

SAN DIEGO POLICE DEPARTMENT

FORENSIC SCIENCE SECTION

FORENSIC BIOLOGY TECHNICAL PROCEDURES

All Procedures Reviewed and Approved by:



DNA Technical Manager

Date: 03-01-2010

List of Forensic Biology Procedures

Screening

Use of the Polilight PL500 Alternate Light Source
Use of the Crime-Lite 80S Alternate Light Source
Screening Evidence for the Presence of Semen
Two-Step Acid Phosphatase Screening Test for Semen
One-Step SERI AP Spot Test Detection of Acid Phosphatase
Identification of Cellular Material Using Xmas Tree Staining
p30 Detection Using the Abacus p30 Diagnostic Card
Leucomalachite Green (LMG) Presumptive Test for Blood
Phenolphthalin Presumptive Test for Blood (Kastle-Meyer Test)
Luminol Detection of Latent Bloodstains
Abacus HemaTrace Detection of Blood
Amylase Radial Diffusion
Detection of Urobilinogen in Fecal Stains

DNA Extraction and Quantitation

General DNA Extraction
DNA Extraction with Microscopic Examination
Differential DNA Extraction
DNA Extraction from Hair
The Processing and DNA Extraction of Bone Samples
Organic DNA purification using Penol/Chloroform/Isoamyl alcohol
DNA Purification/Concentration Using The Microcon-YM100 Centrifugal Filter Devices
DNA Purification/Concentration Using The Centricon-YM100 Centrifugal Filter Devices
BioRobot EZ1 DNA Purification of Evidence Samples
DNA extraction from Reference Samples using the BioRobot EZ1
Quantifiler Setup Using the Qiagen BioRobot® Universal
Quantitative PCR DNA Quantification with the Quantifiler™ Human DNA Quantification Kit
Quantitative PCR DNA Quantification with the Quantifiler™ Duo DNA Quantification Kit

DNA Amplification

Identifiler™ Setup Using the Qiagen BioRobot® Universal
Minifiler™ or Yfiler™ Setup Using the Qiagen BioRobot® Universal
Amplification with the AmpF/STR Identifiler™ Kit
Amplification with the AmpF/STR MiniFiler™ Kit
Amplification with the AmpF/STR Yfiler™ Kit

Electrophoresis of Amplified Samples with the 310 or 3130 Genetic Analyzers

ABI 310 Cleaning and Setup

Preparation of Identifiler™, Minifiler™, or Yfiler™ Samples for Analysis on the ABI 310

CE of Amplified Samples on the 310 Genetic Analyzer

Creating a Matrix with GeneMapperID

ABI 3130 Genetic Analyzer Instrument Preparation and Cleaning

Preparation of Identifiler™, Minifiler™, or Yfiler™ Samples for Analysis on the ABI 3130

Preparation of Amplified Samples for Analysis on the ABI 3130 Using the Automated CAS-1200

Decontaminating the CAS-1200 using the automated UV protocol

CE of Amplified Samples on the Applied Biosystems 3130

Performing Spectral Calibration on the 3130

Performing Spatial Calibration on the 3130

Flushing and Filling the Water Trap on the 3130

GeneMapperID Analysis of Data

Importing Panel and Bin Definitions into GeneMapperID Software

DNA Interpretation Guidelines

General STR Interpretation Guidelines

AmpF/AmpF/STR STR Identifiler™ Interpretation Guidelines

AmpF/STR Minifiler™ Interpretation Guidelines

AmpF/STR Yfiler™ Interpretation Guidelines

Autosomal STR Profile Frequency Calculations

Y-STR haplotype Frequency Calculations

Results and Conclusions in Forensic Biology Unit Reports

Method: **Use of the Polilight PL500 Alternate Light Source**

Date: 3/31/00

Revision Date: 01/01/2007

Approved by: SAM

MATERIALS:

1. Polilight PL500 Alternate Light Source
2. Orange and yellow goggles or filters

PROCEDURES:

Note: The Polilight PL500 is a high intensity light source, which if used inappropriately has the potential to be a hazard to the eyes and skin. Users should use the Polilight PL500 with the awareness that both their own eyes and skin, and those in close proximity should be protected at all times. Even with protective goggles exposure time needs to be considered.

- 1) Make sure the light guide with black focusing lens is connected to the Polilight unit.
- 2) Place the Polilight PL500 on a solid surface and turn the power switch on back panel to the up (ON) position. Ensure the air flow around the unit is not obstructed.
- 3) Conduct a quality control check of the Polilight PL500 using known biological fluid stains prior to examining evidence:
 - a) The known biological stains should be checked with all wavelengths desired in the examination (the 450 and 490 nm wavelengths are recommended for most evidence items) and the results of the quality control check will be recorded in the case notes.
- 4) Filter tuning is achieved by using the wavelength specific numerical buttons located on the front control panel or on the hand held remote control box. (See the figures below)
- 5) Once a filter has been selected it can be fine-tuned. Fine tuning will decrease the wavelength and is used to optimize illumination conditions for specific materials. The fine tuning has a range from t0 - t40.

Figure 1

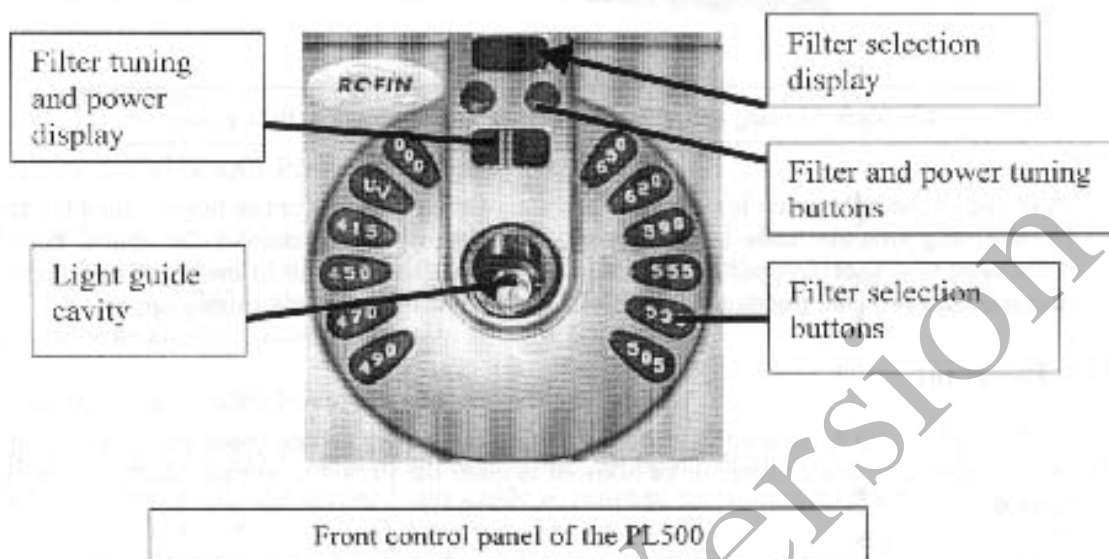
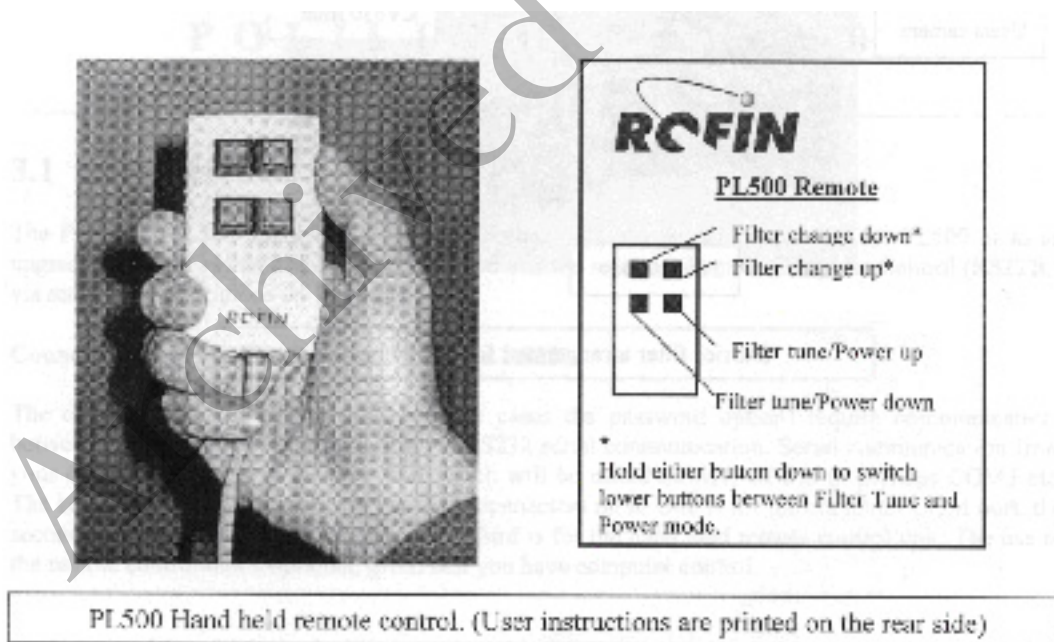


Figure 2



- 6) Once a filter has been selected the intensity can be tuned by changing the power setting. The power setting has a range from P1 (lowest power) - P8 (highest power).
- 7) Systematically pass the light over the entire item to be examined. A fluorescent area

may indicate the presence of a body fluid stain. Note the area for further testing.

- 8) Once the examination is complete, turn the power switch on back panel to the down (OFF) position.
- 9) Secure the Polilight PL500 by either placing it on a stable surface or removing the power cable, remote control, and liquid light guide from the main unit and placing them in the carrying case.

REFERENCES:

1. Auvdel MJ. Comparison of Laser and High-Intensity Quartz Arc Tubes in the Detection of Body Secretions. J Forensic Sci. 1988 Jul;33(4):929-45.
2. Rofin Australia Pty. Ltd. POLILIGHT PL500 (Version 2) Multi-Waveband Tunable Light Source Instruction Manual

Method: **Use of the Crime-lite 80S Alternate Light Source**

Date: 1/12/05

Revision Date: 01/01/2007

Approved by: SAM

MATERIALS:

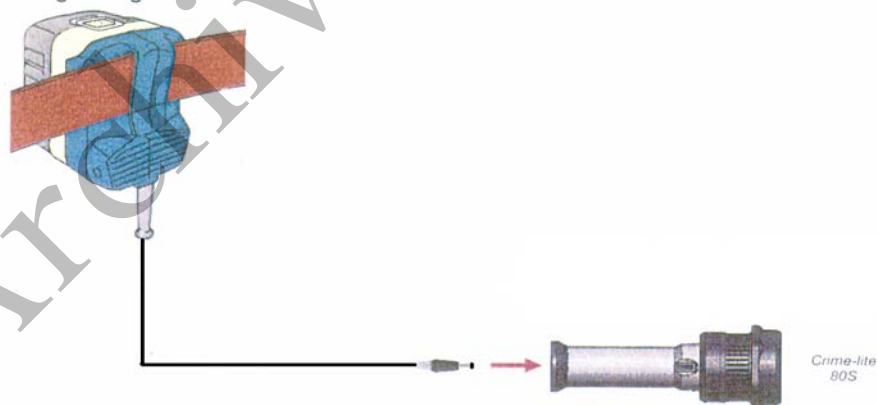
1. Crime-lite™ 80S Blue (450nm) or Crime-lite™ 80S Blue/Green (485nm)
2. Battery Adaptor with Makita® Rechargeable battery
3. Mains Adaptor
4. Orange goggles or filters

PROCEDURES:

Note: The Crime-lite™ 80S is a portable high intensity light source, which if used inappropriately has the potential to be a hazard to the eyes and skin. Users should use the Crime-lite™ 80S with the awareness that both their own eyes and skin, and those in close proximity should be protected at all times. Even with protective goggles exposure time needs to be considered.

1. Connect the output plug holding the Makita® Rechargeable battery with battery adaptor to the Crime-lite™ 80S for portable use.

Connecting the Light Source

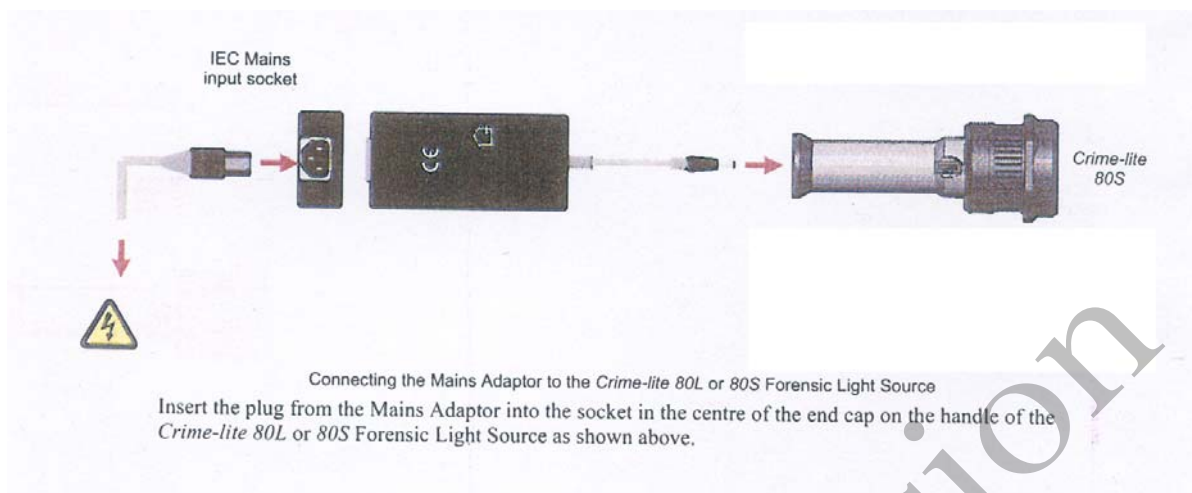


Connecting the Battery Adaptor to a Crime-lite 80L or 80S Forensic Light Source.

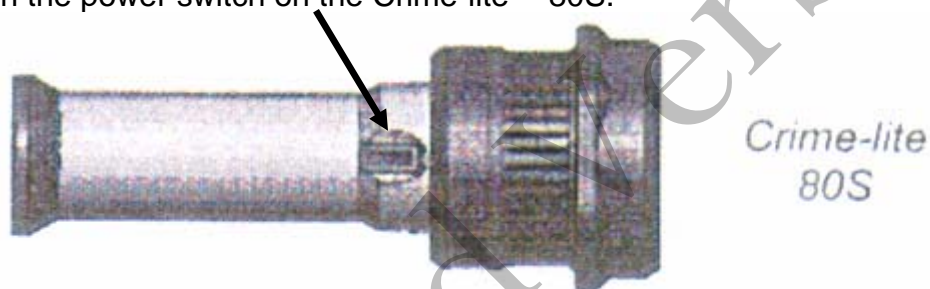
Insert the plug from the Battery Adaptor into the socket in the centre of the end cap on the handle of the Crime-lite 80L or 80S Forensic Light Source.

The light source is now ready for use.

If the battery has a low charge or unavailable, attach the Mains Adaptor to the Crime-lite™ 80S and the nearest outlet.



1. Turn on the power switch on the Crime-lite™ 80S.

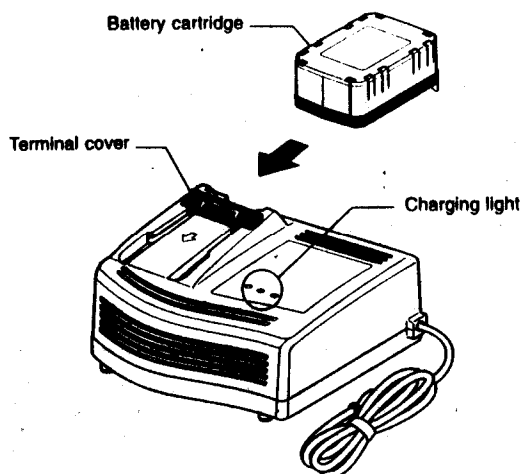


2. Conduct a quality control check of the Crime-Lite™ on known biological stains prior to examining evidence and record the results in the case notes:
4. Systematically pass the light over the entire item to be examined. A fluorescent area may indicate the presence of a body fluid stain. Note the area for further testing.
5. Once the examination is complete, turn off the power switch located on the handle. Caution: the light output head will become warm during use.
6. Secure the Crime-lite™ 80S by either placing it on a stable surface or in its carrying case (for long term storage) and removing the Mains Adaptor or Battery Adaptor.

Charging the *Makita*® rechargeable battery:

The *Makita*® Rechargeable battery is a nickel-metal hydride 24V, DC that supplies a Crime-lite™ 80S sufficient power for up to 50 minutes at a fully charged capacity.

Charging



1. Insert the battery cartridge into the charger until it stops adjusting to the guide of charger. Terminal cover of charger can be opened with inserting and closed with pulling out the battery cartridge.
2. When the battery cartridge is inserted, the charging light color will change from green to red and charging will begin. The charging light will remain steadily during charging.
3. One red charging light indicates charged condition in 0 - 80% and two red ones indicates 80 - 99%.
4. With finish of charge, the charging lights will change from two red ones to two green ones.
5. The charging time is approximately 60 minutes for the *Makita*® Rechargeable battery.
6. If you leave the battery cartridge in the charger after the charging cycle is complete, the charger will switch into its "trickle charge (maintenance charge)" mode which will last approximately 24 hours.
7. After charging, unplug the charger from the power source.

Tips for maintaining maximum battery life:

1. Charge the battery cartridge before completely discharged. Always stop Crime-lite™ 80S operation and charge the battery cartridge when you notice less tool power.

2. Never recharge a fully charged battery cartridge. Overcharging shortens the battery service life.
3. Charge the battery cartridge with room temperature at 10°C - 40°C. Let a hot battery cartridge cool down before charging it.

REFERENCES:

1. Auvdel MJ. Comparison of Laser and High-Intensity Quartz Arc Tubes in the Detection of Body Secretions. J Forensic Sci. 1988 Jul;33(4):929-45.
2. Foster and Freeman- Crime-lite™ Information sheet QCL/80S-B-BG-G. Issue 01 (September 2004)
3. Foster and Freeman- Crime-lite™ Information sheet QCL/83. Issue 01 (September 2004)
4. Foster and Freeman- Crime-lite™ Information sheet QCL/80. Issue 01 (September 2004)
5. Makita®- High Capacity Battery Charger Model DC24SA Instruction Manual.

Method: **Screening Evidence for the Presence of Semen**

Date: 4/2/98

Revision Date: 01/01/2007

Approved by: SAM

Note: This procedure has been specifically written for Analysts involved in screening evidence for the presence of semen but not involved in DNA testing.

1. Remove a very small portion of the putative semen stain and perform a test for the presence of acid phosphatase activity.
2. Remove approximately 1/4 of a swab or a suitable portion of the substrate and place in 1.5mL tube. Add 1mL of nanopure water and incubate for 30 minutes at room temperature. Vortex several times during that 30 minutes. A reagent blank tube should be created at this point which will undergo all of the following manipulations and will be used as a control to monitor potential contamination, if the extracts prepared will be used for DNA testing.
3. Using a pipette tip (or wooden stick) remove as much liquid from the substrate as possible, transfer it to a second microcentrifuge tube, and retain.
4. Microcentrifuge the sample extract at maximum speed for 3-5 minutes.
5. The supernatant may be used for serological tests for blood, semen, saliva, and/or feces. [Consult the specific procedure for details]. Remove an appropriate amount of the supernatant for any desired assays.
6. Carefully pipette off all but 50-100uL of the supernatant being careful not to disturb the pellet. The supernatant can be discarded. At least 50uL of supernatant should be left behind to ensure the cell pellet is not disturbed.
7. Resuspend the cell pellet in the remaining supernatant. Pipette 5-10uL of the sample on to the well of a slide (different amounts may be used). Place the slide in the incubator or on a heat block to dry.
8. Stain the slide with Xmas Tree Stain (refer to the Identification of Cellular Material using Xmas Tree Staining procedure).
9. If only screening is being performed and sperm are present in a sample, no further work on that sample may be necessary and that sample can be referred for DNA testing. If no sperm are observed in a sample then proceed with the following steps.
10. Add back the substrate to what remains of the water extract and add 750uL of digest buffer and 25uL of proteinase K to the tube. Incubate the sample for at least one

hour at 56°C.

11. Retain as much liquid from the substrate as possible and discard the substrate.
12. Microcentrifuge the sample at maximum speed for 3-5 minutes.
13. Carefully pipette off all but 50-100uL of the supernatant being careful not to disturb the pellet. The supernatant can be discarded. At least 50uL of supernatant should be left behind to ensure the cell pellet is not disturbed.
14. Add 1mL of water to the cell pellet and resuspend by lightly vortexing.
15. Microcentrifuge the sample at maximum speed for 3-5 minutes.
16. Carefully pipette off all but 50-100uL of the supernatant being careful not to disturb the pellet. The supernatant can be discarded. At least 50uL of the supernatant should be left behind to ensure the cell pellet is not disturbed.
17. Resuspend the cell pellet in the remaining supernatant. Pipette the remainder (or portion) of the sample on to the well of the slide. Place the slide in the incubator or on a heat block to dry.
18. Stain the slide with Xmas Tree Stain (refer to the Identification of Cellular Material using Xmas Tree Staining procedure).
19. If sufficient sperm are present in a sample, no further work on that sample may be necessary and that sample can be referred for DNA testing. If no sperm were present for a sample even after the second round of microscopy, it may be necessary to prepare an extract of a second sample from the evidence.
20. If warranted, a small portion can be extracted for p30 analysis. Refer to the Detection of Seminal Protein p30 Using the Abacus p30 Diagnostic Card procedure.

REMARKS:

The systematic approach to the analysis of semen provides the analyst with the opportunity to assay for body fluids, to perform microscopy on cells, and to perform DNA testing from a single extract of a potentially limited sized evidence sample. It represents a superior approach for creating individual extracts for each class of testing.

The systematic approach to the analysis of semen is carried out by creating a single water extract from a substrate that may contain semen and other body fluids. Following centrifugation of the water extract, the supernatant can be used to test for the presence of blood, semen, saliva, urine and feces. The cellular portion of the extract, after it has been resuspended in the remaining supernatant, can be analyzed microscopically for

the presence of sperm and other cells. Depending on the results of these initial tests, the remaining sample can be used for DNA testing.

The reagent blank will be subjected to all reagents used in the extraction process and all manipulations carried out on the evidence samples. The reagent blank is necessary to ensure that any contaminant introduced during the extraction process is detected. The use of a reagent blank is mandatory if the samples are to be tested using the extremely sensitive polymerase chain reaction (PCR) DNA tests. If the extracted materials are to be used for DNA analysis, the distilled water and microcentrifuge tubes used in the "Systematic Approach to the Analysis of Semen" must have been autoclaved.

REFERENCE:

Blake, Sensabaugh, Bashinski, "A Systematic Approach to the Analysis of Semen Evidence." CAC Meeting, November, 1980

Method: **Two-Step Acid Phosphatase Screening Test for Semen**

Date: 09/13/2001

Revision Date: 02/06/2009

Approved by: SAM

REAGENTS:

1. SODIUM ALPHA-NAPHTHYL PHOSPHATE SOLUTION (saturated)

Sodium alpha-naphthyl phosphate (a calcium form can also be used) is prepared as a saturated solution in acetate buffer. Prepare fresh or freeze into one use aliquots.

2. Ortho-DIANISIDINE WORKING SOLUTION

Ortho-Dianisidine (fast blue salt BN) is added to acetate buffer until a yellow brown solution is produced. Prepare fresh or freeze into one-use aliquots.

3. ACETATE BUFFER, pH 5

Add 5mL of glacial acetic acid to 10g of sodium acetate (anhydrous) in a suitable container. Bring volume to 500ml with dH₂O. Adjust pH to 5. Good for nine months (refrigerated).

MATERIALS:

1. Spray apparatus
2. Disposable pipettes

PROCEDURES:

A quality control check of the reagents against a known semen sample and a negative of reagents only before use. The results of the quality control test must be recorded in the case notes.

CUTTING METHOD

1. Place a small portion of the sample in a small test tube or on filter paper.
2. Add 1-5 drops of alpha-naphthyl phosphate.
3. Add 1-5 drops of ortho-dianisidine and observe for any color change. Positive tests show a red-purple precipitate.

SWAB METHOD

Wet a cotton swab with dH₂O then rub the sample and proceed as above with step 2 on the swab.

MAPPING

1. Wet a piece of filter paper with dH₂O. Press against the suspected semen stain sample for a few seconds.
2. Drip or spray alpha-naphthyl phosphate onto the paper.
3. Drip or spray ortho-dianisidine onto the paper. Positive tests show a red-purple color on the paper.

INTERPRETATION:

Acid phosphatase is an enzyme found in high concentrations in semen, but is also found in other body fluids and is produced by other organisms such as bacteria, yeast, fungi, and plants. High levels of acid phosphatase can be found in concentrated vaginal discharge or secretions.

Appropriate results from the negative and positive control must be obtained with the reagents prior to their use on unknowns.

No color change should be observed after adding alpha-naphthyl phosphate. If a color occurs prior to adding ortho-dianisidine, then the results are inconclusive.

The color change observed after adding ortho-dianisidine should occur within 45 seconds to be considered a positive reaction. A strong positive reaction will produce an intense color change within a few seconds. A color change within 10 seconds that lacks intensity will be considered a positive reaction. Any color change that occurs after 10 seconds is considered a weak positive reaction, regardless of color intensity. Any weak reactions should be noted in the case notes. Color change reactions observed after 45 seconds are considered negative.

When mapping an item, it is important to remember that as seminal fluid is deposited onto a fabric, the concentration of spermatozoa will usually be highest in the middle of the stain with the greatest concentration of acid phosphatase usually along the outer edges.

REFERENCES:

1. Blake, Sensabaugh, Bashinski. A Systematic Approach to the analysis of Semen Evidence. CAC Meeting, 6 November 1980

2. Gaensslen, R.E. Sourcebook in Forensic Serology, Immunology, and Biochemistry, 1983
3. Saferstein, Baechtel, "The Identification and Individualization of Semen Stains", Forensic Science Handbook, Vol. 2, 1988

Metropolitan Lab. Biology Methods Manual, 1978

Archived Version

Method: **One-Step SERI AP Spot Test Detection of Acid Phosphatase**

Date: 01/11/2001

Revision Date: 02/06/2008

Approved by: SAM

REAGENTS:

1. .26g SERI AP Spot Test (SERI Catalog #R558)
2. 10mL Distilled/Deionized water

Different volumes of the reagent may be prepared. The ratio of reagent to water should not be changed

MATERIALS/EQUIPMENT:

1. Whatman Filter Paper and/or cotton swabs
2. Disposable pipettes

PROCEDURE:

A quality control check of the reagent against a known semen sample and a negative of reagents only before use. The results of the quality control test must be recorded in the case notes.

1. Dissolve the AP Spot Test reagent in water and test the reagent against known positive and negative semen standards before use.
2. Moisten a piece of Whatman filter paper or a cotton swab and vigorously press or rub it against your questioned sample. Alternatively, a small cutting from the questioned sample may be taken and placed on a piece of filter paper.
3. Drip the AP reagent onto the filter paper, swab, or cutting on filter paper.

INTERPRETATION:

Acid phosphatase is an enzyme found in high concentrations in semen, but is also found in other body fluids and is produced by other organisms such as bacteria and yeast. High levels of acid phosphatase can be found in concentrated vaginal discharge or secretions.

Appropriate results from the negative and positive control must be obtained with the reagents prior to their use on unknowns.

The color change observed after adding ortho-dianisidine should occur within 45 seconds to be considered a positive reaction. A strong positive reaction will produce an intense color change within a few seconds. A color change within 10 seconds that lacks intensity will be considered a positive reaction. Any color change that occurs after 10 seconds is considered a weak positive reaction, regardless of color intensity. Any weak reactions should be noted in the case notes. Color change reactions observed after 45 seconds are considered negative.

REMARKS:

Acid phosphatase is an enzyme found in high concentrations in semen, but is also found in other body fluids and is produced by other organisms such as bacteria, yeast, fungi, and plants. High levels of acid phosphatase can be found in concentrated vaginal discharge or secretions.

In the presence of acid phosphatase the SERI AP Spot Test reagent contains all the components necessary to carry out the following reaction: liberation of naphthol from sodium alpha-naphthyl phosphate by the enzyme and the formation of a purple azo dye by the coupling of naphthol with buffered fast blue B.

The reconstituted reagent will remain stable and sensitive for one day's use at room temperature.

REFERENCE:

Serological Research Institute. Laboratory Protocol, September 29, 1989

Method: **Identification of Cellular Material Using Xmas Tree Staining**

Date: 01/20/2000

Revision Date: 01/01/2007

Approved by: SAM

REAGENTS:

1. NUCLEAR FAST RED STAIN

Dissolve 5.0g of aluminum sulfate in 100mL of hot distilled water and add 0.1g of Nuclear Fast Red (C.I. 60760). Stir, cool, and then filter. This solution is stable for many months stored at 8° C.

Stain can be ordered pre-made from Seri, catalog #R540.

2. PICROINDIGOCARMINE STAIN

To 100mL of saturated picric acid solution add 0.33g of Indigo Carmine (C.I. 73015) and stir overnight. Filter and store at 8°C. This solution is stable for many months under these conditions.

Stain can be ordered pre-made from Seri, catalog #R540.

PROCEDURE:

1. Extract the sample according to the procedure for Differential DNA Extraction or Screening for the Presence of Semen and fix the sample(s) to the slide.
2. Cover sample well(s) on the slide(s) with a drop of Nuclear Fast Red stain (Seri Stain A) and leave for approximately 10 minutes.
3. Wash away Nuclear Fast Red stain with distilled water dispensed from a wash bottle.
4. Add one drop of Picroindigocarmine stain (Seri Stain B) to the sample well(s) without drying the slide. Rotate the dye on the slide by hand for 15-30 seconds. Wash the stain from the slide with EtOH dispensed from a wash bottle. Dry the slide and mount a coverslip with Permount.
5. Examine microscopically. The slide can be examined by phase contrast before or after staining with Xmas Tree stain.

REMARKS:

Nuclear material is stained red by the Nuclear Fast Red dye. Sperm heads are usually well differentiated with the acrosome staining less densely than the distal region of the head. Epithelial membranes and sperm tails are stained green by the Picroindigocarmine. Nuclei inside epithelial cells appear red to purple. Yeast cells also stain red, however, the stain is uniform throughout the cells and extends into polyp-like structures which are occasionally observed with yeast cells. Under phase contrast, the sperm heads will appear bright white with a dark acrosome.

REFERENCES:

Gaensslen, R., Sourcebook in Forensic Serology, Immunology and Biochemistry. US Government Printing Office, 1983.

Archived Version

Method: **p30 Detection Using the Abacus p30 Diagnostic Card**

Date: 3/31/00

Revision Date: 04/01/2008

Approved by: SAM

REAGENT:

Sterile Nanopure Water.

MATERIALS/ EQUIPMENT:

1. Abacus p30 Diagnostic Test Card Kit. The kit includes the test cards and plastic droppers. Abacus Diagnostics catalog #308322 (25 tests/kit).
2. Microcentrifuge (capable of 10,000-15,000 x g).

PROCEDURE:

1. Place a cutting of the stain or swab into a sterile 1.5mL tube. Add 75uL or enough sterile nanopure water to just saturate the material leaving a small amount of excess water. Extracts of positive control samples (neat semen and a 1:20 dilution of semen), a concentrated male urine sample, and a negative control (reagents only) should also be run with the unknowns.

Note: The positive control samples can be pre-made stains of neat semen and a 1:20 semen dilution. Alternatively, the 1:20 dilution may be made from the extract of the neat semen stain.

2. Incubate the samples for 45 minutes at room temperature. Vortex or agitate the samples three or four times during the incubation.
3. Remove the substrate or swab and place it into a spin basket. Centrifuge the spin basket for 5 minutes at maximum speed.
4. Remove the spin basket containing the substrate and retain if necessary. The tube will contain a supernatant with water-soluble proteins such as the p30 protein and the cell pellet. The supernatant will be used for the Abacus p30 Diagnostic Test and the cell pellet can be microscopically examined for the presence of cellular material.
5. Remove 20ul of the supernatant and add it to another sterile 1.5mL tube containing 200ul of water.
6. Load the entire contents of tube created in step 5 onto the sample loading "S" area of the test card.

7. Immediately start timing the reaction. The test card result should be read at ten minutes. Record the results as positive or negative on the Abacus p30 Test Worksheet. The test results can be documented by using a document scanner or digital camera. Once a hard copy of the test cards is obtained there is no need to keep a permanent data file of the image.

Note: Under no circumstances should results obtained after 10 minutes be recorded.

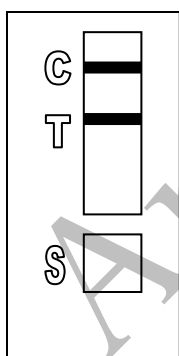
8. If a positive result for the p30 protein is obtained for a given sample it may be appropriate to also test a substrate control for that sample. For positive results from a questioned stain it could prove useful to test additional serial dilutions of the sample to provide information on how concentrated the stain is.

INTERPRETATION:

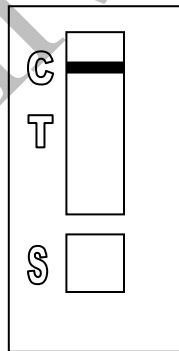
A positive test result for the presence of the protein p30 is indicated by the presence of pink bands at both the control line "C" and the test line "T" on the test card. The presence of a pink band at the control line but the absence of a pink band at the test line indicates a negative result. A pink band will always appear at the control "C" area. The band in the "C" area is an internal control of the test strip. If a pink band does not appear at the control "C" line of the test card the test is invalid.

For a test series to be interpreted the positive and negative controls must yield the expected results.

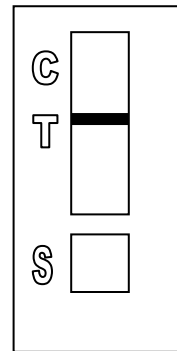
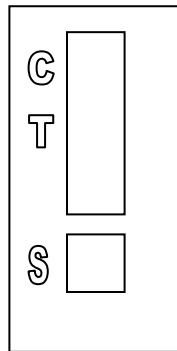
Legend: "S" = Sample loading area "T" = Test line "C" = Control line



POSITIVE



NEGATIVE



INVALID

REMARKS:

The principle behind the Abacus p30 Diagnostic Test Card is that p30 protein present in a sample will react with a mobile monoclonal antihuman p30 antibody creating a mobile antigen-antibody complex. This mobile antigen-antibody complex migrates through the

absorbent test device towards the test area. In the test area "T", a polyclonal antihuman p30 antibody is immobilized. This immobilized antibody captures the above complex creating an antibody-antigen-antibody sandwich. The conjugated pink dye particles concentrate in a narrow zone on the membrane. When the p30 concentration in the samples exceeds 4ng/mL the pink dye particles will form a pink colored band in the test area "T" indicating a positive result. An internal positive control in area (C) consists of an immobilized anti-immunoglobulin antibody that binds all p30 antibody-dye conjugates that are unable to bind to the antibody in the test area (T). The captured dye particles will form a pink band at test area "C" indicating that the test has worked properly.

Validation work on the Abacus p30 Test Card performed at the San Diego Police Department indicates that concentrated male urine can yield a positive test result with the test card. However, the small amount of p30 protein present in male urine was not detected in dried urine stains prepared from urine that had been diluted with water (1:5). Three controls will be prepared for use with Abacus p30 Test card and they consist of an extract prepared from a swatch with undiluted semen, a 1:20 dilution of the semen extract, and an extract prepared from a swatch with undiluted male urine. These controls should be prepared alongside extracts from the questioned samples. The concentrated semen and 1:20 dilution of semen should yield a positive result with the test card and the extract from the male urine should yield a negative result. A reagent blank (negative control) should also be run with the test card.

Analysts should be aware that the p30 protein is used as a diagnostic marker for prostate cancer where it is significantly overexpressed. In males with prostate cancer the p30 protein can be found in very high levels in both urine and in blood.

REFERENCES:

1. Abacus Diagnostics p30 Test Card Product Insert "One step ABACard p30 Test for the Forensic Identification of Semen".
2. Armbruster, D.A. P30: biochemistry, analytical methods, and clinical application. *Clinical Chemistry*. v39(2), p 181-95, 1993.
3. Benton, K.A., Donahue, J.A., and Valdez, Jr., Manuel. Analysis of the ABACard OneStep p30 Test for use in the forensic laboratory. Presented at the Spring Meeting of SWAFS. 1998.
4. Engelmann UH, Schramek, P., Tomamichel, G., Deindl, F., Senge, T.H. Vasectomy reversal in central Europe: results of a questionnaire of urologists in Austria, Germany, and Switzerland. *Journal of Urol*. v143(1), p64-67, 1990.
5. Graves, H.C.B. et al. Postcoital detection of a male-specific semen protein. Application to the investigation of rape. *New England Journal of Medicine*. v312(6), p338-343, 1985.

6. Hochmeister, M., Rudin, O., Borer, U.V., Gehrig, C., Kratzer, A., Dirnhofer, R. Evaluation of prostate-specific antigen (PSA) membrane tests for the forensic identification of semen. *Journal of Forensic Science*. v44, p1057-1060, 1999.
7. Jimenez, Verdejo A., Osana, E. et al. Study of the enzymatic activity of GGT, LDH, PAP, and p30 in semen stains: application to age calculation. *Forensic Science Int*. v68(1), p7-15, 1994.
8. Sensabaugh, G. Isolation and characterization of a semen-specific protein from human seminal plasma: a potential new marker for semen identification. *Journal of Forensic Science*. v23, p106-115, 1978.
9. Sokoll, L.J., Chan, D.W. P30: Its discovery and biochemical characteristics. *Urologic Clinics of North America*. v24(2), p253-9, 1997.
10. Stamey, T.A. et al. Identity of p30 purified from seminal fluid by different methods: comparison by amino acid analysis and assigned extinction coefficients. *Prostate*. v27(4), p198-203, 1995.
11. Stowell LI et al. An enzyme-linked immunabsorbent assay (ELISA) for p30. *Forensic Science Int*. v50(1), p125-38, 1991.
12. Willot, G.M. Frequency of azoospermia. *Forensic Science Int*. v20(1), p9-10, 1982.

Method: **Leucomalachite Green (LMG) Presumptive Test for Blood**

Date: 4/1/99

Revision Date: 08/14/2009

Approved by: SAM

REAGENTS:

1. LEUCOMALACHITE GREEN (LMG) SOLUTION

0.1g LMG (p,p-benzylidene-bis-N,N-dimethylaniline)
66mL glacial acetic acid
33mL distilled H₂O

Store refrigerated over excess zinc. Caution: the reagent will evolve hydrogen gas continuously. Do not cap tightly!!

Expiration: The reagent is usable as long as the LMG is in the clear (reduced) form. The color of the reagent should be evaluated before each use. A reagent which is lightly tinged green is still usable. A reagent that has become dark-green or opaque should be discarded.

2. HYDROGEN PEROXIDE 3%

Prepare by 1:10 dilution of 30% stock H₂O₂ or use a commercial 3% solution.

MATERIALS:

Cotton swabs or filter paper (test substrates)

PROCEDURE:

1. Test the reagents against positive and negative blood standards before using. Record these results in your notes.
2. Moisten a cotton swab or filter paper (the test substrate) with distilled water. Gently press or rub the stain with the substrate.

Alternatively, a portion of the stained material can be cut and used directly as the test substrate.

3. Add 1 or 2 drops LMG solution to the test substrate. Observe briefly for any color change.
4. Add 1 or 2 drops of 3% hydrogen peroxide. Observe for any color change.

INTERPRETATION:

A swiftly developing (within 5 seconds) blue-green color after step 4 is a positive test and presumptively indicates the presence of blood. When evaluating the color change in this reaction, the appearance of the stain, the amount of material being tested, and the condition of the stain should all be taken into consideration. Analysts may denote a reaction as weak if they can articulate their interpretation (the underlying reason for calling a reaction weak - color change that lacks intensity or a delayed color change not to exceed 30 seconds – should be added to the notes).

Lack of a color change before 30 seconds indicates a negative reaction and the absence of blood in detectable quantities.

Any change to a blue-green color after step 3 may indicate the presence of an oxidizer and should not be interpreted as a positive. A color change at this step should be deemed inconclusive.

The test depends on the oxidation of LMG from a colorless to a colored form. This reaction is catalyzed in the presence of heme and its derivatives. The reaction can also take place in the presence of other catalysts, such as peroxidases, or inorganic oxidizers. Therefore, the test cannot be considered completely specific for blood.

REFERENCES:

1. Burdett, P.E. "Presumptive Tests for Blood - A Comparative Survey", CRE Report No. 201, October 1976
2. Cox, M. "A Study of the Sensitivity and Specificity of Four Presumptive Tests for Blood", J Forensic Sci. Vol. 36, no. 5 (Sept. 1991), pp. 1503 - 1511
3. Gaensslen, R.E. Sourcebook in Forensic Serology, Immunology, and Biochemistry, section 6.5
4. Grodsky et al., "Simplified Preliminary Blood Testing - An Improved Technique and Comparison of Methods", J. Criminal Law, Criminology, and Police Science Vol. 42, (1951), pp. 95-104.

Method: **Phenolphthalin Presumptive Test for Blood (Kastle-Meyer Test)**

Date: 4/1/99

Revision Date: 08/14/2009

Approved by: SAM

REAGENTS:

1. PHENOLPHTHALIN STOCK SOLUTION

To a single-neck round-bottom flask, add:

10g phenolphthalein powder
100g potassium hydroxide
100g zinc shavings
500mL distilled H₂O

Place the flask in a heating mantle and attach a reflux unit. Adjust the mantle setting to gently boil the solution. Reflux until clear. Store refrigerated over excess zinc.

Expiration: Stock solution is usable as long as it is in the clear (reduced) form. A pinkish solution has oxidized and should be re-refluxed or discarded.

2. PHENOLPHTHALIN WORKING SOLUTION

Add 4 parts of ethanol to one part phenolphthalin stock solution. The reagent will be cloudy immediately following preparation; let stand refrigerated overnight to clear. Store refrigerated over excess zinc.

Expiration: The same precautions used for the stock solution should be applied. A pinkish solution should be discarded.

3. HYDROGEN PEROXIDE 3%

Prepare by 1:10 dilution of 30% stock H₂O₂ or use a commercial 3% solution.

MATERIALS:

Cotton swabs or filter paper (test substrates)

PROCEDURE:

1. Test reagents against positive and negative blood standards before using. Record these results in your notes.
2. Moisten a cotton swab or filter paper (the test substrate) with distilled water.

Gently press or rub the stain with the substrate. Alternatively, a portion of the stained material can be cut and used directly as the test substrate.

3. Add 1 or 2 drops phenolphthalin working solution to the test substrate. Observe briefly (up to 30 seconds) for any color change.
4. Add 1 or 2 drops of 3% hydrogen peroxide. Observe for any color change.

INTERPRETATION:

A swiftly developing (within 5 seconds) pink to magenta color after step 4 is a positive test and presumptively indicates the presence of blood. When evaluating the color change in this reaction, the appearance of the stain, the amount of material being tested, and the condition of the stain should all be taken into consideration. Analysts may denote a reaction as weak if they can articulate their interpretation (the underlying reason for calling a reaction weak - color change that lacks intensity or a delayed color change not to exceed 30 seconds – should be added to the notes).

Lack of a color change before 30 seconds indicates a negative reaction and the absence of blood in detectable quantities.

Bloodstains may change to a greenish color after step 3. Any change to a pink color at this stage may indicate the presence of an oxidizer and should not be interpreted as a positive.

The test depends on the oxidation of clear phenolphthalin to colored phenolphthalein. This reaction is catalyzed in the presence of heme and its derivatives. The reaction can also take place in the presence of other catalysts, such as peroxidases, or inorganic oxidizers. Therefore, the test cannot be considered completely specific for blood.

REFERENCES:

1. Cox, M. "A Study of the Sensitivity and Specificity of Four Presumptive Tests for Blood", J Forensic Sci. Vol. 36, no. 5 (Sept. 1991), pp. 1503 - 1511
2. Gaensslen, R.E. Sourcebook in Forensic Serology, Immunology, and Biochemistry, section 6.3
3. Grodsky et al., "Simplified Preliminary Blood Testing - An Improved Technique and Comparison of Methods", J. Criminal Law, Criminology, and Police Science Vol. 42, (1951), pp. 95-104.

Method: **Luminol Detection of Latent Bloodstains**

Date: 01/02/2001

Revision Date: 01/01/2007

Approved by: SAM

PURPOSE:

The Luminol test is a presumptive test for the detection of latent bloodstains not visible to the naked eye. The test is based on the peroxidase-like activity of the hemoglobin molecule. Hemoglobin derivatives greatly enhance the chemiluminescence exhibited by luminol when it is oxidized in an alkaline solution.

The luminol test is very well suited for, but not limited to, crime scene applications, especially where there is a suspicion of possible clean-up of bloodstained areas. This test may also be employed to help in the visualization of certain bloodstain patterns that may be only partially visible to the naked eye (i.e. drag marks, shoe impressions, etc.).

POLICY:

This test is not to be used as a substitute for careful visual examination for blood. The area of interest should be searched in daylight or with the aid of high intensity light prior to the use of luminol.

A Criminalist will be called to the scene to take part in the luminol procedure and to interpret any patterns that may develop.

REAGENTS AND MATERIALS:

1. Luminol powder (3-aminophthalhydrazide)
2. Sodium Perborate
3. Sodium Carbonate
4. Distilled water
5. Plastic hand pump spray bottle
6. 1L plastic bottle

CONTROLS:

Positive Control: Known blood (or bloodstained area) or a copper penny.

Negative Control: Area void of any suspected bloodstains.

PROCEDURE:

1. The following proportions of reagents should be employed for 100mL of luminol spray:

0.1g	luminol powder
0.7g	sodium perborate
5.0g	sodium carbonate
100mL	distilled water
2. Weigh out the appropriate amount of the reagents and place them into a plastic 50mL tube. If the reagents are not to be used immediately it is best to store the sodium perborate in a separate 50mL tube. The luminol and the sodium carbonate may be stored together. Once the reagents are mixed together they have a limited shelf life (approximately 24 hours).
3. Measure the appropriate amount of water and place it in a plastic bottle. Immediately prior to luminol application the reagents may be added to the water in the plastic bottle and thoroughly mixed. Once mixed, the luminol reagent should be decanted into the plastic hand pump spray bottle. Undissolved powder may have a tendency to clog the pump mechanism.
4. Prior to the application of the luminol spray, a copper penny should be placed in the general vicinity of the area to be tested. Set up any camera equipment required for documentation of the chemiluminescence at this point, if required.
5. Darken the area suspected of containing latent bloodstains to near or total darkness. It is advisable to allow time for eyes to adjust to darkness.
6. The nozzle of the plastic hand pump spray bottle should be set to the finest mist setting. Lightly spray the area of interest, as well as the copper penny. If applicable lightly spray an area known to contain bloodstains, as well as a known negative area.

INTERPRETATION:

A strong bluish chemiluminescence (positive reaction) should be exhibited by the penny when sprayed with the luminol mixture. This glow results from the penny because the luminol mixture is known to react with certain metal halides of copper and iron. The positive reaction from the penny can be used as a quality control of the luminol mixture.

A positive reaction in the area of interest is also identified by the presence of a strong chemiluminescent "glow", which lasts several seconds. Caution must be exercised in interpreting faint, or weak, reactions as well as "flashes" which could result from the interaction of luminol with metallic halides. Generally, "flashes" will fade to total

darkness immediately after spraying, while a positive bloodstained area will exhibit a longer lasting continuous reaction.

The chemiluminescence exhibited from the oxidation of luminol by a bloodstained area is bluish-white in nature and best visualized under total darkness.

Positive reactions with the luminol reagent should be judged on the color of the reaction, the intensity of the reaction, and the duration of that reaction.

It is important to note that luminol will react to produce a false positive with any agent that can oxidize the chemical directly. Some cleaning agents (i.e. Drano and Pine-sol) as well as metallic halides can produce false positives with the luminol reagent. A positive reaction obtained with the luminol reagent, even a strong, long lasting chemiluminescence, is not enough to confirm the presence of blood.

It should also be noted that luminol will not interfere with further presumptive testing, human origin through testing by HemaTrace, or subsequent DNA testing.

COLLECTION:

General evidence collection guidelines should be used for the collection of luminol positive areas. If a luminol-positive stain is going to be collected using a swab, use the sponge-tipped swab provided specifically for the collection of luminol-positive stains. The preservation steps are the same although drying time is longer.

DOCUMENTATION:

Case notes regarding luminol application should always be taken as with any forensic procedure. Documentation through photography is highly recommended (where possible). Documentation of luminol reactions can be accomplished with digital photography as well as film-based photography. Photographic documentation of the luminol reaction is best accomplished in near or total darkness. It is recommended that a control (available light or flash) photograph be taken of the area to be sprayed. A reliable reference scale should be placed in the area to be photographed. It is important that the camera not be moved between the control photograph and the luminol photograph so that proper orientation and reference can be inferred.

SAFETY CONCERNS:

May be harmful by inhalation, ingestion, or skin absorption. Causes skin and eye irritation. Material is irritating to mucous membranes and upper respiratory tract. Goggles and rubber gloves should be worn. Use of a mask is required if being used in an enclosed room. Do not breathe the chemical dust. In case of contact, immediately flush eyes with water for 15 minutes and wash hands with soap and copious amounts of water.

Do not dispose of excess luminol at the scene. It is to be brought back to the lab for disposal down the drain.

If used at a crime scene, the Department is required to leave behind a list of companies that are available for crimescene clean up.

REFERENCES:

1. Della Manna A and Montpetit S. A novel approach to obtaining reliable PCR results from Luminol treated bloodstains, J. Forensic Sci., 45:886-890, 2000.
2. RRJ Grispino. The effect of Luminol on the serological analysis of dried human bloodstains, Crime Laboratory Digest, Vol. 39(5), pp. 13-23, 1990.
3. DL Laux. Effects of Luminol on the subsequent analysis of bloodstains, J Forensic Sci., 36(5), pp. 1512-1520, 1991.
4. AM Gross, KA Harris, GL Kadlun. The effect of luminol on presumptive tests and DNA analysis using the polymerase chain reaction, J Forensic Sci., 44(4), pp. 837-840, 1999.
5. Sourcebook in Forensic Serology, Immunology and Biochemistry, National institute of Justice (1980).

Method: **Abacus HemaTrace Detection of Blood**

Date: 12/08/1999

Revision Date: 01/01/2007

Approved by: SAM

REAGENTS:

Kit supplied extraction buffer.

MATERIALS/ EQUIPMENT:

Abacus HemaTrace Test Card Kit. The kit includes test cards, plastic droppers, and extraction buffer. Abacus Diagnostics catalog #708424 (25 tests/box).

PROCEDURE:

1. Place a cutting of the stain or swab into a sterile 1.5mL tube. Add 100uL of kit extraction buffer. A solution of 5% ammonia may also be used to extract a stain that is difficult to remove from the substrate. Extracts should also be prepared from a known human blood stain (positive control) and a reagent blank (negative control).

Note: If stains remain in extraction buffer or 5% ammonia in a refrigerator for extraction, they must be warmed to room temperature before proceeding with step 2.

2. Remove enough of the supernatant created in step 1 so that when it is added to a second tube containing 150uL of extraction buffer the color of the solution is a light straw yellow.
3. Load 150uL of the solution from the microcentrifuge tube created in step 2 onto the test card (area S).
4. Immediately start timing the reaction. The test card result(s) should be read at ten minutes. Record the results as positive or negative on the Abacus HemaTrace Test Worksheet. The test results can be documented by using a document scanner or digital camera. Once a hard copy of the test cards is obtained there is no need to keep a permanent data file of the image.

Note: Under no circumstances should results obtained after 10 minutes be recorded.

If a positive result for human blood is obtained for a given sample it may be appropriate to also test a substrate control for that sample.

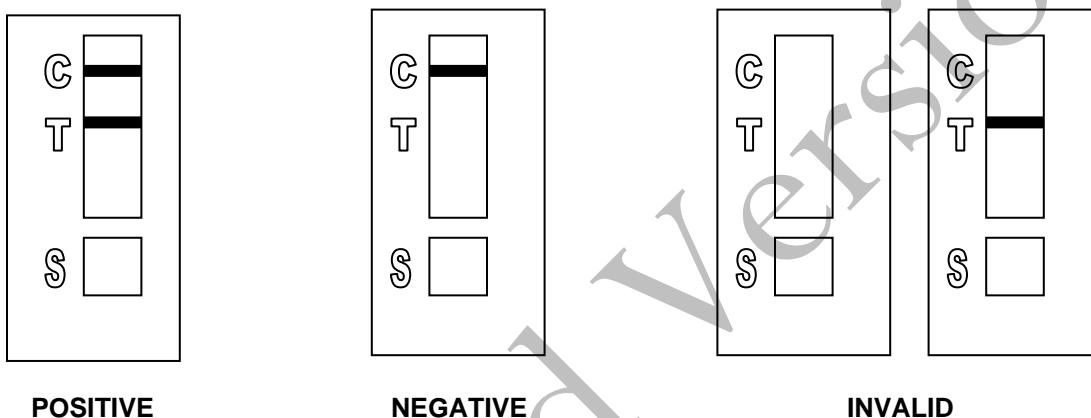
INTERPRETATION:

A positive test result for the presence of human blood is indicated by the presence of

pink bands at both the control line "C" and the test line "T" on the test card. The presence of a pink band at the control line but the absence of a pink band at the test line indicates a negative result. A pink band will always appear at the control "C" area. The band in the "C" area is an internal control of the test strip. If a pink band does not appear at the control "C" line of the test card the test is invalid.

For a test series to be interpreted the positive and negative controls must yield the expected results.

Legend: "S" = Sample loading area "T" = Test line "C" = Control line



REMARKS:

The principle behind the Abacus HemaTrace Test Card is that the human hemoglobin protein present in a sample will react with a mobile monoclonal antihuman hemoglobin antibody creating a mobile antigen-antibody complex. This mobile antigen-antibody complex migrates through the absorbent test device towards the test area. In the test area "T", an antihuman hemoglobin antibody is immobilized. This immobilized antibody captures the above complex creating an antibody-antigen-antibody complex. The conjugated pink dye particles concentrate in a narrow zone on the membrane. When the human hemoglobin concentration in the samples exceeds 0.05ug/mL the pink dye particles will form a pink colored band in the test area "T" indicating a positive result. An internal positive control in area "C" consists of an immobilized anti-immunoglobulin antibody that binds all hemoglobin antibody-dye conjugates that are unable to bind to the antibody in the test area "T". The captured dye particles will form a pink band at test area "C" indicating that the test has worked properly.

Validation work on the Abacus HemaTrace Test Card performed at the San Diego Police Department indicates that the HemaTrace card is specific for human blood, only showing some cross-reactivity (positive results) with certain higher primates. In their very concentrated form, other human body fluids can yield a positive with the

HemaTrace Test Card. This may result from small amounts of hemoglobin being present in these fluids (urine for example).

Due to the positive reaction of the Abacus card with blood of higher primates and published reports of a positive reaction with blood from ferrets, it is required that reports detailing positive reactions with the Abacus card note these possibilities.

Validation of the HemaTrace Test Card by the Abacus Company revealed that treatment with Luminol, Coomassie Blue, Ninhydrin, bleach, or detergent did not interfere with obtaining results from human blood. Human blood present on soil, plant material, leather, or washed jeans also yielded a positive result.

REFERENCES:

1. OneStep ABACard HemaTrace for the Forensic Identification of Human Blood (kit insert), Abacus Diagnostics, 1999.
2. Swander, C.J., Stites, J.G. Evaluation of the ABACard HemaTrace for the Forensic Identification of Blood, MAFS 1998 Annual Meeting.
3. Kristaly, A., Smith, D.A.S. Validation of the One-step ABACard HemaTrace for the Rapid Forensic Identification of Blood, 1999.
4. Culliford, B., The Examination and Typing of Bloodstains in the Crime Laboratory, US Department of Justice, Washington D.C., 1964.
5. Spear, T.F., Binkley, S.A. The Hemeselect Test; a Simple and Sensitive Forensic Species Test, Journal of Forensic Science Society, V34 (1), p 41-46, 1994.
6. Fernando, S.A., Wilson, G.S. Studies of the Hook Effect in the One-Step Sandwich Immunoassay. Journal of Immunology Methods, V151 (1-2), p 47-66, 1992.
7. Laux, D.L. Effects of Luminol on the Subsequent Analysis of Bloodstains, J Forensic Sci, V35 (5), p 1512-20, 1991.
8. Gerberth, V.J. Practical Homicide Investigation, Checklist and Field Guide, p 72-73, 1997.
9. Cox, M.A. A Study of the Sensitivity and Specificity of Four Presumptive Tests for Blood, Journal of Forensic Science, V36 (5), p 1503-1511, 1991.
10. Doherty, P.E., Mooney, D.J., Deciphering Bloody Imprints Through Chemical Enhancement, Journal of Forensic Science, V35 (2), p 457-465, 1990.

Method: **Amylase Radial Diffusion**

Date: 01/27/1998

Revision Date: 01/01/2007

Approved by: SAM

REAGENTS:

1. 0.1M PHOSPHATE BUFFER, PH 6.9

2.7g NaH_2PO_4 , anhydrous (0.045M)
3.9g Na_2HPO_4 , anhydrous (0.055M)
0.2g NaCl (7mM)
500mL deionized H_2O

The buffer is good for nine months when refrigerated.

2. 1% AGAROSE / 0.2% SOLUBLE STARCH GEL

Dissolve 20mg soluble starch and 100mg agarose (e.g. Sigma Type I) in 10mL of 0.1M phosphate buffer. Pour into 100 x 15mm petri dish and allow to cool. Punch wells into the gel at least 1.5cm apart using a glass pipette connected to a vacuum source.

3. IODINE SOLUTION

1.65g KI
1.50g I_2

Dissolve in 30mL warm deionized water. Stir 5 minutes and then filter. Store in an amber bottle at 4°C . This iodine solution is available commercially from Seri catalog #R512 or equivalent.

STANDARDS AND CONTROLS:

1. Saliva stain extract (neat and a 1:10 dilution)

The dried saliva stain can be stored in a freezer but must be used within 30 days.

2. Liquid saliva (1:100 dilution)

3. Blank (de-ionized H_2O)

4. Substrate control (if appropriate)

5. Other body fluid standards may be run as needed.

PROCEDURE:

1. Extract unknown and controls in de-ionized H₂O. Comparable amounts of unknowns and controls should be extracted in comparable amounts of water.
2. Individually pipette 1.5uL or 2uL of unknowns and controls into wells created in the gel plate.
3. Cover the gel plate, invert, and place in a 37°C incubator overnight.
4. Flood gel with 10mL of a 1:100 dilution of iodine solution to stain the starch dark blue.
5. Measure the diameter of the clear circles to the nearest millimeter and record the results.
6. The results should be documented in a photograph with scale.

INTERPRETATION:

- Positive: The presence of saliva is indicated if the diameter of the sample is greater than or equal to the diameter of the 1:10 extracted saliva standard.
- Inconclusive: The test is inconclusive if the diameter of the sample is less than the diameter of the 1:10 extracted saliva standard.
- Negative: The presence of saliva is not indicated if there is no circle around the sample well.

Any amylase activity obtained from substrate controls will be noted in the case notes and report.

REMARKS:

Amylase hydrolyzes starch, first to oligosaccharides, then to maltose and glucose. As the samples diffuse into the gel around each well, starch is hydrolyzed in an area proportional to the concentration of amylase present in the sample. Iodine reacts with starch to produce a deep blue color and clear circles are the result in areas where amylase has hydrolyzed the starch.

Starch----->Oligosaccharides----->Glucose + Maltose
(blue) (red) (colorless)

Amylase is present in saliva at high concentrations, hence the use of amylase as a presumptive test. However, amylase is present in most body fluids and tissues including semen, vaginal fluid, and urine though generally at much lower levels than found in saliva. Perspiration, breast milk, and feces may have high levels of amylase.

REFERENCE:

Gaensslen, R., Sourcebook in Forensic Serology, Immunology, and Biochemistry. US Government Printing Office, 1983.

Archived Version

Method: **Detection of Urobilinogen in Fecal Stains**

Date: 1/27/98

Revision Date: 01/01/2007

Approved by: SAM

REAGENTS:

1. 10 % ALCOHOLIC MERCURIC CHLORIDE (HgCl_2)

Dissolve 0.3 g of mercuric chloride into 3 mL of reagent alcohol.
Freshly prepare this reagent each time this procedure is conducted. Do not store.

2. 10% ALCOHOLIC ZINC CHLORIDE (ZnCl_2)

Dissolve 0.3 g of zinc chloride into 3 mL of reagent alcohol.
Freshly prepare this reagent each time this procedure is conducted. Do not store.

MATERIALS:

1. Ultraviolet (UV) light source
2. 6 x 50mm borosilicate glass test tubes. Note: flint glass tubes will not work for this method.

PROCEDURE:

1. Prepare an aqueous extract of the unknowns and controls. Appropriate controls include positive controls (neat, 1:5, and 1:10 dilutions), a negative control (reagents only) and any substrate controls (if applicable). Cut out approximately 0.5cm square of the stain, and place it into a 6 x 50mm borosilicate glass test tube. Add 0.5mL of distilled water to the substrate.
2. Vortex the extracts, then let the extracts stand for at least 15 minutes.
3. Remove the substrate from each tube.
4. Add 3 drops of the 10% alcoholic mercuric chloride to each tube.
5. Add 3 drops of the 10% alcoholic zinc chloride to each tube and shake the tubes to mix.
6. Examine the extracts under UV light.

INTERPRETATION:

Under UV light the extracts will have a stable apple green fluorescence if urobilinogen is present. The controls should yield the expected results for the test to be interpretable.

A positive urobilinogen result is consistent with the presence of feces.

REFERENCES:

Gaensslen, R.E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, a publication of the National Institute of Justice, U.S. Department of Justice, August 1983, pp. 197-8.

Archived Version

Method: **General DNA Extraction**

Date: 03/30/2000

Revision Date: 02/06/2009

Approved by: SAM

REAGENTS:

1. **DIGEST BUFFER** (10mM Tris-HCL, 10mM EDTA, 50mM NaCl, 2% SDS)

Mix together 1 mL of 1M Tris-HCL, pH= 7.5, 2 mL of .5M EDTA, 1 mL of 5M NaCl, 10 mL of 20% SDS (w/V) and 86 mL of nanopure water. Autoclave and store at room temperature. The solution is good for one year.

2. **10 mg/mL PROTEINASE K**

Dissolve 100mg of proteinase K in 10mL of autoclaved nanopure water. Store at -20°C in 200uL aliquots. The aliquots are good for one year.

3. **DTT** (1M DTT, 10mM Sodium Acetate, pH= 5.2)

Dissolve .77g dithiothreitol in 5mL autoclaved nanopure water. Add 50uL 1M autoclaved sodium acetate pH= 5.2. Store at -20°C in 100uL aliquots. The aliquots are good for one year.

EQUIPMENT:

Microcentrifuge (capable of 10,000-15,000 x g)

PROCEDURE:

1. Place a portion of the stain, substrate, or swab in a sterile 1.5mL microcentrifuge tube and add 400-500uL of digest buffer and 15-20uL of 10mg/mL proteinase K.

[The Large Volume protocol on the EZ1 can accommodate up to 900uL of volume in the sample tubes. Analysts may choose to add more digest buffer to samples to accommodate larger sized substrates. The volume of proteinase K added to the sample should be proportional to the volume of digest buffer.]

[If a bloodstain appears especially dirty, the analyst may add 15uL of 1M DTT to aid in the digestion of the bloodstain. If a tissue sample is embedded in paraffin wax please refer to reference 2 for the process of removing the wax.]

2. Mix gently and incubate at 56°C for at least two hours. Following incubation the samples can be gently centrifuged to remove moisture from the lids.

3. At this point any samples can be purified using either phenol/chloroform/isoamyl alcohol or the BioRobot® EZ1. Please refer to the desired protocol for the procedure.

REFERENCES:

1. Cetus Amplitype User Guide 1990
2. Fregeau CJ *et al.* AmpFISTR Profiler Plus and AmpFISTR COfiler Analysis of Tissue Stored in GenoFix, a New Tissue Preservation Solution for Mass Disaster DNA Identification. J Forensic Sci 2001; 46(5): 1180-1190.

Archived Version

Method: **DNA Extraction with Microscopic Examination**

Date: 03/30/2000

Revision Date: 01/01/2007

Approved by: SAM

REAGENTS:

1. **DIGEST BUFFER** (10mM Tris-HCL, 10mM EDTA, 50mM NaCl, 2% SDS)

Mix together 1mL of 1M Tris-HCL, pH= 7.5, 2mL of .5M EDTA, 1mL of 5M NaCl, 10mL of 20% SDS (w/V) and 86mL of nanopure water. Autoclave and store at room temperature. The solution is good for one year.

2. **10 mg/mL PROTEINASE K**

Dissolve 100 mg of proteinase K in 10 mL of autoclaved nanopure water. Aliquot solution in 200uL aliquots and store in -20°C freezer. The aliquots are good for one year.

EQUIPMENT:

Microcentrifuge (capable of 10,000-15,000 x g)

PROCEDURE:

Note: This procedure is designed for evidence samples. Reference mouth swabs do not require microscopic analysis (see "BioRobot EZ1 DNA Purification of Reference Samples" procedure).

1. Place a portion of the substrate or swab in a 1.5mL microcentrifuge tube and add 0.75 to 1mL of sterile nanopure water.
2. Incubate the sample at room temperature for at least 30 minutes with occasional vortexing.
3. Remove the substrate and place into a second sterile 1.5mL microcentrifuge tube and retain.
4. Centrifuge the sample in a microcentrifuge for 3-5 minutes at maximum speed.
5. Without disturbing the pellet, remove and discard all but 50-100uL of the supernatant. Resuspend the cell pellet.
6. Refer to the procedure "Identification of Cellular Material Using Xmas Tree Staining."

7. Add 0.5mL of digest buffer and 15-20uL of 10 mg/mL proteinase K solution. Vortex briefly. [The original substrate can be added back to increase the DNA yield from the sample].
8. Incubate the sample at 56°C for at least 2 hours.
9. At this point any samples can be purified using either phenol/chloroform/isoamyl alcohol or the BioRobot® EZ1. Please refer to the desired protocol for the procedure.

REFERENCES:

Cetus Amplitype User Guide 1990

Archived Version

Method: **Differential DNA Extraction**

Date: 06/29/2000

Revision Date: 02/06/2009

Approved by: SAM

REAGENTS:

1. **DIGEST BUFFER** (10mM Tris-HCL, 10mM EDTA, 50mM NaCl, 2% SDS)

Mix together 1mL of 1M Tris-HCL, pH= 7.5, 2mL of .5M EDTA, 1mL of 5M NaCl, 10mL of 20% SDS (w/V) and 86mL of nanopure water. Autoclave and store at room temperature. The solution is good for one year.

2. **10 mg/mL PROTEINASE K**

Dissolve 100mg of Proteinase K in 10mL of autoclaved nanopure water. Store at -20°C in 200uL aliquots. The aliquots are good for one year.

3. **DTT** (1M DTT, 10mM Sodium Acetate, pH= 5.2)

Dissolve .77g dithiothreitol in 5mL autoclaved nanopure water. Add 50uL 1M autoclaved sodium acetate pH= 5.2. Store at -20°C in 100uL aliquots. The aliquots are good for one year.

EQUIPMENT:

Microcentrifuge (capable of 10,000-15,000 x g)

PROCEDURE:

Note: The initial dH₂O wash and microscopic analysis of samples that have previously been screened is optional. If this process is skipped analysts may start the differential extraction at step 8.

1. Place the sample (swab or other substrate) to be extracted in a sterile 1.5mL tube and add 1mL of sterile distilled water.
2. Incubate at room temperature for at least 30 minutes.
3. Vortex the tube a couple of times during the incubation to aid in removing the cells from the substrate.
4. Remove the substrate with a pipette tip or plain wood applicator and transfer it to a second sterile microfuge tube and retain.

5. Spin the sample in a microcentrifuge for 3-5 minutes at 10,000 x g (maximum speed) at room temperature.
6. Without disturbing the cell pellet, remove and discard all but 50-100uL of the supernatant. Resuspend the cell pellet in the remaining supernatant. Place 5uL of the sample onto a well of a slide (different amounts may be used). Place the slide in an incubator or on a heat block to fix any cells to the slide.
7. At this point proceed to the procedure "Identification of Cellular Material Using Xmas Tree Staining". Note: In general, 100 sperm cells are needed to obtain a DNA result. The sperm cells observed microscopically, however, provide only an estimate of the total number of sperm present in a sample extract. An analyst should use appropriate judgement when deciding on whether to proceed with DNA analysis on a particular sample.
8. Add 400-500uL of digest buffer and 15-20uL of 10mg/mL proteinase K to the resuspended cell pellet (more digest buffer and proteinase K may be added if the substrate is especially large). [Option: The original substrate can now be added back to increase the amount of cellular material removed from the substrate.]
9. Incubate at 56°C for 1-2 hours to lyse nonsperm cells. [Option: Incubation may be longer to ensure lysing of the nonsperm cells].
10. Spin the sample in a microcentrifuge for 3-5 minutes at maximum speed. Remove the supernatant and place in a sterile 1.5mL microcentrifuge tube. This supernatant contains DNA from any non-sperm cells present in the sample. The sample can be put aside until DNA purification. [The reagent blank supernatant will act as a control for the non-sperm fraction].
11. [Option: A second digest with digest buffer and proteinase K may be performed in order to ensure that the sperm fraction is free of non-sperm cells].

Note: An analyst may elect to skip directly to step 14 in order to immediately perform the microscopic analysis. Steps 12 and 13 should be performed prior to step 16 if sperm cells are observed in the samples.

12. Wash the pellet as follows: Resuspend the pellet in 0.5-1.0mL digest buffer and vortex briefly. Spin the sample in a microcentrifuge for 5 minutes at maximum speed. Remove all but 50-100uL and discard.
13. Repeat wash step 12 at least twice. [Additional washes are critical when the number of sperm is low and the number of non-sperm cells is high].
14. Resuspend the pellet in 0.5-1.0mL sterile distilled water and vortex briefly. Spin the cells in a microcentrifuge for five minutes at maximum speed. Remove and discard

all but 50-100uL of the supernatant. Resuspend the cells in the remaining supernatant.

15. To verify the presence of sperm and the complete digestion of nonsperm cells proceed to the procedure "Identification of Cellular Material Using Xmas Tree Staining".

Note: If the sperm fraction is probative, the entire sample being analyzed should contain approximately 100 sperm to proceed with DNA analysis. In this circumstance both the sperm and non-sperm fractions should be continued through DNA analysis.

If the non-sperm fraction is probative and sufficient sperm cells are present, both the sperm and non-sperm fractions must be DNA typed.

If the non-sperm fraction is probative and insufficient or no sperm cells are found in the sperm fraction, only the non-sperm fraction needs to be typed.

16. Add 400-500uL digest buffer, 20uL 1M DTT and 15-20uL of 10 mg/mL proteinase K solution to the re-suspended sperm cell pellet.
17. Incubate for at least 2 hours at 56°C.
18. At this point any samples (either non-sperm or sperm fraction) can be purified using either phenol/chloroform/isoamyl alcohol or the BioRobot® EZ1. Please refer to the desired protocol for the procedure.

REFERENCES:

Cetus Amplitype User Guide 1990

Method: **DNA Extraction from Hair**

Date: 03/30/2000

Revision Date: 03/01/2010

Approved by: SAM

REAGENTS:

1. **DIGEST BUFFER** (10mM Tris-HCL, 10mM EDTA, 50mM NaCl, 2% SDS)

Mix together 1mL of 1M Tris-HCL, pH= 7.5, 2mL of .5M EDTA, 1mL of 5M NaCl, 10mL of 20% SDS (w/V) and 86mL of nanopure water. Autoclave and store at room temperature. The solution is good for one year.

2. **10 mg/mL PROTEINASE K**

Dissolve 100mg of Proteinase K in 10mL of autoclaved nanopure water. Store at -20°C in 200uL aliquots. The aliquots are good for one year.

3. **DTT** (1M DTT, 10mM Sodium Acetate, pH= 5.2)

Dissolve .77g dithiothreitol in 5mL autoclaved nanopure water. Add 50uL 1M autoclaved sodium acetate pH= 5.2. Store at -20°C in 100uL aliquots. The aliquots are good for one year.

MATERIALS/ EQUIPMENT:

1. Microcentrifuge (capable of 10,000-15,000 x g)
2. Xylene and 100% ethanol (for mounted hairs)
3. Diamond scribe (for mounted hairs)
4. 50mL sterile disposable plastic tubes

PROCEDURE:

1. Wash hair to reduce surface dirt and potential contaminants as follows:
2. For loose hairs, fill a sterile 50mL plastic screw-topped tube with sterile nanopure water and place the hair in the tube using a pair of sterile forceps. Wash each hair separately.

For mounted hairs, freeze the slide in a -20°C freezer for 20 minutes. Remove coverslip by cracking the coverslip with a diamond scribe. Alternatively, the coverslip may be removed by soaking in xylene for several hours after cracking the coverslip with a

diamond scribe. Wash away the mounting medium by squirting the slide with xylene. Place the hair in a 1.5mL microcentrifuge tube and add 100% ethanol. Finally, wash the hair in the microcentrifuge tube by removing and discarding the ethanol and then adding water.

3. Examine the hair under a dissecting microscope. Note the possible presence of body fluids on the hair. Cut off 0.5cm to 1cm of the proximal (root) end for digestion. Because the hair may contain cellular material on the surface that may or may not originate from the hair donor, it is advisable to cut off 0.5 to 1cm of the shaft adjacent to the root as a control. The remaining shaft should be retained. [Note: All evidentiary hairs should be photographed prior to performing DNA testing on them.]
4. Place the hair sample and its control into separate sterile 1.5mL microcentrifuge tubes. Add 0.5mL digest buffer, 20uL 1M DTT and 15-20uL 10mg/mL proteinase K.
5. Incubate at 56°C for at least 6 hours.
6. Add an additional 15-20uL 1M DTT and 15uL of 10 mg/mL proteinase K to the samples.
7. Incubate at least 6 hours or until the hair is dissolved.
8. At this point any samples can be purified using either phenol/chloroform/isoamyl alcohol or the BioRobot® EZ1. Please refer to the desired protocol for the procedure.

REFERENCES:

Cetus Amplitype User Guide 1990

Method: **The Processing and DNA Extraction of Bone Samples**

Date: 06/21/2005

Revision Date: 04/01/2008

Approved by: SAM

REAGENTS:

1. LIQUID NITROGEN (16 liters is needed as a minimum) obtained from the 50 gallon tank located in the Trace Evidence section.

2. 10-20% BLEACH (Sodium Hypochlorite)

Dilute store bought bleach between 1:5 and 1:10 with water.

3. DIGEST BUFFER (10mM Tris-HCL, 10mM EDTA, 50mM NaCl, 2% SDS)

Mix together 1mL of 1M Tris-HCL, pH= 7.5, 2mL of 0.5M EDTA, 1mL of 5M NaCl, 10mL of 20% SDS (w/V) and 86mL of nanopure water. Autoclave and store at room temperature. The solution is good for one year.

4. 10mg/mL PROTEINASE K

Dissolve 100mg of proteinase K in 10mL of autoclaved nanopure water. Store at -20°C in 200uL aliquots. The aliquots are good for one year.

5. DTT (1M DTT, 10mM Sodium Acetate, pH= 5.2)

Dissolve .77g dithiothreitol in 5mL autoclaved nanopure water. Add 50uL 1M sodium acetate pH= 5.2. Store at -20°C in 100uL aliquots. The aliquots are good for one year.

6. AMMONIUM OXALATE (saturated, pH= 3.0)

Make a saturated solution of ammonium oxalate by adding ammonium oxalate to 100mL of nanopure water. Adjust to pH= 3.0 by adding HCL.

7. 0.5M EDTA (pH= 8.0)

Dissolve 186.1g disodium ethylenediamine tetraacetate-2H₂O to 800mL of distilled water. Stir vigorously and adjust the pH to 8.0 by adding NaOH pellets (approximately 20g). Adjust final volume to one liter with distilled water and autoclave.

EQUIPMENT:

1. 6850 Freezer Mill with grinding accessories
2. 50mL plastic sterile tubes

PROCEDURE:

A bone kit has been assembled for the analysts that require the tools needed to process bone samples for casework. The kit includes a vise, clamps to secure the vise to a table, a hacksaw with a supply of blades, sandpaper, and a Dremel™ tool with accessories. It is intended that the vise be used to secure the bone sample while sectioning a portion for analysis.

1. Clean the two metal end plugs, the metal impactor, and the plastic vial with soap and water being careful to remove all of the bone debris. Following this cleaning soak the components in 10% bleach solution for no longer than 30 minutes.
2. Rinse in water and dry all the components (If the metal plugs and impactor are not dried they will rust).
3. Swab all components using a nanopure water moistened cotton-tipped swab. This swab will serve as a control during the extraction procedure and should be extracted alongside the bone sample.
4. Remove a portion of the evidence bone (the amount of bone sectioned will depend on the age and condition of the bone as well as the environmental exposure) using a clean hacksaw blade. Clean the bone piece by removing adhering tissue, decaying marrow, and any other debris. Use the provided sandpaper to remove the approximately 1mm of the outermost surfaces of the bone; including the central area where the marrow was (the bone section may need to be cut in half length-wise to accomplish this).
5. Soak the bone sample in a 10-20% bleach solution for approximately 15 minutes. Rinse the bone section twice with nanopure water.
6. Wrap the bone piece in a latex glove and use a hammer to break the bone into several smaller fragments.
7. Tightly place one steel end plug on the plastic grinding vial. Place bone fragments and metal impactor inside vial. Close the vial with the other metal end plug.
8. Open the freezer mill lid by pressing the Lid Up/Down switch. Lift the metal gate handle up and turn it clockwise to line it up with the slot on the gate. Pull the gate down to insert the vial. Insert the vial, close, and lock the gate (loosely tighten the gate).
9. Wearing insulated gloves and other appropriate protective gear gradually fill the tub

with 16 liters of liquid nitrogen. This requires the carboy that is used to transport liquid nitrogen to be filled completely four times.

10. Lower the lid slowly by pressing the Lid Up/Down switch until the coil enters the liquid nitrogen. Pause to allow boiling of the nitrogen around the warm coil. When the boiling subsides, close the lid the rest of the way.
11. When the vapor stream has slacked off (30 seconds or so) check the liquid nitrogen level with the internal sensor by pressing control keys 2 and 3 simultaneously.
12. When you press the RUN key (with sufficient liquid nitrogen in the tub, a loaded vial in the coil, and the factory settings in place), the Freezer/Mill will perform the following steps:
 - a. The mill cools for 8 min., while the TIME display counts down the minutes remaining in the pre-cooling period. The T3 indicator light should be on.
 - b. The first grinding period begins and lasts for 2 minutes, while the TIME display counts down the time remaining in that grinding period, and the RUN indicator light is lit. The CYCLE display shows 3.
 - c. The first cooling period begins and lasts for 2 minutes, while the TIME display counts down and the T2 indicator light is on. The CYCLE display still shows 3.
 - d. The second grinding/cooling cycle takes place, the same as in steps b and c combined. The TIME display will count down, and the appropriate RUN and COOL indicator lights will be on in turn, while the CYCLE display shows 2.
 - e. The third grinding/cooling cycle is like the second (step d) except that the CYCLE display shows 1.
 - f. At the end of the third grinding cycle, the TIME display will flash END for several seconds, and then the control panel will return to Program Mode, exhibiting the same parameters as when the mill was first started.
13. Wearing protective gear, raise the lid by pressing the Lid Up/Down switch and remove the vial by opening the locked gate.
14. Fit the metal end plug extractor over one end of the vial and turn knob clockwise several turns until the screw engages the metal end plug. Squeeze the lever, then turn the knob clockwise and repeat until the end plug is removed. (If the screw locks up wait briefly for vial to warm up before attempting to open again.)
15. When the end-plug is removed from the vial, empty the contents of the vial into a suitable container. Perhaps the easiest means of gathering the ground bone is first to pour the contents of the vial into a disposable plastic weighboat and then in turn transfer the contents to a 50ml conical plastic tube.
16. Clean the two metal end plugs, the metal impactor, and the plastic vial with soap and water being careful to remove all of the bone debris. Following this cleaning soak the components in 10% bleach solution for no longer than 30 minutes.

17. Rinse in water and dry all the components (If the metal plugs and impactor are not dried they will rust).
18. If running another bone sample ensure the liquid nitrogen level is appropriate and proceed.
19. After the milling of the bone is complete (refer to 6850 Freezer Mill Procedure), pour the ground bone into a clean weighboat and then transfer the ground bone into a 50mL plastic tube.

Steps #20-22 are optional and involve decalcifying the bone powder over a three day period using 0.5M EDTA. In some cases, the decalcifying step will increase the amount of DNA recovered from the ground bone. If a BioRobot® EZ1 extraction is to be performed then the bone sample must be decalcified. A BioRobot® EZ1 extraction is only recommended with relatively fresh bone samples.

20. Decalcifying is accomplished by adding 40mL of 0.5M EDTA, pH = 8.0 to the 50mL plastic tube containing the ground bone. Place the tube on a rocking shaker overnight and the following morning spin the tube in the swinging bucket rotor at approximately 2500rpm for 5 minutes. Pour the supernatant off into another 50mL plastic tube. Save this tube for step #21. Add 40mL of 0.5M EDTA to the bone powder and resuspend the pellet. Place the tube on the rocking shaker. The 0.5M EDTA should be changed approximately 4-5 times over the three day period.
21. The decalcification process should be monitored by the addition of saturated ammonium oxalate pH= 3.0, to the decanted supernatants. The addition of the ammonium oxalate to the decanted supernatant will produce a cloudy precipitate if calcium is present. If the supernatants remains clear after the addition of ammonium oxalate the decalcification is complete. The supernatant can be discarded.
22. Following decalcification, wash the bone pellet three times by adding 40mL of sterile nanopure water, resuspending the pellet and centrifuging the sample for 5 minutes at approximately 2500rpm. Discard the supernatant.
23. To the bone powder (no decalcification) or the bone pellet (decalcification), add 7mL (for organic DNA purification) or 600-700uL (for BioRobot® EZ1 DNA purification) of extraction buffer, 200uL of 10 mg/mL proteinase K ,and 100uL of 1M DTT and mix. Incubate the tube at 56°C for at least 4 hours.
24. To the initial incubation add another 200uL of 10 mg/mL proteinase K and 100uL of 1M DTT. Incubate the tube at 56°C at least 6 hours.

If the samples will be purified using the BioRobot® EZ1, please refer to the BioRobot® EZ1 DNA purification procedure. If phenol/chloroform/isoamyl alcohol DNA purification

is to be performed then continue with step #25 of this procedure.

25. Add 7mL of phenol/chloroform/isoamyl alcohol to the bone sample. Vortex the sample, spin the tube at approximately 3000rpm and transfer the upper aqueous phase to a new 50mL tube. Continue to purify the sample using phenol/chloroform/isoamyl alcohol until the aqueous-organic interface is clear.
26. [Optional] Add 7mL of butanol to the tube, vortex, spin the tube at approximately 3000rpm for 2 minutes and remove the butanol phase (upper) and discard.
27. The purified sample can now be washed and concentrated. Please refer to the protocol for the use of the Centricon-100 centrifugal filter devices (DNA Purification/Concentration Using Centricon YM-100 Centrifugal Filter Devices) to concentrate the purified samples. Each purified bone sample may require several centricon filter devices as the extracts may have fine bone powder in them which tends to clog the devices.

REFERENCES:

Hochmeister MN *et al.* "Typing of Deoxyribonucleic Acid (DNA) Extracted from Compact Bone from Human Remains. J Forensic Sci. 1991 Nov;36(6):1649-61.

Method: **Organic DNA Purification Using Phenol/Chloroform/Isoamyl alcohol**

Date: 05/24/2005

Revision Date: 01/01/2007

Approved by: SAM

REAGENTS:

1. PHENOL/CHLOROFORM/ISOAMYL ALCOHOL (25:24:1)

Add 0.1%(w/v) 8-hydroxyquinoline (Sigma Catalogue #H-6878) to the phenol/chloroform/isoamyl alcohol (Fisher BP1752I-400) to prevent oxidation. Store at 4°C. The solution is good for one year.

NOTE: See additional directions concerning the preparation of phenol/chloroform/isoamyl alcohol as outlined in the Reagent Preparation Log.

2. n-BUTANOL

To 50mL of n-butanol add approximately 50mL of autoclaved nanopure water and shake. Following separation the upper layer is n-butanol which is now saturated with water. The solution is good for one year.

PROCEDURE:

1. Remove the substrate (if applicable), and add 0.5mL buffered phenol/chloroform/isoamyl alcohol solution. Vortex for 15 seconds.

[Phenol/chloroform extractions must be carried out in a hood with a lab coat and eye protection].
2. Spin in a microcentrifuge for 3-5 minutes at maximum speed.
3. Use a sterile pipette tip or equivalent to transfer the upper aqueous phase to a new sterile 1.5mL microcentrifuge tube.
4. Repeat steps 1-3 an additional one or two times or until the aqueous phase is clear. For these additional extractions, the lower phenol-chloroform layer may be removed and discarded, thus eliminating the need for a fresh microcentrifuge tube.
5. The addition of 0.5 to 0.75mL of water saturated n-butanol is an optional step to further purify the extracted sample (n-butanol removes any residual phenol-chloroform). Vortex 15 seconds.
6. Spin in microcentrifuge for one minute at maximum speed. Discard the upper layer which is n-butanol.

7. The purified sample can now be washed and concentrated. Please refer to the protocol for the use of either the Microcon-YM100 centrifugal filter devices (DNA Purification/Concentration Using Microcon YM-100 Centrifugal Filter Devices) or the Centricon-100 centrifugal filter devices (DNA Purification/Concentration Using Centricon YM-100 Centrifugal Filter Devices) to concentrate the purified samples.

REFERENCES:

Cetus Amplitype User Guide 1990

Archived Version

Method: **DNA Purification/Concentration Using Microcon YM-100 Centrifugal Filter Devices**

Date: 05/24/2005

Revision Date: 01/01/2007

Approved by: SAM

REAGENTS:

TE BUFFER (10mM Tris-HCL, 0.1M EDTA pH= 8.0)

Mix together 10mL of 1M Tris-HCL, pH= 8.0 with 0.2mL of 0.5M EDTA and 990mL nanopure water. Aliquot into 100mL bottles, autoclave and store at room temperature. The solution is good for nine months.

MATERIALS:

1. Microcon-YM 100 Centrifugal Filter Devices (Amicon)
2. Microcentrifuge (capable of 10,000-15,000 x g)

PROCEDURE:

Note: The capacity of the Microcon-YM100 reservoir is approximately 500uL. If the purified extract has a larger volume it may require adding any remaining amount of sample that did not fit into the reservoir after the first spin. This protocol may also be used to further concentrate previously purified samples.

1. Assemble the Microcon-YM100 unit by inserting the sample reservoir into the filtrate vial.
2. Add approximately 50uL of TE buffer to the upper Microcon-YM100 reservoir.
3. Add the entire extracted DNA sample on top of the TE buffer. Seal with the attached cap.
4. Centrifuge the assembled Microcon spin-column in a microcentrifuge for 12-15 minutes at 2700rpm (~500 x g). The DNA sample will be concentrated in about 5-15uL of TE buffer in the bottom of the upper Microcon-100 reservoir (molecules with molecular weights of less than 100,000 Daltons will pass through the filter).
5. Discard the effluent in the filtrate vial. Retain the tube for further wash steps.
6. Add approximately 300uL of TE buffer to the concentrated DNA solution in the Microcon reservoir.

7. Centrifuge the sample(s) at 2700rpm for 12-15 minutes. Discard the effluent and repeat the TE wash (steps 5-7) at least one more time.
8. After the last wash, 5-200uL of TE buffer may be added to the Microcon-100 column (depending on desired final volume/concentration). Collect the DNA sample by inverting the reservoir into a new microcon tube and centrifuge at approximately 3800 rpm (approximately 1000 x g) for 3-5 minutes.
9. The concentrated purified DNA now resides in the tube. Cap the tube and store frozen. Alternatively, transfer the purified DNA to a different tube, such as a sterile screw-cap tube for storage.

REFERENCES:

Microcon Centrifugal Filter Device User Guide, Revision J. Millipore 2000

Method: **DNA Purification/Concentration Using Centricon YM-100 Centrifugal Filter Devices**

Date: 01/01/2007

Revision Date: 01/01/2007

Approved by: SAM

REAGENT:

TE BUFFER (10mM Tris-HCL, 0.1M EDTA pH= 8.0)

Mix together 10mL of 1M Tris-HCL, pH= 8.0 with 0.2mL of 0.5M EDTA and 990mL nanopure water. Aliquot into 100mL bottles, autoclave and store at room temperature. The solution is good for nine months.

MATERIALS:

1. Ultracel-YM 100 Centrifugal Filter Devices (Amicon)
2. Table Top Centrifuge with Fixed Angled Rotor (Sorvall RT6000B or RT6000D)

PROCEDURE:

1. Assemble the Ultracel-YM 100 (Centricon) unit by inserting the sample reservoir into the filtrate vial.
2. Add approximately 1.5mL of TE buffer to the upper Centricon reservoir.
3. Add the entire extracted DNA sample on top of the TE buffer. Cover the top of the sample reservoir with the retentate vial.
4. Centrifuge the column in the Sorvall RT6000B (or RT6000D) at approximately 4300 rpm (approximately 2000 x g) at room temperature for 7 minutes. The DNA sample will be concentrated in about 40uL of TE buffer in the bottom of the upper Centricon reservoir (molecules with molecular weights of less than 100,000 Daltons will pass through the filter).
5. Discard the effluent in the filtrate vial.
6. Add approximately 2mL of TE buffer to the concentrated DNA solution in the sample reservoir.
7. Repeat steps 7-8 two more times for a total of three washes.
8. After the last wash, collect the approximately 40uL of concentrated DNA sample by

inverting the sample reservoir into the provided retentate vial and centrifuge at approximately 1800 rpm (approximately 500 x g) for two minutes.

9. The concentrated purified DNA now resides in the retentate vial. Transfer the purified DNA to an appropriate tube and store frozen.

REFERENCES:

Centricon Centrifugal Filter Device User Guide, Revision J. Millipore 2005

Archived Version

Method: **BioRobot® EZ1 DNA purification of Evidence Samples**

Date: 01/01/2007

Revision Date: 04/01/2008

Approved by: SAM

REAGENTS:

BioRobot® EZ1 DNA Investigator reagent cartridges. The cartridges contain all reagents required for the DNA extraction and purification of samples on the BioRobot® EZ1. The reagents in the cartridges includes guanidine thiocyanate/guanidine hydrochloride, the paramagnetic silica coated beads, ethanol, TE buffer, and sterile water.

MATERIALS/EQUIPMENT:

1. EZ1 DNA Investigator kit (QIAGEN Catalogue #952034)
2. BioRobot® EZ1

PROCEDURE:

1. Remove the substrate (if applicable), and transfer the liquid digest to the EZ1 sample tubes provided in the EZ1 DNA Investigator kit.

Optional: The addition of RNA may improve the DNA yields of samples that contain low amounts of DNA. Analysts may add 1ug of RNA to the sample lysates prior to EZ1 purification of the samples.

- a) Add 310uL of TE buffer to the supplied tube of carrier RNA (310ug) to obtain a solution of 1ug/uL.
- b) Dissolve the carrier RNA thoroughly. Add 1uL of the carrier RNA to your sample lysates. Continue following the protocol at step 2.

Note: The dissolved carrier RNA should be stored at -20°C when not being used.

The following steps deal with the set-up and operation of the BioRobot® EZ1.

2. Insert the DNA Investigator v1.0 protocol card into the card slot on the BioRobot® EZ1 and turn the instrument on.
3. Press "START" to display the protocols menu, then select the desired protocol. The Large Volume protocol should be selected if evidence samples are being extracted.
4. Select the desired eluent (TE or water).

5. Select the desired elution volume; 50uL is recommended for most evidence samples; 200uL is recommended for samples with very high expected DNA yields (such as non-sperm fractions from vaginal swabs, etc). Note that the elution volume will be the same for all samples on the same BioRobot® EZ1.
6. Press any key to proceed through the text displayed in the LCD. The text in the LCD prompts the loading of the BioRobot® EZ1.

The following steps deal with the loading of samples and reagents into the BioRobot® EZ1.

7. Invert the reagent cartridges (1-6 per extraction run per instrument) to mix, and then tap the cartridges to deposit the reagents to the bottom of their wells. Insert the appropriate number of reagent cartridges in the cartridge rack. Additional samples can be accommodated in additional instruments or in subsequent instrument runs.
8. Load the cartridge rack (with cartridges) into the BioRobot® EZ1. Ensure that the reagent cartridges are seated properly.
9. Load 1-6 tip holders containing the filter-tips into row 2 of the tip rack.
10. Load 1-6 opened sample tubes (from Step 3.) into row 4 of the tip rack.
11. Load 1-6 uncapped appropriately labeled elution tubes into row 1 of the tip rack. Check to ensure that the elution tubes are in the order corresponding to the sample tube order so that no sample mix-ups occur. Close the workstation door.
12. Press "START" to start the extraction protocol.
13. When the protocol ends, the LCD displays "FINISHED". If you intend to run another extraction protocol, press "ESC" to return to the "Protocols" menu. Otherwise, press "STOP" twice to return the motors to the home positions.
14. Open the workstation door. Remove and cap the elution tubes containing your purified DNA.

The BioRobot® EZ1 can be cleaned by rinsing the tube rack, tip rack, and cartridge rack, briefly, in a solution of 5% Bleach. The UV cross linker may be used to decontaminate the BioRobot® EZ1 components. The BioRobot® EZ1 components may also be cleaned using a diluted neutral soap followed by water.

Unless another extraction protocol is being performed the BioRobot® EZ1 may be switched "OFF".

REFERENCES:

1. BioRobot® EZ1 Genomic DNA Kit Handbook. Qiagen February 2003
2. Magtration® System 6GC Operation Manual Version 1.1. Precision System Science Co. 2002.
3. Montpetit SA, Fitch IT, O'Donnell PT. A simple automated instrument for DNA extraction in forensic casework, J Forensic Sci. 2005 May;50(3):555-63.

Kishor R, *et al.* Optimization of DNA extraction from low yield and degraded samples using the BioRobot EZ1 and BioRobot M48. J Forensic Sci. 2006 Vol. 51(5): 1055.

Archived Version

Method: **DNA Extraction from Reference Samples Using the BioRobot® EZ1**

Date: 06/02/2003

Revised: 04/01/2008

Approved by: SAM

REAGENTS:

1. DIGEST BUFFER (10mM Tris-HCL, 10mM EDTA, 50mM NaCl, 2% SDS)

Mix together 1mL of Tris-HCL, pH = 7.5, 2mL of 0.5M EDTA, 1mL of 5M NaCl, 10mL of 20% SDS (w/v) and 86mL of nanopure water. Autoclave and store at room temperature. The solution is good for one year.

2. 10mg/mL PROTEINASE K

Dissolve 100mg of proteinase K in 10mL of autoclaved nanopure water. Store at -20°C in 200uL aliquots. The aliquots are good for one year.

3. BioRobot EZ1 DNA Investigator reagent cartridges.

The cartridges contain all reagents required for the DNA extraction and purification of samples on the BioRobot EZ1. The reagent in the cartridges included guanidine thiocyanate/guanidine hydrochloride, the paramagnetic silica coated beads, ethanol, TE buffer, and sterile water.

MATERIALS/EQUIPMENT:

1. EZ1 DNA Investigator kit (QIAGEN Catalogue #952034)
2. BioRobot® EZ1

PROCEDURE:

1. To a sterile 1.5mL tube (an EZ1 sample tube is used if the Tip-Dance protocol is used – see step 5) add 190uL of digest buffer, 10uL of 10mg/mL proteinase K and the appropriate amount of the reference sample (bloodstain/liquid blood/reference mouth swab).
2. Mix gently and incubate at 56°C for at least 30 minutes. During the incubation, the robot may be set-up for extraction.
3. Remove the substrate (if applicable), and transfer the liquid digest to the EZ1 sample tubes provided in the EZ1 DNA Extraction kit.

The following steps deal with the set-up and operation of the BioRobot® EZ1.

4. Insert the DNA Investigator v1.0 protocol card into the card slot on the BioRobot® EZ1 and turn the instrument on.
5. Press “START” to display the protocols menu then select the “Trace”, “Trace TD”, or “Normalization” protocol. The Normalization protocol will limit the DNA yield of the samples by employing a limited amount of paramagnetic beads in the extraction. In the Trace TD protocol the protocol uses a Tip-Dance technique that can accommodate the purification of a sample with up to a maximum of ¼ swab in the tube. If this option is selected the substrate, if it is less than or equal to ¼ swab, need not be removed prior to purification on the EZ1.
6. Select the desired eluent (TE or water).
7. Select the desired elution volume; 200uL is recommended for most reference sample extractions.
8. Press any key to proceed through the text displayed in the LCD. The text in the LCD prompts the loading of the BioRobot® EZ1.

The following steps deal with the loading of samples and reagents into the BioRobot® EZ1.

9. Invert the reagent cartridges (1-6 per extraction run per instrument) for your extraction to mix, then tap the cartridges to deposit the reagents to the bottom of their wells. Insert the appropriate number of reagent cartridges in the cartridge rack. Additional samples can be accommodated in additional instruments or in subsequent instrument runs.
10. Load the cartridge rack (with cartridges) into the BioRobot® EZ1. Ensure that the reagent cartridges are seated properly in the cartridge rack.
11. Load 1-6 tip holders containing the filter-tips into row 2 of the tip rack.
12. Load 1-6 opened sample tubes (from Step 3.) into row 4 of the tip rack.
13. Load 1-6 uncapped appropriately labeled elution tubes into row 1 of the tube rack. Check to ensure that the elution tubes are in the order corresponding to the sample tube order so that no sample mix-ups occur. Close the workstation door.
14. Press “START” to start the extraction protocol.
15. When the protocol ends, the LCD displays “FINISHED”. If you intend to run another extraction protocol, press “ESC” to return to the “Protocols” menu. Otherwise, press

“STOP” twice to return the motors to the home positions.

16. Open the workstation door. Remove and cap the elution tubes containing your purified DNA.

The BioRobot® EZ1 can be cleaned by rinsing the tube rack, tip rack, and cartridge rack, briefly, in a solution of 5% Bleach. The UV cross linker may be used to decontaminate the BioRobot® EZ1 components. The BioRobot® EZ1 components may also be cleaned using a diluted neutral soap followed by water.

Unless another extraction protocol is being performed the BioRobot® EZ1 may be switched “OFF”.

REFERENCES:

1. BioRobot® EZ1 Genomic DNA Kit Handbook. Qiagen February 2003
2. Magtration® System 6GC Operation Manual Version 1.1. Precision System Science Co. 2002.
3. Montpetit SA, Fitch IT, O'Donnell PT. A simple automated instrument for DNA extraction in forensic casework, J Forensic Sci. 2005 May;50(3):555-63.

Method: **Quantifiler™ Setup Using the Qiagen BioRobot® Universal**

Date: 10/07/2005

Revision Date: 06/18/2008

Approved by: SAM

REAGENTS:

1. TE Buffer(10mM Tris-HCl, 0.1mM EDTA, pH 8.0):

Mix together 10mL of 1M Tris-HCl, pH = 8.0 with 0.2mL of 0.5M EDTA and 990mL nanopure water. Aliquot into bottles, autoclave and store at room temperature. This solution is good for nine months.

2. Quantifiler Human (or Duo) DNA Quantification Kit: Applied Biosystems (P/N 4343895 or 4387746 (Duo))

- A. Quantifiler Human (or Duo) Primer Mix
- B. Quantifiler (or Duo) PCR Reaction Mix
- C. Quantifiler Human DNA Standard

The Reaction Mix should be removed from the kit upon receipt and stored at 2 to 8°C. The remaining components of the kit should be stored at -15 to -25°C. The primer sets contain a light sensitive dye, and should be stored protected from light. The expiration date is printed on the kit.

MATERIALS:

1. 96- Well Optical Reaction Plates Applied Biosystems (P/N N801-0560)
2. Optical Adhesive Covers Applied Biosystems (P/N 4311971)
3. MicroAmp Splash free Support Base Applied Biosystems (P/N 4312063)

EQUIPMENT:

1. Qiagen Universal BioRobot Universal Liquid Handler Qiagen Corporation
2. ABI Prism 7000/7500 Sequence Detection System Applied Biosystems

PROCEDURE:

1. This protocol is to setup the 96-well optical plate for qPCR DNA quantitation using Quantifiler™ (Human or Duo) quantitation kits. The protocol provides the option to create the DNA standard dilution series manually or have the BioRobot Universal perform the dilution. If the analyst opts to create the DNA standard dilution series manually, the dilutions should be made in 0.2mL tubes using the minimum volume guidelines in Table 3-1 of the Quantifiler User's Manual.
2. Important notes to remember for using the BioRobot Universal for setup:
 - a. Sarstedt screw-cap tubes are required for use on the instrument as they fit securely in the cooling blocks. Caps should be removed after tubes are placed in the cooling blocks and replaced prior to removing tubes from the cooling block.
 - b. If the Universal BioRobot Universal is to prepare the dilution series of the DNA standard it is important that the initial 50ng/uL standard be thoroughly mixed prior to placing it on the deck.
3. Thaw the appropriate Quantifiler Primer Mix and concentrated DNA standard (which is stored at -20°C). Vortex the Primer Mix and the refrigerated qPCR Reaction Mix for 5 seconds. Spin the Primer Mix and DNA standard tubes briefly in a microcentrifuge to remove any liquid from the caps.
4. Prepare a four-fold (1:3) dilution of the 200ng/uL human DNA standard in TE buffer to obtain the initial 50ng/uL standard (see Table 3-1 in the Quantifiler User's Manual for additional details if preparing the standards manually).
5. Determine the total number of samples to be amplified including the set of eight human DNA standards (run in duplicate) and one qPCR blank. Create a master mix by combining the following volumes of reagents into a screw-cap tube:
 - a. 10.5ul of Primer Mix x # of Samples
 - b. 12.5ul of qPCR Reaction Mix x # of Samples

Note: Additional master mix (~10%) should be created to allow for pipeting error.

6. Open the **Universal 8000 Quant Setup** in the SDPD Worksheet maker v2.2 (found on the FB network H:\Worksheets).
 - a. The default spreadsheet shows the locations for the DNA standards (50→0.023ng/uL).
 - b. Fill out the "Sample Name" column with a list of the sample names. Note: Delete any unused rows below your samples (Highlight then **Edit>Delete**).

- c. The Setup Spreadsheet should be saved as a ***.csv** file type. This is accomplished by using the “Save as...” command from the **File** menu. The **.csv** file must be saved in the **ImportData** folder on the **Qiagenuniversal** hard drive. (Note: *.csv file names should begin with the analysts’ initials followed by case # (e.g. initials(XX-XXXXXX).csv).
 - d. When finished, the **qPCR worksheet** should be printed for your case notes.
 - e. Exit the SDPD Worksheet maker v2.2.
7. Open QiaSoft™ v.5 software (on the desktop). Log in as “sdpd” and leave the password blank.
- a. Under “applications” select “Forensic” in the drop-down menu (▼) and then select “**Quantifiler Reaction Setup**”.
 - b. Click “Run” button (▶). Follow the prompts from the protocol for preliminary setup.
 - c. Enter the number of samples (excluding DNA Standards).
 - d. Enter the appropriate **.csv** file name.
 - e. Check the .csv table to ensure correct setup sheet was selected.
 - f. The System Liquid bottle should be filled above the minimum. The protocol will not continue if liquid level is below minimum.
 - g. Enter whether the robot will be creating the DNA standard dilution (Yes/No).
 - h. Empty tip waste (if necessary).
 - i. Refill the tip racks (if necessary). If tips run out the instrument will prompt you to refill later.
 - j. Place the 96-well optical plate on a splash-free support base in the appropriate location on the deck making sure to tape the sides of the plate to the base.
 - k. Place your DNA samples in the appropriate order (according to the setup sheet filled in earlier). You may use your printed copy to ensure the sample positions are correct.

Note: The samples are to be placed in the cooling blocks in a left to right direction, filling each column completely before the next. No tube or an empty tube should be placed in the position for the qPCR blank.

- l. If the Universal BioRobot Universal is to be used to create the dilution series, then eight 0.2mL tubes are required. They should be setup as indicated by the default DNA standard locations. The 50ng/uL DNA standard tube should contain 40uL and the remaining tubes should be empty. If the dilutions were manually created then the dilution tubes can be placed in their appropriate locations (each tube should contain at least 20uL of liquid).
- m. Place the Quantifiler Master Mix and TE Buffer (if the instrument is preparing the DNA Standard serial dilution) in the appropriate places on the deck. Note: if more than 48 samples are being quantified then the master mix will need to be split into two tubes (follow the prompts from the instrument).

8. The BioRobot Universal will now dilute the DNA standard (if selected), add master mix to the 96-well optical plate, and then add 2uL of the samples to the master mix.
9. When the protocol has finished, the tubes can be re-capped and removed, and the 96-well optical plate can be covered with an optical adhesive cover and carried to the amplification room for loading into the ABI 7000/7500 SDS.
10. The qPCR analysis can be continued by following the "Quantitative PCR DNA with the Quantifiler™ Human DNA Quantification Kit" or Quantitative PCR with the Quantifiler® Duo DNA Quantification Kit protocol at step 9.

Reference:

1. BioRobot 8000 Users manual. Qiagen Corporation.
2. QiaSoft 5 Software System Users Guide. Qiagen Corporation.

Method: WellAware Set-up of Samples for Quantitation

Date: 12/06/2010

Approved by: SAM

REAGENTS:

This protocol is for manual setup, using the WellAware as a pipetting assistant, of a 96-well optical plate for qPCR DNA quantitation using Quantifiler™ (Human or Duo) quantitation kits. Please refer to the “Quantitative PCR with the Quantifiler™ Human DNA Quantification Kit” or the Quantitative PCR with the “Quantifiler™ Duo DNA Quantification Kit” for information on DNA standard and master mix preparation.

MATERIALS:

1. 96-Well Optical Reaction Plate Applied Biosystems (P/N N801-0560)
2. Optical Adhesive Covers Applied Biosystems (P/N N801-0560)
3. MicroAmp Splash free Support Base Applied Biosystems (P/N 4312063)
4. Pre-scored 96-well plate sealing tape Nunc (P/N 236701)

EQUIPMENT:

1. WellAware BioTX
2. ABI Prism 7000/7500 Sequence Detection System Applied Biosystems

PROCEDURE:

1. Open the **qPCR Worksheet** in the SDPD Worksheet maker v4.0 (found on the FB network H:\Worksheets). Click on the orange ‘Make WellAware eSOP’ button to generate a WellAware eSOP (electronic standard operating procedure). Save the eSOP as a text file in the WellAware folder on the FB network. This is accomplished by using the “Save as...” command from the File menu. The Text (Tab delimited) file must be saved in the WellAware folder on the FB network.

[Note: The eSOP requires a case number be entered on the worksheet]

2. When finished, the qPCR worksheet should be printed for your case notes.

3. Power on the WellAware computer located in the hood in the amplification set-up room and open the BioTX Lab Suite software.
4. Under the Modules tab, click on 'WellAware' then under the Electronic SOPs tab, click on 'Open Saved eSOP.'
5. Navigate to the WellAware folder on the FB Network and open your saved eSOP.
6. Place a 96-Well Optical Reaction Plate on the bottom right surface of the WellAware screen.
7. Begin the protocol. [It is recommended that when manually pipetting into a 96-well plate that one of the pre-scored tape seals be used to minimize potential contamination.]
8. When the protocol has finished, remove the pre-scored tap seal and the 96-well optical plate can then be covered with an optical adhesive cover and carried to the amplification room for loading into the ABI 7500 SDS instrument.
9. The qPCR analysis can be continued by following the "Quantitative PCR DNA with the QuantifilerTM Human DNA Quantification Kit" or Quantitative PCR with the Quantifiler[®] Duo DNA Quantification Kit protocol at step 9.
10. When the protocol is complete, exit the WellAware software.
11. Power off the WellAware computer.

REFERENCE:

1. WellAware User Guide.

Method: **Quantitative PCR with the Quantifiler™ Human DNA Quantification Kit**

Date: 08/12/2005

Revision Date: 03/01/2010

Approved by: SAM

REAGENTS:

1. TE Buffer (10mM Tris-HCl, 0.1 mM EDTA, pH 8.0)

Mix together 10mL of 1M Tris-HCl, pH = 8.0 with 0.2mL of 0.5M EDTA and 990mL nanopure water. Aliquot into bottles, autoclave and store at room temperature. This solution is good for one year.

2. Quantifiler Human DNA Quantification Kit: Applied Biosystems (P/N 4343895)

- A. Quantifiler Human Primer Mix
- B. Quantifiler PCR Reaction Mix
- C. Quantifiler Human DNA Standard

The Reaction Mix should be removed from the kit upon receipt and stored at 2 to 8°C. The remaining components of the kit should be stored at -15 to -25°C. The primer sets contain a light sensitive dye, and should be stored protected from light. The expiration date is printed on the kit.

MATERIALS:

1. 96- Well Optical Reaction Plates Applied Biosystems (P/N N801-0560)
2. Optical Adhesive Covers Applied Biosystems (P/N 4311971)
3. MicroAmp Splash free Support Base Applied Biosystems (P/N 4312063)

EQUIPMENT:

ABI Prism 7500 Sequence Detection System Applied Biosystems

PROCEDURE:

1. Thaw the Quantifiler Primer Mix and concentrated DNA standard (stored at -20C). Vortex the Quantifiler Primer Mix and the refrigerated Quantifiler PCR Reaction Mix for 5 seconds. Spin the Primer Mix and DNA standard tubes briefly in a microcentrifuge to remove any liquid from the caps.

2. Prepare a three-fold (1:2) dilution series with eight concentration points, ranging from 50ng/uL to 0.023ng/uL, by diluting the 200ng/uL human DNA standard in TE buffer. To obtain the initial 50ng/uL standard a 1:3 dilution is required. (See Table 3-1 in the Quantifiler User's Manual for additional details)
3. Determine the total number of samples to be amplified including the set of eight human DNA standards (run in duplicate) and one qPCR blank. Create a master mix by combining the following volumes of reagents into a 1.5ml microcentrifuge tube:

10.5ul of Quantifiler Primer Mix	x	# of Samples
12.5ul of Quantifiler PCR Reaction Mix	x	# of Samples

Note: Additional master mix (~10%) should be created to allow for pipetting error.

4. Vortex the master mix at medium speed for 5 seconds and spin the tube briefly in a microcentrifuge to remove any liquid from the cap.
5. With the 96-well optical reaction plate in a support base dispense 23uL of the master mix into each sample well.
6. Add 2uL of sample, standard, or control to the appropriate wells.
7. Carefully seal the reaction plate with the optical adhesive cover. Use the applicator to ensure the cover forms a smooth airtight seal over the entire 96-well surface.
8. Transport the reaction plate to the ABI 7500 SDS in the amplification area.
9. Turn on the computer (if necessary) and then the SDS instrument.
10. Push the plate door in and slide it out until it provides room to insert the plate.
11. Position the plate in the thermal block so that well A1 is in the upper left corner and the notched corner is in the upper right corner.
12. Gently close the door to by sliding it backward into position.
13. Launch the 7500 SDS Software and set up a plate document (File> New) either by using a blank document or opening a plate document template. The plate document shows the location of samples/standards on the reaction plate. If a sample import file (.txt) has been created it can be imported by selecting **File>Import Sample Setup** and browsing for the appropriate file. If the sample setup was imported then skip to step 18.
14. If required, add detectors to the plate document. If the detectors for the Quantifiler kit

have been created, they will be listed in the Detector Manager: **Tools>Detector Manager**. Highlight the Quantifiler Human and IPC detectors in order to make them available to the plate. Click "Add to Plate Document".

15. Highlight the entire plate document, then from the Well Inspector (**View>Well Inspector**) add the detectors (Quantifiler Human and IPC) to all the wells of the plate document.
16. Add sample information for each well by double clicking on it (or highlight and **View>Well Inspector**). The Well Inspector box will appear. Add the sample name. If clicking on a well where a quantitation standard is located two changes need to be made to the Quantifiler Human detector. Change task from "unknown" to "standard" and add the concentration of the standard to the "Quantity" column.
17. When finished filling out the plate document save it as a SDS document (*.sds).
18. Select the Instrument tab and review the Quantifiler amplification profile which should consist of:
 - Initial incubation at 95°C for 10 minutes.
 - 40 cycles of the following profile:
 - 95°C for 15 seconds
 - 60°C for 1 minute

If need be, delete the initial 50°C incubation step and change the reaction volume to 25uL. If any changes were made save the file and click "Start" or simply click on "Start".
19. Following completion of the run the plate document can be analyzed. Verify the analysis settings (**Analysis>Analysis Settings**) which should be:
 - Detector: All
 - Threshold: .200000
 - Baseline Start (cycle): 6
 - Baseline End (cycle): 15
20. Once you have verified the settings are correct, analyze your data.
Analysis>Analyze
21. Interpret results by examining the standard curve and the IPCs, which are all accessible from the **Results** tab. Quantitation values for the unknowns are available from the **Plate** tab.
22. Turn the 7500 SDS instrument off.

INTERPRETATION:

The following information can be used to assist in the interpretation of the Quantifiler results:

Quality of the standard curve:

In general, the slope of the standard curve for the Quantifiler Human kit should be between -2.9 and -3.3 with a Y-intercept value of 28.7-29.8.

If the 50ng/uL standard is prepared appropriately the expected Ct value is approximately 23.1-24.1. The Ct values between the subsequent points on the standard curve should be approximately 1.6 cycles, with the Ct value for the 0.023ng/uL expected to be approximately 33.1-35.5. Some variation in these data points is expected.

The r^2 value of your slope should be above 0.98 and approach 1.0. The r^2 value is an indication of how well the points on the standard curve fit the trendline through the points.

The above information should serve to provide some guidance as to the expectations of the values for the standard curve. In general, if the values obtained for the standard curve are within the expected range, the amount of DNA to be amplified should be in the range of the validated target, with case or sample specific exceptions possible. If the values obtained from the standard curve are outside the values listed above, it is an indication that there may be problems relying on the quantitation information obtained, and that your input amount of DNA for amplification may need to be adjusted from the validated target. Quantitation assays yielding slope, intercept, and Ct values outside of those expected may require a new quantitation assay to yield more reliable information. Repeating of analyses is at the discretion of the analyst.

Based on internal validation of the Quantifiler Human kit, the ideal target amount of DNA is approximately 1.8ng for the Identifiler amplification kit, 0.3ng of DNA for the Minifiler amplification kit, and 0.75ng of DNA for the Yfiler amplification kit.

Assessment of sample quality:

If a sample has a high human Ct value and a normal IPC Ct value (~28) the sample has a low amount of DNA.

If a sample has a low human Ct value and a normal or slightly high IPC Ct value the sample has a high amount of DNA. If the sample has a high amount of human DNA it will often out compete the IPC for amplification components thus decreasing its amplification efficiency.

If a sample has a high human Ct value and a high IPC Ct value the sample is likely inhibited. Appropriate troubleshooting measures should be employed with the sample

to overcome the potential inhibitors.

REFERENCE:

Quantifiler™ Human DNA Quantification Kit User's Manual

Archived Version

SDPD FORENSIC BIOLOGY TECHNICAL PROCEDURES

Method: **Quantitative PCR with the Quantifiler® Duo DNA Quantification Kit**

Date: 6/16/08

Revision Date: 03/01/2010

Approved by: SAM

REAGENTS:

1. TE Buffer (10mM Tris-HCl, 0.1 mM EDTA, pH 8.0)

Mix together 10mL of 1M Tris-HCl, pH = 8.0 with 0.2mL of 0.5M EDTA and 990mL nanopure water. Aliquot into 100mL or 250mL bottles, autoclave and store at room temperature. This solution is good for twelve months.

2. Quantifiler Duo DNA Quantification Kit: Applied Biosystems (P/N 4387746)
 - A. Quantifiler Duo Primer Mix
 - B. Quantifiler Duo PCR Reaction Mix
 - C. Quantifiler Duo DNA Standard

The Reaction Mix should be removed from the kit upon receipt and stored at 2 to 8°C. The remaining components of the kit should be stored at -15 to -25°C. The primer sets contain a light sensitive dye, and should be stored protected from light. The expiration date is printed on the kit.

MATERIALS:

1. 96- Well Optical Reaction Plates Applied Biosystems (P/N N801-0560)
2. Optical Adhesive Covers Applied Biosystems (P/N 4311971)
3. MicroAmp Splash free Support Base Applied Biosystems (P/N 4312063)

EQUIPMENT:

ABI Prism 7500 Sequence Detection System Applied Biosystems

PROCEDURE:

1. Thaw the Quantifiler Duo Primer Mix and concentrated DNA standard (stored at -20C). Vortex the Quantifiler Duo Primer Mix and the refrigerated Quantifiler Duo PCR Reaction Mix for 5 seconds. Spin the Primer Mix and DNA standard tubes

briefly in a microcentrifuge to remove any liquid from the caps.

2. Prepare a three-fold (1:2) dilution series with eight concentration points, ranging from 50ng/uL to 0.023ng/uL, by diluting the 200ng/uL human DNA standard in TE buffer. To obtain the initial 50ng/uL standard a 1:3 dilution is required. (See Table 3-1 in the Quantifiler Duo User's Manual for additional details).
3. Determine the total number of samples to be amplified including the set of eight human DNA standards (run in duplicate) and one qPCR blank. Create a master mix by combining the following volumes of reagents into a 1.5ml microcentrifuge tube:

10.5ul of Quantifiler Duo Primer Mix	x	●	# of Samples
12.5ul of Quantifiler Duo PCR Reaction Mix	x		# of Samples

Note: Additional master mix (~10%) should be created to allow for pipetting error.

4. Vortex the master mix at medium speed for 5 seconds and spin the tube briefly in a microcentrifuge to remove any liquid from the cap.
5. With the 96-well optical reaction plate in a support base dispense 23uL of the master mix into each sample well.
6. Add 2uL of sample, standard, or control to the appropriate wells.
7. Carefully seal the reaction plate with the optical adhesive cover. Use the applicator to ensure the cover forms a smooth airtight seal over the entire 96-well surface.
8. Transport the reaction plate to the amplification area.
9. Turn on the computer (if necessary) and then the 7500 SDS instrument.
10. Press the tray door on the SDS instrument to open it.
11. Position the reaction plate in the plate holder so that well A1 is in the upper left corner and the notched corner is in the upper right corner and close the tray door.
12. Launch the 7500 SDS Software and set up a plate document (File> New) either by using a blank document or opening a plate document template. The plate document shows the location of samples/standards on the reaction plate. If a sample import file (.txt) has been created it can be imported by selecting **File>Import Sample Setup** and browsing for the appropriate file. If the sample setup was imported then skip to step 18.
13. If required, add detectors to the plate document. If the detectors for the Quantifiler kit have been created, they will be listed in the Detector Manager: **Tools>Detector**

Manager. Highlight the Dual Quant Human, Dual Quant Y and Dual Quant IPC detectors in order to make them available to the plate. Click "Add to Plate Document".

14. Highlight the entire plate document, then from the Well Inspector (**View>Well Inspector**) add the detectors (Human, Y and IPC) to all the wells of the plate document.
15. Add sample information for each well by double clicking on it (or highlight and **View>Well Inspector**). The Well Inspector box will appear. Add the sample name. If clicking on a well where a quantitation standard is located, two changes need to be made to the Dual Quant Human and Dual Quant Y detectors. Change task from "unknown" to "standard" and add the concentration of the standard to the "Quantity" column.
16. Select the Instrument tab and review the Quantifiler Duo amplification profile which should consist of:
 - Incubation at 50°C for 2 minutes
 - Incubation at 95°C for 10 minutes
 - 40 cycles of the following profile:
 - 95°C for 15 seconds
 - 60°C for 1 minute

Change the sample volume to 25uL and ensure that the 9600 emulation box is checked.
17. When finished filling out the plate document save it as a SDS document (*.sds) and click "Start" to start the run.
18. Following completion of the run the plate document can be analyzed. Verify the analysis settings (**Analysis>Analysis Settings**) which should be:
 - Detector: All
 - Threshold: .200000
 - Baseline Start (cycle): 3
 - Baseline End (cycle): 15
19. Once you have verified the settings are correct, analyze your data.
Analysis>Analyze.
20. Interpret results by examining the standard curves and the IPCs, which are all accessible from the **Results** tab. Quantitation values for the unknowns are available from the **Plate** tab.

21. Turn the 7500 SDS instrument off.

INTERPRETATION:

The following information can be used to assist in the interpretation of the Quantifiler Duo results:

Quality of the standard curve:

In general, the slope of the standard curve for the Quantifiler Duo kit should be between -3.0 and -3.6 with a Y-intercept value between 29 and 30 for the human curve and 30 and 30.8 for the Y curve.

If the 50ng/uL standard is prepared appropriately the expected Ct value is approximately 23.2-24.2 for the human curve and 24.1-25.0 for the Y curve. The Ct values between the subsequent points on the standard curve should be approximately 1.6 cycles, with the Ct value for the 0.023ng/uL expected to be approximately 34.2-35.4 for the human curve and 34.8-37.1 for the Y curve. Some variation in these data points is expected.

The r^2 value of your slope should be above 0.98 and approach 1.0. The r^2 value is an indication of how well the points on the standard curve fit the trendline through the points.

The above information should serve to provide some guidance as to the expectations of the values for the standard curve. In general, if the values obtained for the standard curve are within the expected range, the amount of DNA to be amplified should be in the range of the validated target, with case or sample specific exceptions possible. If the values obtained from the standard curve are outside the values listed above, it is an indication that there may be problems relying on the quantitation information obtained, and that the input amount of DNA for amplification may need to be adjusted from the validated target. Quantitation assays yielding slope, intercept, and Ct values outside of those expected may require a new quantitation assay to yield more reliable information. Repeating of analyses is at the discretion of the analyst.

Based on internal validation of the Quantifiler Duo kit, the ideal target amount of DNA is approximately 1.0ng for the Identifiler amplification kit, 0.3-0.4ng of DNA for the Minifiler amplification kit, and 0.5-0.75ng of DNA for the Yfiler amplification kit.

Assessment of sample quality:

If a sample has a high human Ct value and a normal IPC Ct value (~28) the sample has a low amount of DNA.

If a sample has a low human Ct value and a normal or slightly high IPC Ct value the sample has a high amount of DNA. If the sample has a high amount of human DNA it will often out compete the IPC for amplification components thus decreasing its

amplification efficiency.

If a sample has a high human Ct value and a high IPC Ct value the sample is likely inhibited. Appropriate troubleshooting measures should be employed with the sample to overcome the potential inhibitors.

The relative amount of male and female DNA in a mixture can be determined by dividing the total human DNA quantitation by the male DNA quantitation and subtracting one. As an example, if the total DNA quantitation is four times the male DNA quantitation, there is approximately three times as much female DNA as male DNA.

REFERENCE:

Quantifiler® Duo DNA Quantification Kit User's Manual

Method: **Identifiler™ Setup Using the Qiagen BioRobot® Universal**

Date: 03/05/2007

Revision Date: 07/25/2007

Approved by: SAM

REAGENTS:

1. TE Buffer(10mM Tris-HCl, 0.1mM EDTA, pH 8.0):

Mix together 10mL of 1M Tris-HCl, pH = 8.0 with 0.2mL of 0.5M EDTA and 990mL nanopure water. Aliquot into 100mL bottles, autoclave and store at room temperature. This solution is good for nine months.

2. AmpF/STR Identifiler™ PCR Amplification Kits: Applied Biosystems (P/N 4322288)

- A. AmpF/STR PCR Reaction Mix
- B. AmpF/STR Identifiler™ Primer Set
- C. AmpliTaq Gold™ DNA Polymerase
- D. AmpF/STR Control DNA 9947A

3. Bovine Serum Albumin (4.97mg/mL) Fisher Scientific (P/N CAS 9048-46-8)

The Reaction Mix should be removed from the kit upon receipt and stored at 2 to 8°C. The remaining components of the kit should be stored at -15 to -25°C. The primer sets contain a light sensitive dye, and should be stored protected from light. The expiration date is printed on the kit.

MATERIALS:

1. 96- Well Optical Reaction Plates Applied Biosystems (P/N 4306737)

2. Full Plate Covers Applied Biosystems (P/N N801-0550)

3. Aluminum Seal tape for 96-Well Plates Fisher Scientific (P/N 232698)

EQUIPMENT:

1. Qiagen Universal BioRobot Universal Liquid Handler Qiagen Corporation

2. GeneAmp PCR System 9700 Thermal Cyclers Applied Biosystems

PROCEDURE:

This protocol is to setup the 96-well optical plate for PCR DNA amplification using Identifiler™.

1. Important notes to remember for using the BioRobot Universal for setup:
 - a. Sarstedt screw-cap tubes are required for use on the instrument as they fit securely in the cooling blocks. Caps should be removed after tubes are placed in the cooling blocks and replaced prior to removing tubes from the cooling block.
 - b. The maximum volume of sample is 10uL. If additional Taq DNA polymerase and bovine serum albumin (BSA) are to be added the maximum amount of sample is 8uL. Taq/BSA should be prepared using 0.9uL BSA (4.97mg/mL) and 1.1uL of Taq DNA polymerase per sample with an additional 15-20% added to accommodate for robotic pipetting.
2. Vortex the AmpF/STR PCR Reaction Mix, AmpF/STR Identifiler™ Primer Set, AmpliTaq Gold™ DNA Polymerase, and AmpF/STR Control DNA 9947A for 5 seconds. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.
3. Create a master mix by combining the following volumes of reagents into a 1.5mL Sarstedt screw-cap tube:
 - c. 10.5uL of AmpF/STR PCR Reaction Mix x # of Samples
 - d. 0.5uL of AmpliTaq Gold DNA Polymerase x # of Samples
 - e. 5.5uL of AmpF/STR Identifiler Primer Set x # of Samples

Note: Additional master mix (~10%) should be created to allow for pipetting error.

4. The **Master Amp Sheet** within SDPD Worksheet maker v2.2 (found on the FB network H:\Worksheets) is the primary sheet for using the BioRobot Universal for amplification setup.
 - f. The **Master Amp Sheet** shows the deck positions of all samples and their destination positions.
 - g. Fill out the "Sample Name" column with a list of the sample IDs and the DNA conc. [ng/uL] column. Ensure that the VolumeSample, VolumeTE, and TaqBSA columns are completed appropriately. (Note: The TaqBSA column cannot contain all zeros)
 - h. Delete any unused rows below your samples (Highlight then **Edit>Delete**). The file can be saved as an excel workbook for future use.

- i. The **Master Amp Sheet** should be saved as a ***.csv** file type. This is accomplished by using the “Save as...” command from the **File** menu. The **.csv** file must be saved in the **ImportData** folder on the **Qiagenuniversal** hard drive. (Note: *.csv file names should begin with the analysts’ initials followed by case # (e.g. initials(XX-XXXXXX).csv).
 - j. When finished, the **PCR worksheet(s)** should be printed for the case notes.
 - k. Close the **.csv** file.
5. Open QiaSoft™ v.5 software (on the Qiagenuniversal computer desktop). Log in as “sdpd” and leave the password blank.
- l. Under “applications” select “Forensic” in the drop-down menu (▼) and then select “**SDPD Identifiler Amplification v1.2.2**”.
 - m. Click “Run” button (▶). Follow the prompts from the protocol for preliminary setup.
 - n. Enter the number of samples to be amplified.
 - o. Enter the appropriate **.csv** file name.
 - p. The .csv table will be checked by the software and can be viewed to ensure correct file was selected.
 - q. The System Liquid bottle should be filled above the minimum. The protocol will not continue if liquid level is below minimum.
 - r. Empty tip waste (if necessary).
 - s. Refill the tip racks (if necessary). If tips run out the instrument will prompt you to refill later.
 - t. Place the 96-well optical plate on the appropriate location on the deck making sure to tape the side(s) of the plate to the base.
 - u. Place your DNA samples in the appropriate order (according to the setup sheet filled in earlier). You may use your printed copy to ensure the sample positions are correct.
- Note: The samples are to be placed in the cooling blocks in a left to right direction, filling each column completely before the next.
- v. Four tubes containing TE buffer should be placed in the appropriate location on the deck.
 - w. Taq/BSA should be prepared in the appropriate amount and be placed in the appropriate location on the deck.
 - x. Place the Identifiler Master Mix in the appropriate place(s) on the deck. Note: if more than 48 samples are being quantified then the master mix will need to be split into two tubes (follow the prompts from the instrument).
6. The BioRobot Universal will now add the master mix, TE buffer, Taq/BSA and sample DNA to the 96-well optical plate.

7. When the protocol has finished, the tubes can be re-capped and removed, and the 96-well optical plate can be covered with a rubber plate cover and carried to the amplification room for loading into the GeneAmp 9700 thermocycler.

References:

1. BioRobot 8000 Users manual. Qiagen Corporation.
2. QiaSoft 5 Software System Users Guide. Qiagen Corporation.

Archived Version

Method: **Minifiler™/Yfiler™ Setup Using the Qiagen BioRobot® Universal**

Date: 03/01/2010

Approved by: SAM

REAGENTS:

1. TE Buffer(10mM Tris-HCl, 0.1mM EDTA, pH 8.0):

Mix together 10mL of 1M Tris-HCl, pH = 8.0 with 0.2mL of 0.5M EDTA and 990mL nanopure water. Aliquot into 100mL bottles, autoclave and store at room temperature. This solution is good for nine months.

2. AmpF/STR Yfiler™ PCR Amplification Kits: Applied Biosystems (P/N 4365495)

- A. AmpF/STR PCR Reaction Mix
- B. AmpF/STR Yfiler™ Primer Set
- C. AmpliTaq Gold™ DNA Polymerase
- D. AmpF/STR Control DNA 007

3. AmpF/STR Minifiler™ PCR Amplification Kits: Applied Biosystems (P/N 4373874)

- A. AmpF/STR Minifiler™ Master Mix
- B. AmpF/STR Minifiler™ Primer Set
- C. AmpF/STR Control DNA 007

The Reaction Mix should be removed from the kit upon receipt and stored at 2 to 8°C. The remaining components of the kit should be stored at -15 to -25°C. The primer sets contain a light sensitive dye, and should be stored protected from light. The expiration date is printed on the kit.

MATERIALS:

1. 96- Well Optical Reaction Plates Applied Biosystems (P/N 4306737)

2. Full Plate Covers Applied Biosystems (P/N N801-0550)

3. MicroAmp 8-cap strip Applied Biosystems (P/N N801-0535)

EQUIPMENT:

1. Qiagen Universal BioRobot Universal Liquid Handler Qiagen Corporation

2. GeneAmp PCR System 9700 Thermal Cyclers Applied Biosystems

PROCEDURE:

This protocol is to setup the 96-well optical plate for PCR DNA amplification using Identifiler™.

1. Important notes to remember for using the BioRobot Universal for setup:
 - a. Sarstedt screw-cap tubes are required for use on the instrument as they fit securely in the cooling blocks. Caps should be removed after tubes are placed in the cooling blocks and replaced prior to removing tubes from the cooling block.
 - b. The maximum volume of sample is 10uL. If additional Taq DNA polymerase is to be added, the maximum amount of sample is 8uL.
2. Yfiler - Vortex the AmpF/STR PCR Reaction Mix, AmpF/STR Yfiler™ Primer Set, AmpliTaq Gold™ DNA Polymerase, and AmpF/STR Control DNA 007 for 5 seconds. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.

Minifiler – Vortex the AmpF/STR Minifiler™ Master Mix, AmpF/STR Minifiler™ Primer Set, and AmpF/STR Control DNA 007 for 5 seconds. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps. (Skip to Step 4.)

3. Create a master mix for the appropriate kit by combining the following volumes of reagents into a 1.5mL Sarstedt screw-cap tube:

Yfiler

9.2uL of AmpF/STR Yfiler™ PCR Reaction Mix	x	# of samples
0.8uL of AmpliTaq Gold DNA Polymerase	x	# of samples
5.0uL of AmpF/STR Identifiler Primer Set	x	# of samples

Minifiler

10 µL AmpF/STR MiniFiler™ Master Mix	x	# of samples
5 µL AmpF/STR MiniFiler™ Primer Set	x	# of samples

Note: Additional master mix (~10%) should be created to allow for pipetting error.

4. The **Master Amp Sheet** within SDPD Worksheet maker v3.3 (found on the FB network H:\Worksheets) is the primary sheet for using the BioRobot Universal for amplification setup.
 - a. The **Master Amp Sheet** shows the deck positions of all samples and their destination positions.

- b. Fill out the "Sample Name" column with a list of the sample IDs and the DNA conc. [ng/uL] column. Ensure that the VolumeSample and VolumeTE are completed appropriately.
 - c. Delete any unused rows below your samples (Highlight then **Edit>Delete**). The file can be saved as an excel workbook for future use.
 - d. The **Master Amp Sheet** should be saved as a *.csv file type. This is accomplished by using the "Save as..." command from the **File** menu. The .csv file must be saved in the **ImportData** folder on the **Qiagenuniversal** hard drive. (Note: *.csv file names should begin with the analysts' initials followed by case # (e.g. initials(XX-XXXXXX).csv).
 - e. When finished, the **PCR worksheet(s)** should be printed for the case notes.
 - f. Close the .csv file.
5. Open QiaSoft™ v.5 software (on the Qiagenuniversal computer desktop). Log in as "sdpd" and leave the password blank.
- a. Under "applications" select "Forensic" in the drop-down menu (▼) and then select "**Minifiler – Yfiler Amplification v.1**".
 - b. Click "Run" button (▶). Follow the prompts from the protocol for preliminary setup.
 - c. Enter the number of samples to be amplified.
 - d. Enter the appropriate .csv file name.
 - e. The .csv table will be checked by the software and can be viewed to ensure correct file was selected.
 - f. The System Liquid bottle should be filled above the minimum. The protocol will not continue if liquid level is below minimum.
 - g. Empty tip waste (if necessary).
 - h. Refill the tip racks (if necessary). If tips run out the instrument will prompt you to refill later.
 - i. Place the 96-well optical plate on the appropriate location on the deck making sure to tape the side(s) of the plate to the base.
 - j. Place your DNA samples in the appropriate order (according to the setup sheet filled in earlier). You may use your printed copy to ensure the sample positions are correct.

Note: The samples are to be placed in the cooling blocks in a left to right direction, filling each column completely before the next.

- a. Four tubes containing TE buffer should be placed in the appropriate location on the deck.
- b. Place the Master Mix in the appropriate place(s) on the deck. Note: if more than 48 samples are being quantified then the master mix will need to be split into two tubes (follow the prompts from the instrument).

6. The BioRobot Universal will now add the master mix, TE buffer, and sample DNA to the 96-well optical plate.
7. When the protocol has finished, the tubes can be re-capped and removed, and the 96-well optical plate can be covered with a rubber plate cover and carried to the amplification room for loading into the GeneAmp 9700 thermalcycler.

References:

1. BioRobot 8000 Users manual. Qiagen Corporation.
2. QiaSoft 5 Software System Users Guide. Qiagen Corporation.

Archived Version

Method: WellAware Set-up of Samples for Amplification

Date: 12/06/2010

Approved by: SAM

REAGENTS:

This protocol is for manual setup, using the WellAware as a pipetting assistant, of a 96-well optical plate for PCR amplification using the Identifiler™, Minifiler™, or Yfiler™ amplification kits. Please refer to the Identifiler amplification protocol for information on master mix preparation and other additional information regarding this protocol.

MATERIALS:

1. 96-Well Optical Reaction Plate Applied Biosystems (P/N N801-0560)
2. Full Plate Covers Applied Biosystems (P/N N801-0550)
3. Aluminum Seal tape for 96-Well Plates Fisher Scientific (P/N 232698)
4. Pre-scored 96-well plate sealing tape Nunc (P/N 236701)

EQUIPMENT:

1. WellAware BioTX
2. GeneAmp PCR System 9700 Thermal Cyclers Applied Biosystems

PROCEDURE:

1. Open the **PCR (1-24) worksheet** (or the Minifiler or Yfiler Amp worksheets) in the SDPD Worksheet maker v4.0 (found on the FB network H:\Worksheets). Click on the orange 'Make WellAware eSOP' button to generate a WellAware eSOP (electronic standard operating procedure). Save the eSOP as a text file in the WellAware folder on the FB network. This is accomplished by using the "Save as..." command from the File menu. The Text (Tab delimited) file must be saved in the WellAware folder on the FB network.

[Note: The eSOP requires a case number be entered on the worksheet]

2. When finished, the PCR worksheet should be printed for your case notes.
3. Power on the WellAware computer located in the hood in the amplification set-up room. Open the BioTX Lab Suite software.

4. Under the Modules tab, click on 'WellAware' then under the Electronic SOPs tab, click on 'Open Saved eSOP.' Navigate to the WellAware folder on the FB Network and open your saved eSOP.
5. Place a 96-Well Optical Reaction Plate on the bottom right surface of the WellAware screen.
6. Begin the protocol. [It is recommended that when manually pipetting into a 96-well plate that one of the pre-scored tape seals be used to minimize potential contamination.]
7. When the protocol has finished the 96-well optical plate can be covered with a rubber plate cover and carried to the amplification room for loading into the GeneAmp 9700 thermocycler.
8. When the protocol is complete, exit the WellAware software.
9. Power off the WellAware computer.

REFERENCE:

1. WellAware User Guide.

Method: **Amplification with the AmpF/STR Identifiler™ Kit**

Date: 06/01/2004

Revision Date: 07/25/2007

Approved by: SAM

Information for concentrating sample: Validation studies at the SDPD DNA laboratory have demonstrated that concentrating the amount of EDTA in samples greater than 4-fold will result in a decrease in amplification efficiency. As such, analysts may choose to use water for the final wash during the Microcon/Centricon-100 concentration procedure or elute in water for BioRobot® EZ1 DNA purifications thus eliminating the TE from the sample. Any additional concentration steps may be performed without concerns of altering the solute concentration.

If DNA extracts are to be stored long-term they must be stored frozen.

REAGENTS:

1. TE Buffer (10mM Tris-HCl, 0.1 mM EDTA, pH 8.0)

Mix together 10mL of 1M Tris-HCl, pH = 8.0 with 0.2mL of 0.5M EDTA and 990mL nanopure water. Aliquot into 100mL bottles, autoclave and store at room temperature. This solution is good for nine months.

2. AmpF/STR Identifiler™ PCR Amplification Kits: Applied Biosystems (P/N 4322288)

- A. AmpF/STR PCR Reaction Mix
- B. AmpF/STR Identifiler™ Primer Set
- C. AmpliTaq Gold™ DNA Polymerase
- D. AmpF/STR Control DNA 9947A

The AmpliTaq Gold™ DNA Polymerase enzyme should be removed from the kit upon receipt and stored at -15 to -25°C. The remaining components of the kits should be stored at 2 to 8°C. The primer sets contain a light sensitive dye, and should be stored protected from light. The expiration date is printed on the kit.

3. Bovine Serum Albumin (4.97 mg/mL) Fisher Scientific (P/N CAS 9048-46-8)

MATERIALS:

1. MicroAmp Autoclaved Reaction Tubes with Caps Applied Biosystems (P/N N801-0612)
2. MicroAmp 9700 Tray Applied Biosystems (P/N N801-0541)

EQUIPMENT:

GeneAmp PCR System 9700 Thermal Cyclers Applied Biosystems

PROCEDURE:

1. Fill out an AmpF/STR Identifiler™ Amplification Worksheet and determine the total number of samples to be amplified including the positive control (9947A), the negative amplification control, and all samples.
2. Label a 0.2mL MicroAmp Reaction tube for each of the samples to be amplified.
3. Vortex the AmpF/STR PCR Reaction Mix, AmpF/STR Identifiler™ Primer Set, AmpliTaq Gold™ DNA Polymerase, and AmpF/STR Control DNA 9947A for 5 seconds. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.
4. Create a master mix by combining the following volumes of reagents into a 1.5mL microcentrifuge tube:
 - a. 10.5uL of AmpF/STR PCR Reaction Mix x # of Samples
 - b. 0.5uL of AmpliTaq Gold DNA Polymerase x # of Samples
 - c. 5.5uL of AmpF/STR Identifiler Primer Set x # of Samples

In an effort to reduce the effects of possible inhibitors present in the samples to be amplified, an additional 1.1uL of AmpliTaq Gold DNA Polymerase and 0.9uL of 4.97mg/mL bovine serum albumin (BSA Fraction V) may be added to the samples of interest. This may be accomplished by either adding the additional reagents to the individual reaction tubes or to the PCR Master Mix if all of the amplified samples are to have additional Taq and BSA added. Additional Taq/BSA must also be added to the reagent blanks associated with the samples supplemented with Taq/BSA.

The addition of extra BSA and AmpliTaq Polymerase is meant as a trouble shooting procedure. It is not designed to be a routine alteration of the protocol. The total reaction volume including the additional Taq and BSA should be 25uL.

5. Vortex the master mix at medium speed for 5 seconds and spin the tube briefly in a microcentrifuge to remove any liquid from the cap.

Note: Steps 6 - 8 are performed in a biological hood using dedicated pipettes.

6. Dispense 15uL of the master mix into each labeled 0.2mL reaction tube.

7. If less than 10uL of sample is added for any sample, the difference should be made up by adding TE buffer.

8. Add sample to the labeled 0.2mL reaction tubes as follows:

For all evidence and reference samples, add approximately 1.8ng of target DNA in a volume equal to or less than 10uL. Note: 1.8ng is a suggested target amount of DNA. The actual amount added will depend on the nature of the sample and any additional information obtained from DNA quantitation.

For reagent blanks add a volume that is equal to the greatest volume from any of the evidence samples associated with it.

Add 5-10uL (0.1ng/uL) of AmpF/STR Control DNA 9947A to the positive amplification control tube. If less than 10uL of Control DNA is added, the difference is made up by adding TE buffer.

Add 10uL of TE buffer or water to the negative control tube.

9. Transport the tubes to the Amplification Area. Place a MicroAmp tray containing the PCR reaction tubes one of the thermal cyclers. Use the capping tool to firmly close the caps on all PCR reaction tubes.
10. Turn the thermal cycler on (if not already done).
11. Select the program named **Identifiler**.
 - a. Initial incubation at 95°C for 11 minutes.
 - b. 28 cycles of the following profile:
Denaturing at 94°C for 1 minute
Annealing at 59°C for 1 minute
Extending at 72°C for 1 minute
 - c. Final extension at 60°C for 90 minutes
 - d. Indefinite incubation at 25°C.

The amplified products can be removed from the thermal cycler at any time after reaching 25°C. Amplified samples are stored in the freezer.

REFERENCES:

AmpF/STR Identifiler™ PCR Amplification Kit User's Manual

Method: **Amplification with the AmpF/STR MiniFiler™ Kit**

Date: 07/25/2007

Approved By: SAM

INTRODUCTION:

The AmpF/STR MiniFiler™ PCR Amplification Kit is designed as an adjunct kit to the AmpF/STR Identifier™ kit for genotyping challenged samples that are degraded and/or contain PCR inhibitors. The key procedural differences between the systems are detailed below. All procedures for the AmpF/STR MiniFiler™ PCR Amplification Kit may also be found in the User Guide.

REAGENTS:

1. TE Buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0)

Mix together 10 mL of 1M Tris-HCl, pH= 8.0 with 0.2 mL of 0.5M EDTA and 990 mL nanopure water. Aliquot into 100 mL bottles, autoclave and store at room temperature. This solution is good for nine months.

2. AmpF/STR MiniFiler™ PCR Amplification Kits: Applied BioSystems

- A. AmpF/STR MiniFiler™ Master Mix
- B. AmpF/STR MiniFiler™ Primer Set
- C. AmpF/STR Control DNA 007

The master mix and primer set should be stored at -20°C upon receipt, and at 2 to 8°C after initial use. The control DNA 007 should be stored at 2 to 8°C upon receipt and after initial use. The expiration date is printed on the kit.

MATERIALS:

- 1. MicroAmp Autoclaved Reaction Tubes with Caps Applied Biosystems (P/N N801-0612)
- 2. MicroAmp 9700 Tray Applied Biosystems (P/N N801-0541)
- 3. 96-well Plate Applied Biosystems

EQUIPMENT:

GeneAmp PCR System 9700 Thermal Cyclers Applied Biosystems

10. Turn the thermal cycler on (if not already done).

11. Select the MiniFiler program.

- a. Initial Incubation
95°C, 11 minutes
- b. 30 Cycles
94°C, 20 seconds
59°C, 2 minutes
72°C, 1 minute
- c. Final Extension
60°C, 45 minutes
- d. Final Hold
4°C, ∞

12. Remove the amplified products from the thermal cycler anytime after reaching 4°C, then store in the freezer.

REFERENCE:

AmpF/STR MiniFiler™ PCR Amplification Kit User's Manual

Method: **Amplification with the AmpF/STR Yfiler™ Kit**

Date: 07/25/2007

Approved by: SAM

Amplification and analysis of Y-STR DNA markers using the AmpF/STR Yfiler™ STR kit is performed using similar procedures to those for AmpF/STR Identifiler™ STR analysis. The key procedural differences between the systems are detailed here.

REAGENTS:

1. AmpF/STR Yfiler™ PCR Amplification Kits: Applied Biosystems (P/N 4359513)
 - A. AmpF/STR Yfiler™ PCR Reaction Mix
 - B. AmpF/STR Yfiler™ Primer Set
 - C. AmpliTaq Gold™ DNA Polymerase
 - D. AmpF/STR Control DNA 007 (do not use Control DNA 9947A – female DNA)

PROCEDURE:

1. Fill out an AmpF/STR Yfiler™ Amplification Worksheet and determine the total number of samples to be amplified including the positive control (007), the negative amplification control, and all samples.
2. Label a 0.2mL MicroAmp Reaction tube for each of the samples to be amplified.
3. Vortex the AmpF/STR Yfiler™ PCR Reaction Mix, AmpF/STR Yfiler™ Primer Set, AmpliTaq Gold™ DNA Polymerase, and AmpF/STR Control DNA 007 for 5 seconds. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.
4. Create a master mix by combining the following volumes of reagents into a 1.5mL microcentrifuge tube:
 - a. 9.2uL of AmpF/STR Yfiler™ PCR Reaction Mix x (# of samples +10%)
 - b. 0.8uL of AmpliTaq Gold DNA Polymerase x (# of samples +10%)
 - c. 5.0uL of AmpF/STR Identifiler Primer Set x (# of samples +10%)
5. Vortex the master mix at medium speed for 5 seconds and spin the tube briefly in a microcentrifuge to remove any liquid from the cap.

Note: Steps 6 - 8 are performed in a biological hood using dedicated pipettes.

6. Dispense 15uL of the master mix into each labeled 0.2mL reaction tube.
7. If less than 10uL of sample is added for any sample, the difference should be made up by adding TE buffer.
8. Add sample to the labeled 0.2mL reaction tubes as follows:

For all evidence and reference samples, add approximately 0.75ng of male target DNA in a volume equal to or less than 10uL. Note: 0.75 ng is a suggested target amount of DNA. The actual amount added will depend on the nature of the sample and any additional information obtained from quantitation (or estimation of the concentration) of male DNA in a sample.

For reagent blanks add a volume that is equal to the greatest volume from any of the evidence samples associated with it.

Add 5-10uL (0.1ng/uL) of AmpF/STR Control DNA 007 to the positive amplification control tube. If less than 10uL of Control DNA is added, the difference is made up by adding TE buffer.

Add 10uL of TE buffer to the negative control tube.

9. Transport the tubes to the Amplification Area. Place a MicroAmp tray containing the PCR reaction tubes into one of the thermal cyclers. Use the capping tool to firmly close the caps on all PCR reaction tubes.
10. Turn the thermal cycler on (if not already done).
11. Select the program named **Yfiler**.
 - a. Initial incubation at 95°C for 11 minutes.
 - b. 30 cycles of the following profile:
Denaturing at 94°C for 1 minute
Annealing at 61°C for 1 minute
Extending at 72°C for 1 minute
 - c. Final extension at 60°C for 80 minutes
 - d. Indefinite incubation at 4°C.

The amplified products can be removed from the thermal cycler at any time after reaching 4°C. Amplified samples are stored in the freezer.

REFERENCES:

AmpF/STR Yfiler™ PCR Amplification Kit User's Manual

Archived Version

Method: **ABI 310 Cleaning and Setup**

Date: 9/15/2001

Revision Date: 01/01/2007

Approved by: SAM

It is not necessary to change the polymer and capillary for every run, nor is it necessary to perform all steps of this procedure in every circumstance (e.g., when adding a small volume of polymer in order to complete a long run). Capillaries are changed after approximately 200 injections.

Approximately 5uL of polymer is used per injection. Do not put unused polymer back into the bottle since the polymer in the syringe decomposes over time at room temperature.

REAGENTS:

1. 310 Genetic Analyzer Performance Optimized Polymer 4 (POP-4™):
Applied Biosystems (P/N 402838)
Store at 2-8°C. See bottle for printed expiration date.
2. 310 Genetic Analyzer Buffer with EDTA (10X): Applied Biosystems (P/N 402824)
Store at 2-8°C.

MATERIALS:

1. 310 Genetic Analyzer Capillaries (47cm X 50um): Applied Biosystems (P/N 402839)
2. Genetic Analyzer 4mL Buffer Vials: Applied Biosystems (P/N 401955)
3. Hamilton Gastight® Syringe, 1mL: ABD (P/N 604418-Rev A)
4. ABI PRISM® 310 Data Collection Software

PROCEDURE:

Reminder: If the syringe needs to be filled, remove the POP-4 polymer from the refrigerator and allow it to equilibrate to room temperature. It is useful to open the vial slightly to allow the polymer to de-gas.

1. Power on the ABI 310 instrument.
2. Power on the computer or select **Restart** from the **Start>Shutdown** menu (it is useful but not required to restart the computer on a weekly basis to clear the RAM memory).

Note: The 310 instrument needs to be powered on before the computer is powered on.

3. Launch the ABI Prism 310 Collection Software.
4. Determine if the capillary should be replaced by selecting **Change Capillary...** from the **Instrument** menu and note the number of injections (the capillary should be changed at approximately 200 injections).

If opting not to replace the capillary click **Cancel**.

If the capillary will be replaced click on **Reset Capillary Injection Counter**, or manually change the **Capillary Injection Counter** to zero, and click **OK**.

5. Home the Syringe:
 - Choose **Manual Control** from the **Window** menu.
 - Select **Syringe Home** from the **Function** drop-down menu, and click **Execute**.
6. Remove and clean the syringe:
 - Open the instrument doors.
 - Move the syringe drive toggle to the left.
 - Unscrew the syringe from the pump block.
 - Clean and rinse the syringe with nanopure water.
 - Dry the outside of the syringe with a Kimwipe® and remove excess water from the inside of the syringe by blowing into it with compressed air.
 - Dampen the end of the plunger with nanopure water before inserting the plunger into the syringe.
7. Remove the capillary:
 - Open the door covering the heat plate on the 310 instrument, remove the tape holding the capillary to the heat plate, and pull the capillary through the electrode thumbscrew.
 - Open the laser detector door on the 310 instrument and remove the capillary from the vertical groove of the detector window.
 - Unscrew the ferrule on the right side of the pump block and pull out the capillary.
 - Store the capillary ends in two tubes filled with nanopure water and closed with septa if the capillary is to be used again. Do not allow these tubes to dry out.

Note: Do not use the GS-Wash Capillary and Block module to wash the capillary.

8. Remove and clean the pump block:

- Grasp the pump block with both hands and pull straight out from the instrument.
- Remove all the fittings from the pump block.
- Rinse the anode buffer reservoir with nanopure water, then dry with a Kimwipe®.
- Rinse the outside of the pump block thoroughly with tap water.
- Rinse the inside of the pump block buy using a plastic syringe with nanopure water.
- Dry the pump block and fittings with a Kimwipe® or allow to air dry.
- Replace, but do not tighten, the fittings.

9. Clean the cathode electrode:

- Press the **Tray** button to the left of the pump block to move the tray platform forward.
- Moisten a Kimwipe® with nanopure water and gently wipe the electrode with an up/down motion.
- Dry the electrode with a fresh Kimwipe®.
- Press the **Tray** button again to move the platform back into position.

The POP-4 polymer should be warmed to room temperature before filling the syringe. Precipitate in the bottle should go back into solution at room temperature. Mix the polymer thoroughly by inversion before using.

10. Fill the syringe with POP-4™ polymer:

- Prime the syringe by drawing up a small amount of POP-4™ solution (0.2mL maximum) into the syringe and pulling the plunger to the 1.0mL mark to coat the wall of the syringe with polymer. Discard this polymer solution.
- Fill the syringe manually with a maximum of 0.8mL of POP-4™ polymer. This amount is sufficient for approximately 100 injections if 0.3mL is used to fill the empty pump block.
- Wipe the outside of the syringe with a Kimwipe® to dry.
- Attempt to remove any air bubbles by inverting and tapping on the syringe, and then pushing a small amount of polymer with the air bubbles out of the tip.

11. Replace the pump block:

- Verify that the gold electrode socket at the back of the block is dry.
- Align the steel shafts on the instrument with the holes on the block, and then

gently push the block onto the shafts.

- Ensure that the plunger valve of the buffer reservoir is aligned with the activator arm. It may be necessary to move the activator arm up or down by selecting **Buffer Valve Open** or **Close** from the **Function** drop-down menu in the **Manual Control** window and clicking **Execute**.

12. Install the syringe:

- The syringe toggle drive on the instrument should be moved to the left in order to attach the syringe to the pump block.
- Place the syringe through the right-hand port of the plastic syringe guide plate and screw the syringe into the pump block. The syringe should be finger tight in the block.
- Hand-tighten the valves on the pump block to the left of and below the syringe.

13. Install the capillary:

- Clean the capillary window with a Kimwipe® dampened with 95% ethanol. Do not touch the capillary window after cleaning.
- Insert the end of the capillary that is closest to the window through the center of the plastic ferrule on the right side of the pump block.
- Screw the capillary and ferrule into the block, positioning the capillary directly below the opening to the glass syringe.
- Screw the ferrule to finger tightness to secure the capillary. Test the fitting by gently pulling on the capillary-it should not move. The end of the capillary must visibly protrude out of the ferrule to seal tightly. If the ferrule is tightened without the capillary protruding, the opening in the ferrule will be sealed closed, and a new ferrule will be required.
- Open the laser detector door, and position the capillary in the vertical groove of the detector window.
- Align the green mark on the capillary with the top edge of the detector plate. The central part of the capillary window should rest over the laser detector window without putting strain or tension on the capillary.
- Close the laser detector door. Do not turn the ferrule screw after the detector door is closed. If the screw needs adjustment, first release the capillary from the detector.
- Thread the other end of the capillary through the capillary hole in the electrode thumbscrew until it protrudes past the tip of the electrode by approximately 0.5mm.
- Secure the capillary in place by taping the capillary against the heat plate.
- Close the heat plate door.

14. Filling the buffer reservoirs:

- Dilute 1.5mL of 10X Genetic Analyzer Buffer (with EDTA) to a 1X concentration by adding 13.5mL of nanopure water.
- Fill the anode buffer reservoir to the red line with the 1X Genetic Analyzer Buffer and place the reservoir on the pump block.
- Fill a 4mL glass vial to the fill line with the 1X Genetic Analyzer Buffer. Insert a plastic vial lid with attached septum into the glass vial and place the vial into position 1 on the autosampler platform. This will serve as the cathode buffer.
- Fill a second 4mL glass vial to the fill line with nanopure water. Insert a plastic vial lid with attached septum into the glass vial and place the vial into position 2 on the autosampler.
- Fill a 1.5mL microfuge tube (with the cap cut off) with about 1mL of nanopure water, and place it into position 3 on the autosampler platform.

15. Filling the pump block:

- Select **Buffer Valve Close** from the **Function** drop-down menu in the **Manual Control** window and click **Execute**.
- Partially unscrew the waste valve on the pump block below the syringe.
- Push down on the syringe plunger until the valve space is filled with polymer. This will remove the air bubbles at this valve site and should use about 0.1mL of polymer.
- Tighten the waste valve to close.
- Select **Buffer Valve Open** from the **Function** drop-down menu and click **Execute**.
- Push down on the syringe plunger to push enough polymer through the block so that all of the air bubbles are removed from the polymer channel. Observe the bubbles as they travel through and out of the block and a drop of the polymer falls through the anode buffer. This process should use approximately 0.2mL of polymer.
- Select **Buffer Valve Close** from the **Function** drop-down menu and click **Execute**.

16. Seat the syringe drive:

- Move the syringe toggle drive to the right so that it is positioned over the syringe plunger.
- Select **Syringe Down** from the **Function** pop-up menu in the **Manual Control** window and click **Execute**.
- Enter a number in the **Value** box and click **Execute**. This task can be performed repeatedly until the toggle nears the syringe plunger. Enter smaller numbers in the **Value** box until the toggle makes contact with the syringe plunger.

17. Calibrate the autosampler:

- Choose **Autosampler Calibration...** from the **Instrument** menu.
- Click **Start** on the Autosampler Calibration window.
- Click **Resume** after ensuring that no autosampler tray is present.
- Click on the direction arrows and the page up and page down keys to align the front calibration point on the autosampler with the capillary. To make fine adjustments, press the shift key on the keyboard while clicking the direction arrow. The end of the capillary must be centered nearly touching the calibration point.
- When done, click **Set**.
- Repeat for the rear calibration point.
- When done, click **Set**, then **Resume**, and then **Done**.

18. Set the temperature (optional):

- Close the instrument doors.
- Select **Temperature Set** from the **Function** pop-up menu.
- Enter **60** in the **Value** box and click **Execute**. It takes about 30 minutes for the instrument to reach the run temperature of 60°C.

REFERENCES:

AmpF/STR Profiler Plus™ PCR Amplification Kit User's Manual

AmpF/STR COfiler™ PCR Amplification Kit User Bulletin

ABI PRISM® 310 Genetic Analyzer User's Manual

Method: **Preparation of AmpF/STR Identifiler™, Minifiler™, or Yfiler™
Amplified Samples for Analysis on the ABI 310**

Date: 06/01/2004

Revision Date: 07/25/2007

Approved by: SAM

REAGENTS:

1. HIDI Formamide: Applied Biosystems (P/N 4311320)
Dispense the formamide into 500uL aliquots and store frozen. The frozen formamide can be used up to 1 year after being aliquoted.

Caution: Formamide is a known teratogen and is harmful by inhalation, skin contact, and ingestion. Use in a fume hood and wear chemical resistant gloves and safety glasses when handling.

2. GeneScan-500 [LIZ] Internal Lane Size Standard:
Applied Biosystems (P/N 4322682)
The kit may be stored up to six months at 4°C.
 3. AmpF/STR Identifiler™ Allelic Ladder: (included in the Identifiler™ kit)
Store at 2 to 8°C, protected from light.
- Or
4. AmpF/STR Yfiler™ Allelic Ladder: (included in the Yfiler™ kit)
Store at 2 to 8°C, protected from light.
- Or
5. AmpF/STR Minifiler™ Allelic Ladder: (included in the Minifiler™ kit)
Store at 2 to 8°C, protected from light.

MATERIALS:

1. 0.5mL Genetic Analyzer sample tubes:
Applied Biosystems (P/N 401955)
2. Septa for 0.5mL Genetic Analyzer sample tubes:
Applied Biosystems (P/N 401956)

PROCEDURE:

1. Label a 0.5mL Genetic Analyzer sample tube for each of the samples, allelic ladder(s), and blanks.
2. Aliquot 25uL of formamide into the formamide blank tube.
3. Create a master mix by combining the following volumes of reagents into a 1.5mL

microcentrifuge tube:

- 24.5uL formamide x (# of samples + 10%)
- 0.5uL GeneScan-500 [LIZ] x (# of samples + 10%)

This mixture can be stored at 2-6°C for up to two weeks.

4. Vortex the master mix, then spin briefly in a microcentrifuge. Aliquot 25uL of the master mix into each of the remaining 0.5mL Genetic Analyzer sample tubes.
5. Seal each tube with a septum. Ensure the septa are not sealed shut.
6. Add 1.5-3uL of AmpF/STR Identifiler™ (Minifiler™ or Yfiler™) Allelic Ladder to the appropriate 0.5mL Genetic Analyzer sample tube(s). Mix the contents of the tube by pipetting up and down or by stirring with a pipette tip.
7. Add 1.5uL of amplified DNA to each sample tube. Mix the contents of each tube by pipetting up and down or by stirring with a pipette tip.
8. Denature the samples by placing them in the 95°C heat block for three minutes.
9. Chill the tubes immediately in a freezer block for three minutes.
10. Place the tubes into a 48-well 310 sample tray and check to ensure that no septa became unseated during the heating process.
11. Open the doors of the ABI 310 instrument and press the **Tray** button to the left of the pump block. Place the tray onto the autosampler with position A1 at the upper right. Press the **Tray** button again to return the autosampler. Close the instrument doors.

REFERENCES:

AmpF/STR Identifiler™ PCR Amplification Kit User's Manual

AmpF/STR Minifiler™ PCR Amplification Kit User's Manual

AmpF/STR Yfiler™ PCR Amplification Kit User's Manual

Method: **Capillary Electrophoresis of Samples on the Applied Biosystems 310 Genetic Analyzer**

Date: 06/01/2006

Revision Date: 02/06/2009

Approved by: SAM

MATERIALS:

Applied Biosystems 310 Data Collection Software

PROCEDURE:

1. Launch the **310 Data Collection** software from the icon on the desktop.
2. Under the **File** menu, select **New** and click the **GeneScan Sample Sheet 48 Tube** icon.
3. Fill out the Sample Sheet:
 - For Identifiler, MiniFiler, or Yfiler Samples: Ensure that the 5-dye option is selected. This option can be selected from the drop-down menu in the upper-right corner of the window.
 - In the **Sample Name** column, fill in the appropriate name at each tray position. When finished filling in all sample names, highlight the **Sample Name** column by single clicking in the gray bar at the top and select **Copy** from the **Edit** menu. Alternatively, if a 310 import text file has been created the sample sheet can be automatically populated. Ensure that the 5-dye option is chosen, then select **File>Import Sample Setup** and browse for the appropriate text file.
 - Highlight the **Sample Info** column and select **Paste** from the **Edit** menu.
 - For the 5-dye option (Identifiler, Minifiler, or Yfiler): a diamond symbol should appear next to the orange box in the **Std** column for each sample. If no diamond appears, click in the box next to the orange dye color box placing a diamond symbol there. Highlight the **Std** column and select **Fill Down** from the **Edit** menu to place a diamond symbol in the Orange dye color for each sample.
 - The Collection name column can be left blank.
 - Select **Save** from the **File** menu and save the sample sheet with a unique identifier.
4. Fill out an injection list:
 - Select **File > New**. Then click the **GeneScan Injection List** icon.
 - From the **Sample Sheet** drop-down menu, choose the appropriate sample sheet.
 - For additional injections, highlight the **Inj. #** and select **Insert** or **Add** from the **Edit** menu to insert a blank injection. Highlight the sample you wish to re-inject,
 - Select **Copy** from the **Edit** menu, then highlight the blank row and select **Paste** from the **Edit** menu. The formamide injection blank should be injected at the beginning of the run.

- For Identifiler or Yfiler samples: Select **GS STR POP4 (1mL) G5 v2** from the **Module** drop-down menu for the first sample. Click on the **Module** column heading and choose **Fill Down** from the **Edit** menu.
The run time can be modified by changing the value in the **Run Time** column.
The injection time can be modified by changing it in the **Inj Secs** column.
 - The **Matrix** column for all samples should indicate **<none>**.
 - The **Auto Anlz** column should be deselected for each sample (no "x" in the boxes of this column).
 - No other information is required in the injection list window
5. Fill in the Operator's name and click the **Run** button. Select **Status** from the **Window** menu to view the run parameters.
6. When the run is complete:
- Close the Injection List. Select **Save** at the prompt.
 - Highlight the Sample Sheet in the Sample Sheets folder [D:\Applied Bio\310\Sample Sheets] and move it into the Run Folder [D:\Applied Bio\310\Runs].
 - Rename the Run Folder uniquely following protocol and move it into the individual Analyst's Folder [on the FB network [H:\3130-310 Run Folders\ (Analyst folder)]] prior to any analysis.

REFERENCES:

AMPF/STR Identifiler™ PCR Amplification Kit User's Manual

AMPF/STR Yfiler™ PCR Amplification Kit User's Manual

ABI PRISM® 310 Genetic Analyzer User's Manual

GeneScan® Analysis Software User's Manual

Method: **Creating a Matrix with GeneMapper ID Software**

Date: 12/06/06

Revision Date: 07/25/2007

Approved by: SAM

A new matrix will be made as needed.

REAGENTS:

1. HIDI Formamide: Applied Biosystems (P/N 4311320)
Dispense the formamide into 500ul aliquots and store frozen. The frozen formamide can be used up to 1 year after being aliquoted.
2. Matrix Standard Set DS-33(6-FAM, VIC, NED, PET and LIZ):
PE Applied Biosystems (P/N 4318159)
3. PowerPlex Matrix Standards (JOE, FL, TMR and CXR):
Promega Corporation (Cat #DG3640)

PROCEDURE:

1. Prepare and run matrix samples:
 - Prepare one tube for each matrix standard sample by combining 1-3ul of matrix standard with 25ul deionized formamide.
 - Seal each tube with a septum.
 - Follow steps 9 through 12 of the Preparation of Amplified Samples for Analysis on the ABI 310 procedure.
 - Follow the steps of the Capillary Electrophoresis procedure. Designate appropriate sample names based on the dye color (i.e. FAM, VIC, NED).
2. Create a new matrix:
 - Import the matrix samples into a new GeneMapper ID project.
 - View the raw data to determine the data point range for each of the matrix standards. This range should begin after the primer peak in a flat part of the baseline and extend to include all portions of the sample with a stable baseline and no anomalies (i.e. spikes). You must have at least five peaks to create a matrix.
 - Select **Tools>GeneMapper Manager**. Then click the **Matrices** tab.
 - Click the **New** button.
 - Enter a Matrix name and description (*optional*). The following naming convention should be used when naming the matrix:
5-dye matrix(310#X)date or 4-dye matrix(310#X)date as appropriate.
 - Choose the number of dyes from the **Number of Dyes** drop down list.
 - The buttons represent each of the dye colors (i.e. B = blue, Y = yellow). Click a

button to display a pop-up menu which can be linked to a sample file that represents the dye color for that button.

- Enter the start point in the Start At field.
- Enter the total number of data points to include in order to calculate the matrix in the Points field (*in most cases the default value can be used unless a portion of your data needs to be excluded*).
- Repeat this procedure for the other four matrix files and click **Create**.
- A window appears which contains the matrix values. "1.0000" should appear in the diagonal set of boxes from upper left to lower right. In general, all other numbers should be less than 1.0000.
- Click **OK**.

3. Evaluate the matrix:

- Apply the new matrix to the project created in step 2. For each matrix sample, display all five colors separately on one screen showing only electropherogram data. Visible peaks should be in the color of the matrix standard run in that injection and the electropherograms for the other four colors should be relatively flat.

4. Save the matrix to the FB Network:

- A copy of the new matrix should be saved in the following location: H:\3130-310 Run folders\310 MATRIX\310#X_date.

REFERENCES:

1. AmpF/STR Identifiler™ PCR Amplification Kit User's Manual
2. AmpFISTR Yfiler™ PCR Amplification Kit User's Manual
3. AmpF/STR Minifiler™ PCR Amplification Kit User's Manual
4. GeneMapper® ID Software User's Manual Version 3.1

Method: **ABI 3130 Genetic Analyzer Instrument Preparation and Cleaning**

Date: 06/01/06

Revision Date: 01/01/2007

Approved by: SAM

INTRODUCTION:

Although the 3130 Genetic Analyzer functions by the same principals as the 310 Genetic Analyzer, there are certain fundamental differences including the way in which the instrument is prepared for a run. For example, the pump block does not need to be routinely removed from the instrument for cleaning, but rather is flushed with water while in place on the instrument, and the polymer is not injected via a syringe, but rather is drawn directly from the manufacturer's bottle.

REAGENTS:

3130 Genetic Analyzer Performance Optimized Polymer (POP-4™):

Applied Biosystems (P/N 4352755)

Store at 2-8C. See bottle for expiration date.

(Note: this is not the same as 310 POP-4™ used on the 310)

Genetic Analyzer Buffer (10X) with EDTA:

Applied Biosystems (P/N 402824)

Store at 2-8C.

(Note: this is the same buffer used on the 310)

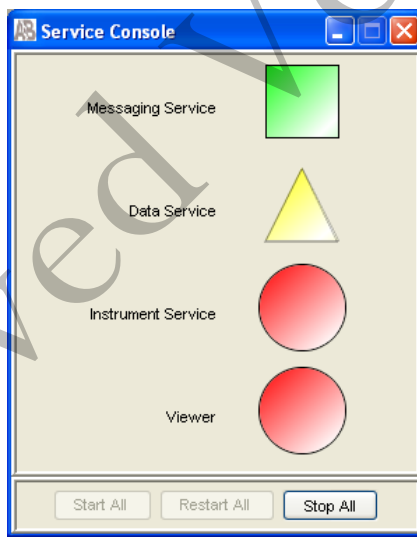
MATERIALS:

1. 3130 & 3100-Avant Capillary Array (36 cm): Applied Biosystems 4-capillary array (P/N 4333464)
2. 96-Well Optical Reaction Plates: Applied Biosystems (P/N 4306737)
3. Plate Septa for 96-well: Applied Biosystems (P/N 4315933)
4. 3130 and 3100 Series Plate Base 96-well: Applied Biosystems (P/N 431723)
5. 3100 Genetic Analyzer Plate Retainer 96-well: Applied Biosystems (P/N 4317241)
6. Buffer/Water Reservoirs: Applied Biosystems (P/N 628-3202)
7. Septa for Buffer/Water Reservoirs: Applied Biosystems (P/N 628-0150)

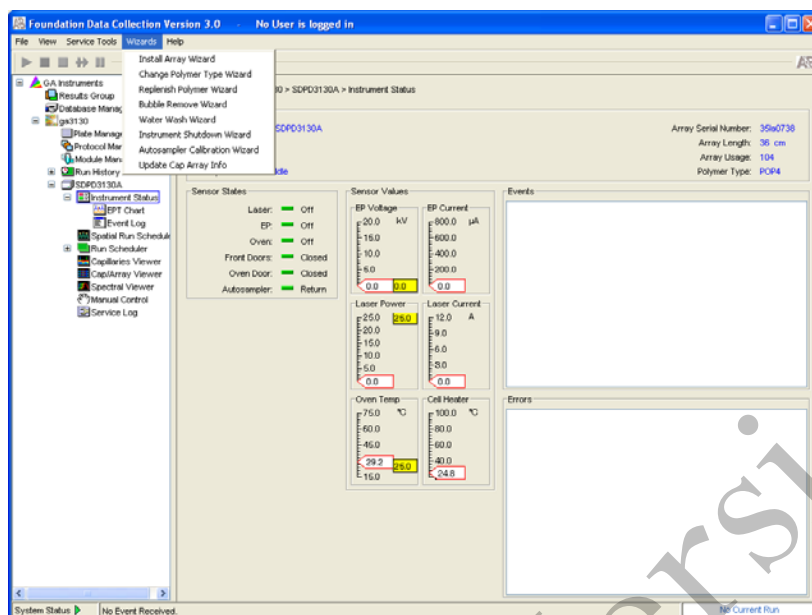
PROCEDURE:

Powering on the 3130 and launching the Data Collection software

1. Power on the computer monitor, then power on the computer. At the Log On prompt, hit **OK** (or Enter) leaving the Password window blank.
2. Before turning on the 3130 instrument, ensure that the oven door is closed and locked, that the instrument doors are closed, and the computer is powered on.
3. Power on the instrument by pressing the on/off button on the front of the instrument. Ensure that the green status light is on and not flashing before proceeding.
4. Click on the Run **3130 Data Collection V3.0** icon on the desktop. As the software launches, the **Service Console** appears. Initially the Service Console displays four red circles, indicating that all applications are off. As each application activates, the red circles (off) change to yellow triangles (activating) and then to green squares (on). This may take several minutes.



Note: the Data Collection Software contains numerous wizards that can be followed to accomplish various tasks. To access these wizards, launch Data Collection and click on **GA Instruments > ga3130 > SDPD3130A > Instrument Status** and select a specific wizard from the **Wizards** drop-down menu.



Installing or replacing the capillary array

The capillary array should be replaced after approximately 250 injections per capillary, or removed from the instrument for storage if the instrument is to be shut down for an extended period of time.

Select the **Install Array Wizard** for guidance.

Wash the pump block

The pump chamber, channels and tubing should be washed on a weekly basis, or before each batch of runs.

Select the **Water Wash Wizard** for guidance.

Replenishing polymer

Polymer should be replenished as needed, but unused polymer should be replaced with fresh polymer after approximately two weeks on the instrument.

1. Remove the polymer from the refrigerator and allow to equilibrate to room temperature. Open the cap of the bottle slightly to allow de-gassing.
2. Select the **Replenish Polymer Wizard** for guidance.

Removing bubbles from the pump block

Periodically, check the pump chamber, channels and tubing for air bubbles. Press the button on the right hand side of the front of the instrument to turn on the lamp inside the instrument. This will help locate any air bubbles.

Select the **Bubble Removal Wizard** for guidance.

Preparing buffer and filling reservoirs

It is recommended that the 1X 3130 buffer in the anode and cathode buffer reservoirs should be replaced before each batch of runs, however, multiple runs have been completed using the same buffers with no adverse affects.

1. Add 5mL of 10X 3130 Genetic Analyzer buffer to a 50mL graduated cylinder and make up to 50mL with nanopure water. Mix.
2. Check that the oven and instrument doors are closed and press the **Tray** button on the front of the instrument. The autosampler will move to the forward position. (Note: the autosampler will not move if the instrument doors are open.)

Wait until the autosampler stops and open the instrument doors.

3. Remove the cathode buffer (position 1), waste (position 2) and water (position 4) reservoirs at the front of the autosampler. (Note: position 3 is and should remain vacant.)
4. Remove and discard the septa, and rinse the reservoirs with nanopure or distilled water. Do not use any detergents when rinsing these reservoirs.
5. Rinse the cathode reservoir with 1X 3130 buffer and then fill to the line with 1X 3130 buffer (approximately 16mL).
6. Fill the waste and water reservoirs to the line with nanopure water (approximately 16mL).
7. Place fresh septa on the reservoirs and place the reservoirs back in their respective positions.
8. Remove the anode buffer reservoir from the bottom of the lower polymer block by firmly pulling down and twisting slowly.
9. Clean and rinse the reservoir with nanopure water, and then rinse with 1X 3130 buffer.
10. Fill the anode reservoir to the line with 1X 3130 buffer (approximately 16mL).
11. Replace the anode reservoir on the lower polymer block.
12. Close the instrument doors and press the **Tray** button to return the autosampler to its original position.

Calibration of the autosampler

Only a trained instrument specialist should calibrate the 3130 autosampler. Under no circumstance should an analyst attempt to calibrate the autosampler.

(See also Procedures for Performing a Spatial Calibration, Spectral Calibration, and Flushing and Filling the Water Trap)

REFERENCES:

Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide.

Archived Version

Method: **Preparation of AmpF/STR Identifiler™ (Minifiler™ or Yfiler™)
Samples for Analysis on the Applied Biosystems 3130 Genetic
Analyzer**

Date: 06/01/2006

Revision Date: 04/01/2008

Approved by: SAM

REAGENTS:

1. HIDI Formamide: Applied Biosystems (P/N 4311320)

Dispense the formamide into 500ul aliquots and store frozen. The frozen formamide can be used up to 1 year after being aliquoted.

Caution: Formamide is a known teratogen and is harmful by inhalation, skin contact, and ingestion. Use in a fume hood and wear chemical resistant gloves and safety glasses when handling.

2. GeneScan-500 [LIZ] Internal Size Standard: Applied Biosystems (P/N 4322682)
The tubes may be stored for up to six months at 4°C.
3. AmpF/STR Identifiler™ (Minifiler™ or Yfiler™) Allelic Ladder: (included in the Identifiler™, Minifiler™, or Yfiler™ kits)
Store at 2 to 8°C, protected from light.

MATERIALS:

1. 96-Well Optical Reaction Plates: Applied Biosystems (P/N 4306737)
2. Plate Septa for 96-Well plates: Applied Biosystems (P/N 4315933)
3. MicroAmp Splash Free Support Base: Applied Biosystems (P/N 4312063)
4. 96-well cooling block: Stored in the reagent freezer

PROCEDURE:

Note: Prior to preparing samples, the 9700 thermal cycler can be turned on so that the top plate can come to temperature.

1. Take out a new 96-well reaction plate.
2. Create a master mix by combining the following volumes of reagents into a 1.5mL microcentrifuge tube.
8.7uL formamide x (# of samples + 10%)

0.3uL GS500 [LIZ] x (# of samples + 10%)

3. Vortex the master mix.
4. Aliquot 10uL of formamide into the formamide blank well (suggestion: A1) if used as a control.
5. Aliquot 9uL of the master mix into each of the appropriate wells on the 96-well reaction plate to accommodate the numbers of amplified samples and allelic ladders samples.
6. Add 1uL of AmpFLSTR Identifier™ (Minifiler™ or Yfiler™) Allelic Ladder to the appropriate well(s) on the reaction plate. A second ladder is recommended when setting up your 3130 plate.
7. Add 1uL of amplified sample to each sample well of the reaction plate.

Note: The 3130 instrument samples wells in sets of four (a through D and E through H). Any extra wells in a set of four should have either formamide or formamide/internal size standard included in them so that the capillaries are not descending into empty wells.

8. Place a 96-well septa mat onto the reaction plate, and place the reaction plate in the 9700 thermal cycler. The plate may be spun down prior to denaturing.
9. Denature the samples by running the **denature 95 3min** protocol. **Important:** Do not clamp down the cover plate of the 9700 onto the septa mat-covered reaction plate as it will melt. Simply slide the cover plate forward to cover the reaction plate without clamping it down.
10. After the denaturation protocol is finished, chill the reaction plate by immediately placing it the 96-well cooling block (-20°C) for three minutes.
11. Place the reaction plate into a 3130 base plate (it will only fit in one orientation) and secure a plate retainer on top of the reaction plate. Check to ensure that the retainer clip is properly seated.
12. Press the tray button on the front of the 3130 Genetic Analyzer. When the tray presents itself, open the doors of the instrument and place the tray onto the right side position (only option available at this time) of the autosampler (the tray will only fit in one orientation). Close the instrument doors.
13. To start a run on the 3130 Genetic Analyzer please see the **Capillary Electrophoresis of Amplified Samples** protocol.

REFERENCES:

AmpF/STR Identifiler™ PCR Amplification Kit User's Manual

AmpF/STR Yfiler™ PCR Amplication Kit User's Manual

AmpF/STR Minifiler™ PCR Amplification Kit User's Manual

Applied Biosystems 3130 User's Bulletin.

Applied Biosystems 3130 User's Manual.

Applied Biosystems 3130 Getting Started Guide.

Archived Version

Method: **Preparation of AmpF/STR Identifiler™, Minifiler™, or Yfiler™
Amplified Samples for Analysis on the ABI 3130 Using the
Automated CAS-1200**

Date: 04/01/2008

Revision Date: 03/01/2010

Approved by: SAM

REAGENTS:

1. HIDI Formamide: Applied Biosystems (P/N 4311320)
Dispense the formamide into 500uL aliquots and store frozen. The frozen formamide can be used up to 1 year after being aliquoted.

Caution: Formamide is a known teratogen and is harmful by inhalation, skin contact, and ingestion. Use in a fume hood and wear chemical resistant gloves and safety glasses when handling.
2. GeneScan-500 [LIZ] Internal Lane Size Standard:
Applied Biosystems (P/N 4322682)
The kit may be stored up to six months at 4°C.
3. AmpF/STR Identifiler™, Yfiler™, or Minifiler™ Allelic Ladder: (included in the Identifiler™, Yfiler™, or Minifiler™ kits)
Store at 2 to 8°C, protected from light.

MATERIALS:

1. 3130 and 3100 Series 96-well Plate Base: Applied Biosystems (P/N 4317237)
2. 3100 Genetic Analyzer 96-well Plate Retainer: Applied Biosystems (P/N 4317241)
3. 96-Well Optical Reaction Plates: Applied Biosystems (P/N N801-0560)
4. Plate Septa for 96-Well Plates: Applied Biosystems (P/N 4315933)
5. 96-Well Cooling Block: Stored in the reagent freezer

EQUIPMENT:

1. CAS-1200 Corbett Robotics
2. GeneAmp PCR System 9700 Thermal Cycler Applied Biosystems

PROCEDURE:

Note: Prior to preparing samples, the 9700 thermal cycler can be turned on so that the top plate can come to temperature.

1. Remove the cover from the 96-well optical reaction plate that contains amplified samples.
2. Place the amplified sample plate on the C1 Sample plate area on the deck of the CAS-1200. Place a new 96-well optical reaction plate on the C2 Reaction plate area on the deck of the CAS-1200.
3. Create a master mix by combining the following volumes of reagents into a 5mL tapered tube:
 - 17.4uL formamide x (# of samples + 20%)
 - 0.6uL GeneScan-500 [LIZ] x (# of samples + 20%)

This mixture can be stored at 2-6°C for up to two weeks.

4. Vortex the master mix. Place the tube of master mix into Well A on the M1 (Mix Plate) deck on the CAS-1200. **See diagram for deck layout and proper tube placement.**
5. Place a 2mL flat tube containing ample AmpF/STR Identifiler™ (Minifiler™ or Yfiler™) Allelic Ladder in Well A on the R1(Reaction Plate) deck on the CAS-1200. Make sure the Ladder has been mixed (via vortexing) and spun down (in a microcentrifuge) prior to loading on the deck.
6. Place a 1.5mL tapered tube containing at least 100uL formamide into Well I on the R1 (Reaction Plate) deck on the CAS-1200.
7. Choose the "3130 Plate Set-up 2uL" protocol (or verify that this is the protocol that is currently open).
8. Look at the C1 Sample Plate on the computer monitor.

8A. If you have more samples than are lit up with the colored indicators on the Sample Plate:

Select the Sample Plate (there should be a red square around the plate). Highlight (click and drag) to add the extra wells that you need to correspond with the number of samples that were amplified, including the positive and negative controls (a red square around the well indicates that the well has been selected).

Right click and select "Add selected wells to sample bank."

In the Update Sample Bank window, select "Existing bank" (the only choice is "Amplified samples Identifiler"). Click "Add selection." The added wells should now light up with color indicators, and the Reaction Plate should automatically correlate with the Sample Plate. There will be seven additional wells that are color indicated on the Reaction Plate: 1 for formamide only, 1 for master mix only, and 2 for ladder (one in the first well and one in the last well).

8B. If you have fewer samples than are lit up with the colored indicators on the Sample Plate:

Select the Sample Plate (there should be a red square around the plate). Highlight (click and drag) to select the wells that you need to remove to correspond with the number of samples that were amplified, including the positive and negative controls (a red square around the well indicates that the well has been selected). Right click and select "Remove selected wells from sample bank."

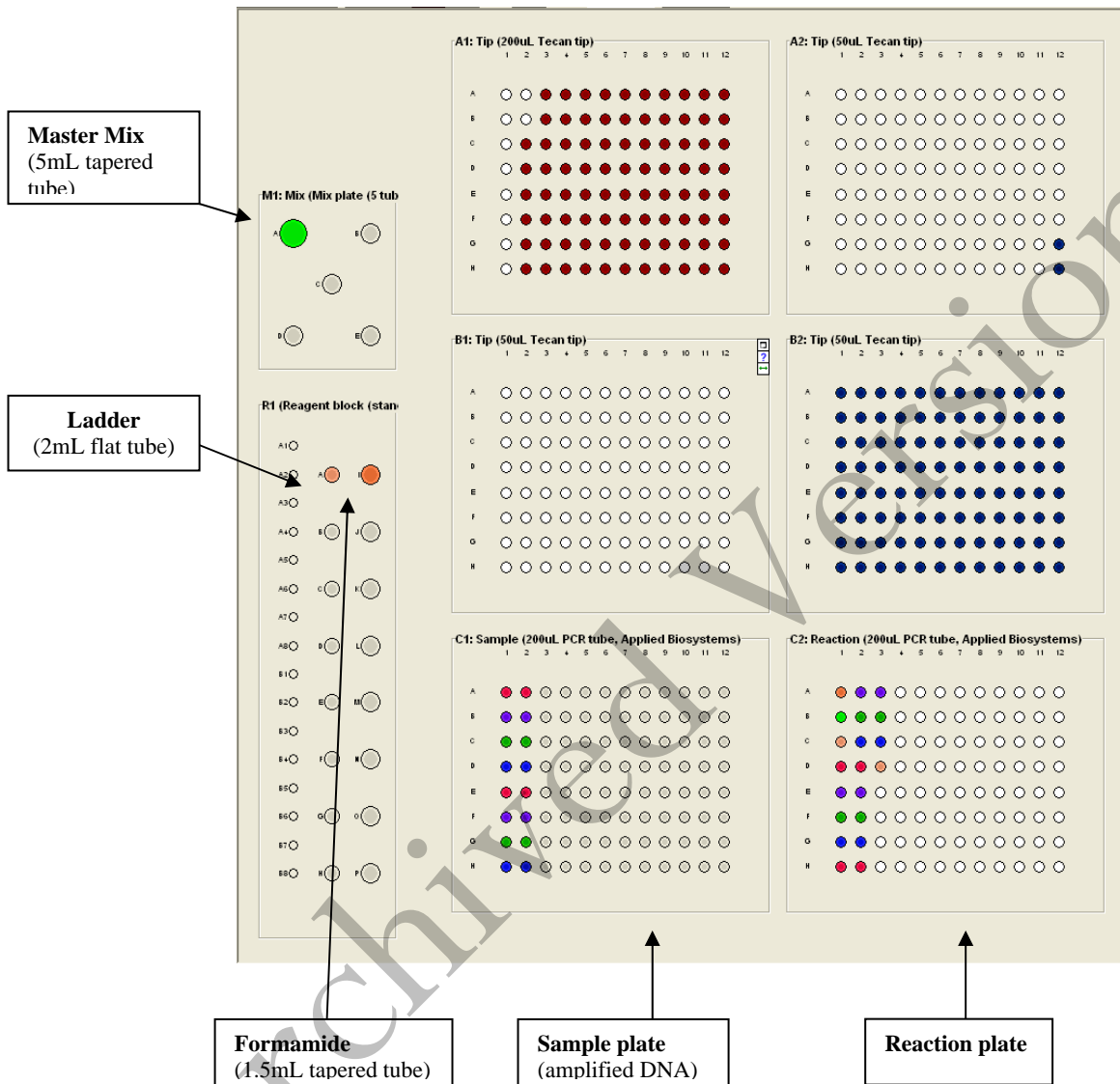
In the Update Sample Bank window, click "Delete selection." The previously lit wells should turn clear, and the Reaction Plate should automatically be updated to correlate with the Sample Plate. There will be seven additional wells that are color indicated on the Reaction Plate: 1 for formamide only, 1 for master mix only, and 2 for ladder (one in the first well and one in the last well).

9. Ensure that there are enough 50uL Tecan tips for the run:

Refill the tip racks in plate positions A2, B1, and B2 (if necessary). Although plate position A1 is for 200uL tips, 200uL tips are not used in this protocol. *If there are not enough tips for the run, the instrument will prompt you in the Checklist window when you attempt to start the run.*

To replace the tips, remove the empty tip rack from the tip rack holder on the instrument, and replace with a full rack of tips (the full tip rack will only seat one way on the tip rack holder). Using the mouse, highlight (click and drag) the tip positions that are now available based on where the tips were just added. Right click and choose "Set selected tips to 'Available'".

Deck Layout Diagram



10. To signal the CAS-1200 to begin the protocol, either click on the green dot with the arrow in it on the shortcut tool bar, or choose "Control" on the tool bar and select "Start." In the Checklist window, click on each message box, such that there's an X in each box. Verify that the tip disposal box is not too full (empty it, if necessary), and click OK.

11. The CAS-1200 will now add the master mix, formamide blank, ladders and DNA samples to the 96-well optical reaction plate.

12. When the protocol has finished, the sample plate on C1 on the deck (the plate containing the original amplified samples) can be covered with a foil cover for freezer

storage.

13. Remove the reaction plate from the C2 deck position and place a plate septa mat on it. Denature the samples by placing the plate in the 95°C heat block for three minutes. Choose the protocol entitled Denature 21uL. **Important:** Do not clamp down the cover plate of the 9700 onto the septa mat-covered reaction plate as it will melt. Simply slide the cover plate forward to cover the reaction plate without clamping it down.
14. Chill the plate immediately in a freezer block for three minutes.
15. Place the plate into a 3130 and 3100 Series 96-well plate base (it will only fit in one orientation) and cover with a 3100 Genetic Analyzer 96-well plate retainer. Check to ensure that the retainer clip is properly seated.
16. Press the **Tray** button on the front of the 3130 CE instrument. Once the autosampler has moved forward, open the doors and place the tray onto the autosampler with position A1 at the upper right. Close the instrument doors.
17. To start a run on the 3130 Genetic Analyzer please see the **Capillary Electrophoresis of Amplified Samples** protocol.

Select sample re-preparation using the CAS-1200:

In order to re-prepare select samples from the 96-well plate of amplified samples, highlight all of the wells that you **do not** want re-prepared and delete them from the sample plate following step 8B.

REFERENCES:

CAS-1200 Liquid Handling System Manual

Method: **Decontaminating the CAS-1200 using the automated UV protocol**

Date: 04/01/2008

Approved by: SAM

EQUIPMENT:

1. CAS-1200 Corbett Robotics

PROCEDURE:

1. Remove the box of ejected tips and use the plug that is attached to the side of the tip ejector chute to plug the tip ejector chute.
2. With the 3130 Plate Setup 2.8uL program open, click on the UV Light icon (the yellow light bulb).
3. In the UV lamp control window, choose the length of time you wish the UV lamp to be active by sliding the flashing cursor right or left (a minimum of 5 minutes is recommended).
4. Select Start.
5. When the UV light operation has completed, click OK, unplug the tip ejector chute, and re-position the ejected tips box.

REFERENCES:

CAS-1200 Liquid Handling System Manual

Method: **Capillary Electrophoresis of Amplified Samples on the Applied Biosystems 3130 Genetic Analyzer**

Date: 06/01/06

Revision Date: 07/25/2007

Approved by: SAM

MATERIALS:

ABI PRISM® 3130 Data Collection Software

PROCEDURE:

1. Launch the **ABI Prism 3130 Collection** Application.
2. In the navigation pane of the Data Collection software click **GA Instruments > ga3130 > Plate Manager**. Click **NEW** to open the New Plate dialog box. Alternatively if a 3130 plate record (*.txt file) has been created using the SDPD Worksheet maker v2.2 it can be imported to the database by clicking on import in the **Plate Manager** then browsing for the appropriate file.
3. Complete the information in the New Plate Dialog box (note: It is suggested that Plate Records be saved with analysts' initials at the beginning of the name so that they can be located more easily in the database.) and hit **OK**. The GeneMapper Plate Editor opens.

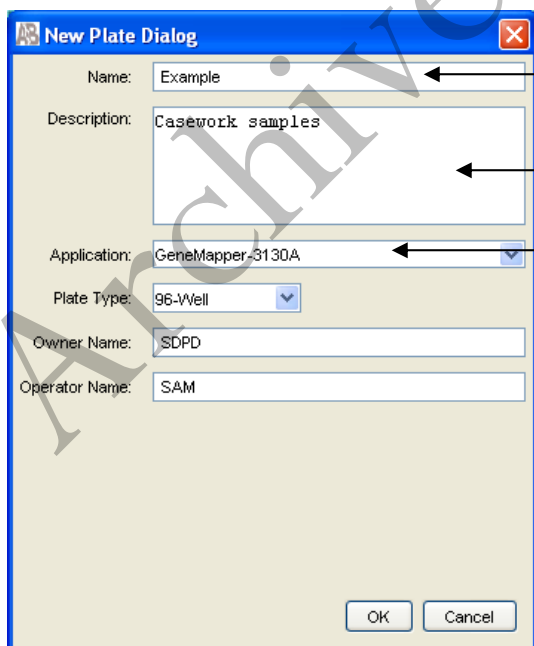


Plate Name (see above note)

Description (optional)

Application will be
SDPD-3130A

- a. GeneMapper Plate Editor:
In the Sample Name column of a row, enter a sample name, then click the next cell. The value of 100 is automatically displayed in the priority column for the entered

San Diego Police Department Crime Lab
Forensic Biology Unit

samples.

Well	Sample Name	Comment	Priority	Sample Type	Size Standard	Panel
A01						
B01						
C01						
D01						
E01						
F01						
G01						
H01						
A02						
B02						
C02						
D02						
E02						
F02						
G02						
H02						
A03						
B03						
C03						
D03						
E03						
F03						
G03						

b. An optional Comment column is available.

Well	User-Defined 3	Results Group 1	Instrument Protocol 1	Results Group 2	Instrument Protocol 2	Results Group 3	Instrument Protocol 3
A01		SDPD_Results_Group	SDPD_Identifier_Protoco				
B01		SDPD_Results_Group	SDPD_Identifier_Protoco				
C01		SDPD_Results_Group	SDPD_Identifier_Protoco				
D01		SDPD_Results_Group	SDPD_Identifier_Protoco	SDPD_Results_Group	SDPD_Identifier_Protoco		
E01		SDPD_Results_Group	SDPD_Identifier_Protoco				
F01		SDPD_Results_Group	SDPD_Identifier_Protoco				
G01		SDPD_Results_Group	SDPD_Identifier_Protoco				
H01		SDPD_Results_Group	SDPD_Identifier_Protoco				
A02		SDPD_Results_Group	SDPD_Identifier_Protoco				
B02		SDPD_Results_Group	SDPD_Identifier_Protoco				
C02		SDPD_Results_Group	SDPD_Identifier_Protoco				
D02		SDPD_Results_Group	SDPD_Identifier_Protoco				
E02		SDPD_Results_Group	SDPD_Identifier_Protoco				
F02		SDPD_Results_Group	SDPD_Identifier_Protoco				
G02		SDPD_Results_Group	SDPD_Identifier_Protoco				
H02		SDPD_Results_Group	SDPD_Identifier_Protoco				
A03		SDPD_Results_Group	SDPD_Identifier_Protoco				
B03		SDPD_Results_Group	SDPD_Identifier_Protoco				
C03		SDPD_Results_Group	SDPD_Identifier_Protoco				
D03		SDPD_Results_Group	SDPD_Identifier_Protoco				
E03		SDPD_Results_Group	SDPD_Identifier_Protoco				
F03		SDPD_Results_Group	SDPD_Identifier_Protoco	SDPD_Results_Group	SDPD_Identifier_Protoco		
G03		SDPD_Results_Group	SDPD_Identifier_Protoco				
H03		SDPD_Results_Group	SDPD_Identifier_Protoco				

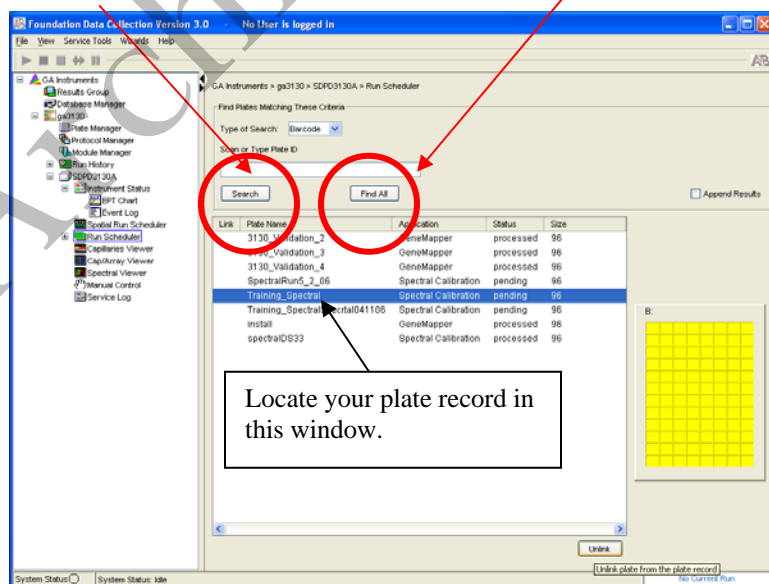
c. In the Sample Type column, select applicable sample type from the drop-

down list. In the Size Standard column, select **CE_G5_HID_GS500** from the drop-down list. In the Panel column, select **Identifiler_v2** from the drop-down list. In the Analysis Methods column, select **SDPD Analysis Methods** from the drop-down list. Scroll across to the Results Group 1 column, select **SDPD_Results_Group** from the drop-down list. In the Instrument Protocol 1 column, select the applicable protocol (e.g. **SDPD_Identifiler_Protocol**) from the drop-down list. For all columns with the exception of Sample Type), fill down to select for all samples. Note: The SDPD_Identifiler_Protocol will be the standard protocol used in Identifiler analysis and is set with a 5 second injection. See below for details on altered injection times and re-injections.

- d. Click **OK** to save your changes and close the plate editor.

Important: After clicking **OK** within the plate editor, the completed plate record is stored in the Plate Manager database. Once in the Plate manager database, the plate record can be searched for, edited, exported, or deleted.

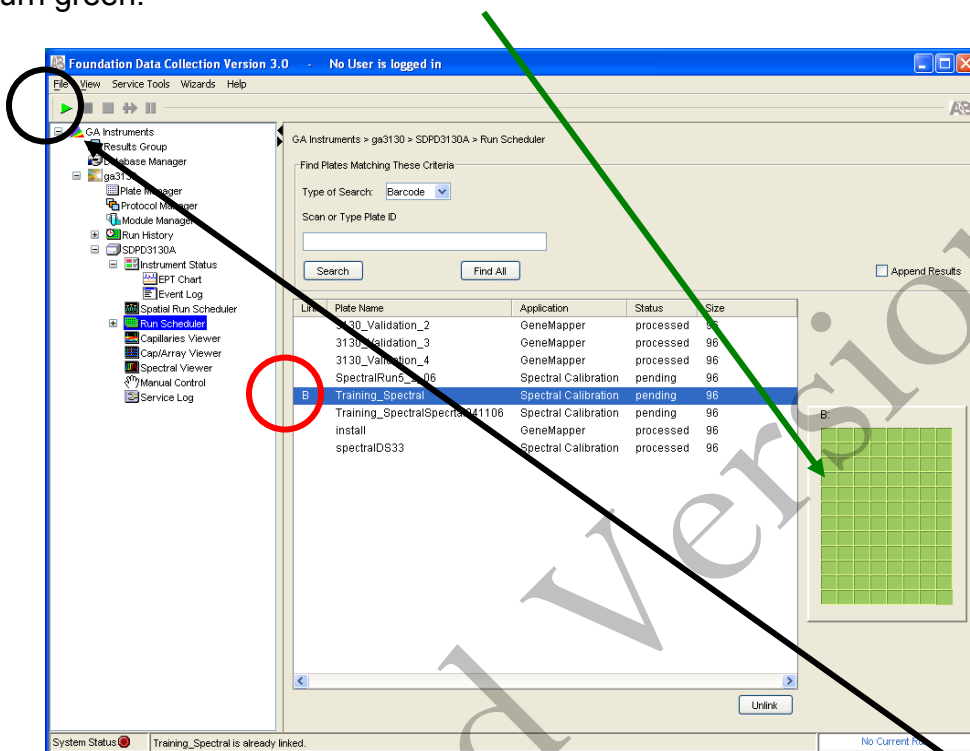
4. If you want to inject a sample more than once within a run:
 - a. Select **Edit > Add Sample Run**. Additional Results Group and Instrument Protocol columns are added to the right on the Plate Record.
 - b. Complete the Results Group and Instrument Protocol columns for the additional injections. Note: If samples are slated to be injected more than once, subsequent injections of these samples will occur immediately after the first injection. Click **OK** to save your changes and close the Plate Record.
5. In the navigation pane of the Data Collection software click **GA Instruments > ga3130 > SDPD3130A > Run Scheduler**. Click **Find All** or locate your plate record using the **Search** button.



6. Link

the plate record

to the 3130 by highlighting your pending plate record, then double-clicking on the yellow plate (B:). A "B" will appear to the left of the linked plate record and the plate will turn green.



7. Once the plate is linked, the run can be started by clicking the green arrow ►. If desired, the oven can be preheated in **GA Instruments > ga3130 > SDPD3130A > Manual Control** prior to starting the run. To turn the oven on you must first send the command to turn the oven on followed by sending the command to heat the oven to 60°C.
8. After the run is complete and you determine that re-injections are necessary you may do the following to start those re-injections.
 - a. Locate your plate record in the plate record manager (**GA Instruments > ga3130 > Plate Manager**) and click **Edit**.
 - b. Select **Edit > Add Sample Run**. Additional Results Group, Instrument Protocol columns are added to the right on the plate record.
 - c. Complete the Results Group and Instrument Protocol columns for the additional injections. Note: If altered injection times are desired instrument protocols have been created for 3 second and 1 second injection times. These can be selected from the drop-down menu. If an injection time other than 3 or 5 seconds is required, a new Protocol will have to be created. Click **OK** to save your changes, and then close the plate record.
 - d. Complete steps 5 and 6 to initiate the re-injection run.

9. When the run is complete:

- Rename the Run Folder uniquely and move it into the individual Analyst's Folder on the FB network [**H:3130-310 Run Folders\Analyst folder**)] prior to any analysis.

REFERENCES:

ABI PRISM® 3130 Genetic Analyzer User's Manual

ABI PRISM® 3130 Genetic Analyzer Getting Started Guide

ABI PRISM® 3130 Genetic Analyzer User's Bulletin

Archived Version

Method: **Performing a Spectral Calibration on the Applied Biosystems 3130 Genetic Analyzer**

Date: 06/01/2006

Revision Date: 03/01/2010

Approved by: SAM

INTRODUCTION:

A spectral calibration creates a "matrix" that is used during a run to spectrally separate the raw data from the instrument into the 5-dye components saved to the sample files. (Note: on the 3130, the spectral is applied to the raw data in real-time whereas on the 310s the matrix is applied upon analysis). A spectral calibration should be performed:

- If a decrease in spectral separation (pull-up and/or pull-down) is observed
- After the laser or CCD camera has been realigned or replaced by a service engineer

REAGENTS:

1. HIDI Formamide: Applied Biosystems (P/N 4311320)

Dispense the formamide into 500ul aliquots and store frozen. The frozen formamide can be used up to 1 year after being aliquoted.

Caution: Formamide is a known teratogen and is harmful by inhalation, skin contact, and ingestion. Use in a fume hood and wear chemical resistant gloves and safety glasses when handling.

2. DS-33 Matrix Standards (Dye Set G5): Applied Biosystems (P/N 4345833)
Store at 2 to 8°C, protected from light. Stable for one year.
(This is the matrix standard set used when analyzing amplified DNA fragments generated using the Identifiler kit, and labeled with 6FAM-, VIC-, NED-, PET-, and LIZ-)

MATERIALS:

1. 96-Well Optical Reaction Plates: Applied Biosystems (P/N 4306737)
2. Plate Septa for 96-well: Applied Biosystems (P/N 4315933)
3. MicroAmp Splash free Support Base PE Applied Biosystems (P/N 4312063)
4. 96-well cooling block: Stored in the reagent freezer

PROCEDURE:

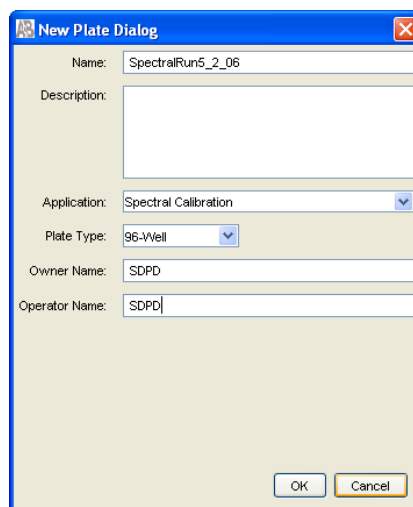
Preparing the Spectral Calibration Chemistry

1. Remove DS-33 Matrix Standard tube from the refrigerator, vortex to mix, then spin briefly in a microcentrifuge.
2. For a 36cm array, combine 5-7uL of standard with 193-195uL formamide (for a total volume of 200uL) in a 1.5mL microfuge tube. Vortex to mix, then spin briefly in a microcentrifuge.
3. Aliquot 10uL of standard/formamide mix into 4 wells (sugg: A1-D1) of a 96-well optical reaction plate.
4. Place a 96-well septa mat onto the reaction plate, and place the reaction plate in the 9700 thermal cycler.
5. Denature the samples by running the **spectraldenature** protocol. **Important:** Do not clamp down the cover plate of the thermal cycler on the septa covered reaction plate as it will melt. Simply slide the cover plate forward to cover the reaction plate without clamping it down.
6. After the denaturation protocol is finished, chill the reaction plate immediately in the 96-well cooling block (-20°C) for two minutes.
7. Place the reaction plate into a 3130 base plate (it will only fit in one orientation) and secure a plate retainer on top of the reaction plate. Check to ensure that the retainer clip is properly seated.
8. Press the **Tray** button on the exterior of the 3130 Genetic Analyzer. When the tray presents itself, open the doors of the instrument and place the tray onto the right side position of the autosampler (the tray will fit on the autosampler in one orientation only). Close the instrument doors.

Performing a Spectral Calibration

1. In the navigation pane of the Data Collection software, click: **GA Instruments>ga3130>Plate Manager**.
2. Click **New**. The New Plate Dialog box opens. Enter the details shown below (add the appropriate date to the name):

San Diego Police Department Crime Lab
Forensic Biology Unit



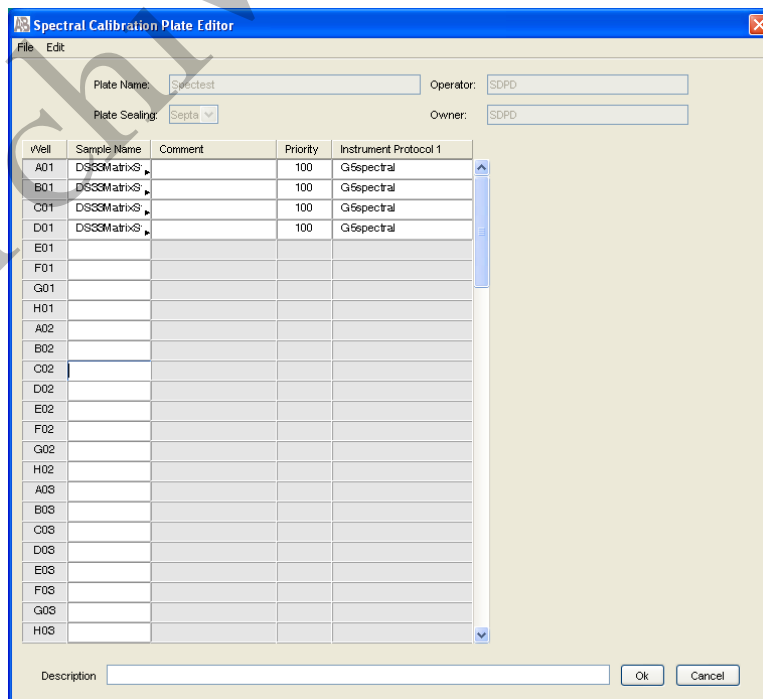
The 'New Plate Dialog' box contains the following fields and options:

- Name: SpectralRun5_2_06
- Description: (empty text box)
- Application: Spectral Calibration (dropdown menu)
- Plate Type: 96-Well (dropdown menu)
- Owner Name: SDPD
- Operator Name: SDPD
- Buttons: OK, Cancel

3. Click **OK**.

4. The Spectral Calibration Plate Editor dialog box opens.

- In the **Sample Name** column of A01, enter sample name (e.g. G5MatrixStd)
- Click on the **Comment** cell. The value 100 automatically displays in the **Priority** column
- In the **Instrument Protocol 1** column, select the spectral instrument protocol **G5Spectral** from the drop-down menu
- Highlight the entire row and select **Edit > Fill Down Special**. The software automatically fills in the appropriate well numbers (i.e. A1-D1)

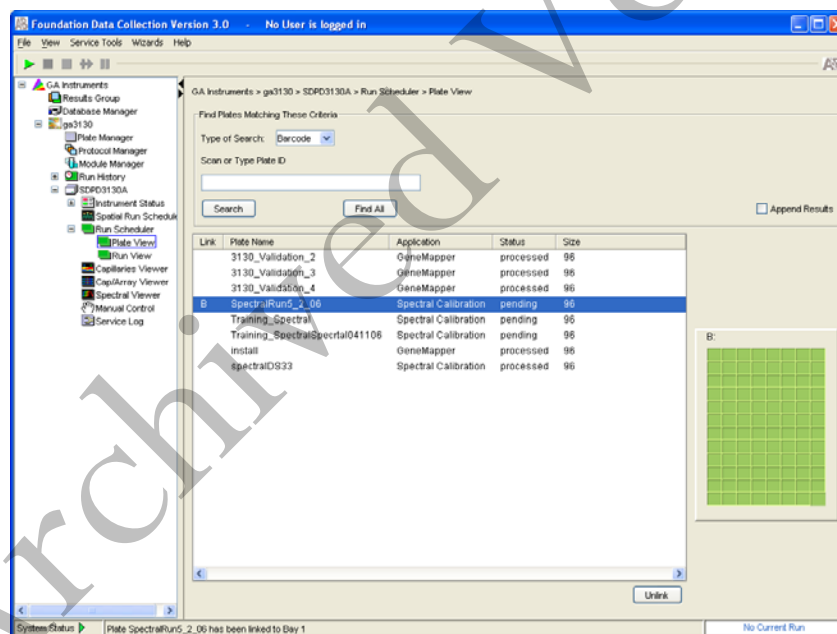


The 'Spectral Calibration Plate Editor' dialog box features a table with the following columns: Well, Sample Name, Comment, Priority, and Instrument Protocol 1. The table is populated with rows A01 through H03. The first four rows (A01-D01) are pre-filled with 'DSSMatrixS', '100', and 'G5Spectral' respectively. The 'Well' column lists wells in 12-column blocks (A-D, E-H, A-D, E-H, A-D, E-H, A-D, E-H). The 'Description' field at the bottom is empty. Buttons for 'Ok' and 'Cancel' are at the bottom right.

Well	Sample Name	Comment	Priority	Instrument Protocol 1
A01	DSSMatrixS		100	G5Spectral
B01	DSSMatrixS		100	G5Spectral
C01	DSSMatrixS		100	G5Spectral
D01	DSSMatrixS		100	G5Spectral
E01				
F01				
G01				
H01				
A02				
B02				
C02				
D02				
E02				
F02				
G02				
H02				
A03				
B03				
C03				
D03				
E03				
F03				
G03				
H03				

Click **OK**.

5. In the navigation pane of the Data Collection software, click:
GA Instruments > ga3130 > SDPD3130A > Run Scheduler > Plate View.
6. Search for your plate record. Select **Barcode** in the Type of Search drop-down list and type in the plate name then click **Search**. The plate record will appear in the lower box. Alternatively, you can select **Find All** and all previous plate records will appear. (You can also perform an **Advanced** search for the plate record. See page 41 of the Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide.)
7. Highlight the desired plate record and then click on the plate position indicator to the right to link the plate. The plate map color will change from yellow to green when it is successfully linked and a "b" will appear beside the linked plate record.



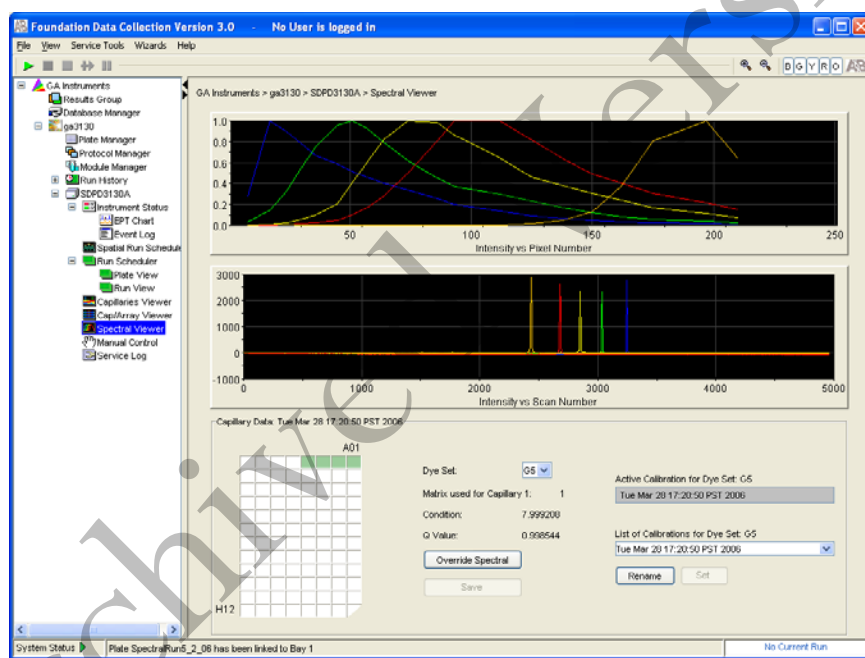
8. In the toolbar of the Data Collection software window, click ► to begin the run.
9. The **Processing Plates** dialog box opens. Click **OK**. The Spectral Calibration run time is approximately 35 min.
10. After the run, the pass or fail status of each capillary is recorded in the **Event Log** section of the **Instrument Status** window:
 - Click **GA Instruments > ga3130 > SDPD3130A > Instrument Status > Event Log**.

- **Event Messages** will indicate whether or not each capillary was successfully calibrated.
- Each capillary should have a **Q-value** above 0.95 and a **Condition Number** in the range 7 to 12.

Evaluating the Spectral Calibration Data

Note: the spectral calibration of each capillary should be evaluated even if the Spectral Calibration Results box indicated that they passed. If the spectral calibration failed, see the Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting and Reference Guide.

1. Click **GA Instruments > ga3130 > SDPD3130A > Spectral Viewer**.



2. Select G5 from the Dye Set drop-down list (if it is not already displayed).
3. In the plate diagram, dark green boxes should be present at A1-D1. The dark green boxes confirm that the four capillaries passed the spectral calibration. Tan colored boxes in the plate diagram indicate failed spectral calibration.
4. Select a well in the plate diagram. The dark green box will turn light green, and the spectral profile and raw data corresponding to that well/capillary will be displayed. No spectral profile will be displayed if the spectral calibration failed. Evaluate each well/capillary for the following:
 - a. Verify that the order of the peaks in the spectral profile from left to right are: Blue-Green-Yellow-Red-Orange [In the top window]

- b. Verify that the order of the peaks in the raw data from left to right are:
Orange-Red-Yellow-Green-Blue [In the bottom window]
5. Verify that the peaks in the spectral profile do not contain gross overlaps, dips or other irregularities
6. The spectral run name will default to the day, date and time of the run. The name may be changed by clicking **Rename**.

Activating the Spectral Calibration

Note: a run cannot start unless a calibration file that matches the dye set and capillary array length to be used for the run is active.

1. In the navigation pane of the Data Collection software, click: GA Instruments > ga3130 > SDPD3130A > Spectral Viewer.
2. Select G5 from the **Dye Set** drop-down menu.
3. Select a spectral calibration from the **List of Calibrations**.
4. Click **Set**.

REFERENCES:

1. Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide.
2. Applied Biosystems DS-33 (Dye Set G5) Matrix Standard Kit product insert

Method: **Performing a Spatial Calibration on the Applied Biosystems 3130 Genetic Analyzer**

Date: 06/01/2006

Revision Date: 01/01/2007

Approved by: SAM

INTRODUCTION:

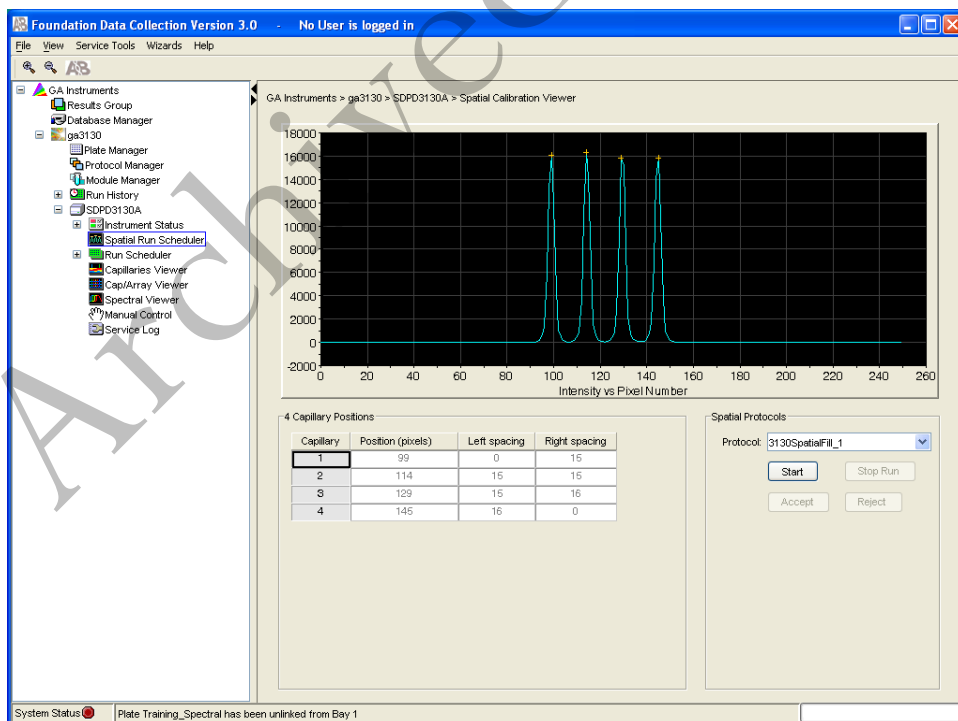
The 3130 Genetic Analyzer Data Collection software uses images collected during the spatial calibration to establish a relationship between the signal emitted by each capillary and the position where the signal is detected on the CCD camera. A spatial calibration should be performed when you:

- Install or replace a capillary array
- Temporarily remove the capillary array from the detection block
- Move the instrument

PROCEDURE:

Creating a Spatial Calibration file

1. In the navigation pane of the Data Collection software, click: **GA Instruments > ga3130 > SDPD3130A > Spatial Run Scheduler.**



2. In the Spatial Protocol section, select one of the following:

- **Protocol > 3130SpatialNoFill_1**, if the capillary contains fresh polymer, otherwise select;
- **Protocol > 3130SpatialFill_1**

(Note: you do not need to fill the capillaries each time you perform a spatial calibration.)

3. Click **Start**.

The calibration run lasts approximately:

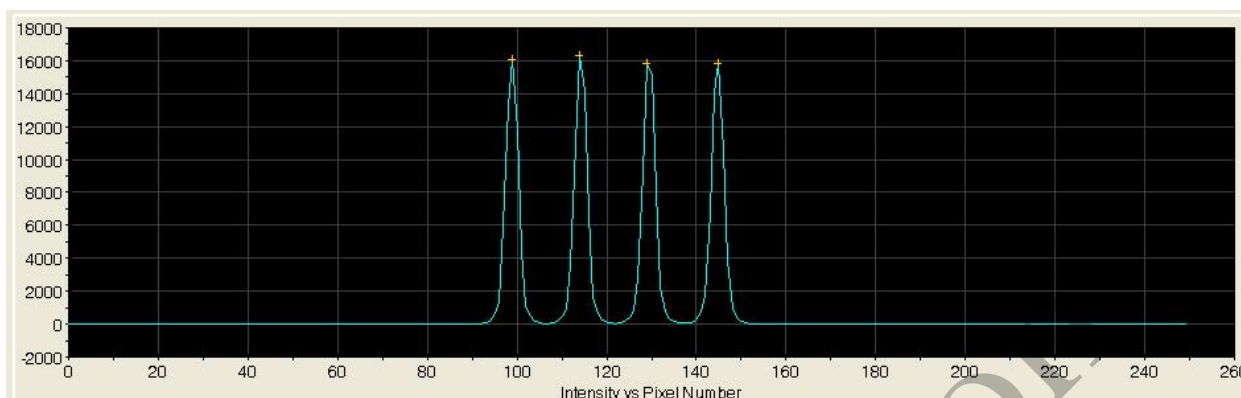
- 2 min. without filling the capillaries, or;
- 6 min. when filling the capillaries

(Note: the spatial profile window turns black when you start a spatial calibration.)

Evaluation the Spatial Calibration file

1. Evaluate the spatial calibration profile using the following criteria:

Peak Attribute	Acceptable Criteria
Height	Similar height for all peaks.
Orange crosses	One orange cross marking the top of every peak. No misplaced crosses.
Shape	Single sharp peak for each capillary. Small shoulders are acceptable.
Spacing	The difference between adjacent positions is 13 to 16 pixels. Theoretical spacing between capillaries is 15.



2. Examine each row in the 4 Capillary Positions table and verify that the values in both the Left spacing and Right spacing columns range between 13 and 16 pixels. The “+” sign denotes the top of each peak. If a “+” is out of alignment, it may be manually moved as follows:

To move the cross:

Type a new value in the Positions (pixels) box for the capillary of interest.

Click outside of the box or press **Enter**.

4 Capillary Positions			
Capillary	Position (pixels)	Left spacing	Right spacing
1	99	0	15
2	114	15	15
3	129	15	16
4	145	16	0

Accepting or Rejecting a Spatial Calibration

Based on the above criteria (Height, + position, shape, and spacing), if the calibration:

- Passed, click **Accept** to write the calibration data to the database and .ini file. This will irrevocably replace the current spatial file.
- Failed, click **Reject** then see the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting and Reference Guide*.

Spatial Protocols

Protocol: 3130SpatialFill_1

Start Stop Run

Accept Reject

REFERENCES:

Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide.

Archived Version

Method: **Flushing and Filling the Water Trap on the Applied Biosystems 3130 Genetic Analyzer**

Date: 06/01/2006

Revision Date: 01/01/2007

Approved by: SAM

INTRODUCTION:

The PDP water trap at the top of the pump block should be flushed with distilled or deionized water once a week to remove any diluted polymer and to clear air bubbles. The trap should be filled with water at all times.

MATERIALS:

1. A 20mL syringe, preferably the all-plastic Luer lock syringe (Applied Biosystems P/N 4324463) that was supplied with the instrument.

(Note: syringes smaller than 20mL should not be used, as they may generate excessive pressure within the trap.)

2. Distilled or deionized water.

PROCEDURE:

1. Fill the syringe with nanopure water.
2. Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting clockwise with the other hand.
3. Open the Luer fitting by grasping the body of the fitting and turning it and the attached syringe approximately one-half turn counterclockwise.
4. Open the exit fitting at the top left side of the pump block by turning it approximately one-half turn counterclockwise.
5. Hold an empty tube or beaker under the exit fitting to receive approximately 5mL of flushed water waste. Flush the trap by pushing steadily on the syringe plunger.

(Note: do not use excessive force. Take approximately 30 seconds to flush 5mL.)

6. Once flushing is complete, re-tighten Luer fittings and remove the syringe.

REFERENCES:

Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide.

Archived Version

Method: **GeneMapper ID™ Analysis of Data**

Date: 06/01/2006

Revision Date: 02/06/2009

Approved by: SAM

INTRODUCTION:

GeneMapper™ ID software is an automated genotyping software package that combines the precision-sizing capabilities of the GeneScan® Analysis software with the allele-calling power of Genotyper® software, but includes additional features and enhancements. The software will genotype samples based on allelic bin definitions stored within the Panel Manager. The AmpFISTR panels and bins are pre-installed in the Panel Manager within the software. Custom SDPD Analysis Methods have also been created in the GeneMapper Manager for use in analyzing raw data files for Identifiler, Yfiler, and Minifiler amplifications, respectively. An analysis method defines the analysis parameters to be used for the analysis of samples imported into the Project Window. When the GeneMapper™ ID software is installed on any computer, the Panel, Bin Set and Analysis Method have to be defined prior to the analysis of raw data files. See also the procedure for importing panels and bins.

MATERIALS:

Applied Biosystems GeneMapper™ ID Software

PROCEDURE:

1. Open the GeneMapper™ ID software by clicking on the icon located on the computer's desktop and log-in. Using the drop-down menu select **SDPD** as the User Name. The password for the GeneMapper™ ID software on all instruments and computers is "**sdpdfb**". The password is case sensitive. Upon successful log-in the **Project Window** will open. The project window is the primary window for the user interface.
2. To add samples to the Project Window. Select **File>Add Samples to Project**. For samples run on the 3130:
 - Navigate to Forensic Biology on FB server H:\3130-310 Run Folders to locate the desired run folder.
 - Select the desired run folder and click **Add To List**. The selected folder will appear in the **Samples To Add** box on the right. Note: Multiple run folders can be added to the same project. Click **Add** to import the files into the project and close the dialog box.
3. For each sample within the **Project Window** select the appropriate **Sample Type**, **Analysis Method**, **Panel**, **Size Standard**, and **Matrix** (if appropriate). Unknowns should be designated Sample, Allelic Ladders should be designated Allelic Ladder, the positive PCR control should be designated as Positive Control, and any reagent

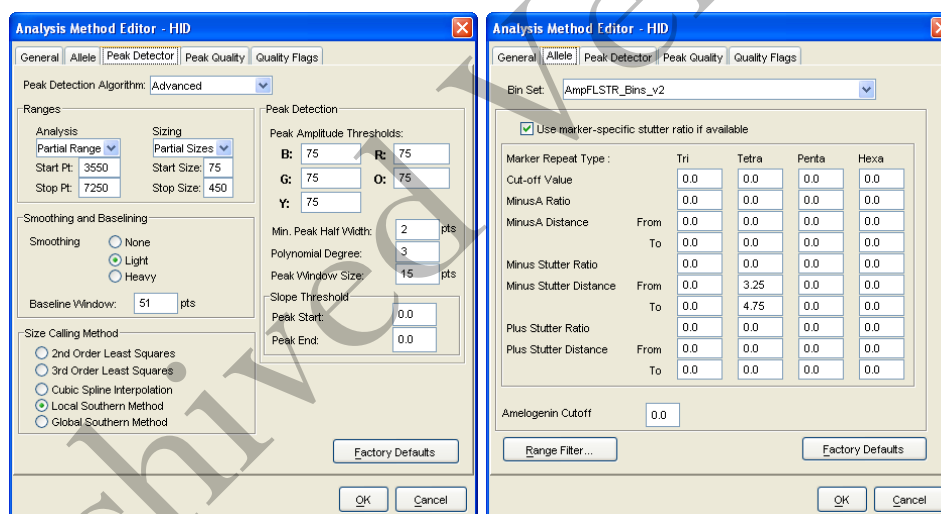
blanks, amplification blanks, or formamide blanks should be designated as Negative Controls.

For Identifiler analyses, the analysis method will be **SDPD v2 Analysis Method**, the panel will be **Identifiler_v2**, and the size standard will be **CE_G5_HID_GS500**.

For Minifiler analyses, the analysis method will be **Minifiler Analysis Method**, the panel will be **Minifiler_GS500_v1**, and the size standard will be **CE-G5_HID_GS500**.

For Yfiler analyses, the analysis method will be **SDPD Yfiler Analysis Method**, the panel will be **Yfiler_v2**, and the size standard will be **CE_G5_HID_GS500**.

Samples run on the 310s will require a Matrix file, samples run on the 3130 will not require a Matrix file as the Spectral is applied to the sample in real time on the 3130. The analysis parameters for the **SDPD Analysis Method v2** are as follows:



Note: In general, these analysis parameters will not change with the exception of the analysis range which will be dependent on the electrophoresis of each run. If peak amplitude thresholds greater than 75rfu are required for analysis of specific samples, a separate Analysis Method should be used.

The SDPD Y-STR Analysis method, and the parameters for any method, can be viewed from the **Analysis Method** tab in the **GeneMapper Manager** window.

- Click ► (Analyze), and the Save project dialog box opens. Save the project with a uniquely identifying name [e.g. initials(Case #)]. After analysis, the project is automatically saved, and the Genotypes Tab becomes available. Analyzing the data sizes and genotypes the samples in the project. There must be at least one ladder in each run folder analyzed for the analysis to be successful.

5. Accurate application of the size standard to the samples can be verified in a variety of ways. Using the Process Component Quality Values (PQVs) such as the **Sizing Quality** flag is an efficient method of verifying the sizing of individual samples. In the Samples tab of the Project Window the sizing quality of each sample is displayed as a symbol (■ = Pass, ▲ = Check sizing for problems, or ● = Low Quality, sizing failed). Alternatively, individual or multiple samples can be selected in the project window and the **Size Match Editor** used to view the size standard for each sample. It is possible to overwrite the Sizing Quality PQV in this window for individual samples. This could be done, for example, if the 450bp size standard peak was not detected for a particular sample, but it was determined that the absence of this standard would not affect the sizing of any peaks within the sample.
6. The migration of the samples within the run can also be verified in a number of ways. For Identifiler, Minifiler, and Yfiler, one method is to select all the samples within the **Project Window** and then select **Display Plots**. From the plot settings drop-down menu, select **Overlay LIZ**. Using the cursor, zoom in on the 250bp peak in the plot window, and select all the 250bp peaks by clicking within them. Select **View>Table Filter>Show Selected Rows** (or press CTRL+S). The 250bp peak should size consistently and overlap. The difference in the migration of this fragment should be <1bp across the entire run. Note: If it is >1bp the project may require some troubleshooting to obtain reliable genotyping of the samples. Close the sample plot.
7. The individual sample files can be reviewed and printed for the analytical record. If needed, labels can be edited within the samples. To edit labels, select the labeled peak to be edited. With the desired peak highlighted, right-click and select the applicable option of changing or deleting the label. An Edit Allele Comment dialog box will open and a reason for the change can be input. Sample plots with edited labels should be printed for the analytical record with the edit displayed in the plot and with the Allele Edit Comment displayed in the Table.
8. PQVs can be used to assess the quality of a variety of aspects of each sample. Below is a list of some of the PQVs that can be used to assist in the analysis of the sample data:
 - OS = Off-Scale data. If off-scale data is present in the sample, ▲ is automatically displayed within the size standard region of the signal. Otherwise a ■ is displayed
 - SQ = Sizing Quality. The sizing quality is calculated based on the similarity of the size standard fragment pattern and the actual size standard peak distribution pattern in the sample.
 - AE = Allele Edit. This box will be checked when the marker allele calls have been edited.
 - ADO= Allele Display Overflow. This box is checked when the number of alleles at any locus exceeds the number that can be displayed (this number is

selected by the user.

AN = Allele Number. ▲ is automatically displayed when the number of alleles at any locus is greater than the user defined expected allele number.

Note: Currently set to 4 in the SDPD Analysis Method.

Bin= Out of Bin Allele. ▲ is automatically displayed when the apex of a called peak is outside the boundary of a bin.

PHR =Peak Height Ratio. ▲ is automatically displayed when there are two alleles present at any locus and the ratio between the lower allele height and the higher allele height is below the user defined threshold. Note: Currently set to 0.6 in the SDPD Analysis Method.

LPH = Low Peak Height. ▲ is automatically displayed when alleles are lower than the specified values and do not result in the proper intensity. Note: Currently set to 200rfu for homozygotes and 75rfu for heterozygotes in the SDPD Analysis Method.

SPU = Spectral Pull-up. ▲ is automatically displayed when any locus displays pull-up. Pull-up is defined by the system to be when the height of a labeled peak is less than 5% of any larger peak within +/- 1 data point.

BP = Broad Peak. ▲ is automatically displayed when the peak width of a labeled peak is wider than 1.5bp.

SP = Split Peak. ▲ is automatically displayed when the GeneMapper™ ID software detects a split peak.

CC = Control Concordance. ▲ is automatically displayed when the result for any positive control sample does not match the defined alleles for any locus. ▲ is also automatically displayed when peaks are detected in negative controls.

GQ = Genotype Quality. Is an overall assessment of the quality of an individual locus based on a mathematical formula using the previously mentioned PQVs as variables. The user can define the weights applied to the above PQVs in this formula in the Analysis Method.

Note: Other PQVs exist but not all are applicable to human identification testing. For more information please see the GeneMapper™ ID software user's guide.

9. When the analysis of the GeneMapperID project is completed the project file should be exported to the appropriate folder on the Forensic Biology network. To export the project: highlight the project in the **Projects** tab in **GeneMapper Manager** and click the **Export** button. Browse for the appropriate folder on the network (**Forensic Biology on FB server (H:)>GeneMapper ID Projects>Analysts folder**) and save the exported project using the Project name. Use of analysts initials at the start of the file name will be useful in locating projects as the number of files increases and is highly recommended [e.g. initials(XX-XXXXXX;etc)] .

10. The original project should be deleted from the GeneMapperID database once the project is exported. To delete the original project: highlight the project in the **Projects** tab in **GeneMapper Manager** and click the **Delete** button. Prior to deleting

the project it is recommended to check that the file was exported to the appropriate folder.

11. To view exported GeneMapperID projects. Open **GeneMapper Manager** and from the **Projects** tab click **Import**. Browse to **Forensic Biology on FB server (H:) > GeneMapper ID Projects** and select the desired project. Click **OK**. The project will have been imported to the GeneMapperID database and can be opened as usual. When the imported project is no longer needed it should be deleted from the database (see step 10.).

REFERENCES:

AmpF/STR Identifier™ PCR Amplification Kit User's Manual

AmpF/STR Yfiler™ PCR Amplification Kit User's Manual

AmpF/STR Minifiler™ PCR Amplification Kit User's Manual

PowerPlex®Y Amplification Kit User's Manual

GeneMapper™ ID Software version 3.1 User Manual.

GeneMapper™ ID Software Tutorial.

Method: **Importing Panel and Bin Definitions into GeneMapper™ ID Software**

Date: 06/01/2006

Revision Date: 02/06/2009

Approved by: SAM

INTRODUCTION:

The GeneMapper™ ID software will genotype samples based on allelic bin definitions stored within the Panel Manager. Custom SDPD Analysis Methods have also been created in the GeneMapper Manager for use in analyzing raw data files for Identifier, Minifiler, and Yfiler amplifications, respectively. An analysis method defines the analysis parameters to be used for the analysis of samples imported into the Project Window. When the GeneMapper™ ID software is installed on any computer, the Panel, Bin and Analysis Method have to be defined prior to the analysis of raw data files. Follow the procedure below to import panel and bin definitions.

MATERIALS:

Applied Biosystems GeneMapper™ ID Software

PROCEDURE:

1. Open the GeneMapper™ ID software by clicking on the icon located on the computer's desktop and log-in. Using the drop-down menu select **SDPD** as the User Name. The password for the GeneMapper™ ID software on all instruments and computers is "**sdpdfb**". The password is case sensitive. Upon successful log-in the **Project Window** will open. The project window is the primary window for the user interface.
2. Open the Panel Manager by selecting **Tools>Panel Manager**.
3. In the navigation pane select **Panel Manager**. From the **File** menu select **Import Panels**. This will open the Import Panels dialog box.
4. In the Import Panels dialog box select **AmpFISTR_Panels_v2.txt** for Identifier analysis, **AmpFISTR_Yfiler_Panel_v2.txt** for Yfiler analysis, or **AmpFISTR_Minifiler_GS500_Panels_v1.txt** for Minifiler analysis and click **Import**.
5. In the navigation pane select the **AmpFISTR_Panels_v2** folder for Identifier analysis, **AmpFISTR_Yfiler_Panel_v2** folder for Yfiler analysis, or **AmpFISTR_Minifiler_GS500_Panels_v1.txt** for Minifiler analysis. From the **File** menu select **Import Bin Set**. This will open the Import Bin Set dialog box.
6. In the Import Bin Set dialog box select **AmpFISTR_Bins_v2.txt** for Identifier analysis, **AmpFISTR_Yfiler_Bin_v2.txt** for Yfiler analysis, or

AmpFISTR_Minifiler_GS500_Bins_v1.txt for Minifiler analysis and click **Import**.
To complete this process click **OK** or **Apply**.

Note: The panel and bin definitions can be viewed while in the Panel Manager by selecting the desired panel (e.g. Identifiler_v1) in the navigation pane and expanding it so that the individual loci can be viewed. Once a locus is selected, the bin definitions are visible in the right pane.

REFERENCES:

AmpF/STR Identifiler™ PCR Amplification Kit User's Manual

AmpF/STR Yfiler™ PCR Amplification Kit User's Manual

AmpF/STR Minifiler™ PCR Amplification Kit User's Manual

GeneMapper™ ID Software version 3.1 User Manual.

GeneMapper™ ID Software Tutorial.

Method: **GeneMapper® ID-X Analysis of Data**

Date: 06/01/2006

Revision Date: 08/14/2009

Approved by: SAM

INTRODUCTION:

GeneMapper™ ID-X software is an automated genotyping software package that builds on the capabilities of the GeneMapper ID 3.2 including several additional features and enhancements. The software will genotype samples based on allelic bin definitions stored within the Panel Manager. The AmpFISTR panels and bins are pre-installed in the Panel Manager within the software. Custom SDPD Analysis Methods have also been created in the GeneMapper Manager for use in analyzing raw data files for Identifiler, Yfiler, and Minifiler amplifications, respectively. An analysis method defines the analysis parameters to be used for the analysis of samples imported into the Project Window.

MATERIALS:

Applied Biosystems GeneMapper™ ID-X Software

PROCEDURE:

1. Open the GeneMapper™ ID-X software by clicking on the icon located on the computer's desktop and log-in. Using the drop-down menu select **your personal login** (or type it manually if it is your first time logging on to a particular instrument) as the User Name. The password for the GeneMapper™ ID-X will be user specific. The password is case sensitive. Upon successful log-in the **Project Window** will open. The project window is the primary window for the user interface.
2. To add samples to the Project Window. Select **File>Add Samples to Project** (or use the icon).
 - Navigate to Forensic Biology on FB server H:\3130-310 Run Folders to locate the desired run folder.
 - Select the desired run folder and click **Add To List**. The selected folder will appear in the **Samples To Add** box on the right. Note: Multiple run folders can be added to the same project. Click **Add** to import the files into the project and close the dialog box.
3. For each sample within the **Project Window** select the appropriate **Sample Type, Analysis Method, Panel, Size Standard, and Matrix** (if applicable). Unknowns should be designated Sample, Allelic Ladders should be designated Allelic Ladder, the positive PCR control should be designated as Positive Control, and any reagent blanks, amplification blanks, or formamide blanks should be designated as Negative Controls.

For Identifiler analyses, the analysis method will be **ID-X Identifiler Analysis**

Method, the panel will be **Identifiler_v1X**, and the size standard will be **CE-G5_HID_GS500**.

For Minifiler analyses, the analysis method will be **ID-X Minifiler Analysis Method**, the panel will be **Minifiler_GS500_v1X**, and the size standard will be **CE-G5_HID_GS500**.

For Yfiler analyses, the analysis method will be **ID-X Yfiler Analysis Method**, the panel will be **Yfiler_v1X**, and the size standard will be **CE-G5_HID_GS500**.

Samples run on the 310s will require a Matrix file, samples run on the 3130 will not require a Matrix file as the Spectral is applied to the sample in real time on the 3130. The analysis parameters for the **ID-X Identifiler Analysis Method** are as follows:

Analysis Method Editor - HID

General | Allele | Peak Detector | Peak Quality | Quality Flags

Peak Detection Algorithm: Advanced

Ranges

Analysis: Partial Range | Sizing: Partial Sizes

Start Pt: 3550 | Start Size: 75

Stop Pt: 7250 | Stop Size: 450

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

Factory Defaults

OK Cancel

Analysis Method Editor

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

Bin Set: AmpFLSTR_Bins_v1X

☒ Use marker-specific stutter ratio and distance if available

Marker Repeat Type	Tri	Tetra	Penta	Hexa
Global Cut-off Value	0.0	0.0	0.0	0.0
MinusA Ratio	0.0	0.0	0.0	0.0
MinusA Distance	From: 0.0	0.0	0.0	0.0
	To: 0.0	0.0	0.0	0.0
Global Minus Stutter Ratio	0.0	0.0	0.0	0.0
Global Minus Stutter Distance	From: 0.0	3.25	0.0	0.0
	To: 0.0	4.75	0.0	0.0
Global Plus Stutter Ratio	0.0	0.0	0.0	0.0
Global Plus Stutter Distance	From: 0.0	0.0	0.0	0.0
	To: 0.0	0.0	0.0	0.0
Amelogenin Cutoff	0.0			

Range Filter ... Factory Defaults

Save As Save Cancel Help

Note: In general, these analysis parameters will not change with the exception of the analysis range which will be dependent on the electrophoresis of each run. If peak amplitude thresholds greater than 75rfu are required for analysis of specific samples, a separate Analysis Method should be used.

The SDPD Y-STR Analysis method, and the parameters for any method, can be viewed from the **Analysis Method** tab in the **GeneMapper Manager** window.

4. Click ► (Analyze), and the Save project dialog box opens. Save the project with a uniquely identifying name [e.g. initials(Case #)]. After analysis, the project is automatically saved, and the Genotypes and Analysis Summary Tabs becomes available. Analyzing the data sizes and genotypes the samples in the project. There must be at least one ladder in each run folder analyzed for the analysis to be successful.

5. Accurate application of the size standard to the samples can be verified in a variety

of ways. Using the Process Component Quality Values (PQVs) such as the **Sizing Quality (SQ)** flag is an efficient method of verifying the sizing of individual samples. In the Samples tab of the Project Window the sizing quality of each sample is displayed as a symbol (■ = Pass, ▲ = Check sizing for problems, or ● = Low Quality, sizing failed). Alternatively, individual or multiple samples can be selected in the project window and the **Size Match Editor** used to view the size standard for each sample. It is possible to overwrite the Sizing Quality PQV in this window for individual samples. This could be done, for example, if the 450bp size standard peak was not detected for a particular sample, but it was determined that the absence of this standard would not affect the sizing of any peaks within the sample.

6. The migration of the samples within the run can also be verified in a number of ways. For Identifiler, Minifiler, and Yfiler, one method is to select all the samples within the **Project Window** and then select **Display Plots**. From the plot settings drop-down menu, select **Check LIZ**.
7. The individual sample files can be reviewed and printed for the analytical record. If needed, labels can be edited within the samples. To edit labels, select the labeled peak **to be edited**. With the desired **peak highlighted**, right-click and select the applicable option of changing or deleting the label. An Edit Allele Comment dialog box will open and a reason for the change can be input.
8. PQVs can be used to assess the quality of a variety of aspects of each sample. Below is a list of some of the PQVs that can be used to assist in the analysis of the sample data:
 - SOS = Sample Off-Scale. If off-scale data (>7000rfu) is present in the sample, ▲ is automatically displayed within the size standard region of the signal. Otherwise a ■ is displayed
 - SQ = Sizing Quality. Evaluates the similarity between the fragment pattern for the size standard dye specified in the size standard definition and the actual distribution of size standard peaks in the sample, calculates an interim SQ.
 - SE = Sample Edit. This box will be checked when the marker allele calls have been edited.
 - MIX= Mixed Sample. Indicates a potential mixed-source sample. A sample is considered a potential mixture if it meets either of the following conditions:
Condition 1: Two or more markers contain three or more called alleles **or**
Condition 2: One or more markers contain three or more called alleles **and** another two or more markers have yellow PHR values
 - AN = Allele Number. Indicates if the software detects within a marker size range: No alleles **or** more than the Max Expected Alleles **or** no X allele in Amelogenin.
 - Bin= Out of Bin Allele. ▲ is automatically displayed when the apex of a called peak is outside the boundary of a bin.

PHR = Peak Height Ratio. Indicates if the peak height ratio between the lowest and highest peaks within a marker size range is less than the Min Peak Height Ratio. Note: Currently set to 0.6 in the ID-X Identifier Analysis Method.

LPH = Low Peak Height. ▲ is automatically displayed when alleles are lower than the specified values and do not result in the proper intensity. Note: Currently set to 200rfu for homozygotes and 75rfu for heterozygotes in the SDPD Analysis Method.

SPU = Spectral Pull-up. ▲ is automatically displayed when any locus displays pull-up. Pull-up is defined by the system to be when the height of a labeled peak is less than 5% of any larger peak within +/- 1 data point.

BP = Broad Peak. ▲ is automatically displayed when the peak width of a labeled peak is wider than 1.5bp.

SP = Split Peak. ▲ is automatically displayed when the GeneMapper™ ID software detects a split peak.

CC = Control Concordance. Indicates if a positive control, custom control, or negative control produces the expected profile.

GQ = Genotype Quality. Marker genotype quality indicator. Determined differently for samples and allelic ladders. The GQ is used to determine the CGQ.

Samples: Considers the individual marker GQ (Genotype Quality) results. See GQ Calculation, GQ Weighting, and GQ

Ladders: Considers the allelic ladder quality assessment (below):

Rule	Description
1	All ladder alleles (specified in the panel used to analyze) are detected.
2	In each marker, the peak height ratio between the first and second peak is greater than 50%. This rule eliminates allelic ladders if the stutter peak before the first true allele peak is labeled as an allele.
3	No spikes are detected above 20% (default) of the highest allele peak in the same dye color. Note: Spike detection for Allelic Ladders is performed within each extended marker range (no gaps are present between markers; the end point of each marker is extended past the marker definition in the panel to the beginning of the next marker). Note: The Allelic Ladder Spike Cut-off value is user-definable.
4	The peak height ratio between the lowest and

highest peak is equal to or greater than 15%.

- 5 In each marker, the base pair spacing between any two ladder alleles (specified in the panel used to analyze) is within the expected range.
- 6 No off-scale (OS) fluorescent signal is detected within each extended marker range.
Note: The Allelic Ladder GQ Weighting for Off-scale is user definable.

CGQ =Composite Genotype Quality. Indicates overall genotype quality. Determined differently for samples and allelic ladders. For samples it considers the individual marker GQ (Genotype Quality) results. For ladders considers the allelic ladder quality. Other than SPK and OS, does not consider individual marker GQ (Genotype Quality) values.

Note: Other PQVs exist and definitions and explanations for them can be found within the GeneMapper ID-X Software Help.

9. To view old exported GeneMapperID 3.2.1 projects. Open **GeneMapper Manager** and from the **Projects** tab click **Import**. Browse to **Forensic Biology on FB server (H:) > GeneMapper ID Projects** and select the desired project. Click **OK**. The project will have been imported to the GeneMapperID database and can be opened as usual. Allele edits cannot be done on old projects.

GeneMapper ID-X contains several other features that can assist the analyst in reviewing and interpreting the data. GeneMapper ID-X has a profile comparison tool and a Mixture Analysis tool that can both be found under the **Tools** menu.

The Profile Comparison tool does the following for all samples in a project:

- Groups samples with 100% concordant profiles
- Compares samples in the project against all other samples in the project
- Compares samples in the project against lab reference, custom control, and QC sample profiles stored in the Profile Manager.

The mixture analysis tool contains specific functionality, including two-person mixture deconvolution, to assist in the analysis, interpretation and statistical analysis of DNA mixtures.

REFERENCES:

AmpF/STR Identifier™ PCR Amplification Kit User's Manual

AmpF/STR Yfiler™ PCR Amplification Kit User's Manual

AmpF/STR Minifiler™ PCR Amplification Kit User's Manual

GeneMapper™ ID-X Software Manuals.

Archived Version

Method: **General STR Interpretation Guidelines**

Date: 06/01/2004

Revision Date: 10/04/2010

Approved by: SAM

INTRODUCTION:

When performing short tandem repeat (STR) analysis using the AmpF/STR Identifiler™ Amplification kit, the AmpF/STR Yfiler Amplification kit, or the AmpF/STR Minifiler Amplification kit in conjunction with the ABI 310 or 3130 Genetic Analyzers in casework, the interpretation of results is a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-set rule. However, it is important that a laboratory employ defined criteria for the interpretation of data. Here we present a number of general interpretation guidelines that our laboratory has adopted for using these systems in forensic DNA casework. They are based on manufacturer's recommendations, available literature, and our own validation studies. It is anticipated that these guidelines will continue to evolve as the collective experience of the laboratory grows.

The rationale behind these guidelines is to establish a set of standards to ensure:

- That conclusions are supported by reliable data, and derived using a scientific approach, and;
- That interpretation of results is as objective as possible, and is consistent from one analyst to another.

The evaluation of inclusions or exclusions of questioned individuals should take into account all the loci where DNA types were detected, the nature of the sample, the condition of the profile (inhibited/degraded), and should be scientifically justifiable and based on the collected experience and knowledge of the laboratory. These guidelines are designed to provide a general foundation for interpretation and are not a substitute for an analyst's judgment and reasoning in a particular case. As with all guidelines, they may not anticipate all crime scene scenarios and DNA typing results seen in forensic casework.

EVALUATION OF DATA:

The goal of the DNA analysis is to generate accurate and representative STR profiles (or Y-STR haplotypes), for extracted evidence and reference samples, for comparison purposes. General guidelines and criteria for evaluating STR data generated from the ABI 310 or 3130 Genetic Analyzers are presented below, and artifacts commonly encountered in the course of such analysis are discussed:

General

- A peak is defined as a distinct, icicle-shaped elevation on an electropherogram.

- Allele assignments are made by calculating the base pair size of the peaks in the amplified samples and comparing them with the base pair size of the alleles within the allelic ladder(s).
- Raw data peaks reaching 8191rfu on 310 instruments saturate the CCD camera and are considered “off-scale”. On 3130 instruments the camera will become saturated by peaks with high rfu levels, although there is no defined maximum rfu value. These peaks result from over-amplification and can cause several interpretational difficulties:
 - Due to their broad bases, the sizing of off-scale allele peaks may result in GeneMapper ID assigning them as off-ladder (i.e., “OL”).
 - Off-scale peaks may be “split peaks” due to prominent “minus-A” products (see below).
 - The “stutter” peak height relative to an off-scale peak height will appear artificially enlarged (see below).
 - Off-scale peaks can result in significant “pull-up” (see below).
 - Depending upon the severity of an off-scale data-associated problem, it may be necessary to re-amplify using less input DNA, dilute amplified material for re-injection, or decrease the injection time.
- The analysis of at least one of the injected ladders must produce the known allele peaks associated with the ladder in order for the GeneMapper ID software to type the other samples of the run.
- Analysis of the positive amplification control (9947A or 007 DNA depending on the testing kit) must consistently generate the known STR profile. If the correct profile is not obtained, the results for all samples amplified with that control are considered inconclusive. All samples associated with the failed positive control will be re-amplified, or re-extracted, if possible. Samples associated with the failed positive control for which no extracted DNA, or original evidence, remains will be deemed inconclusive. Please refer to the “Results Interpretation Policy” in the Unit Policy Manual for additional information regarding failed positive amplification controls.
- If allele peaks are detected in the “No DNA” negative amplification control, in the relevant reagent blank, or in a formamide blank, this is an indication of a contamination event. Based on an evaluation of the contamination the test may be deemed inconclusive and require some or all of the analysis to be repeated for any conclusions to be drawn. Not all contamination events will necessitate reanalysis and will be judged on a case-by-case basis.
- If the authenticity of a detected peak is of concern, the sample may be re-injected to confirm the nature of the detected peak.

Known Artifacts

Stutter

- Stutter peaks are a common reproducible artifact of the PCR DNA amplification process and are believed to result from core repeat “slippage” during amplification. For STR loci these stutter peaks are most commonly at one repeat shorter than the full-length allele peak. For tetranucleotide repeats this will be at n-4bp. The amount of stutter varies from locus to locus but has been evaluated as part of SDPD validations of the amplification kits used. Stutter values for every marker and most alleles have been established and are available in the validation record for each of the testing kits employed.
- Peaks in stutter positions that are larger than the stutter cutoff value for a specific locus should be interpreted with caution, and may result from the following:
 - An off-scale allele peak (see above).
 - DNA from a secondary source.
 - Stutter peaks overlapping an area of elevated baseline may exhibit an apparent increased percentage stutter. Elevated baselines may be indicative of an unsuitable matrix (310) or spectral (3130) (see below).
 - Amplification that produces a larger stutter peak than is usually expected. A new amplification of the sample may reduce the size of the stutter peak.
 - Stutter percentages generally increase for larger alleles within a given locus. Larger alleles may produce stutter percentages greater than the cutoff value.
- Other “stutter-like” peaks have been observed. See the individual kit interpretation guidelines for additional details.

Minus-A

- One property of AmpliTaq Gold™ DNA polymerase is the non-template addition of adenosine (A) residues to the 3' ends of double-stranded DNA. The PCR amplification programs include a post-cycle incubation to allow for this A addition to the amplified products. Incomplete A additions may result in “split peaks” due to “minus-A” products one nucleotide shorter than the full-length “plus-A” products. As mentioned above, over-amplification can result in prominent “minus-A” products and “split peaks”. These may be eliminated either by incubating amplified products at 60°C for an additional time period, or alternatively, re-amplifying using less input DNA.

Spikes

- “Spikes” are artifacts of electrophoresis, which may resemble DNA peaks. The exact nature of spikes is unclear. They are thought to result from electrical surges and/or solid residues derived from either formamide or polymer. Multi-color spikes can be identified by the presence of peaks occurring in several colors at the same data point location. Single-color spikes can be identified by the presence of a sharp peak occurring in a single color in the raw data, with no spectral overlap into the other colors. Since spikes are generally random, reinjection of a sample should eliminate a questioned spike.

Pull-up Peaks

- Artfactual peaks can result from a phenomenon known as “pull-up”. This is where bone fide allele peaks in one color dye result in identically sized peaks in one or more of the other color dyes, due to spectral overlap. Pull-up is normally avoided by applying a suitable matrix to 310 data during GeneMapper ID analysis. It may be necessary to create and apply a new matrix or spectral in order to achieve optimal GeneMapper ID analysis and this should be done as needed. Pull-up that is associated with off-scale data may not be eliminated by applying a new matrix. 3130 sample runs apply the spectral as the data is collected, therefore samples will need to be re-run if a new spectral is required.

Degradation and Inhibition

- Inhibition of the PCR process can be caused by a variety of substances and is generally described as a reduction in efficiency of the PCR process. The effects of inhibition will vary based on the inhibitor itself as well as the amount present in a sample. Inhibition can affect different loci within the typing kits used at the SDPD and examples of the effects of inhibition can be observed in the SDPD Inhibition presentation on the FB network in the presentations folder.
- DNA is generally stable if stored properly, however, degradation of DNA can occur over time, through exposure to the elements, or through the action of bacteria. Degradation can be identified by a classic pattern in samples where rather robust results are obtained at the smaller (base pair) loci while little to no results are obtained at the larger (base pair) loci. The overall results have been described as being in a ski slope pattern.
- In general, inhibition should affect all contributors to a DNA mixture equally and therefore raising the concern of actually detecting all alleles that should be present in the mixture. Degradation, however, may not affect all contributors equally and mixtures containing degraded DNA can pose a challenge to the analyst attempting to interpret them. Analysts are encouraged to exercise caution when interpreting a mixture that is thought to contain, or is known to contain, degraded DNA.

GENERATING COMPOSITE DNA PROFILES

When generating a composite profile from multiple amplifications of the same sample of extracted DNA, or multiple injections of the same sample preparation, analysts must indicate in the table of results that the represented result is a composite of multiple amplifications/or injections (whichever is applicable). When generating a composite DNA profile the analyst must use the highest rfu value of a reproducible peak when determinations of contribution level are done.

MANUAL DESIGNATIONS OF ALLELES

Off ladder alleles that are deemed to be true DNA peaks will be given allele designations by the analyst. Alleles that fall above or below the ladder alleles (even

those in virtual bins) will be designated as greater than (>) or less than (<) the respective ladder allele. Off ladder alleles that occur within the ladder ranges (i.e. microvariants that contain an incomplete repeat motif) should be designated by the number of complete repeats and, separated by a decimal point, the number of base pairs in the incomplete repeat (e.g. FGA 18.2 allele).

CONFIRMING MICROVARIANTS AND TRI-ALLELIC PATTERNS

Microvariants will be confirmed through a re-injection of the sample as a means of demonstrating the reproducibility of the retention time and sizing of the peak. If a microvariant is observed in multiple samples from the same case, re-injection for confirmation purposes is unnecessary.

Tri-allelic patterns (or bi-allelic patterns in Y-STR profiles in any locus other than DYS385) will be confirmed through re-amplification. The re-amplification of the sample serves to demonstrate that the tri-allelic pattern is not due to some amplification related phenomenon. If the same tri-allelic pattern in multiple samples, re-amplification to confirm the pattern is unnecessary.

UNINTERPRETABLE SAMPLES

In general, any mixed DNA samples that an analyst determines have a significant amount of missing information (e.g. a mixture that appears to be of two or more individuals where only a few DNA types rise above the detection threshold) and/or where a majority of DNA peaks are less than 200rfu may be considered uninterpretable. In these instances, a statement will be added to the report that due to the limited information obtained for the sample no comparisons will be made.

SECOND ANALYST REVIEW:

In casework, all GeneMapper ID analysis data will be reviewed by a second, qualified DNA analyst.

REFERENCES:

AmpF/STR Identifier™ PCR Amplification Kit User's Manual
AmpF/STR Identifier™ Validation Studies
AmpF/STR Yfiler™ PCR Amplification Kit User's Manual
AmpF/STR Yfiler™ PCR Validation Studies
AmpF/STR Minifiler™ PCR Amplification Kit User's Manual
AmpF/STR Minifiler™ PCR Validation Studies

Method: **AmpF/STR Identifiler Interpretation Thresholds**

Date: 06/01/2004

Revision Date: 08/14/2009

Approved by: SAM

- A peak detection threshold of 75rfu will be used for analysis of AmpF/STR Identifiler data. Peaks below 75rfu are not reported.
- Homozygous allele peaks are often approximately twice the height of heterozygous allele peaks as a direct result of allele dosage.
- At heterozygous loci, the ratio of the height of the shorter peak to the height of the higher peak is generally above 60% (see Peak Height Ratio validation study). Occasionally, however, heterozygous allele peak pairs may be outside this range.
- n-4bp stutter peaks will be filtered by the software when they are below the percent cutoff value for a locus. The stutter cutoff values used by the software are presented below:

Marker	Stutter Cut-off Value
D8S1179	8.2%
D21S11	9.4%
D7S820	8.2%
CSF1PO	9.2%
D3S1358	10.7%
TH01	5.1%
D13S317	8.0%
D16S539	10.4%
D2S1338	11.1%
D19S433	13.3%
vWA	12.6%
TPOX	4.8%
D18S51	17.0%
D5S818	6.8%
FGA	14.7%

- The homozygote peak height threshold is the level of detection of a DNA peak where an analysts may have confidence that the detected allele is a true homozygote and does not have an undetected peak that it is paired with. Our validation efforts determined that both alleles of a heterozygote pair will be detected when one of the alleles reaches at least 200rfu (>99% of the time). When evaluating an apparent single source evidence profile, care should be taken when interpreting a single DNA peak below 200rfu as allelic drop-out may have occurred.

Mixtures

- A mixed DNA profile will have more than two alleles present at a locus, and/or a significant imbalance in peak height between peaks at a locus. Generally, evidence of a mixed DNA sample exists at multiple loci.
- In most cases involving DNA mixtures in which biological fluids have been identified,

caution is warranted in attributing a particular biological fluid to one of the DNA contributors in the mixture, as either source could be the origin of that body fluid in question (sperm fractions involving male:female mixtures may be an exception). Instead, the presence of the body fluid will be noted in the report and a statement given as to whether the subject is included or excluded as one of the potential sources of the DNA mixture.

- Please refer to the "Interpreting DNA Mixtures" protocol for additional information with regards to mixtures.

Probability of Identity using the Applied Biosystems Identifiler kit.

The Probability of Identity (P_I) [Sensabaugh 1982] is the probability that two individuals selected at random will have an identical Identifiler kit genotype. For the Identifiler kit the P_I are as follows:

1 in 200 quadrillion in the U.S. Caucasian population,
1 in 760 quadrillion in the African American population,
1 in 130 quadrillion in the Hispanic population.

The P_I expresses the average 15 locus DNA profile frequencies using the Applied Biosystems Identifiler kit. This data was taken from the Identifiler user's manual page 4-55.

REFERENCES:

SDPD Identifiler Validation studies

AmpF/STR® Identifiler™ User's Manual

Method: **AmpF/STR Minifiler Interpretation Thresholds**

Date: 07/25/2007

Revision Date: 08/14/2009

Approved by: SAM

- A peak detection threshold of 75rfu will be used for AmpF/STR Minifiler data. Peaks below 75rfu are not reported.
- Homozygous allele peaks are often approximately twice the height of heterozygous allele peaks as a direct result of allele dosage.
- At heterozygous loci, the ratio of the height of the shorter peak to the height of the higher peak is generally above 78% (see Peak Height Ratio validation study). Occasionally, however, heterozygous allele peak pairs have been observed with an imbalance as low as 35%.
- n-4bp stutter peaks will be filtered by the software when they are below the percent cutoff value for a locus. The stutter cutoff values used by the software are presented below:

Marker	Stutter Cut-off Value
D13S317	14.0%
D7S820	11.0%
D2S1338	18.0%
D21S11	16.0%
D16S539	15.0%
D18S51	18.0%
CSF1PO	14.0%
FGA	15.0%

Apparent Single Source Samples

- An apparent single source profile consists of no more than two relatively balanced alleles at each locus. (Single source samples with three allele peaks at a single locus have been reported, though are extremely rare.)
- Allelic dropout is a phenomenon observed commonly when amplifying low levels of DNA, and is defined as having only one allele of a heterozygote pair (or possibly no alleles) detected above the peak detection threshold at a given locus. Our validation efforts determined that both alleles of a heterozygote pair will be detected when one of the alleles reaches at least 450rfu (>99% of the time, however allelic drop-out was observed when the larger peak was approximately 680rfu). When evaluating an apparent single source evidence profile, care should be taken when interpreting a single DNA peak below 450rfu as allelic drop-out may have occurred. As a conservative measure of interpreting inclusions, when single allele peaks are detected below 450rfu at any locus, that locus (those loci) will be excluded from the calculation. In an apparent single source sample, when two alleles are present at a locus below 450rfu, those alleles may be used in a statistical calculation, as allelic drop-out is not a concern.

Mixtures

- A mixed DNA profile will have more than two alleles present at a locus, and/or a significant imbalance in peak height between peaks at a locus. Generally, evidence of a mixed DNA sample exists at multiple loci.
- In most cases involving DNA mixtures in which biological fluids have been identified, caution is warranted in attributing a particular biological fluid to one of the DNA contributors in the mixture, as either source could be the origin of that body fluid in question (sperm fractions involving male:female mixtures may be an exception). Instead, the presence of the body fluid will be noted in the report and a statement given as to whether the subject is included or excluded as one of the potential sources of the DNA mixture.
- Please refer to the "Interpreting DNA Mixtures" protocol for additional information with regards to mixtures. In general, due to the increased imbalance observed in single source samples with the Minifiler kit, analysts should use a more conservative approach when interpreting mixed DNA samples generated with the Minifiler amplification kit.

Probability of Identity using the Applied Biosystems Minifiler kit.

The Probability of Identity (P_I) [Sensabaugh 1982] is the probability that two individuals selected at random will have an identical Identifiler kit genotype. For the Identifiler kit the P_I are as follows:

1 in 12 billion in the U.S. Caucasian population,
1 in 15 billion in the African American population,
1 in 4.8 billion in the Hispanic population.

The P_I expresses the average 8 locus DNA profile frequencies using the Applied Biosystems Minifiler kit. This data was taken from the Identifiler user's manual page 5-46.

REFERENCES:

SDPD Minifiler Validation studies

AmpF/STR® Minifiler™ User's Manual

Method: **AmpF/STR Yfiler Interpretation Guidelines**

Date: 07/25/2007

Approved by: SAM

- A peak detection threshold of 100rfu will be used for AmpF/STR Yfiler amplification kit. Peaks below 100rfu are not reported.
- n+3bp stutter products have been observed in the DYS392 locus and n-2/n+2bp stutter at DYS19 in the AmpF/STR Yfiler system. The GeneMapperID software product does not currently have the capacity to filter such stutter products, but data for these artifacts is contained within the validation studies. The stutter cutoff values used by the software are presented below:

Marker	Stutter Cut-off Value
DYS456	13.21%
DYS389I	11.79%
DYS390	10.4%
DYS389II	13.85%
DYS458	12.2%
DYS19	11.4%
DYS385	13.9%
DYS393	12.58%
DYS391	11.62%
DYS439	11.18%
DYS635	10.75%
DYS392 (note n-3 stutter position)	16.22%
Y GATA H4	11.08%
DYS437	8.59%
DYS438 (note n-5 stutter position)	4.28%
DYS448 (note n-6 stutter position)	4.96%

Apparent single source profiles

- An apparent single source Y-STR DNA profile (or haplotype) consists usually of one allele at each Y-STR locus, except for DYS385 where gene duplication results in two alleles. (Note: single source samples with additional allele peaks have been reported.)
- In a Y-STR DNA profile that consists of peaks heights 100rfu or above, the results at all loci will be used to determine the frequency of that haplotype in a given database (see Y-STR Haplotype Frequency Calculations).

Mixed DNA profiles

- Mixed Y-STR DNA profiles are apparent when more than one allele is present at some or all of the loci (in a male DNA mixture, more alleles may be expected at DYS385 than at other loci). Generally, evidence of a mixed DNA sample exists at multiple loci.
- If a significant difference in allele peak height exists in a male DNA mixture, a predominant Y-STR DNA profile may be deduced. Under these circumstances, allele peaks from secondary donor(s) may be reported as “weak” alleles. Weak is to be used when the height difference is less than 1/3. The frequency of the predominant haplotype in a given database may be determined using the rules for apparent single source profiles, above.

REFERENCES:

SDPD Yfiler Validation studies

AmpF/STR® Yfiler™ User's Manual

Method: **Autosomal STR Profile Frequency Calculations**

Date: 07/25/2007

Revision Date: 10/04/2010

Approved by: SAM

DNA profile frequency calculations, unless otherwise specified, will be calculated based on the DNA profile of the evidence using the most recent version of Popstats software available. DNA profile frequency calculations will be performed using population data taken from the Journal of Forensic Sciences 1999, 44(6), 1277-1286 and, for the D2S1338 and D19S433 loci, from Forensic Science Communications 2001, 3(3). When applicable other population databases may be used for DNA profile frequency estimates. The use of any additional population databases will be documented in the case notes.

Apparent single source profiles

An apparent single source profile consists of no more than two relatively balanced alleles at each locus. (Single source samples with three allele peaks at a single locus have been reported, though are extremely rare.)

The DNA profile frequency estimates for single source DNA profiles will be calculated using National Research Council Recommendation 4.1 (The Evaluation of Forensic DNA Evidence, National Academy Press 1996) using a theta (θ) value of 0.01.

Single source DNA profile frequency estimates (random match probabilities) will be calculated as follows:

- Homozygote frequencies are calculated using the National Research Council (NRCII) Recommendation 4.1 formula: $f(A_1A_1) = p^2 + p(1 - p)\theta$. In general, a theta (θ) value of 0.01 will be used for DNA profile frequency estimates, except in cases involving small, isolated subpopulations (e.g., certain Native American populations) when it will be 0.03.

Single allele peaks detected below 200rfu at any locus are considered in the stochastic range where allelic drop-out is a concern. As a conservative measure for interpreting matches when single allele peaks are detected at less than 200rfu, the alleles at that locus (those loci) can be included in the random match probability estimate using the $(2p - p^2)$ calculation for the genotype frequency. Alternatively, the loci with single alleles less than 200rfu can be excluded from the calculation.

- Heterozygote frequencies are calculated using the Hardy-Weinberg formula: $f(A_1A_2) = 2pq$ (where p and q are the population allele frequencies of A_1 and A_2 , respectively).

In an apparent single source sample, when two alleles are present at a locus below 200rfu, the locus may be used in a statistical calculation as allelic drop-out is not a

concern.

- In the event a rare allele is encountered in a DNA profile a minimum allele frequency of $5/2n$ (where n is the number of individuals in the database) will be used in the genotype frequency calculation as a conservative measure against underestimating the frequency of the rare allele in the population.
- All DNA profile frequencies will be truncated to two significant figures.

Mixed DNA profiles

- In mixtures, the significance of an inclusion can be expressed using the statistical approaches of Random-Match Probability, Combined Probability of Inclusion, and/or Likelihood Ratios. The circumstances of the case may suggest which is more appropriate.
- If in a DNA mixture from apparently two sources, one of those sources is known, then a statistical approach to the mixture that theoretically subtracts the known individual's contribution to the mixture can be used (e.g. in a sexual assault case, the victim is a possible contributor on a DNA mixture from her vaginal swab).
- Please refer to the "Interpreting DNA Mixtures" protocol for additional information with regards to mixtures.

PARENTAGE AND KINSHIP CALCULATIONS:

In cases involving disputed parentage or kinship, a parentage/kinship calculation will be provided using the formulae integral in the Popstats software.

Parentage

When the parent of a child is disputed, and the other parent is known, a Parentage Index (PI) will be calculated. This is the Likelihood Ratio of the probability of the genetic evidence given that the alleged parent is the biological parent of the child versus the probability of the genetic evidence given that the alleged parent is not the biological parent of the child.

Reverse Parentage

When the child of two parents is disputed, a Reverse Parentage Index (RPI) will be calculated. This is the Likelihood Ratio of the probability of the genetic evidence given that the alleged child is the biological child of the parents versus the probability of the genetic evidence given that the alleged child is not the biological child of the parents.

Kinship

When the biological relationship, or kinship, of two individuals is disputed, a Kinship Index (KI) will be calculated. This is the Likelihood Ratio of the probability that the two individuals' genotypes are associated by kinship, versus the probability that the two individuals' genotypes are associated by chance.

REFERENCES:

1. AmpF/STR Identifiler™ PCR Amplification Kit User's Manual
2. AmpF/STR Minifiler™ PCR Amplification Kit User's Manual
3. The Evaluation of Forensic DNA Evidence. National Research Council (U.S.) National Academy Press, Washington, D.C., 1996 (NRCII)
4. Budowle, B., Moretti, T.R., Baumstark, A.L., Defenbaugh, D.A., and Keys, K.M. (1999). Population Data on the Thirteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. *Journal of Forensic Sciences* 44, 1277-1286.
5. Budowle B *et al.* Source Attribution of a Forensic DNA Profile. *Forensic Science Communication* July 2000 Vol 2(3).

Method: **Y-STR Haplotype Frequencies Calculations**

Date: 07/25/2007

Approved by: SAM

Y-STR loci are linked on the Y-chromosome and do not undergo linkage disequilibrium. Therefore neither Recommendation 4.1 (NRC 1996) nor the Product Rule can be applied to Y-STR haplotypes. The significance of a haplotype match in casework must be expressed in terms of the observed frequency of the particular haplotype in the population (the counting method). Numerous Y-STR haplotype databases are available on-line.

Apparent single source profiles

- For the purposes of expressing the significance of a Y-STR haplotype match all applicable alleles will be searched in the database.

Mixed DNA profiles

- If a significant difference in allele peak height exists in a male DNA mixture, a predominant Y-STR DNA haplotype may be deduced. If a predominant haplotype is deduced, all applicable alleles from the predominant haplotype will be searched in the database. For the purposes of expressing the significance of a Y-STR haplotype match all applicable alleles will be searched in the database.
- If a predominant Y-STR DNA profile cannot be deduced, the frequencies in a given database may be determined for each possible haplotype and the sum of all combinations of possible haplotypes may be reported.

Y-STR haplotype frequencies can be calculated from a number of established databases. Applied Biosystems generated a database of searchable haplotypes incorporating all of the Yfiler loci at:

www.appliedbiosystems.com/yfilerdatabase

The website displays each of the Yfiler loci next to drop down lists and text boxes. Common alleles should be selected from the drop down list. Microvariant alleles and loci with multiple alleles should be inputted into the text boxes. Upon searching the questioned haplotype, the website will indicate how many haplotypes in the database match. The number of matching haplotypes is listed for eight different population groups and for all samples in the database. The number listed on the website for each group in the database is a link that will display the full haplotypes for all matches. This can be helpful when searching partial profiles or a subset of alleles in a mixture. The results page can be printed for the case notes, and the haplotype frequencies can be expressed as the number of matches divided by the number of samples in the database.

Numerous other Y-STR haplotype databases are available on-line and their use is at the discretion of the analyst. The Promega Corporation has a haplotype database at:

www.promega.com/techserv/tools/pplexyl/

Upon visiting this website, launch the PowerPlex®Y Haplotype Database and enter the alleles from the questioned haplotype. Choose the sample population of interest (e.g. Caucasian, Asian, Hispanic, African-American or Native American), or select "All Populations". Upon submitting the questioned haplotype, the website will indicate how many haplotypes in the selected population database match and the percentage of matching samples in the selected population. The results page can be printed for the case notes, and the haplotype frequency can be expressed as the number of matches divided by the number of samples in the database.

Other Y-STR haplotype databases are available at:

www.usystrdatabase.org

www.ystr.org/index_usa_gr.html

www.reliagene.com

REFERENCES:

1. AmpF/STR Yfiler™ PCR Amplification Kit User's Manual
2. SDPD AmpF/STR Yfiler™ validation studies.
3. www.usystrdatabase.org
4. www.ystr.org/index_usa_gr.html.
5. www.reliagene.com.

Method: **Interpretation of DNA Mixtures**

Date: 08/14/2009

Approved by: SAM

IDENTIFICATION OF MIXED DNA PROFILES

A mixed DNA profile possesses certain common attributes that will tend to indicate the presence of more than one contributor to the observed results. The attributes may include:

- Greater than two alleles present at a locus, unattributable to a mutation or a tri-allelic pattern.
- Significant imbalance in peak height between peaks at a locus.
- Generally, evidence of a mixed DNA sample exists at multiple loci.

To determine whether a mixed DNA profile exists, the DNA profile must be evaluated in its entirety. If a DNA mixture exists, a conclusion to that fact should be stated in the Forensic Biology report, and may be stated independently of the conclusion(s) surrounding potential individual contributors.

ESTIMATING THE MINIMUM NUMBER OF DNA CONTRIBUTORS

A conclusion with regard to the minimum number of DNA contributors to a mixture can provide important quantitative information that may help convey the general nature of the DNA typing results from a given sample. As such, conclusions with respect to the minimum number of contributors should be routinely included in a report and should be used as a general statement to introduce the detection of a mixture for a given specimen. Generally, an estimate of the minimum number of contributors is based on the locus that exhibits the greatest number of allelic peaks. As an example, if at most five alleles are detected at one or more loci of a multi-locus profile, the DNA typing results are consistent with have arisen from three or more individuals (although a tri-allelic pattern could be present, but is extremely rare). A recommended statement that conveys this observation is given below:

"The DNA results obtained from the _____ (Item X) indicate a mixture of DNA from at least three people."

While the true number of contributors to a mixture can be established with a high probability and absolute determination cannot be ascertained. This estimation is determined by the analyst and provided in the report to define the fewest number of individuals who must have contributed DNA to a mixture.

DEFINITIONS OF TERMINOLOGY USED FOR DNA CONCLUSIONS AND REPORTING OF MIXTURES

DNA contributor:

An individual is considered a DNA contributor to a mixture when it appears that types consistent with that individual are present at multiple DNA markers and that any types from that individual missing in the mixture can be scientifically explained. If no major or minor contributors can be determined in a mixture then this conclusion will be used for inclusions.

Predominant DNA contributor:

If the overwhelming contribution to the DNA in a mixture appears to originate from a single individual then the term "predominant" will apply. A predominant DNA profile will be one where a single source genotype from the single strongest contributor to the mixture is able to be easily deduced from all of the DNA markers tested. In general, all alleles from predominant DNA contributors will fall above 33.3% of the tallest peak at a marker. Factors that will affect deducing a predominant DNA profile such as the genotype of an assumed additional contributor, or other issues commonly observed in PCR systems (stutter, peak height ratios, drop-out, preferential amplification, etc) should all be considered in the interpretation of a predominant genotype.

Major DNA contributor:

An individual (or individuals) who appears to contribute a significant portion of the DNA to a mixture. A DNA contributor should be contributing more DNA than at least one of the other contributors to the mixture to be considered a major contributor. There can be more than one major DNA contributor to a mixture. Major DNA contributors should show proportional contribution across the DNA mixture.

Minor DNA contributor:

An individual who appears to contribute a small portion of the DNA to a mixture. A DNA contributor should be contributing less DNA than at least one of the other contributors to the mixture to be considered a minor contributor. Minor DNA contributors should show proportional contribution across the DNA mixture. There can be more than one minor DNA contributor to a mixture. In general, minor DNA contributors will possess weaker DNA types across several loci.

Masking and allelic drop-out are more likely with minor DNA contributors. Merely having one or two weaker DNA types in a complex mixture is not enough to define a minor DNA contributor. The validation data indicates that it is common to have weaker DNA alleles in mixtures with relatively equivalent contributors.

Obligate minor alleles:

In general there are two ways to determine alleles from minor contributors. The differentiation is between mixtures that are unbalanced between the contributors and mixtures where there exists some balance between at least two major contributors.

In unbalanced mixtures a DNA type is considered to be from a minor contributor if it is less than $\frac{1}{3}$ the intensity of the tallest peak at a marker. The presence of these minor contributor DNA types can assist in making determinations as to the level of DNA contribution in a mixture.

Validation data has shown in mixtures that have varying contribution levels from the DNA donors that individuals that contribute over 50% of the DNA in a sample will not possess any alleles designated as "minor" in the mixture. In these situations "minor" alleles can be used as an interpretational tool for determining major vs. minor contributors to a mixture.

In mixtures that have relative equal contributions from two or more contributors (balanced mixtures) DNA types $\frac{1}{3}$ the height of the tallest peak lose their interpretational significance and should not be used to determine major vs. minor contributors. In balanced mixtures minor contributor alleles will generally be less than 10% of the total rfu at a locus. 10% is meant as guidelines and phenomenon such as stutter and baseline artifacts may affect the percentage from locus to locus, however, deviations from this should be rare and generally used for more conservative interpretations of mixtures. If the percentage of an allele is such that it is suggestive that it may be from a more significant contributor it should not be considered minor.

Alleles identified as being from minor contributors by either method (less than $\frac{1}{3}$ the tallest peak in unbalanced mixtures, or less than 10% the total rfu at a locus when balance between contributors is indicated) will be denoted in the table of results. Weak (wk) will be used to identify DNA types from minor contributors in unbalanced mixtures. Brackets [] will identify DNA types from minor contributors in mixtures where balance has been indicated.

DETERMINING DNA CONTRIBUTION LEVEL IN A MIXED DNA SAMPLE

When mixtures are unbalanced in relative contributions from the donors, the $<\frac{1}{3}$ the height of the tallest allele at a locus criteria is very useful as a predictor of contribution level and in identifying alleles from minor contributors.

When mixtures approach relative equal contributions from at least two of the DNA donors, the $<\frac{1}{3}$ criteria become less diagnostic in determining specific contribution levels. In instances where balance between contributors exists in a mixture a more

conservative standard for identifying alleles from minor contributors should be employed. 10% the total rfu at a locus will be the general guideline for determining minor contributor alleles in mixed DNA samples that have an indication of balance between at least two contributors.

In general, balance between at least two contributors in a mixed DNA profile can be determined by examining the results for the following criteria:

- One locus with at least 5 alleles greater in height than $\frac{1}{3}$ the tallest peak at that locus (five including the tallest peak) and/or three loci with four alleles within 50% of the tallest allele at those loci (four including the tallest peak).

If no alleles from minor contributors can be identified in the mixed DNA sample then the mixture should be considered unresolvable in terms of determining major and minor DNA contributors.

Other factors or phenomenon such as inhibition or degradation within the sample may lead an analyst to adopt a more conservative interpretation and statistical assessment of any potential inclusions. Analysts should be able to articulate the basis for their interpretation.

STATISTICAL CALCULATIONS USED IN MIXTURE INCLUSIONS

Predominant DNA contributor:

A single source statistic will apply for predominant contributors.

DNA contributor:

All alleles at loci where the individual is included (drop-out is possible) will be used in the calculation. If drop-out is occurring then analysts will use either the "size-exclusion model" (e.g. if a possible minor contributor to the mixture is dropping out at D13, loci that are larger than D13 will not be used in the inclusion calculation. FGA may be an exception to this rule) or the "total rfu model" (i.e. only loci with higher total rfu content than all loci where the individual is thought to be dropping out will be used in the calculation) for determining which loci will be used in the calculation. Analysts will indicate on the electropherogram or the profile frequency calculation pages which method they are employing for determining which loci will be used in the calculation.

Major DNA contributor:

Analysts can provide a statistical calculation for major contributors that excludes alleles that had to come from minor DNA contributors. Alleles $<\frac{1}{3}$ the tallest peak at a locus can be excluded in unbalanced mixtures. In mixtures where balance is indicated between at least two contributors, alleles $<10\%$ the total rfu at a locus can be excluded if there is an indication that they came from minor contributors. As the number of

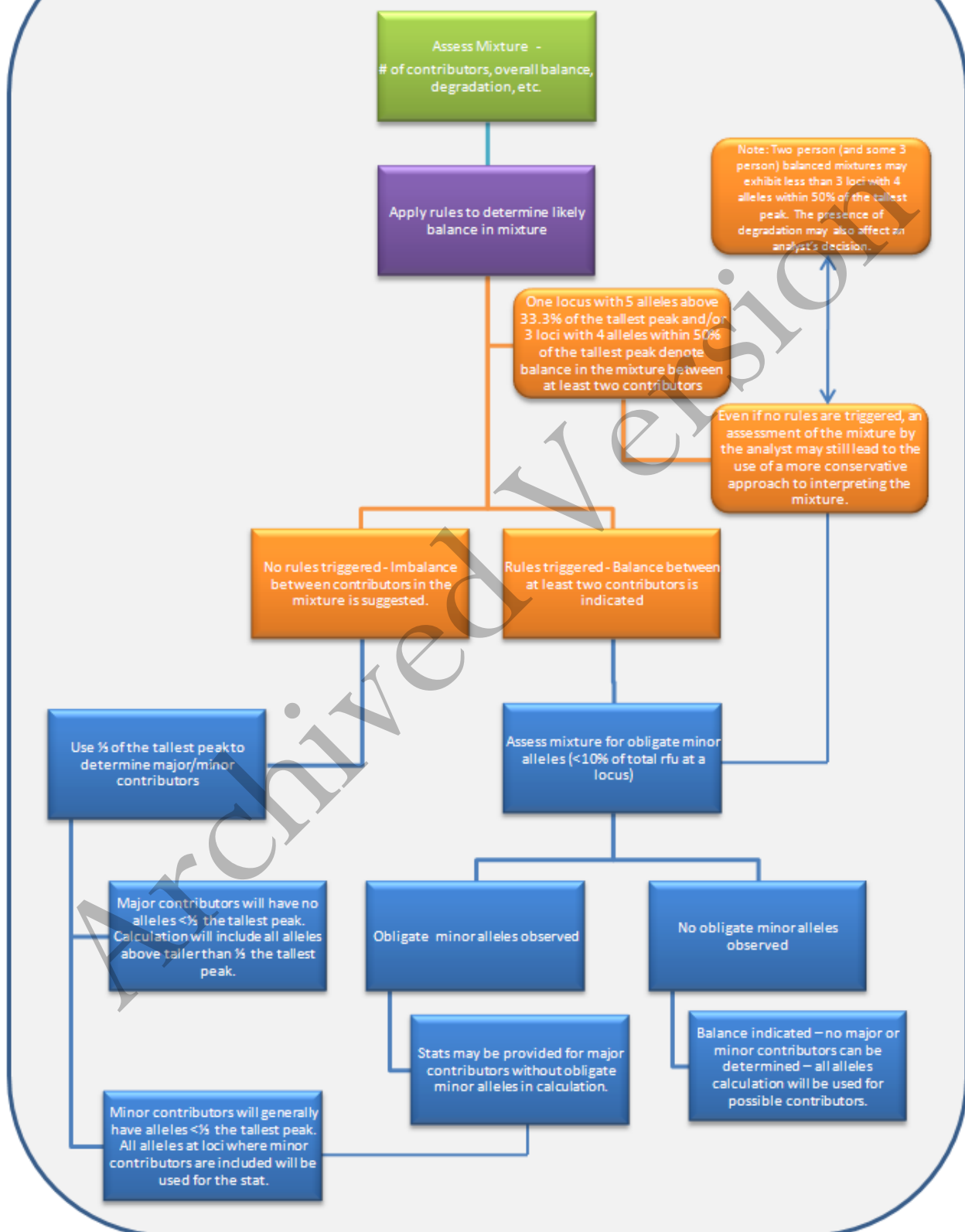
contributors goes up the % cutoff for minor contributor alleles will tend to decrease.

Minor DNA contributor:

All alleles at loci where the individual is included will be used for statistical calculations. If drop-out is occurring then analysts will use either the "size-exclusion model" or the "total rfu" model for determining which loci will be used in the calculation. Analysts will indicate on the electropherogram or the profile frequency calculation pages which method they are employing for determining which loci will be used in the calculation.

Archived Version

SDPD Mixture Interpretation Flow Chart



Method: **Results and Conclusions in Forensic Biology Unit Reports**

Date: 07/25/2007

Approved by: SAM

The following are meant as guidelines for reporting conclusions for DNA testing in Forensic Biology Unit reports. There are many ways to state a conclusion in a DNA report and the suggestions listed below do not cover every possible scenario. Alternative wording may be used to express a conclusion so long as the conclusion is correct, supported by the data, and is appropriate based on the statistical probability limits employed by the Unit.

INCLUSIONS

As a general rule, inclusions on samples that are (or are potentially) probative in nature require the significance of the inclusion to be supported with a probability of inclusion statement.

Single Source or Predominant Autosomal DNA profile

If the profile frequency is rarer than 1 in 300 million:

Conclusions using the words “match” or “very likely the source” may be used. For example:

The (predominant) DNA profile from the evidence matches the DNA profile obtained from John Doe. The approximate probabilities that a person selected at random would possess the same (predominant) DNA profile as the evidence are:

If the profile frequency is more common than 1 in 300 million:

The words “match” and “very likely” are not considered appropriate in these instances.

John Doe is included/could not be excluded as being the source of the DNA from the evidence. The approximate probabilities that a person selected at random would possess the same DNA types as the evidence are:

Mixtures

Two or more person mixture where no assumptions can be made

A mixture of at least two (or however many) people was recovered from the evidence. John Doe is included as being a possible DNA contributor to the mixture from the evidence. The approximate probabilities that a person selected at random would be included as a possible DNA contributor to the mixture are:

Two person mixture with one assumed contributor: (sexual assault type scenario)

A mixture of at least two people was recovered from the evidence. John Doe and Jane Victim are both included as possible DNA contributors to this mixture. Assuming that Jane Victim is a DNA contributor to the mixture and there is only one additional DNA contributor, then John Doe is (included as/very likely) the source of the DNA foreign to Jane Victim. The probabilities that a person selected at random would be included as a contributor to the foreign DNA are:

Paternity

John Doe cannot be excluded as being the biological father of Baby Doe based on the DNA evidence. It is (X/Y) more likely to have these DNA results if John Doe was the true biological father than if a random (ethnicity) man was the father. The probability of John Doe being the biological father is greater than 99.9999%.

Y-STRs - Apparent Single Source or predominant Y-STR Haplotype

Male specific Y-STR testing of the evidence indicated the presence of DNA from a single male or male lineage. John Doe is included as a possible source of the male DNA from this sample. The number of times the Y-STR DNA types from the evidence were observed in the ... Database (website of appropriate database) is:

Y-STR - Mixtures

During male specific Y-STR analysis of the evidence, a mixture of DNA from at least two males was detected. John Doe is included as a possible contributor to the mixture of male DNA from this sample. The approximate number of times the possible combinations of Y-STR haplotypes from this mixture were observed in the ... Database (website of appropriate database) is:

INCLUSIONS ON NON-PROBATIVE SAMPLES

As a general rule, inclusions on samples that have no apparent probative value do not require the significance of the inclusion to be supported with a probability of inclusion statement, as long as the wording chosen for the conclusion is appropriate based on the statistical probability limits employed by the Unit.

Single Source or Predominant Autosomal DNA profile

The words "match" and "very likely the source" may be used for conclusions on non-probative samples if a 15 locus match is obtained given the minimum 15 locus profile frequencies using the Applied Biosystems Identifiler kit (see below). The term "consistent with originating from" is also acceptable but is not subject to statistical

limitations so long as the data supports its use.

If less than a 15 locus match is obtained and an analyst still wishes to use “match” or very likely the source” it may be necessary to have a profile frequency calculation in the analytical record to support the application of those terms, but that does not have to appear in the report.

Given the minimum 8 locus profile frequencies using the Applied Biosystems Minifiler kit, the words “match” and “very likely the source” may **only** be used for conclusions on non-probative samples if their use is supported by a profile frequency calculation in the analytical record, which does not have to appear in the report. (see below).

Mixtures

A mixture of at least two (or however many) people was recovered from the evidence. John Doe is included as being a possible DNA contributor to the mixture from the evidence. There is at least one other unknown DNA contributor to the mixture.

In instances of unknown DNA being present it may be necessary to state that:

DNA from the evidence will be searched against the databases of the Combined DNA Index System (CODIS). A supplemental report will be issued in the event of a match.

Minimum 15 locus profile frequencies using the Applied Biosystems Identifiler kit.

The minimum 15 locus profile frequencies using the Applied Biosystems Identifiler kit were generated using the most common genotype at each locus for each population group and calculating the resulting profile frequency for that population. They are as follows:

Population Group	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
CAU	13,14	29,30	10,11	11,12	15,16	6,9.3	11,12	11,12	17,20	13,14	17,18	8	14,17	11,12	21,22
BLK	13,14	28,29	10,11	10,12	15,16	7	12	9,11	21,22	13,14	15,16	8,11	15,16	11,12	22,24
HIS	13,14	29,30	10,11	11,12	15,16	7,9.3	9,12	11,12	17,19	14	16,17	8	13,14	11,12	22,23

1 in 16 trillion in the U.S. Caucasian population,
1 in 42 trillion in the African American population,
1 in 4.6 trillion in the Hispanic population.

Minimum 8 locus profile frequencies using the Applied Biosystems Minifiler kit.

The minimum 8 locus profile frequencies using the Applied Biosystems Minifiler kit were generated using the most common genotype at each locus for each population group and calculating the resulting profile frequency for that population. They are as follows:

Population Group	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
CAU	11,12	10,11	17,20	29,30	11,12	14,17	11,12	21,22
BLK	12	10,11	21,22	28,29	9,11	15,16	10,12	22,24
HIS	9,12	10,11	17,19	29,30	11,12	13,14	11,12	22,23

1 in 67 million in the U.S. Caucasian population,
1 in 84 million in the African American population,
1 in 35 million in the Hispanic population.

INCONCLUSIVE RESULTS/CONCLUSIONS

Results

DNA analysis results may be considered inconclusive or uninterpretable when allele peaks and/or internal standard peaks do not meet the expected morphology or size requirements due to loss of resolution during electrophoresis. Such results may require sample re-injection and/or re-preparation.

(See also the General STR Interpretation Guidelines Evaluation of Data section regarding the positive and negative amplification controls.)

Conclusions

In certain samples, such as complex DNA mixtures or samples with a low level of DNA, where allelic drop-out is suspected, it may be inconclusive as to whether or not a known individual is included or excluded as a possible contributor to the mixture. This situation arises when matching alleles are too few to support an inclusion, but sufficiently present whereby a definitive exclusion cannot be made. There is no strict rule governing such interpretations, and inconclusive determinations will be made on a sample-by-sample basis.

Suggested wording for inconclusive:

It is inconclusive whether John Doe could be a possible DNA contributor to this mixture/evidence/sample.

Due to the low level of DNA in the evidence no conclusions could be made regarding this sample.

EXCLUSIONS

Single Source or Predominant DNA profile

The (predominant) DNA recovered from the evidence is from an unknown male/female. John Doe is excluded as the source of the (predominant) DNA from the evidence.

If minor DNA types are present in a mixture scenario where a predominant profile is deduced, it may be required to state John Doe's possible inclusion as a possible minor contributor.

Mixtures

A mixture of at least two (or however many) people was recovered from the evidence. John Doe is excluded as a possible DNA contributor to the mixture.

In instances of exclusions it may be necessary to state that:

DNA from the evidence will be searched against the databases of the Combined DNA Index System (CODIS). A supplemental report will be issued in the event of a match.

REFERENCES

1. AmpF/STR Identifier™ PCR Amplification Kit User's Manual
2. The Evaluation of Forensic DNA Evidence. National Research Council (U.S.) National Academy Press, Washington, D.C., 1996 (NRCII)
3. Budowle, B., Moretti, T.R., Baumstark, A.L., Defenbaugh, D.A., and Keys, K.M. (1999). Population Data on the Thirteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. *Journal of Forensic Sciences* 44, 1277-1286.
4. Budowle B *et al.* Source Attribution of a Forensic DNA Profile. *Forensic Science Communication* July 2000 Vol 2(3).
5. Sensabaugh, GF. Biochemical markers of individuality. In Saferstein, R. *Forensic Science handbook*. Prentice hall, Inc., New York 1982:pp338-415.