed.¹

John Smith and Sally Smith were evaluated as full siblings by considering the following propositions:

- H1: John Smith and Sally Smith are related as full siblings.
- H2: John Smith and Sally Smith are unrelated.
- The genetic results are 10 times more likely if John Smith and Sally Smith are unrelated than if John Smith and Sally Smith are related as full siblings.
- This statistical analysis provides limited support for the proposition that John Smith and Sally Smith are unrelated.

The statistical results for sibship are in a range where the alleged relationship is not supported despite there being a potential biological relationship. These results should be considered in the context of other information known in the case when making determinations of the identity of the individual. Additional genetic testing is recommended if further information is needed. Please contact the reporting analyst if desired.

Example 4: Pedigree, same nuclear family

Item 001: DNA standard – John Smith

The DNA profile was developed.¹

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Item 002: DNA standard – Sally Smith

The DNA profile was developed.¹

Item 003: DNA standard – Jane Smith

The DNA profile was developed.¹

John Smith, Sally Smith, and Jane Smith were evaluated for relatedness by considering the following propositions:

- ▶ H1: John Smith is the father of Sally Smith and Jane Smith; Sally Smith is the full sibling of Jane Smith.
- ▶ H2: John Smith is the father of Sally Smith; Jane Smith is unrelated.
- ▶ The genetic results are 1,000,000 times more likely if John Smith, Sally Smith, and Jane Smith are related as alleged (H1) than if Jane Smith is unrelated to John Smith and Sally Smith (H2).
- ▶ This statistical analysis provides very strong support for the proposition that includes the alleged relationship (H1).

Example 5: Pedigree, extended family

Item 001: DNA standard – John Smith

The DNA profile was developed.¹

Item 002: DNA standard – Sally Smith

The DNA profile was developed.¹

Item 003: DNA standard – Jane Smith

The DNA profile was developed.¹

John Smith, Sally Smith, and Jane Smith were evaluated for relatedness by considering the following propositions:

- ▶ H1: John Smith is the grandfather of Sally Smith and Jane Smith; Sally Smith is the cousin of Jane Smith.
- ▶ H2: John Smith is the grandfather of Sally Smith; Jane Smith is unrelated.

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- ▶ The genetic results are 99 times more likely if John Smith is the grandfather of Sally Smith and Jane Smith, and Sally Smith is the cousin of Jane Smith, than if Jane Smith is unrelated to John Smith and Sally Smith.
- ▶ This statistical analysis provides limited support for the proposition that John Smith is the grandfather of Sally Smith, and Jane Smith and Sally Smith is the cousin of Jane Smith.

The statistical results for the familial relationships are in a range where adventitious support with unrelated individuals can occur. These results should be considered in the context of other information known in the case when making determinations of the identity of the individual. Additional genetic testing is recommended if further information is needed. Please contact the reporting analyst if desired.

Example 6: Trash pull, single parentage

Item 001: DNA standard – Sally Smith

The DNA profile was developed.¹

Item 002: Water bottle

The DNA profile [from the water bottle] was interpreted as originating from a single individual. A male profile was determined from the sample and was purported to belong to Ron Smith.¹

The contributor of the DNA profile from item 002A and Sally Smith were evaluated as biological father and child by considering the following propositions:

- ▶ H1: The contributor of the DNA profile from item 002A is the father of Sally Smith.
 - ▶ H2: An unknown, unrelated man is the father of Sally Smith.
- ▶ The genetic results are 1,000,000 times more likely if the contributor of the DNA profile from item 002A is the father of Sally Smith than if an unknown, unrelated man is the father of Sally Smith.
- ▶ This statistical analysis provides very strong support for the proposition that the contributor of the DNA profile from item 002A and Sally Smith are related as biological father and child.

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MEMO FOR Y-STR TESTING TO SUPPORT INVESTIGATIVE GENETIC GENEALOGY:

Investigator Name

Agency

Address

City, IN Zip

Date

Agency Case Number:

ISP Forensic Services Division Case Number:

This notice is to inform you of potential investigative information. Y-STR testing utilizing PowerPlex® Y23 has been performed to aid in the genealogical investigation for the above referenced case.

This information is for investigative purposes only. No statistical analysis has been performed to give statistical weight to any potential familial relationships or the frequency of the Y-STR profile(s) in the population and is therefore not appropriate to support criminal prosecution. It is recommended that standards of any persons of interest be submitted for direct comparison to the profile in question.

The genetic results are based only on the data developed at the time of reporting and do not supersede any additional genetic testing that may be performed. Male individuals in the same paternal line are expected to share the same Y-STR profile. Differences in Y-STR profiles may indicate distantly related male individuals due to mutations or it may indicate a non-relationship. As the differences accumulate, this indicates more distant relationships or provides more support for a non-relationship. A variety of factors, including the number of Y-STR loci analyzed and mutation rate estimates, currently impede the ability to make specific conclusions on suspected familial relationships. Additional kinship analysis may provide more information.

DNA results for these items have been described in ISP issued Certificate of Analysis Request XXX. The Y-STR profile from *sample description* (item #) was compared to the Y-STR profile developed from *John Doe* (item #). # mismatches were observed out of # evaluated Y-STR loci. Based on this comparison, it is likely that the donors of the Y-STR profiles are *paternally related/probably paternally related/possibly paternally related/not related by a recent common paternal ancestor*. These results should be utilized with caution.

Analyst

Biology Section

Indiana State Police Forensic Services Division

Year/Lab/Memo#

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

Y-STR Report Wording

THE BEGINNING OF RESULTS SECTION (if applicable)

SPERM/NON-SPERM FRACTION STATEMENT

To be placed at the beginning of the results section if sperm and non-sperm fractions are reported.

In the results detailed below, [a portion of the] samples were processed utilizing a differential extraction procedure. The terms "sperm fraction" and "non-sperm fraction" refer to the two portions of the extract generated during the differential extraction procedure. The presence of DNA in the "sperm fraction" does not confirm the presence of seminal material and is not directly correlated to the cell types from which the DNA originated.

EXCLUSION

PERSON is **excluded**.

SINGLE SOURCE STATEMENTS

UNKNOWN PROFILE

Standard is excluded or no standard is present

The Y-STR profile [from ITEMDESCRIPTION] was interpreted as originating from a single male or male lineage.

CONSISTENT STATEMENT

Profile obtained from sample; no differences from standard

The Y-STR profile [from ITEMDESCRIPTION] was interpreted as originating from a single male or male lineage.

- ▶ The [Y-STR] profile is LR times more likely if it originated from PERSON or any of his male paternal relatives than if it originated from an unknown, unrelated male individual.
- ▶ This statistical analysis provides limited/moderate support for the proposition that PERSON is **included**.

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CONSISTENT STATEMENT + ADDITIONAL ALLELE

Profile obtained from sample; no differences from standard with additional allele

The Y-STR profile [from ITEMDESCRIPTION] was interpreted as originating from a single male or male lineage. However, one additional allele was detected from which no conclusion can be drawn.

- ▶ The [Y-STR] profile is LR times more likely if it originated from PERSON or any of his male paternal relatives than if it originated from an unknown, unrelated male individual.
- ▶ This statistical analysis provides limited/moderate support for the proposition that PERSON is **included**.

CONSISTENT WITH MALE VICTIM

The Y-STR profile [from ITEMDESCRIPTION] was interpreted as originating from a single male or male lineage. Assuming PERSON as a contributor, no additional [Y-STR] alleles were detected.

MIXTURE STATEMENTS

INDISTINGUISHABLE MIXTURE

The Y-STR profile is a mixture in which a major profile could not be determined. The results do not qualify for statistical calculations; therefore, no further conclusions can be drawn.

MAJOR/MINOR COMPONENTS

The Y-STR profile [from ITEMDESCRIPTION] demonstrated the presence of a mixture with a major profile assumed to be a single male or male lineage. No conclusion can be drawn regarding the additional [Y-STR] alleles.

- ▶ The major [Y-STR] profile is LR times more likely if it originated from PERSON or any of his male paternal relatives than if it originated from an unknown, unrelated male individual.
- ▶ This statistical analysis provides limited/moderate support for the proposition that PERSON is **included**.

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MAJOR/MINOR COMPONENTS, NO STANDARD:

The Y-STR profile [from ITEMDESCRIPTION] demonstrated the presence of a mixture with a major profile assumed to be a single unknown male or male lineage. No conclusion can be drawn regarding the additional [Y-STR] alleles.

MAJOR CONSISTENT WITH MALE VICTIM

The Y-STR profile [from ITEMDESCRIPTION] demonstrated the presence of a mixture with a major profile assumed to be a single male or male lineage. Assuming PERSON as a contributor, no conclusion can be drawn regarding the additional [Y-STR] alleles.

INCONCLUSIVE/NOT EXAMINED

LIMITED DATA

The Y-STR profile has limited data; therefore, no further conclusions can be drawn.

NO PROFILE

ITEMDESCRIPTION failed to demonstrate a Y-STR profile.

FAILED CONTROL

No conclusion can be drawn regarding ITEMDESCRIPTION because the/a test control[s] did not meet required parameters.

SAMPLE NOT EXAMINED

No Y-STR analysis was performed on # additional samples at this time.

SAMPLE NOT SUITABLE

Upon evaluation, no samples were determined to be suitable for Y-STR analysis.

STANDARDS

STANDARD PROFILE DEVELOPED

The [autosomal and] Y-STR profile [of PERSON] [was/were] developed.

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STANDARD NOT EXAMINED

No Y-STR analysis was performed [on the DNA standard of PERSON] due to a lack of evidence for comparison.

REQUESTS FOR STANDARDS

If [additional] Y-STR comparisons are desired, please submit an appropriate male DNA standard (such as an oral/buccal standard) from any suspect(s).

CONTAMINATION FROM LABORATORY PERSONNEL

Not all possible situations are given, consultation with the Technical Leader and/or Supervisor is recommended

SINGLE SOURCE

No Y-STR profile of apparent value was identified on ITEMDESCRIPTION. The Y-STR profile of an individual from the Indiana State Police Forensic Services Division Quality Assurance DNA Database was identified.

MIXTURE

The Y-STR profile obtained from ITEMDESCRIPTION demonstrated the presence of a mixture with a major profile. The major [Y-STR] profile is consistent with the Y-STR profile of an individual from the Indiana State Police Laboratory Forensic Services Quality Assurance DNA Database. No conclusion can be drawn regarding the additional [Y-STR] alleles.

REQUIRED STATEMENTS (PLACED AFTER RESULTS):

WORDING OF STATISTICAL ANALYSIS

Y-STR results for item[s] XA have been searched using the Y Chromosome Haplotype Reference Database (<https://yhrd.org>), Release R# on date.

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The Appendix below will be added to all Y-STR requests in Justice Trax after the analyst's signature, formatted as a stand-alone page.

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Y-STR Report Appendix

The following includes supplemental information on biological analysis performed at the Indiana State Police Forensic Services Division (FSD) and definitions of some commonly used terms. Not all terms referenced in the appendix may apply to the testing in the associated case report. Please contact the reporting analyst with specific questions regarding the DNA analysis in this case.

- I. This report and evaluation only provide information that helps address who may be the source of the DNA - not how or when the DNA was deposited. This report cannot contribute any information regarding the mechanisms, timings, or actions that led to the deposition of the recovered biological material.
- II. Biological (DNA-based) sex information reported here is based solely on the detection of Y chromosome markers. Reference to a male in the report should not be misinterpreted to mean that an individual must appear as male based upon this indication alone.
- III. **Insufficient Male DNA:** Following extraction and quantitation, Y-STR profiles are not developed from samples which do not yield enough male DNA based on the FSD's established threshold. Male DNA quantitation at low levels can be unreliable and does not confirm the presence of male DNA.
- IV. An **unknown male** may be given a designation (e.g., "Unknown Male #1") to distinguish between multiple unknown male profiles within a case or to indicate multiple samples consistent with the same unknown profile.
- V. A **mixture** is a sample that contains DNA from more than one male. Samples in which a major contributor cannot be determined, minor portions of mixtures, or samples with limited data are not suitable for comparison by the FSD's current methods.
- VI. Statistics are presented in the form of a **likelihood ratio** (LR), which is a comparison between two competing hypotheses to explain the evidence profile. If all parties agree that the DNA profile originated from the same individual(s), the LR would equal 1. Calculations for the Caucasian, African American, Hispanic, and Asian populations are performed and a LR is reported for the combined populations. Any statistical estimates performed are based on the recommended concepts endorsed by the Scientific Working Group on DNA Analysis Methods (3/2/22). The LR is based upon the case information provided. If additional information should be considered, please contact the reporting analyst.
- VII. Profiles are searched against the Y-Chromosome Haplotype Reference Database (YHRD). <https://yhrd.org>
- VIII. Statistical calculations are reported with a **verbal qualifying statement** to express the strength of statistical support. These statements follow the verbal scale recommended by the Scientific Working Group on DNA Analysis Methods (7/12/18). The scale reflects stronger degrees of support as the likelihood ratio increases. Reported exclusion(s) may include statistical calculations to support the verbal qualifying statement.

Likelihood Ratio	Support Level	Support for:
$LR \geq 1,000,000$	Very strong	Inclusion
$10,000 \leq LR < 1,000,000$	Strong	Inclusion
$100 \leq LR < 10,000$	Moderate	Inclusion
$2 \leq LR < 100$	Limited	Inclusion
1	Uninformative	Uninformative
$2 \leq (1/LR) < 100$	Limited	Exclusion
$100 \leq (1/LR)$	--	Exclusion

- IX. **Evidence disposition:** Unless otherwise noted, all portions of the evidence not consumed during analysis will be returned to the submitting agency. Retained items will remain at the Indiana State Police Forensic Services Division. The remainder of the evidence or the original packaging will be returned.
- X. At this time, Y-STR profiles cannot be entered into the Combined DNA Index System (CODIS).
- XI. **DNA chemistry:** Y-STR profiles are developed using Polymerase Chain Reaction (PCR) with the PowerPlex® Y23 System by Promega Corporation.

INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS APPENDIX 8 DEFINITIONS

1. 99% 1-Sided Lower HPD – A confidence interval is applied to the point estimate to account for uncertainty. The interval gives a range of where the true allele probabilities lie. The lower end of the HPD is reported to be the most conservative to the person of interest for an inclusionary LR.
2. Acid Phosphatase – a chemical component of semen; also found in other body fluids at significantly lower concentrations.
3. Allele – the alternative form of a gene.
4. Allele Frequency – the proportion of a particular allele found in a population.
5. Allelic Ladder – a set of DNA fragments of the commonly known alleles for each locus. By comparing the samples to the allelic ladder, the correct allele designation may be assigned.
- 6.
7. Amelogenin – the marker for determining the biological sex of the individual contributor to a DNA profile.
8. Amplification – using the PCR process to create many copies of a specific DNA sequence(s). An increase in the number of copies of a specific DNA fragment.
9. Analyst Discretion - the use of individual judgment, based on an analyst's training and experience to determine the optimum modes of analysis for an item of evidence.
10. Artifact – non-allelic product of the amplification process, an anomaly of the detection process, or a by-product of primer synthesis. A data peak that does not represent a true allele.
11. Assumed reference – A standard from an individual whose DNA is reasonably expected based upon the context of the sample and the presence of their DNA is not in question. This may include samples recovered from the individual's own body or from an item they regularly use. It may also include a consensual partner of an individual.
12. Base Pairs – paired nucleotides which make up the DNA molecule. Two complementary nucleotides joined by a hydrogen bond.
13. Blood – the fluid that circulates through the body, containing red and white blood cells carrying oxygen and nutrients to cells throughout the body; and carrying away waste and carbon dioxide.
14. Burn-in – The initial 10,000 accepts during the MCMC process that allow the chains to converge on a "good space". These iterations are discarded.
15. Capillary Electrophoresis – a method to separate DNA fragments based on size using electrical current. The DNA sample is placed in a thin tube (capillary) containing polymer and subjected to high voltage current allowing the DNA fragments to migrate through the tube.
16. Chromosome – the structure on which genes are naturally arranged and how DNA is organized.
17. Combined DNA Index System (CODIS) – refers to the DNA database and its software. It is composed of National (NDIS), State (SDIS), and Local (LDIS) components. It contains DNA profiles from offenders, crime scenes and includes a missing person database.
18. Conditioning – A likelihood ratio in which an individual is included in both H_1 and H_2 .
19. Contamination – When DNA is present in a sample that is not endogenous to the sample.

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20. DBLR™ (Database Likelihood Ratios) – Software designed for the calculation of LR_s, performing any conceivable kinship calculation, rapidly searching databases, evaluating the value of individual mixture components, and assessing samples for a common donor.
21. Deconvolution – Process to determine the potential contributor genotypes to a mixed DNA profile.
22. Degradation – the breaking down of the DNA molecule into smaller fragments.
23. Denaturation – the separation of the double stranded DNA molecule into two single strands.
24. Deoxyribonucleic Acid (DNA) - the genetic material present in the nucleus of most cells. Also located in cell mitochondria.
25. DNA Sequence - a specific order of base pairs.
26. Electropherogram – the visual representation of the DNA fragments contained in each sample; generated by the analysis software of the Capillary Electrophoresis Instrument.
27. Electrophoresis – the method to separate molecules based on their size by placing them in a medium and applying an electrical current. The molecules will travel through the medium at different rates, the smaller molecules traveling through the medium more quickly than the larger ones.
28. Enzyme – a protein which acts as a catalyst, speeding up a specific chemical reaction without being changed or consumed in the process.
29. Epithelial Cells – skin cells and other surface cells such as from the mouth (buccal cells) or the vaginal cavity.
30. Sub-Source LR – Likelihood ratio which compares the person of interest to the total mixture independent of contributor order. Formerly known as the Factor of N! LR.
31. Gelman-Rubin – A value which informs the user whether the MCMC chains have converged.
32. Gene – the basic functional unit of heredity. Most genes determine the structure and function of proteins.
33. Genotype – the specific genetic sequence of a person's DNA.
34. Genotype Probability Distribution – A summary of the genotype combinations accepted at each locus and the percentage of accepts per genotype.
35. H_1/H_p – Interchangeable terms for the most likely proposition that includes a person of interest.
36. H_2/H_d – Interchangeable terms for the most likely proposition that excludes a person of interest.
37. Hair – a slender outgrowth from the skin of mammals.
38. Heme – the iron containing complex of the hemoglobin molecule.
39. Hemin – the oxidized heme molecule.
40. Hemoglobin - the iron containing protein in blood.
41. Heterozygous – having different alleles at a particular locus.
42. Homozygous – having two of the same allele at a particular locus.
43. Internal Lane Standard (ILS) – a set of DNA fragments of known length(s). The ILS is simultaneously injected with all DNA samples during electrophoresis. This allows accurate measurement of the length of each allele in a DNA sample.
44. Iteration – A proposed genotype combination and mass parameters that are either accepted or rejected during the MCMC process.
45. Likelihood Ratio – A ratio of the probability of the evidence given two different propositions (H_1 and H_2).
46. Locus (loci) – the physical location of a gene on a chromosome.
47. LUS – Longest uninterrupted stretch for an allele variation.
48. Marker – a gene of known location and phenotype used as a point of reference.

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- 49. Mass Parameters – Collection of parameters (degradation rate per contributor, amplification efficiencies per locus, replicate amplification strength, template per contributor, and kit efficiency), that are used to determine expected peak heights during deconvolution. Collectively referred to as DARTB.
- 50. Maximum Likelihood Estimate (MLE) LR – A likelihood ratio calculated when a variable number of contributors deconvolution was utilized. The MLE assigns an LR using the most probable number of contributors under H_1 and H_2 . This means the number of contributors in the numerator and denominator may be the same or they may be different. The number of contributors will depend on which number of contributor analysis has the highest likelihood given the proposition for each.
- 51. MCMC (Markov Chain Monte Carlo) – A mathematical method that uses a process of random re-sampling to give the best explanation of observed data.
- 52. Meaningful profile – a profile that is developed from an item or location where the individual's profile would not reasonably be expected. A person's profile may be expected on an item or sample from their own body, their own clothing, or their property such as their house or vehicle. All other samples will generally be considered meaningful.
- 53. Minimum allele frequency - a conservative frequency correction for alleles observed less than 5 times in the STR population frequency tables.
- 54. Mitochondria - a part of a cell used in energy production. Contains circular DNA inherited from the mother only.
- 55. Nucleus - the part of a cell that contains the double stranded DNA inherited from both parents.
- 56. Phenotype – the expressed genotype.
- 57. Polymerase – an enzyme that initiates the duplication of a DNA molecule.
- 58. Polymerase Chain Reaction (PCR) – a process for amplifying (copying) the DNA molecule.
- 59. Polymorphism – the presence of more than one possible allele set for a specific gene.
- 60. Population – a stable group of random individuals, chosen for genetic analysis.
- 61. Primer – a short nucleotide fragment of known sequence used to locate its complementary sequence on the DNA molecule for the initiation of PCR. Primers target the specific loci to be amplified.
- 62. Proficiency Testing – a test to evaluate the competency of an analyst in a specific procedure.
- 63. Property crime – a category of crime that includes, among other crimes, burglary, theft, arson, drug offenses, vandalism, and criminal mischief. Property crimes do not involve force or threat of force.
- 64. Proposition – A proposed explanation of the data. Interchangeable with “hypothesis”.
- 65. Post Burn-in – The iterations after burn-in from which the deconvolution is determined.
- 66. Replicate – A reamplification of the same extract.
- 67. Saliva – digestive fluid from the mouth.
- 68. Semen/Seminal Fluid – the male ejaculate. In a dried state may be referred to as seminal material. Semen normally contains spermatozoa.
- 69. Short Tandem Repeat (STR) – small sections of DNA that contain short segments (2, 3, 4 or more base pairs) which repeat several times. The number of repeat units may vary between individuals. STRs are located between specific genes and are considered non-functional.
- 70. Spermatozoa – the male reproductive cell, sperm cell.
- 71. Stochastic effect – peak imbalance observed in a locus and/or allele drop-out due to random, disproportionate amplification of alleles in low quality/quantity template samples.

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- 72. STRmix™ - A fully continuous probabilistic genotyping software which combines biological modeling with mathematical processes to deconvolute DNA profiles and then compare them to reference standards and provide statistical weight in the form of a likelihood ratio.
- 73. Stutter – an artifact that occurs as a by-product of the PCR process. It is observed as a minor peak typically observed one repeat unit smaller than a primary STR allele caused by strand slippage during amplification. Stutter has been observed and documented in other positions.
- 74. Substrate – the material on which a biological sample is deposited such as at a crime scene.
- 75. SWGDAM – Scientific Working Group on DNA Analysis Methods.
- 76. Unified Likelihood Ratio – LR that takes into account that the unknown contributors are made up of both relatives and unrelated people.
- 77. Validation – a study to assess whether a particular procedure (or instrument) can obtain a desired result reliably and reproducibly. The study includes looking at the conditions necessary to obtain those results and the limitations of the procedure/instrument. All DNA analysis methods shall be validated prior to implementation.
- 78. Verbal Scale – A verbal representation of the relative strength of the likelihood ratio results.
- 79. Work Product – the material that is generated as a function of analysis, which may include extracts and amplified product, in tubes or plates, and any aliquots thereof.
- 80. X chromosome – a sex chromosome, present twice in female cells and once in male cells.
- 81. Y chromosome – a sex chromosome, present once in male cells (and absent in female cells), paired with the X chromosome.

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The DNA data drive (“DNA Data”) will be used for storage and archiving of case data and photos. Real Time PCR files, 3500 run folders, GeneMapper™ ID-X projects, genotypes table .txt files, STRmix™ files, DBLR™ files, photos, and NGS files shall be saved to DNA Data. Any worksheets, statistic sheets, typed notes pages, or workbooks shall NOT be saved to DNA Data. The completed case record in LIMS will be the official, tracked copy. We want to avoid duplication of items between LIMS and the server.

In DNA Data, each analyst will have a folder to organize and store their data files. Other analysts will have access to the items in the folder for purposes of review (read-only), but they shall not change, delete, or add items within the folder.

Within the analyst’s folder there will be one folder for each year of the analyst’s casework. The analyst will create one folder for each case number and request, which will contain all associated electronic data for that request. If no electronic data is generated for a request, the analyst does not need to create a case folder. It is recommended to organize each case folder within the year folder that matches the case number, not the year the case was completed.

Batched data:

Any projects, quant data, or run folders that contain multiple cases shall be saved individually within each of the associated case folders. Batch data generated by the Automation team is an exception. Automation batch data shall be stored only in the zAutomation folder and not duplicated per individual case.

The following nomenclature should be used:

- Laboratory case and request folder – “case number_request number” – ex. “1011234_1” – the request number corresponds to the laboratory request number assigned in LIMS.
 - RT-PCR projects: “1st case on the plate_date_plate#” – ex. “1011234_01Jan11_01”
 - 3500 run folder: “1st case on the plate_date_plateinjection#” – ex. “1011234_01Jan11_01” – plate injection number indicates how many times that plate preparation has been put in the instrument for injection; if the same plate preparation is run on a different date, the original date should still be used from when the plate was prepared. If a second preparation of a plate is made on the same date as the first, it shall be designated with a “-2” after the date. Ex. “1011234_01Jan11-2_01”
 - GeneMapper™ ID-X project name: must contain the date the 3500 plate was prepared, so the project matches the run folder. If there were multiple injections of the plate or multiple preparations of the plate on the same date, a number shall be added to clarify which run folder the data was pulled from. - ex. “01Jan11”, “1011234_01Jan11”, “Controls_01Jan11” or “1011234_01Jan11 (02)”. The exported genotypes table .txt file will maintain the same name as the project with the automatic extension “Genotypes Table” added during export.
 - STRmix™ results folders: The deconvolution results folder should include “case number_item number_Decon number”. The LR from previous results folder should include “case number_item number_Decon number-LRPrev number”. If additional characters are added to the name of the either the deconvolution results folder or the LR from previous results folder, those characters shall be added after the specified naming convention.

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- DBLR™ Kinship analysis name: At a minimum, the name shall include the case number, followed by an underscore and the DBLR run number, increasing sequentially (e.g., 23L1234_DBLR1, 23L1234_DBLR2). If additional characters are added to the “Analysis Name” designation, those characters shall be added after the specified naming convention.
- Photos: create a folder per item photographed – folder name “case_item#” ex. “10I1234_item1”. The individual photo file names shall, at a minimum, have the laboratory case number and the Forensic Scientist’s or Forensic Technician’s initials, name, or permanent employee (PE) number.

Note: RT-PCR and 3500 Plates generated by the automation team may substitute Batch ID # for Lab Case #. RT-PCR plate names generated by the automation team shall also include the automation analyst initials and PE number.

Note: For RT-PCR projects and 3500 run folders – only the first case on the plate should be included in the name. Do not write more than one case number and do not write “et al.” after the name.

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RELATIONSHIP COMPARISON STATISTICAL REFERENCE SHEET

Formulas for Paternity Index (PI) and Random Man Not Excluded (RMNE) (Table below adapted from the AABB, *Standards for Relationship Testing Laboratories*, 9th Edition, Appendix 7 and 8)

Mother	Child	Alleged Father	PI	RMNE	PE
BD	AB	AC	$1/2a$	$1-(1-a)^2$	$(1-a)^2$
BC	AB	AC	$1/2a$	$1-(1-a)^2$	$(1-a)^2$
BC	AB	AB	$1/2a$	$1-(1-a)^2$	$(1-a)^2$
BC	AB	A	$1/a$	$1-(1-a)^2$	$(1-a)^2$
B	AB	AC	$1/2a$	$1-(1-a)^2$	$(1-a)^2$
B	AB	AB	$1/2a$	$1-(1-a)^2$	$(1-a)^2$
B	AB	A	$1/a$	$1-(1-a)^2$	$(1-a)^2$
AB	AB	AC	$1/[2(a+b)]$	$1-(1-a-b)^2$	$(1-a-b)^2$
AB	AB	AB	$1/(a+b)$	$1-(1-a-b)^2$	$(1-a-b)^2$
AB	AB	A	$1/(a+b)$	$1-(1-a-b)^2$	$(1-a-b)^2$
AB	A	AC	$1/2a$	$1-(1-a)^2$	$(1-a)^2$
AB	A	AB	$1/2a$	$1-(1-a)^2$	$(1-a)^2$
AB	A	A	$1/a$	$1-(1-a)^2$	$(1-a)^2$
A	A	AB	$1/2a$	$1-(1-a)^2$	$(1-a)^2$
A	A	A	$1/a$	$1-(1-a)^2$	$(1-a)^2$
	AB	AC	$1/4a$	$1-(1-a-b)^2$	$(1-a-b)^2$
	AB	AB	$(a+b)/4ab$	$1-(1-a-b)^2$	$(1-a-b)^2$
	AB	A	$1/2a$	$1-(1-a-b)^2$	$(1-a-b)^2$
	A	AC	$1/2a$	$1-(1-a)^2$	$(1-a)^2$
	A	A	$1/a$	$1-(1-a)^2$	$(1-a)^2$

Paternity Index for Null allele in standard trio comparisons =

Known parent = AB, Child = AA or AS, Alleged parent = CC or CS

a = known parent shared allele frequency

c = alleged parent unshared allele frequency

s = silent allele frequency = 0.001

$$\frac{CS}{(2cs + c^2) \times (a + s)}$$

Adapted from ISFG 2022 Kinship Analysis Workshop and California Criminalistics Institute Kinship Analysis Course

Combined Paternity Index (CPI) = $PI_1 \times PI_2 \times PI_3 \dots$

Probability of Paternity (W) = $[CPI \times Pr]/[CPI \times Pr + (1-PR)]$

Pr = Prior Probability

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Formulas for Reverse Paternity

(Table below adapted from the AABB, Guidelines for Mass Fatality DNA Identification Operations, 2010, Appendix B)

Mother	Possible Child	Father	Formula
A	A	AB	$1/2a^2$
A	AB	AB	$1/4ab$
A	AB	BC	$1/4ab$
AB	A	AB	$1/4a^2$
AB	A	AC	$1/4a^2$
BC	AB	AB	$1/8ab$
BC	AB	AC	$1/8ab$
BD	AB	AC	$1/8ab$
A	A	A	$1/a^2$
AB	A	A	$1/2a^2$
B	AB	A	$1/2ab$
BC	AB	A	$1/4ab$
AB	AB	AC	$1/8ab$
AB	AB	A	$1/4ab$
AB	AB	AB	$1/4ab$

Formulas for Sibship

(Table below adapted from the AABB, Guidelines for Mass Fatality DNA Identification Operations, 2010, Appendix B)

Sibling	Possible Full Sibling	Formula
AB	AB	$(1+a+b+2ab)/8ab$
A	A	$(1+a)^2/(2a)^2$
A	AB	$(1+a)/4a$
AB	AC	$(1+2a)/8a$
AB	CD	$1/4$

Half Sibling	Possible Half Sibling	Formula
AB	AB	$(a+b+4ab)/8ab$
A	A	$(1+a)/2a$
A	AB	$(1+2a)/4a$
AB	AC	$(1+4a)/8a$
AB	CD	$1/2$

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Mutation Rates

Mutation rates for all autosomal loci present in the PowerPlex® Fusion 6C kit and D17S1301 and D6S1043 present in the ForenSeq® DNA Signature Plus Kit Primer Set B were reported in AABB, Relationship Testing Technical Report For Testing in 2023: As compared to the reported data from 2021 and 2022.

D20S482, D4S2408, and D9S1122 utilize the average paternal and maternal mutation rates from the tetranucleotide markers.

<u>LOCUS</u>	<u>PATERNAL</u>	<u>MATERNAL</u>
CSF1PO	0.000847	0.000086
D10S1248	0.00045	0.000116
D12S391	0.004373	0.000564
D13S317	0.000782	0.000152
D16S539	0.000556	0.000137
D18S51	0.001199	0.000251
D19S433	0.000411	0.00021
D1S1656	0.000628	0.000108
D21S11	0.00073	0.000486
D22S1045	0.000117	0.00004
D2S1338	0.000788	0.000081
D2S441	0.00025	0.000082
D3S1358	0.000736	0.000072
D5S818	0.000588	0.0001
D7S820	0.000562	0.000088
D8S1179	0.000909	0.000121
FGA	0.001761	0.00024
Penta D	0.001357	0.000407
Penta E	0.0034	0.000476
SE33	0.002501	0.000577
TH01	0.000057	0.000022
TPOX	0.000107	0.000038
vWA	0.001363	0.000158
D17S1301	0.000196	0.000588
D20S482	0.000969	0.000209
D4S2408	0.000969	0.000209
D6S1043	0.001519	0.000322
D9S1122	0.000969	0.000209

The average mutation rate used for iiSNP loci is set to 1×10^{-8} , the default parameter set in DBLR™ and supported by Nachman, M.W., Crowell, S. (2000) Estimate of the Mutation Rate per Nucleotide in Humans. Genetics. 156: 297-304.

The mutation factor for a single discordant Y-STR locus contained in the kit shall be incorporated as a haplotype Relationship Index and incorporated manually into the CRI by multiplying by 0.001 for all Y-STR loci except DYS570, DYS576, DYS612, and DYF387S1. This mutation rate value is supported by Ballantyne, K.N., et. al., (2012) A New Future of Forensic Y-chromosome Analysis: Rapidly Mutating Y-STRs for Differentiating Male Relatives and Paternal Lineages. Forensic Science International: Genetics. 6: 208-218. DYS570, DYS576, DYS612, and DYF387S1 are identified as rapidly mutating Y-STR loci. The mutation factor for a single discordant Y-STR locus defined as rapidly mutating will be incorporated as a haplotype Relationship Index and incorporated manually into the CRI by multiplying by 0.01. This rapidly mutation Y-STR definition and mutation rate value is supported by Boattini, A., et. al., (2016) Mutation

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Rates and Discriminating Power for 13 Rapidly-Mutating Y-STRs between Related and Unrelated Individuals. PLoS ONE.

NIST 1036 Revised U.S. Population Dataset (July 2017)

Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) U.S. population data for 29 autosomal STR loci. *Forensic Sci. Int. Genet.* 7: e82-e83.

Steffen, C.R., Coble, M.D., Gettings, K.B., Valone, P.M. (2017) Corrigendum to ‘U.S. Population Data for 29 Autosomal STR Loci’. *Forensic Sci. Int. Genet.* 31: e36-e40.

NIST 1036 Expanded U.S. Population Dataset

Gettings, K.B., Borsuk, L.A., Steffen, C.R., Kiesler, K.M., Vallone, P.M. (2018). Sequence-based U.S. population data for 27 autosomal STR loci. *Forensic Sci. Int. Genet.* 37: 106-115.

SNP Allele Frequencies

Churchill, J.D., Novroski, N.M.M., King, J.L., Seah L.H., & Budowle, B. (2017). Population and performance analyses of four major populations with Illumina’s FGx Forensic Genomics System. *Forensic Sci. Int. Genet.* 30: 81-92.

Linkage Map for use in DBLR™

Matisse, T.C. *et al.* (2007). A second-generation combined linkage physical map of the human genome. *Genome Res.* 17:1783-6.

Nato, A.Q., Buyske, S., and Matisse, T.C. The Rutgers map: A third-generation combined linkage-physical map of the human genome. *Human Genetics Institute of New Jersey Second Research Day*. Life Sciences Building, Rutgers University, Piscataway, NJ, USA. (Manuscript in Process)

Phillips, C., Ballard, D., Gill, P., Syndercombe Court, D., Carracedo, Á. & Lareu, M.V. (2012). The recombination landscape around forensic STRs: Accurate measurement of genetic distances between syntenic STR pairs using HapMap high density SNP data. *Forensic Sci. Int. Genet.* 6. 354–365.

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APPENDIX 11

Applied Biosystems® 3500 Genetic Analyzer - Data Collection Software version 2.0 - Instrument

(only necessary the first time PowerPlex® Y23 samples are run or if the parameters change)

See [Appendix 3](#) for security, user roles, and user account settings.

1.0 Create an Instrument Protocol

- 1.1 Navigate to the **Library** and select “Instrument Protocols”.
- 1.2 Select “Create”.
- 1.3 Select “HID” for the Application Type.
- 1.4 Select “36” for the Capillary Length.
- 1.5 Select “POP4” for the Polymer.
- 1.6 Select the appropriate Dye Set: “Promega G5 WEN”.
- 1.7 Select “HID36_POP4” for the Run Module.
- 1.8 Enter the desired Injection Time and Injection Voltage.
- 1.9 Name the protocol with the kit name and the injection voltage and time (e.g., “PPY23_1.2kV15s”).
- 1.10 Ensure settings match the figure below.
- 1.11 Mark “Locked” and click “Save”.

Instrument Protocol:

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Edit Instrument Protocol PPY23_1.2kV15s

Setup an Instrument Protocol

Application Type: Capillary Length: cm Polymer:

Dye Set:

Instrument Protocol Properties

* Run Module:

* Protocol Name: ☒ Locked

Description:

Oven Temperature (°C):	60	Run Voltage (kVolts):	15.0	PreRun Voltage (kVolts):	15	Injection Voltage (kVolts):	1.2
Run Time (sec.):	1210	PreRun Time (sec.):	180	Injection Time (sec.):	15	Data Delay (sec.):	1

▶ Advanced Options

Close Save

2.0 Create a Size Standard

- 2.1 Navigate to the **Library** and select "Size Standard".
- 2.2 Select "Create".
- 2.3 Assign the appropriate name: "WEN ILS 500 Y23".
- 2.4 Select "Orange" for the Dye Color.
- 2.5 Type the following into the left column: 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, and 500. Click "Add Size(s)".
- 2.6 Mark "Locked" and click "Save".

Size Standard:

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Edit Size Standard WEN ILS 500 Y23

Setup a Size Standard

* Size Standard: WEN ILS 500 Y23 ☐ Locked

Description:

* Dye Color: Orange

Enter sizes in the field below separated by a comma, space, or return then click the "Add Size(s) >>" button to add them to the current size standard definition.

Enter new Size Standard definition: (e.g. 11.0, 34.2, 55)

Add Size(s) >>

* Current Size Standard definition:

60.0
65.0
80.0
100.0
120.0
140.0
160.0
180.0
200.0
225.0
250.0
275.0
300.0
325.0
350.0
375.0
400.0
425.0
450.0
475.0
500.0

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3.0 Create a QC Protocol

- 3.1 Navigate to the **Library** and select “QC Protocols”.
- 3.2 Select “Create”.
- 3.3 Assign the appropriate name: “WEN_ILS_500_Y23”.
- 3.4 Change settings to match the figure below.
- 3.5 Mark “Locked” and click “Save”.

QC Protocol:

Edit QC Protocol WEN_ILS_500_Y23

Setup a QC Protocol

* Protocol Name: WEN_ILS_500_Y23 ☒ Locked

Description:

Size Standard: WEN ILS 500 Y23

Sizecaller: SizeCaller v1.1.0

Analysis Settings QC Settings

Analysis Range: Full Sizing Range: Partial Size Calling Method: Local Southern

Analysis Start Point: 0 Sizing Start Size: 60

Analysis Stop Point: 1000000 Sizing Stop Size: 500

	Blue	Green	Yellow	Red	Purple	Orange
Peak Amplitude Threshold	75	75	75	75	175	75

Common Settings

Use Smoothing: Light

Use Baseline (Baseline Window (Pts)) ☒ 51

Minimum Peak Half Width: 2

Peak Window Size: 15

Polynomial Degree: 3

Slope Threshold Peak Start: 0.0

Slope Threshold Peak End: 0.0

Close Save

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4.0 Create an Assay

- 4.1 Navigate to the **Library** and Select “Assays”.
- 4.2 Select “Create”.
- 4.3 Assign a descriptive assay name (e.g., “PPY23_1.2kV15s”).
- 4.4 Select “HID” for the Application Type.
- 4.5 Select the appropriate QC Protocol: “WEN_ILS_500_Y23”.
- 4.6 Select the appropriate instrument protocol.
- 4.7 Mark “Locked” and click “Save”.

Assay:

Edit Assay PPY23_1.2kV15s

Setup an Assay

* Assay Name: ☒ Locked Color:

Application Type:

Protocols

Do you wish to assign multiple instrument protocols to this assay? ☒ No ☐ Yes

* Instrument Protocol:

* QC Protocol:

GeneMapper IDX Protocol:

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5.0 Create a Naming Convention

5.1 Navigate to the **Library** and Select “File Name Convention”.

5.2 Select “Create”.

5.3 Assign a descriptive name (e.g., PPY23_FNC).

5.4 Select desired attributes and click “Add”.

5.5 Select desired delimiters and click “Add”.

5.6 Leave file location as the default.

5.7 Look at the “Preview of File Name” field for accuracy.

5.8 Mark “Locked” and click “Save”.

File Name Convention:

The screenshot shows the 'Edit File Name Convention' dialog box for 'PPY23_FNC'. The dialog is titled 'Setup a File Name Convention'. At the top, the 'Name' field is 'PPY23_FNC', it is 'Locked', and the 'Color' is 'Blue'. Below this is the 'Select File Name Attributes' section. The 'Preview of File Name' field shows '<Sample Name>_<Instrument Protocol>_<Date of Run>_<Well Position>'. The 'Available Attributes' list includes 'Amplicon Name', 'Analysis Protocol Name', 'Assay Name', 'Capillary Number', 'Custom Text1', 'Custom Text2', and 'Custom Text3'. The 'Delimiters' section shows 'Underscore (_)' selected. The 'Selected Attributes' list includes 'Sample Name', 'Underscore (_)', 'Instrument Protocol', 'Underscore (_)', 'Date of Run', 'Underscore (_)', and 'Well Position'. At the bottom, the 'Select File Location' section shows 'Default File Location' as 'D:\Applied Biosystems\3500\Data'. The 'Close' button is highlighted in blue, and the 'Save' button is at the bottom right.

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6.0 Create a Results Group

- 6.1 Navigate to the **Library** and Select “Results Group”.
- 6.2 Select “Create”.
- 6.3 Assign a descriptive assay name (e.g., . PPY_ResultsGroup).
- 6.4 Select the desired attributes and click “Add”.
- 6.5 Select the desired delimiters, if there are multiple delimiters click the “Add between attributes” box and click “Add”.
 - 6.5.1 The order of the attributes and delimiters can be changed using the “Move Up” and “Move Down” buttons.
- 6.6 Ensure that “Store reinjection sample file with original sample files (same level)” is selected.
- 6.7 Leave file location as the default.
- 6.8 Click so the “Include a Result Group Name folder” box is selected.
- 6.9 Look at the “Preview of “Results Group Name” field for accuracy.
- 6.10 Mark “Locked” and click “Save”.

Results Group:

Edit Results Group PPY_ResultsGroup

Setup a Results Group

* Name: PPY_ResultsGroup ☐ Locked Color: Dark Magenta

Select Results Group Attributes

Preview of Results Group Name: <Plate Name>

Available Attributes

- Assay Name
- Injection Number
- Delimiters

Select a delimiter: Dash (-)

☐ Add between attributes

Add >> << Remove Move Up Move Down

Selected Attributes

- Plate Name

Enter a custom value as either the Prefix or Suffix (optional)

Prefix: Suffix:

Select Reinjection Folder Option

☐ Store reinjection sample files in a separate Reinjection folder (same level as Injection folders)

☒ Store reinjection sample files with original sample files (same level)

Select Folder Option

☒ Default file location D:\Applied Biosystems\3500\Data\<Plate Name>

☐ Custom file location Browse...

☐ Include an Instrument Run Name folder

☒ Include a Result Group Name folder

☐ Include an Injection folder

Close Save

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APPENDIX 12**

**GeneMapper™ ID-X Version 1.6 Software - PowerPlex®
Y23 Software Settings**

1.0 Importing Panel and Bin Files for use with WEN ILS 500

- 1.1 Open the GeneMapper™ ID-X Version 1.6 software.
- 1.2 Select **Tools** → **Panel Manager**.
- 1.3 Highlight the “Panel Manager” icon in the navigation pane.
- 1.4 Select **File** → **Import Panels**.
- 1.5 Navigate to the saved panel, bin, and stutter files. Select “PowerPlexY23_Panels_IDX_v2.0” then click “Import”.
- 1.6 Select the “ISP Casework Security Group”. Click “OK”.
- 1.7 In the navigation pane, highlight the “PowerPlexY23_Panels_IDX_v2.0” folder.
- 1.8 Select **File** → **Import Bin Set**.
- 1.9 Select “PowerPlexY23_Bins_IDX_v2.0” then click “Import”.
- 1.10 In the navigation pane, highlight the “PowerPlexY23_Panels_IDX_v2.0” folder.
- 1.11 Select **File** → **Import Marker Stutter**. A warning box will appear asking to overwrite the current values. Select “Yes”.
- 1.12 Select “PowerPlexY23_Stutter_IDX_v2.0” then click “Import”. This will import the Promega Marker Stutter file that has been modified to include the Indiana State Police PowerPlex® Y23 stutter percentages for filtering out stutter as determined by the internal validation studies.
- 1.13 In the Panel Manager window, select “Apply”, then “OK”.

2.0 Creating a Casework Analysis Method

- 2.1 Select **Tools** → **GeneMapper Manager**.
- 2.2 Select the “Analysis Methods” tab.
- 2.3 Select “New” and a new analysis method dialog box will open.
- 2.4 Enter the name: “PowerPlex Y23 WEN”.
- 2.5 Select the “ISP Casework Security Group”.
- 2.6 Select the “Allele” tab. In the “Bin Set” drop-down menu select “PowerPlexY23_Bins_IDX_v2.0”. Ensure that the “Use marker-specific stutter ratio and distance if available” box is checked. The table values may be left as the factory defaults.

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The Allele Tab:

The screenshot shows the 'Analysis Method Editor' window with the 'Allele' tab selected. The 'Bin Set' is 'PowerPlexY23_Bins_IDX_v2.0'. The checkbox 'Use marker-specific stutter ratio and distance if available' is checked. Below this is a table for 'Marker Repeat Type' with columns for 'Tri', 'Tetra', 'Penta', and 'Hexa'. The table contains settings for 'Global Cut-off Value', 'MinusA Ratio', 'MinusA Distance' (From/To), 'Global Minus Stutter Ratio', 'Global Minus Stutter Distance' (From/To), 'Global Plus Stutter Ratio', and 'Global Plus Stutter Distance' (From/To). The 'Amelogenin Cutoff' is set to 0.0. At the bottom are buttons for 'Range Filter...', 'Factory Defaults', 'Save', 'Cancel', and 'Help'.

Marker Repeat Type:	Tri	Tetra	Penta	Hexa
Global Cut-off Value	0.0	0.0	0.0	0.0
MinusA Ratio	0.0	0.0	0.0	0.0
MinusA Distance				
From	0.0	0.0	0.0	0.0
To	0.0	0.0	0.0	0.0
Global Minus Stutter Ratio	0.0	0.0	0.0	0.0
Global Minus Stutter Distance				
From	0.0	3.25	0.0	0.0
To	0.0	4.75	0.0	0.0
Global Plus Stutter Ratio	0.0	0.0	0.0	0.0
Global Plus Stutter Distance				
From	0.0	0.0	0.0	0.0
To	0.0	0.0	0.0	0.0

Amelogenin Cutoff: 0.0

Buttons: Range Filter..., Factory Defaults, Save, Cancel, Help

2.7 Select the "Peak Detector" tab. Change the settings to match the figure below.

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The Peak Detector Tab:

The screenshot displays the 'Analysis Method Editor' window with the 'Peak Detector' tab selected. The interface is divided into several sections for configuring peak detection parameters.

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

Peak Detection Algorithm: Advanced

Ranges

Analysis	Sizing
Full Range	Partial Sizes
Start Pt: 0	Start Size: 60
Stop Pt: 10000	Stop Size: 500

Smoothing and Baseline

Smoothing: ☐ None ☒ Light ☐ Heavy

Baseline Window: 51 pts

Size Calling Method

☐ 2nd Order Least Squares
☐ 3rd Order Least Squares
☐ Cubic Spline Interpolation
☒ Local Southern Method
☐ Global Southern Method

Peak Detection

Peak Amplitude Thresholds:

B:	R:
100	100
G:	P:
100	100
Y:	O:
100	100

Min. Peak Half Width: 2 pts
Polynomial Degree: 3
Peak Window Size: 15 pts

Slope Threshold

Peak Start: 0.0
Peak End: 0.0

Normalization

☐ Use Normalization, if applicable

Factory Defaults

Save As **Save** **Cancel** **Help**

2.8 The Peak Amplitude Threshold (analytical threshold) values were determined during the internal validation by calculating the noise level of each instrument and determining the cut-off in which the majority of the noise peaks would be below.

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2.9 Select the “Peak Quality” tab. Change the settings to match the image below.

The Peak Quality Tab:

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Quality' tab selected. The dialog has a title bar with a close button (X). Below the title bar are five tabs: 'General', 'Allele', 'Peak Detector', 'Peak Quality' (selected), and 'SQ & GQ Settings'. The 'Peak Quality' tab contains several sections with settings:

- Min/Max Peak Height (LPH/MPH)**
 - Homozygous min peak height: 225.0
 - Heterozygous min peak height: 100.0
 - Max Peak Height (MPH): 28000.0
- Peak Height Ratio (PHR)**
 - Min peak height ratio: 0.7
- Broad Peak (BD)**
 - Max peak width (basepairs): 1.5
- Allele Number (AN)**
 - Max expected alleles:
 - For autosomal markers & AMEL: 2
 - For Y markers: 1
- Allelic Ladder Spike**
 - Spike Detection: Enable (dropdown)
 - Cut-off value: 0.2
- Sample Spike Detection**
 - Spike Detection: Enable (dropdown)

At the bottom right of the dialog is a 'Factory Defaults' button. At the very bottom are four buttons: 'Save As', 'Save', 'Cancel', and 'Help'.

2.10 Leave the “SQ and GQ Settings” tab set to the factory defaults.

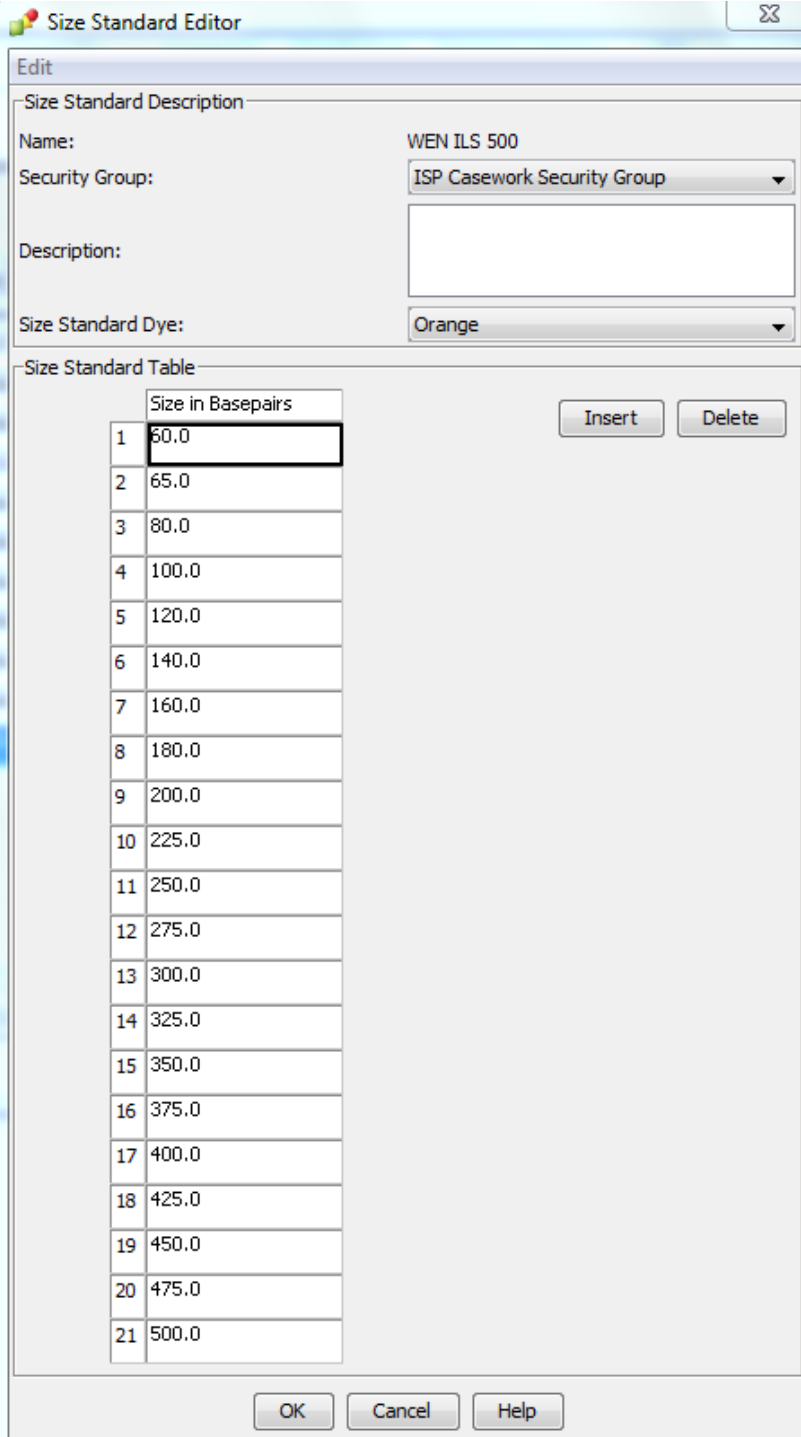
2.11 Select “Save”.

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3.0 Creating a Size Standard

- 3.1 Select **Tools** → **GeneMapper Manager**.
- 3.2 Select the “Size Standard” tab and click “New”
- 3.3 Create the “WEN ILS 500” size standard (see figure below for exact settings and sizes).
- 3.4 Select “OK”.

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The image shows a software window titled "Size Standard Editor". It has a menu bar with "Edit" and a window control button. The main area is divided into two sections. The top section, "Size Standard Description", contains fields for "Name:" (WEN ILS 500), "Security Group:" (ISP Casework Security Group), "Description:" (empty), and "Size Standard Dye:" (Orange). The bottom section, "Size Standard Table", contains a table with 21 rows. The first row is selected, showing "1" in the first column and "60.0" in the second column. To the right of the table are "Insert" and "Delete" buttons. At the bottom of the window are "OK", "Cancel", and "Help" buttons.

Size Standard Editor

Edit

Size Standard Description

Name: WEN ILS 500

Security Group: ISP Casework Security Group

Description:

Size Standard Dye: Orange

Size Standard Table

	Size in Basepairs
1	60.0
2	65.0
3	80.0
4	100.0
5	120.0
6	140.0
7	160.0
8	180.0
9	200.0
10	225.0
11	250.0
12	275.0
13	300.0
14	325.0
15	350.0
16	375.0
17	400.0
18	425.0
19	450.0
20	475.0
21	500.0

Insert Delete

OK Cancel Help

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4.0 Creating a Table Setting

- 4.1 Select **Tools** → **GeneMapper Manager**.
- 4.2 Select the “Table Setting” tab and click “New”.
- 4.3 Under the “General” tab, name the Table Setting “PPY23” and select the “ISP Casework Security Group”.
- 4.4 Under the “Samples” tab, ensure that check marks are located next to only the following: Status, Sample File, Sample Name, Sample Type, Analysis Method, Panel, Size Standard, Sizing Quality Overridden, Sample File Not Found, Sample Off-Scale, and Sizing Quality. Leave the font “Arial” and the size “11”. Sort by “Sample Type” and then by “Sample Name”. Select the “Ascending” option for both, and leave the final sort option to “None”.
- 4.5 Under the “Genotypes” tab, ensure that check marks are located next to only the following: Sample Name, Marker, Allele, and Height. Sort by “Sample Name”, then by “Marker”, and then by “None”. Select the “Ascending” option for all three. Change the “Show number of alleles” to 8 and check “Keep Allele, Size, Height, Area, Data Point, Mutation and Comment together”. (If the option to “Keep Allele, Size, Height...” is not visible in the window, expand the window size until the option appears.) Leave the font “Arial” and the size “11”.
- 4.6 Select “OK”.

5.0 Creating a Plot Setting – Samples, Controls, and Ladders

- 5.1 Select **Tools** → **GeneMapper Manager**.
- 5.2 Select the “Plot Settings” tab and click “New”.
- 5.3 Under the “General” tab, name the Plot Setting “PPY23” and select the “ISP Casework Security Group”.
- 5.4 Under the “Sample Header” tab, ensure that check marks are located next to the following: Sample File, Sample Name, Panel, Sizing Quality Overridden, Sample Off-Scale and Sizing Quality.
- 5.5 Under the “Genotype Header” tab, ensure that check marks are located next to the following: Sample File, Sample Name, Panel, Marker, Sample Off-Scale, Out of Bin Allele, Peak Height Ratio, Control Concordance, and Genotype Quality.
- 5.6 Under the “Sizing Table” tab, ensure that check marks are located next to the following: Dye/Sample Peak, Sample File Name, Marker, Allele, Size, Height, Area, and Data Point. Leave the font “Arial” and the size “11”.
- 5.7 Select the “Labels” tab. Change the settings to match the following figure. Alternatively, if the allele edit comment” function is being used, “Label 4” for “Assigned Allele”, “Custom Allele”, and “Artifact” can be changed to “AE Reason for Change”.

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Labels Tab:

upper™ ID-X Manager X

Plot Settings Editor X

General Sample Header Genotype Header Sizing Table **Labels** Display Settings

Show Labels on Samples and Genotypes Plot:

Labels

	Assigned Allele	Custom Allele	Allelic Ladder	Artifact
Label 1:	Allele Call	Allele Call	Allele Call	Artifact Label
Label 2:	Height	Height	NONE	Height
Label 3:	Size	Size	NONE	Size
Label 4:	NONE	NONE	NONE	NONE

Font

Font: Times New Roman

Size: 9

When opening the Plot Window:

☐ Show PQV trigger peak (LPH,MPH,BD,OS)

☐ Show data type prefixes

☒ Display virtual allele label in black

☐ Show type of edit

Label Color: Dye Color-Border

OK Cancel Help

5.8 Under the “Display Settings” tab, change the display settings so that they match the following figure and select “OK”.

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Display Settings Tab:

Plot Settings Editor

✕

General Sample Header Genotype Header Sizing Table Labels Display Settings

When opening the Plot Window:

☐ Use the display settings last used for this plot

☒ Use these display settings:

For both Sample and Genotype plots:

Panes: 2

Labels

☐ No Labels

☐ Horizontal Labels

☒ Vertical Labels

Show

☒ Plot Header
☒ Marker Range
☐ Marker Indicators
☒ Bins
☒ Toolbar

☐ Peak Positions
☐ Bring Ctrl's to Top
☐ Bring Ladders to Top
☒ Allele Changes
☐ Off-scale

Axes

Y-Axis Scale individually

X-Axis * Basepairs

For Sample plot only:

Select Dyes

☒ Blue

☒ Green

☒ Yellow

☒ Red

☐ Purple

☒ Orange

☐ All Dyes

All-Dye Range (bp): *

Start Range 0.0

End Range 1000.0

Labels

☒ Size Std Labels

Print

☐ Quality Value Details

Tables

☒ No Table

☐ Sizing Table

☐ Genotypes Table

☐ Label Edit Viewer

Dye Layout

☐ Combine Dyes

☒ Separate Dyes

☐ Overlay All

Custom Colors

For Genotype plot only:

Marker Margin: 5 bp

* Will be overridden if Retain X-axis Zoom Range is enabled on Plots ->Zoom menu

OK
Cancel
Help

6.0 GeneMapper™ ID-X Maintenance

- 6.1 The number of audit records on the GeneMapper™ ID-X database should be routinely checked. Audit records should be backed-up, saved to the DNA server, and then deleted from the GeneMapper™ ID-X database monthly or if the number of records exceeds 40,000. If the number of audit records exceeds 60,000, the performance of the software may be affected.

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- 6.2 The amount of database space in the GeneMapper™ ID-X software should be routinely checked. If the occupied space exceeds 80%, additional disk space should be allocated.

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POWERPLEX® Y23 STUTTER TABLE

Stutter Guidelines for PowerPlex® Y23						
	reverse (e.g., n-4)		double reverse (e.g., n-8)		forward (e.g., n+4)	
	Mean	Mean + 3SD	Mean	Mean + 3SD	Mean	Mean + 3SD
DYS576	9.82%	12.57%	0.74%	1.78%	1.39%	4.00%
DYS389 I	5.51%	7.14%	0.57%	1.50%	0.44%	1.12%
DYS448	2.17%	2.55%	n/a	n/a	n/a	n/a
DYS389 II	11.32%	14.80%	1.23%	2.30%	1.05%	2.44%
DYS19	5.71%	8.48%	n/a	n/a	0.66%	1.55%
DYS391	7.00%	9.74%	0.71%	2.19%	0.28%	1.02%
DYS481	17.78%	25.93%	3.05%	5.04%	2.52%	5.12%
DYS549	6.61%	9.66%	0.99%	1.69%	1.11%	2.02%
DYS533	6.68%	9.05%	0.69%	1.68%	0.99%	2.19%
DYS438	3.03%	5.10%	n/a	n/a	0.66%	1.61%
DYS437	4.94%	7.96%	0.69%	0.98%	0.69%	1.75%
DYS570	9.69%	12.25%	0.69%	1.30%	0.90%	1.88%
DYS635	6.27%	10.67%	0.69%	1.74%	0.48%	1.26%
DYS390	8.55%	12.80%	1.01%	2.67%	0.44%	1.14%
DYS439	6.06%	9.19%	0.51%	1.02%	0.87%	2.31%
DYS392	9.64%	14.09%	1.09%	2.23%	5.93%	12.11%
DYS643	2.33%	5.41%	n/a	n/a	0.50%	1.31%
DYS393	9.78%	13.05%	1.03%	1.97%	1.44%	2.71%
DYS458	9.73%	12.71%	0.84%	1.31%	0.80%	1.88%
DYS385	7.89%	14.14%	1.09%	2.22%	0.82%	1.58%
DYS456	10.71%	15.71%	1.37%	2.93%	1.85%	2.68%
YGATAH4	7.41%	10.05%	1.09%	2.10%	1.44%	2.25%
n-2			n+2			
	Mean	Mean + 3SD		Mean	Mean + 3SD	
DYS19	7.76%	9.09%	DYS19	2.81%	4.36%	
DYS391	0.50%	1.05%				

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APPENDIX 14
OUTSOURCED SAMPLE ANALYSIS**

Samples may be sent to an external laboratory for analysis at the request and authorization of the investigating agency, prosecutor or by court order. Payment arrangements for additional analysis shall be agreed upon by the appropriate entity prior to sending the samples. Authorization to send the samples shall be documented in the case record.

If possible, only a portion of the evidence should be sent with the remainder retained by the Forensic Services Division or returned to the contributing agency. A subitem (or sub-subitem) of the evidence shall be generated for this purpose for tracking in LIMS. Items and/or subitems sent for analysis shall be tracked in the Chain of Custody, including extracts. Even though extracts are defined as work product, they shall be tracked in the Chain of Custody if being sent outside of the Forensic Services Division for the purpose of testing. The Chain of Custody shall document "Carrier Delivery" and the laboratory name of where the evidence is being sent. Copies of the shipping paperwork and paperwork filled out for the laboratory doing the testing shall be documented in the case record.

A Certificate of Analysis to the original submitting agency shall be generated to document the sample was sent out utilizing the wording specified in the report wording guidelines.

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**APPENDIX 15
Electronic Case Record Generation and Review**

1. Electronic Generation of Case Record

- 1.1. Analysts may electronically generate case notes using software (e.g., Microsoft Word), provided that the notes are clear, legible, and may be easily printed (if a paper record is being generated) or incorporated into PDF file format (if an electronic copy is being generated).
 - 1.1.1. If an official worksheet exists for the examination of a particular evidence workflow (e.g., the sexual assault kit worksheets), it shall be used (an analyst may not create their own). If a table is included in an analyst's electronic case notes to record serology results and reagent lot numbers, only the approved table shall be used.
 - 1.1.1.1. Official worksheets and approved tables are available on SharePoint.
- 1.2. Results of serological testing shall be recorded on the official stain identification worksheets or in the analyst's generated case notes.
 - 1.2.1. All required controls, lot numbers, etc. shall be documented.

2. Electronic Technical and Administrative Review

- 2.1. At the reporting case analyst's discretion, a case record may be created in or converted to an electronic (PDF) format prior to technical/administrative review.
 - 2.1.1. Exceptions shall be approved by a Unit Supervisor and documented in the case record.
- 2.2. The electronic copy of the case record becomes the official, tracked copy, and is the copy utilized for technical and administrative review.
 - 2.2.1. All data sheets, notes, and other generated information which are normally kept in the paper case record shall be included in the electronic file.
 - 2.2.2. Every page shall include analyst's initials and case number.
 - 2.2.2.1. These may be inserted by software (e.g., typed) or hand-written.
 - 2.2.3. Scanned documents from an original paper case record may be merged with electronically generated files to create the electronic copy.
 - 2.2.4. When the case has been administratively reviewed and uploaded into LIMS, any remaining paper copies should be destroyed.
 - 2.2.4.1. Paper copies should be retained at least 1 week prior to destruction in order to allow for a LIMS server backup.
 - 2.2.4.2. Paper copies should be destroyed within 2 months of administrative review.
- 2.3. The analyst may make alterations to their electronic case record using software (e.g., Adobe), including but not limited to merging files, inserting/rearranging pages, adding text, strikethroughs, highlighting, and drawing lines.
 - 2.3.1. All alterations (except those with no data gain/loss, such as rearrangement) shall be initialed and dated.
- 2.4. If a page of the electronic copy needs to be added, recreated, or removed, a watermark or other notation shall be placed on the original page stating the reason (e.g. "not used" or "page added") with the case analyst's initials and date. When data is rejected, the reason shall be listed. New pages should be inserted before or after original pages when appropriate or as best organizes the data.

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- 2.4.1. Examples: additional deconvolution worksheets generated after first review, or removal of a CODIS sheet for a profile deemed ineligible
- 2.5. Completed case records ready for technical review shall be placed into a folder on the reporting analyst's personal working drive, in a subfolder marked "Electronic Reviews".
 - 2.5.1. Example: \ DNAworking \ IRL \ JDoe \ Electronic Reviews.
 - 2.5.2. The case report printed to PDF from LIMS should be saved in the same folder as a separate electronic document. The case analyst shall verify the most up to date copy of the report is ready present for technical review.
 - 2.5.3. This folder shall be separate from the case folder and should include only temporary case records for technical and administrative reviews. Case data shall be stored on the secure DNA data drive.
 - 2.5.4. The analyst shall also upload the un-reviewed electronic copy to LIMS prior to technical review. This will preserve the record prior to changes.
 - 2.5.5. If changes are made to the electronic case record by the analyst after a technical reviewer has been assigned, the casework analyst shall notify the technical reviewer of such changes.
- 2.6. Electronic records shall be retained as indicated in Appendix 9 unless otherwise indicated below.
 - 2.6.1. The original case record PDF file shall be named as follows:
DNA_CASE#_REQUEST#_NOTES_ PE#_V0
 - 2.6.2. The final, technically reviewed case record PDF file shall be named as follows:
DNA_CASE#_REQUEST#_NOTES_ PE#_V1
- 2.7. If the case analyst is converting their case record to electronic format, they shall notify the appropriate supervisor/manager when their electronic case record is ready for technical review.
 - 2.7.1. If someone other than the case analyst has converted the case record to electronic format, they shall notify the case analyst and supervisor/manager (as appropriate). The individual who scanned the case record shall place it in the case analyst's data drive as indicated above. The case analyst shall then upload the electronic record to LIMS as indicated above.
- 2.8. The assigned technical reviewer shall use the electronic copy of the case record in the case analyst's personal working folder for review.
 - 2.8.1. The technical reviewer shall save a copy of the technical review worksheet to the case analyst's personal working folder to document the review.
 - 2.8.2. The technical reviewer shall check to ensure that the V0 copy is uploaded to LIMS prior to review.
 - 2.8.3. The technical reviewer should save comments to the analyst's electronic copy.
 - 2.8.4. The technical reviewer's electronic alterations to the case record should be limited to drawings with the drawing tool and/or comment boxes (i.e., "sticky notes") within the comment tool in Adobe.
 - 2.8.4.1. All temporary alterations/comments shall be deleted by the technical reviewer before the casefile is finalized.
 - 2.8.4.2. Technical review notation made using the comment tool may be removed by printing the PDF file as a PDF file; there is an option in the print menu that allows keeping/omitting such markups and shall be selected as appropriate.
 - 2.8.5. A technical reviewer may opt to print the case record for their own review, at their discretion, but any generated paper copies shall be destroyed upon completion of review.

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- 2.9. When technical review is complete, the case analyst shall add the completed technical review worksheet as the first page of the PDF case notes, save the electronic copy as the final copy, named as outlined above and upload it to LIMS. The case analyst shall notify the appropriate supervisor/individual that it is ready for administrative review.
- 2.10. If an additional request for lab exam is generated as part of the case exam (e.g., a Y-STR request), the case analyst is responsible for creating a print copy and forwarding that request to the case manager following administrative review.
 - 2.10.1. A copy of additional request form(s) should be retained in the original case notes.

3. CODIS Upload and Review

- 3.1. When a DNA profile is being submitted to CODIS for upload, the corresponding CMF file(s) shall be generated from the current version of STRmix and saved in the appropriate CMF server folder.
 - 3.1.1. Paper submissions may be accepted in place of CMF files, pending the Local CODIS Administrator's approval.
- 3.2. When ready for CODIS upload, the technical reviewer shall send an email to all appropriate CODIS administrators which indicates the case number, and case analyst associated with the upload. Multiple cases may be sent in one email.
- 3.3. The CODIS administrators will access the case record and CODIS sheet via the case analyst's data review folder.
 - 3.3.1. A PDF file of the Specimen Detail Sheet (SDS) will be placed in the case analyst's data review folder. The SDS will be labeled with the case number and "spec detail". This becomes the official, tracked copy of the SDS.
 - 3.3.2. Paper submissions will be returned as a paper copy.
- 3.4. A CODIS administrator will notify the case analyst via email when the upload is completed.
- 3.5. The case analyst shall merge any electronic copies of the SDS with the electronic copy of the case record during review.

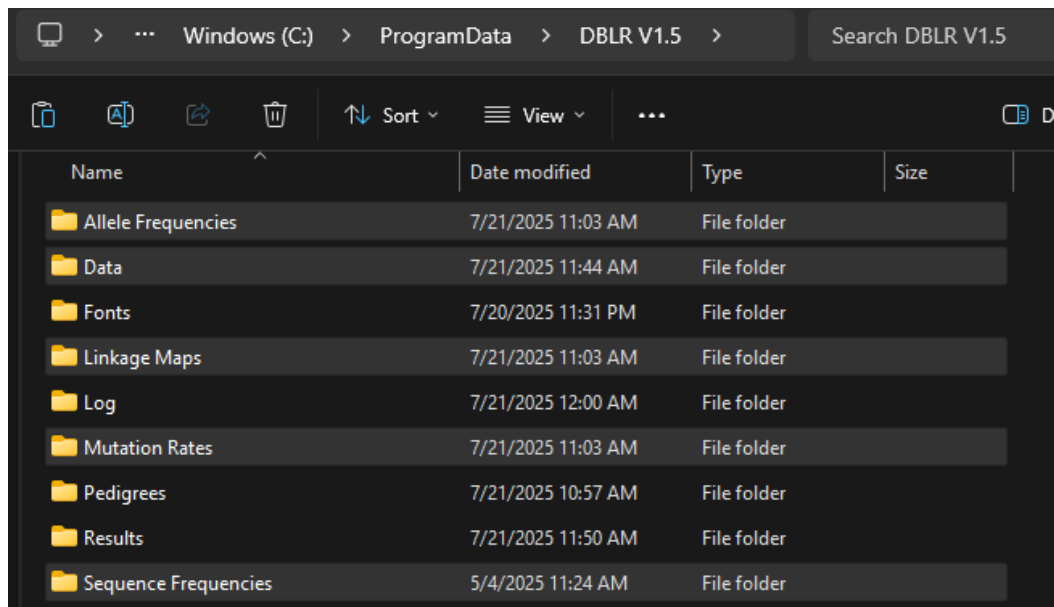
INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS APPENDIX 16 DBLR™ SOFTWARE SETTINGS

1. DBLR™ Installation and Set-up

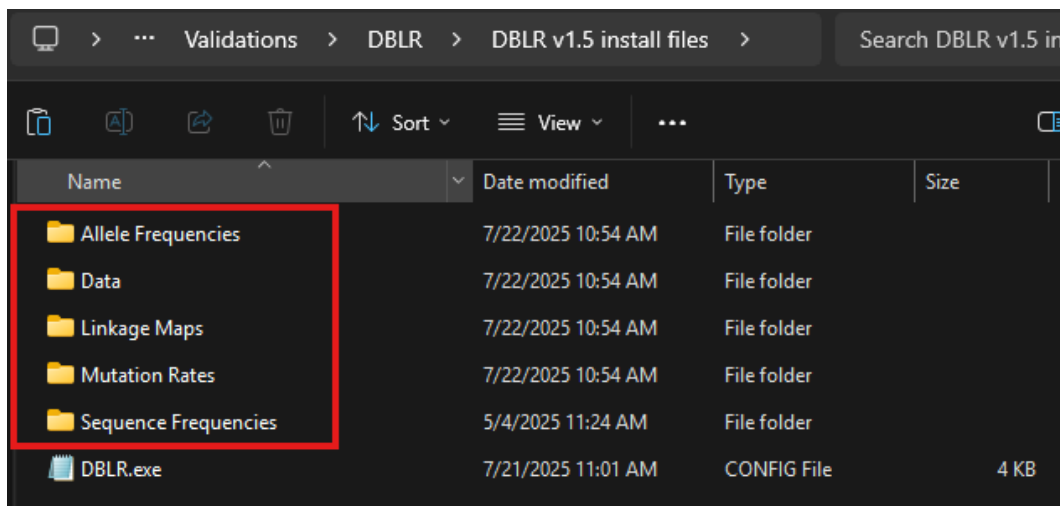
1.1. Install the current version of DBLR™ (v1.5).

1.2. Install ISP settings.

1.2.1. Open **C:\ProgramData\DBLR V1.5**. DELETE the following folders: Allele frequencies, Data, Linkage Maps, Mutation Rates, Sequence Frequencies



1.2.2. Navigate to BiologyQA→Validations→DBLR→DBLR v1.5 install files and COPY the following folders to **C:\ProgramData\DBLR V1.5**: Allele frequencies, Data, Linkage Maps, Mutation Rates, Sequence Frequencies

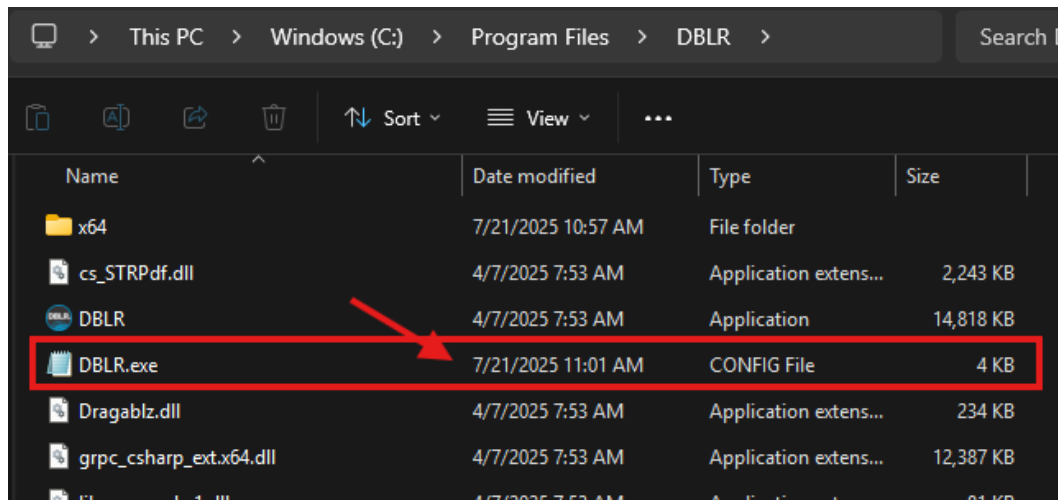


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1.2.2.1. Optionally, for analysts trained to perform relationship comparisons, previously developed pedigrees may be imported into the Pedigrees folder.

1.3. Open *C:\ProgramFiles\DBLR*. Copy and replace the DBLR.exe CONFIG File from the BiologyQA drive. (Note: you may need to first copy the CONFIG file to your desktop and then drag it into the *C:\ProgramFiles\DBLR* folder.

1.3.1. Confirm the Date modified matches the image below.

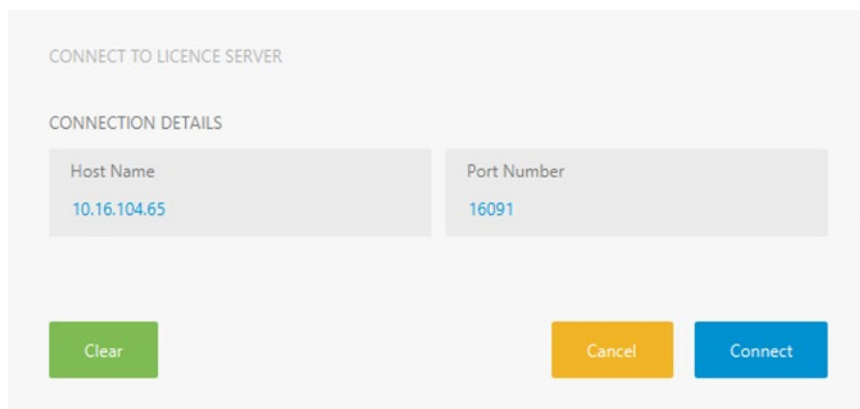


1.4. Open DBLR™ to connect to the floating license server:

1.4.1. A warning window may open that indicates “The fingerprint of settings.xml does not match...”. Select “OK”.

1.4.2. Enter Host Name: 10.16.104.65

1.4.3. Confirm Port Number: 16091



1.4.4. Select “Connect”.

1.4.5. Review and agree to Software License Agreement.

1.5. Confirm the settings update.

1.5.1. Select the “Settings” tab in the main menu. Open the drop-down menus to confirm the images below.

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1.5.1.1. When finished, close the settings window by selecting “Close without saving”.

DBLR
— □

Allele Frequencies

Name	Default?	Sequences?
NIST_2017_African American w iSNP_020723.csv	☐	☐
NIST_2017_Asian w iSNP_020723.csv	☐	☐
NIST_2017_Caucasian w iSNP_020723.csv	☐	☐
NIST_2017_Combined w iSNP_020723.csv	☒	☐
NIST_2017_Hispanic w iSNP_020723.csv	☐	☐

Populations

Name	Default?	Subpopulations
ISP_NIST_African American w iSNP	☐	NIST_2017_African American w iSNP_020723.csv (100%, Fst=0.01, FstSNP=0.01)
ISP_NIST_Asian w iSNP	☐	NIST_2017_Asian w iSNP_020723.csv (100%, Fst=0.01, FstSNP=0.01)
ISP_NIST_Caucasian w iSNP	☐	NIST_2017_Caucasian w iSNP_020723.csv (100%, Fst=0.01, FstSNP=0.01)
ISP_NIST_Combined w iSNP	☒	NIST_2017_Combined w iSNP_020723.csv (100%, Fst=0.01, FstSNP=0.01)
ISP_NIST_Hispanic w iSNP	☐	NIST_2017_Hispanic w iSNP_020723.csv (100%, Fst=0.01, FstSNP=0.01)

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DBLR

Populations

Search Strategies

Loci

Special allele labels

General

Name	Non-Autosomal?	Sex?	Do not count towards minimum # loci for database upload?	Alternative spellings
AMEL	☒	☒	☒	n/a 
DYS391	☒	☐	☒	n/a 
DYS570	☒	☐	☒	n/a 
DYS576	☒	☐	☒	n/a 
Yindel	☒	☐	☒	n/a 