

# FORENSIC BIOLOGY SECTION



# CASEWORK TEST METHODS

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

**FOREWORD**

**The Laboratory 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 the various biological materials. The Laboratory reserves the right to evaluate and prioritize the items submitted and limit the total number in order to expedite service. 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. DNA analysts shall have successfully completed college course work covering the subject areas of genetics, biochemistry, molecular biology and statistics. All analysts undergo an intensive formalized training program dealing with forensic techniques and instrumentation. Completion of the Training Program is required before analysis of evidence is performed. Additionally, all analysts participate in proficiency testing utilizing open trials, blind trials, and/or re-examination techniques. The accuracy and specificity of test results are ensured by running known controls with each set of tests.**

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## 1. Serology Methods:

- 1.1. Scope:** This test method is designed for the guidance of laboratory 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 collected from evidence submitted for serological examination. Collected 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 required, hairs shall be evaluated for the possibility of human origin and the potential for future analysis.
- 1.1.2.** A search is made of submitted items for the presence of biological material.
- 1.1.3.** If applicable, stain cards from whole blood samples, cuttings from body fluid stains and other DNA evidence may be retained by the laboratory for possible future testing. See flow charts.
- 1.1.4.** The 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 the 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 laboratory 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 cm<sup>2</sup>.
- 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% H<sub>2</sub>O<sub>2</sub>
- 1.5.8. Luminol Reagent
- 1.5.9. Phenolphthalein Stock Solution
- 1.5.10. Phenolphthalein Working Solution
- 1.5.11. 10% Sodium Hydroxide
- 1.5.12. Takayama Solution

#### 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. The spraying of Luminol reagent in the laboratory shall be confined to a chemical fume hood whenever practical.
- 1.6.4. Universal Precautions shall be in use whenever biological materials are being handled.
- 1.6.5. Extreme caution shall be used when handling liquid body fluid samples.
- 1.6.6. 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.

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## 1.8. Procedures/Instructions:

### 1.8.1. Examination Procedure

#### 1.8.1.1. Standards for Comparison

- 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 Laboratory, 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. Elimination/Victim standards in property crime cases should be returned to the submitting agency.
- 1.8.1.1.8. Suspect standards in all cases and elimination/victim standards in crimes against person cases shall be retained as a subitem for future reference and analysis.
- 1.8.1.1.9. 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 case report.
  - 1.8.1.1.9.1. 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).
  - 1.8.1.1.9.2. In the case of a missing person, personal items such as toothbrush, hairbrush, razor or other item may be submitted.
  - 1.8.1.1.9.3. 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.

#### 1.8.1.2. 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 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.

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- 1.8.1.2.4. Analyst 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. In cases where there is no other DNA evidence of value, the prosecutor/investigator shall first be contacted for approval of consuming the hair sample if further analysis of the hair is performed.
- 1.8.1.2.8. When appropriate, examination of the hair collected shall be performed to determine if hair consistent with human origin is present.
- 1.8.1.2.9. Human hairs can be submitted for DNA analysis when beneficial.
- 1.8.1.3. **Dried Stains** (See flow charts)
  - 1.8.1.3.1. 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.
  - 1.8.1.3.2. If phenolphthalein is being performed on stains which demonstrated positive luminol result and phenolphthalein is negative, no further testing should be performed due to the possibility of false positives for luminol. These results should be reported out as inconclusive.
  - 1.8.1.3.3. Confirmatory testing for semen and blood/human blood is at the discretion of the analyst.
- 1.8.1.4. **Stain Evaluation – Sexual Assault kits**
  - 1.8.1.4.1. No serological testing shall be performed on samples within a kit.
  - 1.8.1.4.2. Exceptions require permission from a supervisor with documentation in the case record.
  - 1.8.1.4.3. Acid Phosphatase testing and sperm searches may be done at the request of a Prosecutor with proper justification and documentation in the case record.
- 1.8.1.5. **Stain Evaluation – All other**
  - 1.8.1.5.1. After attempting to identify the stain, the stain shall be evaluated for further testing.
  - 1.8.1.5.2. If no biological material is identified on the item, no further testing shall be performed, except for instances where touch DNA or handler/wearer analysis is appropriate.
    - 1.8.1.5.2.1. Underwear submitted in sexual assault cases that are submitted outside of a kit shall be tested in the same manner as if they were located inside of the kit. No serological testing is required and at a minimum DNA analysis shall be performed on a sample of the crotch area.
  - 1.8.1.5.3. If the stain is found to be biological but no standards are available for comparison, the stain or a portion thereof may be retained by the laboratory for future testing.

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DNA testing may be performed and results entered into the Combined DNA Index System (CODIS) when eligible. See flow charts.

1.8.1.5.4. If the stain is found to be biological material and all necessary standards have been submitted, the analyst shall determine if DNA testing shall be 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.

## 1.8.1.6. Collection and labeling of swabs

1.8.1.6.1. Each swab in a submitted item or collected and retained by the analyst shall have a unique identifier if serological testing is performed on any of the swabs for that item. If different testing results are obtained between swabs in the same item each result will be reported.

1.8.1.6.2. 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 may be labeled together as one item.

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

## 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. A fume hood and appropriate personal protective equipment shall be utilized when sampling evidence. Every effort should be made to prevent exposure to potentially hazardous materials. Analysts shall use the designated fume hood.

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

1.8.2.3. Place any utensils and consumables to be used inside the hood so that it will not be necessary to reach outside the hood during item examination.

1.8.2.4. Open and examine the item(s) within the designated hood.

1.8.2.5. Change gloves frequently. When changing gloves, dispose of used gloves in a biohazard bin located inside the hood.

1.8.2.6. Collection of possible DNA by swabbing the item shall occur inside the designated hood. Allow the swab to dry in the designated hood.

1.8.2.7. Seal the evidence within the hood. An additional outer container may be used if the packaging cannot be fully cleaned.

1.8.2.8. Once the evidence examination is complete, clean the hood thoroughly.

1.8.2.9. Dispose of any unused consumables.

1.8.2.10. The hood biohazard bag shall be closed and placed inside the larger biohazard bin.

## 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.

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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

- 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 the 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. Luminol, Presumptive Test for Blood

- 1.8.4.1. **Principle:** Luminol is a presumptive test designed to process large areas where stains are not readily visible. Luminol works best on old stains (where the heme group has been converted to hemin) and while it does soak the area tested, it should not interfere with additional testing. In the luminol test, the hemin acts as a catalyst, triggering the oxidation of luminol by hydrogen peroxide in an alkaline solution.

Confirmatory testing shall be performed for conclusive identification. Luminol has been shown to give false positives with certain metal compounds, plant peroxidases and some cleansers, especially cleansers which contain hypochlorite (bleach). Luminol is an alternative method that can be used on items where bloodstains are not easily visible.

#### 1.8.4.2. Procedure

- 1.8.4.2.1. Prepare the luminol solution by adding the contents of the powdered luminol tube (8 oz size) to 8 oz (250 ml) of distilled water in a plastic reagent bottle. Alternate powdered luminol tubes are available in 4 oz and 16 oz sizes. If these are used, add the corresponding amount of distilled water, 4 oz (125 ml) or 16 oz (500 ml).
- 1.8.4.2.2. Gently mix the powder and distilled water with a gentle swirling action in order to avoid mixing an excessive amount of air or oxygen into the solution.
- 1.8.4.2.3. Transfer the luminol solution into a fine mist spray bottle. Any unmixed powder remnants should be left in the bottom of the plastic reagent bottle and not transferred into the spray bottle to avoid clogging the sprayer.
- 1.8.4.2.4. The mixed luminol solution should be used within about 20 - 30 minutes.
- 1.8.4.2.5. All individuals present during testing shall wear gloves, eye protection and a surgical mask or equivalent.
- 1.8.4.2.6. Spray the area of interest in the dark until well-soaked. Luminol's reaction with a true bloodstain produces a luminescent glow, frequently in patterns such as spatters, smears, wipes or drag marks, or even footwear impressions.

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- 1.8.4.3. Any remaining luminol solution cannot be stored for later use because the reagent will lose potency. The remaining luminol solution can be disposed by washing it into the sink with a large quantity of water. Also, be sure to clean out the spray bottle and nozzle with clean water, as any luminol remaining in the sprayer will form a difficult-to-remove clog.
- 1.8.4.4. 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 or at a crime scene. Lot numbers of reagents shall also be recorded.

#### 1.8.5. HemDirect Hemoglobin Test, Confirmatory Test for Human Blood

- 1.8.5.1. **Principle:** Human blood reacts with an anti-human hemoglobin antibody, which forms a red line in the “test” region of the HemDirect cassette.
- 1.8.5.2. **Procedure**
  - 1.8.5.2.1. Place a ~2mm<sup>2</sup> 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.
  - 1.8.5.2.2. Add three drops of the extraction buffer/sample mix with the provided pipette to the sample well of the cassette.
  - 1.8.5.2.3. A positive result may be recorded as soon as red lines are observed in both the “C” (control) and “T” (test) regions.
  - 1.8.5.2.4. Negative results (a red line in only the “C” region; no line in the “T” region) should be confirmed after 10 minutes.
  - 1.8.5.2.5. If a line in the “C” region does not appear after 10 minutes, test results are invalid and shall be repeated.
- 1.8.5.3. 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.

#### 1.8.6. Takayama, Confirmatory Test for Blood

- 1.8.6.1. **Principle:** Takayama reacts with the heme portion of the blood to form characteristic hemochromogen microcrystals.

Some vegetable peroxidases may form Takayama crystals similar to those formed by blood; however, these substances do not have the same appearance or physical properties as blood.
- 1.8.6.2. **Procedure**
  - 1.8.6.2.1. Prepare working solution by combining glucose, sodium hydroxide, pyridine, and distilled water. The working solution may be made up and stored in the refrigerator for up to one month.
  - 1.8.6.2.2. Place a small cutting, few threads or flakes on a microscope slide and cover with a cover slip. Alternatively, a drop of stain extract may be dried onto the slide.
  - 1.8.6.2.3. Flood under the cover slip with the prepared Takayama reagent.
  - 1.8.6.2.4. Gently heat the microscope slide on a hot plate or equivalent heat source. Optionally, if the stain is weak, the test may be allowed to sit at room temperature.

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- 1.8.6.2.5. When cutting turns bright pink/red, remove from heat and allow to cool to room temperature before observing under the microscope. Negative samples may never turn to the bright pink color.
- 1.8.6.2.6. Bright red, spiky crystals should be apparent in the presence of a sufficient concentration of blood. Tapping of the cover slip to separate crystals from the cutting may be necessary to observe crystals.
- 1.8.6.3. 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.

#### 1.8.7. Acid Phosphatase, Presumptive Test for Semen

- 1.8.7.1. **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. Confirmatory testing shall be performed for conclusive identification. Possible sources of acid phosphatase other than semen include vaginal secretions, urine, sweat or fecal material.

- 1.8.7.2. **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.
  - 1.8.7.2.1. Weigh out about 20 µg of alpha naphthyl phosphate and place in shell vial or small test tube.
  - 1.8.7.2.2. 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.
  - 1.8.7.2.3. Add about 1 ml of acetate buffer to each and mix.
  - 1.8.7.2.4. 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.
  - 1.8.7.2.5. Wet the cutting with the alpha naphthyl phosphate solution. Wait about 1 minute.
  - 1.8.7.2.6. Add 1-2 drops of the dye solution to the cutting.
  - 1.8.7.2.7. 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.
  - 1.8.7.2.8. 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.
  - 1.8.7.2.9. The interpretation of the results shall be reported by the analyst.
- 1.8.7.3. 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.

#### 1.8.8. Extraction of Body Fluid Stains

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1.8.8.1. **Principle:** Evidentiary stains can be extracted and the extracts used for body fluid stain identification.

#### 1.8.8.2. **Procedure**

1.8.8.2.1. 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.

1.8.8.2.2. After incubation, the cutting should be removed, placed into the cap with holes for drainage or a spinex basket, and the extract spun down.

#### 1.8.9. **Visual Observation of Spermatozoa**

1.8.9.1. **Principle:** The visual identification of spermatozoa is a scientifically accepted method of positively identifying the presence of seminal material.

#### 1.8.9.2. **Procedure**

1.8.9.2.1. Place ~10 µl of the previously extracted sample on a microscope slide and cover. 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.

1.8.9.2.2. 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. 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.

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

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

1.8.10.1. **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.

#### 1.8.10.2. **Procedure**

1.8.10.2.1. 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.

1.8.10.2.2. 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.

1.8.10.2.3. Heat fix the sample onto the slide.

1.8.10.2.4. 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.

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

1.8.10.2.6. 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.

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

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

1.8.10.3. 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.

## 1.8.10.4. Interpretation

1.8.10.4.1. 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

1.8.10.4.1.1. 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.

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

## 1.8.11. Visual Observation of Human Hair

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

### 1.8.11.2. Procedure

1.8.11.2.1. 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 the 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.

1.8.11.2.2. 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

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

- 1.8.11.2.3. 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.

#### 1.8.12. Crosslinker Procedure

1.8.12.1. 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.

1.8.12.2. 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 all items retained for possible DNA analysis 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.

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- 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.
- 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 Laboratory 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 be retained by the laboratory for any possible future testing. For amplified DNA see 2.8.1.2.7. For subitems and extracts see flow charts.

**2.1.2.2.** The 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 Laboratory.

**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 laboratory personnel.

## 2.3. Related Information:

**2.3.1.** Worksheet Manual

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

## 2.4. Instruments:

**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.

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- 2.4.5. BIOMEK NX<sup>P</sup> Automation Platform – Robotic liquid handling system.
  - 2.4.6. BIOMEK 3000 Automation Platform – Robotic liquid handling system.
  - 2.4.7. 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.
  - 2.4.8. Crosslinker - Preprogrammed ultraviolet exposure is factory set to 120,000 microjoules per cm<sup>2</sup>. Preset ultraviolet time exposure is factory set to 2 minutes.
  - 2.4.9. 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.
  - 2.4.10. Maxwell® 16 – Robotic extraction system.
  - 2.4.11. 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.
  - 2.4.12. 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.
  - 2.4.13. pH Meter – An instrument capable of manual or automatic temperature compensation and reading +/- 0.01 pH units. Used for preparation of buffers and reagents.
  - 2.4.14. 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).
  - 2.4.15. 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 (NFH<sub>2</sub>O)
  - 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
  - 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<sup>-4</sup>
  - 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

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## 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. The manual handling of lysis buffer should be confined to a chemical fume hood.
- 2.6.5. Universal Precautions shall be in use whenever biological materials are being handled.
- 2.6.6. Extreme caution shall be used when handling liquid body fluid samples.
- 2.6.7. 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.

#### 2.8.1.2. DNA Analysis

- 2.8.1.2.1. All extracts are considered work product. Extracts should only be retained if needed for Y-STR analysis or if from an unknown item in a crimes against a person case where the original, retained subitem has been consumed. Any transfer of an extract to another analyst, shall be properly documented as to chain of custody in the case file. Retained extract tubes shall be labeled with the case number and unique sample designation and shall be sealed within a zip lock bag or plastic sleeve, keeping different samples separated by heat sealing or in separate bags. The extracts shall be maintained for long term storage within the original item or subitem. Extraction controls for those associated samples shall also be retained in separate compartments or bags within the item or subitem(s). All other extracts shall be discarded. See flow charts.

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- 2.8.1.2.1.1. Evidence collection kit samples (where a male suspect is indicated) that demonstrate no amplifiable male DNA shall be returned, and their extracts shall be discarded.
- 2.8.1.2.1.2. Once any Y-STR analysis is complete, associated sample extract(s) may be discarded unless the original subitem was consumed.
- 2.8.1.2.2. 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 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.
- 2.8.1.2.3. When multiple samples have been retained from one item, the analyst can limit the number of samples per item to be extracted. A representative sample shall be tested initially. On an initial examination, 5 samples per piece of clothing or bedding should be sufficient to be extracted. Additional samples may be extracted, as necessary.
- 2.8.1.2.4. Sexual Assault kits: Four to six samples/body areas may be chosen for the first tier of analysis based on the priority list below. If foreign allele at eight or more loci are 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.
  - 2.8.1.2.4.1. If the kit contains the following samples, these samples shall be selected for testing utilizing differential extraction methods:
    - 2.8.1.2.4.1.1. Either vaginal/cervical swabs or vaginal wash
    - 2.8.1.2.4.1.2. Anal/rectal swabs
    - 2.8.1.2.4.1.3. External genital swabs
  - 2.8.1.2.4.2. 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.
    - 2.8.1.2.4.2.1. Breast swabs
    - 2.8.1.2.4.2.2. Dried secretions/bitemark swabs (including other body area swabs)
    - 2.8.1.2.4.2.3. Internal genital swabs
    - 2.8.1.2.4.2.4. Underwear
    - 2.8.1.2.4.2.5. Speculum swabs
    - 2.8.1.2.4.2.6. Bra
    - 2.8.1.2.4.2.7. Oral swabs
  - 2.8.1.2.4.3. Differential extractions should be performed on samples considered intimate or 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

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secretions/bitemark swabs or fingernail scrapings), the analyst may choose to perform a regular extraction.

- 2.8.1.2.4.3.1. 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.
- 2.8.1.2.4.4. Up to four swabs from each selected envelope/body area shall be combined for extraction and evaluated for the presence of male DNA. The combined amount of swabs shall not exceed the volume equivalent of one swab. Representative areas from underwear shall be extracted as one extract.
- 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.
- 2.8.1.2.5. Underwear submitted in sexual assault cases that are submitted outside of a kit shall be tested in the same manner as if they were located inside of the kit. No serological testing is required and at a minimum DNA analysis shall be performed on a sample of the crotch area.
- 2.8.1.2.6. A quantification procedure shall be performed to estimate the quantity of extracted DNA, human and male.
  - 2.8.1.2.6.1. In a sexual assault case, if there are no informative autosomal STR results, samples with male DNA at  $\geq 0.006$  ng/ $\mu$ l shall be retained. A Y-STR analysis case should be created if meaningful information could be gained from the additional testing
- 2.8.1.2.7. In order to be used in a comparison with evidentiary samples, any standard (primary or secondary) shall demonstrate a single source profile. Any exception to this rule shall only be made with the 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 has a quantity of DNA that would require concentration and another sample/cutting from the same item has an appropriate quantity for analysis without additional treatment; it shall be left to the analyst's discretion as to whether or not 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 should have more than one reagent 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

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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.
- 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.

#### 2.8.2. 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 shall consume only as much of the available sample as is needed. Normally not more than half of the available sample will be used for testing. If it is necessary to consume an entire sample in testing or an amount such that the remaining portion is insufficient for additional testing, the analyst shall first receive permission from the prosecutor or investigator and document such in the case record. This may be done at the time of submission.
- 2.8.3.3. **Sampling for Regular Extraction**
  - 2.8.3.3.1. **Blood:** Sample approximately 3 X 3 mm from a blood stain or 3 µl of whole blood.
  - 2.8.3.3.2. **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.
  - 2.8.3.3.3. **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

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extracting with Maxwell® 16. Cut sample into smaller pieces before placing in extraction tube.

- 2.8.3.3.4. **Epithelial Swabs:** Sample from 1/4 to entire swab used to collect epithelial cells. For automated extractions, the total sample addition should not exceed the equivalent of one swab and the reagent volume shall not be adjusted/increased.
  - 2.8.3.3.4.1. For swabs from a sexual assault kit, samples from multiple swabs may be combined into one tube with the total sample not to exceed the equivalent of one swab.
- 2.8.3.3.5. **Hair:** Rinse root end of hair in 100% ethanol and then dH<sub>2</sub>O before sampling 1 cm of root end. If desired a second 1 cm of hair may be extracted separately as a sample blank.
- 2.8.3.3.6. **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.
- 2.8.3.3.7. **Combining extracts:**
  - 2.8.3.3.7.1. Two or more swabs/samples may be extracted in separate extraction tubes, when using an organic extraction, combining at step 2.8.9.2. Multiple completed automation extracts may be combined before concentrating at step 2.8.9.2. The number and volume of the associated RBs shall be the same as the samples.

#### 2.8.3.4. Sampling for Semen (Differential Extraction)

- 2.8.3.4.1. **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 with the total sample not to exceed the equivalent of one swab.
- 2.8.3.4.2. **Clothing:** Sample approximately a 5 X 5 mm cutting.
- 2.8.3.4.3. **Smear Slides:** Use a moistened swab and swab entire smear, including underside of coverslip. Sample swab as above.
- 2.8.3.4.4. **Vaginal Wash:** Spin down vaginal wash and collect pellet on one or more swabs. Sample swab(s) as above.

#### 2.8.3.5. Bone, Teeth and Fingernails

- 2.8.3.5.1. Sample cleaning/preparation methods (e.g., scraping, heating, etc.) that best address the variability of sample quality may be used, as necessary.
- 2.8.3.5.2. Remove and separate any soft tissue adhering to sample with a sterilized scalpel or other appropriate tool.
- 2.8.3.5.3. Immerse and agitate at least 1 cubic inch of bone in cold distilled water.
  - 2.8.3.5.3.1. A small brush or toothbrush may be used to gently remove any dirt/debris from the outer surfaces and exposed inner surfaces of sample.
  - 2.8.3.5.3.2. 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.

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2.8.3.5.3.3. If necessary, the specimen can be further cleaned by immersion in ethanol and ethyl ether for about 15 minutes each.

2.8.3.5.3.4. If surface staining is present, it is recommended to remove the outer table of bone by scraping or sanding.

2.8.3.5.4. Rinse sample in distilled water and allow the sample to dry.

2.8.3.5.5. Pulverize sample into a fine powder by manual mechanical crushing or by use of a freezer/mill. 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.

#### 2.8.3.5.5.1. **Manual Mechanical Crushing with Tissue Pulverizer**

2.8.3.5.5.1.1. Larger samples may initially need to be reduced to ~0.5 in. fragments or smaller to accommodate the physical size of the pulverizer.

2.8.3.5.5.1.2. Crush bone sample using the pulverizer and mallet. Optionally, sample may be immersed in liquid nitrogen for ~1–10 minutes to aid in crushing.

#### 2.8.3.5.5.2. **SPEX SamplePrep® 6770 Freezer/Mill**

2.8.3.5.5.2.1. 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.

2.8.3.5.5.2.2. Insert a sterilized blunt end cap into a sterilized grinding cylinder.

2.8.3.5.5.2.3. Add the sample to the cylinder. Do not add sample in excess of 1/3 total volume of the cylinder.

2.8.3.5.5.2.4. Insert a steel impactor into the cylinder and seal the cylinder with a sterilized flanged end cap.

2.8.3.5.5.2.5. 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.

2.8.3.5.5.2.5.1. Always handle liquid nitrogen containers or any item exposed to liquid nitrogen (including grinding cylinders) with cryogenic gloves.

2.8.3.5.5.2.6. 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.

2.8.3.5.5.2.7. 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

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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.

2.8.3.5.5.2.8. 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.

2.8.3.5.5.2.9. 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.

2.8.3.5.6. 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.

2.8.3.5.7. 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 grams of powder is recommended for the bone demineralization procedure.

#### **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 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.

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. 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. For samples lysed in a 1.5 ml microcentrifuge tube:**

2.8.4.4.1. 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.

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- 2.8.4.4.2. Using a wooden applicator stick, remove the cutting and proceed to 2.8.4.6 or
- 2.8.4.4.3. 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.

#### **2.8.4.5. For samples lysed in a Qiagen® Investigator® Lyse&Spin basket:**

- 2.8.4.5.1. Spin in a microcentrifuge at 10,000 – 14,000 rcf for at least 1 minute. 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 2.8.9 for Microcon® procedure.

#### **2.8.5. Demineralization and Organic Extraction of Bone, 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.
- 2.8.5.3. Incubate the samples in an orbital shaker at 56°C and 150 rpm overnight (18 hours minimum/24 hours maximum). A shortened extraction time 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 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 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.

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- 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 at 4°C or proceed to QIAquick® purification.

## 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.
- 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 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.

## 2.8.7. 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 NFH<sub>2</sub>O
  - 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.

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- 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.
- 2.8.7.11. To the tube containing the washed pellet add:
  - 150 µl Tris/EDTA/NaCl
  - 50 µl 20% Sarkosyl
  - 150 µl NFH<sub>2</sub>O
  - 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 to which buffer has been added (p/c/i) 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 2.8.9 for Microcon® procedure.

#### 2.8.8. QIAcube® Workstation Differential Extraction

- 2.8.8.1. For each sample, combine 240 µl Tris/EDTA/NaCl, 6 µl 20% Sarkosyl, 54 µl NFH<sub>2</sub>O and 3 µl Proteinase K (10 mg/ml). Example: for 16 samples, combine 3840 µl Tris/EDTA/NaCl, 96 µl 20% Sarkosyl, 864 µl NFH<sub>2</sub>O, and 48 µl Pro K.
- 2.8.8.2. Place each sample in a 1.5 ml microcentrifuge tube. Each group of samples being extracted shall include reagent blanks (sperm and non-sperm fraction) as the last samples in the batch.

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2.8.8.2.1. For differential fractionation on the QIAcube® workstation, samples shall be loaded specifically into properly labelled QIAGEN 1.5 ml tubes.

- 2.8.8.3. 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.
- 2.8.8.4. Incubate the sample at 37°C for 2 hours.
- 2.8.8.5. Spin briefly in a microcentrifuge to force the condensate into the bottom of the tube.
- 2.8.8.6. Using a wooden applicator stick, remove the cutting and spin in a microcentrifuge for about 5 minutes at approximately 14,000 rcf.
- 2.8.8.7. 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.
- 2.8.8.8. Place the 1.5 ml tube containing the sample in Position L3 of the QIAcube® rotor adapter with cap open and flipped into holder. Place the rotor adapter with sample into the appropriate position on the QIAcube® centrifuge.
- 2.8.8.9. Prepare lysis buffer master mix with the volumes of Lysis buffer and 1M DTT indicated below:

# Samples	Lysis buffer (µl)	1 M DTT (µl)	Buffer Bottle 2 (µl)
2	4250	43	3850
3	4880	49	4280
4	5110	52	4710
5	6255	63	5255
6	6885	69	5685
7	7515	76	6115
8	8145	82	6545
9	8775	88	6975
10	9520	96	7520
12	10800	110	8400

- 2.8.8.10. Place 200 µl of lysis buffer master mix into labelled 2 ml tubes and place them in the appropriate location in the QIAcube® shaker. Add the appropriate amount of Lysis Buffer master mix to a 30ml reservoir bottle (Buffer Bottle 2) as indicated above and load onto the QIAcube® instrument at Reagent Bottle Rack Position 2.
- 2.8.8.11. Fill both tip racks with QIAGEN® “Disposable Filter-Tips, 1000 µl, wide bore.”
- 2.8.8.12. Add Digest Wash buffer to a 30 ml reservoir bottle and place in the QIAcube® deck at Reagent Bottle Rack Position 1 as follows:

# Samples	Digest Wash Buffer (µl)
2	6620
3	8680
4	10740
5	12800
6	14860

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7	16920
8	18980
9	21040
10	23100
12	27220

- 2.8.8.13. For ≤ 6 samples, run “Custom Separate + Lyse 6” protocol.
- 2.8.8.14. For > 6 samples (cannot run 11 samples), run “Custom Separate + Lyse 12A” protocol. After protocol ends, re-fill both racks with “Disposable Filter-Tips, 1000 µl, wide bore.” Run “Custom Separate + Lyse 12B” protocol.
- 2.8.8.15. When the final protocol is finished, remove samples from QIAcube®.
- 2.8.8.16. For automation processing, transfer samples into 1.5ml Click-Fit tubes and proceed to step 5.8.2.2.12.
- 2.8.8.17. For Maxwell 16 processing, continue to step 2.8.11.3.

## 2.8.9. Microcon® Concentration Of Extracted DNA (Microcon® 100 or Fast Flow)

- 2.8.9.1. To a Microcon® Concentrator add 100 µl TE<sup>-4</sup>.
- 2.8.9.2. 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.
- 2.8.9.3. 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.
- 2.8.9.4. 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.
- 2.8.9.5. Remove the cap and add 200 µl nuclease free water (NFH<sub>2</sub>O) or TE<sup>-4</sup> 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.
- 2.8.9.6. Remove the cap and add a measured volume of NFH<sub>2</sub>O or TE<sup>-4</sup> (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.
- 2.8.9.7. 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.
- 2.8.9.8. 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.
- 2.8.9.9. 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.
- 2.8.9.10. 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.

## 2.8.10. Centri-sep Clean-Up Of Extracts

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- 2.8.10.1. 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.
  - 2.8.10.2. Remove the top column cap and reconstitute the column by adding 800 µl of nuclease free water (NFH<sub>2</sub>O). 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.
  - 2.8.10.3. Allow the column to hydrate for at least 30 minutes at room temperature. Reconstituted columns may be refrigerated at 4°C overnight. Allow to warm up to room temperature before proceeding.
  - 2.8.10.4. 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.
  - 2.8.10.5. First remove the column cap, and then remove the column end stopper from the bottom.
  - 2.8.10.6. 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.
  - 2.8.10.7. 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.
  - 2.8.10.8. Optionally: discard eluate and rinse column with 150-200 µl NFH<sub>2</sub>O and spin for 2 minutes at 750 rcf.
  - 2.8.10.9. Blot any drop at the end of the column. Discard the wash tube. Do not allow the gel material to dry excessively.
  - 2.8.10.10. 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).
  - 2.8.10.11. 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.
  - 2.8.10.12. Discard the spin column and cap the sample collection tube.
  - 2.8.10.13. 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.
- 2.8.11. Maxwell® Protocol**
- 2.8.11.1. **Maxwell® Preparation of Samples For Any Cellular Material (except bone):**
    - 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

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batch can be no larger than the capacity of the Maxwell Unit being utilized. For a Maxwell® 16 or 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:**

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. Add 200 µl of Lysis Buffer to each sample.

2.8.11.1.5.3. Vortex samples briefly and spin down.

2.8.11.1.5.4. Using a wooden applicator stick, remove the cutting and proceed to 2.8.11.1.7 or

2.8.11.1.5.5. 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. 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 2.8.11.3 for Maxwell® 16 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 NFH<sub>2</sub>O 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® 16 or 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.

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- 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.
- 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 2.8.11.2.11. 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 2.8.11.2.9 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 2.8.11.3 for Maxwell® 16 automated extraction.

## **2.8.11.3. Maxwell® 16 Instrument Automated DNA Purification:**

- 2.8.11.3.1. Place the number of cartridges to be used into the cartridge preparation rack. Each cartridge corresponds to one sample. Place each cartridge into the rack. Hold the cartridge firmly and remove the seal.
- 2.8.11.3.2. Place one plunger into well #8 of each cartridge.
- 2.8.11.3.3. Transfer the entire sample into well #1.
- 2.8.11.3.4. Place 0.5 ml elution tubes (properly labeled) into the elution tube slot. Add 50 µl of Elution Buffer.
- 2.8.11.3.5. Turn on the Maxwell® 16 Instrument. Verify “LEV” and “FNCS” modes are displayed.
- 2.8.11.3.6. Scroll to “Run” on the Menu screen and press the “Run/Stop” button.
- 2.8.11.3.7. Open the door when prompted to do so on the LCD display. Press the “Run/Stop” button to extend the platform.

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- 2.8.11.3.8. Place the cartridge rack into the Maxwell® 16 Instrument.
- 2.8.11.3.9. Press the “Run/Stop” button. The platform will retract. Close the door.
- 2.8.11.3.10. Upon method completion, open the instrument door. The plungers should be located in Well #8 at the end of the run. Check to make sure that all of the plungers have been removed from the magnetic rod assembly. If the plungers have not been removed, push them down gently by hand and remove them from the magnetic rod assembly.
- 2.8.11.3.11. Press the “Run/Stop” button to extend the platform.
- 2.8.11.3.12. Remove the elution tubes from the heated elution tube slots, as soon as possible and close the top on each tube. (Leaving the heated elution tubes in the instrument for an extended period of time can result in evaporation of the sample.) Samples can be stored at 4°C or frozen. Prior to use after storage, the samples shall be vortexed briefly and spun in a centrifuge for about 5 seconds.
- 2.8.11.3.13. Remove cartridges and plungers from the instrument platform and discard.
- 2.8.11.3.14. When completed, clean the Maxwell® 16 Instrument and cartridge rack with 70% ethanol and turn off machine.

## **2.8.11.4 Maxwell® FSC 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 seals.
- 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 50µl of Elution Buffer to each elution tube.
- 2.8.11.4.5 DO NOT WEAR GLOVES WHEN TOUCHING THE TABLET! 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® FCS 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 “Start” 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 rack, 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

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appropriate kit on the screen. Touch “Proceed” on the right side of the highlighted line.

- 2.8.11.4.10 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.
- 2.8.11.4.11 “The door will now open” box will appear. Touch “OK”.
- 2.8.11.4.12 Load cartridge rack with cartridges, plungers, samples and **open** elution tubes. Touch “Start”.
- 2.8.11.4.13 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 2.8.11.4.16. Samples shall not be restarted and will be lost. Proceed to 2.8.11.4.17.
- 2.8.11.4.14 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.
- 2.8.11.4.15 If all plungers have been properly ejected from the bar into the cartridges, proceed to 2.8.11.4.17. If plungers have not properly ejected from the bar, you **MUST** run a clean-up. Do not remove cartridge rack from instrument.
- 2.8.11.4.16 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.
- 2.8.11.4.17 Remove cartridge rack from instrument.
- 2.8.11.4.18 Touch the door icon in the upper right to close the instrument door.
- 2.8.11.4.19 Close all elution tubes and remove from cartridge rack.
- 2.8.11.4.20 Remove all plungers from cartridges and dispose.
- 2.8.11.4.21 Remove all cartridges from cartridge rack and dispose.
- 2.8.11.4.22 Clean Maxwell® instrument and cartridge rack with 70% ethanol – never bleach! Tablet screens may be wiped with microfiber screen cleaning cloths.
- 2.8.11.4.23 Close out of the Maxwell® FSC software using the X icon in the upper left corner then touch “Yes”.
- 2.8.11.4.24 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”.
- 2.8.11.4.25 Tablet will go to sleep after 1 hour. DO NOT turn off the instrument.

#### **2.8.11.5 Maxwell ® RSC 48 Instrument Automated DNA Purification:**

- 2.8.11.5.1 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.
- 2.8.11.5.2 Place one plunger into well #8 of each cartridge (closest well to elution tube).

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- 2.8.11.5.3 Transfer entire sample into well #1 of respective cartridge (furthest well from elution tube).
- 2.8.11.5.4 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.
- 2.8.11.5.5 DO NOT WEAR GLOVES WHEN TOUCHING THE TABLET! Use the power button on the far left top edge of the tablet to wake up the tablet.
- 2.8.11.5.6 Swipe up on the tablet screen to select “Analyst” account on the tablet. Touch “Sign In” (no password necessary).
- 2.8.11.5.7 Double tap the Maxwell® RCS 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 “Start” 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”.
- 2.8.11.5.8 To begin a sample run select “Start” from the Maxwell® software home screen.
- 2.8.11.5.9 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.
- 2.8.11.5.10 “The door will now open” box will appear. Touch “OK”.
- 2.8.11.5.11 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.
- 2.8.11.5.12 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. Samples shall not be restarted and will be lost. Proceed to 2.8.11.5.16.
- 2.8.11.5.13 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.
- 2.8.11.5.14 If all plungers have been properly ejected from the bar into the cartridges, proceed to 2.8.11.5.15. If plungers have not properly ejected from the bar, you MUST run a clean-up. Do not remove cartridge rack from instrument.
- 2.8.11.5.15 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.

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- 2.8.11.5.16 Remove cartridge rack from instrument.
- 2.8.11.5.17 Touch the door icon in the upper right to close the instrument door.
- 2.8.11.5.18 Close all elution tubes and remove from cartridge rack.
- 2.8.11.5.19 Remove all plungers from cartridges and dispose.
- 2.8.11.5.20 Remove all cartridges from cartridge rack and dispose.
- 2.8.11.5.21 Clean Maxwell® instrument and cartridge rack with 70% ethanol – never bleach! Tablet screens may be wiped with a microfiber screen cleaning cloth.
- 2.8.11.5.22 Close out of the Maxwell® RSC 48 software using the X icon in the upper left corner then touch “Yes”.
- 2.8.11.5.23 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”.
- 2.8.11.5.24 Tablet will go to sleep after 1 hour. 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<sup>-4</sup>.
  - 2.8.12.4.2 Add TE<sup>-4</sup> 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.
  - 2.8.12.4.4 If the reagent blank did not dehydrate, an additional reagent blank with the same volume of TE<sup>-4</sup> 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®.

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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 kit shall be stored at -15 to -25°C upon receipt. All kit components shall be maintained together in the kit sleeve.

**2.8.13.1.5** Allow the entire kit to thaw prior to first use after which all components shall be stored at 2 to 8°C.

**2.8.13.1.6** The Quantifiler® Trio Primer Mix and the Quantifiler® THP PCR Reaction Mix shall be protected from excessive exposure to light.

**2.8.13.1.7** 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.

Standard	Concentration (ng/µl)	Dilution Buffer	DNA Standard	Dilution Factor
Std 1	50	15 µl dilution buffer (Option 1)	15 µl DNA stock (Option 1)	2X

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		25 µl dilution buffer (Option 2) 35 µl dilution buffer (Option 3)	25 µl DNA stock (Option 2) 35 µl DNA stock (Option 3)	
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
<b>Note:</b>	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.

## 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.

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**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](#)

**2.8.13.6** Experiment Analysis

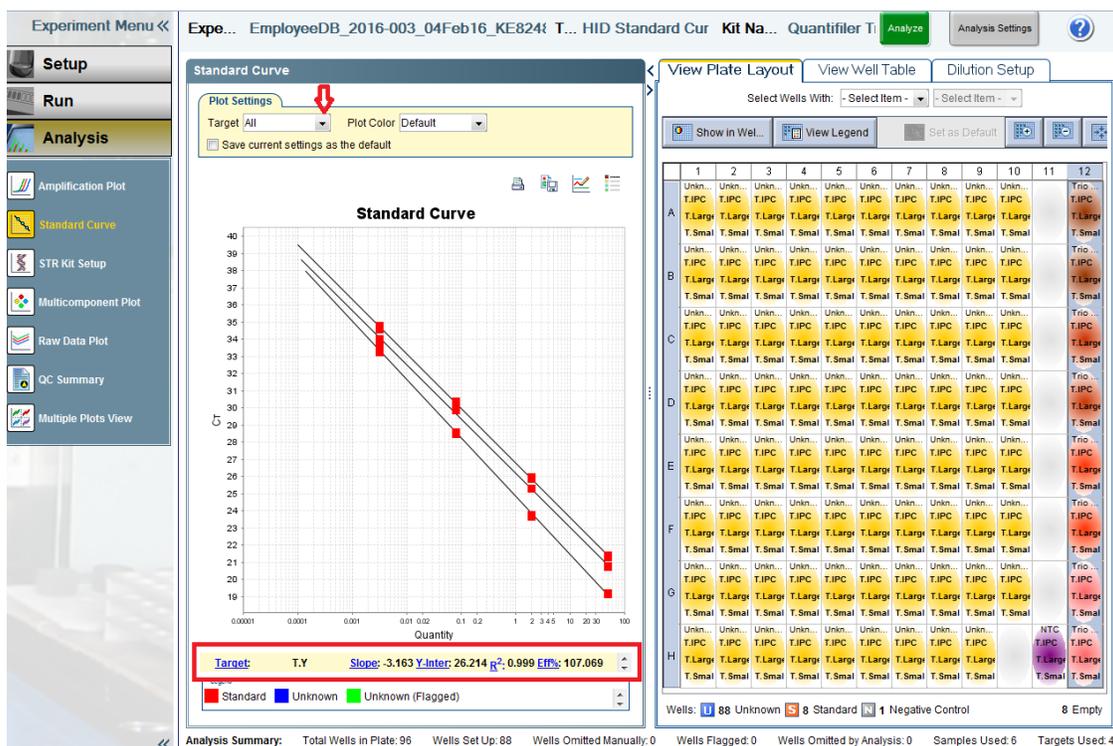
**2.8.13.6.1** Analyze the experiment by clicking 

**2.8.13.6.2** Evaluate the slope, Y-intercept, and R<sup>2</sup> values of all three standard curves as described under **2.8.12.7 Interpretation Guidelines**.

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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 **Trio Raw Data** tab.

2.8.13.6.5 View and Print the **Quant Results** and **Quant Std Curve** tabs. The case notes shall contain the quantification values, IPC C<sub>T</sub> values, degradation indices, and a graph containing all three standard curves with slope, R<sup>2</sup>, and y-intercept values for each curve.

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.

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.

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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.

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/ $\mu$ l 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.

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<sup>2</sup> 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  $\geq 0.980$ . If the R<sup>2</sup> value is  $<0.98$  the experiment is inconclusive and shall be repeated with notification to the Technical Leader documented in the case record.

**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.

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
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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 <sub>T</sub> )	Undetected or Elevated	Inconclusive IPC result	Dilute and re-quantify (optional)
Detected (High C <sub>T</sub> )	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)  $\geq 0.001$  ng/ $\mu$ l 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/ $\mu$ l 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/ $\mu$ l. 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/ $\mu$ l, 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/ $\mu$ l, 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/ $\mu$ l during extraction. If a sample quantifies  $> 50$  ng/ $\mu$ l 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 is determined by subtracting the quantity of male DNA from the quantity of small autosomal DNA (equation: [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.

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.

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- 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 sufficient (i.e.  $\geq 0.025$  ng amplification input). The relative value of autosomal STR analysis of samples in this range should be weighed against other related samples.
- 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 sufficient (i.e.  $\geq 0.025$  ng amplification input). The relative value of autosomal STR or Y-STR analysis of samples in this range should be weighed against other related samples.
- 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. Y-STR analysis may be recommended if appropriate. If a sperm fraction is amplified, the corresponding non-sperm fraction may be amplified regardless of the FTMR and amount of male DNA present.
- 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)  $\geq 0.001$  ng/ $\mu$ 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 The analyst may re-extract the associated samples if contamination is suspected.
- 2.8.13.7.7.1.2 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)  $< 0.001$  ng/ $\mu$ l may be due to background fluorescence or low level contamination.
- 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/ $\mu$ l for autosomal STRs;  $< 0.006$  ng/ $\mu$ 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.

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- 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/μl for autosomal STRs; <0.006 ng/μl for Y-STRs) shall be reported as failed to demonstrate a sufficient quantity of (male) DNA for further/autosomal STR analysis.

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

##### 2.8.14.1 Introduction

- 2.8.14.1.1** 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).
- 2.8.14.1.2** 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.
- 2.8.14.1.3** 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.
- 2.8.14.1.4** The PowerPlex® Fusion 6C System locus-specific and allelic ladder information is available at [www.promega.com/protocols/](http://www.promega.com/protocols/) in the PowerPlex® Fusion 6C System Technical Manual TMD045.
- 2.8.14.1.5** All PowerPlex® Fusion 6C components should be stored at -10°C to -30°C upon receipt in the appropriate laboratory locations. The PowerPlex® Fusion 6C Allelic Ladder is packaged separately for shipping and should be stored in the post-amplification kit. Once thawed all components are stable at 2-10°C for 6 months. Do not refreeze.
- 2.8.14.1.6** 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.
- 2.8.14.1.7** The preparation of the amplification reactions shall be performed in the PCR amplification set-up area.

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## 2.8.14.2 PowerPlex® Fusion 6C Amplification Set-Up

- 2.8.14.2.1** 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. The Master Mix and Primer Pair Mix shall be stored at 2-10°C after first use.
- 2.8.14.2.2** 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.
- 2.8.14.2.3** Place one 0.2 ml PCR reaction tube for each sample into a rack and label appropriately. A 96-well plate may be used.
- 2.8.14.2.3.1** 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.
- 2.8.14.2.4** 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.
- 2.8.14.2.5** For samples that will amplify 5 µl of template DNA, use the **PowerPlex® Fusion 6C Amplification Worksheet - 5 µl Input** 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 H <sub>2</sub> O	10
PowerPlex® Fusion 6C 5X Master Mix	5
PowerPlex® Fusion 6C 5X Primer Pair Mix	5
Total Volume	20

- 2.8.14.2.6** Combine the calculated volume of each component into a 1.5 ml tube and vortex ~5 seconds.
- 2.8.14.2.7** Pipette 20 µl of the PCR master mix to each sample tube or well.
- 2.8.14.2.8** Add 5 µl of sample, reagent blank, positive amplification control, or negative amplification control to the appropriate tube or well.
- 2.8.14.2.8.1** 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 cycler. The diluted positive control should be stored at 2-10°C.

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2.8.14.2.8.2 Amplification Grade H<sub>2</sub>O or NF H<sub>2</sub>O shall be used as a negative amplification control included with each thermal cycler and will be the last sample in the batch.

**2.8.14.2.9** For samples requiring more than 5 µl of template DNA, use the **PowerPlex® Fusion 6C Amplification – Variable Input** 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

**2.8.14.2.10** Combine the calculated volume of each component into a 1.5 ml tube and vortex ~5 seconds.

**2.8.14.2.11** Pipette 10 µl of the PCR master mix to each sample tube or well.

**2.8.14.2.12** Add the calculated volume of Amplification Grade H<sub>2</sub>O or NF H<sub>2</sub>O (0-10 µl) to each sample tube or well.

**2.8.14.2.13** Add the calculated volume of sample, reagent blank, positive amplification control, or negative amplification control (5-15 µl) to each sample tube or well.

2.8.14.2.13.1 If the template DNA is stored in TE<sup>-4</sup> buffer, the volume of the DNA sample added should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl) or available magnesium concentration (due to chelation by EDTA). DNA samples stored (or diluted) in NF H<sub>2</sub>O are not subject to this caution, but may contain other PCR inhibitors at low concentrations depending on the source of the template DNA and the extraction procedure employed.

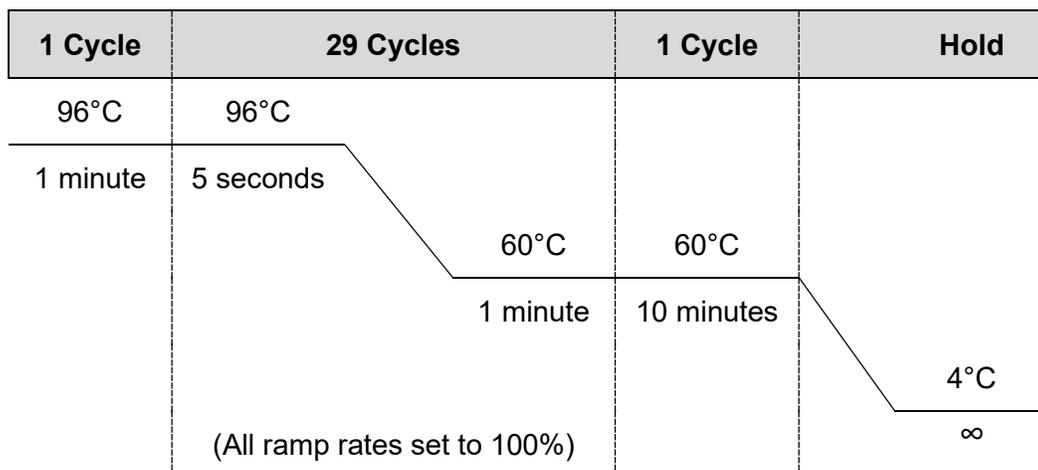
**2.8.14.2.14** For samples amplified in a 96-well plate, seal the plate with adhesive foil. Press the foil well using a roller tool.

## **2.8.14.3 PowerPlex® Fusion 6C Amplification**

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

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

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**2.8.14.3.3** Remove samples after the amplification process is completed and proceed with capillary electrophoresis or store the amplified samples at -10°C to -30°C. Alternatively, amplified samples may be stored at 2-10°C if they are to be used within 2 days.

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

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

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

### 2.8.15.1 Sample Preparation

**2.8.15.1.1 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. The formamide shall be frozen in aliquots at -20°C and the remainder of each aliquot shall be discarded after it is thawed. Multiple freeze-thaw cycles or long-term storage at 4°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.

**2.8.15.1.2 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.

**2.8.15.1.3** 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. The ILS and allelic ladder shall be stored at 2-10°C after first use.

**2.8.15.1.4** 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.

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- 2.8.15.1.5** 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.
- 2.8.15.1.5.1 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.
- 2.8.15.1.6** Vortex to mix.
- 2.8.15.1.7** 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.
- 2.8.15.1.8** 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).
- 2.8.15.1.8.1 If amplified sample is in a 96-well plate, seal the amplification plate with a new aluminum foil seal for storage.
- 2.8.15.1.9** Cover the wells with the plate septa and briefly centrifuge to remove air bubbles.
- 2.8.15.1.10** 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.
- 2.8.15.2 Create a Plate Record**
- 2.8.15.2.1** 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.
- 2.8.15.2.2** Select **Start Pre-Heat**. This should be done at least 30 minutes prior to the first injection.

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## Dashboard:

**Common Operations**

- Quick Start Run
- Create New Plate
- Create Plate from Template
- Edit Existing Plate
- View Run Results
- Maintain Instrument

**Quick View**

**Gauges**

- POP4 Polymer:** 240 Samples Remaining (14 Injections Remaining)
- ABC - (Anode):** 7 Days Remaining (49 Injections Remaining)
- CBC - (Cathode):** 7 Days Remaining (49 Injections Remaining)
- 36cm - 24 cap Array:** 130 Injections Performed

**Instrument Status:**

- Instrument: 3500 Instrument
- Laser: Off
- EP: Off
- State: Idle
- Oven: Off
- Oven Door: Close
- Instrument Door: Close
- Oven Temperature (°C): 27.2
- Detection Cell Temperature (°C): 22.05

**Pre-Heat the Oven**

Set Temperature to: 60 (°C)

**Consumables Information**

Consumable	Name	Status	Days on Instrument	Expiration Date	Lot Number	Part Number
Polymer	POP4	240 Samples Remaining	7	03-Nov-2012 08...	1203033	4393715
Anode Buffer	ABC	7 Days Remaining	0	01-Aug-2012 08...	1112065	4393927
Cathode Buffer	CBC	7 Days Remaining	0	10-Nov-2012 07...	1203094	4408256
Capillary Array	36cm - 24 cap	30 Injections Remaining	133	19-Oct-2012 04...	16	4404687 - Serial # M311U1612

**Maintenance Notifications**

### 2.8.15.2.3 Select **Create New Plate** or **Create Plate from Template**.

2.8.15.2.3.1 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).

2.8.15.2.3.1.1 If the plate is re-injected the "01" number shall increase sequentially (00A1234\_01Jan16\_02).

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

2.8.15.2.3.1.3 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).

2.8.15.2.4 Select "96" for Number of Wells.

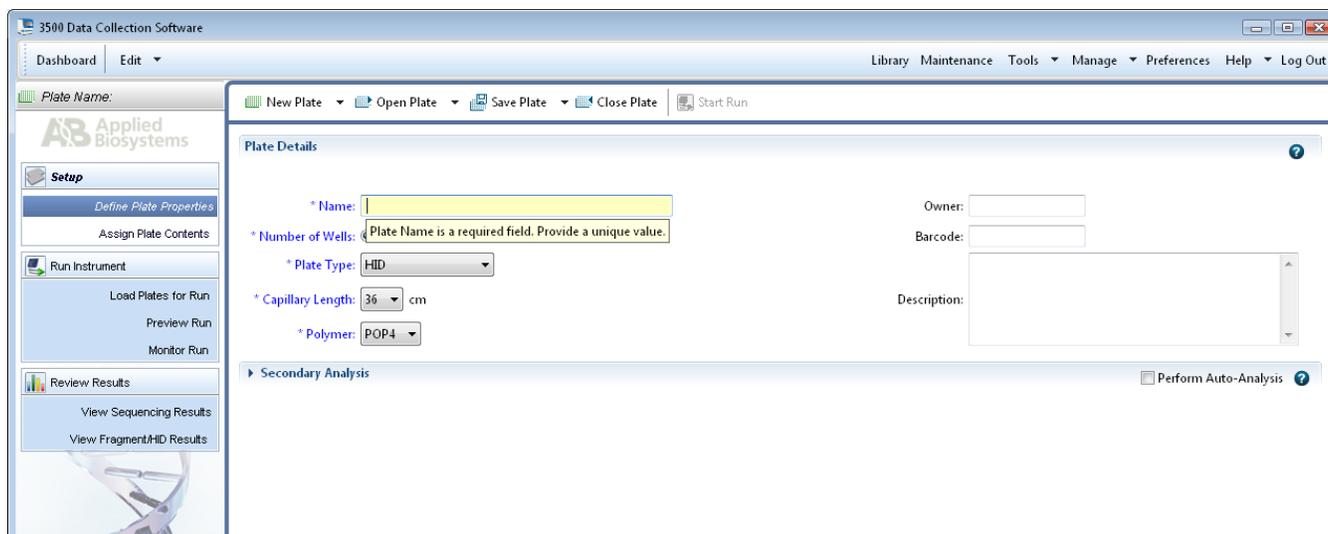
2.8.15.2.5 Select "HID" for Plate Type.

2.8.15.2.6 Select "36"cm for Capillary Length.

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2.8.15.2.7 Select “POP4” for Polymer.

## Create A Plate:



2.8.15.2.8 Click “Assign Plate Contents”.

2.8.15.2.9 Enter sample information or import a plate record.

2.8.15.2.9.1 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 sub-item number is required.

2.8.15.2.9.2 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.

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

2.8.15.2.11 Under **File Name Convention**, click “Add from Library” and select “Fusion6C\_FNC”. Double-click or click “Add to Plate” then “Close”.

2.8.15.2.12 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:

The screenshot displays the software interface for assigning plate contents. At the top, there is a menu bar with options like 'New Plate', 'Open Plate', 'Save Plate', 'Close Plate', 'Import', 'Export', 'Find/Replace', 'View Plate Grid Report', and 'Print'. Below the menu is a toolbar with icons for 'Show In Wells', 'Select Wells', 'Array Selection', 'Row', 'Column', 'Zoom In', 'Zoom Out', and 'Fit'. The main area is a 12x12 grid representing a plate. The first column (A) is labeled with letters A through H. The first row (1) is labeled with numbers 1 through 12. The grid contains sample information for wells A1 through H8. For example, well A1 contains 'Allelic Ladder', well B1 contains '001A 00A-1234', and well H8 contains 'RB 00A-6789'. Below the grid is a yellow bar with a search field containing 'HID' and a 'Name: 00A-1234\_01JANI6\_01' field. At the bottom, there are three panels: 'Assays' (with 'Fusion6C\_1.2kv15s' selected), 'File Name Conventions' (with 'Fusion6C\_FNC' selected), and 'Results Groups' (with 'Fusion6C\_RG' selected). To the right is a 'Customize Sample Info' window with a table of properties and values. A 'Link Plate for Run' button is located at the bottom center.

Row	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										

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

**2.8.15.2.13.1** 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.

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

**2.8.15.2.15** 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”.

**2.8.15.2.16** 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”.

### 2.8.15.3 Start a Plate Run

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

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

**2.8.15.3.3** Click “Start Run”.

**2.8.15.3.4** 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 at -10°C to -30°C. The plate shall be destroyed after the case has been administratively reviewed.

2.8.15.3.4.1 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.

2.8.15.3.5 A copy of the CE Plate Record Worksheet shall be maintained with each case record.

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

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

#### 2.8.17.1 Processing Sample Data

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

2.8.17.1.2 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.

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

2.8.17.1.3 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”.

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

2.8.17.1.4.1 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.

2.8.17.1.5 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.

2.8.17.1.6 Set the **Table Setting** at the top of the screen to “STRmix\_Fusion6C”.

2.8.17.1.7 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.

2.8.17.1.8 In the **Analysis Method** column,

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- 2.8.17.1.8.1 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.
  - 2.8.17.1.8.2 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.
  - 2.8.17.1.8.3 Positive controls can be analyzed with either analysis method; however, stutter will need to be manually deleted from positive control profiles. Negative controls shall be analyzed with the lowest or lower analytical threshold of any associated samples.
  - 2.8.17.1.8.4 A project may contain samples with different analysis methods. The project shall contain a separate ladder for each analysis method used.
  - 2.8.17.1.8.5 A project does not need to be created for standards if no items of evidence in that case are amplified or demonstrate conclusive results.
- 2.8.17.1.9** In the **Panel** column,
- 2.8.17.1.9.1 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.
  - 2.8.17.1.9.2 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.
  - 2.8.17.1.9.3 Ladders, positive and negative controls shall utilize the panel that corresponds to the analysis method used.
- 2.8.17.1.10** 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.
- 2.8.17.1.11** 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 appropriate settings as the default in the drop-down menus for Analysis Method, Size Standard, and Panel. Click “OK”.
- 2.8.17.1.12** 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 for additional clarification. The case number is recommended but not required. Select the “ISP Casework Security Group”. Click “OK”.
- 2.8.17.2 Evaluating Sample Data**
- 2.8.17.2.1** 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.
  - 2.8.17.2.2** Highlight all sample rows containing **Allelic Ladders** and click “Display Plots”. In the Samples Plot screen, change the “Plot Setting” drop-down box to “Fusion6C”.

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Magnify the area from about 50 to 500 bp and verify that the correct allele calls are made for each peak. Print the electropherograms.

- 2.8.17.2.3 Highlight all sample rows containing **Negative Controls** and click “Display Plots”. Verify the Plot Setting is set to “Fusion6C” and check the negative controls to ensure that no peaks above analytical threshold are present. Print the electropherograms ensuring that the primer peak is visible.
- 2.8.17.2.4 Highlight all remaining sample rows and click “Display Plots”. Magnify the area from about 50 to 500 bp. Verify the Plot Setting is set to “Fusion6C”, evaluate all allele calls, and print the electropherograms. Optionally, the remaining sample rows may be viewed, evaluated, and printed with the Allelic Ladders.
- 2.8.17.2.5 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.
- 2.8.17.2.6 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.
- 2.8.17.2.7 When exporting the GeneMapper™ ID-X project, ensure that the “Export with analysis settings” box is checked.

#### 2.8.18 General Rules For PowerPlex® Fusion 6C Analysis On The Applied Biosystems® 3500 Genetic Analyzer

- 2.8.18.1 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.
- 2.8.18.2 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.
- 2.8.18.3 A signal range of 5,000 to 20,000 RFU will produce optimal results.
- 2.8.18.4 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).
- 2.8.18.5 Only the data used for interpretation needs to be printed for 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.

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

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- 2.8.19.1** 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.
- 2.8.19.2** A copy of all electropherograms used in interpretation as well as a printout of the plate record shall be placed in the case record.

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

#### **2.8.21 Interpretation Guidelines For PowerPlex® Fusion 6C and STRmix™ Deconvolutions**

##### **2.8.21.1 Scope**

- 2.8.21.1.1** The following interpretation guidelines shall aid in peak/true allele determinations and profile interpretations.
- 2.8.21.1.2** 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™.
- 2.8.21.1.3** 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.
- 2.8.21.1.4** Final allele calls shall be documented on the STRlite printout.
- 2.8.21.1.4.1** 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 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.
- 2.8.21.1.5** 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.

2.8.21.1.5.1 The ISP Laboratory has validated STRmix™ v2.7.0 for casework.

##### **2.8.21.2 DNA Profile Interpretation**

- 2.8.21.2.1** Assumptions used in the interpretation of mixtures and interpretation decisions shall be documented in the case record.
- 2.8.21.2.2** Analysts shall consider the additive effects of allele sharing during interpretation.
- 2.8.21.2.3** 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.

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Statistical analysis (if necessary).

**2.8.21.2.4** 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.

#### **2.8.21.2.5 Identify If The Profile Is A Mixture**

##### **2.8.21.2.5.1 Single Contributor DNA Profile Determination**

2.8.21.2.5.1.1 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:

2.8.21.2.5.1.1.1 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.

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

2.8.21.2.5.1.1.2 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.

2.8.21.2.5.1.2 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.

2.8.21.2.5.1.2.1 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.

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

##### **2.8.21.2.5.2 Mixed DNA Profile Determination**

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- 2.8.21.2.5.2.1 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.
- 2.8.21.2.5.2.2 A peak height ratio less than 0.65 at a locus may indicate a mixed profile and that allele sharing may be occurring.
  - 2.8.21.2.5.2.2.1 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.
- 2.8.21.2.5.2.3 Peaks in stutter position that exceed the mean +3 SD stutter percentage may indicate the presence of a mixture.
- 2.8.21.2.5.2.4 All loci shall be considered when determining the presence of a mixture.
- 2.8.21.2.5.3 **Profiles In Which A Single Additional Allele is Detected (not tri-allelic)**
  - 2.8.21.2.5.3.1 If an additional allele is present at Amelogenin or the Y-STR (and therefore cannot be modeled by STRmix™), the sample shall be reported with the additional allele wording outlined in the Report Writing Guidelines (Appendix 7).
  - 2.8.21.2.5.3.2 If the additional allele is possible drop-in at an autosomal locus ( $\leq 200$  RFU), the assumed number of contributors should not be increased and STRmix™ shall be allowed to model the peak.
  - 2.8.21.2.5.3.3 If the additional allele 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 account for that additional contributor.

#### 2.8.21.3 Preliminary Evaluation of Allele Peaks

- 2.8.21.3.1 An analyst is required to visually confirm that all allelic ladders used for allele designation performed correctly.
- 2.8.21.3.2 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 all unknown samples.
  - 2.8.21.3.2.1 Peaks below analytical threshold shall not be interpreted, but may be considered when deciding if the possible number of contributors can be reasonably assumed.
  - 2.8.21.3.2.2 For reference standards, 150 RFU shall be used as the analytical threshold for all Fusion 6C dye channels.
- 2.8.21.3.3 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.
  - 2.8.21.3.3.1 If a reference sample has a locus with a single peak present  $\leq 950$  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

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approval is not necessary for ignoring more than one locus due to possible allelic drop-out of reference sample.

- 2.8.21.3.4** 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.
- 2.8.21.3.4.1 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.
  - 2.8.21.3.4.2 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.
- 2.8.21.3.5** 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.
- 2.8.21.3.5.1 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 printed 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
  - 2.8.21.3.5.2 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 printed 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.
  - 2.8.21.3.5.3 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 printed 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.

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- 2.8.21.3.6** 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 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](#).
- 2.8.21.3.6.1 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.
- 2.8.21.3.6.2 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 printed electropherogram should indicate the reason for deletion, either manually or labeled in the software. Examples include:
- 2.8.21.3.6.2.1 Stutter peaks in the double-backward position (n-6/n-8/n-10).
- 2.8.21.3.6.2.2 Partial repeat (n-2/n+2) stutter peaks not included above (most common for D1S1656, D19S433, SE33 n+2 only and FGA).
- 2.8.21.3.6.2.3 All stutter peaks at the Y-STR loci.
- 2.8.21.3.6.3 For reference profiles, all stutter peaks shall be deleted.
- 2.8.21.3.7** 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.
- 2.8.21.3.7.1 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.
- 2.8.21.3.7.1.1 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).
- 2.8.21.3.7.1.2 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.

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2.8.21.3.7.1.2.1 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.

2.8.21.3.7.1.3 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 ignored from the STRmix™ deconvolution.

2.8.21.3.7.1.4 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.

## 2.8.21.4 Evaluation of Controls

**2.8.21.4.1** Failed controls require the documented notification to the Technical leader with appropriate documentation in the case record.

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

### 2.8.21.4.3 Reagent Blank:

2.8.21.4.3.1 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.

2.8.21.4.3.2 A reagent blank with peaks below the Analytical Threshold shall not prevent associated samples from being interpreted.

2.8.21.4.3.3 A reagent blank with peaks above the Analytical Threshold shall be considered a failed negative control. All associated samples shall be inconclusive. All of the samples shall be repeated when appropriate.

### 2.8.21.4.4 Positive Control:

2.8.21.4.4.1 The 2800M DNA is used as a positive control to demonstrate that the kit is performing properly. If the expected alleles are not detected in the positive control well, then the test is considered inconclusive.

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

<b>X, Y</b>	<b>17, 18</b>	<b>12, 13</b>	<b>10, 14</b>	<b>13, 15</b>	<b>9, 11</b>	<b>7, 14</b>
Amelogenin	D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E
<b>9, 13</b>	<b>16, 18</b>	<b>22, 25</b>	<b>12</b>	<b>12, 13</b>		
D16S539	D18S51	D2S1338	CSF1PO	Penta D		
<b>6, 9, 3</b>	<b>16, 19</b>	<b>29, 31.2</b>	<b>8, 11</b>	<b>12</b>	<b>11</b>	
TH01	vWA	D21S11	D7S820	D5S818	TPOX	

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

10	20, 23	18	17
DYS391	FGA	DYS576	DYS570

## 2.8.21.4.5 Amplification Blank:

- 2.8.21.4.5.1 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.
- 2.8.21.4.5.2 An amplification blank with peaks below the Analytical Threshold shall not prevent associated samples from being interpreted.
- 2.8.21.4.5.3 An amplification blank with peaks above the Analytical Threshold shall be considered a failed negative control. All associated samples shall be inconclusive. All of the samples shall be repeated when appropriate.

## 2.8.21.5 Determination of the Assumed Number of Contributors

- 2.8.21.5.1** 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 may be used to aid in the determination of number of contributors for that sample.
- 2.8.21.5.1.1 The profile from the corresponding fraction of a differential extraction may also be used to aid in the determination of the number of contributors.
- 2.8.21.5.2** 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.
- 2.8.21.5.3** Depending on sample data, an analyst may assign the number of contributors to a sample as one, two, three, four, or at least five.
- 2.8.21.5.4** 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
- 2.8.21.5.5** The following steps should be followed in assigning the number of contributors to a profile:
- 2.8.21.5.5.1 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.

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- 2.8.21.5.5.2 If replicate amplifications have been undertaken, all qualifying replicate profiles should be reviewed when determining the number of contributors.
- 2.8.21.5.5.3 Identify possible stutter peaks (both forward and backward) by reference to stutter table (see [Appendix 6](#)).
- 2.8.21.5.5.4 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.
- 2.8.21.5.5.5 If one or more contributors at this locus is a clear major, check that this pattern is represented at other loci.
- 2.8.21.5.5.6 Apply the general pattern of contributors (e.g., number of alleles, peak height ratios) to other loci in the profile.
- 2.8.21.5.5.7 Discriminating loci such as FGA and SE33 are likely to be the most informative when determining the number of likely contributors.
- 2.8.21.5.5.8 The presence of a single minor peak or elevated stutter may indicate the presence of drop-in and not an additional contributor.
- 2.8.21.5.5.9 After initial assignment of the number of contributors and subsequent STRmix™ analysis, and prior to comparison with the person of interest reference profiles, analysis 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 on the STRmix™ output and the electronic file of the first deconvolution shall be maintained in the case record.
- 2.8.21.5.5.10 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.
  - 2.8.21.5.5.10.1 It is recommended that the analyst utilize other troubleshooting methods to resolve the number of contributors (such a reamplification) prior to utilization of varNOC.
  - 2.8.21.5.5.10.2 Technical Leader notification is required if varNOC is utilized and the notification shall be documented in the case record.
  - 2.8.21.5.5.10.3 The range of contributors evaluated in varNOC shall not exceed a difference of one contributor.

#### **2.8.21.6 General Interpretation Considerations for STRmix™**

- 2.8.21.6.1** STRmix™ should be used to deconvolute all qualifying samples, where a qualifying sample is defined as:
  - 2.8.21.6.1.1 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.

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- 2.8.21.6.1.1.1 A sample demonstrating a partial profile may be interpreted if the number of contributors can be reasonably assumed. STRmix™ requires a minimum of two autosomal loci for interpretation.
- 2.8.21.6.2** Evidence samples with identical profiles shall be analyzed independently with STRmix™.
- 2.8.21.6.3** 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.
- 2.8.21.6.4** 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:
- 2.8.21.6.4.1 Tri-allelic loci
  - 2.8.21.6.4.2 Somatic and primer binding site mutations
  - 2.8.21.6.4.3 Technical issues (saturated or poorly-resolved GeneMapper™ ID-X data)
- 2.8.21.6.5** Non numeric values such as OL, <, or > are not permitted within the STRmix™ input files.
- 2.8.21.6.6** 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.
- 2.8.21.6.6.1 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.
  - 2.8.21.6.6.2 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.
- 2.8.21.6.7** 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.
- 2.8.21.6.7.1 Replicates may only be analyzed together through STRmix™ with Technical Leader approval documented in the case record.
  - 2.8.21.6.7.2 Replicates shall be performed under identical conditions (e.g., template amount) for joint analysis in STRmix™.
- 2.8.21.6.8** 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:
- 2.8.21.6.8.1 No results: No peaks were detected in the electropherogram.

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2.8.21.6.8.2 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.

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

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

2.8.21.6.9.2 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.

2.8.21.6.9.3 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.

**2.8.21.6.10** 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.

#### **2.8.21.7 Setting Up Propositions for Calculating the Likelihood Ratio (LR)**

**2.8.21.7.1** 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.

**2.8.21.7.2** 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.

2.8.21.7.2.1 Examples:

2.8.21.7.2.1.1 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.

$H_1$ : DNA originates from victim and POI

$H_2$ : DNA originates from victim and unknown contributor

2.8.21.7.2.1.2 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

2.8.21.7.2.1.3 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-

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$H_{1-2}$ : 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

**2.8.21.7.3** 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.

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

2.8.21.7.3.2 The reference profiles shall have at least 20 autosomal complete loci (loci with no possible allelic drop-out).

2.8.21.7.3.3 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 ignored from the STRmix™ deconvolution.

2.8.21.7.3.4 Intimate samples should 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).

2.8.21.7.3.5 Non-intimate samples may 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).

## **2.8.21.8 Initial STRmix™ Analysis for Evidentiary Sample Data (Deconvolution)**

**2.8.21.8.1** To start a new STRmix™ deconvolution, from the STRmix™ main menu select **Interpretation**.

2.8.21.8.1.1 Alternately, a **Batch Mode** for deconvolutions may be utilized for multiple samples, which is accessed from the main menu and functions as below.

**2.8.21.8.2** 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.

**2.8.21.8.3** Enter the proposed number of contributors for the sample (1, 2, 3, or 4), and select **Confirm**.

**2.8.21.8.4** Ensure the **Contributor Range** box is not checked if varNOC is not being utilized.

2.8.21.8.4.1 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\_Caucasian** as the population for the range.

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- 2.8.21.8.5** 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**.
- 2.8.21.8.5.1 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.
- 2.8.21.8.5.2 Aside from number of accepts, no other changes should be made in **Run Settings** without Technical Leader approval.
- 2.8.21.8.6** Verify that **ISP\_Fusion6C\_v3** is the selected DNA kit used.
- 2.8.21.8.7** 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.
- 2.8.21.8.8** 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.
- 2.8.21.8.9** After the reference profile is added, check the **Contributor to: Hd** box to reflect that it is a conditioned sample.
- 2.8.21.8.10** Ensure that the **Perform Database Search** box is checked.
- 2.8.21.8.11** To ignore loci from deconvolution, select **Kit Settings**, then select **Loci**, then select **Ignore?** for appropriate loci, then select **Apply**.
- 2.8.21.8.12** Select **Start**. This will begin a deconvolution analysis of the chosen sample and search the results against the ISP QA DNA Database.
- 2.8.21.8.13** When the deconvolution is complete, STRmix™ will generate several data files and save them to the STRmix™ Results folder on the C: drive.
- 2.8.21.8.14** Save each deconvolution folder to the DNA server for the appropriate case.

#### **2.8.21.9 Review of Run Diagnostics**

- 2.8.21.9.1** 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.
- 2.8.21.9.1.1 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).
- 2.8.21.9.1.1.1 Rarely, due to limitations in stutter modeling for loci with complex repeat structures, STRmix™ may not model stutter peaks as

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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 ([http://strbase.nist.gov/str\\_fact.htm](http://strbase.nist.gov/str_fact.htm)) 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.

## 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

### 2.8.21.9.1.2 Template (RFU) and Mixture Proportions

2.8.21.9.1.2.1 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.

### 2.8.21.9.1.3 Degradation

2.8.21.9.1.3.1 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

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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.

## 2.8.21.9.1.4 Total Iterations and Acceptance Rate

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

2.8.21.9.1.4.2 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

2.8.21.9.1.5 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.

2.8.21.9.1.6 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.

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- 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™.

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

2.8.21.9.1.6.2 Good quality mixed DNA profiles are likely to give higher average log(likelihood) values than good quality single source profiles.

2.8.21.9.1.6.3 Low average log(likelihood) values alone are not necessarily an indicator of an issue, especially if the profile is single source.

2.8.21.9.1.7 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.

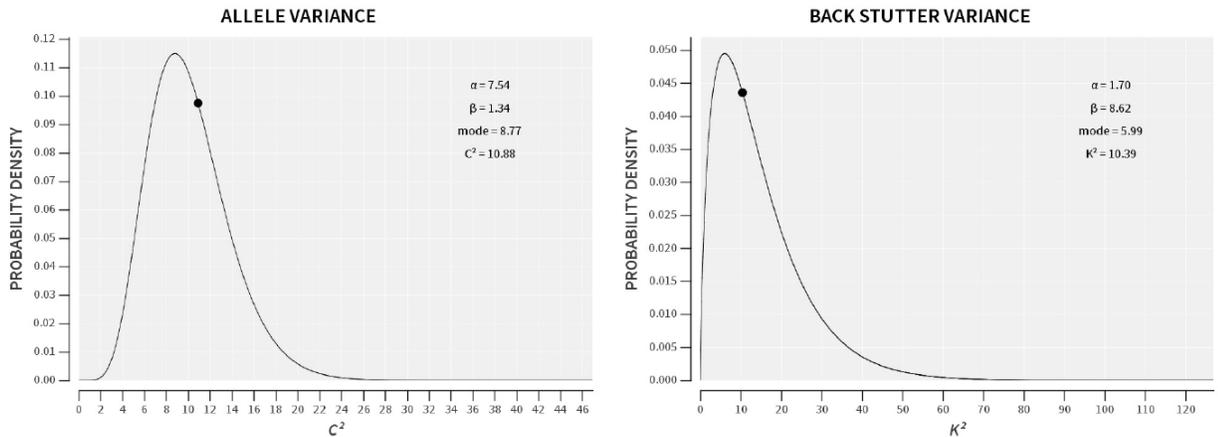
2.8.21.9.1.7.1 If the GR is above 1.2 then there exists, the possibility that the analysis hasn't converged. If the GR value is above 1.2, STRmix™ will automatically run an additional 50,000 post burn-in accepts. This may decrease the GR to below 1.2, yielding a clearer deconvolution.

2.8.21.9.1.7.2 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.

2.8.21.9.1.8 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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## VARIANCE CHARTS



2.8.21.9.1.8.1 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.

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

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

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

## 2.8.21.10 Indiana State Police Laboratory Division Quality Assurance DNA Database Search Review

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

2.8.21.10.2 Any database matches with a  $LR \geq 10,000$  should be evaluated for the likelihood of potential contamination. The Technical Leader shall be notified, with documentation in the case file. Appropriate report wording shall be utilized if contamination is suspected.

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

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

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

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

## 2.8.21.11 Comparing Sample Deconvolutions to Reference Standards (LR From Previous)

2.8.21.11.1 A reference standard may be visually excluded without STRmix™ analysis.

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- 2.8.21.11.2** To compare reference STR profiles to a previously-run STRmix™ deconvolution, from the STRmix™ main menu, select **Investigation**, then **LR from Previous**.
- 2.8.21.11.2.1 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.
- 2.8.21.11.3** 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.
- 2.8.21.11.4** 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” designation at this step, those characters shall be added after the specified naming convention. Select **Next**.
- 2.8.21.11.4.1 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**.
- 2.8.21.11.5** 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.
- 2.8.21.11.6** To ignore loci from deconvolution, select **Kit Settings**, then select **Loci**, then select **Ignore?** for appropriate loci, then select **Apply**.
- 2.8.21.11.6.1 If a locus in the reference profile has potential allelic drop-out (a single allele  $\leq 950$  RFU), the locus shall be omitted from the LR from Previous calculation.
- 2.8.21.11.7** If a varNOC deconvolution was performed, select **LR Settings**. Under **Contributor Range**, select **MLE** for the priors method, then select **Apply**.
- 2.8.21.11.7.1 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.
- 2.8.21.11.7.1.1 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**.
- 2.8.21.11.8** Select **Start**. This will begin the LR calculation of the chosen sample.
- 2.8.21.11.9** When the analysis is complete, STRmix™ will generate several data files and save them to the Results folder on the C: drive.
- 2.8.21.11.10** 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.

#### 2.8.21.12 LR Calculations

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- 2.8.21.12.1** 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, a 99% 1-Sided Lower HPD Interval, and a Stratified LR.
- 2.8.21.12.2** 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.
- 2.8.21.12.2.1 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.
- 2.8.21.12.2.1.1 Homozygous  $\frac{[2\theta + (1-\theta)p][3\theta + (1-\theta)p]}{[1+\theta][1+2\theta]}$
- 2.8.21.12.2.1.2 Heterozygous  $\frac{2[\theta + (1-\theta)p][\theta + (1-\theta)q]}{[1+\theta][1+2\theta]}$
- 2.8.21.12.2.1.3 Each locus LR shall be multiplied together to form the multi-locus LR for the profile.
- 2.8.21.12.3** 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.
- 2.8.21.12.4** 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.
- 2.8.21.12.5** The Stratified LR utilizes the HPD calculations and population settings to produce a single LR that samples across all the calculated populations.
- 2.8.21.12.6** 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.
- 2.8.21.12.7** The varNOC Maximum Likelihood Estimate LR (MLE) assigns an LR using the most probable number of contributors under H<sub>1</sub> and H<sub>2</sub>. 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.

## **2.8.21.13 Troubleshooting STRmix™ Results**

- 2.8.21.13.1** 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 LRs (greater than 1) are obtained for each locus, except one where the LR is 0.
  - The mixture proportions do not reflect what is observed.

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- The interpreted contributor genotypes are not intuitively correct.

**2.8.21.13.2** 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.

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

2.8.21.13.3.1 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.

**2.8.21.13.4** 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 his 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.

2.8.21.13.4.1 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. 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.

**2.8.21.13.5** 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.

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

## **2.8.21.14 Use of STRmix™ to aid in deconvolution of mixtures for CODIS entry**

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

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- 2.8.21.14.1.1 If a mixture includes support for inclusion of a suspect 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.
- 2.8.21.14.1.1.1 The case documentation of this deconvolution shall be clearly marked as for CODIS purposes only.
  - 2.8.21.14.1.1.2 The analyst shall ensure all alleles to be entered in CODIS are present in the original mixture.
  - 2.8.21.14.1.1.3 The original deconvolution should generally be utilized for comparison if an evidentiary reference standard is submitted at a later date.
- 2.8.21.14.1.2 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.
- 2.8.21.14.1.2.1 The single source unknown profile may be reanalyzed in GeneMapper™ ID-X utilizing reference profile settings.
  - 2.8.21.14.1.2.2 The mixture may be deconvoluted conditioning on the reanalyzed single source unknown profile.
  - 2.8.21.14.1.2.3 The analyst shall ensure all alleles to be entered in CODIS are present in the original mixture.
  - 2.8.21.14.1.2.4 The case documentation of the reanalysis and the deconvolution shall be clearly marked as for CODIS purposes only.
  - 2.8.21.14.1.2.5 The original deconvolution with no conditioning on the unknown profile shall be utilized for all LR calculations.

#### 2.8.21.15 Use of the STRlite Excel program

- 2.8.21.15.1 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.
- 2.8.21.15.1.1 STRlite v2.0 (or most current version as approved by the Technical Leader) shall be utilized.
  - 2.8.21.15.1.2 Printed 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.
    - 2.8.21.15.1.2.1 The STRlite Deconvolution page or a 1P/2P Combo worksheet shall be printed for all deconvolutions that represent a valid interpretation of the profile.

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2.8.21.15.1.2.1.1 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 printed for that run, but the STRmix™ deconvolution files shall be maintained electronically and the case record shall document why the run was not used.

2.8.21.15.1.2.2 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.

2.8.21.15.1.2.3 For single source profiles, the analyst shall manually add results for Amelogenin to the CODIS worksheet.

2.8.21.15.1.2.4 For mixed profiles, the analyst may manually add results for Amelogenin to the CODIS worksheet if they feel the data supports the determination.

2.8.21.15.1.3 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® Matrix Standards, 3100/3130 Technical Bulletin. Part No. TBD022. 1/06 or most current issue.

2.11.7 Promega Corporation. Maxwell® 16 Instrument Operation Manual. Part No. TM295. 2008 or most current issue

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- 2.11.8 Promega Corporation. DNA IQ™ Casework Sample Kit for Maxwell® 16. Part No. TB 354. 2009 or most current issue.
- 2.11.9 Promega Corporation. Tissue and Hair Extraction Kit (for use with the DNA IQ™). Part No. TB307. 2006 or most current issue.
- 2.11.10 Promega Corporation. DNA IQ™ Reference Sample Kit for Maxwell® 16. Part No. TB347. 2009 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
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- 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 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.
- 2.11.19 Gill, P. et al. Considerations from the European DNA profiling group (EDNAP) concerning STR nomenclature. *Forensic Science International* (1997) 87, 185-192.
- 2.11.20 SWGDAM (2017). SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories. Available at <https://www.swgdam.org/publications>.
- 2.11.21 Clayton, T.M., Whitaker, J.P., Sparkes, R., Gill, P., Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Science International* (1998) 91, 55-70.
- 2.11.22 Institute of Environmental Science and Research Limited. STRmix™ V2.7 Operation Manual. 11 October 2019 or most current version.
- 2.11.23 Institute of Environmental Science and Research Limited. STRmix™ V2.7 Installation Manual. 4 September 2018 or most current version.
- 2.11.24 Institute of Environmental Science and Research Limited. STRmix™ V2.7 User's Manual. 8 September 2019 or most current version.
- 2.11.25 Hill, C.R. et al. U.S. population data for 29 autosomal STR loci. *Forensic Sci. Int. Genet.* (2013) 7, e82-e83. Letter to the Editor regarding 2017 corrections pending publication. Corrected allele frequencies available at: <https://strbase.nist.gov/NISTpop.htm>.
- 2.11.26 Committee on DNA Technology in Forensic Science, National Research Council. *DNA Technology in Forensic Science*. Washington, D.C: National Academy, 1992.
- 2.11.27 Committee on DNA Forensic Science: An Update, National Research Council. *The Evaluation of Forensic DNA Evidence*. Washington, DC: National Academy, 1996.

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- 2.11.28 SWGDAM (2018). Recommendations of the SWGDAM Ad Hoc Working Group on Genotyping Results Reported as Likelihood Ratios. Available at <https://www.swgdam.org/publications>.

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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 to 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.

### 3.3. 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. Interpretations 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 parentage relationship cases:

3.9.1.2.1. The genetic results strongly support the hypothesis of the alleged relationship; therefore, the alleged individual cannot be excluded from the relationship. [Combined Parentage Index (CPI) >100]

3.9.1.2.2. The genetic results do not strongly support the hypothesis of the alleged relationship nor does it indicate no relationship; therefore, it is deemed inconclusive. An inconclusive result may also be derived if a genetic profile cannot be interpreted or is of poor quality. [100 > Combined Parentage Index (CPI) > 1]

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3.9.1.2.3. The genetic results support the hypothesis of no relationship; therefore, the alleged individual can be excluded from the relationship. [Combined Parentage Index (CPI) <1]

3.9.1.3. For non-parentage relationship cases:

3.9.1.3.1. The genetic results support the hypothesis of the alleged relationship. [Combined Relationship Index (CRI) >10]

3.9.1.3.2. The genetic results do not strongly support the hypothesis of the alleged relationship nor does it indicate no relationship; therefore, it is deemed inconclusive. [ $10 > \text{CRI} > 0.05$ ]

3.9.1.3.3. The genetic results support the hypothesis of no relationship. [CRI <0.05]

#### 3.9.2. Inconsistent Profiles In Parentage Relationship Cases

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 could account for the inconsistencies, then the mutation shall be included into the statistical calculations.

#### 3.9.3. Mixtures

3.9.3.1. With product of conception/fetal samples, it is necessary to have a standard from the mother. Only loci where the obligate paternal allele can be determined shall be used for statistical purposes.

3.9.3.1.1. The obligate paternal alleles may be deduced when appropriate.

3.9.3.2. With personal effects believed to belong to a missing person, the degree of the mixture shall be evaluated to determine whether conclusive results can be obtained. Additional standards may be requested to aid in interpretation.

#### 3.10. Rules for Relationship Statistical Calculations:

3.10.1. Calculations shall be performed using the most current available version of Popstats (the version shall be documented in the case record), a computer software program designed by the FBI for statistical calculations.

3.10.2. Statistical calculations shall be calculated and reported in forensic relationship comparison cases where Results/Opinions/Interpretations are being given in regards to relationship. However, statistical calculations shall not be performed in parentage cases with conclusions of no relationship/exclusion.

3.10.3. D12S391 and vWA may demonstrate linkage disequilibrium for some relationships (i.e. sibships and incestuous parentage) and may not be eligible for incorporation into the Combined Relationship Index using the product rule. When genetic information is available from both loci, only D12S391 shall be used for statistical evaluation; unless a possible mutation has occurred at vWA, in which case only the mutation calculation for vWA shall be used; or in the case of sibship where at least one allele is shared at D12S391 but no allele sharing occurs at vWA, then vWA shall be used.

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- 3.10.4. 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.
- 3.10.5. See Relationship Comparison Statistical Reference Sheet ([Appendix 10](#)) for a list of formulas for calculations.
- 3.10.6. 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.
- 3.10.7. Relationship statistical calculations utilize the race of the alleged individual. The calculations shall be performed with the appropriate population data using the racial information provided by the contributing agency. If racial information is unknown or unobtainable, calculations shall be performed using each population group.
- 3.10.8. Manual calculations performed by an analyst shall be verified by a qualified technical reviewer and/or a qualified unit supervisor.
- 3.10.9. A Prior Probability (Pr) of 0.5 shall be used for all relationship calculations.
- 3.10.10. The Paternity Index for a locus demonstrating a possible mutation shall be calculated using the average locus mutation rate ( $\mu$ ) and the Probability of Exclusion (PE) determined by the obligate paternal allele. See [Appendix 10](#) for a list of mutation rates and PE formulae.
  - 3.10.10.1. In the event that a mutation is suspected at one Y-STR locus, a mutation factor of 0.001 shall be used as a haplotype Relationship Index and incorporated into the Combined Relationship Index. If a mutation is suspected at more than one Y-STR locus, the analyst may recommend additional genetic testing with consultation from the Technical Leader.
- 3.10.11. Mutation rates for historical loci (PowerPlex® 16 HS and SE33) have been reported in the AABB (American Association of Blood Banks) Annual Summary. For any locus without a published mutation rate, the rate shall be calculated by averaging the available tetranucleotide STR mutation rates (excluding SE33).

### 3.11. Report Writing for Relationship Comparisons General Guidelines:

- 3.11.1. The DNA profiles shall be reported in table format in the Results/Opinions/Interpretations area of the Certificate of Analysis. The Paternity Index for each locus, the Combined Paternity Index, and the Probability of Paternity shall be reported in the table when applicable.
- 3.11.2. All reports shall include the required loci analyzed/kit utilized statement and retention statement listed in the report wording section ([Appendix 7](#)).
- 3.11.3. The items used for comparison will be reported in the format listed in the report wording section ([Appendix 7](#)).
- 3.11.4. Alternate report wording may be used depending on the type of relationship and calculation performed.
- 3.11.5. Terminology of Parentage, Paternity, Maternity, Relationship, Kinship, and Sibling Relationship (Sibship) may be used interchangeably where applicable.

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- 3.11.6. The Combined Paternity Index and the Paternity Index shall be reported as calculated by Popstats. However, any Popstats calculation of the Combined Paternity Index with a decimal value shall be rounded down to the nearest whole number.
- 3.11.7. The Probability of Paternity shall be reported to four decimal places. Paternity Index calculations shall be truncated to four significant figures for manual calculations.
- 3.11.8. Wording of Relationship Comparisons for Results/Opinions/Interpretations are located in [Appendix 7](#).

#### 3.12. Definitions:

- 3.12.1. **Random Man Not Excluded (RMNE)** – The frequency of selecting a random man from the population that could not be excluded as the biological father.
- 3.12.2. **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.
- 3.12.3. **Parentage Index (PI)/Relatedness Index (RI)** – A likelihood ratio based on two different conditional probabilities; PI/RI is calculated for each locus in a system.
- 3.12.4. **Combined Parentage Index (CPI)/Combined Relatedness 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.
- 3.12.5. **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.
- 3.12.6. **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 to 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:

4.2.1.1. Cases that have not provided meaningful autosomal results should be considered for analysis.

4.2.1.2. Cases from sexual assault/misconduct should be considered for analysis.

4.2.1.2.1. Due to the limitations of the technology, property crimes generally will not be considered for analysis.

## 4.3. Related Information:

4.3.1. Worksheet Manual

## 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

## 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 may be performed on extracts previously used for autosomal STR testing or 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 and four-color detection of twenty-three loci. The amplification occurs in a single reaction tube and detection occurs by a single capillary electrophoresis injection.

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

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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.

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## The PowerPlex® Y23 System Locus-Specific and Allelic Ladder Information:

STR Locus	Chromosomal Location <sup>1</sup>	Repeat Sequence <sup>2</sup> 5'→3'	Label	Size Range of Allelic Ladder Components <sup>3,4</sup> (bases)	Repeat Numbers of Allelic Ladder Components <sup>5</sup>
DYS576	Y	AAAG	Fluorescein	97-145	11-23
DYS389 I	Y	(TCTG) (TCTA)	Fluorescein	147-179	9-17
DYS448	Y	AGAGAT	Fluorescein	196-256	14-24
DYS389 II	Y	(TCTG) (TCTA)	Fluorescein	259-303	24-35
DYS19	Y	TAGA	Fluorescein	312-352	9-19
DYS391	Y	TCTA	JOE	86-130	5-16
DYS481	Y	CTT	JOE	139-184	17-32
DYS549	Y	GATA	JOE	198-238	7-17
DYS533	Y	ATCT	JOE	245-285	7-17
DYS438	Y	TTTTTC	JOE	293-343	6-16
DYS437	Y	TCTA	JOE	344-380	11-18
DYS570	Y	TTTTC	TMR-ET	90-150	10-25
DYS635	Y	TSTA compound	TMR-ET	150-202	15-28
DYS390	Y	(TCTA) (TCTG)	TMR-ET	207-255	17-29
DYS439	Y	AGAT	TMR-ET	263-307	6-17
DYS392	Y	TAT	TMR-ET	314-362	4-20
DYS643	Y	CTTTT	TMR-ET	368-423	6-17
DYS393	Y	AGAT	CXR-ET	101-145	7-18
DYS485	Y	GAAA	CXR-ET	159-215	10-24
DYS385 a/b	Y	GAAA	CXR-ET	223-307	7-28
DYS456	Y	AGAT	CXR-ET	316-364	11-23
Y-GATA-H4	Y	TAGA	CXR-ET	374-414	8-18

<sup>1</sup>Information about most of these loci can be found at: [www.cstl.nist.gov/biotech/strbase/chrom.htm](http://www.cstl.nist.gov/biotech/strbase/chrom.htm)

<sup>2</sup>The August 1997 report (30,31) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, "1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5' nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used".

<sup>3</sup>The length of each allele in the allelic ladder has been confirmed by sequence analysis.

<sup>4</sup>When using an internal lane standard, such as the CC5 Internal Lane Standard 500 Y23, the calculated sizes of allelic ladder components may differ from those listed. This occurs because different sequences in allelic ladder and ILS components may cause differences in migration. The dye label also affects migration of alleles.

<sup>5</sup>For a current list of microvariants, see the Variant Allele Report published at the U.S. National Institute of Standards and Technology (NIST) web site at: [www.cstl.nist.gov/div831/strbase/](http://www.cstl.nist.gov/div831/strbase/) and the Y Chromosome Haplotype Reference Database at: [www.yhrd.org](http://www.yhrd.org)

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- 4.8.2.2. **PowerPlex® Y23 Amplification Set-Up** (Note: The following steps shall be performed in the PCR amplification set-up area.)
- 4.8.2.2.1. Thaw the Amplification Grade Water, PowerPlex® Y23 5X Master Mix, and PowerPlex® Y23 10X Primer Pair Mix. When thawed, it is important to vortex the PowerPlex® Y23 5X Master Mix and PowerPlex® Y23 10X Primer Pair Mix tubes for 5 to 10 seconds. (Do not centrifuge after vortexing as this may cause the primers/dNTPs to be concentrated at the bottom of the tube.) The Amplification Grade Water may be stored at 2-10° C or at room temperature for extended periods.
  - 4.8.2.2.2. Determine the number of samples to be amplified, including controls (reagent blank, a positive control [PC], and an amplification blank [AB]). 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.
  - 4.8.2.2.5. Using the Master Mix worksheet, calculate the required amount of each component of the PCR master mix. Multiply the volume (µl) per sample by the total number of reactions to obtain the final volume (µl).

**Components of Master Mix/sample:**  
12.5 µl Nuclease Free or Amplification Grade Water  
5.0 µl PowerPlex® Y23 5X Master Mix  
2.5 µl PowerPlex® Y23 10X Primer Pair Mix  
20 µl Total Volume (w/o sample)
  - 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.
  - 4.8.2.2.8. Pipette 5 µl of sample, reagent blank, positive amplification control, or negative amplification control into the respective tube containing Master Mix.
    - 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. Pipette 5.0 µl (0.1 ng to 0.5 ng) of diluted male DNA into a 0.2 µl reaction tube containing 20 µl of PCR master mix. A positive control shall be included in each thermal cycler. (NOTE: The diluted positive control should be stored at 2-10°C.)

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4.8.2.2.8.2. Amplification grade H<sub>2</sub>O or NF H<sub>2</sub>O 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 variable PPY23 Amplification Worksheet to calculate the required amount for each component of the PCR master mix. Sample volumes above 5 µl shall be subtracted from the volume of water in the master mix.

**Components of the Master Mix/sample:**

- Up to 12.5 µl Nuclease Free or Amplification Grade Water
- 5.0 µl PowerPlex® Y23 5X Master Mix
- 2.5 µl PowerPlex® Y23 10X Primer Pair Mix
- Between 7.5 and 20 µl Total Volume (without sample)

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 up to 20 µl of PCR master mix to each sample tube or well.

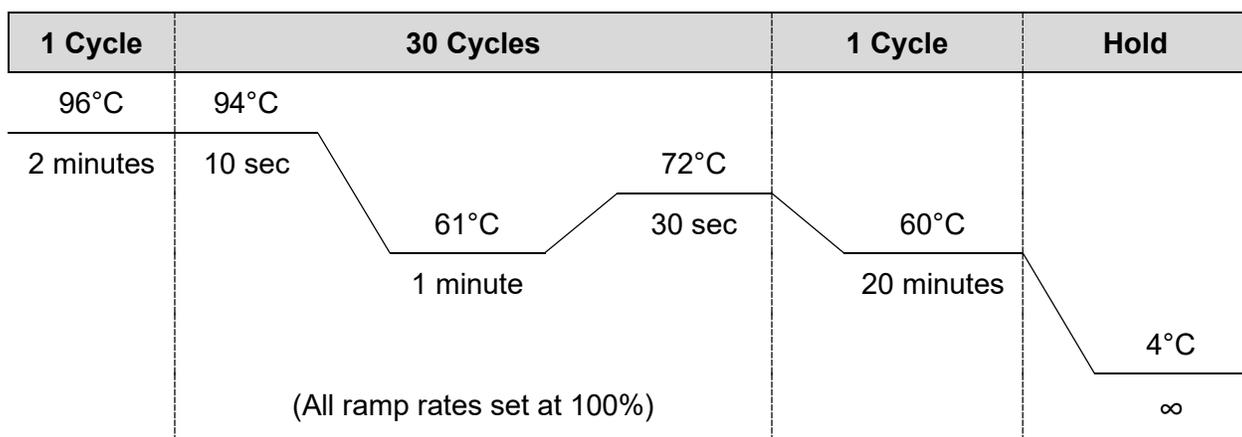
4.8.2.2.12. Pipette the appropriate amount of each sample (up to 17.5 µl) into the respective tube containing master mix. For organic extractions if the template DNA is stored in TE<sup>-4</sup> buffer, the volume of the DNA sample added should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl) or available magnesium concentration (due to chelation by EDTA). DNA samples stored (or diluted) in NFH<sub>2</sub>O are not subject to this caution, but may contain other PCR inhibitors at low concentrations depending on the source of the template DNA and the extraction procedure employed.

**4.8.2.3. PowerPlex® Y23 Amplification**

4.8.2.3.1. Assemble the tubes in a thermal cycler.

4.8.2.3.2. Select and run the thermal cycling protocol below:

4.8.2.3.3. **Veriti™ Thermal Cycler:**



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

4.8.2.3.3.2. Remove samples after the amplification process is completed and proceed with capillary electrophoresis or store the amplified samples at -10°C to -30°C. Alternatively, amplified samples may be stored at 2-10°C if they are to be used within 2 days.

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

#### 4.8.2.4. **PowerPlex® Y23 Direct Amplification Sampling for Standards**

- 4.8.2.4.1. (Oral swabs only) Place the head of one swab in a deep well plate or a 1.5ml microcentrifuge tube.
- 4.8.2.4.2. Using a fresh pipette tip every time, place 1ml SwabSolution™ into each well/tube containing a swab head.
- 4.8.2.4.3. For the reagent blank, place solution only into an empty well/tube.
- 4.8.2.4.4. Incubate at 70° C for approximately 1 hour.
- 4.8.2.4.5. Store the samples in the refrigerator at 2-10°C. Samples may be stored for up to three weeks prior to direct amplification.

#### 4.8.2.5. **PowerPlex® Y23 Direct Amplification Set-Up for Standards** (Note: The following steps shall be performed in the PCR amplification set-up area.)

- 4.8.2.5.1. Thaw the Amplification Grade Water, PowerPlex® Y23 5X Master Mix, and PowerPlex® Y23 10X Primer Pair Mix. When thawed, it is important to vortex the PowerPlex® Y23 5X Master Mix and PowerPlex® Y23 10X Primer Pair Mix tubes for 5 to 10 seconds. (Do not centrifuge after vortexing as this may cause the primers/dNTPs to be concentrated at the bottom of the tube.) The Amplification Grade Water may be stored at 2-10° C or at room temperature for extended periods.
- 4.8.2.5.2. Determine the number of samples to be amplified, including controls (reagent blank, a positive control [PC], and an amplification blank [AB]). Add 2 to 4 reactions to this number to compensate for the loss that occurs during reagent transfers.
- 4.8.2.5.3. Place one 0.2 ml PCR reaction tube for each sample into a rack and label appropriately. A 96-well plate may be used.
  - 4.8.2.5.3.1. 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.
- 4.8.2.5.4. For samples and controls, calculate the required amount of each component of the PCR master mix. Multiply the volume (µl) per sample by the total number of reactions (from 4.8.2.5.2) to obtain the final volume (µl).

#### **Components of Master Mix/sample:**

15.5 µl Nuclease Free or Amplification Grade Water  
5.0 µl PowerPlex® Y23 5X Master Mix  
2.5 µl PowerPlex® Y23 10X Primer Pair Mix  
23 µl Total Volume (w/o sample)

- 4.8.2.5.5. Add the calculated volume of each component to a 1.5 ml tube. Mix gently.
- 4.8.2.5.6. Add 23 µl of PCR master mix to each sample tube/well.
- 4.8.2.5.7. Pipette 2 µl of sample, reagent blank, positive amplification control, or negative amplification control into the respective tube/well containing Master Mix.

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4.8.2.5.7.1. 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. Pipette 2 μl (2 ng to 3 ng) of diluted male DNA into a 0.2 μl reaction tube containing 23 μl of PCR master mix. A positive control shall be included in each thermal cycler. (NOTE: The diluted positive control should be stored at 2-10°C.)

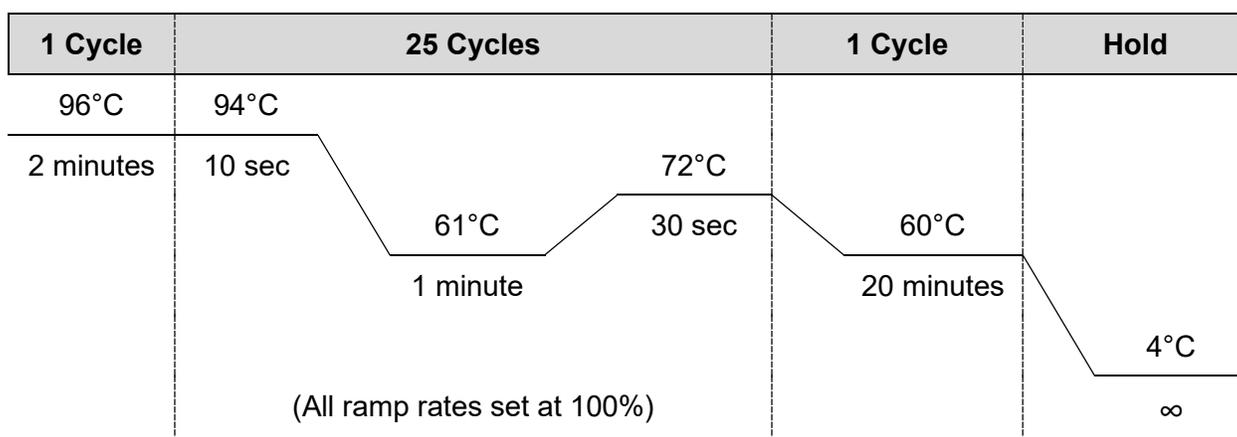
4.8.2.5.7.2. Amplification grade H<sub>2</sub>O or NF H<sub>2</sub>O shall be used as a negative amplification control included with each thermal cycler as the last sample in the batch.

## 4.8.2.6. PowerPlex® Y23 Direct Amplification for Standards

4.8.2.6.1. Assemble the tubes in a thermal cycler.

4.8.2.6.2. Select and run the thermal cycling protocol below:

4.8.2.6.3. Veriti™ Thermal Cycler:



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

4.8.2.6.3.2. Remove samples after the amplification process is completed and proceed with capillary electrophoresis or store the amplified samples at -10°C to -30°C. Alternatively, amplified samples may be stored at 2-10°C if they are to be used within 2 days.

4.8.2.6.3.3. 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. The formamide shall be frozen in aliquots at -20°C and the remainder of each aliquot shall be discarded after it is thawed. Multiple freeze-thaw cycles or long-term storage at 4°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.

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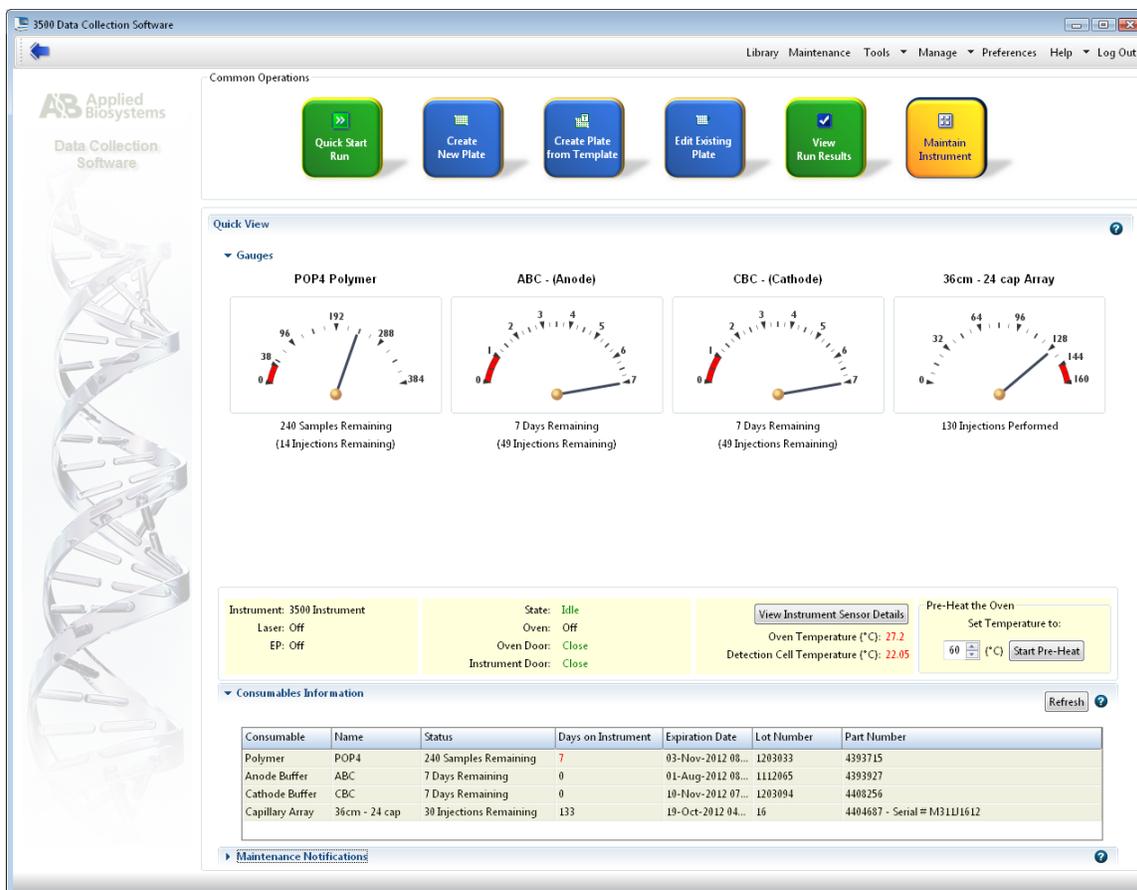
- 4.8.4.1.2. **Caution:** Formamide is an irritant and teratogen; therefore, universal precautions and a fume hood shall be utilized when manually working with formamide to avoid inhalation and contact with the skin.
- 4.8.4.1.3. Thaw the ILS (WEN ILS 500), the allelic ladder, and an aliquot of Hi-Di™ Formamide. When thawed, centrifuge briefly then vortex to mix (do not centrifuge the ILS after vortexing).
- 4.8.4.1.4. Determine the number of samples to be injected, including controls (reagent blanks, positive control and amplification blank) 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 1 µl of the allelic ladder to each well. It is recommended that one allelic ladder is injected within each set of 3 injections on the 8 capillary Applied Biosystems® 3500 instruments to ensure that a usable ladder injection occurs. At least one allelic ladder is required within each run folder.
- 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).

4.8.4.2.4. Select “96” for Number of Wells.

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- 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 shows the '3500 Data Collection Software' interface. The main window is titled 'Plate Details' and contains the following fields and options:

- Name:** A text input field with a yellow highlight and a message: "Plate Name is a required field. Provide a unique value."
- Number of Wells:** A dropdown menu.
- Plate Type:** A dropdown menu set to "HID".
- Capillary Length:** A dropdown menu set to "36" with "cm" next to it.
- Polymer:** A dropdown menu set to "POP4".
- Owner:** A text input field.
- Barcode:** A text input field.
- Description:** A large text area.
- Secondary Analysis:** A section with a checkbox for "Perform Auto-Analysis".

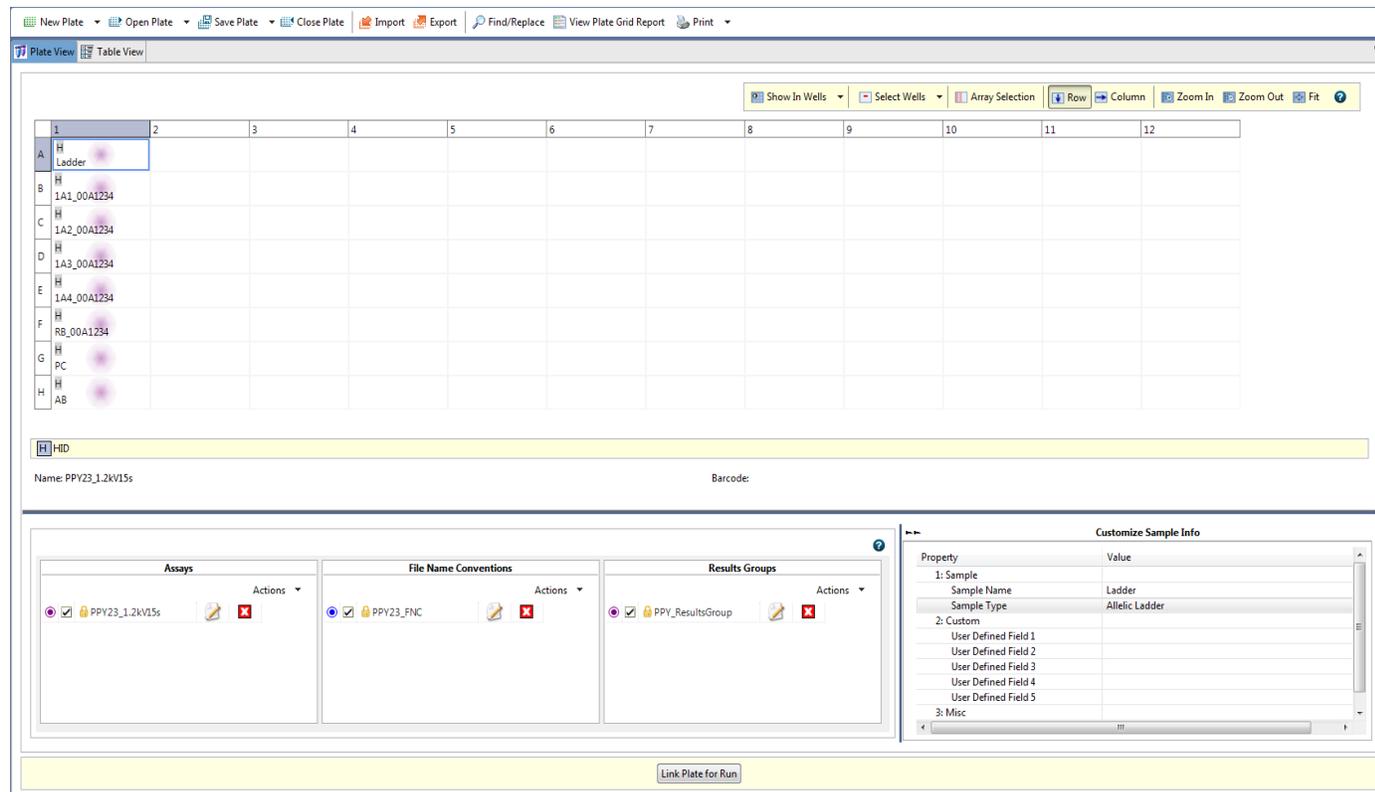
At the bottom of the form, there is a yellow bar containing the button "Assign Plate Contents".

- 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”.
- 4.8.4.2.11. Under **File Name Convention**, click “Add from Library” and select “PPY23\_FNC”. Click “Add to Plate” then “Close”.

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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 (ex. 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

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

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- 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 at -10°C to -30°C. 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. A copy of 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.**
- 4.8.6. 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.
- 4.8.7.1.3. 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 (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.
- 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 run was started on the instrument at a minimum. 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. Highlight all sample rows containing **Allelic Ladders**. Then 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 and verify the correct allele calls are made for each peak. Print the electropherograms.
- 4.8.7.2.3. Highlight all sample rows containing **Negative Controls** (ex. amplification blanks and reagent blanks). Then click "View", then "Display Plots". In the Samples Plot screen, change the "Plot Setting" drop-down box to "PPY23". Check the negative controls to ensure that no peaks above analytical threshold are present. Print the electropherograms ensuring that the primer peak is visible.
- 4.8.7.2.4. Highlight all remaining sample rows. Then 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. After evaluating all allele calls, click "File">"Print">"Print" to print off all sample electropherograms. Optionally, the remaining sample rows may be viewed, evaluated, and printed with the Allelic Ladders.
- 4.8.7.2.5. After all analysis is complete, save the 3500 Data Collection Run Folder and associated GeneMapper™ ID-X projects under each associated laboratory case number and request folder located in the analysts' folders on the server. Projects

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should be deleted monthly from the “GeneMapper Manager” to maintain database space.

- 4.8.7.2.5.1. When exporting the GeneMapper™ ID-X project, ensure that the “Export with analysis settings” box is checked.
- 4.8.7.2.6. 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.
- 4.8.7.2.7. 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.

## 4.8.8. Interpretation Guidelines For PowerPlex® Y23

- 4.8.8.1. Once a determination has been made whether a peak is to be considered a true allele, the following interpretation guidelines shall be used. The minimum peak height threshold is established at 100 relative fluorescent units (RFU) for GeneMapper™ ID-X software. The analytical threshold for data interpretation is 100 RFU. The stochastic threshold for data interpretation of the DYS385 a/b locus is 225 RFU.
- 4.8.8.2. Peaks below 100 RFU shall not be interpreted or marked on the STR summary sheet.
- 4.8.8.3. Interpretation of peaks with RFU from 100 to 225 RFU at the DYS385 a/b locus is a qualitative assessment. It shall be interpreted with care. 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 and it will be reported with a bracket (ex. [13]) on the Y-STR summary sheet. If the DYS385 a/b locus demonstrates two alleles in which one or both alleles are above 100 RFU and below 225 RFU, and the sample appears to be single source, the allele(s) below 225 RFU will be reported with a bracket. Both alleles are interpretable and may be used in a Y-STR database search.
- 4.8.8.4. Peaks at the remaining loci with RFU of 100 and above are reportable based on both qualitative and quantitative assessment of data.
- 4.8.8.5. **Controls:**
  - 4.8.8.5.1. The appearance of pull-up or known artifact peaks does not render the following controls inconclusive.
  - 4.8.8.5.2. **Reagent Blank:** 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.
    - 4.8.8.5.2.1. A reagent blank with peaks below 100 RFU shall not prevent associated samples from being interpreted.
    - 4.8.8.5.2.2. A reagent blank with peaks of 100 RFU and above shall be considered a failed negative control. All associated samples shall be inconclusive. All the samples shall be repeated when reasonable and appropriate.
  - 4.8.8.5.3. **Positive Control:**

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4.8.8.5.3.1. The 2800M or other appropriate male DNA standard supplied with the PowerPlex® Y23 kit is used as a positive control to demonstrate that the kit is performing properly. If the expected alleles are not detected in the positive control well, then the test is considered inconclusive.

Y-STR Locus	2800M
DYS576	18
DYS389 I	14
DYS448	19
DYS389 II	31
DYS19	14
DYS391	10
DYS481	22
DYS549	13
DYS533	12
DYS438	9
DYS437	14
DYS570	17
DYS635	21
DYS390	24
DYS439	12
DYS392	13
DYS643	10
DYS393	13
DYS485	17
DYS385 a/b	13,16
DYS456	17
Y-GATA-H4	11

#### 4.8.8.5.4. Amplification blank:

4.8.8.5.4.1. 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 tube, the sample well should be blank except for the internal lane standard peaks. If amplified product is detected in the amplification blank well, the test is considered inconclusive.

4.8.8.5.4.2. An amplification blank with peaks below 100 RFU shall not prevent associated samples from being interpreted.

4.8.8.5.4.3. An amplification blank with peaks of 100 RFU and above shall be considered failed negative controls. All associated samples shall be inconclusive. All the samples shall be repeated when reasonable and appropriate.

4.8.8.6. The analytical threshold shall be determined during validation. 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 can delete the allele call label in either the software file or manually on the printed electropherograms. The GeneMapper™ ID-X software is set to

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display all allele changes. Therefore, any change or deletion in an allele call shall be visible on the electropherogram print-out.

- 4.8.8.7. An analyst is required to visually confirm all allelic ladders used for allele designation and the allele calls for all positive controls.
- 4.8.8.8. **Stutter peaks** are artifacts of the amplification process. These peaks will typically be observed in the n-4 position of major peaks for tetranucleotide repeat loci, in the n-5 position of major peaks for the pentanucleotide repeat locus, in the n-6 position of major peaks for the hexanucleotide repeat locus, or in the n-3 position of the major peaks for the trinucleotide repeat loci. The peak heights of stutter peaks will be less intense than that of the major peak. The average observed percent stutter for each locus and the mean + 3 SD values are listed in the table in [Appendix 13](#). The mean stutter value for each locus is used as the stutter cut-off value in the marker stutter file "PowerPlexY23\_IDX\_v1.2" for GeneMapper™ ID-X analysis. Therefore, any peaks in the n-4 (for tetranucleotide repeats), the n-3 (for trinucleotide repeats), the n-5 (for pentanucleotide repeats), or the n-6 (for hexanucleotide repeats); and n-2/n+2 associated with the DYS19 locus stutter positions that are below these values when compared to the major peak will be automatically filtered out by the software and will not be labeled. However, these values may vary slightly. There are also stutter-like peaks at the n-9 and n-2 positions and are also listed on the table below. This column may be used as a guideline to the analyst for determining stutter peaks that were not filtered out by the software. It is the analyst's discretion to determine which allele calls may be renamed as stutter in GeneMapper™ ID-X analysis. For samples which have been over-loaded, the percent stutter calculation will not be accurate due to the saturation effect of the major peak. In addition to stutter peaks, several other stutter-like peaks can be observed at some PowerPlex® Y23 loci, especially when samples are amplified at high concentrations.
- 4.8.8.8.1. **Note:** Stutter peaks have also been observed during the validation process at the n+4 (tetranucleotide) and n+3 (trinucleotide) positions as well as the n-8 (tetranucleotide) and n-6 (trinucleotide) positions. These values are listed in Appendix 13. Other artifacts of less intensity have been reported which may not line up with the ladder. The interpretation of these peaks, similar to the other artifact peaks, shall be at the discretion of the analyst based on their training and experience.
- Stutter Table: See [Appendix 13](#)**
- 4.8.8.9. **Artifacts** have been observed utilizing the PowerPlex® Y23 amplification kit. The intensity of these peaks is directly related to signal intensity; therefore, reducing the signal intensity to less than 15,000 RFU should minimize the appearance of these types of artifacts. If an analyst renames the allele call of any artifact in GeneMapper™ ID-X, it shall be labeled appropriately. Artifact peaks have been documented at 66-69 base pairs in the Fluorescein dye channel and at 58-60 base pairs in the JOE dye channel. The following artifacts have been observed: DYS448 in the n-9 to n-15 position; DYS635 at 160 bases; DYS481 at 164 bases; DYS549 at 187 bases; DYS458 at 201 bases; DYS533 at 253 bases and at 272 bases; and DYS643 at 427 bases and 440 bases.
- 4.8.8.10. **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 should be labeled on the electropherogram as pull-up. 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.

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- 4.8.8.11. **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 should be clearly labeled as spikes on the electropherogram printout. 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.
- 4.8.8.12. **Rare variants** have been described in the literature. The causes of these rare variants are microvariants or chromosomal mutations (duplication or deletion).
- 4.8.8.12.1. Microvariants: alleles one, two or three nucleotides shorter than the common four base repeat alleles (one or two nucleotides shorter in the case of three base repeat alleles, up to four nucleotides shorter in the case of five base repeat alleles, and up to five nucleotides shorter in the case of six base repeat 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., a 0.1, 0.2, 0.3, or 0.4 in the case of a pentanucleotide repeat). Therefore, if a peak is 1 base pair larger than the 5 allele it shall be designated as 5.1. The precision of sizing at a 99.7% confidence level is less than 0.25 bp which is precise enough to be confident in the sizing of microvariants. A microvariant 4 base pairs larger than an allele (or 5 base pairs for a pentanucleotide) on the ladder 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). A current list of microvariants is available on the "Variant Allele Report" published at the U.S. National Institute of Standards and Technology (NIST) website: <http://www.cstl.nist.gov/div831/strbase> and on the Y Chromosome Haplotype Reference Database at <http://www.yhrd.org>
- 4.8.8.12.2. Alleles which are located outside the range of the ladder or bin set (above or below) shall 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)". This should be clear when used and can be verified with a locus review of the electropherogram.
- 4.8.8.12.3. A duplication occurs when multiple alleles are present at a locus/loci. 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 has been observed.
- 4.8.8.12.3.1. Steps to distinguish between a mixture and a duplication: determine the number of loci containing more than one peak; consider the position on the Y-chromosome if multiple loci have two peaks; determine if the repeat spread is >1 repeat unit; examine DYS385 a/b for the presence of >2 peaks; and consider autosomal testing to confirm single-source sample.
- 4.8.8.12.4. A deletion occurs when there is a lack of a peak at a single locus/loci. 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.
- 4.8.8.13. **Mixed DNA Samples.** All loci shall be taken into consideration when interpreting a mixture. A two-peak pattern at two or more loci (except for DYS385 a/b; this locus may

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exhibit three or more peaks) may be an indication of a mixture. However, two-peak patterns have been reported for single-source samples, but these instances are extremely rare. If a two-peak pattern is observed for a single-source unknown sample and is believed to be a rare variant or duplication, the profile should be re-amplified and re-analyzed to confirm the profile.

- 4.8.8.14. The “phenotype” of each profile shall be recorded.
- 4.8.8.15. 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.
- 4.8.8.16. During interpretation, the analyst and technical reviewer shall each compare all single source and major Y-STR profiles to available staff profiles to ensure that samples have not been contaminated. All instances of profiles consistent with a staff member shall be reported to the Technical Leader.
- 4.8.8.17. There are four possible outcomes of Y-STR analysis:
  - 4.8.8.17.1. No results: No peaks were detected on the electropherogram.
  - 4.8.8.17.2. Inconclusive: Peaks were observed at one or more loci; however no conclusive results can be drawn from them.
  - 4.8.8.17.3. 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. At least twelve loci eligible for statistical calculations shall be needed to draw this conclusion.
  - 4.8.8.17.4. Exclusion: The profile obtained from the unknown sample was not the same as the reference sample.

#### **4.8.9. General Rules For PowerPlex® Y23 Analysis On The Applied Biosystems® 3500 Genetic Analyzer**

- 4.8.9.1. 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.
- 4.8.9.2. Only the data used for interpretation need to be printed for 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.
- 4.8.9.3. 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.
- 4.8.9.4. 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.

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#### 4.8.10. Archiving Applied Biosystems® 3500 and GeneMapper™ ID-X Projects

- 4.8.10.1. 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.
- 4.8.10.2. A hard copy of all electropherograms used in interpretation as well as a print-out of the plate record shall be placed in the case record.
- 4.8.10.3. All processed plate records shall be deleted from the Data Collection Software database weekly. Run folders containing the sample files and the GeneMapper™ ID-X projects shall be deleted on or after the 15<sup>th</sup> of each month on the instrument computers. It shall be each analyst's responsibility to ensure that all data is backed up prior to the 15<sup>th</sup> of the month.

#### 4.8.11. Mixture Interpretation

- 4.8.11.1. A profile is defined as a mixture between two or more males when two or more loci demonstrate two or more alleles, except DYS385 a/b. A profile where only one locus demonstrates two alleles could be an indication of:
  - 4.8.11.1.1. A mixture where only one allele from a minor contributor is detected above analytical threshold;
  - 4.8.11.1.2. Extraneous DNA;
  - 4.8.11.1.3. A duplication.
- 4.8.11.2. In situations 1 and 2 above, report wording to the effect that one lone allele was detected and no conclusion can be reached is appropriate. In situation 3, no mention is required in the Certificate of Analysis.
- 4.8.11.3. If the profile is determined to be a mixture, each locus should be evaluated and classified as below:
  - 4.8.11.3.1. Indistinguishable mixture between two or more males; in this case, since no statistical interpretations can be made, no conclusions will be made for these samples.
  - 4.8.11.3.2. Mixture demonstrating a major contributor.
  - 4.8.11.3.3. Demonstrates the potential for allelic drop-out.
- 4.8.11.4. For mixtures of two male individuals, a profile demonstrating twelve or more loci classified as a mixture demonstrating major and minor components shall have a major single source profile calculated based on the following criteria:
  - 4.8.11.4.1. If the peak height ratio between the peaks is less than 20%, then the highest peak can be called a major at that locus.
  - 4.8.11.4.2. 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.
  - 4.8.11.4.3. 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.

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- 4.8.11.4.4. 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.
- 4.8.11.4.5. If the sample does not meet these criteria, do not call a major at this locus.
- 4.8.11.4.6. Mixtures of three males which meet the above criteria should be interpreted with caution.
- 4.8.11.4.7. 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.
  - 4.8.11.4.7.1. 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.
  - 4.8.11.4.7.2. A mixture threshold of 1000 RFU shall be used to interpret single peaks and major peaks at a locus.
  - 4.8.11.4.7.3. 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.

#### 4.8.12. Y-STR Database Searching

- 4.8.12.1. A Y-STR profile is treated as a single locus because it is a haplotype.
- 4.8.12.2. 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.
- 4.8.12.3. 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>.
- 4.8.12.4. The unknown haplotype shall be searched using the appropriate loci from the PowerPlex® Y23 kit (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 Y-GATA-H4).
- 4.8.12.5. The feature National Database (with Subpopulations, 2014 SWGDAM- compliant) shall be added to the statistical report after the Y-STR profile has been searched.
- 4.8.12.6. The statistic result sheet shall be printed out for every sample.
- 4.8.12.7. The Theta-corrected Likelihood Ratio (without Native American) shall be included in the Certificate of Analysis as a frequency estimate. A verbal scale shall accompany the likelihood ratio result in an effort to provide additional explanation of the weight of the evidence.

Likelihood Ratio	Support for Inclusion
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

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$1,000,000 \leq LR$
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Provides very strong support
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4.8.12.8. The Certificate of Analysis shall acknowledge the date of the search and the release number of YHRD at the time of the search.

## 4.9. Records:

- 4.9.1. The appropriate worksheets or the equivalent workbooks shall be used to record all procedures. These will be found in the Worksheet Manual.
- 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. When test results from an unknown sample are compared with a reference standard the following conclusions may be reported:
  - 4.10.1.1. The unknown sample and the reference sample may have common origin. If this is indicated, it shall be reported that all paternally related males will share the same profile and a frequency of occurrence in the Y-Chromosome Haplotype Reference Database shall be calculated and the match probability shall be reported out.
  - 4.10.1.2. The reference sample may be excluded as a possible source of the unknown sample.
  - 4.10.1.3. No conclusion may be reached with regard to the unknown sample and the reference sample.
- 4.10.2. If test results from a sample cannot be clearly interpreted, the results shall be reported as inconclusive.
- 4.10.3. See Y-STR Interpretation guidelines.

## 4.11. Reporting of Y-STR Analysis (See [1.11](#) for formatting):

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

## 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. Butler, J.M. 2011. Y Chromosome DNA Testing. Advanced Topics in Forensic DNA Typing: Methodology. pp 371-403. Elsevier Academic Press. Burlington, MA.
- 4.12.3. Butler, J.M. 2010. Lineage Markers: Y Chromosome and mtDNA testing. Fundamentals of Forensic DNA Typing. pp 363-374. Academic Press. Burlington, MA.
- 4.12.4. 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.

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- 4.12.5.** SWGDAM (2014). SWGDAM Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Testing Laboratories. Available at [http://swgdam.org/SWGDAM\\_YSTR\\_Guidelines\\_APPROVED\\_01092014\\_v\\_02112014\\_FINAL.pdf](http://swgdam.org/SWGDAM_YSTR_Guidelines_APPROVED_01092014_v_02112014_FINAL.pdf)
- 4.12.6.** Ballantyne, J. and Fatolitis, L. The U.S. Y-STR database. Profiles in DNA. 2008. 11(1): 13 – 14.
- 4.12.7.** Y-Chromosome Haplotype Reference Database (YHRD). <http://yhrd.org>

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#### 5. DNA Sample Processing using the Biomek NX<sup>P</sup> and Biomek 3000 Automated Workstations:

**5.1. Scope:** This test method is designed for the direction of laboratory personnel who will operate the Biomek NX<sup>P</sup> and Biomek 3000 automation workstations to process DNA samples from extraction/purification through CE (capillary electrophoresis) setup. The Biomek NX<sup>P</sup> workstation is capable of extracting up to 87 samples and blanks in two hours, setting up quantification plates, normalizing samples and setting up amplification. The Biomek 3000 workstation then transfers amplified product to a CE plate to be run on a genetic analyzer. 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 NX<sup>P</sup> Automated Laboratory Workstation— robotic DNA processing system.

**5.4.2.** Biomek 3000— 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 (NFH<sub>2</sub>O)

**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<sup>TM</sup> 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:

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- 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.
- 5.6.3. Biological waste shall be disposed of in the appropriate waste receptacle.
- 5.6.4. On the Biomek NX<sup>P</sup>, a light curtain prevents interaction with the deck or the robotic arm while a procedure is running.
- 5.6.5. There is an emergency stop button on the front of the Biomek 3000.

## 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. Reagent blanks shall contain “RB” in the name on the pre-extraction worksheet.
- 5.8.1.5. 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](mailto: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. To maximize efficiency of each run, the automation team may process samples up to 14 days from their submission date.
- 5.8.1.7. 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 printed and retained in the case record are: emails documenting submission of samples and return of samples to the analyst, the pre-extraction worksheet, the

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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.
- 5.8.2.1.2. 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.*
- 5.8.2.1.4. 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;

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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 no 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 three 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.
  - 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.*

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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 three 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 five samples marked code M. Of these, one is P1, three are P2, and one is P3. The P1 sample is amplified, and the P2 samples are compared to find the best two. The remaining P2 sample and the P3 sample are not amplified.*

5.8.2.4.4. After the best three 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.*

5.8.3.1.2. Place sample at the bottom in a microcentrifuge tube (see sampling protocols). Each group of samples being extracted shall include a reagent blank as the last sample in the batch.

5.8.3.1.2.1. For extraction on the Biomek NX<sup>P</sup> workstation, samples shall be loaded specifically into Qiagen® Investigator® Lyse&Spin 2 ml collection tubes. Do not use “dolphin-style” or other microcentrifuge tubes.

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.

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- 5.8.3.1.5. 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).
- 5.8.3.1.6. Using a wooden applicator stick, remove the cutting and proceed to 5.8.2.1.8 or:
- 5.8.3.1.7. 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.
- 5.8.3.1.8. Close the lid of the sample tube and cut the hinge.
- 5.8.3.1.9. 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 NFH<sub>2</sub>O and 3 µl Proteinase K (10 mg/ml). *Example: for 16 samples, combine 3840 µl Tris/EDTA/NaCl, 96 µl 20% Sarkosyl, 864 µl NFH<sub>2</sub>O, and 48 µl Pro K.*
- 5.8.3.2.2. Place the sample in 1.5 ml microcentrifuge tube (see sampling protocols). 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 NX<sup>P</sup> workstation, samples shall be loaded specifically into Qiagen® Investigator® Lyse&Spin 2 ml collection tubes. Do not use “dolphin-style” or other microcentrifuge tubes.
- 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.
- 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.

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- 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.

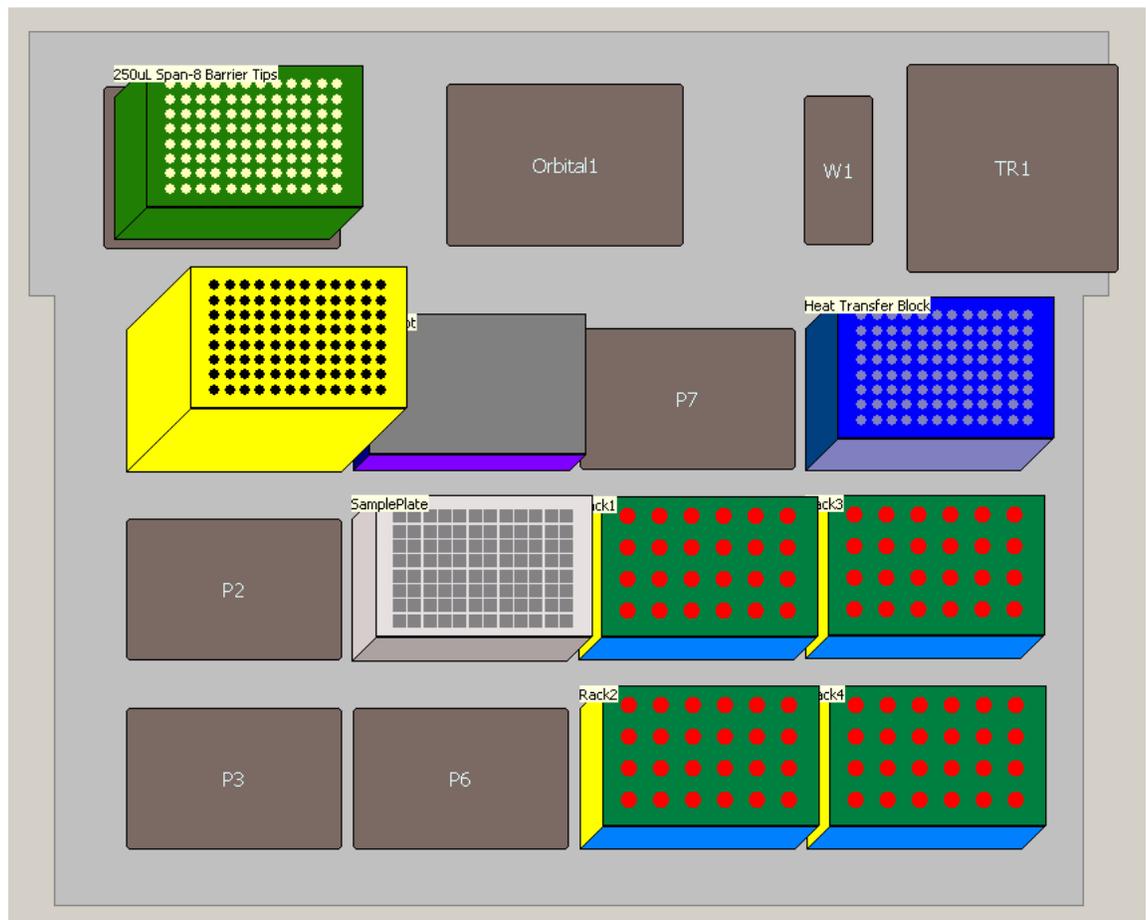
## 5.8.4. Biomek NX<sup>P</sup> Preparation

- 5.8.4.1. Wipe down the deck with 70% ethanol 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. “Instrument” menu → “Hardware Setup”.
  - 5.8.4.5.2. Set Port option to “COM1”.
  - 5.8.4.5.3. Click “Reconnect” and then “Accept”.
- 5.8.4.6. Run “Home All Axes” under the Instrument menu.
  - 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.

## 5.8.5. Initial Tube Transfer

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- 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 P250 Span-8 Barrier Tips (Cat. no. 379503) on the Rapid-Shuck module (top left).



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- 5.8.5.5.** Open the “Initial Tube Transfer” method contained in the Promega project.
- 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  $\mu$ l.
    - 5.8.5.7.2.1. *Example: Most of the sample tubes contain 300  $\mu$ l, but one tube contains 340  $\mu$ 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 P250 Span-8 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.
- 5.8.5.10.** Remove the 2.2 ml Square-Well Deep Well Plate [containing sample transferred during Initial Tube Transfer (5.8.4)] and cover with a temporary seal. Spin the plate using either a centrifuge or a salad spinner to remove any bubbles and force samples into the bottom of each well.

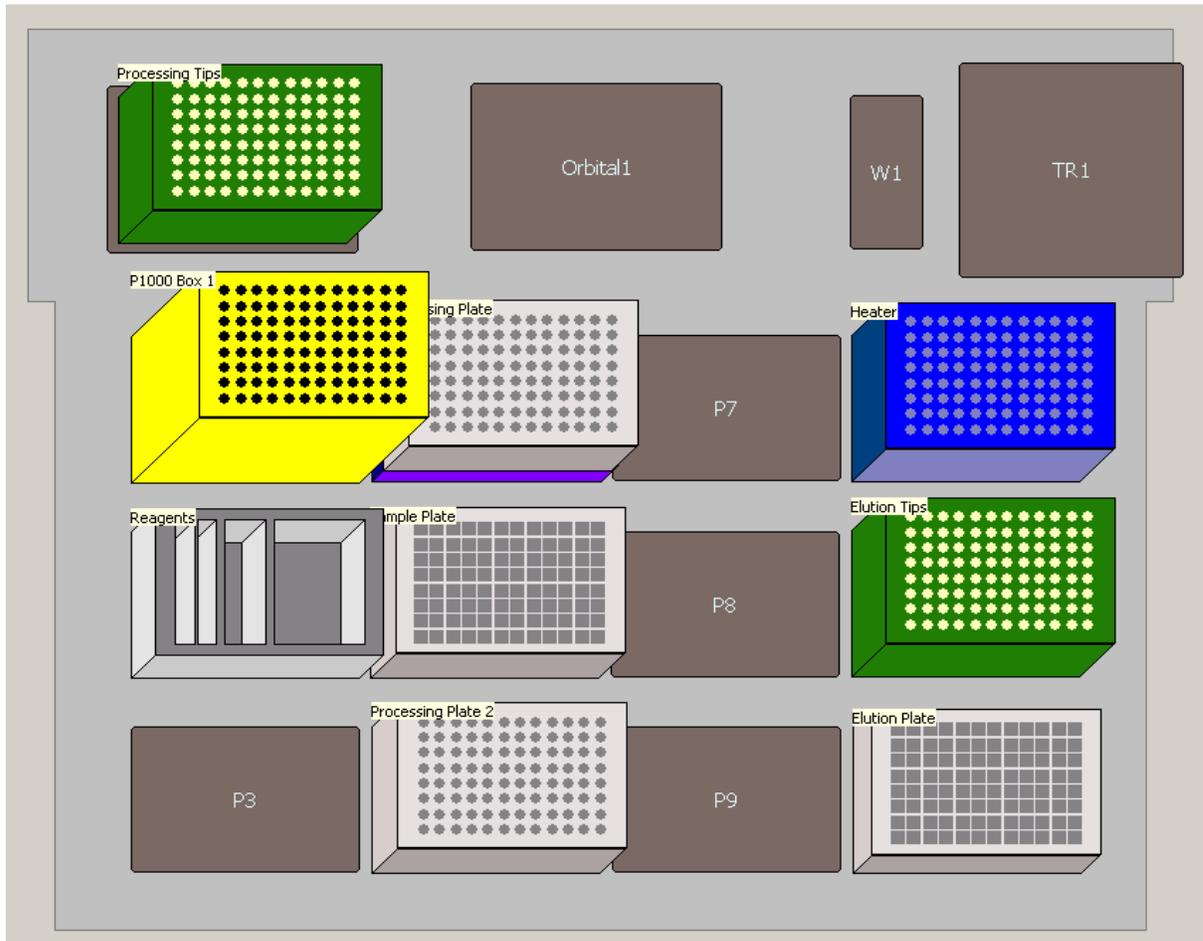
#### **5.8.6. DNA IQ™ Automated Extraction Procedure**

##### **5.8.6.1. Instrument Deck Setup**

- 5.8.6.1.1. Turn on the heating block via the switch to the right of the Biomek NX<sup>P</sup>, 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 P250 Span-8 Barrier Tips used in the Initial Tube 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 P1000 Span-8 Tips (Cat. no. 987925) on position P1. If there are fewer than five columns of tips available, the program will prompt for a full box of P1000 tips on position P3, otherwise, a partial box may be used.

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- 5.8.6.1.4.2. Box of new P250 Span-8 Barrier Tips on position P11.
- 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 P12. Orient the plate so that the clipped corner is on the bottom left.



## 5.8.6.2. Reagent Setup

- 5.8.6.2.1. Open the Automation Workbook from the “Robot Team” network folder.
- 5.8.6.2.2. The “Reagents” 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  $\mu$ l per sample).
  - 5.8.6.2.2.3. Do not count the No Template Control (NTC) or quantification standards.

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- 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 height, 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 “Reagents” 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 “Extraction\_v2.0.0.ISP” method, contained in the Promega project.
- 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 two 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 250 µl tip box on the Rapid-Shuck module. Assuming a new box was used during the Initial Tube Transfer (5.8.4), the value should be 0.
- 5.8.6.3.4. A plate map interface will appear.
  - 5.8.6.3.4.1. Enter the approximate sample volume.
    - 5.8.6.3.4.1.1. If a previously-extracted lysate plate is to be re-extracted/purified, increase the sample volume accordingly.
  - 5.8.6.3.4.2. Enter the desired elution volume between 50 and 100 µl (should match the elution volume entered in the Automation Workbook, above).
  - 5.8.6.3.4.3. Set “Sample Type” to “Aqueous”.
    - 5.8.6.3.4.3.1. If a previously-extracted lysate plate is to be re-extracted/purified, set “Sample Type” to “Lysis”
  - 5.8.6.3.4.4. Set “Perform Lysis Wash?” to “Yes”.
  - 5.8.6.3.4.5. Select the wells to be processed.
    - 5.8.6.3.4.5.1. Click on any well in a column to select the entire column.

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5.8.6.3.4.5.2. Click/hold on any well and drag the cursor out of the well to select that well only.

5.8.6.3.4.5.3. Hold "Ctrl" to select separate wells/columns and "Shift" to select consecutive wells/columns.

5.8.6.3.4.5.4. Do not select the last column (#12). This will always be used for quantification standard.

5.8.6.3.4.5.5. Click "Enter". The wells you selected will change color.

5.8.6.3.4.6. Step 5.8.5.3.4.5 may be repeated to specify different sample volumes or elution volumes, but doing so will change the amount of reagents required for the run (see Reagent Preparation section and step 5.8.5.3.5.1, below).

5.8.6.3.4.7. When all samples have been selected and specified, click "Finished".

5.8.6.3.5. A map of the reagent reservoir will appear.

5.8.6.3.5.1. Unless the sample volumes have been altered, the reagent volumes should match what the Biomek Workbook displayed in the Reagent Setup section above.

5.8.6.3.5.2. Click "Close Window".

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

5.8.6.3.7. When the run is finished, reservoirs may be carefully rinsed, labeled, and reused. Discard partial tip box from position P11. 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.

5.8.6.3.8. 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.

5.8.6.3.9. 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. REAL-TIME PCR QUANTIFICATION USING QUANTIFILER® TRIO Prepared on the Biomek NX<sup>P</sup> Laboratory Automation Workstation**

##### **5.8.7.1. Instrument Deck Setup**

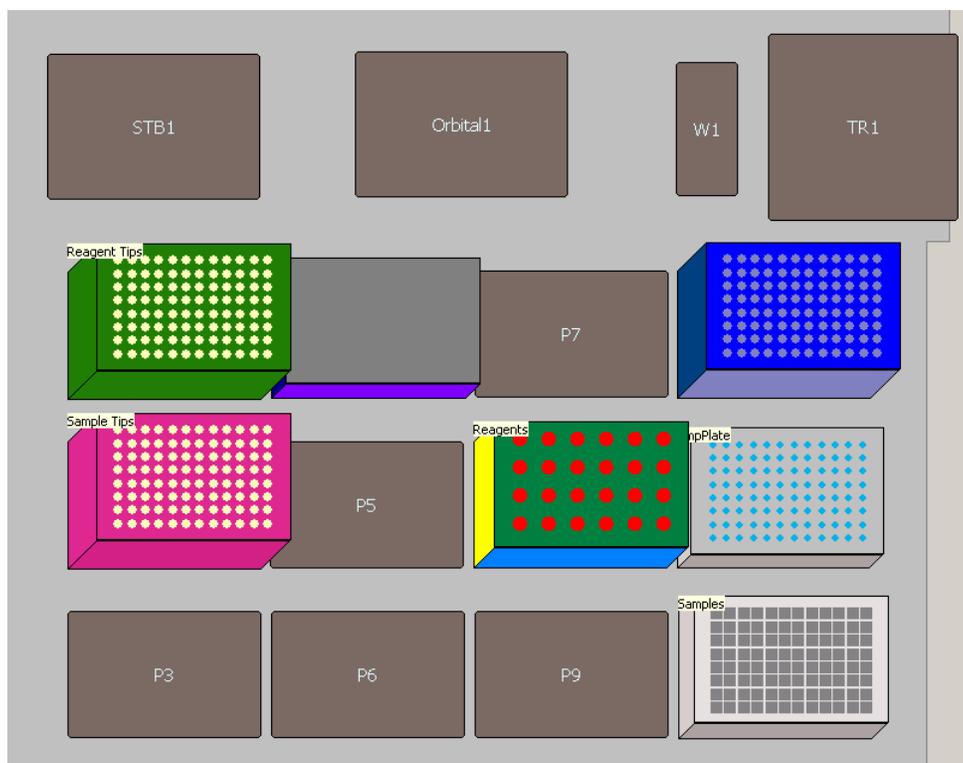
5.8.7.1.1. In the Biomek Software, open the "Trio Quant" method contained in the ABI project.

5.8.7.1.2. Click on the "User Interface" line within the method.

5.8.7.1.2.1. To the right, check that "Quantifiler Trio" is selected.

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- 5.8.7.1.2.2. Enter the number of samples to process (equal to the number of elution plate wells that contain extract).
- 5.8.7.1.2.3. Do not count the NTC or quantification standards.
- 5.8.7.1.2.4. Do not add extra samples for excess volume; it is already calculated into the program.
- 5.8.7.1.3. Place the following labware on the deck as shown in the figure below:
- 5.8.7.1.3.1. New box of Beckman Coulter Biomek P50 Barrier Tips (Cat. no. A21586) 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.
- 5.8.7.1.3.2. Box of P250 Span-8 Barrier Tips on position P1. A partial box may be used (as above) if at least two columns are available.
- 5.8.7.1.3.3. New Applied Biosystems (Foster City, CA) MicroAmp® Optical 96-Well reaction plate (Cat. no. N801-0560) on position P11.
- 5.8.7.1.3.4. The elution plate containing extracted samples on position P12. This plate must be centrifuged prior to quantification to prevent bubbles in the sample wells.



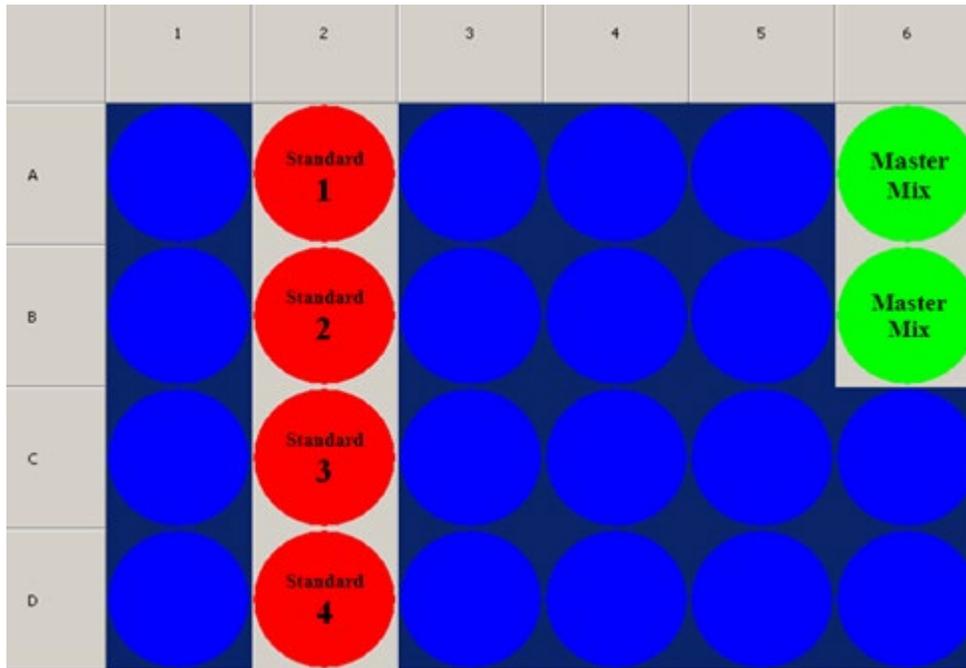
## 5.8.7.2. Reagent Preparation

- 5.8.7.2.1. Open the Automation Workbook from the “Robot Team” network folder.

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- 5.8.7.2.2. The “Reagents” tab has a description of the reagents and supplies needed to run automated quantification setup.
  - 5.8.7.2.2.1. Enter the number of samples on the plate, the same as entered into the “User Interface” above.
- 5.8.7.2.3. When a new Quantifiler® Trio kit is opened, Quantification Enhancer may be added to the reaction mix as follows:
  - 5.8.7.2.3.1. 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.
  - 5.8.7.2.3.2. Mark each tube of reaction mix with “QE” to indicate that Quantification Enhancer has been added.
  - 5.8.7.2.3.3. Record the lot number of the Quantification Enhancer on the Automated Extraction/Quantification Worksheet.
  - 5.8.7.2.3.4. Quantification Enhancer shall be stored at room temperature.
- 5.8.7.2.4. 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.
- 5.8.7.2.5. Place the master mix tube(s) in wells A6 (and B6) of the Reagent Tube Rack as indicated below.
- 5.8.7.2.6. 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.
- 5.8.7.2.7. Place the standard tubes in column 2 of the Reagent Tube Rack as indicated below.
- 5.8.7.2.8. Open and fold back all tube caps and place the Reagent Tube Rack on deck position P8.

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## 5.8.7.3. Running the Automated Quant Trio Setup Method

- 5.8.7.3.1. With the “Quant Trio” method open and the number of samples specified, click the “Run” button at the top of the screen.
- 5.8.7.3.2. A window will appear to confirm the amount and placement of the master mix tubes. Click “OK”.
- 5.8.7.3.3. A window will appear to confirm the deck layout as shown by the software. Click “OK”.
- 5.8.7.3.4. 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”.
- 5.8.7.3.5. 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.
- 5.8.7.3.6. 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.
- 5.8.7.3.7. Spin the plate using either a centrifuge or a salad spinner to remove any bubbles and force samples into the bottom of each well.
- 5.8.7.3.8. Place the plate in the real-time PCR instrument, turn on the instrument, and open the 7500 System software.

## 5.8.7.4. Create A Plate Document

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- 5.8.7.4.1. 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.
- 5.8.7.4.2. 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.
  - 5.8.7.4.2.1. 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.
- 5.8.7.4.3. 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.
- 5.8.7.4.4. Import the text file you just created. The wells should populate with the names of the samples and standards.
- 5.8.7.4.5. 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.

## **5.8.8. PowerPlex® Fusion 6C Amplification Prepared on the Biomek NX<sup>P</sup> Laboratory Automation Workstation**

### **5.8.8.1. Vortexing Extracts**

- 5.8.8.1.1. Prior to amplification, the extract plate (the elution plate from the DNA IQ™ procedure) shall be vortexed briefly at low speed to ensure uniform pipetting of sample.
- 5.8.8.1.2. 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.

### **5.8.8.2. Normalization Data Setup**

- 5.8.8.2.1. Open the Automation Workbook file containing the batch to be amplified.
- 5.8.8.2.2. On the “Extraction-Quant” tab, click the “Import & Analyze Quant Results” button.
- 5.8.8.2.3. Browse for the appropriate text import file containing quantification data for this batch. Click “Open”.
- 5.8.8.2.4. 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.

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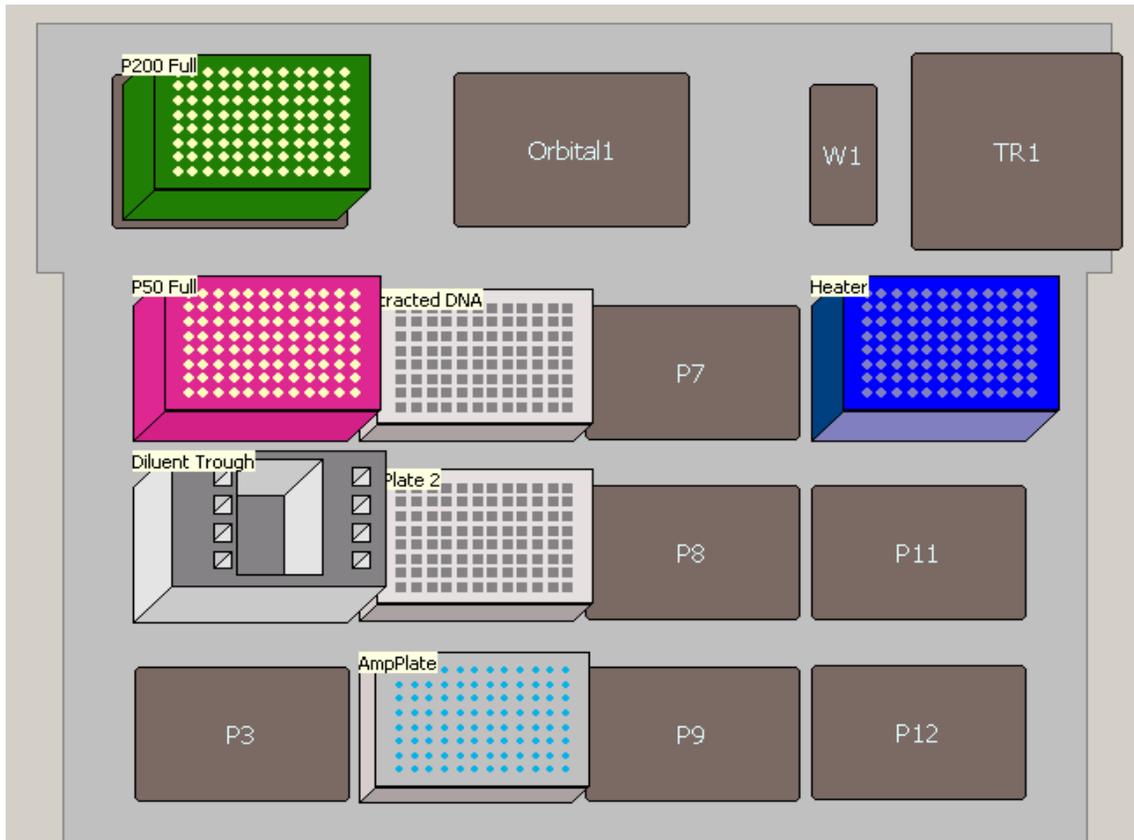
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- 5.8.8.2.4.1. If any standard curve parameter fails, or if the NTC contains a value, or if a standard curve well has an undetected IPC, an error message will appear and the batch will not be analyzed until the standard curve is fixed. Points should be omitted in the Real-Time PCR software and a new results file exported.
- 5.8.8.2.5. 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.
- 5.8.8.2.6. Move or copy this AmpSetup file to the folder //IN PROGRESS/Amp Setups on the "Robot Team" drive.

#### 5.8.8.3. Instrument Deck Setup

- 5.8.8.3.1. In the Biomek Software, open the "Normalize-Amp\_Fusion6C" method contained in the Promega project.
- 5.8.8.3.2. Place the following labware on the deck as shown in the figure below:
  - 5.8.8.3.2.1. Box of P250 Span-8 Barrier Tips on the Rapid-Shuck module.
  - 5.8.8.3.2.2. Box of P50 Barrier Tips on position P1. A second box of P50 Barrier Tips may be required on position P3.
  - 5.8.8.3.2.3. 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.
  - 5.8.8.3.2.4. New 96-well optical amplification plate on position P6.
  - 5.8.8.3.2.5. Extract plate containing samples on position P4.
    - 5.8.8.3.2.5.1. CAUTION: ensure that the MagnaBot module has been removed.

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5.8.8.3.3. Click “Run”.

5.8.8.3.4. The program will prompt for three values:

5.8.8.3.4.1. 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.

5.8.8.3.4.2. First\_P250\_Tip = the number of the first unused tip in the 250 µl tip box on the Rapid-Shuck module, numbering down columns and then across. For a new box, enter 1.

5.8.8.3.4.2.1. Example: If two and a half columns (twenty tips) of P250 tips have been used, enter “First\_P250\_Tip” as 21.

5.8.8.3.4.3. 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.

## 5.8.8.4. Reagent Preparation

5.8.8.4.1. 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.

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- 5.8.8.4.2. Place the STR Master Mix tube in position 4 (front-most) of the Left Tube Holder on the reagent rack.
- 5.8.8.4.3. 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”.
- 5.8.8.4.4. 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.

## **5.8.8.5. Running the Normalization/Amp Set-up Method**

- 5.8.8.5.1. Check that the deck layout matches the layout indicated in the next prompt. Click “OK”.
- 5.8.8.5.2. The run will begin, performing normalization and amplification setup in one step.
- 5.8.8.5.3. When the run is finished, remove the amplification plate and cover with adhesive foil. Press the foil well using a roller tool.
- 5.8.8.5.4. Remove the elution plate containing extracted samples and cover with a temporary seal and refrigerate.

## **5.8.8.6. Create CE Plate Record**

- 5.8.8.6.1. The Amp-CE Plate Record in the Automation Workbook is automatically populated with the amplified samples when the batch is analyzed with quantification results.
- 5.8.8.6.2. Open the Automation Workbook containing the batch of interest and click on the “Amp-CE” tab.
- 5.8.8.6.3. 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.
  - 5.8.8.6.3.1. 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.
- 5.8.8.6.4. 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.
- 5.8.8.6.5. Fill out the rest of the worksheet as designated, including dates, reagent lot numbers, amplification reaction type, and injection protocols.

## **5.8.9. Preparing a 3500 plate on the Biomek 3000**

### **5.8.9.1. Biomek 3000 Preparation**

- 5.8.9.1.1. Open the “Biomek Software” located on the desktop.
- 5.8.9.1.2. Calibrate the Biomek 3000 prior to operation.

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- 5.8.9.1.3. All tubes of allelic ladder in the post-amplification kit shall be combined into a single 1.5 ml Eppendorf microcentrifuge tube or Promega 1.5 ml ClickFit microtube (do not use a “dolphin”-style tube), marked as “ladder” with associated lot numbers recorded on the tube, and stored with the kit when not in use.

## 5.8.9.2. Instrument Deck Setup

- 5.8.9.2.1. Click File → Open, select “3500\_Direct\_transfer\_with\_ladder” for 3500xL setup.

- 5.8.9.2.1.1. This will open the designated program that will transfer a plate with dedicated ladder positions into the A6, A12, H3, and H9 positions for 3500xL setup.

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

- 5.8.9.2.2.1. Box of Beckman Coulter Biomek AP96 P250 Tips (Cat. no. 717253) on the ML1 position.

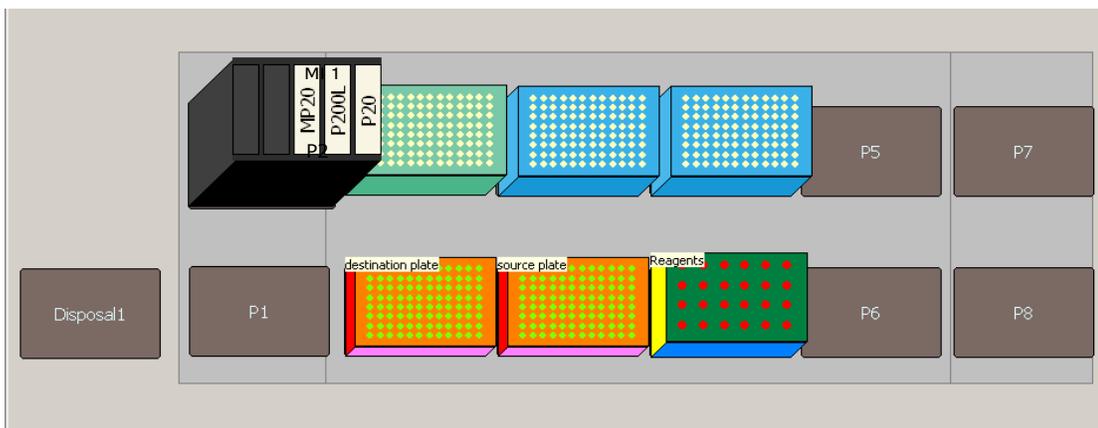
- 5.8.9.2.2.2. Two boxes of Beckman Coulter Biomek AP96 P20 Tips (Cat. no. 717256) on the ML2 and ML3 positions.

- 5.8.9.2.2.2.1. Note: A typical plate setup will utilize one P250 tip, eight P20 tips per column transferred, and between one and six P20 tips for ladder transfers. Ensure that enough tips are available before proceeding.

- 5.8.9.2.2.3. One new 96-well optical plate at position P2.

- 5.8.9.2.2.3.1. This is the CE plate where master mix and samples will be loaded when the program has finished.

- 5.8.9.2.2.4. Spin down the amplification plate and ensure that no bubbles remain. Place the plate in deck position P3. Secure the amplified plate to the plate rack with tape.



- 5.8.9.2.3. In the software program, click the “Instrument Setup” step, which will bring up the Deck Display.

- 5.8.9.2.4. Right-click on P250 box in position ML1 and select “Properties” to open the “Labware Properties” menu as shown below.

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- 5.8.9.2.5. Ensure that the “Unload Tips Into:” drop down menu is selected as “Disposal1”, the “When empty, send to:” drop down menu is selected as “<Home>”, and the “Load no more than” drop down menu is designated as “1”.

- 5.8.9.2.6. Select “Show Available Tips” as shown below.

- 5.8.9.2.7. By clicking on individual tip locations, you may designate whether tips are present (blue) or absent (white). For every location where a tip is absent from the physical deck, click the appropriate location to turn that location white. You may reference the bottom left corner of the menu to determine the number of available tips that are being designated as usable.

- 5.8.9.2.8. Configure Labware Properties and available tips for the P20 boxes located at positions ML2 and ML3 in the Deck Layout.

## 5.8.9.3. Reagent Preparation

- 5.8.9.3.1. To prepare master mix for automated dispensing by the Biomek 3000:

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5.8.9.3.2. In a 1.5 ml Eppendorf microcentrifuge tube or Promega 1.5 ml ClickFit Microtube, prepare the appropriate volume of master mix (Hi-Di™ and ILS) for the associated number of samples and/or columns (see example chart below for 0.5 µl ILS/sample).

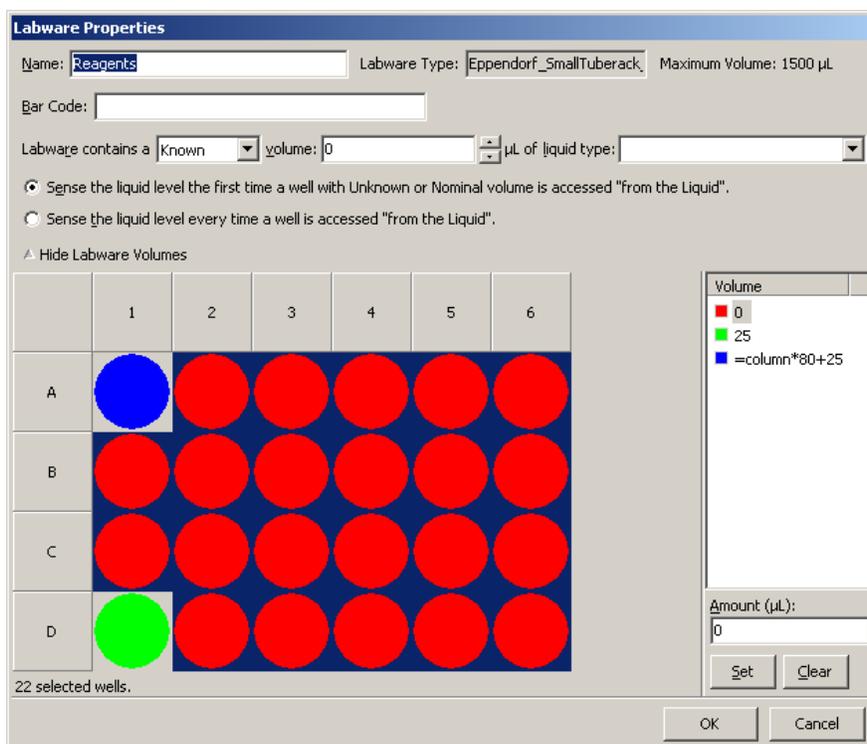
Number of Samples+Ladders	Number of Columns	ILS (µl) (0.5 µl/sample)	Hi-Di (µl)	Total (µl)
1-24 (3500xL)	3	13	247	260
25-48 (3500xL)	6	25	475	500
49-72 (3500xL)	9	38	712	750
73-96 (3500xL)	12	49	931	980

5.8.9.3.2.1. Do not use a “dolphin”-style microcentrifuge tube, or the robot may fail to load master mix accurately to the columns.

5.8.9.3.2.2. Ensure that sufficient master mix is prepared to cover all capillaries during electrophoresis, or manually pipet Hi-Di™ into empty wells after the plate is complete.

5.8.9.3.3. Vortex and spin down, ensuring that no bubbles remain.

5.8.9.3.4. Place the prepared master mix tube in position A1 of the 24-well tube rack located in position P4 on the Deck, as shown in blue below.



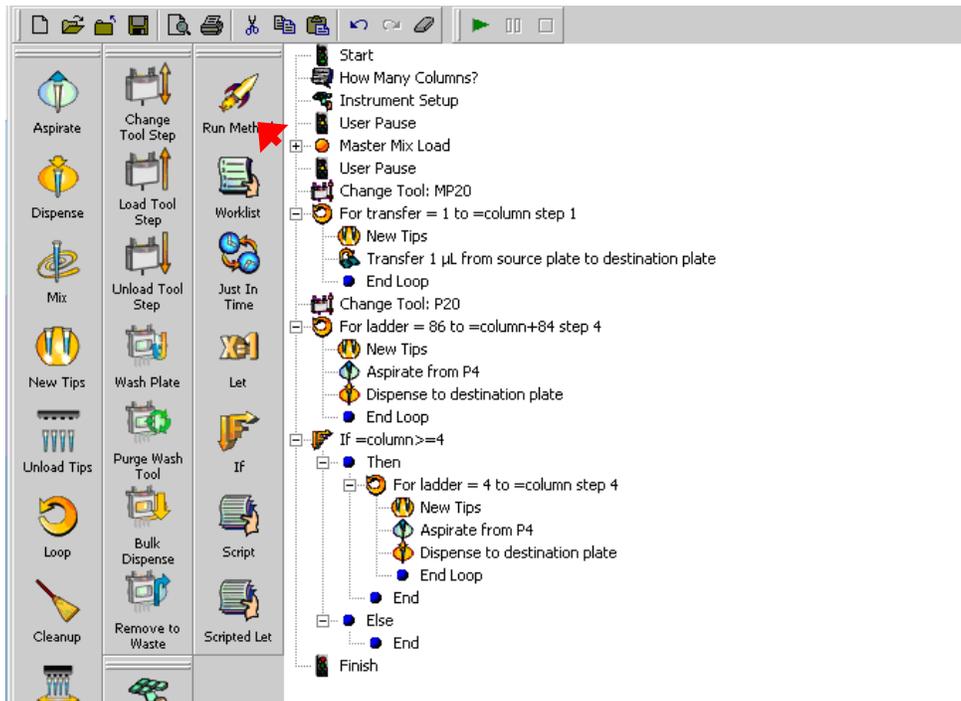
5.8.9.3.5. Vortex and quickly spin down the combined tube of allelic ladder and ensure that no bubbles remain.

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- 5.8.9.3.6. Place the tube of ladder in position D1 of the 24-well tube rack located in position P4 on the Deck, as shown in green above.

## 5.8.9.4. Starting the CE Plate Set-up Run

- 5.8.9.4.1. Remove all lids from tip boxes and from the tubes of prepared master mix and ladder and place them clear of the deck.
- 5.8.9.4.2. In the software program, click on the green “run” arrow located on the top command bar, as shown below.



- 5.8.9.4.3. Follow the prompts as they appear. You will be asked to input the number of columns for the plate setup. This number should be the same as originally determined when preparing the master mix (see chart above).
- 5.8.9.4.4. When the program has completed, remove and discard any empty tip boxes.
- 5.8.9.4.5. Discard the master mix tube, and store the allelic ladder tube with the post-amplification kit.
- 5.8.9.4.6. Seal the amplification plate with aluminum foil plate seal and store frozen.
- 5.8.9.4.7. Add a septa cover to the CE plate, and load onto genetic analyzer as described in section 2 of the Forensic Biology Section Casework Test Method.

## 5.8.10. Final Tube Transfer

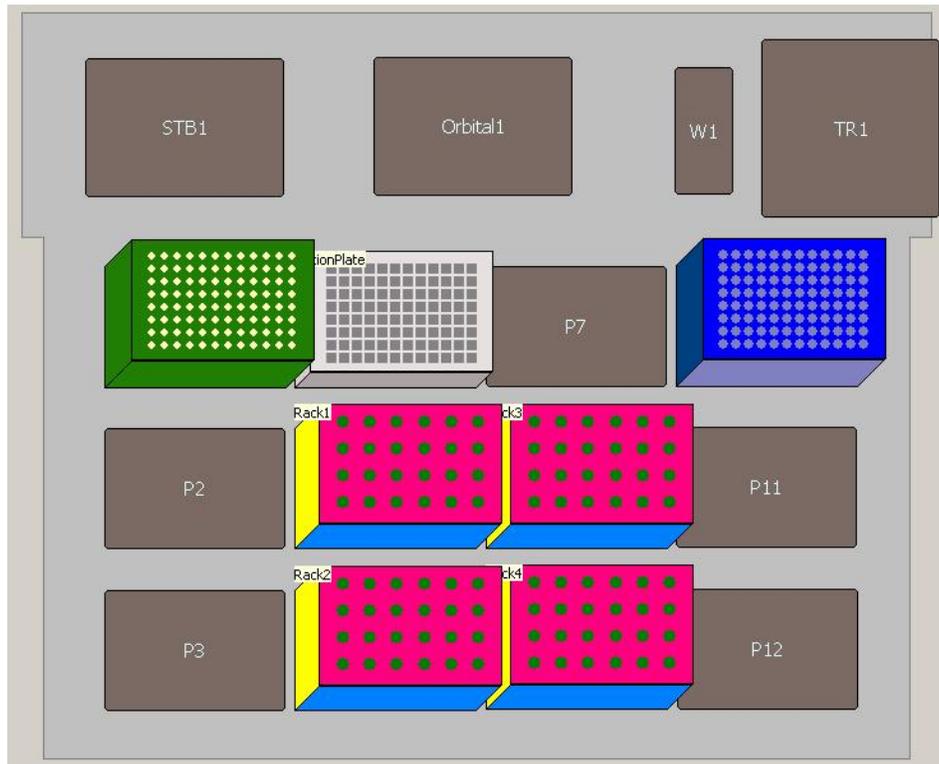
- 5.8.10.1. Open the Automation Workbook containing the set of samples that have been run, and click on the “FTT Layout” tab. Click the “Consolidate Samples” button on the upper right.

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Only code T samples, which have been designated by the analyst to be returned to tubes, will be included in the final tube transfer. The remaining samples will remain in the elution plate.

- 5.8.10.1.1. 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.
  - 5.8.10.1.1.1. You cannot use a partial sheet if the # of used labels + # of tubes required is greater than 80.
- 5.8.10.2. 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.
- 5.8.10.3. Move or copy the .csv file to the folder //IN PROGRESS/FTT Setups on the “Robot Team” drive.
- 5.8.10.4. 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.
- 5.8.10.5. Arrange the deck layout as shown in the figure below:
  - 5.8.10.5.1. Place sample tube racks on positions P5, P6, P8, and P9, again paying attention to sample order according to the FTT Layout.
  - 5.8.10.5.2. Place the plate containing extracted samples on position P4.
  - 5.8.10.5.3. Place a new box of Beckman Coulter Biomek P250 Span-8 Barrier Tips on position P1.

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**5.8.10.6.** Open the “Final Plate to Tube Transfer” method contained in the Promega project.

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

5.8.10.7.1. 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.

**5.8.10.8.** 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.

5.8.10.8.1. 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.

**5.8.11.** 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. Chain of Custody for Automated Processing of Casework Samples**

**5.9.1.1.** 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](mailto:DAutomation@isp.in.gov).

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## FORENSIC BIOLOGY SECTION

### TEST METHODS

- 5.9.1.2.** This form, as well as the email that included it, shall be retained by the analyst in the case record.
- 5.9.1.3.** 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. The extracts leave the analyst's custody and are transferred to a storage area where they will be retrieved for automated processing.
- 5.9.2.** Within the same work day that the Robot Submission Form is submitted, the analyst shall deliver all associated samples to the IRL S201A robot room storage area.
- 5.9.3.** 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 NX Automated Extraction/Quantitation Worksheet.
  - 5.9.3.1.** The name (or initials) of robot operator receiving custody of samples, as well as the date of transfer, shall be marked on the worksheet.
- 5.9.4.** 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.
  - 5.9.4.1.** 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.
  - 5.9.4.2.** After approximately 60 days post-processing, the lysis plate shall be discarded.
- 5.9.5.** 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.
  - 5.9.5.1.** 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.
  - 5.9.5.2.** If processing requires repeating steps or deviation from the test methods which results 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.
- 5.9.6.** 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.
- 5.9.7.** 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 individual tubes and return these extracts to the IRL S201A robot room storage area within the same workday that the notification email is delivered.
  - 5.9.7.1.** 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.
  - 5.9.7.2.** 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.
  - 5.9.7.3.** 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.

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**5.9.8.** 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).

**5.9.8.1.** After approximately 60 days post-processing, any remaining extracts not designated for retention shall be discarded.

**5.9.9.** 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.

**5.9.9.1.** 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:**

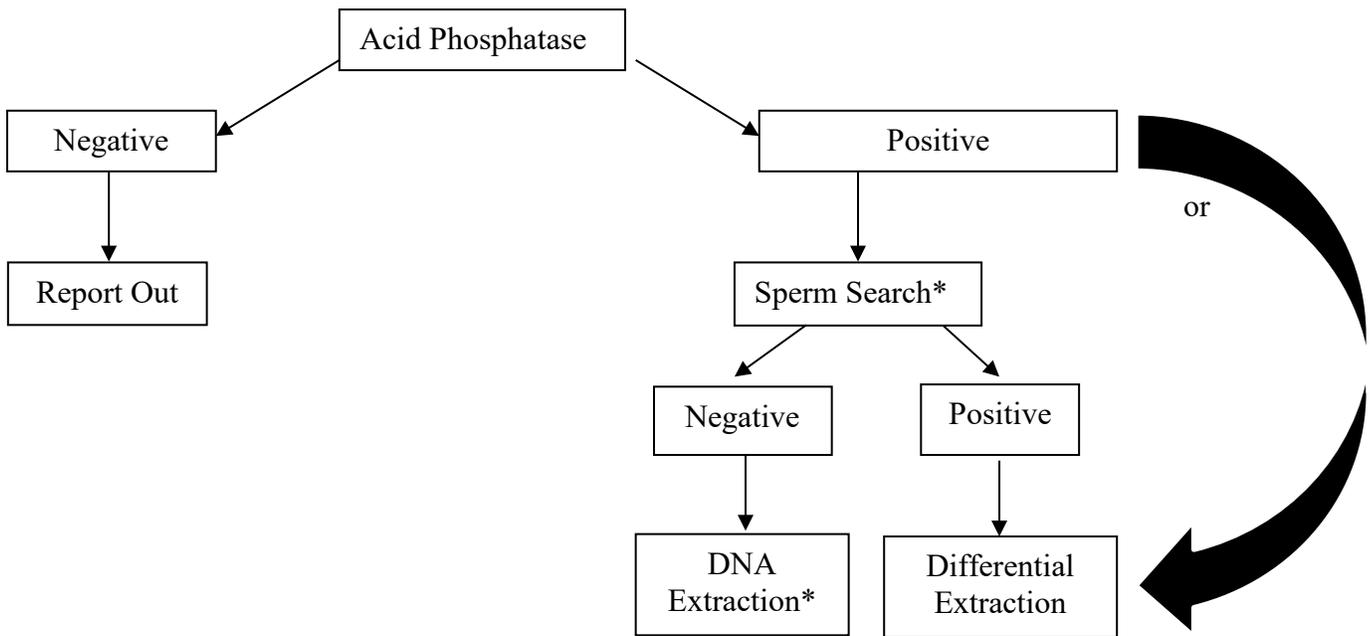
**5.11.1.** N/A

**5.12. References:**

**5.12.1.** N/A

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FORENSIC BIOLOGY SECTION  
TEST METHODS  
APPENDIX 1  
FLOW CHARTS**

**Items outside of a Sexual Assault kit\*\* (e.g., clothing, bedding, etc.) for Semen Identification**

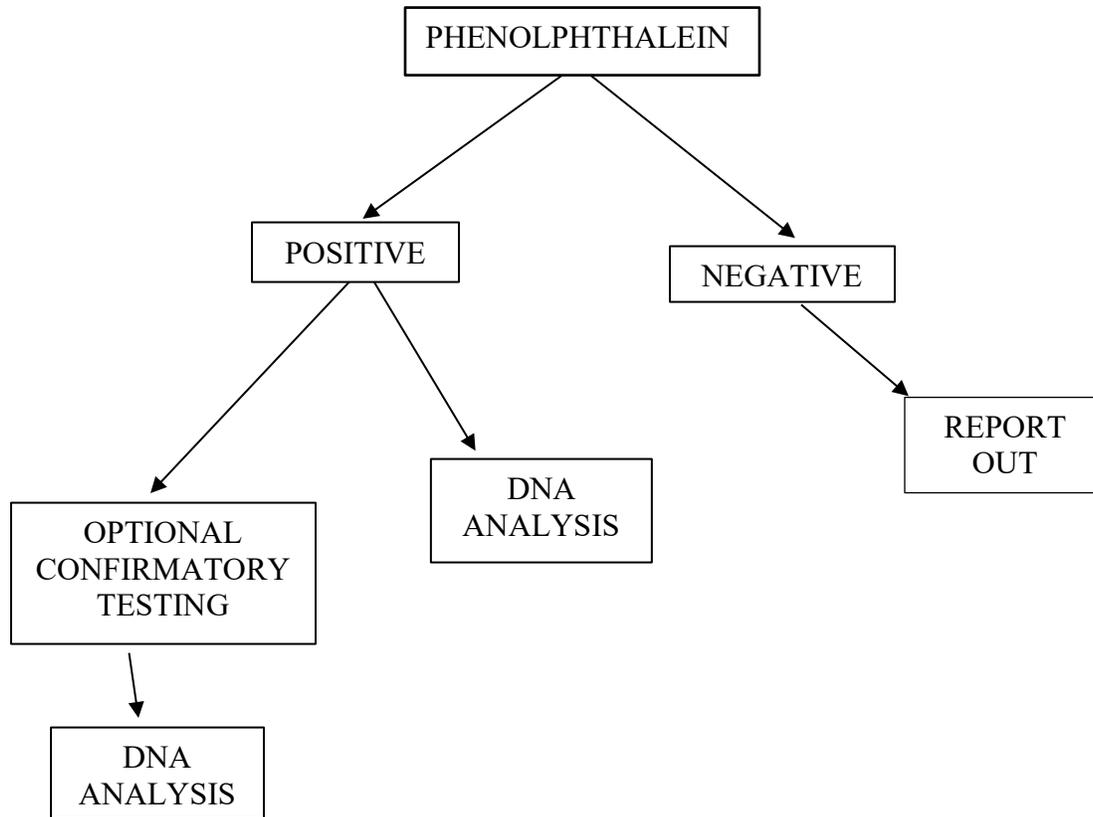


\*Optional/Analyst's discretion

\*\*Underwear submitted separately from a sexual assault kit shall be examined with kit protocols.

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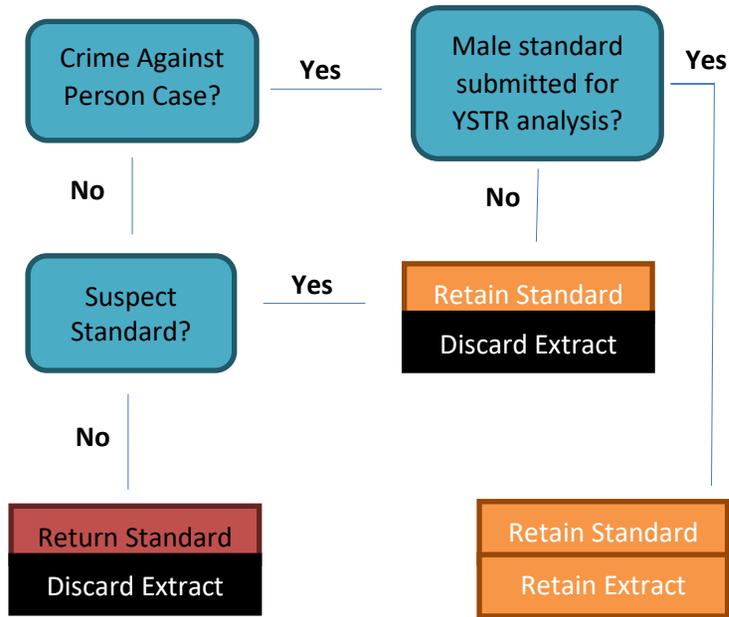
## BLOOD IDENTIFICATION



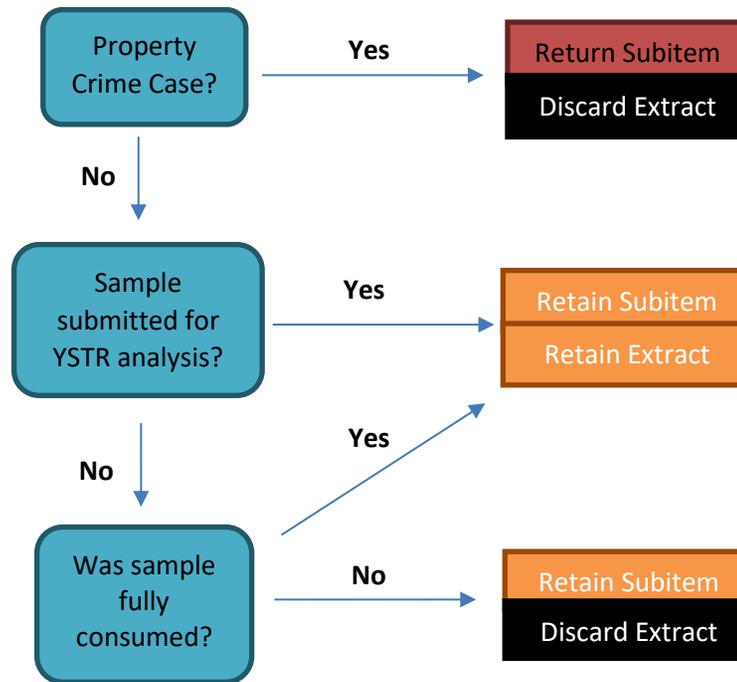
Any stain identification testing may be omitted if sample would be consumed before DNA analysis.

# SUBITEM RETENTION

## Standards



## Evidence\*



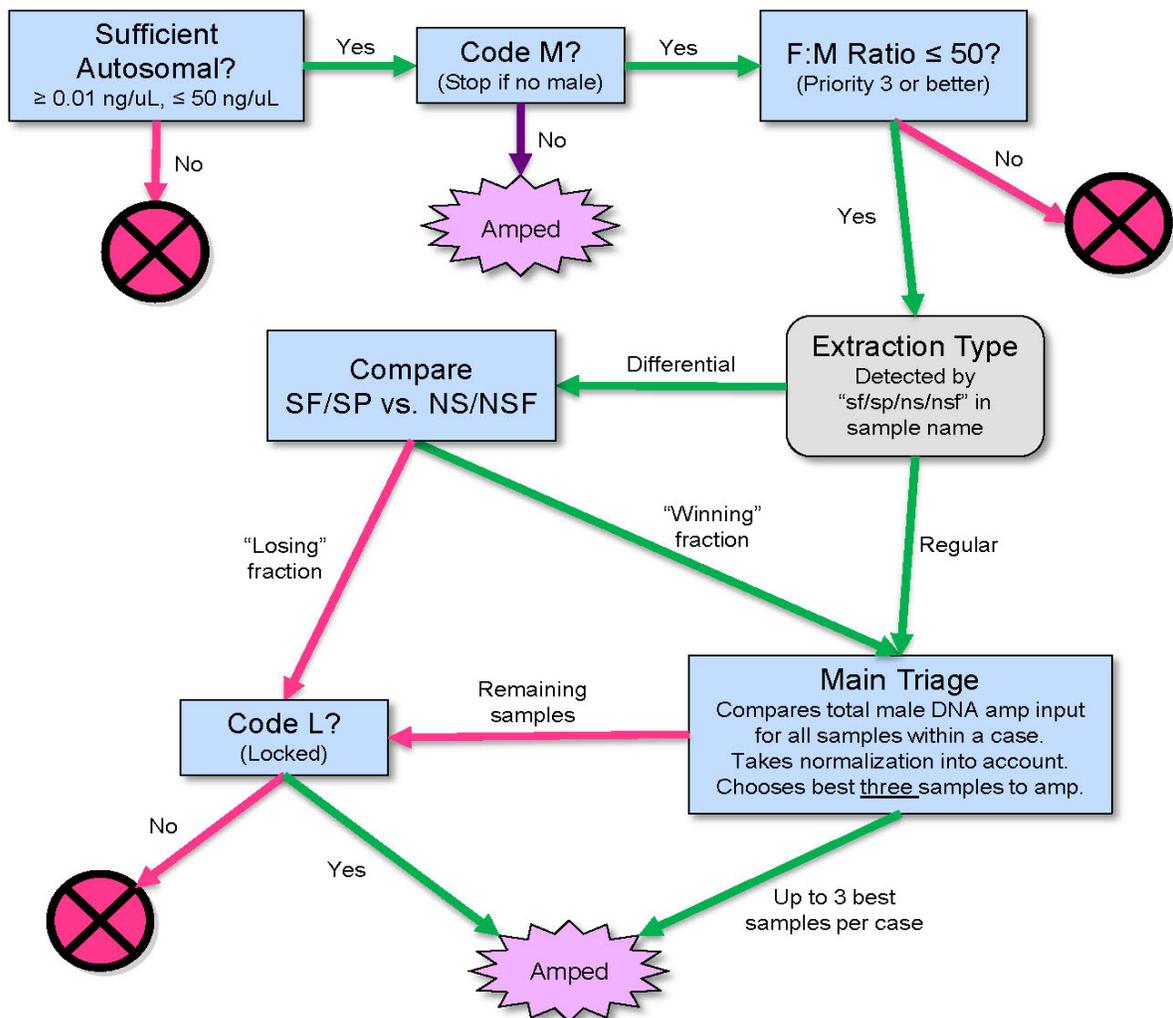
Above flowcharts reflect typical evidence but cannot account for all case scenarios. Deviation from above may be obtained with supervisor approval.  
\*Evidence collection kit samples that demonstrate no amplifiable male DNA shall be returned, and their extracts shall be discarded.

# Automation Sample Triage

## Sample Property Codes:

<b>S</b>	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.
<b>M</b>	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).
<b>L</b>	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.
<b>T</b>	Return to Tube	Marks any sample or reagent blank to be returned to a tube for retention. No effect on other codes. Only samples marked T will be returned to tubes.

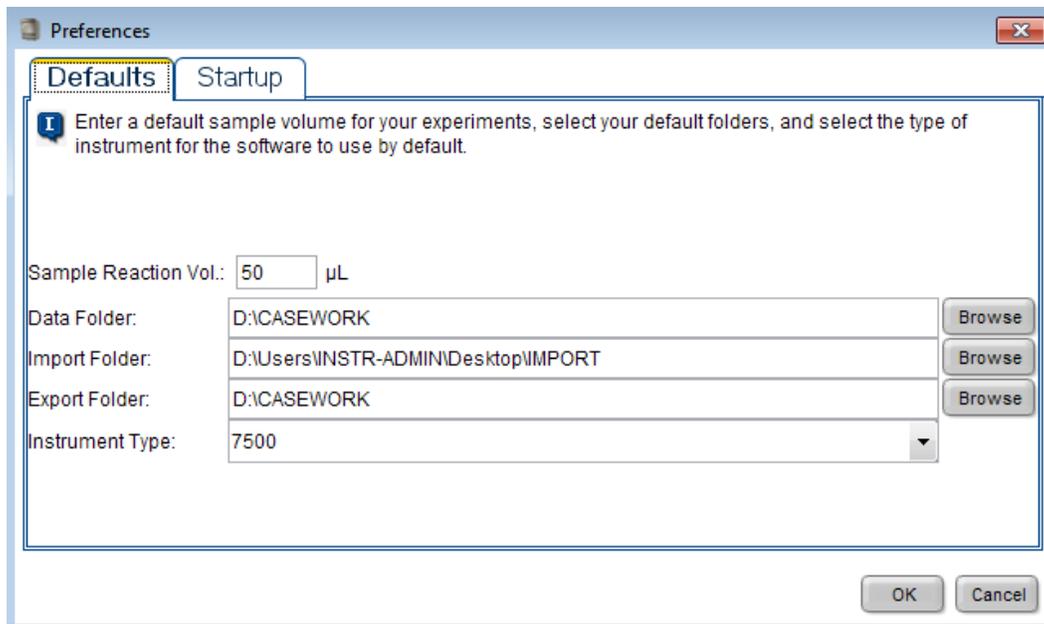
## Triage Process:



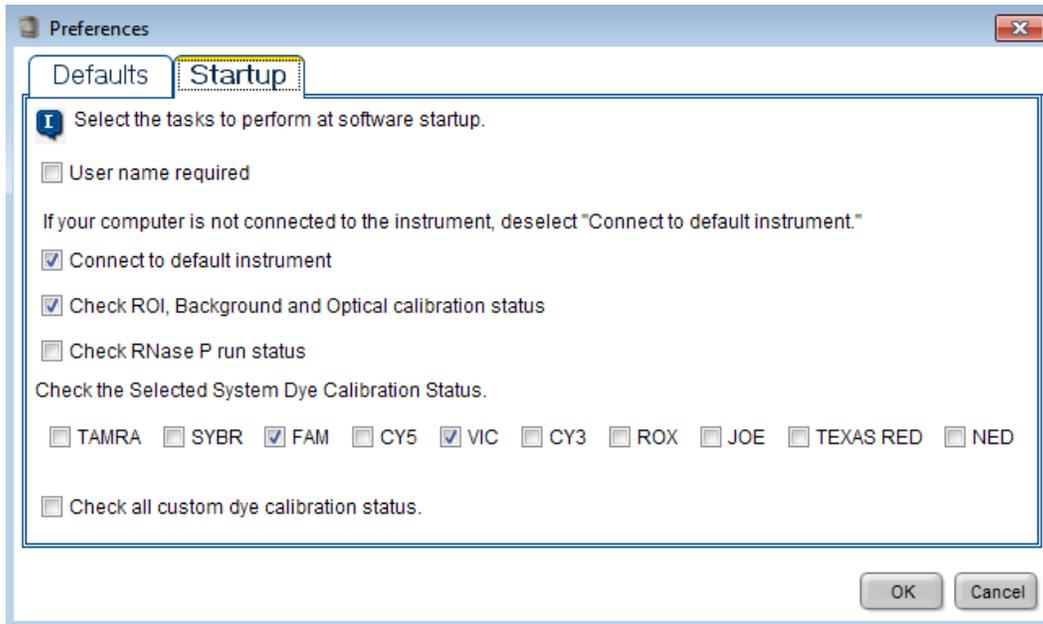
## APPENDIX 2

# 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.

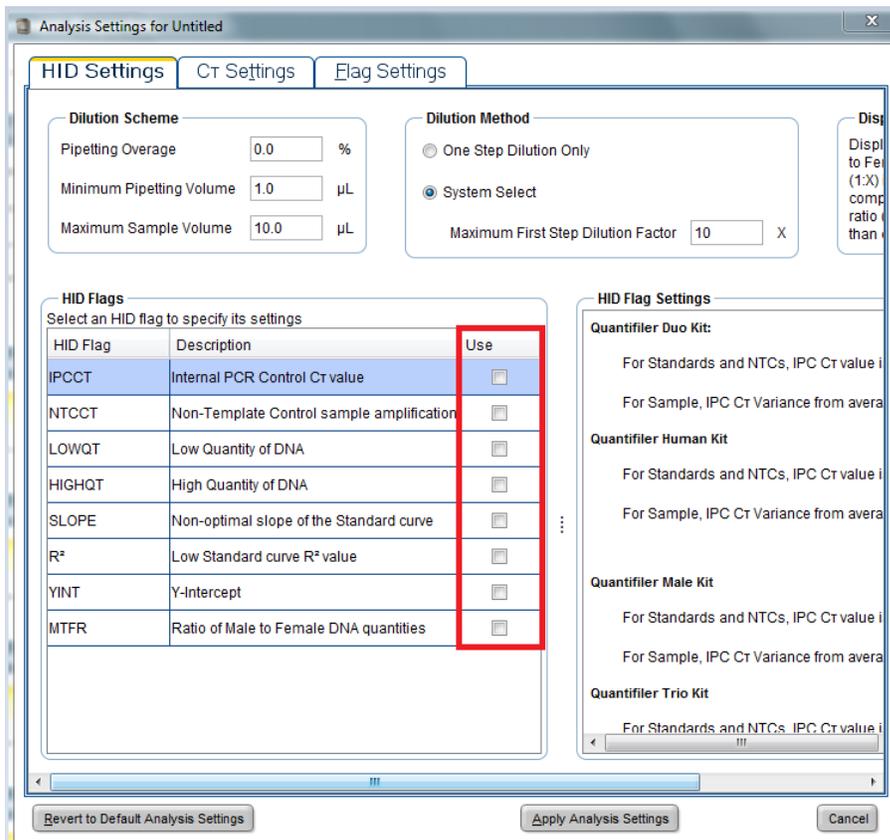


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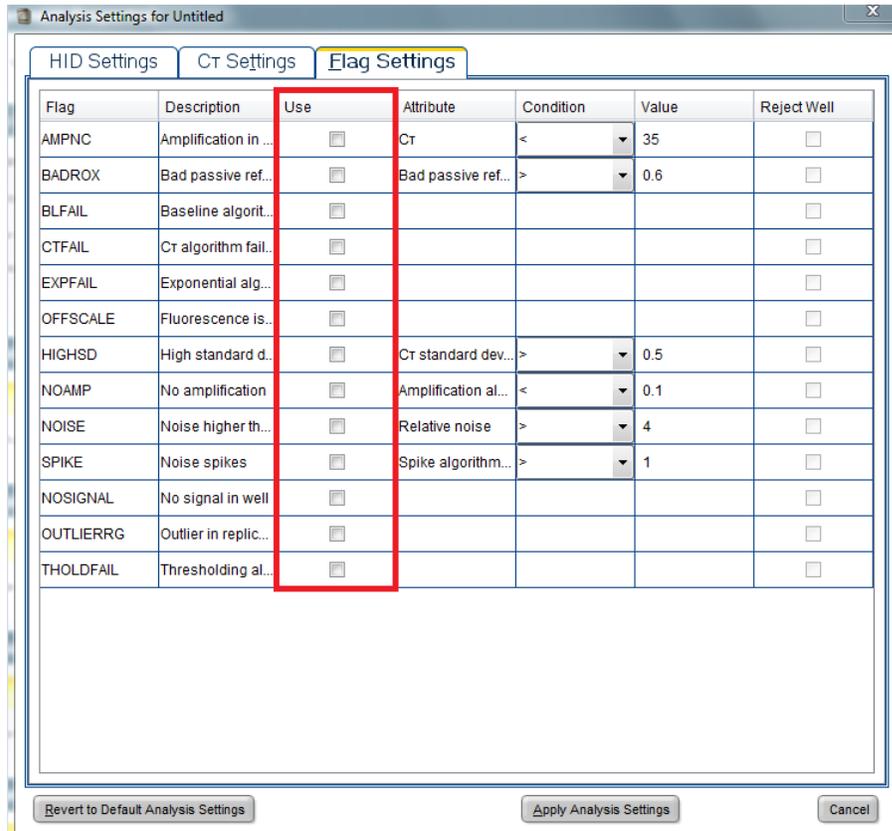


1.5 Select the **Quantifiler® Trio** icon on the Home screen.

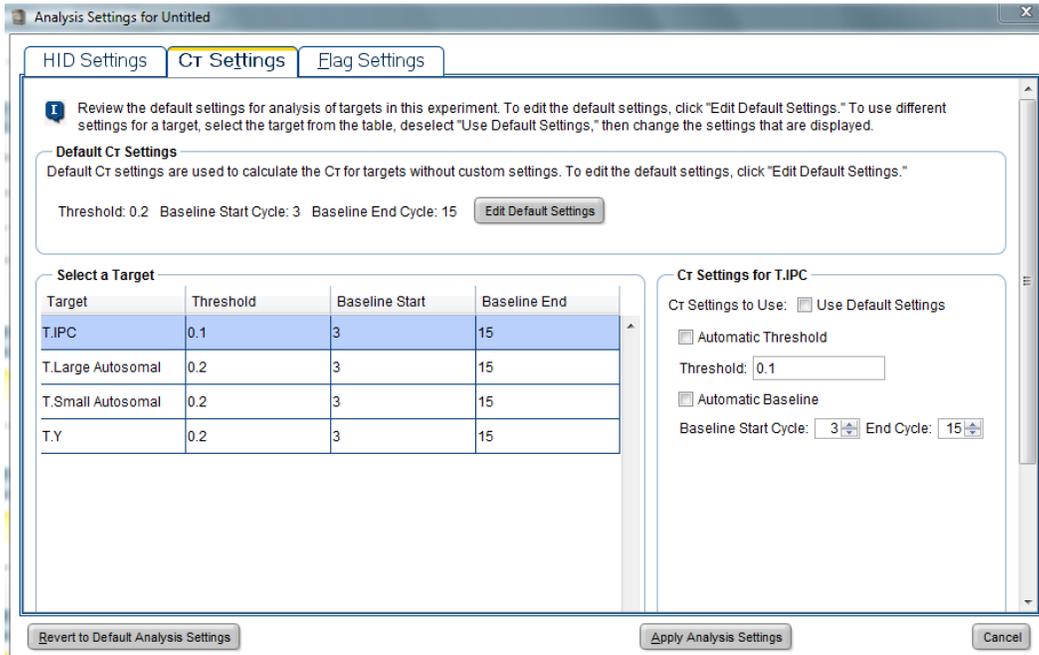
1.6 Select **Analysis** → **Analysis Settings** to remove all flags from the “HID Settings” and “Flag Settings” tabs.



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1.7 Verify the CT Settings in the image below.



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**

**I** Instructions: Standards and NTC are set by default. Select wells, then assign targets if applicable.

**Assign sample(s) to the selected wells.**

Assign	Sample
<input checked="" type="checkbox"/>	Trio Standard 1
<input type="checkbox"/>	Trio Standard 2
<input type="checkbox"/>	Trio Standard 3
<input type="checkbox"/>	Trio Standard 4
<input type="checkbox"/>	NTC

**Assign target(s) to the selected wells.**

Assign	Target	Task	Quantity
<input checked="" type="checkbox"/>	T.Large A...	<input checked="" type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	50
<input checked="" type="checkbox"/>	T.Small A...	<input checked="" type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	50
<input checked="" type="checkbox"/>	T.IPC	<input type="checkbox"/> <input checked="" type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
<input checked="" type="checkbox"/>	T.Y	<input type="checkbox"/> <input checked="" type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	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  

	1	2	3	4	5	6	7	8	9	10	11	12
A												T.IPC T.Large A T.Small A
B												T.IPC T.Large A T.Small A
C												T.IPC T.Large A T.Small A
D												T.IPC T.Large A T.Small A
E												T.IPC T.Large A T.Small A
F												T.IPC T.Large A T.Small A
G												T.IPC T.Large A T.Small A
H											T.IPC T.Large A T.Small A	T.IPC T.Large A T.Small A

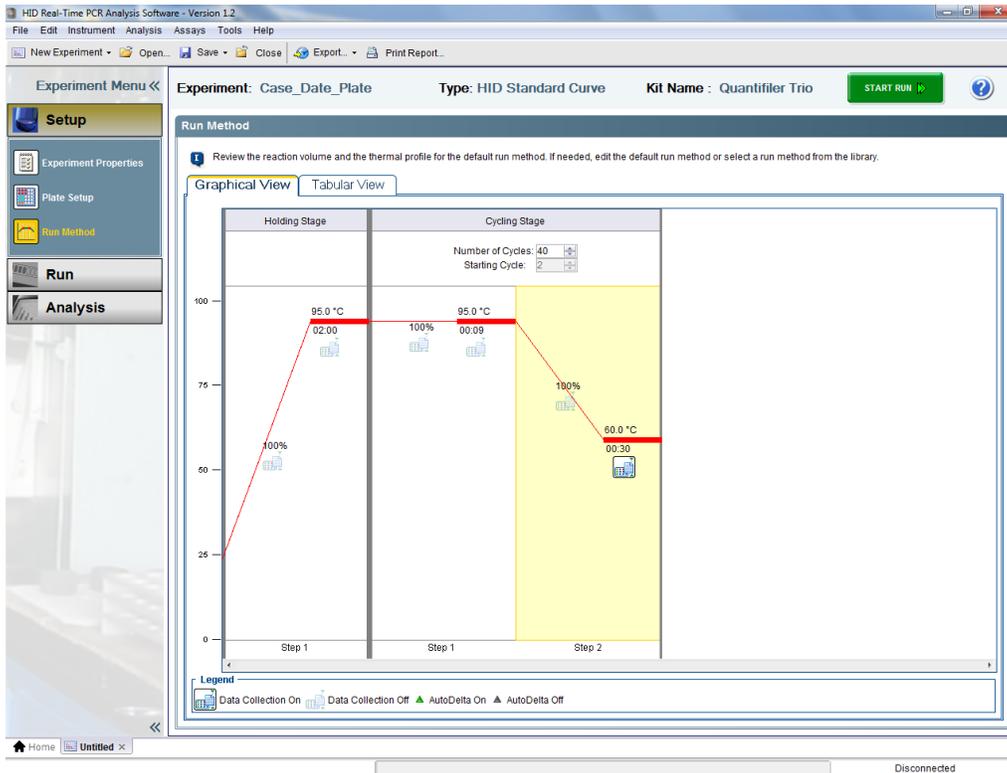
Wells:  9 Unknown    8 Standard    1 Negative Control      87 Empty

1.11 Verify the **Run Method**.

Holding Stage: 95.0°C for 2.00 minutes.

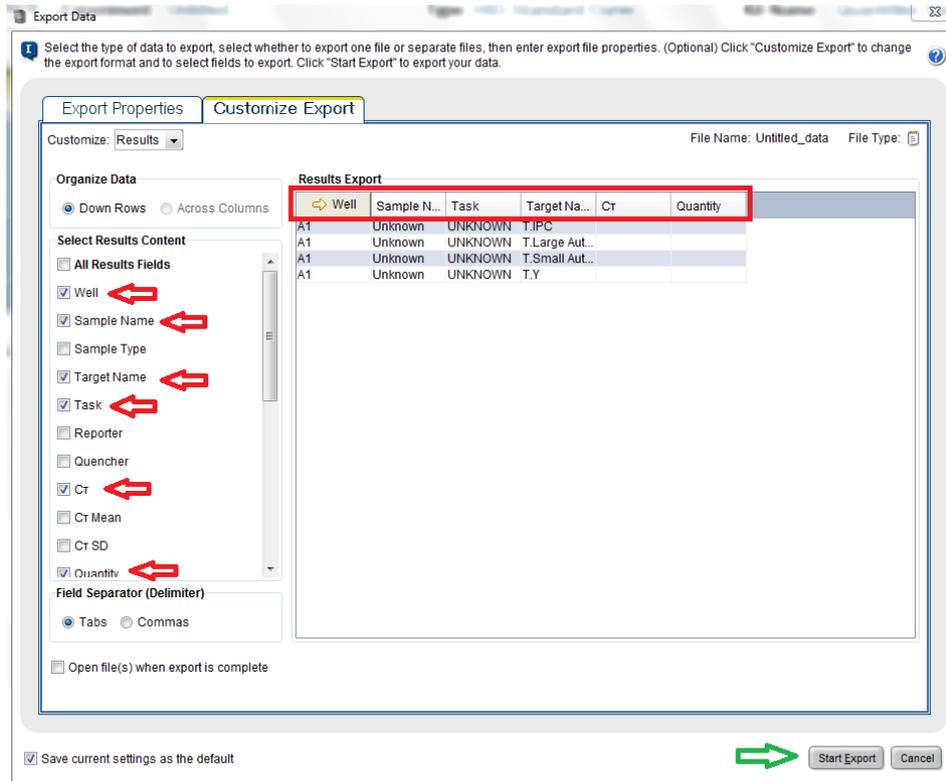
Cycling stage: 40 cycles of 95°C for 9 seconds, 60.0°C for 30 seconds.

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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<sub>T</sub>, and Quantity. Select **Save as default** at the bottom and **Start Export**.

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1.13 Overwrite the QuantifierTrio.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 **QuantifierTrio.edt**.

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS APPENDIX 3

## 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:

The screenshot shows the 'Security Screen' in the Applied Biosystems Data Collection Software. The interface includes a top navigation bar with 'Dashboard' and 'Edit' options, and a right-side menu with 'Library', 'Maintenance', 'Tools', 'Manage', 'Preferences', 'Help', and 'Log Out'. The main content area is titled 'Settings Resources' and features a sidebar with 'Manage Reports', 'Manage Users', and 'Manage Settings' sections. The 'Manage Settings' section is expanded to show 'Security' settings. The 'Security' settings are divided into two main sections: 'Account Setup' and 'Security Policies'. The 'Account Setup' section includes 'User Name' and 'User Password' configuration options. The 'User Name' section has a text box for 'Define name spacing' with 'Leading' and 'Trailing' checkboxes (unchecked) and a 'Consecutive' checkbox (checked). Below it is a 'Define name characteristics' section with checkboxes for 'Alpha' (checked), 'Numeric' (checked), 'Uppercase' (checked), 'Lowercase' (checked), and 'Special' (checked). The 'User Password' section has a text box for 'Define password spacing' with 'Leading' and 'Trailing' checkboxes (unchecked) and a 'Consecutive' checkbox (checked). Below it is a 'Define password characteristics' section with checkboxes for 'Alpha' (checked), 'Numeric' (checked), 'Uppercase' (checked), 'Lowercase' (checked), and 'Special' (checked). The 'Security Policies' section includes three sub-sections: 'Password Expiration', 'Account Suspension', and 'Session Timeout'. The 'Password Expiration' section has radio buttons for 'Yes' (unchecked) and 'No' (checked), with a text box for 'every 60 days' and 'Notify the user 3 days before expiration'. The 'Account Suspension' section has radio buttons for 'Yes' (unchecked) and 'No' (checked), with a dropdown for 'for the next 24 Hours' and a text box for 'if consecutively failing 3 time(s) within any 60 minute span'. The 'Session Timeout' section has radio buttons for 'Yes' (checked) and 'No' (unchecked), with a text box for 'for 60 minutes' and a note '(An instrument run is not considered user activity.)'. At the bottom of the screen, there are buttons for 'Setup Messaging Notifications', 'Set Defaults', and '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 a web application window titled "Edit Role". At the top, there is an "Instruction" box that reads: "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".

Below the instruction, there are two main sections:

1. Define New Role
  - \* Role Name:
  - 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

At the bottom of the window, there are two buttons: "Close" and "Save Role".

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**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

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

## 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:

The screenshot shows a window titled "New User:" with a close button in the top right corner. The main content area is titled "Setup a User" and contains the following fields and controls:

- \* User Name:** Text box with placeholder "first initial, last name, PE#"
- \* Password:** Password field with 7 dots
- \* Re-Enter Password:** Password field with 7 dots
- \* First Name:** Text box with value "Jane"
- \* User Role:** Dropdown menu with "Scientist" selected
- \* Dx User:** Check box (unchecked)
- Email:** Text box
- Comments:** Text area
- Created By Admin On:** Text box
- Last Updated On:** Text box
- Pre-expired**
- Password Expires On:** Text box
- MI:** Text box
- \* Last Name:** Text box with value "Doe"
- \* Status:** Dropdown menu with "Active" selected
- Electronic Signature:** Radio buttons for "Enable" (unchecked) and "Disable" (checked)
- Phone:** Text box

At the bottom of the window, there are two buttons: "Close" on the left and "Save" on the right.

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

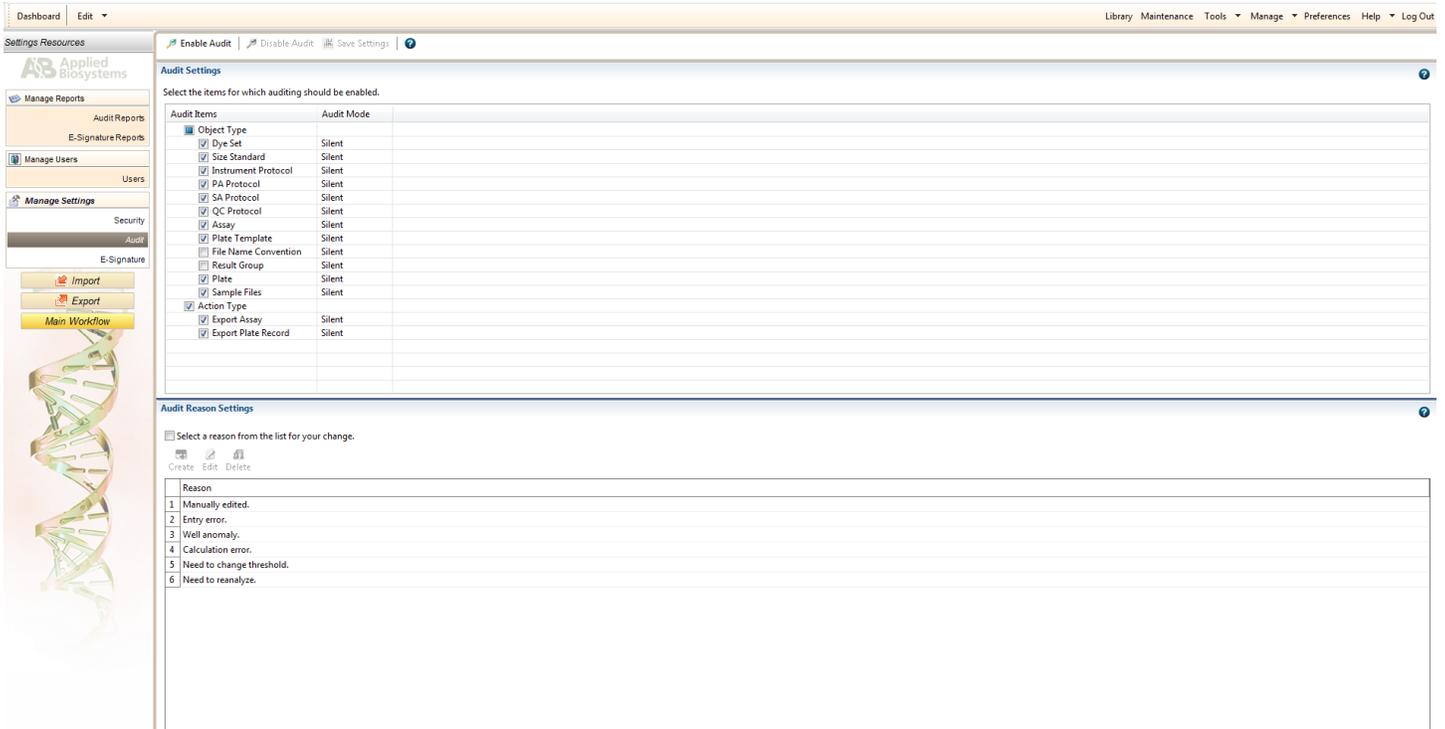
## 4.0 Manage Audit Settings

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

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
<input checked="" type="checkbox"/> Object Type	
<input checked="" type="checkbox"/> Dye Set	Silent
<input checked="" type="checkbox"/> Size Standard	Silent
<input checked="" type="checkbox"/> Instrument Protocol	Silent
<input checked="" type="checkbox"/> PA Protocol	Silent
<input checked="" type="checkbox"/> SA Protocol	Silent
<input checked="" type="checkbox"/> QC Protocol	Silent
<input checked="" type="checkbox"/> Assay	Silent
<input checked="" type="checkbox"/> Plate Template	Silent
<input type="checkbox"/> File Name Convention	Silent
<input type="checkbox"/> Result Group	Silent
<input checked="" type="checkbox"/> Plate	Silent
<input checked="" type="checkbox"/> Sample Files	Silent
<input checked="" type="checkbox"/> Action Type	
<input checked="" type="checkbox"/> Export Assay	Silent
<input checked="" type="checkbox"/> 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.



# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

## 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:  Capillary Length:  cm Polymer:

Dye Set:

Instrument Protocol Properties

\* Run Module:

\* Protocol Name:   Locked

Description:

Oven Temperature (°C):  Run Voltage (kVolts):  PreRun Voltage (kVolts):  Injection Voltage (kVolts):

Run Time (sec.):  PreRun Time (sec.):  Injection Time (sec.):  Data Delay (sec.):

▶ Advanced Options

Close Save

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

## 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:

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)

\* 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

Add Size(s) >>

Close Save

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

## 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:

**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 | 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	150	150	150	150	150	150

Common Settings

Use Smoothing: Light

Use Baselining (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

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

## 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:

**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

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

## 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:

Edit File Name Convention Fusion6C\_FNC

#### Setup a File Name Convention

\* Name: Fusion6C\_FNC     Locked    Color: Cyan

Select File Name Attributes

Preview of File Name: <Sample Name>\_<Instrument Protocol>\_<Date of Run>\_<Well Position>

Available Attributes	Selected Attributes
Capillary Number	Sample Name
Custom Text1	Underscore ( _ )
Custom Text2	Instrument Protocol
Custom Text3	Underscore ( _ )
Injection Number	Date of Run
Instrument Name	Underscore ( _ )
Owner Name	Well Position

Delimiters

Select a delimiter: Underscore ( \_ )    Add >>

Add between attributes    Add >>

Add a custom value to available attributes (optional)

Custom Text 1:    Custom Text 2:    Custom Text 3:

Select File Location

Default File Location    D:\Applied Biosystems\3500\Data

Custom File Location    Browse...

Close    Save

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

## 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 >>

Add >>

<< Remove

Move Up

Move Down

Selected Attributes

Plate Name

Underscore (\_)

IP 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>\_<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: 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

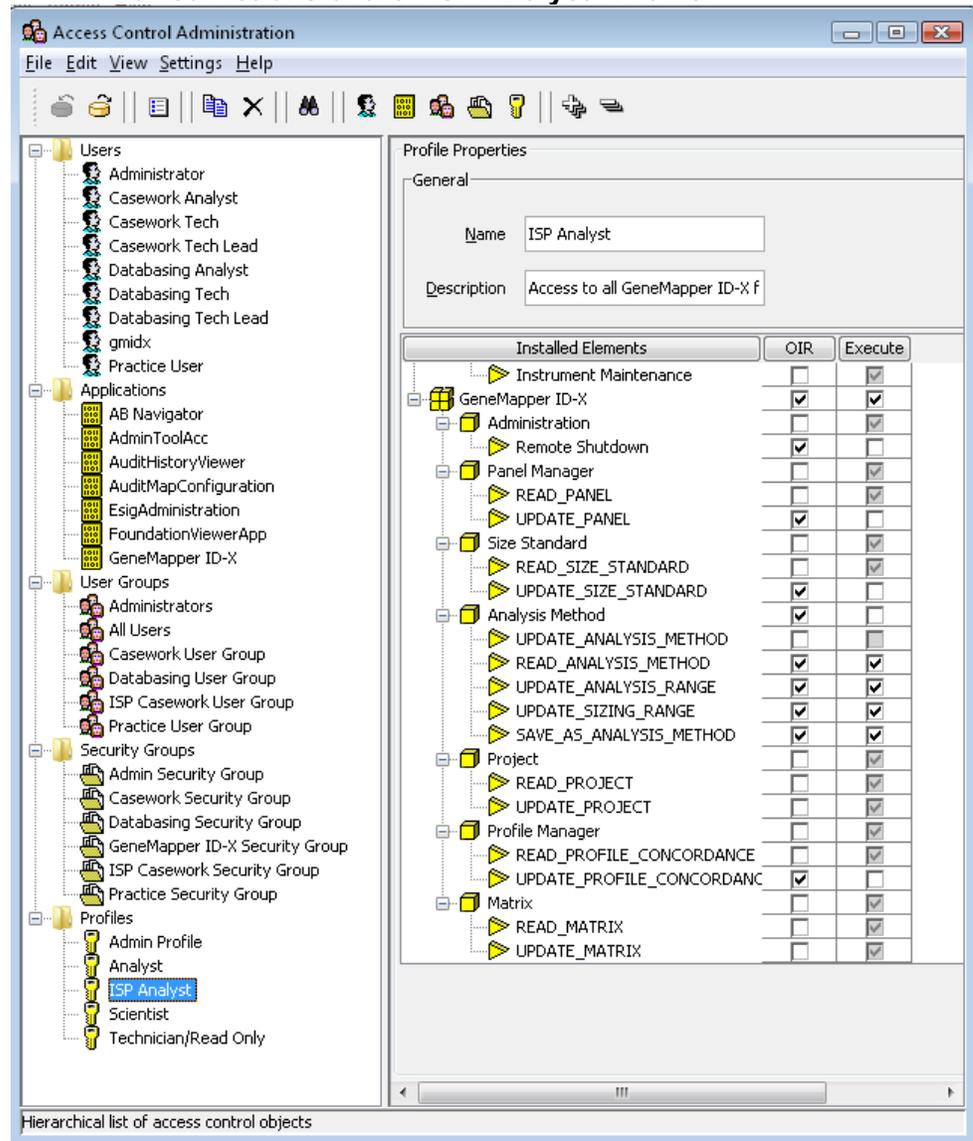
- 1.3.1 Open the Security Manager.
- 1.3.2 Select the appropriate default security group in the left navigation pane (e.g., “Casework Security Group”).
- 1.3.3 Select “Edit” then “Duplicate”.
- 1.3.4 In the “General” section, enter a name for the security group (e.g., “ISP Casework Security Group”).
- 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: 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: 150 | R: 150  
G: 150 | P: 150  
Y: 150 | O: 150

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 settings are as follows:

Section	Parameter	Value
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
	Cut-off value	0.2
Sample Spike Detection	Spike Detection	Enable

Buttons at the bottom: Save As, Save, Cancel, Help, and Factory Defaults.

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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 window title is 'Analysis Method Editor' and it has a close button (X) in the top right corner. The dialog is divided into several sections:

- Quality weights are between 0 and 1.**
- Sample and Control GQ Weighting:**
  - Broad Peak (BD): 0.8
  - Out of Bin Allele (BIN): 0.8
  - Overlap (OVL): 0.8
  - Marker Spike (SPK): 0.3
  - AMEL Cross Check (ACC): 0.0
  - Allele Number (AN): 1.0
  - Low Peak Height (LPH): 0.3
  - Max Peak Height (MPH): 0.3
  - Off-scale (OS): 0.8
  - 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 (dropdown)
  - Off-scale (OS): 1 (dropdown)
- SQ & GQ Ranges:**
  - Pass Range: (highlighted in green)
  - Low Quality Range: (highlighted in red)
  - Sizing Quality: From 0.75 to 1.0 (Pass) and From 0.0 to 0.25 (Low Quality)
  - Genotype Quality: From 0.75 to 1.0 (Pass) and From 0.0 to 0.25 (Low Quality)

At the bottom of the dialog, there are buttons for 'Save As', 'Save', 'Cancel', 'Help', and 'Reset Defaults'.

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:

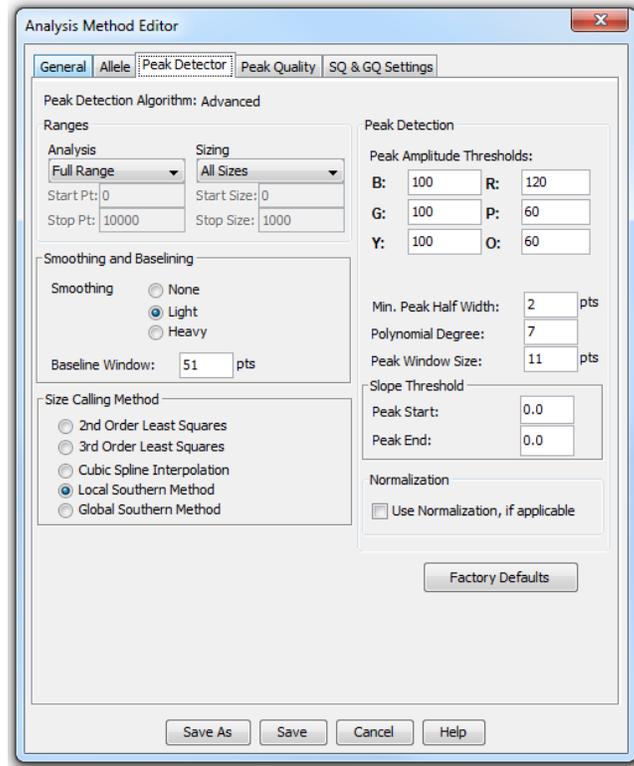
2.2.11.1 Enter the name "STRmix\_Fusion6C\_v3".

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:



2.2.11.4 Select the **Peak Quality** tab. Change the settings to match the following figure.

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

## Peak Quality Tab:

The screenshot shows the 'Analysis Method Editor' window with the 'Peak Quality' tab selected. The settings are as follows:

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

Buttons at the bottom: Save As, Save, Cancel, Help, and Factory Defaults.

## 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.
- 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”.
- 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”.

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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

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

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** [Close]

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.

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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:

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

The screenshot shows the 'STRmix' application window titled 'MANAGE KITS'. On the left, a sidebar lists 'Profiling Kits' with three entries: 'ISP\_Fusion6C', 'ISP\_Fusion6C\_v2', and 'ISP\_Fusion6C\_v3'. The 'ISP\_Fusion6C\_v3' kit is selected. The main panel has four tabs: 'GENERAL', 'STUTTERS', 'LOCI', and 'IMPORT'. The 'GENERAL' tab is active, showing the following settings:

- Kit Type:** Fusion6C
- Size Regression File:** Fusion6C\_SizeRegression\_ISP.csv (with an 'Edit' button)
- VARIANCE:**
  - Allelic Variance: 8.249, 1.296
  - Locus Amplification Variance: 0.008
  - Minimum Variance Factor: 0.5
  - Variance Minimization Parameter: 1,000

At the bottom right, there are 'Cancel' and 'Save' buttons.

This screenshot shows the same 'STRmix' application window, but with the 'STUTTERS' tab selected. The 'Profiling Kits' sidebar remains the same. The 'STUTTERS' tab displays the following settings:

- 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
  - Degradation Start Point:  Use Smallest Peak
  - Saturation Threshold: 28,000

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

GENERAL   STUTTERS   LOCI   IMPORT

---

## BACK STUTTER

<input checked="" type="checkbox"/> Stutter Enabled	Position Relative to Parent -1, 0
Inversely Proportional To Observed Height of Parent Allele	
Maximum Stutter Ratio <input type="checkbox"/> No Maximum   0.3	Variance 1.825, 9.576
Stutter Regression File Indiana_Fusion_6C_backward_stutter_v2.csv	
Stutter Exceptions File Indiana_stutter_exception_table_v2.csv	

# 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	<input checked="" type="checkbox"/>						
D3S1358	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D1S1656	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D2S441	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D10S1248	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D13S317	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Penta E	<input type="checkbox"/>	5	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D16S539	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
LOCUS NAME	GENDER?	REPEAT LENGTH	IGNORE?	DETECTION THRESHOLD	BACK STUTTER	FORWARD STUTTER	SE33 -2 STUTTER
D18S51	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D2S1338	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
CSF1PO	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Penta D	<input type="checkbox"/>	5	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
TH01	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
vWA	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D21S11	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D7S820	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

LOCUS NAME	GENDER?	REPEAT LENGTH	IGNORE?	DETECTION THRESHOLD	BACK STUTTER	FORWARD STUTTER	SE33 -2 STUTTER
D5S818	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
TPOX	<input type="checkbox"/>	4	<input type="checkbox"/>	100	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D8S1179	<input type="checkbox"/>	4	<input type="checkbox"/>	120	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D12S391	<input type="checkbox"/>	4	<input type="checkbox"/>	120	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D19S433	<input type="checkbox"/>	4	<input type="checkbox"/>	120	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
SE33	<input type="checkbox"/>	4	<input type="checkbox"/>	120	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
D22S1045	<input type="checkbox"/>	3	<input type="checkbox"/>	120	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
DYS391	<input checked="" type="checkbox"/>						
FGA	<input type="checkbox"/>	4	<input type="checkbox"/>	60	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
DYS576	<input checked="" type="checkbox"/>						
DYS570	<input checked="" type="checkbox"/>						

- 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\_African American  
NIST\_2017\_Asian  
NIST\_2017\_Caucasian  
NIST\_2017\_Hispanic

### 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

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

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\_Caucasian ▼

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 following four population databases shall be used to perform statistical calculations: NIST\_2017\_Caucasian, NIST\_2017\_African\_American, NIST\_2017\_Hispanic, and NIST\_2017\_Asian.
- 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 ( $\theta$ ): 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 <a href="#">NIST_2017_African American.csv</a> <span style="float: right;">▼ <span style="background-color: #76b82a; color: white; padding: 2px 5px; border-radius: 3px;">Edit</span></span>		
Population Proportion 0.096	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

Allele Frequency File <a href="#">NIST_2017_Asian.csv</a> <span style="float: right;">▼ <span style="background-color: #76b82a; color: white; padding: 2px 5px; border-radius: 3px;">Edit</span></span>		
Population Proportion 0.021	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

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

Allele Frequency File NIST_2017_Caucasian.csv <span style="float: right;">▼ <span style="background-color: #76b82a; color: white; padding: 2px 5px; border-radius: 3px;">Edit</span></span>		
Population Proportion 0.8	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

Allele Frequency File NIST_2017_Hispanic.csv <span style="float: right;">▼ <span style="background-color: #76b82a; color: white; padding: 2px 5px; border-radius: 3px;">Edit</span></span>		
Population Proportion 0.067	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

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

## 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.

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	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Post Burn-In Summary	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Evidence Peak Issues	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Variance Charts	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Contributor Degradation Curves	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Locus Efficiencies	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Evidence Files	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Reference Files	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Settings	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Kit Files	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Interpretation Details	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Weights	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Component Interpretation	<input checked="" type="checkbox"/>	<input type="checkbox"/>
LR Summary	<input checked="" type="checkbox"/>	<input type="checkbox"/>

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

< **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**.

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

**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 %
<b>D3S1358</b>	9.13	<b>14.1</b>	0.74	<b>1.43</b>	0.64	<b>1.39</b>
<b>D1S1656</b>	8.51	<b>14.3</b>	0.91	<b>1.87</b>	0.97	<b>1.84</b>
<b>D2S441</b>	5.03	<b>8.55</b>	0.38	<b>0.69</b>	0.78	<b>1.49</b>
<b>D10S1248</b>	8.31	<b>13.8</b>	0.73	<b>1.46</b>	0.35	<b>1.04</b>
<b>D13S317</b>	5.05	<b>10.9</b>	0.39	<b>0.65</b>	0.87	<b>2.01</b>
<b>Penta E</b>	2.75	<b>6.99</b>	0.41	<b>0.92</b>	0.37	<b>0.84</b>
<b>D16S539</b>	7.04	<b>12.6</b>	0.61	<b>1.27</b>	1.07	<b>2.37</b>
<b>D18S51</b>	8.08	<b>14.9</b>	0.80	<b>1.59</b>	1.07	<b>3.11</b>
<b>D2S1338</b>	8.23	<b>13.8</b>	0.78	<b>1.49</b>	0.80	<b>1.54</b>
<b>CSF1PO</b>	6.50	<b>12.1</b>	0.51	<b>0.81</b>	1.08	<b>2.49</b>
<b>Penta D</b>	1.90	<b>3.91</b>	0.43	<b>1.10</b>	0.56	<b>1.37</b>
<b>TH01</b>	2.07	<b>4.60</b>	0.34	<b>0.82</b>	0.38	<b>0.79</b>
<b>vWA</b>	7.43	<b>16.8</b>	0.75	<b>1.57</b>	0.66	<b>1.74</b>
<b>D21S11</b>	8.51	<b>12.3</b>	0.72	<b>1.42</b>	1.07	<b>1.90</b>
<b>D7S820</b>	5.38	<b>11.1</b>	0.53	<b>1.12</b>	0.76	<b>1.76</b>
<b>D5S818</b>	6.53	<b>11.1</b>	0.55	<b>1.12</b>	1.00	<b>1.88</b>
<b>TPOX</b>	3.01	<b>6.42</b>	0.31	<b>0.50</b>	0.23	<b>0.31</b>
<b>D8S1179</b>	6.96	<b>10.7</b>	0.60	<b>1.08</b>	0.70	<b>1.28</b>
<b>D12S391</b>	9.68	<b>17.9</b>	1.01	<b>2.40</b>	0.81	<b>2.38</b>
<b>D19S433</b>	7.85	<b>12.5</b>	0.81	<b>2.37</b>	1.06	<b>2.58</b>
<b>SE33</b>	10.5	<b>18.1</b>	1.05	<b>2.02</b>	1.04	<b>2.25</b>
<b>D22S1045</b>	7.55	<b>18.1</b>	1.01	<b>1.86</b>	4.50	<b>9.48</b>
<b>DYS391</b>	6.79	<b>8.71</b>	0.77	<b>1.64</b>	0.73	<b>2.02</b>
<b>FGA</b>	7.21	<b>12.4</b>	0.81	<b>1.86</b>	0.77	<b>2.21</b>
<b>DYS576</b>	8.89	<b>12.1</b>	0.89	<b>2.27</b>	1.28	<b>2.83</b>
<b>DYS570</b>	8.96	<b>12.9</b>	0.97	<b>1.62</b>	1.07	<b>1.82</b>

	Half Backward n-2 bp	
	Mean %	Mean + 3SD %
<b>D1S1656</b>	1.68	<b>2.97</b>
<b>SE33</b>	4.46	<b>5.53</b>
<b>FGA</b>	0.47	<b>0.97</b>

	Half Forward n+2 bp	
	Mean %	Mean + 3SD %
<b>SE33</b>	1.26	<b>2.25</b>

**INDIANA STATE POLICE  
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APPENDIX 7  
REPORT WORDING**

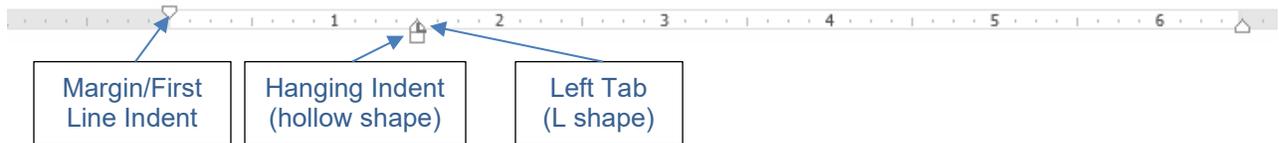
**REPORT WORDING RULES**

These are general wording guidelines for commonly occurring test results. Alterations must be approved by a supervisor. Any words in these guidelines that are in italics should be changed to reflect your results. 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. A margin (first line indent) shall be set at 0. For item results, a hanging indent and a left tab will each be set at 1.5 (see below). General results have no indent.



<b>Item 001</b>	<b>Evidence Collection Kit – Jane Smith:</b> Items 001A-001J were present in or prepared from items in the kit. Items 001A1, 001C and 001D were retained.
Item 001A	Blood standard: A stain card was prepared as item 001A1.
Item 001A1	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): <u>Sperm fraction</u> The DNA profile from the combined swabs was interpreted

3. Original items and their description shall be bolded. All other information shall be in normal Verdana 12. When a subitem is reported it shall be bolded if the original item is not present and was previously reported.

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4. Subitems shall be listed to the left (no indent) regardless of whether they are retained or not (see examples [2](#) and [3](#)).
5. Sub-subitems shall be grouped by results under underlined headings (see example [5](#)).
6. Sperm and Non-sperm fractions, where DNA results are different, shall be separated under underlined headings (see item 001C in [example 1](#)).
7. Exclusions and comparisons without LR's (inclusions of unknown individuals) shall be listed with the contributor assumption before the bullets (see item 002A in [example 5](#)).
8. Likelihood ratios:
  - Likelihood ratios shall be formatted with bullets.
  - Clicking the Bulleted List button while tabbed at 1.5 should insert the correct formatting:
    - First Line Indent at 1.75
    - Hanging/Left Indent (NOT Left Tab) at 2.0
  - 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. An appendix with appropriate definitions and explanations shall be attached to the end of each report with a "Biology" designation in LIMS.
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. Retention statements generally will be in the opening paragraph for an item.
9. The significant contents of an item shall be listed in the results section.

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10. 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.
11. Individual items from another case should not be listed in the item descriptions or as separate items in the body of the report.
12. Similar results within an item may be grouped together.
13. Swabs created for DNA sampling and not retained shall be reported as a "sample" of the item being tested.
14. If two or more swabs are collected by the analyst as one sample, they do not need to be itemized separately.
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. Untested swabs do not need to be itemized separately.
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 Y target.
18. When differential extractions are employed, the terminology "sperm [cell] fraction" and "non-sperm [cell] fraction" shall be used in the report, except where the following situations apply:
  - When both fractions of a differential extraction exhibit the same testing result, 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 the appropriate insufficient male DNA wording that indicates the largest amount of male DNA present between the two fractions.

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- If sperm/non-sperm terminology is not used in the results section, the sperm/non-sperm terminology disclaimer shall not be included.

## 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, they do not have to be mentioned at every sample from which they are visually excluded.
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.

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## STATISTICAL EVALUATIONS

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 more likely for all four population groups the phrase "at least 1 trillion" shall be utilized in place of the calculated LR number.
  - o A verbal scale shall accompany the LR result in an effort to provide additional explanation of the weight of the evidence.

<b>HPD Likelihood Ratio (or 1/LR)</b>	<b>Support for Inclusion</b>	<b>Support for Exclusion</b>
1	uninformative	uninformative
$2 \leq \text{HPD}_{\text{LR}} < 100$	limited	limited
$100 \leq \text{HPD}_{\text{LR}} < 10,000$	moderate	exclusion
$10,000 \leq \text{HPD}_{\text{LR}} < 1,000,000$	strong	exclusion
$1,000,000 \leq \text{HPD}_{\text{LR}}$	very strong	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.
  - o 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

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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 LR 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 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 LR 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. 99% 1-sided lower HPD LR values will be calculated for the four subpopulations (African American, Asian, Caucasian and Hispanic).
- When all four LR values are in the support of H1, the lowest LR of the four calculated populations will be reported, rounded down to two significant figures.
    - If the lowest LR in support of H1 is less than 2, it shall be rounded down to 1.0 (uninformative).
  - If any of the four LR values is in support of H2, the highest 1/LR value favoring H2 will be reported, rounded up to two significant figures.
    - If the highest LR in support of H2 is less than 2, it shall be rounded up to 2.0 (limited support for exclusion).

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- If any of the four calculated populations have a LR of zero, the LR shall be reported as zero.
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.
  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 (2.11.1.23.1) or amended (2.11.1.23.2 and .3) 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, the new statistical worksheets shall be uploaded under the original request number 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**

Superscripts shall refer to the Report Appendix to reflect the test(s) supporting the result.

1 = presumptive tests, 2 = confirmatory tests. a = seminal material, b = blood. c = Takayama test.  
3 = hair. 4 = trace

### **NEGATIVE RESULT**

*No seminal material/blood was detected.<sup>1a/1b</sup>*

### **POSITIVE CONFIRMATORY RESULT**

[Human] Blood was confirmed.<sup>1b, 2b/2c</sup> X samples were retained as item XA.

Seminal material was confirmed.<sup>1a, 2a</sup> X samples were retained as item XA.

### **POSITIVE PRESUMPTIVE RESULT**

*Blood/seminal material was indicated by presumptive testing.<sup>1b/1a</sup> [X samples were retained as item XA.]*

### **NEGATIVE CONFIRMATORY RESULT**

In addition to positive presumptive result.

Confirmatory testing was negative [for *blood/seminal material*].<sup>2b/2c/2a</sup>

### **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.

### **TRACE EVIDENCE**

The reporting term "collected" shall only be used if the evidence is being retained within a subitem.

Potential trace evidence was observed/collected and retained as item XA.<sup>4</sup>

### **HAIR STATEMENTS**

The reporting term "collected" shall only be used if the hair is being retained within a subitem.

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Hair demonstrating characteristics of human hair with the potential for nuclear DNA analysis was *observed/collected and retained as item XA*.<sup>3</sup>

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.<sup>3</sup>

Hair demonstrating characteristics not consistent with human hair was observed.<sup>3</sup>

## **SUBITEMS NOT EXAMINED**

Placed at the end of the item.

The *item description, item description and item description* (items XA-XZ) were not examined.

## **EXCLUSION**

Excluded visually or with an individual STRmix™ calculated LR=0 or 1/LR ≥100.

To be placed with the appropriate interpretation statement and any other inclusions, before the LR bullet points.

*PERSON* is excluded [as a contributor to the DNA profile/mixture].

## **DESIGNATED UNKNOWN INDIVIDUAL**

Determined at >12 loci in another profile and included in a mixture at the analyst's discretion.

*Unknown female/male/individual #1* cannot be excluded as a contributor to the mixture.

## **IDENTICAL TWINS**

To be placed at the beginning of the results section.

The DNA profiles obtained from *Jane Doe* and *Susan Doe* demonstrated the same DNA profile. This is typically associated with identical twins. The conclusions reflect the comparisons with the provided standards.

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## SINGLE SOURCE STATEMENTS

The DNA profile [from *item description*] was interpreted as originating from a single individual.

- 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.
- Statistical analysis provides *limited/moderate/strong/very strong* support for the **inclusion** of *PERSON*.

### ONE ADDITIONAL ALLELE

One additional allele may be detected at Amelogenin or a Y-STR locus.

Add the following to the interpretation paragraph above, after “from a single individual.”

[However, one additional allele was detected from which no conclusion can be drawn.]

### UNKNOWN PROFILE

STRmix™ deconvolution performed, but no LR calculated.

The DNA profile [from *item description*] was interpreted as originating from a single unknown *male/female/individual* [(Unknown *Male/Female/Individual #*)].

### UNKNOWN PROFILE + 1 ADDITIONAL ALLELE

STRmix™ deconvolution performed, but no LR calculated.

One additional allele may be detected at Amelogenin or a Y-STR locus.

The DNA profile [from *item description*] was interpreted as originating from a single unknown *male/female/individual* [(Unknown *Male/Female/Individual #*)]; however, one additional allele was detected from which no conclusion can be drawn.

### CONSISTENT WITH ELIMINATION STANDARD

No deconvolution performed.

The DNA profile [from *item description*] was interpreted as originating from a single individual. Assuming *PERSON* as a contributor, no additional alleles were detected.

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## **CONSISTENT WITH ELIMINATION + 1 ADDITIONAL ALLELE**

One additional allele may be detected at Amelogenin or a Y-STR locus. No deconvolution performed.

The DNA profile [from *item description*] was interpreted as originating from a single individual. Assuming *PERSON* as a contributor, one additional allele was detected from which no conclusion can be drawn.

## **MIXTURE STATEMENTS**

### **UNKNOWN PROFILES ONLY**

STRmix™ deconvolution performed, but no LR calculated. The sex and number of specific unknown individuals may be adjusted (or omitted) as appropriate.

The DNA profile [from *item description*] was interpreted as a mixture of *two/three/four* unknown individuals [, including at least one male].

### **UNKNOWN PROFILES ONLY WHEN VARNOC UTILIZED**

The DNA profile [from *item description*] was interpreted as a mixture of *N* or *N+1* individuals [, including at least one male].

### **CONDITIONED PROFILE + UNKNOWN(S)**

STRmix™ deconvolution performed, but no LR calculated. The sex and number of specific unknown individuals may be adjusted (or omitted) as appropriate.

The DNA profile [from *item description*] was interpreted as a mixture of two individuals. Assuming *PERSON* as a contributor, the mixture contains an unknown *male/female/individual* [(Unknown *Male/Female/Individual #*)].

The DNA profile [from *item description*] was interpreted as a mixture of *three/four* individuals [, including at least one male]. Assuming *PERSON* as a contributor, the mixture contains *two/three* unknown individuals.

## **CONSISTENT WITH MULTIPLE ELIMINATION STANDARDS**

No deconvolution performed.

The DNA profile [from *item description*] was interpreted as a mixture of two individuals. Assuming *PERSON1* and *PERSON2* as contributors, no additional alleles were detected.

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## 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 item description] was interpreted as a mixture of two individuals. Assuming *PERSON* and the corresponding *sperm/non-sperm* fraction as contributors, no additional alleles were detected.

## GENERAL MIXTURE STATEMENT

$LR \geq 2$ , result supports H1, inclusion of one person of interest.

The DNA profile [from *item description*] was interpreted as a mixture of  $N$  individuals.

- 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.
- Statistical analysis provides *limited/moderate/strong/very strong* support for the **inclusion** of *PERSON*.

## MIXTURE WITH CONDITIONING

$LR \geq 2$ , result supports H1, inclusion of one person of interest.

The DNA profile [from *item description*] was interpreted as a mixture of two individuals. *PERSON2* was assumed 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.
- Statistical analysis provides *limited/moderate/strong/very strong* support for the **inclusion** of *PERSON1*.

## LIMITED EXCLUSIONARY LR

STRmix™ LR calculated,  $2 \leq 1/LR < 100$ , result supports H2.

The DNA profile [from *item description*] was interpreted as a mixture of  $N$  individuals.

- 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].

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- Statistical analysis provides limited support for the **exclusion** of *PERSON*.

## 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 DNA profile originated from *Jane Doe* or *John Doe* individually. However, there is no scientific support for the proposition that the DNA profile originated from *Jane Doe* and *John Doe* together. Refer to the independent inclusionary statistics reported above.

## INCONCLUSIVE LR

STRmix™ LR calculated, LR rounds to 1.0.

The DNA profile [from *item description*] was interpreted as a mixture of  $N$  individuals.

- 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**.

## ALTERNATE PROPOSITIONS

The DNA profile [from *item description*] was interpreted as a mixture of two individuals. The probability of the evidence has been calculated by considering multiple propositions.

### **Person1 and Person2 were considered as contributors:**

- The DNA profile is *at least 1 trillion/LR* times more likely if it originated from *PERSON1* and *PERSON2* than if it originated from two unknown, unrelated individuals.
- Statistical analysis provides *limited/moderate/strong/very strong* support for the **inclusion** of both *PERSON1* and *PERSON2*.

**Assuming Person2 as a contributor, Person1 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 *PERSON2* and *PERSON1* than if it originated from *PERSON2* and an unknown, unrelated individual.
- Statistical analysis provides *limited/moderate/strong/very strong* support for the **inclusion** of *PERSON1*.

## VARIABLE NUMBER OF CONTRIBUTORS

The appropriate mixture wording should be adjusted to accurately reflect results. One example below.

The DNA profile [from *item description*] was interpreted as a mixture of *N* or *N+1* individuals.

- 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.
- Statistical analysis provides *limited/moderate/strong/very strong* support for the **inclusion** of *PERSON*.

## INCONCLUSIVE SAMPLES

### NO ALLELES DETECTED

[Item description] failed to demonstrate a DNA profile.

### INCONCLUSIVE NUMBER OF CONTRIBUTORS

The DNA profile *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 is a mixture. *PERSON* was assumed as a contributor. No further conclusions can be drawn due to the uncertainty in the number of contributors.

### >4 CONTRIBUTORS

No conclusion can be drawn because the DNA profile is a mixture of more than four individuals.

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## FAILED CONTROL

No conclusion can be drawn because a test control did not meet required parameters.

## 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 *item description*] was interpreted as originating from a single identified individual from the Indiana State Police Laboratory 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 Laboratory Division Quality Assurance DNA Database was present and assumed as a contributor.

- The DNA profile is *at least 1 trillion/LR* times more likely if it originated from the Database individual and *PERSON1* than if it originated from the Database individual and an unknown, unrelated individual.
- Statistical analysis provides *limited/moderate/strong/very strong* support for the **inclusion** of *PERSON1*.

## 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

*Item description* demonstrated an insufficient quantity of DNA for further analysis.

## INSUFFICIENT MALE DNA for AUTOSOMAL ANALYSIS

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*Item description* demonstrated an insufficient quantity of male DNA for autosomal STR analysis.

**INSUFFICIENT MALE DNA**

*Item description* demonstrated an insufficient quantity of male DNA for further analysis.

**UNDETECTED MALE DNA**

No male DNA was detected [on *item description*].

**SAMPLE TRIAGED**

*Item description* 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 EXAMINED**

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 – *John Smith*:** [The standard was retained as item *XA*.]

**STAIN CARD**

A stain card was prepared [and retained as *item XA*].

**PROFILE DEVELOPED**

The DNA profile [of *PERSON*] was [previously] developed.

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## PROFILE DEVELOPED BUT NOT INTERPRETED

The sample was processed but not interpreted due to a lack of evidence for comparison.

## MULTIPLE STANDARDS

The standard was not *retained/examined* due to the presence of multiple standards.

## STANDARD COMPARED TO EVIDENCE IN OTHER CASE

For the case that contains the standard

The DNA profile of *PERSON* (item *XXA*) was compared to the evidence in Indiana State Police Laboratory 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 Laboratory 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

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Additional information is required regarding *item number* 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).

## **ALTERNATE CALCULATIONS**

To be used additional/alternate LR's were calculated but not included in report.

Alternate calculations have been performed for item(s) *XA, XB and XC* and are available upon request.

## **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).*

## **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.

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## 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.

## 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* or] any suspect(s).

## Y-STR ANALYSIS PENDING

This case will be evaluated for possible Y-STR analysis. Any results from additional testing will be provided separately.

## PATERNITY/KINSHIP ANALYSIS PENDING

When completed, *Paternity/Kinship* analysis of the DNA profiles determined for *John Doe, Jane Doe, and Baby Doe* will be provided separately.

## SAMPLES SENT TO ANOTHER LABORATORY

Item(s) XA [were/was] sent to *XYZ Laboratory, city, state* for [additional] analysis as per the authority of *John Smith, Police Department*.

## SAMPLES SENT TO UNT

[A portion of] The *sample/bone* (item XA) was sent to the University of North Texas Center for Human Identification Laboratory for [additional] DNA analysis and entry into the National Missing Persons DNA Database on *date* as per the authority of *John Smith, Police Department*.

## REQUEST FOR FAMILY REFERENCE STANDARDS

Upon submission of an appropriate DNA standard (such as an oral/buccal swab standard) from *John Doe* and the required Family Reference Standard paperwork,

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samples will be sent to the University of North Texas Center for Human Identification Laboratory for analysis and entry into the Biological Relatives of Missing Persons Database.

## 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 a buccal/oral swab standard) is required before analysis can be completed. The evidence will be returned to the submitting agency.

## EXAMPLES

### Example 1: Homicide with Kit

- Item 001**      **Evidence Collection Kit – Jane Smith:** Items 001A-001J were present in or prepared from items in the kit. Items 001A1, 001C and 001D were retained.
- Item 001A      Blood standard: A stain card was prepared as item 001A1.
- Item 001A1     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.
- 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.
  - Statistical analysis provides very strong support for the **inclusion** of John Doe.
- Non-sperm fraction  
The DNA profile from the combined swabs was interpreted as a mixture of two individuals. Assuming Jane Smith and the

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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. Assuming Jane Smith as a contributor, an unknown male profile was determined (Unknown Male #1).
- 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.

The pubic hair combings, head hair combings, foreign material collection, and underwear (items 001G through 001J) were not examined.

**Item 002 DNA standard – John Doe:** The DNA standard was retained as item 002A.

Item 002A The DNA profile was developed.

**Item 003 Knife:** Human blood was confirmed.<sup>1b, 2b</sup> Two samples were retained as item 003A.

Item 003A Item 003A1  
The DNA profile from the knife blade was interpreted as a mixture of two individuals. John Doe is excluded as a contributor. Unknown Male #1 cannot be excluded 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.
- Statistical analysis provides moderate support for the **inclusion** of Jane Smith.

#### Item 003A2

The DNA profile from the knife handle was interpreted as a single unknown male (Unknown Male #1).

**Item 004 Shirt:** Human blood was confirmed.<sup>1b, 2b</sup> Seminal material was indicated by presumptive testing.<sup>1a</sup> Four samples were retained as item 004A.

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Item 004A

## Item 004A1 Sperm Fraction

The DNA profile was interpreted as originating from a single unknown male (Unknown Male #2).

## Item 004A1 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 004A2 and 004A4

The DNA profiles were each interpreted as originating from a single individual.

- 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 **inclusion** of Jane Smith.

## Item 004A3

The sample demonstrated an insufficient quantity of DNA for further analysis.

DNA profiles from items 001D and 004A1 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. All extracted subitems are retained.

**Item 005**

**Bag of trash:** Items 005A-005D were present in the bag of trash. Items 005A and 005B were retained.

Item 005A

Condom wrapper: The DNA profile was interpreted as originating from a single individual. Bob Roberts is excluded as a contributor to the DNA profile.

- The DNA profile is 17 times more likely if it originated from Jane Smith than if it originated from an unknown, unrelated individual.

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- Statistical analysis provides limited support for the **inclusion** of Jane Smith.

Item 005B Piece of gum: Seminal material was indicated by presumptive testing.<sup>1a</sup>

## Sperm Fraction

The DNA profile was interpreted as a single individual.

- The DNA profile is 17 times more likely if it originated from Bob Roberts than if it originated from an unknown, unrelated individual.
- Statistical analysis provides limited support for the **inclusion** of Bob Roberts.

## Non-Sperm Fraction

The DNA profile was interpreted as a single individual.

- 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.
- Statistical analysis provides very strong support for the **inclusion** of Jane Smith.

The banana peel and apple core (items 005C and 005D) were not examined.

### **Example 3: Property crime with serological testing**

Submitted swabs that are considered distinctly separate (due to case circumstances, sample purpose, serology results, etc.), but not retained, still 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.<sup>1b</sup> Confirmatory testing was negative.<sup>2c</sup>

The DNA profile was interpreted as originating from a single unknown female (unknown female #1).

Item 001B No blood was detected on the remaining swab.<sup>1b</sup> No DNA analysis was performed.

#### **Item 002 Swabs of floor (2):**

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Blood was indicated on the two swabs. <sup>1b</sup> Blood was confirmed on one swab. <sup>1c</sup> No confirmatory testing was performed on the remaining swab.

The DNA profile from the combined swabs was interpreted as originating from a single unknown male (unknown male #1).

**Item 003**      **Buccal swab standard – Robert Jones:** The standard was retained as item 003A.

Item 003A      The DNA profile was developed.

## Example 4: Property crime – no serological testing

**Item 001**      **Crowbar:** A sample of the crowbar demonstrated an insufficient quantity of DNA for further analysis.

**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 5: Crime against person – many areas on one item

**Item 001**      **Victim's Blanket:** Human blood was confirmed. <sup>1b, 2b</sup> Thirty samples were retained as item 001A.

Item 001A      Items 001A4 and 001A12  
Each sample demonstrated an insufficient quantity of DNA for further analysis.

Items 001A19 and 001A25  
Each DNA profile was interpreted as originating from a single individual. Assuming Mary Doe as a contributor, no additional alleles were detected.

Items 001A1-3, 5-11, 13-18, 20-24, and 26-30  
No DNA analysis was performed.

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**Item 002**      **Shirt:** Human blood was confirmed.<sup>1b, 2b</sup> A sample was retained as item 002A.

Item 002A      The DNA profile was interpreted as a mixture of two individuals. The probability of the evidence has been calculated by considering multiple propositions. Bob Doe is excluded as a contributor.

**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.
- Statistical analysis provides strong support for the **inclusion** of Mary Doe.

**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.
- Statistical analysis provides moderate support for the **inclusion** of John Doe.

**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.

**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 Laboratory 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. **Serological testing:** Superscripts in the report refer to the test(s) listed below.
  1. Presumptive testing indicates the possible presence of the substance in question.
    - a) Seminal material: Acid Phosphatase chemical test
    - b) Blood: Kastle-Meyer/phenolphthalein chemical test
  2. Confirmatory testing identifies the substance in question.
    - a) Seminal material: microscopic sperm search
    - b) Human blood: HemDirect™ immunological test
    - c) Blood: Takayama microcrystalline test
  3. Potential human hair is detected with a macroscopic and microscopic examination.
  4. Trace evidence may include but is not limited to paint, glass, hair, and fibers.
  
- II. The terms "**sperm fraction**" and "**non-sperm fraction**" refer to the two portions of the extract generated during a differential extraction procedure. The presence of DNA in the "sperm fraction" does not confirm the presence of seminal material and may or may not indicate the type of cells from which the DNA originated.
  
- III. **Insufficient DNA:** Following extraction and quantitation, DNA profiles are not developed from samples which do not yield enough DNA based on the laboratory'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 laboratory's established threshold. These samples may still be eligible for Y-STR analysis.
  
- 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. Mixtures with limited or low quality data and mixtures of more than four contributors are not interpretable by the laboratory'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 for the Caucasian, African American, Hispanic, and Asian populations are performed. The lowest LR is reported unless all calculated LRs exceed 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.
  
- X. The 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 for Inclusion	Support for Exclusion
1	uninformative	uninformative
$2 \leq LR < 100$	limited	limited
$100 \leq LR < 10,000$	moderate	exclusion
$10,000 \leq LR < 1,000,000$	strong	exclusion
$1,000,000 \leq LR$	very strong	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 Laboratory. The remainder of the evidence or the original packaging will be returned.

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- 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.

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**Relationship Testing Wording**

<b>DNA STR Locus</b>	<b>Mother Item 1A</b>	<b>Child Item 2A</b>	<b>Alleged Father Item 3A</b>	<b>Paternity Index (Race)</b>
AMELOGENIN				
D3S1358				
D1S1656				
D2S441				
D10S1248				
D13S317				
Penta E				
D16S539				
D18S51				
D2S1338				
CSF1PO				
Penta D				
TH01				
vWA				
D21S11				
D7S820				
D5S818				
TPOX				
D8S1179				
D12S391				
D19S433				
SE33				
D22S1045				
DYS391				
FGA				
DYS576				
DYS570				

**Combined Paternity Index =**  
**Probability of Paternity = %**

All tested loci shall be displayed on the Certificate of Analysis, even for loci not used in statistical evaluations.

**PARENTAGE RESULTS:**

**A parentage case where alleged individual cannot be excluded:**

*John Doe* (item *XA*), the alleged father, cannot be excluded as the biological father of *the child* (item *XA*). The genetic results listed below are *CPI* times more likely if *John Doe* is the biological father of *the child* than a random, untested, unrelated man in the

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*Caucasian/African American/Hispanic* population. The probability of paternity (assuming a prior probability of 0.5) is [W] %.

## **A parentage case where alleged individual can be excluded:**

Given the lack of genetic markers that must be contributed to the child by the biological father in the genetic results listed below, *John Doe* (item XA), the alleged father, can be excluded as the biological father of *the child* (item XA).

## **A parentage case with inconclusive results:**

The genetic results listed below do not strongly favor the hypothesis of *John Doe* (item XA), the alleged father, being the biological father of *the child* (item XA). The Combined Paternity Index of *CPI* is low and does not meet our laboratory standards in order to establish paternity; therefore, these results are inconclusive. The genetic results are based only on the current data and additional genetic testing is recommended.

## **A reverse parentage case where alleged individuals cannot be excluded:**

*Jane Doe* (item XA), the alleged mother, and *John Doe* (item XA), the alleged father, cannot be excluded as the biological parents of *the child*. The genetic results listed below are *CPI* times more likely if *Jane Doe* and *John Doe* are the biological parents of *the child* than random, untested, unrelated individuals in the *Caucasian/African American/Hispanic* population. The probability of parentage (assuming a prior probability of 0.5) is [W] %.

## **SIBSHIP RESULTS:**

### **A sibship comparison with evidence of relationship:**

The genetic results listed below support the conclusion that *John Doe* (item XA) and *Joe Doe* (item XA) are related as full siblings. These genetic results are *CRI* times more likely if *John Doe* is a full sibling of *Joe Doe* than if they are unrelated. The probability of relationship (assuming a prior probability of 0.5) is [W] %.

### **A sibship comparison with no evidence of relationship:**

The genetic results listed below support the hypothesis that *John Doe* (item XA) and *Joe Doe* (item XA) have different biological parents over the hypothesis that *John Doe* and *Joe Doe* share biological parents. These genetic results are *CRI* times more likely if *John Doe* is unrelated to *Joe Doe* than if they are full siblings. The genetic results are based only on the current data and do not supersede any additional genetic testing.

### **A sibship comparison with inconclusive results:**

The genetic results listed below do not strongly support the hypothesis that *John Doe* (item XA) and *Joe Doe* (item XA) share the same biological parents nor does it indicate that *John Doe* and *Joe Doe* have different biological parents; therefore, it is deemed inconclusive. The Combined Relationship Index for full siblings of *CRI/* is low and does not meet the laboratory standards necessary to establish a relationship. The genetic

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results are based only on the current data and additional genetic testing is recommended. Please contact the reporting analyst in regards to further genetic testing.

## **CASES WITH AN OBSERVED MUTATION:**

A single genetic inconsistency between the child and the alleged father was observed at *Locus*. This has been incorporated into the calculation as a mutation.

## **CASES WITH AN OBSERVED MIXTURE:**

A mixture was observed in the sample of *John Doe* (item *XA*). The following loci were not suitable for statistical calculations as the obligate paternal allele was not able to be determined: (list loci).

## **CASES WITH MULTIPLE EVIDENTIARY OR STANDARD SAMPLES:**

DNA profiles were developed for [two] additional samples of *item description* (item *XA*); however, these profiles were not used for statistical purposes.

## **WHEN A STATISTICAL CALCULATION IS BEING REPORTED:**

Statistical calculations were performed based on the recommendations of the AABB (American Association of Blood Banks).

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**Y-STR WORDING**

**REQUIRED STATEMENTS (PLACED AFTER RESULTS):**

**DNA Profiles Generated Using PowerPlex® Y23:**

In the DNA analysis detailed above, Y-STR profiles were developed using Polymerase Chain Reaction (PCR) with the PowerPlex® Y23 System by Promega Corporation.

**OR**

**No Samples Amplified:**

Extraction and quantification were performed in the Y-STR analysis of the sample(s) detailed above.

**SINGLE SOURCE STATEMENTS:**

**Use Of Exclusionary Statement (Full Profile Obtained From Sample; Standard Is Excluded):**

Item XA           The Y-STR profile obtained [from *item description* (item XA)] is consistent with an unknown male. [*John Doe* (item XA) can be excluded as a contributor to the sample.]

**Use Of Consistent Statement (Full Profile Obtained From Sample; No Differences From Standard):**

Item XA           The Y-STR profile obtained [from *item description* (item XA)] was interpreted as originating from a single male individual. The Y-STR profile is *LR* times more likely if it originated from *John Doe* (item XA) or any of his male paternal relatives than if it originated from an unknown, unrelated male individual. This analysis provides *moderate/limited* support that *John Doe* is a contributor to the Y-STR profile.

**Use Of Consistent Statement (Full Profile Obtained From Sample; No Differences From Standard with additional allele):**

Item XA           The Y-STR profile obtained [from *item description* (item XA)] was interpreted as originating from a single male individual; however, one additional allele was detected. The Y-STR profile is *LR* times more likely if it originated from *John Doe* (item XA) or any of his male paternal relatives than if it originated from an unknown, unrelated male individual. This analysis provides *moderate/limited* support that *John Doe* is a contributor to the Y-STR profile. No conclusion can be made regarding the additional allele.

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## MIXTURE STATEMENTS:

### **Indistinguishable Mixture:**

Item XA The Y-STR result obtained [from *item description* (item XA)] demonstrated the presence of a mixture in which the number of contributors cannot reasonably be assumed. Therefore, no further conclusions were drawn.

Item XA The Y-STR result obtained [from *item description* (item XA)] demonstrated the presence of a mixture of at least *two/three* male individuals. The results do not qualify for statistical calculations; therefore, no further conclusions were drawn.

### **Distinguishable Major And Minor Components:**

Item XA The Y-STR result obtained [from *item description* (item XA)] demonstrated the presence of a mixture with a major and minor profile[s]. The major Y-STR profile is *LR* times more likely if it originated from *John Doe* (item XA) or any of his male paternal relatives than if it originated from an unknown, unrelated male individual. This analysis provides *moderate/limited* support that *John Doe* is a contributor to the major Y-STR profile. No conclusion can be made regarding the minor [Y-STR] profile.

## SAMPLES UNSUITABLE FOR COMPARISON:

Item XA Due to the limited quantity and/or quality of the sample, the Y-STR profile obtained [from *item description* (item XA)] failed to demonstrate conclusive results.

*Item XA* *Item description* failed to demonstrate a Y-STR profile.

Item XA No conclusion can be drawn regarding *item description* (item XA) because *the/a* test control(s) did not meet required parameters.

## SAMPLE/SUBITEM NOT EXAMINED:

Item XA No Y-STR analysis was performed on # additional samples at this time.

Item XA No Y-STR analysis was performed on the DNA standard of *John Doe* (item XA) due to a lack of evidence for comparison.

Upon evaluation, no samples were determined to be suitable for Y-STR analysis.

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**IDENTIFICATION OF CONTAMINATION FROM LABORATORY PERSONNEL** (Not all possible continuations are given, consultation with the Technical Leader and/or Supervisor is recommended):

Item XA No Y-STR profile of apparent value was identified on *item description* (item XA). The Y-STR profile of a *laboratory analyst/non-laboratory person* was identified.

Item XA The Y-STR result obtained from *item description* (item XA) demonstrated the presence of a mixture with a major profile. The major Y-STR profile is consistent with the Y-STR profile of a *laboratory analyst/non-laboratory person*.

## **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 suspects.

## **WORDING OF STATISTICAL ANALYSIS:**

When A Calculation With A Frequency Is Reported The Following Paragraph Shall Be Added:

Statistical estimates were performed based on the recommended concepts endorsed by the Scientific Working Group on DNA Analysis Methods (1/9/14). Y-STR results for item[s] XA have been searched using the Y Chromosome Haplotype Reference Database (<https://yhrd.org>), Release R# on *date*. Calculations for the Caucasian, African American, Hispanic, and Asian populations have been performed and a likelihood ratio has been reported for the combined populations.

The statistical calculations are reported in conjunction with a verbal scale qualifying statement used 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). This scale reflects stronger degrees of support as the likelihood ratio increases in magnitude.

<b><u>Likelihood Ratio</u></b>	<b><u>Support for Inclusion</u></b>
<b>1</b>	Uninformative
<b>2 ≤ LR &lt; 100</b>	Provides limited support
<b>100 ≤ LR &lt; 10,000</b>	Provides moderate support
<b>10,000 ≤ LR &lt; 1,000,000</b>	Provides strong support
<b>1,000,000 ≤ LR</b>	Provides very strong support

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**CODIS STATEMENT (Placed At The End of Any Report Detailing New Profiles):**

At this time, Y-STR profiles cannot be entered or searched in the Combined DNA Index System (CODIS).

**EVIDENCE DISPOSITION STATEMENT:**

Unless otherwise noted, all items tested for Y-STR analysis will be retained by the Indiana State Police Laboratory for the possibility of future analysis.

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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.
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. Ambiguous Zygosity – a locus where a single allele is present in which it is unclear if the locus is homozygous or if the sister allele is dropped out or masked.
7. Amelogenin – the marker for determining the gender 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. Base Pairs – paired nucleotides which make up the DNA molecule. Two complementary nucleotides joined by a hydrogen bond.
12. 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.
13. Burn-in – The initial 100,000 accepts during the MCMC process that allow the chains to converge on a “good space”. These iterations are discarded.
14. 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.
15. Chromosome – the structure on which genes are naturally arranged and how DNA is organized.
16. 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.
17. Conditioning – A likelihood ratio in which an individual is included in both  $H_1$  and  $H_2$ .
18. Contamination – the process of making a sample impure or unusable.
19. Deconvolution – Process to determine the potential contributor genotypes to a mixed DNA profile.
20. Deduced – the inference of an unknown contributor's DNA profile after taking into consideration the contribution of an assumed contributor's DNA profile in an intimate sample based on quantitative peak height information.
21. Degradation – the breaking down of the DNA molecule into smaller fragments.

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22. Denaturation – the separation of the double stranded DNA molecule into two single strands.
23. Deoxyribonucleic Acid (DNA) - the genetic material present in the nucleus of most cells. Also located in cell mitochondria.
24. DNA Sequence - a specific order of base pairs.
25. Electropherogram – the visual representation of the DNA fragments contained in each sample; generated by the analysis software of the Capillary Electrophoresis Instrument.
26. 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.
27. Enzyme – a protein which acts as a catalyst, speeding up a specific chemical reaction without being changed or consumed in the process.
28. Epithelial Cells – skin cells and other surface cells such as from the mouth (buccal cells) or the vaginal cavity.
29. Factor of NI – Likelihood ratio which compares the person of interest to the total mixture at the sub-source level, independent of contributor order.
30. Gelman-Rubin – A value which informs the user whether the MCMC chains have converged.
31. Gene – the basic functional unit of heredity. Most genes determine the structure and function of proteins.
32. Genotype – the specific genetic sequence of a person's DNA.
33. Genotype Probability Distribution – A summary of the genotype combinations accepted at each locus and the percentage of accepts per genotype.
34.  $H_1/H_p$  – Interchangeable terms for the most likely proposition assigned to the prosecution.
35.  $H_2/H_d$  – Interchangeable terms for the most likely proposition assigned to the defense.
36. Hair – a slender outgrowth from the skin of mammals.
37. Heme – the iron containing complex of the hemoglobin molecule.
38. Hemin – the oxidized heme molecule.
39. Hemoglobin - the iron containing protein in blood.
40. Heterozygous – having different alleles at a particular locus.
41. Homozygous – having two of the same allele at a particular locus.
42. 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.
43. Intimate samples – biological sample that is obtained directly from an individual's body.
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 sequence for an allele variation.
48. Marker – a gene of known location and phenotype used as a point of reference.
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

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

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. Modified Random Match Probability - an estimate based on the assumption of the number of contributors within a sample, of the probability of selecting an unrelated individual at random that would be included as a potential contributor to the mixed profile.
56. Nucleus - the part of a cell that contains the double stranded DNA inherited from both parents.
57. Obligate allele – an allele in a mixed DNA typing result that is a) foreign to an assumed contributor, or b) based on quantitative peak height information, determined to be shared with the assumed contributor.
58. Partial profile – DNA profile for which typing results were not obtained for all tested loci.
59. Phenotype – the expressed genotype.
60. Polymerase – an enzyme that initiates the duplication of a DNA molecule.
61. Polymerase Chain Reaction (PCR) – a process for amplifying (copying) the DNA molecule.
62. Polymorphism – the presence of more than one possible allele set for a specific gene.
63. Population – a stable group of random individuals, chosen for genetic analysis.
64. 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.
65. Proficiency Testing – a test to evaluate the competency of an analyst in a specific procedure.
66. 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.
67. Proposition – A proposed explanation of the data. Interchangeable with “hypothesis”.
68. Post Burn-in – The iterations after burn-in from which the deconvolution is determined.
69. Random Match Probability (RMP) - the probability of obtaining a match between two distinct and unrelated individuals.
70. Replicate – An identical reamplification of an extract.
71. Saliva – digestive fluid from the mouth.
72. Semen/Seminal Fluid – the male ejaculate. In a dried state may be referred to as seminal material. Semen normally contains spermatozoa.
73. 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.
74. Source attribution - a RMP threshold at which a degree of confidence can be assured that an unknown sample can be attributed to a known reference sample.

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75. Spermatozoa – the male reproductive cell, sperm cell.
76. 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.
77. 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.
78. Stratified Likelihood Ratio – A single LR that samples across all populations.
79. 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.
80. Substrate – the material on which a biological sample is deposited such as at a crime scene.
81. SWGDAM – Scientific Working Group on DNA Analysis Methods.
82. Theta - a value to adjust for population substructure in homozygous loci.
83. Unified Likelihood Ratio – LR that takes in to account that the unknown contributors are made up of both relatives and unrelated people.
84. 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.
85. Verbal Scale – A verbal representation of the relative strength of the likelihood ratio results.
86. 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.
87. X chromosome – a sex chromosome, present twice in female cells and once in male cells.
88. Y chromosome – a sex chromosome, present once in male cells (and absent in female cells), paired with the X chromosome.

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS APPENDIX 9 DNA SERVER INSTRUCTIONS

The DNA servers will be used in place of CDs for storage and archiving of data and photos. Real Time PCR files, 3500 run folders, GeneMapper™ ID-X projects, genotypes table .txt files, STRmix™ files, and photos will be saved to the server. Any worksheets, statistic sheets, typed notes pages, or workbooks shall NOT be saved to the server. The printed copy that will be scanned into LIMS upon completion of the case will be the official, tracked copy. We want to avoid duplication of items between LIMS and the server.

On the server, 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, but they shall not change, delete or add items within the folder. Within the analyst's folder there will be one folder for each laboratory case number and request. Each folder will contain all associated data for that request. Any projects or run folders that contain batched data will be saved individually within each of the associated case folders. The following nomenclature shall be used:

Analyst's Folder (created by Lab IT)

- 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: “1<sup>st</sup> case on the plate\_date\_plate#” – ex. “1011234\_01Jan11\_01”
  - 3500 run folder: “1<sup>st</sup> 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.
  - Photos: create a folder per item photographed – folder name “case\_item#” ex. “1011234\_item1”. The individual photo file names are left to the analyst's discretion.

**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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APPENDIX 10**

**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, 9<sup>th</sup> Edition, Appendix 7 and 8)

Mother	Child	Alleged Father	PI	RMNE	PE
BD	AB	AC	1/2a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
BC	AB	AC	1/2a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
BC	AB	AB	1/2a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
BC	AB	A	1/a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
B	AB	AC	1/2a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
B	AB	AB	1/2a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
B	AB	A	1/a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
AB	AB	AC	1/[2(a+b)]	1-(1-a-b) <sup>2</sup>	(1-a-b) <sup>2</sup>
AB	AB	AB	1/(a+b)	1-(1-a-b) <sup>2</sup>	(1-a-b) <sup>2</sup>
AB	AB	A	1/(a+b)	1-(1-a-b) <sup>2</sup>	(1-a-b) <sup>2</sup>
AB	A	AC	1/2a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
AB	A	AB	1/2a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
AB	A	A	1/a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
A	A	AB	1/2a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
A	A	A	1/a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
	AB	AC	1/4a	1-(1-a-b) <sup>2</sup>	(1-a-b) <sup>2</sup>
	AB	AB	(a+b)/4ab	1-(1-a-b) <sup>2</sup>	(1-a-b) <sup>2</sup>
	AB	A	1/2a	1-(1-a-b) <sup>2</sup>	(1-a-b) <sup>2</sup>
	A	AC	1/2a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>
	A	A	1/a	1-(1-a) <sup>2</sup>	(1-a) <sup>2</sup>

**Paternity Index for Autosomal Mutations =  $\mu$ /[PE]**

$\mu$  = mutation rate for the locus

PE is calculated from the above table using the obligate paternal allele

**Paternity Index for Y-STR Mutation = 0.001**

**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	$1/2$

## Mutation Rates

Mutation rates for CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, Penta D, Penta E, TH01, TPOX, and vWA reported in AABB, Standards for Relationship Testing Laboratories, 10<sup>th</sup> Edition, Appendix 6.

Average paternal and maternal mutation rates calculated from tetranucleotide markers listed in AABB, Standards for Relationship Testing Laboratories, 10<sup>th</sup> Edition, Appendix 6 for D1S1656, D2S441, D10S1248, D12S391, D22S1045.

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*Mutation rates for SE33 reported in AABB Annual Report 2003.*

<u>LOCUS</u>	<u>PATERNAL</u>	<u>MATERNAL</u>
CSF1PO	0.002021	0.000283
D10S1248	0.001692	0.000409
D12S391	0.001692	0.000409
D13S317	0.001743	0.000436
D16S539	0.001127	0.000481
D18S51	0.002530	0.000748
D19S433	0.000745	0.000596
D1S1656	0.001692	0.000409
D21S11	0.001709	0.001295
D22S1045	0.001692	0.000409
D2S1338	0.001526	0.000245
D2S441	0.001692	0.000409
D3S1358	0.001691	0.000211
D5S818	0.001742	0.000300
D7S820	0.001348	0.000073
D8S1179	0.002031	0.000333
FGA	0.003713	0.000522
Penta D	0.000259	<0.000253
Penta E	0.000260	<0.000253
SE33	0.006400	<0.003000
THO1	0.000070	0.000043
TPOX	0.000130	0.000081
vWA	0.003258	0.000494

## **PowerPlex® Fusion 6C STR Population Data**

Moretti, Tamyra R. et al. Population Data on the Expanded CODIS Core STR Loci for Eleven Populations of Significance for Forensic DNA Analyses in the United States. Forensic Science International: Genetics. November 2016, Volume 25: 175-181.

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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 Change the run time to 1500 seconds.
- 1.10 Name the protocol with the kit name and the injection voltage and time (ex. “PPY23\_1.2kV15s”).
- 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):  Run Voltage (kVolts):  PreRun Voltage (kVolts):  Injection Voltage (kVolts):

Run Time (sec.):  PreRun Time (sec.):  Injection Time (sec.):  Data Delay (sec.):

▶ Advanced Options

## 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:   Locked

Description:

\* Dye Color:

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)

\* 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:

**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 Baselining (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 (ex. “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 created in 4.7.3.2.
- 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 (ex. 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 a software window titled "Edit File Name Convention PPY23\_FNC". The window contains the following elements:

- Title Bar:** Edit File Name Convention PPY23\_FNC
- Header:** Setup a File Name Convention
- Name Field:** \* Name: PPY23\_FNC, with a checked "Locked" box and a "Color: Blue" dropdown.
- Select File Name Attributes:**
  - Preview of File Name:** <Sample Name>\_<Instrument Protocol>\_<Date of Run>\_<Well Position>
  - Available Attributes:** Amplicon Name, Analysis Protocol Name, Assay Name, Capillary Number, Custom Text1, Custom Text2, Custom Text3.
  - Delimiters:** Select a delimiter: Underscore (\_), with an "Add >>" button and a checkbox for "Add between attributes".
  - Selected Attributes:** Sample Name, Underscore (\_), Instrument Protocol, Underscore (\_), Date of Run, Underscore (\_), Well Position.
  - Buttons:** Add >>, << Remove, Move Up, Move Down.
- Add a custom value to available attributes (optional):** Custom Text 1, Custom Text 2, Custom Text 3 (all empty text boxes).
- Select File Location:**
  - Default File Location: D:\Applied Biosystems\3500\Data
  - Custom File Location: [Empty text box] with a "Browse..." button.
- Buttons:** Close, Save.

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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 (ex. PPY\_ResultsGroup).

6.4 Select the desired attributes and click “Add”.

6.5 Select the desired 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:

**Setup a Results Group**

\* Name:   Locked Color:

Select Results Group Attributes

Preview of Results Group Name:

Available Attributes

- Assay Name
- Injection Number
- ...

Delimiters

Select a delimiter:

Add between attributes

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  - Custom file location
- Include an Instrument Run Name folder
- Include a Result Group Name folder
- Include an Injection folder

**INDIANA STATE POLICE  
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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 Enter 3500 as the instrument.
- 2.7 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'. A checkbox 'Use marker-specific stutter ratio and distance if available' is checked. Below this is a table for 'Marker Repeat Type' settings for Tri, Tetra, Penta, and Hexa alleles. The 'Amelogenin Cutoff' is set to 0.0. Buttons for 'Range Filter...', 'Factory Defaults', 'Save', 'Cancel', and 'Help' are visible.

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
	To	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
	To	0.0	4.75	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
	To	0.0	0.0	0.0
Amelogenin Cutoff	0.0			

2.8 Select the “Peak Detector” tab. Change the settings to match the figure below.

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## The Peak Detector Tab:

Analysis Method Editor

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

Peak Detection Algorithm: Advanced

Ranges

Analysis: Full Range (dropdown)  
Sizing: Partial Sizes (dropdown)

Start Pt: 0  
Stop Pt: 10000

Start Size: 60  
Stop Size: 500

Smoothing and Baselineing

Smoothing:  None  Light  Heavy

Baseline Window: 51 pts

Size Calling Method

2nd Order Least Squares  
 3rd Order Least Squares  
 Cubic Spline Interpolation  
 Local Southern Method  
 Global Southern Method

Peak Detection

Peak Amplitude Thresholds:

B: 100 R: 100  
G: 100 P: 100  
Y: 100 O: 100

Min. Peak Half Width: 2 pts  
Polynomial Degree: 3  
Peak Window Size: 15 pts

Slope Threshold

Peak Start: 0.0  
Peak End: 0.0

Normalization

Use Normalization, if applicable

Factory Defaults

Save As Save Cancel Help

2.9 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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2.10 Select the “Peak Quality” tab. Change the settings to match the image below.

## The Peak Quality Tab:

The screenshot shows the 'Analysis Method Editor' window with the 'Peak Quality' tab selected. The settings are as follows:

Section	Parameter	Value
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
	Cut-off value	0.2
Sample Spike Detection	Spike Detection	Enable

Buttons at the bottom: Save As, Save, Cancel, Help, and a Factory Defaults button.

2.11 Leave the “SQ and GQ Settings” tab set to the factory defaults.

2.12 Select “Save”.

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## 3.0 Creating a Size Standard

3.1 Create the "WEN ILS 500" size standard (see figure below for exact settings and sizes).

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 not being used, “Label 4” for “Assigned Allele”, “Custom Allele”, and “Artifact” can be changed to “NONE”.

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## Labels Tab:

anner™ ID-X Manager

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:	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”.

# INDIANA STATE POLICE FORENSIC BIOLOGY SECTION TEST METHODS

## 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  Peak Positions  
 Marker Range  Bring Ctrl's to Top  
 Marker Indicators  Bring Ladders to Top  
 Bins  Allele Changes  
 Toolbar  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

Tables

No Table  
 Sizing Table  
 Genotypes Table  
 Label Edit Viewer

Dye Layout

Combine Dyes  
 Separate Dyes  
 Overlay All

Labels

Size Std Labels

Print

Quality Value Details

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

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TEST METHODS  
APPENDIX 13  
POWERPLEX® Y23 STUTTER TABLE**

Stutter Guidelines for PowerPlex® Y23						
	n-3/n-4/n-5/n-6		n-6/n-8/n-12		n+3/n+4/n+5/n+6	
	Mean	Mean + 3SD	Mean	Mean + 3SD	Mean	Mean + 3SD
DYS576	10.03%	<b>11.89%</b>	<b>0.93%</b>	n/a	1.53%	<b>4.54%</b>
DYS389 I	5.17%	<b>7.40%</b>	n/a	n/a	n/a	n/a
DYS448	2.37%	<b>3.68%</b>	n/a	n/a	n/a	n/a
DYS389 II	10.12%	<b>13.33%</b>	n/a	n/a	2.46%	<b>3.11%</b>
DYS19	5.67%	<b>8.00%</b>	n/a	n/a	n/a	n/a
DYS391	6.47%	<b>8.95%</b>	0.92%	<b>1.50%</b>	<b>0.66%</b>	n/a
DYS481	17.83%	<b>25.08%</b>	3.18%	<b>4.99%</b>	2.35%	<b>4.28%</b>
DYS549	6.80%	<b>10.52%</b>	1.08%	<b>1.49%</b>	1.11%	<b>1.45%</b>
DYS533	6.24%	<b>8.12%</b>	n/a	n/a	1.58%	<b>3.48%</b>
DYS438	3.12%	<b>6.03%</b>	n/a	n/a	<b>1.42%</b>	n/a
DYS437	5.06%	<b>7.13%</b>	n/a	n/a	n/a	n/a
DYS570	9.34%	<b>13.61%</b>	0.84%	<b>1.09%</b>	0.89%	<b>1.98%</b>
DYS635	6.50%	<b>10.90%</b>	<b>1.26%</b>	n/a	<b>1.78%</b>	n/a
DYS390	8.14%	<b>11.32%</b>	n/a	n/a	n/a	n/a
DYS439	6.30%	<b>9.81%</b>	n/a	n/a	1.20%	<b>2.27%</b>
DYS392	8.84%	<b>13.95%</b>	1.04%	<b>1.23%</b>	4.38%	<b>7.82%</b>
DYS643	2.29%	<b>4.26%</b>	n/a	n/a	<b>3.06%</b>	n/a
DYS393	9.50%	<b>13.30%</b>	0.96%	<b>1.62%</b>	1.34%	<b>2.27%</b>
DYS458	9.55%	<b>13.06%</b>	0.93%	<b>1.37%</b>	1.33%	<b>4.07%</b>
DYS385	8.55%	<b>14.91%</b>	1.56%	<b>2.28%</b>	n/a	n/a
DYS456	10.78%	<b>14.29%</b>	1.23%	<b>2.87%</b>	1.70%	<b>3.24%</b>
YGATAH4	7.98%	<b>11.70%</b>	<b>1.03%</b>	n/a	1.39%	<b>2.36%</b>

n-2			n+2		
	Mean	Mean + 3SD		Mean	Mean + 3SD
DYS19	7.57%	<b>9.82%</b>	DYS19	2.98%	<b>4.68%</b>

**INDIANA STATE POLICE  
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TEST METHODS  
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 laboratory. 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 laboratory 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.