

Unit Technical Manual

Forensic Biology

SAN DIEGO POLICE DEPARTMENT

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Issuing Authority: J. Simms, Quality Assurance Manager

Unit Technical Manual

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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.

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Figure 1

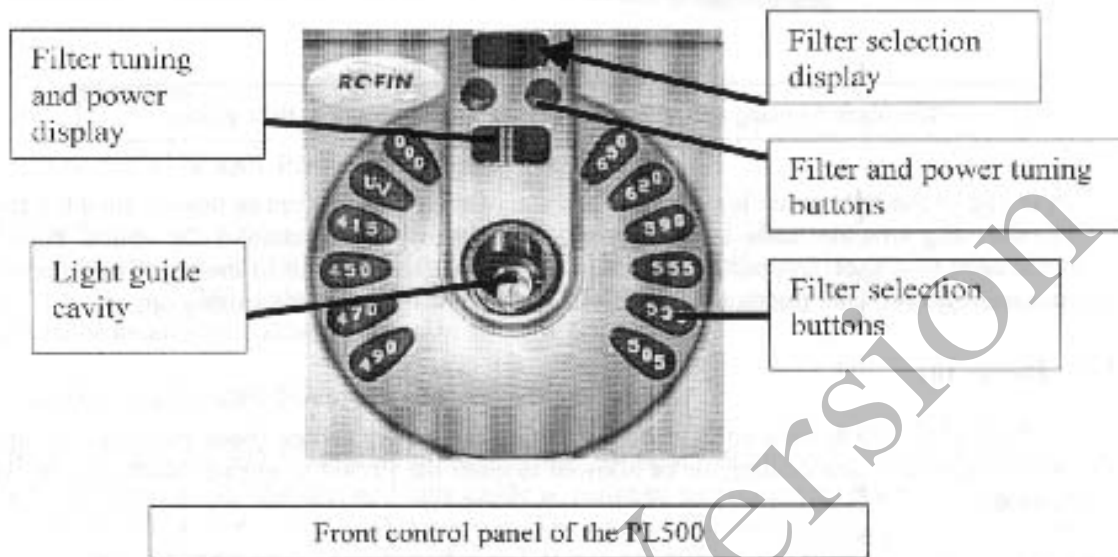
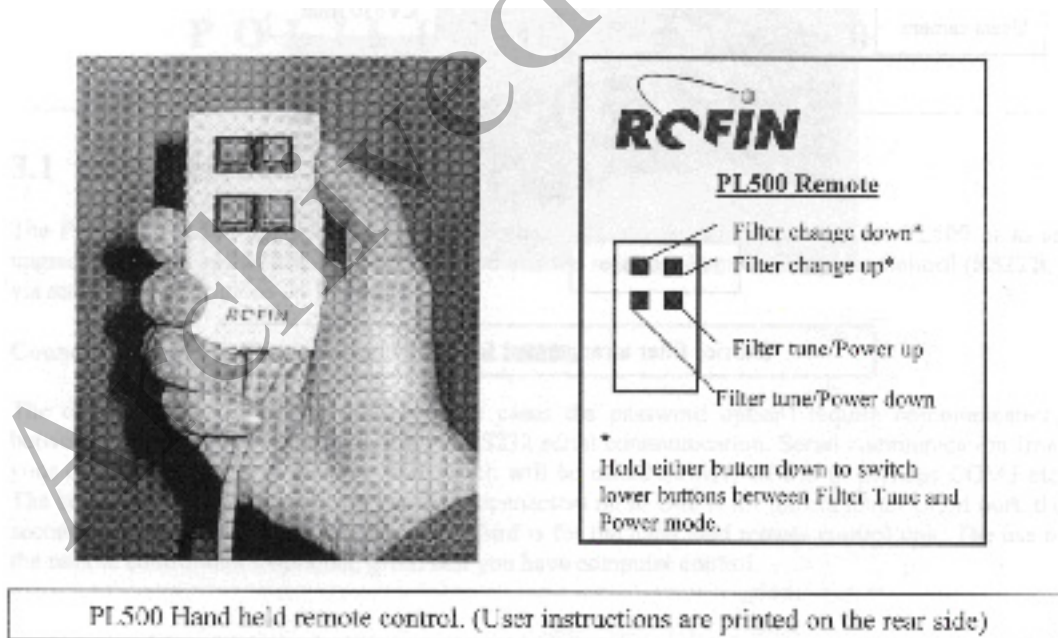


Figure 2



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- 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

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

Date: 1/12/05

Revision Date: 01/01/2007

Approved by: SAM

MATERIALS:

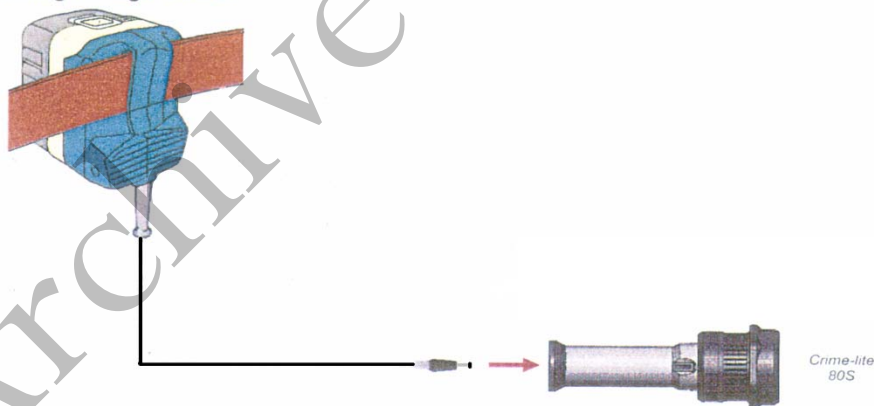
1. Crime-lite™ 80S Blue (450nm) or Crime-lite™ 80S Blue/Green (485nm)
2. Battery Adaptor with Makita® Rechargeable battery
3. Mains Adaptor
4. Orange goggles or filters

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.

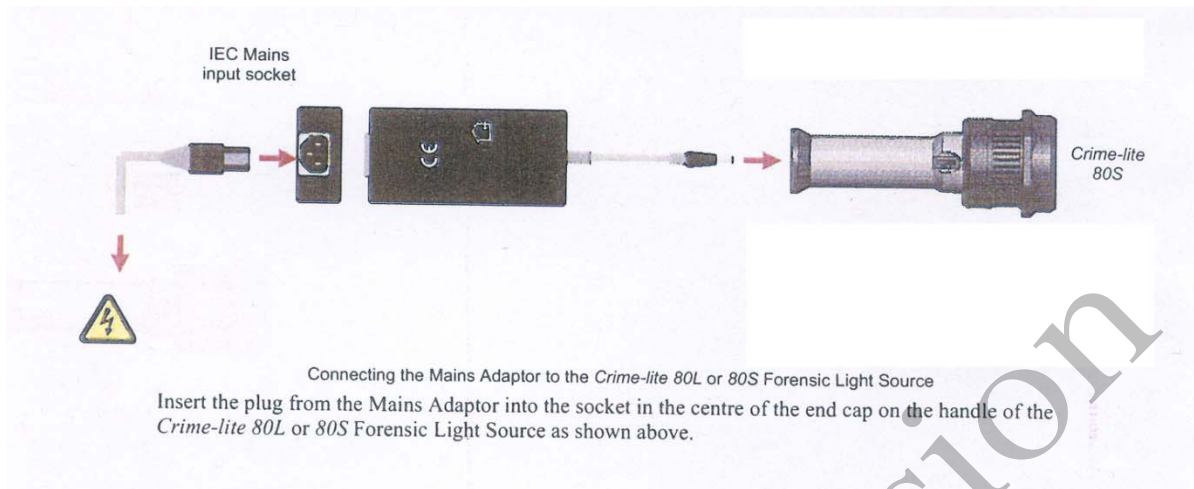
Insert the plug from the Battery Adaptor into the socket in the centre of the end cap on the handle of the Crime-lite 80L or 80S Forensic Light Source.

The light source is now ready for use.

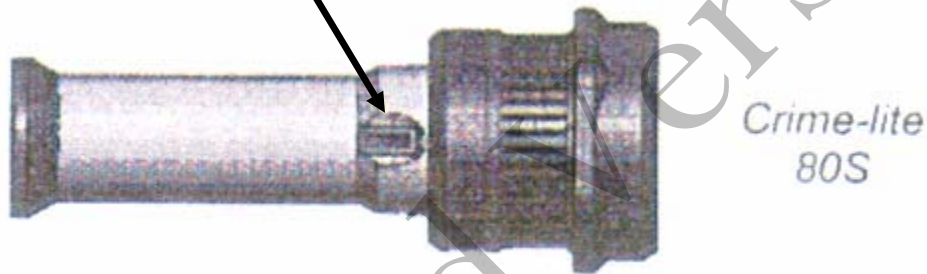
If the battery has a low charge or unavailable, attach the Mains Adaptor to the Crime-lite™ 80S and the nearest outlet.

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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.

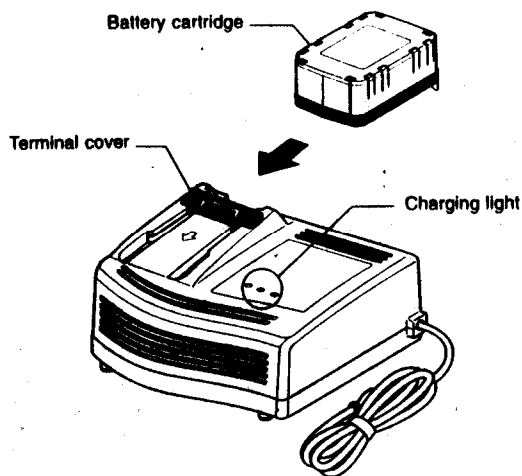
Charging the Makita® rechargeable battery:

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.

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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

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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.

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Method: **Microscopic Screening of 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 enough sperm are present in a sample, no further work on that sample may be necessary and that sample (along with the reagent blank) can be referred for DNA testing. In general, at least 100 sperm cells in the sample are needed for successful DNA typing. 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.

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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.

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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

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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.

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MAPPING

1. Wet a piece of filter paper with dH_2O . 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
4. Metropolitan Lab. Biology Methods Manual, 1978

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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.

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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

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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 Permunt.
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

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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.

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Method: p30 Detection Using the Abacus p30 Diagnostic Card

Date: 3/31/00

Revision Date: 05-13-2013

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. If one swab or less is tested, add 250µL nanopure water to saturate the material. If 1¾ swabs is used, then 300-350uL nanopure water should be enough to saturate the material. Extracts of positive control samples (neat semen and a 1:20 dilution of semen), and a negative control (reagents only) should also be run with the unknowns.

* If a high amount of semen is expected, add 75µL or enough nanopure water to just saturate the material, leaving a small amount of excess water. A 1:10 dilution of the extract will be generated prior to its addition to the test card.

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. If a high amount of semen is expected, create a 1:10 dilution of the supernatant, otherwise skip to Step 6. To generate the 1:10 dilution, add 20ul of the supernatant to a sterile 1.5mL tube containing 200ul of water.

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6. Load 200 μ L of the supernatant from Step 4 onto the sample loading “S” area of the test card. If the supernatant was diluted, load the entire contents of tube created in step 5 onto 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.

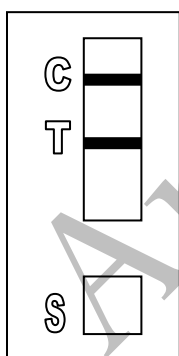
8. If a positive result for the p30 protein is obtained for a given sample it may be appropriate to also test a substrate control for that sample. For positive results from a questioned stain it could prove useful to test additional serial dilutions of the sample to provide information on how concentrated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

INTERPRETATION:

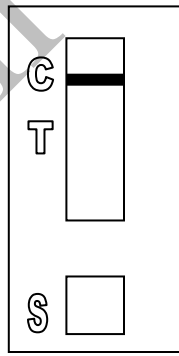
A positive test result for the presence of the protein p30 is indicated by the presence of pink bands at both the control line “C” and the test line “T” on the test card. The presence of a pink band at the control line but the absence of a pink band at the test line indicates a negative result. A pink 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.

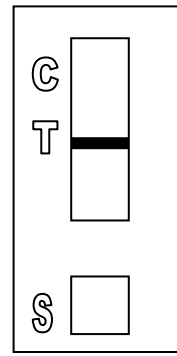
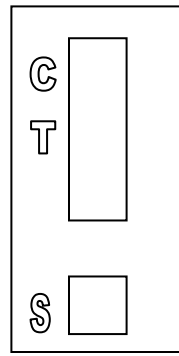
Legend: “S” = Sample loading area “T” = Test line “C” = Control line



POSITIVE



NEGATIVE



INVALID

REMARKS:

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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.

During the initial validation work on the Abacus p30 Test Card performed at the San Diego Police Department, a single concentrated male urine sample gave 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 were previously prepared for use with Abacus p30 Test card consisting 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. Additional experiments demonstrated that dried male urine did not produce positive results with the test cards. As such, the undiluted male urine control is unnecessary and the only controls required are the neat and 1:20 diluted semen controls. These controls should be prepared alongside extracts from the questioned samples. The neat semen and 1:20 dilution of semen should yield a positive result with the test card. 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., Dirnhofner, R. Evaluation of prostate-specific antigen (PSA) membrane tests for the forensic identification of semen. *Journal of Forensic Science*. v44, p1057-1060, 1999.

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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.
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Unit Technical Manual

Forensic Biology

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

Alternative amounts of the above reagents (in the same proportions) may be used depending on the amount of desired reagent. 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.

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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:

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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.

Unit Technical Manual

Forensic Biology

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.

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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.

Unit Technical Manual

Forensic Biology

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.

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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.

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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.

Unit Technical Manual

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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.
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Unit Technical Manual

Forensic Biology

Method: Abacus HemaTrace Detection of Blood

Date: 12/08/1999

Revision Date: 11/01/2011

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.

Note: If the supernatant created in step 1 is clear or light straw yellow, dilution in additional extraction buffer is not necessary. Alternative volumes of the kit extraction buffer may also be used to obtain the light straw yellow color.

3. Load 150uL of the extraction solution 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:

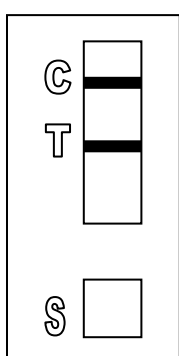
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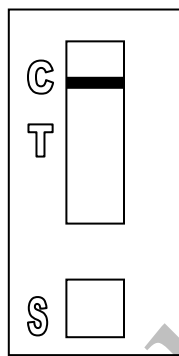
A positive test result for the presence of human blood is indicated by the presence of pink bands at both the control line “C” and the test line “T” on the test card. The presence of a pink band at the control line but the absence of a pink band at the test line indicates a negative result. A pink band 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.

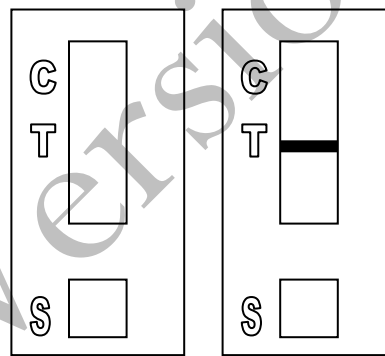
Legend: “S” = Sample loading area “T” = Test line “C” = Control line



POSITIVE



NEGATIVE



INVALID

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).

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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.

Unit Technical Manual

Forensic Biology

Method: Fluid Identification Interpretation Guidelines

Date: 12/08/1999

Revision Date: 09/06/2011

Approved by: SAM

The following is meant as a guide for what conclusions can be drawn based on a given combination of serological test results.

IDENTIFICATION OF SEMEN

Catalytic Test	Sperm	p30	Conclusion
Negative	Not Done	Not Done	Negative for the presumptive test for semen
Negative	Negative	Not Done	No semen was found.
Negative	Negative	Negative	No semen was found.
Positive	Negative	Negative	No semen was found.
Negative	Positive	Negative	Sperm cells were found.
Positive	Positive	Not Done	Semen was found.
Positive	Negative	Positive	Seminal fluid / semen was found.
Negative	Positive	Positive	Semen was found.
Positive	Positive	Positive	Semen was found.
Positive	Positive	Negative	Semen was found.
Positive/Negative	Inconclusive	Inconclusive	Inconclusive
Positive/Negative	Inconclusive	Inconclusive/Negative	Inconclusive

When weak AP positive results are obtained, the disclosure statement, "Acid phosphatase is an enzyme found in high concentrations in semen, but can also be detected in other body fluids and is produced by other organisms" needs to appear in the report.

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IDENTIFICATION OF BLOOD

Catalytic test	HemaTrace	Conclusions
Negative	Not Done	No blood was found.
Positive	Not Done	Presumptive test indicated the possible presence of blood.*
Positive	Negative	Presumptive test indicated the possible presence of blood; however the presence of human blood could not be confirmed.
Positive	Positive	Blood was found.**
Negative	Positive	Blood was found.**

*Analysts may come to the reasonable conclusion that a stain is apparently blood (to be stated in report as such) assuming the stain appears visually consistent with the analyst's expectation for blood, a positive catalytic test result, or the stain's existence is obvious given the context of the crime. An example would be a bloodstained t-shirt belonging to a stabbing victim. A positive HemaTrace result is mandated for an analyst to conclude blood (or human blood) is present if or when the stain is diffuse, the catalytic test result for the stain is suspect because it is difficult to visualize, the stain is on a substrate, such as rust, known to possibly cause a weak positive reaction with the catalytic test, or triggers a weak catalytic test. Analysts are encouraged where there is any doubt, or where the probative value of stain is critical, to employ the HemaTrace test to conclusively demonstrate the presence of blood.

**May also be blood of ferret or higher primate in origin. This statement must appear in report or testimony as qualifier if conclusion is presented as to human blood being present.

Unit Technical Manual

Forensic Biology

Method: **Phadebas Amylase Test**

Date: 5/1/2012

Approved by: SAM

REAGENTS:

1. PHADEBAS WORKING SOLUTION

0.02g crushed Phadebas tablet

550µL Nanopure water

This recipe is for 5 sample tests, but can be scaled up as needed.

STANDARDS AND CONTROLS:

1. Human salivary α -amylase standard, Sigma-Aldrich #A1031 (500 µL of 1:100,000 dilution of stock standard is required for the test)
[The stock standard was reconstituted to ~2mg/mL with Nanopure water and is stored frozen. 1/100 dilutions of this stock have been prepared for use when analysts run this test.]
2. Neat saliva. This is generally prepared fresh on a swab (of which a ~1/4 swab is used) or on filter paper (of which an ~5x5mm cutting is used) for each test.
3. Blank (Nanopure water)
4. Substrate control (if appropriate)

PROCEDURE:

1. Clear 1.5mL microcentrifuge tubes should be used for this test. For unknowns, add 500 µL of nanopure water to an ~5x5 mm cutting or an ~1/4 swab. The frozen amylase standard aliquot should be diluted 1/1000 prior to use (suggested dilution is 5uL in 5mL). For the controls, 500 µL of the amylase standard dilution should be used for the test, 500uL nanopure water should be used as a negative, and 500uL of nanopure water should be added to the neat saliva sample as the positive control.
2. Add 100µL of the Phadebas working solution to each sample and vortex.
3. Incubate the samples at 37°C for 30 minutes.
4. Vortex, then centrifuge the samples at maximum speed for 2 minutes.

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INTERPRETATION:

Positive: The presence of α -amylase is indicated if the blue color in the supernatant is darker than that of the 1:100,000 human salivary α -amylase standard.

NOTE: Blue substrates may produce blue supernatants during the extraction process even though no α -amylase is present on them. If this is expected, results may be inconclusive (see below).

Inconclusive: The test is inconclusive if the blue color in the supernatant is lighter than that of the 1:100,000 human salivary α -amylase standard, but darker than that of the reagent blank. The test may also be inconclusive if the blue color in the supernatant is masked by another color generated during the extraction process (e.g. red-brown supernatant from a bloodstain).

Negative: The absence of α -amylase activity is indicated if the supernatant is clear.

REMARKS:

Amylase is an enzyme present in saliva at high concentrations, hence the use of amylase as a presumptive test for the presence of saliva. Amylase is responsible for the digestion of starches, first to oligosaccharides, then to maltose and glucose. The Phadebas tablets are composed of starch polymers conjugated to a blue dye. In the presence of amylase, the blue dye is liberated into the supernatant, generating a blue color in proportion to the level of amylase activity.

Amylase is also present in most other body fluids (e.g. urine) and tissues, albeit at much lower concentrations than that found in saliva. Feces, breast milk, and nasal secretions may have high levels of amylase.

Unit Technical Manual

Forensic Biology

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 0.25mL 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 and transfer the supernatant into a 6 x 50mm borosilicate glass test 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:

Unit Technical Manual

Forensic Biology

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.

Method:

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Unit Technical Manual

Forensic Biology

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 (or a 2.0mL collection 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.

Unit Technical Manual

Forensic Biology

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.* AmpFlSTR Profiler Plus and AmpFlSTR 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.

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Unit Technical Manual

Forensic Biology

Method: **DNA Extraction in Preparation for Y-Screen**

Date: 03/15/2012

Revision Date: 03/15/2012

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 -20oC 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 -20oC 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 or a 2.0mL collection tube and add 500 -750uL of digest buffer, 20-30uL of 10mg/mL proteinase K, and 20-30uL of 1M DTT.
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 the BioRobot® EZ1 and eluted in 40uL. Please refer to the desired protocol for the procedure.

REFERENCES:

1. Cetus Amplitype User Guide 1990

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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].

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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

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Unit Technical Manual

Forensic Biology

Method: **Differential DNA Extraction**

Date: 06/29/2000

Revision Date: 11/01/2011

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

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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. In this instance step 14 does not need to be repeated.

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].

Note: The number of total washes (both digest buffer and distilled water) performed on the sperm fractions should be appropriate for the nucleated epithelial and sperm cell counts observed during microscopic examination. For example: a sample with high sperm cell counts should have a

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minimum of three digest buffer washes and one water wash performed. A sample with low sperm cell counts and high epithelial cell counts should have more digest buffer washes performed (e.g. five) in an attempt to obtain a sperm fraction free of non-sperm DNA.

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

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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.

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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

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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:

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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.

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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

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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

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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.

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Forensic Biology

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

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.

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7. The purified sample can now be washed and concentrated in TE buffer. 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 Amplitye User Guide 1990

Archived Version

Unit Technical Manual

Forensic Biology

Method: **Organic DNA Purification Using PCIA with the Phase Lock Gel (PLG) Tubes**

Date: 10/10/2011

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.

PHASE LOCK GEL TUBES Phase Lock Gel Heavy 2mL tubes (Fisher Scientific P/N #FP2302830).

PROCEDURE:

1. Immediately prior to use, pellet Phase Lock Gel (PLG) at 12,000 -16,000 x g in a microcentrifuge for 20-30 seconds.
2. Transfer the digested solution (max volume 750uL) to a Phase Lock Gel 2mL tube.

[Phenol/chloroform extractions must be carried out in a hood with a lab coat and eye protection].
3. Add 0.5mL buffered phenol/chloroform/isoamyl alcohol solution. Mix thoroughly by inversion or by repeated pipetting. Do not vortex.
4. Spin in a microcentrifuge for 5 minutes at 16,000 x g (12,900rpm) to separate the phases.
5. Use a sterile pipette tip or equivalent to transfer the upper aqueous phase to a new sterile 2mL PLG tube.

NOTE: In order to dispose of the liquid in the organic phase, puncture the gel with a pipette tip to release the liquid below it.
6. Repeat steps 3-5 an additional one or two times or until the aqueous phase is clear.
7. The purified sample can now be washed and concentrated in TE buffer. 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

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the purified samples.

REFERENCES:

1. Manual Phase Lock Gel™ (PLG) User's Manual. 5Prime Gaithersburg, MD
2. Penn State University Forensic Science Program Organic Extraction Procedure (Protocol No. PSU 031)

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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 2300rpm (~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 2300rpm for 12-15 minutes. Discard the effluent and repeat the TE wash (steps 5-7) at least one more time.

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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 3200rpm (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

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Forensic Biology

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.

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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

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Method: **DNA Purification/Concentration Using Vivacon 2 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. Forensic Grade Vivacon 2 Centrifugal Filter Devices (Sartorius Stedim P/N VN02H41ETO)
2. Table Top Centrifuge with Fixed Angled Rotor (Sorvall RT6000B or RT6000D)

PROCEDURE:

1. Assemble the Vivacon 2 unit by inserting the sample reservoir into the filtrate vial.
2. Add approximately 1.5mL of TE buffer to the upper Vivacon 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 sample may need to be spun for a longer duration (up to 30minutes) to achieve the desire volume. The DNA sample will be concentrated in about 40uL of TE buffer in the bottom of the upper Vivacon 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. Nanopure water may also be used in the wash steps if desired.
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

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(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:

Technical data and Operating Instructions for the Vivacon 2 PCR Grade

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

Date: 01/01/2007

Revision Date: 12/28/2012

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 or BioRobot® EZ1 Advanced XL

PROCEDURE:

1. Remove the substrate (if applicable), and transfer the liquid digest to the EZ1 sample tubes provided in the EZ1 DNA Investigator kit (alternatively, the 2.0ml collection tube can be used in the instrument).

If the 2.0mL collection tubes are used, centrifuge the tubes at maximum speed to collect the lysate.

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 v1.2 (EZ1) or EZ1 DNA Investigator Advanced XL v1.1 (EZ1 Advanced XL) 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.

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4. Select the desired eluent (TE or water).
5. Select the desired elution volume based on the expected yield; 40, 50, 100, and 200uL are options. 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 on the EZ1 or 1-14 per extraction run on the EZ1 Advanced XL) 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 (or 1-14) tip holders containing the filter-tips into row 2 of the tip rack.
10. Load 1-6 (or 1-14) opened sample or 2.0mL collection tubes (from Step 3.) into row 4 of the tip rack.
11. Load 1-6 (or 1-14) 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 EZ1 Advanced XL has a built in UV cross linker that can be used to decontaminate the instrument for between 20 and 60 minutes. 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:

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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.
4. 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.

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

Date: 06/02/2003

Revised: 10/28/2011

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 or BioRobot® EZ1 Advanced XL

PROCEDURE:

1. To a sterile 1.5mL tube or to a 2.0ml collection tube 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. If the 2.0mL collection tubes were used, centrifuge them at maximum speed for 5min to collect the lysate.

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

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4. Insert the DNA Investigator v1.2 (EZ1) or EZ1 DNA Investigator Advanced XL v1.1 (EZ1 Advanced XL) 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”, “Norm”, or “Norm TD” protocol. The Normalization protocols will limit the DNA yield of the samples by employing a limited amount of paramagnetic beads in the extraction. In the TD protocols 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 (additional elution volumes available are 40, 50, and 100 uL).
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 on the EZ1 or 1-14 per extraction run on the EZ1 Advanced XL) 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 (or 1-14) tip holders containing the filter-tips into row 2 of the tip rack.
12. Load 1-6 (or 1-14) opened sample or collection tubes (from Step 3.) into row 4 of the tip rack.
13. Load 1-6 (or 1-14) 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.

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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 EZ1 Advanced XL has a built in UV cross linker that can be used to decontaminate the instrument for between 20 and 60 minutes. 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.

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Method: **NIST Quantitation Setup Using the Qiagen BioRobot® Universal**

Date: 06/18/2012

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 and the NIST Quantitation Standard Curve.

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Important notes to remember for using the BioRobot Universal for setup:

- a. It is recommended that the system liquid carboy be well above the minimum level prior to commencing a run.
 - b. It is recommended that the system be re-initialized and *at least one* 100mL flush (available from the Tools drop-down menu) be performed prior to commencing a run.
 - c. 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.
2. 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.
 3. Prepare a 1/10 and a 1/50 dilution of the human DNA standard in TE buffer. These dilutions will serve as calibrators for the quantitation assay.
 4. Determine the total number of samples to be amplified including the calibrators 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 (~5-10%) should be created to allow for pipeting error.

5. Open the **Quant Sheet** in the most current version of the SDPD SIMS (found on the FB network H:\Worksheets).
 - a. The default spreadsheet shows the locations for the DNA calibrators and blank.
 - b. Fill out the "Sample Name" column with a list of the sample names. Acceptable volumes for quantitation are 2 or 8uL (do not change the volume of the standards). For Y-screen analysis, the volume for the samples should be 8uL (do not change the volume of the standards).
 - 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 SIMS.
6. Open QiaSoft™ v.5 software (on the desktop). Log in as "sdpd" and leave the password blank.

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- a. Under “applications” select “Forensic” in the drop-down menu (▼) and then select “SDPD Quantifiler NIST Setup v1.2”.
- 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. The load check is optional.
- h. Enter whether the robot will be creating the DNA standard dilution (Yes/No).
- i. Empty tip waste (if necessary).
- j. Refill the tip racks (if necessary). If tips run out the instrument will prompt you to refill later.
- k. 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.
- l. 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.

- 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).
7. The BioRobot Universal will now add master mix to the 96-well optical plate, and then add 2 (or 8)uL of the samples to the master mix.
 8. 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 7500 SDS.
 9. 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.

References:

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

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Method: **qPCR with the Quantifiler™ Human DNA Quantification Kit using the NIST SRM Human DNA Quantitation Standard**

Date: 05/01/2012

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.

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2. Prepare a 1/10 and a 1/50 dilution of the human DNA standard in TE buffer. These dilutions will serve as calibrators for the quantitation assay.
3. Determine the total number of samples to be amplified including the set of DNA calibrators 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% suggested) 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 or 8uL of sample, and 2uL calibrators, and the appropriate volume of controls (2 or 8uL) 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

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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. Save your file..
22. Export the Ct values of your samples by selecting **File>Export>Ct**. The 7500 software can be closed at this point and the instrument can be turned off..
23. Import the Ct values into the SDPD Worksheet Maker's "**Quant Data**" worksheet. The data is automatically compared against the NIST standard curve in the workbook and the results are available in the "**Quant Results**" worksheet of the workbook. The "Quant Results" worksheet should be printed (File>Print or use the print icon) and included in the analytical record.

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Assessment of DNA calibrators:

The concentration of the Human DNA standard received with each lot of kit will be determined during the QC process. The values obtained during this QC will determine the expected values for the calibrators. Values obtained that are significantly outside the expected quantitation values of calibrators may indicate a problem with the qPCR run. If problems are indicated the quantitation assay should be interpreted with caution and a second assay may be required.

Assessment of sample quality:

If a sample has a high human Ct value and a normal IPC Ct value (generally less than 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 (slightly greater than 28) 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 (>>28) 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

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Method: **qPCR with the Quantifiler[®] Duo DNA Quantification Kit using the NIST SRM Human DNA Quantitation Standard**

Date: 05/01/2012

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.

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2. Prepare a 1/10 and a 1/50 dilution of the human DNA standard in TE buffer. These dilutions will serve as calibrators for the quantitation assay.
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% suggested) 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 or 8uL of sample, and 2uL calibrators, and the appropriate volume of controls (2 or 8uL) to the appropriate wells. Note: For quantitation for Y-Screen DNA analysis, add 8uL of sample 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

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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. Save your file.
21. Export the Ct values of your samples by selecting **File>Export>Ct**. The 7500 software can be closed at this point and the instrument can be turned off.
22. Import the Ct values into the SDPD Worksheet Maker's "**Quant Data**" worksheet. The data is automatically compared against the NIST standard curve in the workbook and the results are available in the "**Quant Results**" worksheet of the workbook. The "Quant Results" worksheet should be printed (File>Print or use the print icon) and included in the analytical record.

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Assessment of DNA calibrators:

The concentration of the Human DNA standard received with each lot of kit will be determined during the QC process. The values obtained during this QC will determine the expected values for the calibrators. Values obtained that are significantly outside the expected quantitation values of calibrators may indicate a problem with the qPCR run. If problems are indicated the quantitation assay should be interpreted with caution and a second assay may be required.

Assessment of sample quality:

If a sample has a high human Ct value and a normal IPC Ct value (generally less than 30.75) 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 (slightly higher than 30.75) 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 (≥ 30.75) 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.

Assessment of samples quantified for Y-Screen analysis:

Based on the results of the Y-screen validation, the following general guidelines should be taken into account for submission of screened samples for DNA testing. Although higher amounts are more likely to provide complete and robust results, in general, samples having as low as 0.06ng of total male DNA may provide some informative results using Identifiler Plus and samples having as low as 0.03ng of total male DNA may provide some informative results using Yfiler. If $\frac{1}{4}$ swab was tested for Y-screen analysis, the following are recommended (assuming ng/4ul, not ng/ul):

Quant	Submission recommendations
0.005ng/4uL	1 $\frac{3}{4}$ swabs should provide informative Yfiler results
0.013ng/4uL	$\frac{3}{4}$ swab for Yfiler (minimum) and/or 1 $\frac{3}{4}$ swab for Identifiler Plus
0.033ng/4uL	$\frac{3}{4}$ swab for Identifiler Plus (minimum) and/or remaining Y-screen extract may provide Yfiler information

Note that other factors, such as the likelihood of multiple semen donors and the ratio of male to female DNA in the sample, may require the submission of a greater amount of sample for DNA testing.

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REFERENCE:

Quantifiler® Duo DNA Quantification Kit User's Manual

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Method: **Quantifiler™ Setup Using the Hamilton Microlab® Nimbus 4**

Date: 04/17/2013

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 Human (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 4306737)
2. Optical Adhesive Covers: Applied Biosystems (P/N 4311971)

EQUIPMENT:

1. Hamilton Microlab Nimbus 4 Liquid Handling Workstation: Hamilton Robotics
2. ABI Prism 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 and the NIST Quantitation Standard Curve.

Important notes to remember when using the Microlab Nimbus for setup:

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- a. The pre-run maintenance should be performed prior to the first run of the day (refer to maintenance protocol in the FB Unit Policy Manual).
 - b. It is highly recommended that all tip racks are full prior to the start of a run. At minimum, complete columns of tips must be present sufficient to perform the run.
 - c. Sarstedt 1.5mL screw-cap tubes are required for use on the instrument for all samples and controls. 2.0mL screw-cap tubes are required for master mix. Caps should be removed after tubes are placed in the racks and replaced prior to removing tubes from the racks.
2. Thaw the appropriate Quantifiler Primer Mix and concentrated DNA standard (which is stored at -20°C). Vortex the Primer Mix and 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.
3. Prepare a 1/10 and a 1/50 dilution of the human DNA standard in TE buffer. These dilutions will serve as calibrators for the quantitation assay.
4. Determine the total number of samples to be amplified including the calibrators and one qPCR blank. Create a master mix by combining the following volumes of reagents in a 2.0mL screw-cap tube.
 - a. 10.5µL of Primer Mix x # of Samples
 - b. 12.5µL of qPCR Reaction Mix x # of Samples

Note: The above is the minimum volume required per sample. The Nimbus requires additional master mix to perform the setup. The volumes calculated in the current version of the SDPD SIMS should be prepared.

Note: If the total number of samples is greater than 72, then two tubes of master mix are required. The first 2.0mL screw-cap tube should contain 1205µL of master mix and the second tube should contain the remaining volume.

5. Open the **Quant Sheet** in the most current version of the SDPD SIMS (found on the FB network H:\Worksheets).
 - a. The default spreadsheet shows the locations for the DNA calibrators and blank.
 - b. Fill out the "Sample Name" column with a list of the sample names. If appropriate, edit the volume for the samples from 2 or 8µL (do not change the volume of the standards).
 - c. Save the SDPD SIMS workbook to a location accessible on the FB network (the Nimbus cannot read from a password protected Excel file).
 - d. When finished, the **qPCR worksheet** should be printed for the case notes.
 - e. Exit the SDPD SIMS.
6. Empty the tip waste (if necessary).

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7. Refill the tip racks on the Nimbus.
8. Place the 96-well optical plate in the appropriate location on the deck.
9. Place the 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 racks in a left to right direction, filling each column completely before the next.

10. Place the Quantifiler Master Mix in the appropriate place on the deck.

Note: If more than 72 samples are being quantified then the master mix will need to be split into two tubes (see step 4 above).

11. Remove the caps from the samples and master mix.
12. Close the instrument door (the door must remain closed during operation).
13. Open the Hamilton Run Control software (on the desktop).
 - a. Open the current version of the **SDPD qPCR and PCR Setup** method within the software.
 - b. Click the **“Run”** button (▶).
 - c. In the user prompt, navigate to the worklist input file (from step 5 above), select **“qPCR Setup”**, and click **“Continue”**.
 - d. Verify that sufficient 300µL and 50µL tips are loaded onto the deck and click **“Continue”**.
 - e. Enter the first and last position of the 300µL tips (1=A1, 2=A2, ..., 96=H12) and click **“OK”**.
 - f. Enter the first and last position of the 50µL tips (1=A1₁, 2=A2₁, ..., 96=H12₁, 97=A1₂, 98=A2₂, ..., 192=H12₂) and click **“OK”**.
14. The Nimbus will now add master mix to the 96-well optical plate, and then add 2 (or 8)µL of the samples to the master mix.
15. Click **“OK”** to acknowledge completion of the run.
16. 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 7500 SDS.

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17. 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.

REFERENCES:

Microlab® Nimbus Independent Channel Operator's Manual. Hamilton Robotics.
Microlab® Nimbus Enclosed Version Operator's Manual. Hamilton Robotics.
Microlab® Nimbus Independent Channel Programmer's Manual. Hamilton Robotics.
Quantifiler™ Human DNA Quantification Kit User's Manual. Applied Biosystems.
Quantifiler® Duo DNA Quantification Kit User's Manual. Applied Biosystems.

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Method: **Target Ranges for Single Source Samples Using NIST Standard based Quantitation**

Date: 05/01/2012

Approved by: SAM

The chart below represents the results obtained for the sensitivity study of the Identifiler Plus, Yfiler, and Minifiler kits using data obtained with the NIST standard. The highlighted areas represent the range for which results were obtained with peak heights between 100 and 3000rfu

This information is presented for analysts as a guide to determine the amplification targets for single source samples. Sample type and the expectation of obtaining a mixture will affect the amount of DNA amplified in casework samples.

QF Human Value	QF Duo Value	ID Plus Results	Yfiler Results	Minifiler Result
0.010	0.012	4 low level types	4 low level types	7 low level types
0.031	0.035	Stochastic level profile w/ drop-out	Partial profile (11 loci)	Complete low level profile (82-841rfu)
0.052	0.058	Full profile w/ stochastic level types	Complete low level profile (140-417rfu)	Complete profile (299-1870(H)rfu)
0.105	0.116	Full profile w/ stochastic level types	Complete profile (250-630rfu)	Complete profile (345-2079(H)rfu)
0.157	0.174	Full profile (546 lowest locus rfu)	Complete profile (415-902rfu)	Complete profile (470-4900(H)rfu)
0.209	0.232	Full profile (897 lowest locus rfu)	Complete profile (580-1500rfu)	Complete profile (1261-6900(H)rfu)
0.314	0.347	Full profile (700-3500rfu)	Complete profile (1000-1800rfu)	Complete profile (1500-7300(H)rfu) w/ minor artifacts
0.418	0.463	Full profile (700-4200rfu)	Complete profile (2000-3600rfu)	Complete profile (1700-7900(H)rfu) w/ artifacts
0.523	0.579	Full profile (800-3200rfu)	Complete profile (2100-4600rfu)	Complete profile (2400-8700rfu) w/ pull-up
0.785	0.869	Manageable artifacts (pull-up)	Complete profile (2700-6000rfu) w/ pull-up	Complete profile (2900-8800rfu) w/ pull-up
1.046	1.158	Numerous Artifacts	Complete profile (2800-7000rfu) w/ pull-up	Complete profile (300-8800rfu) w/ pull-up/pref amp
0.017	0.019	6 low level types	3 low level types	6 low level types
0.051	0.058	Stochastic level profile w/ drop-out	Partial profile (13.5 loci)	Complete low level profile (76-650rfu)
0.085	0.096	Full profile just above stochastic level	Complete low level profile (150-650rfu)	Complete profile (210-1870(H)rfu)
0.169	0.192	Full profile w/ 1 stochastic level type	Complete profile (330-1000rfu)	Complete profile (430-2200(H)rfu)
0.254	0.288	Full profile (1021 lowest locus rfu)	Complete profile (640-1700rfu)	Complete profile (500-4000(H)rfu)
0.338	0.384	Full profile (919 lowest locus rfu)	Complete profile (580-1500rfu)	Complete profile (470-4100(H)rfu)
0.507	0.576	Full profile (800-1900 rfu)	Complete profile (1000-2700rfu)	Complete profile (1100-8200(H)rfu) w/ minor artifacts
0.676	0.768	Full profile (1100-3000 rfu)	Complete profile (1500-3600rfu)	Complete profile (1050-8600(H)rfu) w/ artifacts
0.845	0.960	Full profile (900-3200 rfu)	Complete profile (1300-4600rfu)	Complete profile (11500-8700rfu) w/ artifacts
1.268	1.440	Manageable artifacts (pull-up)	Complete profile (2300-7700rfu) w/ pull-up	Complete profile (3100-9100rfu) w/ pull-up/pref amp
1.690	1.920	Numerous Artifacts	Complete profile (2900-8400rfu) w/ pull-up	not amplified

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Method: **Concentrating DNA Extracts with Microcon YM-100 Filters**

Date: 04/01/2011

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 at 1,000 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 appropriate amount of extracted DNA sample to be concentrated 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 2300rpm (~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). Check the microcon assembly periodically during the spin to ensure that the filter has not dried out.

Important: Spinning the filter to dryness can cause irreversible binding of the DNA to the membrane.

5. Discard the effluent in the filtrate vial. Place the Microcon spin-column right-side up into a new into filtrate vial.

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8. Add 3-6uL of TE buffer to the Microcon-100 column (depending on desired final volume). Gently vortex the filter assembly.
9. Collect the DNA sample by inverting the reservoir into a new microcon tube and centrifuge at approximately 3200 rpm (approximately 1000 x g) for 3-5 minutes.
10. The concentrated purified DNA now resides in the tube. The volume can be further adjusted as necessary to achieve the desired concentration. Cap the tube and store frozen. Alternatively, transfer the purified DNA to a different tube, such as a sterile screw-cap tube for storage.

REFERENCE:

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

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Method: **DNA Concentration Using the DNA Speed-Vac**

Date: 05/01/2012

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.

EQUIPMENT:

Savant DNA Speed Vac

NOTES:

Based on an internal study conducted on concentrating samples containing TE buffer, it is not recommended to concentrate samples containing TE to less than a fourth ($\frac{1}{4}$) their original volume. Samples whose volume was reduced to less than $\frac{1}{4}$ the original volume started exhibiting signs of inhibition to the polymerase chain reaction using the Identifiler kit. Total inhibition of PCR was observed when the volume was reduced to less than $\frac{1}{8}$ the original volume. It is presumed that the concentration of the EDTA in the TE inhibits PCR by chelating the magnesium in the reaction. Concentration of samples eluted in water generally do not exhibit PCR inhibition at any level of volume reduction.

PROCEDURE:

1. Place the appropriately labeled open sample extract tubes in the Savant DNA Speed-Vac.
2. Turn on the DNA Speed-Vac. Select one of the three heat level options (low, medium, and high). Generally either medium or high are selected. The heat level will influence how quickly evaporation occurs.
3. Close the lid firmly and the DNA Speed-Vac will commence concentrating the samples through a combination of heat and the application of a vacuum to the inner chamber of the Speed-Vac.
4. After the appropriate amount of volume reduction has occurred, remove and cap your sample tubes. Any samples whose volume was reduced more than desired can be brought up to the appropriate volume using either TE buffer or nanopure water.
5. Turn of the DNA Speed-Vac.

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Method: **Amplification with the AmpF/STR® Identifiler Plus® kit**

Date: 10/19/2010

Approved By: SAM

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

REAGENTS:

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

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

4. AmpF/STR® Identifiler Plus® PCR Amplification Kits: Applied BioSystems

- A. AmpF/STR® Identifiler Plus® Master Mix
- B. AmpF/STR® Identifiler Plus® Primer Set
- C. AmpF/STR® Control DNA 9947A

Store all kit components at -15 to -25 °C upon receipt. Store all components at 2 to 8 °C after initial use. The primers contain light-sensitive dyes, and should be stored protected from light. The expiration date is printed on the kit.

MATERIALS:

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

EQUIPMENT:

GeneAmp PCR System 9700 Thermal Cyclers - Applied Biosystems

PROCEDURE:

18. Fill out an AmpF/STR® Identifiler Plus® Amplification Worksheet and determine the total number of samples to be amplified including the positive control (9947A DNA), the negative amplification control, and all samples.
19. Label a 0.2 mL MicroAmp Reaction Tube for each of the samples to be amplified.

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20. Vortex the AmpF/STR® Identifiler Plus® Master Mix, AmpF/STR® Identifiler Plus® Primer Set, and AmpF/STR® Control DNA 9947A. Vortex the three tubes for 5 seconds and spin briefly in a microcentrifuge to remove any liquid from the caps.
21. Create a master mix by combining the following volumes of reagents into a 1.5 mL microcentrifuge tube:
 - a. 10 µL AmpF/STR® Identifiler Plus® Master Mix x (# samples) + 10 %
 - b. 5 µL AmpF/STR® Identifiler Plus® Primer Set x (# samples) + 10 %

22. Vortex the master mix for 5 seconds and then spin briefly in a microcentrifuge to remove any liquid from the cap.

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

23. Aliquot 15 µL of the master mix into each labeled 0.2 mL reaction tube.
24. If less than 10 µL of sample is added for any sample, the difference should be made up by adding TE buffer for a final volume of 10 µL.
25. Add sample to the labeled 0.2 mL reaction tubes as follows:

For all evidence and reference samples, add approximately 1.5 to 2.0 ng of target DNA in a volume equal to or less than 10 µL. *Note:* 1.5 to 2.0 ng is a suggested target amount. 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 6-7 µL (0.1 ng/µL) of AmpF/STR® Control DNA 9947A to the positive amplification control tube. Add TE buffer to obtain a final volume of 10 µL.

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

26. Transport the tubes to the Amplification Room. 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.
27. Turn the thermal cycler on (if not already done).
28. Select the ID Plus x28 program.

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- a. Initial Incubation
95 °C, 11 minutes
- b. 28 Cycles
94 °C, 20 seconds
59 °C, 3 minutes
- c. Final Extension
60 °C, 10 minutes
- d. Final Hold
4 °C, ∞

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

REFERENCES:

AmpF/STR® Identifier Plus® PCR Amplification Kit User's Manual

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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

PROCEDURE:

1. Fill out an AmpF/STR MiniFiler™ Amplification Worksheet and determine the total number of samples to be amplified including the positive control (007 DNA), the negative amplification control, and all samples.

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2. Label a 0.2 mL MicroAmp Reaction Tube for each of the samples to be amplified.
3. Vortex the AmpF/STR MiniFiler™ Master Mix, AmpF/STR MiniFiler™ Primer Set, and AmpF/STR Control DNA 007. Vortex the three tubes for 5 seconds and spin 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 1.5 mL microcentrifuge tube:
 - a. 10 µL AmpF/STR MiniFiler™ Master Mix x (# samples) + 10%
 - b. 5 µL AmpF/STR MiniFiler™ Primer Set x (# samples) + 10%
5. Vortex the master mix for 5 seconds and then spin briefly in a microcentrifuge to remove any liquid from the cap.

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

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

For all evidence and reference samples, add approximately 0.3ng of target DNA in a volume equal to or less than 10µL. *Note:* 0.3ng is a suggested target amount. 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 2-4µL (0.1ng/µL) of AmpF/STR Control DNA 007 to the positive amplification control tube.
Add TE buffer to obtain a final volume of 10 µL.

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

9. Transport the tubes to the Amplification Room. Place a MicroAmp tray containing the PCR reaction tubes onto a thermal cycler .
10. Turn the thermal cycler on (if not already done).
11. Select the MiniFiler program.

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- 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

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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 Identifier™ 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.

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

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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

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Method: **Amplification with the AmpF/STR® Identifiler Direct® kit**

Date: 05/01/2012

Approved by: SAM

REAGENTS:

1. Prep-n-Go™ Buffer: Applied Biosystems

Store at room temperature. The expiration date is printed on the packaging.

2. AmpF/STR® Identifiler Direct® PCR Amplification Kits: Applied BioSystems

- D. AmpF/STR® Identifiler Direct® Master Mix
- E. AmpF/STR® Identifiler Direct® Primer Set
- F. AmpF/STR® Control DNA 9947A (2ng/μL)

Store all kit components at -15 to -25°C upon receipt. Store all components at 2 to 8°C after initial use. The primers contain light-sensitive dyes, and should be stored protected from light. The expiration date is printed on the kit.

MATERIALS:

1. 96 Deep Well Plate: Multiple vendors
2. 96 Well Reaction Plate: Applied Biosystems
3. 96 Well Full Plate Cover: Applied Biosystems

EQUIPMENT:

3. GeneAmp PCR System 9700 Thermal Cyclers: Applied Biosystems

PROCEDURE:

1. Fill out an AmpF/STR® Identifiler Direct® Amplification Worksheet.
2. Remove the cotton tip from a buccal swab and place it into a well of a 96 deep well plate. Repeat for each buccal swab to be analyzed.

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3. Add 400 μL of Prep-n-Go™ Buffer to each well of the deep well plate that contains a swab.
4. Incubate the deep well plate (containing swabs and buffer) for 20 minutes at room temperature.
5. Vortex the AmpF/STR® Identifiler Direct® Master Mix, AmpF/STR® Identifiler Direct® Primer Set, and AmpF/STR® Control DNA 9947A. Vortex the three tubes for 5 seconds and spin briefly in a microcentrifuge to remove any liquid from the caps.
6. Create a master mix by combining the following volumes of reagents into a 1.5 mL microcentrifuge tube:
 - a. 8.5 μL AmpF/STR® Identifiler Direct® Master Mix x (# samples) + 10 %
 - b. 8.5 μL AmpF/STR® Identifiler Direct® Primer Set x (# samples) + 10 %

7. Vortex the master mix for 5 seconds and then spin briefly in a microcentrifuge to remove any liquid from the cap.

Note: Steps 8-9 must be performed in a biological hood using dedicated pipettes.

8. Aliquot 17 μL of the master mix into the appropriate wells of a 96 well reaction plate.
9. Add samples to the appropriate wells of the 96 well reaction plate as follows:

For all reference samples, add 2 μL of lysate from the 96 deep well plate.

For the positive control, add 1 μL of Control DNA 9947A (2 ng/ μL).

For the amplification blank, add 2 μL of Prep-n-Go™ Buffer.

10. Cover the 96 well reaction plate with a 96 well full plate cover and transport the plate to the Amplification Room. Place the plate into one of the thermal cyclers.
11. Turn the thermal cycler on (if not already done).
12. Select the "ID Direct x27" program.

- e. Initial Incubation
95°C, 11 minutes

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f. 27 Cycles

94°C, 20 seconds

59°C, 2 minutes

72°C, 1 minute

g. Final Extension

60°C, 25 minutes

h. Final Hold

4°C, ∞

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

Note: The remaining sample lysates may be left in the deep well plate or transferred to separate tubes and stored frozen. Sample lysates have been successfully amplified after more than five months of being stored frozen at the SDPD. Once acceptable results have been obtained, sample lysates may be discarded. In the event that additional analysis is deemed necessary, the remaining unanalyzed buccal swab could be used or a new reference could be collected.

REFERENCES:

AmpF/STR® Identifiler Direct® PCR Amplification Kit User's Manual

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Method: **Identifiler Plus™, Minifiler™, Yfiler™ Setup Using the Hamilton Microlab® Nimbus 4**

Date: 04/17/2013

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. AmpF/STR® Identifiler Plus™ PCR Amplification Kit: Applied Biosystems (P/N 4427485)

- D. AmpF/STR® Identifiler Plus™ Master Mix
- E. AmpF/STR® Identifiler Plus™ Primer Set
- F. AmpF/STR® Control DNA 9947A

3. AmpF/STR® Yfiler™ PCR Amplification Kit: Applied Biosystems (P/N 4365495)

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

4. AmpF/STR® Minifiler™ PCR Amplification Kit: Applied Biosystems (P/N 4373874)

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

Store all kit components at -15 to -25°C upon receipt. Store AmpliTaq Gold™ DNA Polymerase at -15 to -25°C indefinitely. Store all other components at 2 to 8°C after initial use. The primer sets contain a light sensitive dye, and should be stored protected from light. The expiration date is printed on the kits.

MATERIALS:

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

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2. Full Plate Covers: Applied Biosystems (P/N N801-0550)
3. MicroAmp 8-cap strips: Applied Biosystems (P/N N801-0535)

EQUIPMENT:

1. Hamilton Microlab Nimbus 4 Liquid Handling Workstation: Hamilton Robotics
2. GeneAmp PCR System 9700 Thermal Cyclers: Applied Biosystems

PROCEDURE:

1. This protocol is to setup the 96-well optical plate for PCR DNA amplification using Identifiler Plus™, Minifiler™, and Yfiler™.

Important notes to remember when using the Microlab Nimbus for setup:

- d. The pre-run maintenance should be performed prior to the first run of the day (refer to maintenance protocol in the FB Unit Policy Manual).
 - e. It is highly recommended that all tip racks are full prior to the start of a run. At minimum, complete columns of tips must be present sufficient to perform the run.
 - f. Sarstedt 1.5mL screw-cap tubes are required for use on the instrument for all samples, controls, and TE buffer. 2.0mL screw-cap tubes are required for master mix. Caps should be removed after tubes are placed in the racks and replaced prior to removing tubes from the racks.
2. Identifiler Plus – Vortex the AmpF/STR® Identifiler Plus™ Master Mix, AmpF/STR® Identifiler Plus™ Primer Set, and AmpF/STR® Control DNA 9947A for 5 seconds. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.

Yfiler – 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.

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.

3. Determine the total number of samples to be amplified including the positive control and one amplification blank (Note: The Nimbus requires at least 8 samples to run the amplification protocol). Create a master mix by combining the following volumes of reagents in a 2.0mL screw-cap tube.

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Identifiler Plus

10µL AmpF/STR® Identifiler Plus™ Master Mix	x	# of Samples
5µL AmpF/STR® Identifiler Plus™ Primer Set	x	# of Samples

Yfiler

9.2µL AmpF/STR® Yfiler™ PCR Reaction Mix	x	# of Samples
0.8µL AmpliTaq Gold™ DNA Polymerase	x	# of Samples
5µL AmpF/STR® Yfiler™ Primer Set	x	# of Samples

Minifiler

10µL AmpF/STR® Minifiler™ Master Mix	x	# of Samples
5µL AmpF/STR® Minifiler™ Primer Set	x	# of Samples

Note: The above is the minimum volume required per sample. The Nimbus requires additional master mix to perform the setup. The volumes calculated in the current version of the SDPD SIMS should be prepared.

4. Open the **Amp setup** sheet in the most current version of the SDPD SIMS (found on the FB network H:\Worksheets).
 - f. The default spreadsheet shows the deck position 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/µL] column. Ensure that the VolumeSample and VolumeTE are completed appropriately.
 - h. Save the SDPD SIMS workbook to a location accessible on the FB network (the Nimbus cannot read from a password protected Excel file).
 - i. When finished, the **PCR worksheet(s)** should be printed for the case notes.
 - j. Exit the SDPD SIMS.
5. Empty the tip waste (if necessary).
6. Refill the tip racks on the Nimbus.
7. Place the 96-well optical plate in the appropriate location on the deck.
8. Place the 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.

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

9. Place the master mix in the appropriate place on the deck.
10. Place four 1.5mL screw-cap tubes each containing 300µL of TE in the appropriate place on the deck.
11. Remove the caps from the samples, master mix, and TE.
12. Close the instrument door (the door must remain closed during operation).
13. Open the Hamilton Run Control software (on the desktop).
 - g. Open the current version of the **SDPD qPCR and PCR Setup** method within the software.
 - h. Click the **“Run”** button (▶).
 - i. In the user prompt, navigate to the worklist input file (from step 4 above), select **“PCR Setup”**, and click **“Continue”**.
 - j. Verify that sufficient 300µL and 50µL tips are loaded onto the deck and click **“Continue”**.
 - k. Enter the first and last position of the 300µL tips (1=A1, 2=A2, ..., 96=H12) and click **“OK”**.
 - l. Enter the first and last position of the 50µL tips (1=A1₁, 2=A2₁, ..., 96=H12₁, 97=A1₂, 98=A2₂, ..., 192=H12₂) and click **“OK”**.
14. The Nimbus will now add master mix, TE buffer, and sample DNA to the 96-well optical plate.
15. Click **“OK”** to acknowledge completion of the run.
16. 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.
17. The PCR analysis can be continued by following the “Amplification with the AmpF/STR® Identifier Plus™ Kit” protocol at step 9, the “Amplification with the AmpF/STR® Yfiler™ Kit” protocol at step 10, and the “Amplification with the AmpF/STR® Minifiler™ Kit” protocol at step 10.

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REFERENCES:

Microlab[®] Nimbus Independent Channel Operator's Manual. Hamilton Robotics.
Microlab[®] Nimbus Enclosed Version Operator's Manual. Hamilton Robotics.
Microlab[®] Nimbus Independent Channel Programmer's Manual. Hamilton Robotics.
AmpF/STR[®] Identifiler Plus[™] PCR Amplification Kit User's Manual. Applied Biosystems.
AmpF/STR[®] Yfiler[™] PCR Amplification Kit User's Manual. Applied Biosystems.
AmpF/STR[®] Minifiler[™] PCR Amplification Kit User's Manual. Applied Biosystems.

Archived Version

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Method: **Identifiler Plus™, 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.

3. AmpF/STR® Identifiler Plus® PCR Amplification Kits: Applied BioSystems
 - A. AmpF/STR® Identifiler Plus® Master Mix
 - B. AmpF/STR® Identifiler Plus® Primer Set
 - C. AmpF/STR® Control DNA 9947A
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

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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. It is recommended that the system liquid carboy be well above the minimum level prior to commencing a run.
 - b. It is recommended that the system be re-initialized and a 100mL flush (available from the Tools drop-down menu) be performed prior to commencing a run.
 - c. 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.
 - d. 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:

Identifiler Plus

10 µL AmpF/STR® Identifiler Plus® Master Mix	x	# samples + 10 %
5 µL AmpF/STR® Identifiler Plus® Primer Set	x	# samples + 10 %

Yfiler

9.2uL of AmpF/STR Yfiler™ PCR Reaction Mix	x	# of samples + 10 %
0.8uL of AmpliTaq Gold DNA Polymerase	x	# of samples + 10 %
5.0uL of AmpF/STR Identifiler Primer Set	x	# of samples + 10 %

Minifiler

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

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

4. The **Amp Setup** within the current version of the SDPD Worksheet maker (found on the FB network

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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 either the “**SDPD Identifiler Plus Amplification v.1**” or the “**Minifiler – Yfiler Amplification v.1**” protocols.
 - 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.

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- b. Place the Master Mix in the appropriate place(s) on the deck. Note: if more than 48 samples are being amplified 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 thermocycler.

References:

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

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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 Identifier™, Minifiler™, or Yfiler™ amplification kits. Please refer to the Identifier 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:

[Note: fractions of uLs are incompatible with the protocol for WellAware setup of samples. Please round to the nearest whole number prior to creating your WellAware eSOP file.]

1. Open the **PCR (1-24) worksheet** (or the Minifiler or Yfiler Amp worksheets) in the current version of the SDPD SIMS (found on the FB network H:\Worksheets). Click on the 'Make WellAware eSOP' button to generate a WellAware eSOP (electronic standard operating procedure). The eSOP is automatically saved on the WellAware folder on the FB networks.
2. Navigate to the newly saved file and save it as a text file (.txt) in the appropriate 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]

3. When finished, the PCR worksheet should be printed for your case notes.

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4. Power on the WellAware computer (if needed) located in the hood in the amplification set-up room. Plug in the keyboard to the laptop (if not currently connected). To log into the computer, the username is BioTx. Open the BioTX Lab Suite software.
5. 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.
6. Place a 96-Well Optical Reaction Plate on the bottom right surface of the WellAware screen.
7. Plug the hand sensor into the USB port on the computer.
8. 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.]
9. 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 thermalcycler.
10. When the protocol is complete, exit the WellAware software.
11. Unplug the hand sensor from the USB port.
12. Power off (shut down, do not select hibernate) the WellAware computer.

REFERENCE:

WellAware User Guide.

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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

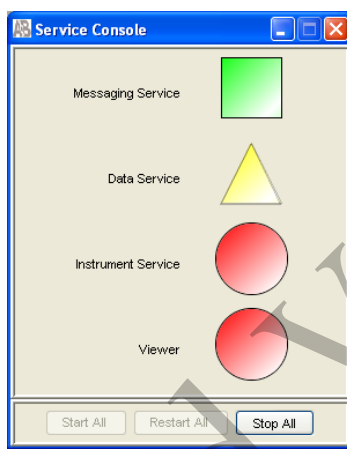
1. Power on the computer monitor, then power on the computer. At the Log On prompt, hit **OK** (or Enter) leaving the Password window blank.
2. Before turning on the 3130 instrument, ensure that the oven door is closed and locked, that the

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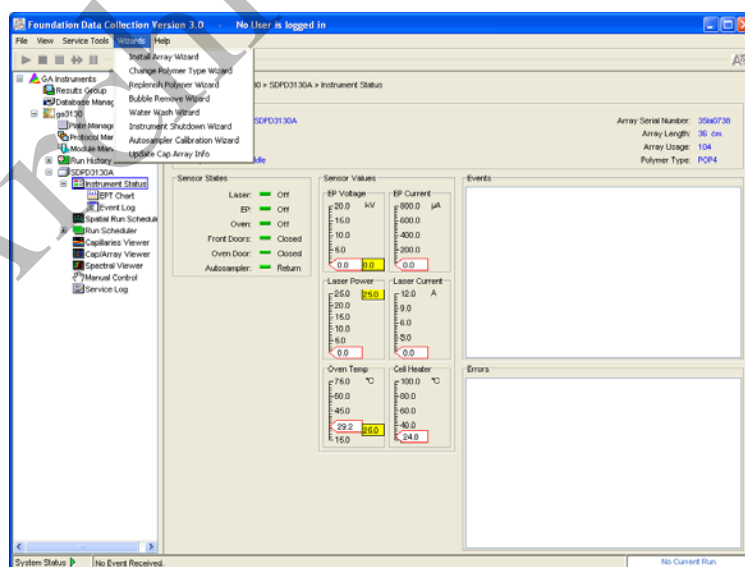
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instrument doors are closed, and the computer is powered on.

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.



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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.)

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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.

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

Date: 06/01/2006

Revision Date: 04/01/2011

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 Plus™ (Identifiler Direct, Minifiler™ or Yfiler™) Allelic Ladder: (included in the Identifiler Plus™, Identifiler Direct, 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%)

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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 Identifiler Plus™ (Identifiler Direct, 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 Plus™ PCR Amplification Kit User's Manual
AmpF/STR Identifiler Direct™ PCR Amplification Kit User's Manual
AmpF/STR Yfiler™ PCR Amplification Kit User's Manual
AmpF/STR Minifiler™ PCR Amplification Kit User's Manual
Applied Biosystems 3130 User's Bulletin.

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Applied Biosystems 3130 User's Manual.

Applied Biosystems 3130 Getting Started Guide.

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

Date: 04/01/2008

Revision Date: 04/01/2011

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 Plus™, Identifiler Direct™, Yfiler™, or Minifiler™ Allelic Ladder: (included in the Identifiler Plus™, Identifiler Direct™, 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.

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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 Plus™ (Identifiler Direct, 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).

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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'".

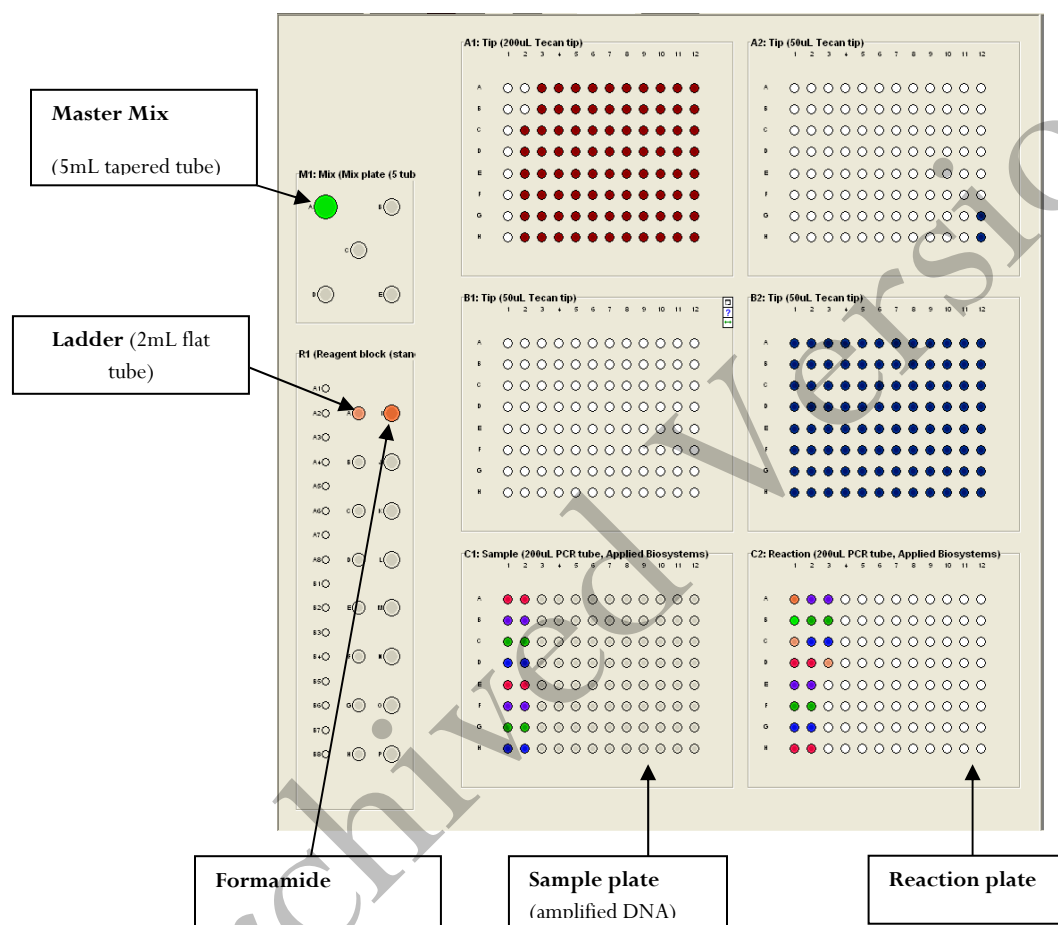
10. To signal the CAS-1200 to begin the protocol, either click on the green dot with the arrow in it on the shortcut tool bar, or choose "Control" on the tool bar and select "Start." In the Checklist window, click on each message box, such that there's an X in each box. Verify that the tip disposal box 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.

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- 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.

Deck Layout Diagram



- 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.

- 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:

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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

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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

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

Date: 06/01/06

Revision Date: 05/01/2012

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.

The screenshot shows the 'New Plate Dialog' window. It contains the following fields and values:

- Name: Example
- Description: Casework samples
- Application: GeneMapper-3130A
- Plate Type: 96-Well
- Owner Name: SDPD
- Operator Name: SAM

At the bottom are 'OK' and 'Cancel' buttons. Arrows from the text on the right point to the Name, Description, and Application fields.

Plate Name (see above note)

Description (optional)

Application will be

GeneMapper 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

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for the entered samples.

GeneMapper Plate Editor

File Edit

Plate Name: Example Operator: SAM

Plate Sealing: Septa Owner: SDPD

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						

Description: Casework samples

Ok Cancel

b. An optional Comment column is available.

GeneMapper Plate Editor

File Edit

Plate Name: 3130_Vandenberg Operator: SAM

Plate Sealing: Septa Owner: SDPD

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_Protocol				
B01		SDPD_Results_Group	SDPD_Identifier_Protocol				
C01		SDPD_Results_Group	SDPD_Identifier_Protocol				
D01		SDPD_Results_Group	SDPD_Identifier_Protocol	SDPD_Results_Group	SDPD_Identifier_Protocol		
E01		SDPD_Results_Group	SDPD_Identifier_Protocol				
F01		SDPD_Results_Group	SDPD_Identifier_Protocol				
G01		SDPD_Results_Group	SDPD_Identifier_Protocol				
H01		SDPD_Results_Group	SDPD_Identifier_Protocol				
A02		SDPD_Results_Group	SDPD_Identifier_Protocol				
B02		SDPD_Results_Group	SDPD_Identifier_Protocol				
C02		SDPD_Results_Group	SDPD_Identifier_Protocol				
D02		SDPD_Results_Group	SDPD_Identifier_Protocol				
E02		SDPD_Results_Group	SDPD_Identifier_Protocol				
F02		SDPD_Results_Group	SDPD_Identifier_Protocol				
G02		SDPD_Results_Group	SDPD_Identifier_Protocol				
H02		SDPD_Results_Group	SDPD_Identifier_Protocol				
A03		SDPD_Results_Group	SDPD_Identifier_Protocol				
B03		SDPD_Results_Group	SDPD_Identifier_Protocol				
C03		SDPD_Results_Group	SDPD_Identifier_Protocol				
D03		SDPD_Results_Group	SDPD_Identifier_Protocol				
E03		SDPD_Results_Group	SDPD_Identifier_Protocol				
F03		SDPD_Results_Group	SDPD_Identifier_Protocol	SDPD_Results_Group	SDPD_Identifier_Protocol		
G03		SDPD_Results_Group	SDPD_Identifier_Protocol				
H03		SDPD_Results_Group	SDPD_Identifier_Protocol				

Description: Low level stochastic experiment
Randomized blind decision

Ok Cancel

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- c. In the Sample Type column, the Size Standard column, the Panel column, and the Analysis Methods column, select the appropriate selection from the drop-down list for the kit being used. Scroll across to the Results Group 1 column, select **SDPD_Results_Group** from the drop-down list which is the same regardless of kit type. In the Instrument Protocol 1 column, select the appropriate protocol from the drop-down list. Note: The **SDPD_Identifier_Protocol** will be the standard protocol used for most kits and is set with a 5 second injection (see the Table below for the appropriate selections for each kit). For all columns with the exception of Sample Type, fill down to select for all samples. See below for details on altered injection times and re-injections.

Kit	Analysis Method	Panel	Size Standard	Protocol
ID Plus	ID-X Identifier Plus	Identifier_Plus_Panels_v1X	CE_G5_Identifier_Plus_GS500	SDPD_Identifier_Protocol
Minifiler	ID-X Minifiler	Minifiler_GS500_v1X	CE_G5_HID_GS500	SDPD_Identifier_Protocol
Yfiler	ID-X Yfiler	Yfiler_v1X	CE_G5_HID_GS500	SDPD_Identifier_Protocol
ID Direct	ID-X Identifier Direct	IdentifierDirect_GS500_v1X	CE_G5_IdentifierDirect_GS500	SDPD_Identifier_Protocol

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

Important: For amplification blanks and reagent blanks the injection time used should be equal to the greatest injection time from any of the evidence samples associated with it.

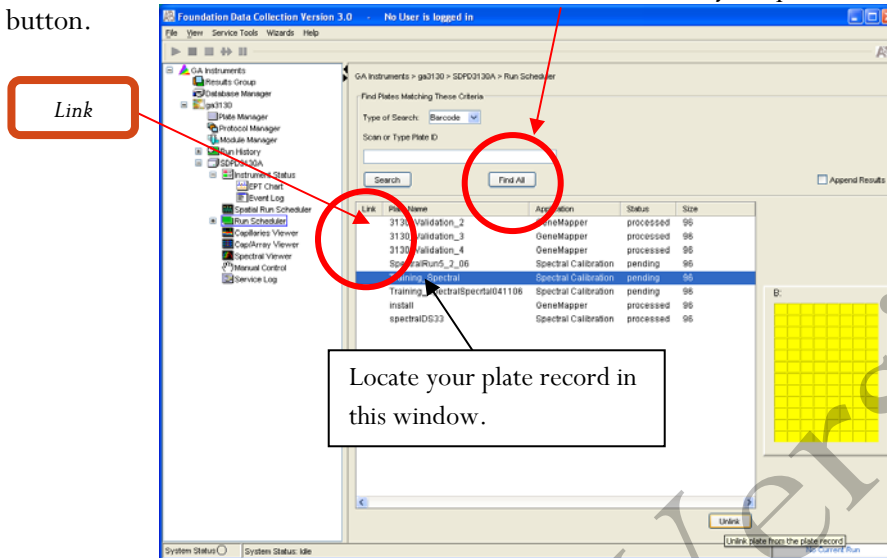
After clicking **OK** within the plate editor, the completed plate record is stored in the Plate Manager database. Once in the Plate manager database, the plate record can be searched for, edited, exported, or deleted.

4. If you want to inject a sample more than once within a run:
- Select **Edit > Add Sample Run**. Additional Results Group and Instrument Protocol columns are added to the right on the Plate Record.
 - 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.

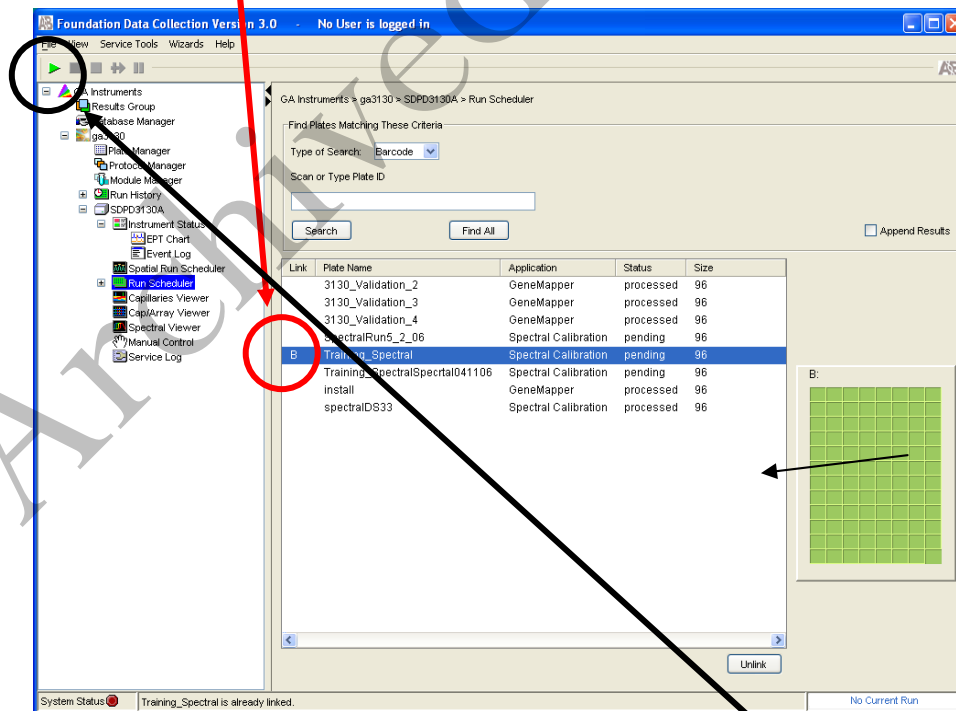
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5. In the navigation pane of the Data Collection software click **GA Instruments > ga3130 > SDPD3130A > Run Scheduler**. Click **Find All** or locate your plate record using the **Search** button.



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.



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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 additional injection times. These can be selected from the drop-down menu. If an injection time other than those available 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

Unit Technical Manual

Forensic Biology

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

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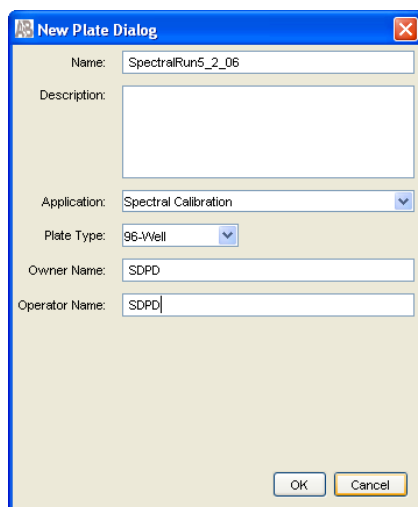
1. Remove DS-33 Matrix Standard tube from the refrigerator, vortex to mix, then spin briefly in a microcentrifuge.
2. For a 36cm array, combine 5-7uL of standard with 193-195uL formamide (for a total volume of 200uL) in a 1.5mL microfuge tube. Vortex to mix, then spin briefly in a microcentrifuge.
3. 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):

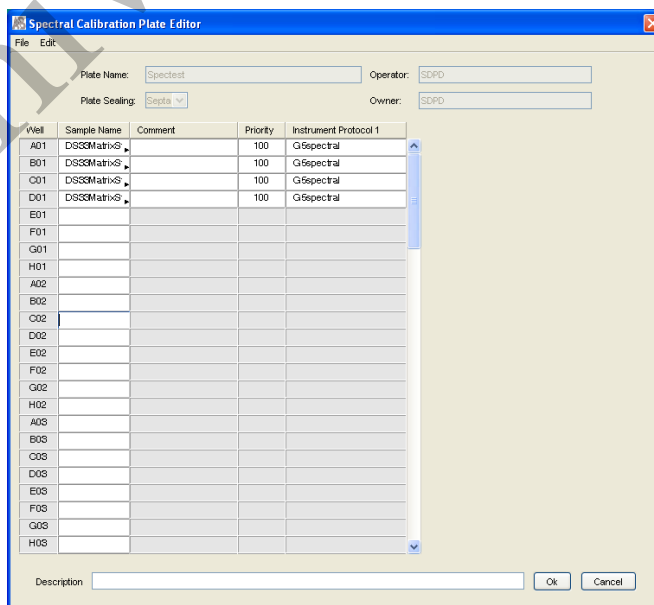
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The 'New Plate Dialog' box is a standard Windows-style dialog with a blue title bar. It contains several input fields: 'Name' (text box with 'SpectralRun5_2_06'), 'Description' (empty text box), 'Application' (dropdown menu with 'Spectral Calibration'), 'Plate Type' (dropdown menu with '96-Well'), 'Owner Name' (text box with 'SDPD'), and 'Operator Name' (text box with 'SDPD'). At the bottom right are 'OK' and 'Cancel' buttons.

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 menu bar with 'File' and 'Edit'. It has fields for 'Plate Name' (text box with 'Spectest'), 'Operator' (text box with 'SDPD'), 'Plate Sealing' (dropdown menu with 'Seals'), and 'Owner' (text box with 'SDPD'). The main area is a table with columns: 'Well', 'Sample Name', 'Comment', 'Priority', and 'Instrument Protocol 1'. The table lists wells from A01 to H05. Rows A01 through D01 are populated with 'DSSMatrixS', '100', and 'G5Spectral'. Below the table is a 'Description' text box and 'OK' and 'Cancel' buttons.

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				

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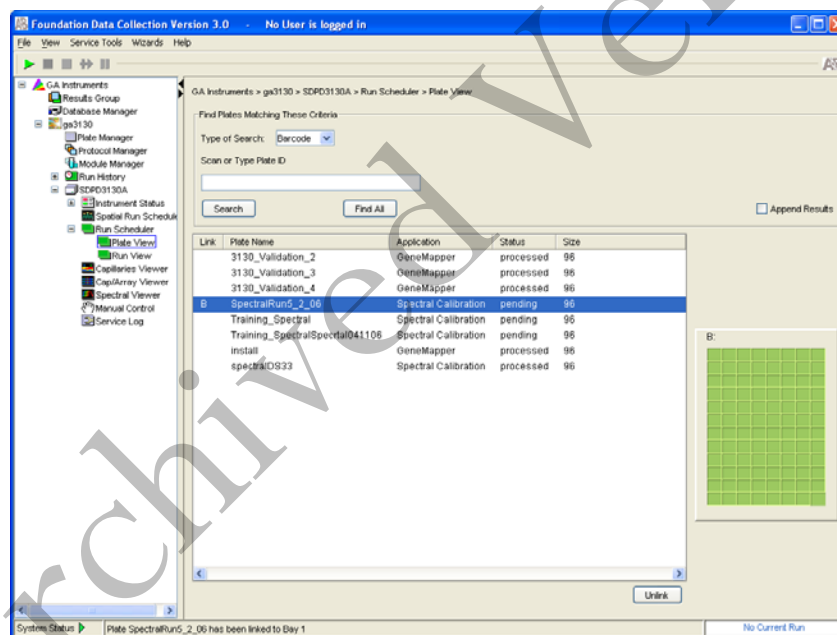
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Click **OK**.

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**

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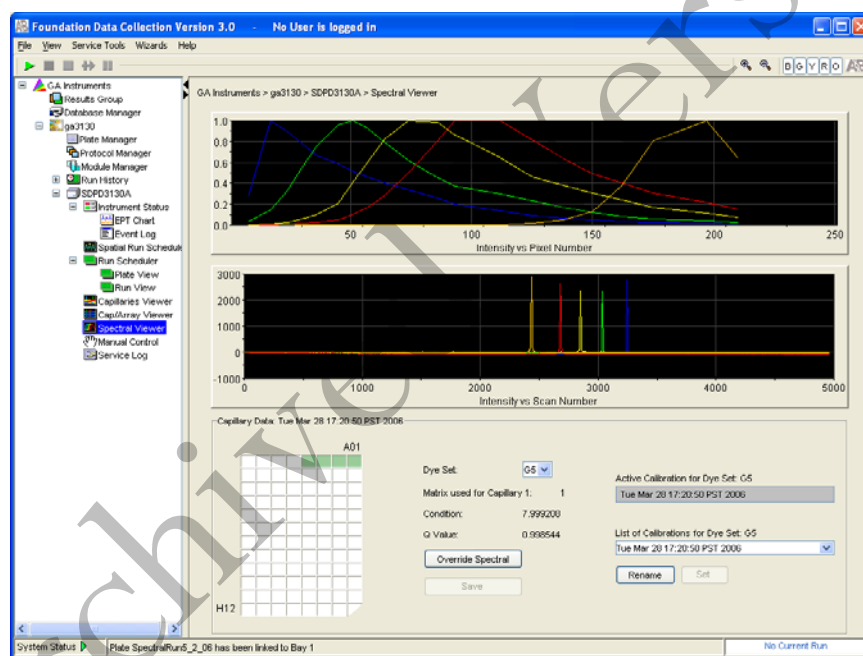
Log.

- **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:

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- 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 are sufficiently robust ($>750\text{rfu}$) and 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**.

Evaluating a Spectral

The manufacturer's engineering specifications state that pull-up between 1-5% is within tolerable limits. In general the average pull-up expected is approximately 3% with some outliers being around 5%. If the average pull-up observed is in excess of 3%, a new spectral should be created. If the average pull-up observed is consistently above 5%, even after attempts at new spectrals, it may indicate a problem with the instrumentation and the DNA Technical Manager needs to be informed.

REFERENCES:

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

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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:

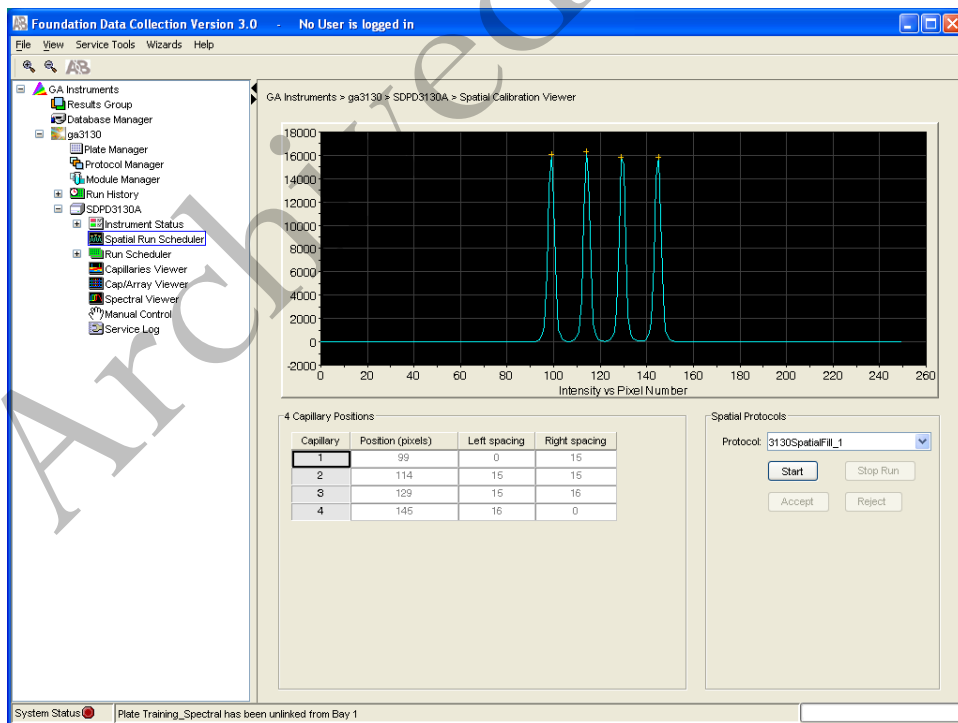
The 3130 Genetic Analyzer Data Collection software uses images collected during the spatial calibration to establish a relationship between the signal emitted by each capillary and the position where the signal is detected on the CCD camera. A spatial calibration should be performed when you:

- 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**.



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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:

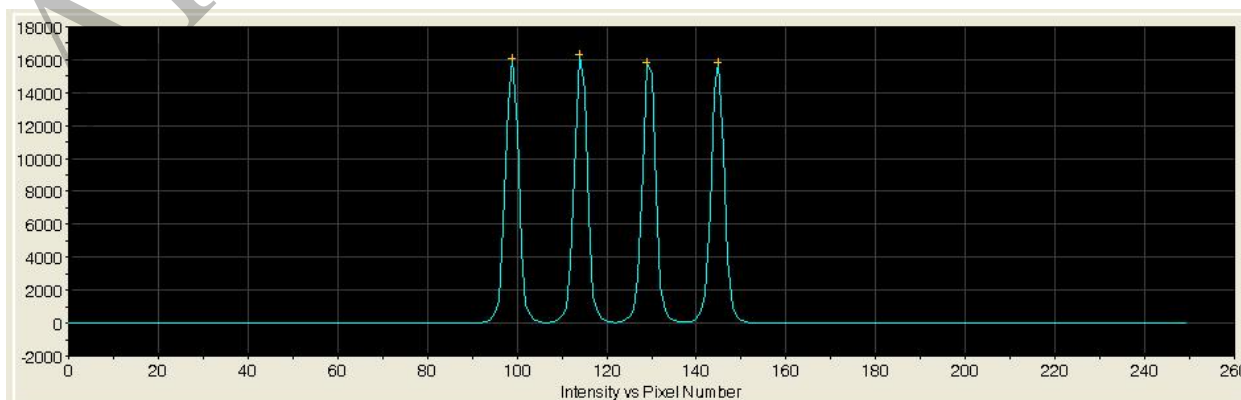
- 2 min. without filling the capillaries, or;
- 6 min. when filling the capillaries

(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, robust 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.



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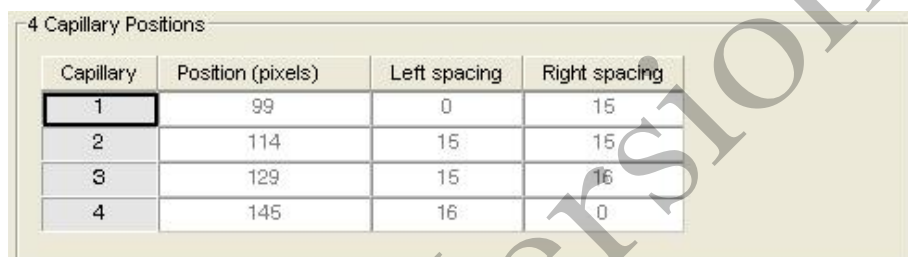
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2. Examine each row in the 4 Capillary Positions table and verify that the values in both the Left spacing and Right spacing columns range between 13 and 16 pixels. The “+” sign denotes the top of each peak. If a “+” is out of alignment, it may be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



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*.



REFERENCES:

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

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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.

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Forensic Biology

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

Date: 06/01/2006

Revision Date: **05/01/2012**

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 Plus, 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-X software by clicking on the icon located on the computer's desktop and log-in. Using the drop-down menu select **your user ID** as the User Name. 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** (see below). 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.

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Kit	Analysis Method	Panel	Size Standard	Protocol
ID Plus	ID-X Identifiler Plus	Identifiler_Plus_Panels_v1X	CE_G5_Identifiler_Plus_GS500	SDPD_Identifiler_Protocol
Minifiler	ID-X Minifiler	Minifiler_GS500_v1X	CE_G5_HID_GS500	SDPD_Identifiler_Protocol
Yfiler	ID-X Yfiler	Yfiler_v1X	CE_G5_HID_GS500	SDPD_Identifiler_Protocol
ID Direct	ID-X Identifiler Direct	IdentifilerDirect_GS500_v1X	CE_G5_IdentifilerDirect_GS500	SDPD_Identifiler_Protocol

Note: In general, analysis parameters will not change with the exception of the analysis range which will be dependent on the electrophoresis of each run.

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 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.

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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

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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. inits(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 Identifiler Plus™ PCR Amplification Kit User's Manual
AmpF/STR Identifiler Direct™ 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.

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

Date: 04/01/2011

Approved by: SAM

- A peak detection threshold of 50rfu will be used for analysis of AmpF/STR Identifiler Plus data. Peaks below 50rfu are not reported, but can be used qualitatively to determine the possible presence of a mixture.
- 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 Table below), however, peak height ratios tend towards larger imbalances as the height of the tallest peak decreases toward the stochastic threshold.

<u>Max.</u> <u>PH</u>	<u>Min.</u> <u>Ratio</u>	<u>Avg.</u> <u>Ratio</u>	<u>Std.</u> <u>Dev.</u>
0-100	33.33	71.94	16.81
100-200	22.16	66.97	18.92
200-300	21.29	68.89	20.64
300-400	21.50	73.93	20.11
400-500	49.21	83.61	12.41
500-600	52.88	87.15	10.83
600-700	57.72	88.25	8.25
700-800	62.91	89.35	8.52
800-900	48.30	86.52	9.12
900-1000	66.73	86.91	8.04
1000+	63.32	88.10	7.40

- 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	10.32%
D21S11	10.67%
D7S820	9.69%
CSF1PO	9.2%
D3S1358	12.27%
TH01	4.08%
D13S317	9.93%
D16S539	10.39%
D2S1338	12.44%
D19S433	11.21%
vWA	12.45%
TPOX	6.38%
D18S51	13.68%
D5S818	10.06%
FGA	13.03%

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- N+4 Stutter does occur more frequently than in past AB kits due to the different reaction mix formulation. On average, n+4 stutter products are less than 2% of the parent peak, however n+4 stutter peaks as high as 3% have been observed at several loci.
- Validation efforts have established the stochastic thresholds (or homozygote peak height threshold) above which both alleles of a heterozygote pair will most likely be detected. When evaluating an evidence profile, care should be taken when interpreting DNA peaks less than the designated rfu values as allelic drop-out may have occurred.

≤5 second injection	200rfu
7 second injection	280rfu
10 second injection	400rfu

Probability of Identity using the Applied Biosystems Identifiler Plus 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™ Plus User's Manual

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Forensic Biology

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

Date: 07/25/2007
SAM

Revision Date: 08/14/2009

Approved by:

- 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.

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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

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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).

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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

Unit Technical Manual

Forensic Biology

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

Date: 04/01/2012

Approved by: **SAM**

- A peak detection threshold of 50rfu will be used for analysis of AmpF/STR® Identifiler Direct® data. Peaks below 50rfu 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 70% (see Peak Height validation study), however, peak height ratios tend towards larger imbalances as the height of the tallest peak decreases toward the stochastic threshold.
- A global filter of 20.00% will be applied to data as opposed to marker specific stutter percentages.
- Validation efforts have established the stochastic thresholds (or homozygote peak height threshold) above which both alleles of a heterozygote pair will most likely be detected.

≤5 second injection	150rfu
7 second injection	210rfu
10 second injection	300rfu

Probability of Identity using the Applied Biosystems Identifiler Direct® kit.

The Probability of Identity (P_i) [Sensabaugh 1982] is the probability that two individuals selected at random will have an identical Identifiler Direct® kit genotype. For the Identifiler Kit® 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 Direct® kit. This data was taken from the Identifiler Direct® user's manual page 105.

REFERENCES:

SDPD Identifiler Direct Validation studies
AmpF/STR® Identifiler Direct® User's Manual

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METHOD: **Autosomal STR Interpretation Guidelines (Summary Document)**

Date: 06/01/2004

Revision Date: 11/01/2011

Approved by: SAM

Introduction

The Scientific Working Group on DNA Analysis Methods (SWGDM) states that:

“The interpretation of DNA typing results for human identification purposes requires professional judgment and expertise. Additionally, laboratories that analyze DNA samples for forensic casework purposes are required by the Quality Assurance Standards for Forensic DNA Testing Laboratories (effective July 1, 2009) to establish and follow documented procedures for the interpretation of DNA typing results and reporting. Due to the multiplicity of forensic sample types and the potential complexity of DNA typing results, it is impractical and infeasible to cover every aspect of DNA interpretation by a preset rule. However, the laboratory should utilize written procedures for interpretation of analytical results with the understanding that specificity in the standard operating protocols will enable greater consistency and accuracy among analysts within a laboratory. It is recommended that standard operating procedures for the interpretation of DNA typing results be sufficiently detailed that other forensic DNA analysts can review, understand in full, and assess the laboratory’s policies and practices. The laboratory’s interpretation guidelines should be based upon validation studies, scientific literature, and experience.”

Here we present a number of general interpretation guidelines for interpreting samples in forensic DNA casework. The rationale behind these guidelines is to establish a set of standards to ensure that conclusions are supported by reliable data, derived using a scientific approach, and that interpretation of results is as objective as possible and is consistent from one analyst to another.

The interpretation of DNA results should take into account the DNA results obtained, the nature of the sample, the condition of the profile, any forensically valid assumptions made, and should be scientifically justifiable and based on the collected experience and knowledge of the laboratory and the scientific community. These guidelines are designed to provide a general foundation for interpretation.

Please refer to the complete Autosomal STR Interpretation Guidelines document if more information is desired on any section within this summary document. See Appendix A for a glossary of terms.

DNA interpretation should take place in the following order:

- Preliminary Evaluation of Data and Allele Designation (section 1)
- Interpretation of DNA Typing Results (section 2)
- Comparison of DNA Typing Results (section 3)
- Statistical Analysis of DNA Typing Results (section 4)
- Reporting of DNA Typing Results and Conclusions (section 5)

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Section 1 – Preliminary Evaluation of Data and Allele Designation

1.1 Detection of peaks

The detection of peaks in the data is performed by the GeneMapper ID-X software. The analytical thresholds (peak detection thresholds) for the autosomal STR kits are listed within documents specific to each individual kit.

1.2 Evaluation of internal standards

The internal size standard should be evaluated to ensure that for each sample it has produced the expected pattern of peaks. These peaks should generally be between 1000 and 2000rfu for a 5 second injection and not show signs of poor resolution. If a sample has issues in the size standard that prevent proper sizing, or has signs of poor resolution, the sample should be reinjected or reprepared.

For Identifiler Plus and Minifiler analysis, GS500 LIZ peaks from 75-450bp are required. If no DNA peaks larger than 350bp are detected, it is possible to adjust the analysis range to 75-400bp.

1.3 Evaluation of allelic ladders

The GeneMapper ID-X software compares the ladder injections in the run with the expected sizes. GeneMapper ID-X analysis of at least one of the injected allelic ladders must produce the known allele peaks associated with the ladder in order for the software to type the other samples of the run. If a ladder injection passes the evaluation by the software, the ladder can be relied upon without further manual evaluation or reinjection.

1.4 Designation of alleles

Allele assignments are made by comparing the base pair size of the peaks in the amplified samples with the base pair size of the alleles within the allelic ladder(s). Allele assignments are performed automatically by GeneMapper ID-X. Analysts may on occasion need to manually edit “off-ladder” peaks to designate them as true alleles, or to redesignate peaks originally called as alleles by the software as artifacts.

1.4.1 If **multiple allelic peaks** are **improperly called as off-ladder alleles**, it may be necessary to only designate a subset of the allelic ladder injections as ladders, create separate projects using only those samples and ladder combinations that eliminate off-ladder alleles, or reinject samples and allelic ladders.

1.4.2 Occasionally an **allele** will be detected that is **outside the ladder range** for a given locus. 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.

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Occasionally an *allele* will fall *outside the ladder range between two loci*. An analyst should consider the size of the apparent allele and the expected zygosity in an attempt to determine to which locus the allele belongs. A list of variant alleles is maintained by NIST on the STRBase website (www.cstl.nist.gov/strbase/var_tab.htm). This list can be consulted to assist in determining to which locus the allele belongs. The allele will then be renamed with the appropriate (>) or (<) allele designation for the appropriate locus. Supporting documentation should be included in the administrative documents for the case.

- 1.4.3 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. D21S11 29.1 allele). *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* should be confirmed through reamplification.

1.5 Designation of artifacts

Some data contained within the electropherograms may not represent actual alleles that originate in the sample. Non-allelic peaks such as stutter, non-template dependent nucleotide addition (minus-A), nonspecific amplification products, spikes, raised baseline, pull-up, or disassociated primer dyes should be identified prior to the comparisons of reference samples to the data. Generally, non-allelic data such as stutter, non-template dependent nucleotide addition, disassociated dye, and incomplete spectral separation are reproducible; spikes and raised baseline are generally non-reproducible.

- 1.5.1 *Off-scale data* is often the result of over-amplification and can cause several other interpretational difficulties, such as: “minus-A” products, increased stutter, and pull-up (see below). If off-scale data is present in a sample, the sample may be re-injected with a decreased injection time, reamplified using less input DNA, or the amplified product may be diluted for reinjection.
- 1.5.2 *Pull-up* results from poor spectral separation and is characterized by signal from a peak in one fluorescent dye color producing a smaller, artifactual peak in another, often adjacent, dye color. Amplification of less sample, injection of a sample for less time, dilution of amplified product, or running the samples after creating a new spectral for the instrument can reduce pull-up peaks.
- 1.5.3 *Minus-A* (-A) is a form of PCR product that does not possess an extra nucleotide at the 3' end. Over-amplification can result in prominent “minus-A” products. Re-amplifying using less input DNA will likely reduce minus-A peaks. Incubating amplified products at 60°C for an additional time period may also reduce minus A.

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- 1.5.4 **Stutter** peaks are minor peaks appearing one repeat unit smaller (e.g. $n-4$ in tetranucleotide loci) or larger ($n+4$ in tetranucleotide loci) than a primary STR allele. If a called peak is suspected to be stutter, the validation study should be consulted to determine the expected stutter percentage for that allele. If the observed percentage is within the expected stutter percentage range for that allele, but above the filter threshold, an analyst may determine the peak to be stutter. For alleles that are at the larger base pair range of a locus, if the suspected stutter peak is above the stutter filter, but based on the validation consistent with the expected percentage of stutter for an allele of that size an analyst may determine the peak to be stutter. Additional factors such as spectral overlap or the presence of a potential stutter peak between two true alleles may enhance stutter peaks above the expected values. Ultimately, re-amplification can help determine if a peak is truly stutter or if it may be DNA from a secondary source. Unfiltered stutter peaks that are more than two standard deviations from the mean observed for an allele during the validation study will be listed in the table of results if the sample is not reamplified or if the unfiltered stutter is determined to be reproducible through re-amplification.
- 1.5.5 **Spikes** are artifacts of electrophoresis, which may resemble DNA peaks. Often spikes will be narrower than true DNA peaks and will be filtered by the GeneMapper ID-X software. 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.

1.6 Evaluation of analytical controls

If the analytical controls are properly designated, the GeneMapper ID-X software will flag controls that fail to meet expectations. Controls that have passed GeneMapper ID-X verification can be relied upon without further evaluation.

- 1.6.1 The **reagent blank** is a check for possible contamination of the sample preparation reagents by extraneous DNA. If DNA types are detected in the reagent blank this is an indication of a possible contamination event or that extraneous DNA was introduced into the sample from some unintended source. Based on an evaluation of the DNA types in the reagent blank, the results of associated samples may be deemed inconclusive. The analyst and the DNA Technical Manager will confer on an appropriate action based on the particular circumstance.

Samples extracted in a batch where the reagent blank shows DNA types greater than the interpretation threshold may need to be re-extracted. Not all contamination events will necessitate reanalysis and will be judged on a case-by-case basis.

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In some cases, re-extraction of samples affected by a reagent blank containing detectable DNA may not be possible. Results for these samples may be reported if any possible concerns related to the reagent blank are adequately explained.

- 1.6.2 The **negative amplification control** (Amplification Blank, NAC, or No DNA Control) is a check for possible contamination of samples during the amplification process. The appearance of DNA types in this control indicates that problems similar to those described for the reagent blank may have occurred during the amplification.

The interpretation of detectable DNA in the amplification blank should take into account the results obtained from any samples and reagent blanks amplified in the same batch. A batch where both the amplification blank and the reagent blank give the same typing result, or where only the amplification blank gives a result, may be caused by a problem limited to the amplification process. In this case, re-amplification of the samples may resolve the problem. A batch that shows a typing result for the reagent blank, but not for the amplification blank is more likely to be caused by an extraction-related problem.

If the reagent blank and amplification blank give different typing results, both the extraction and amplification processes are potentially suspect and should be repeated.

- 1.6.3 The **positive amplification control** (PAC) is a check for effectiveness of the amplification and the Short Tandem Repeat (STR) analytical processes. This control should always show interpretable results consistent with the known genotype published in the DNA kit literature. No other interpretable peaks should be present. Peaks that are determined to be artifacts (elevated stutter, pull-up, etc) are not considered interpretable peaks.

Results from samples associated with a positive amplification control for which these criteria are NOT met must be jointly evaluated by the DNA analyst and the DNA Technical Manager (or his/her designee). On a case-by-case basis, the DNA Technical Manager will evaluate the results obtained and determine the appropriate interpretation, taking the observed control results into account. If the observed control results bring the reliability of a sample result into question (as determined by the DNA Technical Manager), the sample result will not be interpreted or reported. The evaluation of the control results and the DNA Technical Manager's approval (or his/her designee's) of the process must be documented in the analyst's case notes and, in an Unexpected Results Summary (see FB policy manual section 1.6). If the results from samples associated with the failed positive control are deemed usable, the failure of the positive control must be included in the final report.

If the cause of the positive amplification control cannot be determined, samples associated with the failed positive amplification control should be reamplified. Samples associated with the failed positive control that were consumed for the original amplification would require re-examination (re-extraction) of the original evidence. If no evidence remains then the samples will be reported as inconclusive.

If the cause of the positive amplification control cannot be determined, the only time the results from samples associated with a failed positive amplification control that were consumed

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for the original amplification will be reported is when they form the basis of an exclusion. In this instance, troubleshooting of the problem must lead to the determination that the amplification for that sample is reliable and full disclosure of the unexpected result (failed positive amplification control) as well as the exclusion will be documented in the final report.

1.7 Evaluation of samples that have been reinjected, reamplified, and/or reextracted

- 1.7.1 Analysts should evaluate *multiple data sets for an item amplified with the same autosomal STR kit* to determine if there are indications of differences in peak proportions between the injections/amplifications for the sample. Analysts must indicate in the table of results if the represented result is a composite of multiple amplifications/or injections (see section 5). 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. If significant differences exist between the data generated from reamplification of the same DNA extract the analyst may choose to consider the data as separate results instead of generating a composite profile.
- 1.7.2 Analysts should evaluate data for an *item amplified with multiple autosomal STR kits* to verify that no unexplainable discordances exist between loci common to the kits. Possible reasons for discordances include; but are not limited to; degradation or inhibition affecting one of the amplifications to a greater degree than the other, variation in primer sequence, stochastic effects of low level samples, and the slight differences in peak proportions typically encountered during multiple amplifications of mixed samples.

Section 2 – Interpretation of DNA Typing Results

In general, interpretation of DNA samples will be completed prior to comparison of reference samples. All interpretation of samples will be performed using the actual electrophoresis data and not the table of results. DNA interpretation will include the following steps:

- Determine whether a sample is a mixture (section 2.1)
- Estimate the number of contributors to a sample (section 2.2)
- Determine whether stochastic effects are likely (section 2.3)
- Identify whether a sample is degraded and/or inhibited (section 2.4)
- Determine contribution level in mixed DNA samples (section 2.5)
- Document relevant assumptions (section 2.6)
- Determine which loci and combinations of DNA types are useful for statistical support of inclusions (section 2.7).

These steps will be documented in the analytical record on an interpretation worksheet prior to comparison to reference samples.

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2.1 Determination of whether a sample is a mixture

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 (peak height ratios <60%).
- 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 (see section 5).

2.2 Estimation of the number of contributors to a sample

Conclusions with respect to the minimum number of contributors must be included in a report. Generally, an estimate of the minimum number of contributors is based on the locus that exhibits the greatest number of allelic peaks and the fact that each contributor would contribute at most two alleles to the locus (although tri-allelic patterns could be present, but are extremely rare). Additional information such as expected peak height ratios can be used to determine minimum number of contributors. While the minimum number of contributors to a mixture can be established with a high probability, an absolute determination of the number of contributors cannot be ascertained. Assumptions as to the number of contributors can be made if the data appears consistent with a given number of contributors (see section 2.6)

2.3 Determination of whether stochastic effects are likely

Our validation efforts determined that for single source samples amplified with Identifiler Plus injected for five seconds, both alleles of a heterozygote pair will be detected when one of the alleles reaches at least 200 RFU (greater than 99% of the time). With Minifiler, the stochastic threshold was determined to be 450 RFU. Please refer to the Identifiler Plus Interpretation Thresholds document for the stochastic thresholds for altered injection times.

The analyst should consider the possibility that one or more DNA types may have dropped out if data is detected below the stochastic threshold. DNA types below the stochastic threshold will be designated with an asterisk (e.g. 16*) in the table of results (see section 5).

2.4 Determination of whether inhibition and/or degradation is present

2.4.1 **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. Examples of the effects of inhibition

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can be observed in the SDPD Inhibition presentation on the FB network in the presentations folder.

Evaluation of the IPC in the Quantifiler assays can be helpful to determine whether inhibitors may be present. Because a larger amount of DNA may be amplified than used for quantitation, a sample with an IPC result within expectations may still show evidence of inhibition when amplified.

Several options exist to reduce inhibition. Purifying the sample using the QIAGEN BioRobot EZ1 reduces some inhibitors as compared to organic purification. Reducing the amount of template DNA amplified can also reduce the amount of copurified inhibitors introduced into the PCR process. The Identifiler Plus and Minifiler kits have been optimized to reduce the effects of PCR inhibitors and can be used to improve previous results from kits (Profiler Plus, Cofiler, or Identifiler) more susceptible to inhibition.

- 2.4.2 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 will affect all contributors to a DNA mixture equally and could raise the concern of actually detecting all alleles that should be present in the mixture. Degradation, however, may not affect all contributors equally (**differential degradation**) 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.

Amplification of single source samples with Minifiler may provide better results than Identifiler or Identifiler Plus if degradation is suspected.

2.5 Determination of contribution levels in mixed DNA samples

The individual peak heights of alleles within a mixture can provide information regarding the relative contribution level (major/minor) to the overall mixture for people possessing those types. This assessment begins by determining whether there appears to be multiple significant contributors (section 2.5.1). Once balance has been evaluated, the mechanism for identifying DNA types more likely from minor DNA contributors can be determined. The determination of the appropriate method for assigning DNA types likely from minor contributors is critical in the interpretation of complex mixtures.

Validation studies conducted on 2-, 3-, and 4-person mixtures indicates that the partitioning model presented below can be extremely helpful in the interpretation of complex mixtures. This model will

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not apply to all mixtures and consideration should be taken in applying the partitioning model, especially for complex mixtures outside the scope of the validation (i.e. mixtures of 5- or more people). In mixtures known to contain at least 5- or more people, for instance, if the loci with the most DNA types detected contain data indicating that any DNA type could pair with any other at the marker (based on validated peak height ratio expectations), there may not be any contributors contributing more DNA than another. Not applying the partitioning model would be the best approach to interpreting mixtures in these instances.

The two methods for assigning minor DNA types in a mixture are by using 1/3 the tallest peak at a marker or using a threshold of <10% the total rfu detected at a locus (these are explained further in sections 2.5.1.1 and 2.5.1.2). Using a threshold of 1/3 the tallest peak at a marker will generally provide a subset of DNA types that includes the genotype of the single strongest DNA contributor in a mixture and is used when there is an indication that the mixture is imbalanced in contributions levels between the contributors. Using the threshold of <10% the total rfu detected a marker will provide the subset of DNA types that include the genotypes for the two or three strongest contributors to the mixture.

DNA types identified as more likely from minor DNA contributors will be designated by brackets (e.g. [16]) in the table of results (see section 5).

In general, a **major DNA contributor** is an individual 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. Major contributors to a DNA mixture do not possess DNA types that are designated as minor alleles unless a significant amount of stacking of alleles is likely.

In general, a **minor DNA contributor** is an individual who appears to contribute a small portion of the DNA to a mixture. Minor contributors possess multiple DNA types that are designated as minor alleles. If no alleles from minor contributors are identified in the mixed DNA sample then the mixture should be considered unresolvable in terms of determining major and minor DNA contributors.

When a DNA profile presents as a mixture of three or more individuals with at least one major DNA contributor, at least one minor DNA contributor and a contributor at a level somewhere between the major and minor contributor(s), that contributor will be a **mid-level contributor** or **second significant contributor**. Additional terms such as “significant minor” may be used to further clarify assumptions about the contribution levels in the mixture provided they are clearly defined in the case notes (see section 2.7.1.4 c). The interpretation of the presence of a significant second contributor to a mixture should be based on a discernable difference in peak intensities between the second significant contributor and any other minor alleles present in the mixture. Alleles designated as likely originating from the significant second contributor should be documented in the analytical record.

2.5.1 In general, mixtures having multiple major contributors (a balanced mixture) in a mixed DNA profile can be determined by examining the results using the following criteria:

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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 the above indicators are not met, but the overall information in the profile indicates that the situation of at least two significant contributors is likely, analysts should adopt the <10% the total rfu at a marker for the designation of minor contributor alleles. Some two person mixtures may not have 3 loci with 4 DNA types and therefore peak proportions at the other loci should be evaluated for indications of balance. Loci with two alleles that have close to 1:1 or 3:1 ratio, or three alleles with some combination close to a 2:1:1 ratio is indicative of balance between significant contributors.

Additional factors or phenomena 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, such as low level mixtures that may have peaks below the detection threshold that suggest balance in the mixture.

2.5.1.1 Minor contributor types in *mixtures with a single major contributor indicated*

In mixtures with a single major DNA contributor indicated (imbalanced 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.

Validation data has shown in mixtures that have varying contribution levels from the DNA donors, individuals that generally contribute greater than 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 some circumstances the imbalance in a sample will be so extreme, that the genotype from a single major contributor (predominant) see 2.7.1.4a can clearly be deduced.

2.5.1.2 Minor contributor types in *mixtures with multiple major contributors*

In mixtures with multiple major contributors (balanced mixtures), minor contributor alleles will generally be less than 10% of the total RFU at a locus. 10% is meant as a guideline and phenomena such as the overall intensity of the types in the mixture, stutter and baseline artifacts may affect the percentage from locus to locus. 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. When the overall peak heights in a mixture are low, it is more likely to have stochastic issues affect expected peak height

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ratios of the contributors, as such, 7% will be used as the threshold for loci with less than 1600 total rfu in mixtures with multiple major contributors (balanced mixtures).

2.5.1.3 Minor contributor types in mixtures obtained using the *Minifiler amplification kit*

The Minifiler amplification kit inherently has more peak imbalance than the Identifiler Plus DNA testing kit with peak height ratios between paired peaks sometime observed as low as 32%. As such using $<1/3$ the tallest peak as an indicator of a DNA type from a minor DNA contributor is inappropriate. In Minifiler derived mixtures, minor contributor alleles will generally be less than 10% of the total RFU at a locus. 10% is again meant as a guideline and phenomena 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.

- 2.6 When identifying DNA types in a mixture that are more likely from minor contributors, the robustness of the DNA profile should be taken into consideration in evaluating and interpreting a DNA profile. As results approach the stochastic range (e.g. 200 RFU for Identifiler Plus), peak height ratios of less than 30% may be observed. Based on the increased imbalance expected with lower levels of DNA, loci where the tallest peak is less than or equal to 350rfu should not be used to determine major/minor contributor DNA types. In some mixtures where the majority of the types detected are stochastic level and only a few DNA types are above 350rfu, analysts may determine that assigning major vs minor alleles should not be done due to the low level of the data.

2.7 Documenting Assumptions

In certain circumstances, *assumptions regarding DNA profiles* may be made to assist the analyst in drawing conclusions and to put the evidence in the proper context. All assumptions regarding any interpretation will be presented in the report and should also be in the notes (see section 5).

- 2.7.1 While the absolute *number of contributors* to a DNA mixture cannot be determined in most instances. In certain circumstances, especially fairly robust samples with few contributors, factors such as allelic dropout, degradation, inhibition, masking of alleles, partial profiles, etc. are believed to play a less significant role and the number of contributors to a mixture or the number of major contributors to a mixture can be assumed with confidence and should be supported by the data.
- 2.7.2 In certain circumstances *assumptions regarding the presence of a contributor* in a DNA mixture is permissible (i.e. intimate samples – see complete document for additional instances where it may be done). In these circumstances the DNA profile from the assumed

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contributor(s) can be used to assist interpretation of the additional contributor(s) DNA profile/types (section 2.7).

The assumption of the presence of one or more contributors may require the analyst to change the minimum number of contributors to a mixture. It also may be possible that the assumption of the presence of a contributor may change the confidence with which an assumed number of contributors can be made.

2.8 Determining the Loci to be used for Statistics/Comparisons

Prior to making comparisons, the profile should be evaluated to ***determine what loci and/or combinations of DNA types are acceptable to support a conclusion statistically.*** Much of the evaluation to determine what parts of the DNA results can be used for statistical weight takes into account the presence of DNA types below the stochastic threshold and whether assumptions regarding the number of contributors have been made. If all DNA types are above the stochastic threshold, all DNA types can be used for comparison, but it may be useful to further refine the genotypes for comparison if a limited number of combinations are likely.

If an analyst believes that ***filtered stutter peaks*** should be evaluated for comparison, the mean stutter percentage observed for the allele during validation and other studies should be used to assess whether excess stutter is likely. Peaks in stutter positions that are more than 2 standard deviations above the mean observed stutter indicate the presence of excess stutter and that DNA contribution from a source other than stutter is likely. If a peak in a stutter position is deemed to have originated from a separate contributor to the sample, then all stutter peaks in the mixture should be evaluated in the same manner and included in the assessment of the mixture. After the evaluation of stutter peaks in the mixture it may be necessary to reassess the minimum number of DNA contributors to the sample.

2.8.1 Samples with an assumed number of contributors.

2.8.1.1 An apparent ***single source sample*** consists of no more than two relatively balanced alleles at each locus. Single source samples with three allele peaks at a single locus and/or partial null alleles have been reported, though are extremely rare. For single source samples, allele number and peak height ratios should be appropriate for the level of the detected DNA.

Alleles used for comparison:

When two alleles, or one allele detected above the stochastic threshold, are detected at a locus, allelic dropout is not a concern. These alleles are used for comparison. When one allele is detected and the allele is below the stochastic threshold, allelic dropout is possible. The allele is used for comparison, but the analyst should consider that any other allele could be paired with the detected allele.

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- 2.8.1.2 When the data supports it, an analyst may make an ***assumption as to the number of DNA contributors in a mixture***. It is not recommended that analysts make assumptions on mixtures of four or more individuals as the data in those mixtures becomes much harder to interpret.

When the number of contributors to a DNA mixture is assumed to be two or three people, there are restrictions on the genotypes that could have contributed to the profile. Given the number of assumed contributors, the number of detected alleles, and the ratio of the contributors, it is possible to remove certain allele combinations from consideration.

Alleles used for comparison:

All alleles at loci where the analyst is confident that all DNA types are detected will be used for comparison for mixtures of two or three people.

Loci with DNA types below the stochastic threshold can be used in some circumstances in mixtures of two or three people:

- a) If all DNA types from all contributors appear to be present at a locus, all alleles at the locus, including those below the stochastic threshold, will be used for comparison.
- b) If DNA types below the stochastic threshold are observed and at least one allele is present from each contributor (three alleles in a two person mixture or five alleles in a three person mixture), all alleles can be used for comparison if the analyst considers the possibility that the DNA type(s) below the stochastic threshold can be paired with an undetected allele.
- c) If the analyst does not have confidence that at least one allele has been detected from each contributor, the locus should not be used for comparison if DNA types below the stochastic threshold are detected unless a predominant contributor or major contributors are present (see section 2.7.1.4a).

- 2.8.1.3 Mixtures of two or three contributors where a forensically valid assumption regarding a contributor can be made

In addition to the guidelines in section 2.7.1.2, when the number of contributors is assumed and a forensically valid assumption regarding a contributor is made, further refinement of the mixture is possible. In these circumstances, possible combinations representing the source(s) of the DNA foreign to the assumed contributor(s) should be considered for comparison as described below. DNA types foreign to the assumed contributor(s) are referred to as obligate foreign alleles.

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Loci used for comparison:

Loci where the analyst is confident that all DNA types are detected can be used for comparison. Loci with DNA types below the stochastic threshold can be used if all of the types below the stochastic threshold are due to the assumed contributor and/or if at least one DNA type from the foreign contributor(s) is present, in a similar fashion to 2.7.1.2a and 2.7.1.2b.

2.8.1.4 Some samples may not allow for confident assumption as to the number of total contributors, but do allow for an ***assumption as to the number of major contributors***.

- a) ***Assumption of one major contributor.*** If the overwhelming contribution to the DNA in a mixture appears to originate from a single individual then the term predominant DNA contributor 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 deduced from all of the DNA markers tested. In general, all alleles from predominant DNA contributors will be designated as major. 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, preferential amplification, etc) should all be considered in the interpretation of a predominant genotype.

In some circumstances a single major contributor will be apparent, but some ambiguity may exist for the genotype of the major contributor at a few loci due to stacking of minor contributor alleles. Peak height ratio expectations may limit the ambiguity, but if uncertainty still exists, an analyst may choose all combinations of types that are not designated as minor at the ambiguous loci or all loci (see example in the complete document).

Alleles/loci used for comparison:

The DNA types for a predominant contributor will be considered for comparison in a similar fashion as single source samples (section 2.7.1.1).

Minor contributors to a mixture with a single major contributor, but no assumption as to the number of total contributors will be evaluated in a similar fashion as in section 2.7.2.2

- b) ***Assumption of two major contributors.*** In balanced mixtures, it is possible that two DNA contributors will contribute significantly more DNA than any other contributors. In this circumstance, an analyst can treat the two major contributors in a fashion similar to section 2.7.1.2.

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Minor contributors to a mixture with two major contributors, but no assumption as to the number of total contributors will be evaluated in a similar fashion as in section 2.7.2.2

- c) ***Assumption of one major contributor, one mid-level DNA contributor, and at least one minor DNA contributor.*** In certain mixtures, it is possible to identify a “mid-level” contributor that is contributing more than at least one other contributor but less than another. In these circumstances, the assumptions of the analyst should be documented in the case packet. Additional terms such as “mid-level” or “significant” can be used provided they are clearly defined in the case notes. Alleles designated as likely originating from the significant second contributor should be documented in the analytical record.

Alleles/loci used for comparison:

The interpretation of the presence of a significant second contributor to a mixture should be based on a discernable difference in peak intensities between the second significant contributor and any other minor alleles present in the mixture. Alleles designated as likely originating from the significant second contributor should be documented in the analytical record. Care should be taken with any alleles thought to be from a second significant contributor if those alleles are either in the stochastic range.

- 2.8.2 Even when the ***number of total contributors and/or the number of major contributors cannot be confidently assumed***, the sample may be evaluated for the presence of possible major contributors (section 2.7.2.1) and minor contributors (section 2.7.2.2). In some circumstances, the mixture cannot be resolved into major and minor components (section 2.7.2.3) or is not useful for comparison (section 2.7.2.4).

- 2.8.2.1 ***Major contributors with no assumption of the number of contributors.*** Major contributors should not have types that are designated as minor contributor alleles (see section 2.5); the subset of alleles in a DNA mixture above the level of minor contributor alleles are considered possible major contributor DNA types. It may be possible that the number of major contributors can be assumed even if the number of total contributors cannot be confidently determined (see section 2.7.1.4).

Loci used for comparison:

Loci with all possible major contributor DNA types above the stochastic threshold will be used for comparison to the major contributor DNA types. If the mixture contains multiple major contributors (i.e. a balanced mixture), caution should be exercised in using loci for comparison where the total signal detected is less than 700rfu. At this level it is possible that major contributor alleles may be below the detection threshold of 50rfu (using the 7% threshold). Variables including the peak heights, number of

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detected alleles, and the minimum number of contributors will all play a part in determining whether a locus should be used for comparisons.

If possible major contributor DNA types at a locus are detected below the stochastic threshold, in a mixture evaluated without assumptions; the locus will not be used for comparison purposes to possible major contributors. The locus will be not be used for comparison due to the concern that one or more major contributors may have DNA types that were not detected.

2.8.2.2 Minor contributors with no assumption of the number of contributors. 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 (see section 2.5). Masking and allelic drop-out are more likely with minor DNA contributors. Caution should be taken when interpreting a mixture with only one or two minor DNA types in a *complex* mixture as allele sharing may have caused the allele designation to be a “minor” type(s). The validation data indicates that it is possible to have alleles less than 1/3 the height of the tallest in mixtures with relatively equivalent contributors due to allele stacking effects.

If no assumptions are made about the number of minor DNA contributors then all combinations of alleles at a locus should be considered as possibly originating from a minor contributor and not just genotypes containing the designated minor alleles.

Loci used for comparison:

Loci with minor contributor alleles present and where the analyst is confident that all DNA types are detected will be used for comparison to possible minor contributors. Analysts should be able to articulate their reasoning for the use of loci for comparison to potential minor contributors. Peak heights, number of detected peaks, minimum number of potential DNA contributors may all play a role in an analysts decision use a locus for comparison purposes. Loci with DNA types below the stochastic threshold will not be used for comparison purposes to possible minor contributors due to concern that one or more minor contributors may have DNA types that were not detected. If fewer than four loci are useful for comparison to possible minor contributors, the profile will be considered **uninterpretable** with respect to minor contributors.

2.8.2.3 Mixtures with no major contributors and no assumption of the number of contributors. Samples with no DNA types designated as minor contributor alleles (see section 2.5) are not resolvable into major or minor contributors. Mixtures, such as those of more than four contributors, may have DNA types designated as minor contributor alleles, but ambiguity may still exist as to whether one or more

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contributors is donating more DNA than others in the mixture. Such samples will be evaluated for possible **DNA contributors**.

Loci used for comparison:

Loci where the analyst is confident that all DNA types are detected will use all alleles for comparison to possible DNA contributors. Loci with DNA types below the stochastic threshold will not be used for comparison purposes to possible DNA contributors due to concern that one or more DNA contributors may have DNA types that were not detected. If fewer than four loci are useful for comparison to possible DNA contributors, the profile will be considered uninterpretable.

- 2.8.2.4 **Uninterpretable mixtures** are mixtures where no assumptions can be confidently made and where all observed alleles are below the stochastic threshold are not useful for comparison and are considered uninterpretable. A mixture may be useful for comparison to possible major contributors and may be uninterpretable with respect to possible minor contributors.

3. Comparison of DNA Typing Results

- 3.1 **Inclusion.** If a person of interest has no unexplainable differences from the alleles or allele combinations determined to be useful for statistical support of inclusions, and thus comparison, they are included as a source of the DNA or possible contributor to the DNA mixture.
- 3.2 **Exclusion.** If a person of interest has unexplainable differences from the alleles or allele combinations determined to be useful for comparisons, they are excluded as a source of the DNA or possible contributor to the DNA mixture.
- 3.3 **Inconclusive.** Some mixtures such as those with five or more contributors may not be suitable for comparison, unless a minimal number of major contributors exist. Comparisons to these mixtures will be considered inconclusive.

An analyst may determine that due to a combination of variables including (but not limited to) the number of DNA contributors as well as a lack of information in the results that no comparisons can be made to the profile (or comparison for possible minor contributors).

- 3.4 **Uninterpretable.** Mixtures with no assumptions made to the number of contributors, that have less than 4 loci available for comparison, will be deemed uninterpretable

If the positive amplification control does not provide the expected results, or if DNA types are detected in the negative amplification control or the relevant reagent blank, the sample may not be useful for comparison (see section 1.6) and would thus be uninterpretable.

4. Statistical Analysis of DNA Typing Results

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When a comparison leads to a conclusion that an individual is included as a possible source of the DNA or a possible contributor to a DNA mixture, an assessment of the significance of that inclusion should be performed by calculating the rarity of such an inclusion. If the inclusion has little probative value a calculation may not be necessary. If an individual is included as a possible contributor to the DNA from multiple items in a case, calculations may only be necessary for the most probative item(s) and/or the item(s) with the highest discrimination potential.

Calculations will only be performed on loci and/or allele combinations that have been deemed acceptable for comparison purposes (section 2.7).

All calculations will be generated for the Caucasian, African American and Southwestern Hispanic populations using the DNA allele frequency data taken from J Forensic Sci 1999, 44(6): 1277-1286 and for the D2S1338 and D19S433 loci from Forensic Science Communications 2001, 3(3) unless noted. If a different database is used for calculations, it should be included in the administrative documents with the case file and noted in the report.

The product rule will be used for calculations by determining the probability at each locus and multiplying the results for each locus together.

In general, Popstats is used for statistical calculations. In some circumstances the SDPD 2p calculator can be used for low level samples. There is also the SDPD RMP calculator, which can be used for mixtures, that allows for the exclusion of certain genotypes from the calculation.

4.1 For *single source samples* and samples with a ***predominant DNA profile*** the significance of a match should use the random match probability (RMP) formulae for single source samples. Loci with two alleles (heterozygotes) or with a single allele above the stochastic threshold (homozygotes) can be calculated using Popstats. For loci with a single allele below the stochastic threshold the analyst can use the SDPD 2p calculator in combination with the Popstats results or can decide not to use the locus for statistical weight if the added value would be insignificant to the overall conclusion. The formulae for the Random Match Probability for single source samples are shown below:

$f = 2pq$	(heterozygote)
$f = p^2 + p(1 - p)\theta$	(homozygote) ($\theta=0.01$ in most instances)
$f = 2p - p^2$	(single peak below stochastic threshold)

4.2 There are two methods used for calculations when an ***assumption is made regarding the number of contributors or the number of major contributors*** to a mixture: the likelihood ratio (section 4.2.1) and the random match probability (section 4.2.2). Both methods can be restricted (taking into account peak height ratios and mixture ratios) or unrestricted. The RMP can also be modified based on an assumed number of contributors to exclude unreasonable genotypes from consideration. Both methods can take into account the presence of an assumed contributor.

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- 4.2.1 If the number of contributors can be assumed with confidence, the **likelihood ratio** takes into account all of the information in the sample. The likelihood ratio is the ratio of two probabilities of the same event under two mutually exclusive hypotheses. Given the same data, it is possible to calculate more than one likelihood ratio under a different set of assumptions and hypotheses.

$$L = \frac{P(E|C_x)}{P(E|C_y)}$$

In general, the likelihood ratio formula is:

Typically the calculation in the numerator is the probability of obtaining the evidence under the hypothesis that a person of interest is one of the contributors to the DNA mixture along with additional known or unknown people. The calculation in the denominator is the probability of obtaining the evidence under the hypothesis that a randomly selected individual is contributing to the DNA mixture along with additional known (generally the same as the numerator) or unknown contributors. If the result of the ratio is a number greater than one, the DNA results are more probable if the person of interest is a contributor to the mixture. If the result of the ratio is less than one, the DNA results are more probable if the evidence is a mixture of DNA from randomly selected individuals.

In the SDPD lab, the likelihood ratio has been more commonly calculated when a contributor is assumed and the number of contributors is also assumed, but it can also be calculated without the assumption of a contributor. Likelihood ratios are also used in parentage and kinship calculations, but these will be dealt with later (sections 4.4 and 4.5).

Please see the complete document for additional scenarios where likelihood ratios could be useful. Popstats can calculate likelihood ratios; however it does not employ formulas to deal with restricted likelihood ratios or scenarios where allelic dropout is possible.

- 4.2.2 Although commonly used for single source samples, **random match probabilities (RMP)** calculations can also be performed for mixtures with an assumed number of contributors or for major contributors to a mixture with an assumed number of major contributors. The RMP calculates the probability of each genotype that could reasonably represent a contributor. The RMP differs from a combined probability of inclusion (see section 4.3) in that fewer possible genotypes exist due to the assumption regarding the number of contributors.

When a mixture is encountered that appears to have a single major contributor, but where some ambiguity exists in the genotype at one or more loci (see section 2.7.1.4a for an

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example), an RMP incorporating multiple genotypes at the ambiguous loci can be performed.

In balanced mixtures of DNA from two people, the RMP could be calculated by using only the possible combinations that could have contributed to the mixture (e.g. heterozygote combinations where four alleles are detected).

In circumstances where DNA types below the stochastic threshold are detected and where dropout has possibly occurred, a modified version of the $2p - p^2$ calculation in section 4.1 can be performed using the following formula for the alleles below the stochastic threshold (p, q, r, etc):

$$2(p + q + r + \dots) - (p + q + r + \dots)^2$$

In mixtures of DNA where the presence of a specific contributor is assumed, an RMP could be calculated for the combinations of DNA types foreign to the assumed contributor. Such calculations focus on the obligate alleles in the mixture.

4.3 Combined Probability of Inclusion

When no assumptions regarding the number of contributors can be made with confidence, the **combined probability of inclusion (CPI)** is used to express the significance of an inclusion. The probabilities of all possible combinations at each locus are calculated and summed to generate a probability of inclusion or PI. The PI for each of the loci acceptable for comparison are multiplied together to generate the CPI. The PI for each marker and the CPI are calculated as:

$$\begin{aligned} \text{PI} &= (p + q + r + \dots)^2 \\ \text{CPI} &= \text{PI}_1 * \text{PI}_2 * \text{PI}_3 * \dots \end{aligned}$$

A CPI using the possible major contributor DNA types (e.g. types above minor) can be calculated to express the significance of an inclusion as a major contributor. A separate CPI calculation using all DNA types can be performed to express the weight of an inclusion of a minor contributor. For samples without major and minor contributors, a CPI using all alleles can be performed to express the significance of an inclusion as a possible DNA contributor.

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4.4 Parentage

In cases involving disputed parentage, a parentage calculation will be provided using the formulae integral in the Popstats software. If manual calculations are necessary because the parents and child are from a racial group for which Popstats does not have relevant population data, the formulae can be found in the “Help” section of Popstats. Calculations for three parentage scenarios are performed: when one parent is alleged and the other is known (section 4.4.1), when one parent is alleged and the other is unknown (section 4.4.2), and when both parents are known and the child is alleged (section 4.4.3).

- 4.4.1 The scenario when an allegation of parentage exists and DNA types from the child, the known parent, and the alleged parent exist is referred to as a **parentage trio**. In this circumstance, a Parentage Index (PI) is calculated. The PI 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 a randomly selected, unrelated individual is the biological parent of the child. See the complete document for information on considering mutations.
- 4.4.2 In cases where one parent is alleged and the other parent is either unknown or for which DNA results are unavailable, a **single parent case** exists. These are often referred to as a “motherless paternity” or “fatherless maternity” cases. These calculations are performed by Popstats within its kinship tools.
- 4.4.3 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.
- 4.5 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. In cases involving disputed kinship, a kinship calculation will be provided using the formulae integral in the Popstats software.

5. Reporting of DNA Typing Results and Conclusions

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.

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5.1 Reporting of DNA typing results

If all samples tested gave no DNA typing results, an analyst may convey these results with a written statement to that effect in the results section of the report.

If DNA typing results were obtained for one or more samples, the DNA typing results for all samples will be reported in tabular form. If a low number of samples were tested, the table may be placed in the results section of the report. If a larger number of samples were tested, or if the analyst desires, the table should be placed at the end of the report with a statement in the results section directing the reader to the location of the table. If multiple items are tested, the table should include a clear indication of the item being presented. A brief description of each item is desired. An example of a table of results is shown below:

Description	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	Amel	D5S818	FGA
100XXXX Stain from pants	13, 14	30	9, 10*	ND	14, 15	9	8, 11	13*, 14	24*	13	14, 15	8*, 11	ND	X, Y	10, 12	21*
100XXXY Baseball cap	13, 14, 15, 16 [10]	28, 30 [32.2*]	8, 9, 10	11, 12 [13*]	14, 15, 17 [18]	6, 9 [9.3]	8, 11 [13]	11, 12, 13, 14 [10*]	19, 24, 26 [22]	13, 14, 14.2 [15.2]	14, 15, 16	8, 11	15, 17, 18, 20 [12*]	X, Y	10, 11, 12	20, 21, 24 [19*], [27*]
100XXYY John Smith	13, 14	30	9, 10	NT	14, 15	NT	8, 11	NT	NT	NT	14, 15	NT	15, 17	X, Y	10, 12	20, 21

1 = DNA results are a composite of two injections

2 = DNA results taken from Criminalist Joe Blow's report for case #XX-XXXXXX dated April 7, 2002

[] = DNA type(s) likely from minor contributor(s).

* = DNA type detected below the stochastic threshold

ND = No DNA types detected

NT = Not tested

If results from previous analyses are reported with results from current analysis, a reference to the report from which the previously obtained results are taken should be included in the table.

The table of results will indicate which types are minor contributors alleles and which types were detected below the stochastic threshold. If a composite profile is used, a note to that effect should be placed in the table. The table should specify if no results were obtained or if testing was not performed at a locus. If abbreviations or symbols are used in the table, a key to the symbols should be provided with the table.

In an effort to streamline the reporting process, especially in cases without suspects, uninterpretable samples may be omitted from the table of results. Victim profiles may also be omitted from the tabular results unless the profile comprises an element of a potentially probative evidence sample.

Asterisks indicating DNA types below the stochastic threshold are not required for reference samples containing heterozygous genotypes with one or more DNA types below the stochastic threshold.

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5.2 Reporting of conclusions

Conclusions for each item should be present in the conclusions section of the report. It is acceptable to group similar conclusions for multiple items together.

- 5.2.1 In general, conclusions should state whether a mixture was obtained, and if a mixture was obtained, the minimum number of contributors should be stated. The conclusion should indicate if the sample is not suitable for comparison purposes. A brief description of the reason that it is not suitable should be included. In the case of a mixture that is only suitable for comparison to possible major contributors, an indication should be included that the sample is not suitable for minor contributor statistical assessments and therefore unsuitable for comparison to possible minor contributors (see 5.2.1.1). It may in some circumstances be relevant to indicate whether male or female DNA is present in the sample even if no statistics/comparisons can be made to the male DNA.

If the sample is suitable for comparison and if comparisons are made, the conclusion should indicate whether the persons of interest are included or excluded. If the comparison is determined to be inconclusive, a statement giving reasons leading to the inconclusive determination should be included in the report.

If an inclusion is made, and if the inclusion warrants a probability calculation (see section 4), the applicable probabilities should be listed.

Any assumptions made regarding the comparisons and/or the probability calculations will be listed in the conclusion.

If the inclusion of an individual requires a larger number of contributors than the minimum based on allele number and peak height ratios, a statement to that effect should be included.

A statement as to whether DNA types or a subset of the DNA types from an item will be uploaded into CODIS should be included, if applicable.

- 5.2.1.1 Statement when the mixture, or a portion of the mixture (i.e. minors), is unsuitable for statistical calculations or comparisons:

Due to the number of possible DNA contributors and the low level of the detected DNA types, the results in this mixture do not meet the laboratory's criteria for use in statistical calculations and therefore no comparisons can be made to reference samples.

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Statement when the mixture, or a portion (i.e. minors) of the mixture, is unsuitable for statistical calculations or comparisons, but a search of the CODIS databases will be attempted:

Due to the number of possible DNA contributors and the low level of the detected DNA types, the results in this mixture do not meet the laboratory's criteria for use in statistical calculations and therefore no comparisons can be made to reference samples. Sufficient information exists in the mixture to allow for searching against the CODIS databases. Any hits obtained should only be treated as investigative leads as no statistical weight can be applied to possible matches.

5.2.2 Examples of inclusions in common scenarios

5.2.2.1 Examples of inclusions as a possible source of a single source or predominant sample

If the profile frequency is rarer than 1 in 300 million, the terms "match" or "very likely the source" may be used to describe the inclusion:

The (predominant) DNA profile from the evidence (Item 1) 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, a term such as "is included as a possible source" may be used:

John Doe is included (or could not be excluded) as being the source of the DNA from the evidence (Item 1). The approximate probabilities that a person selected at random would possess the same DNA types as the evidence are:

5.2.2.2 Example of an inclusion as a contributor to a mixture with no assumption as to the number of contributors

A mixture DNA from at least two (or however many) people was recovered from the evidence (Item 1). John Doe is included as being a possible DNA (or major or minor) contributor to the DNA mixture from the evidence. The approximate probabilities that a person selected at random would be included as a possible DNA (or major or minor) contributor to the mixture are:

5.2.2.3 Example of a conclusion where an assumption is made

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A mixture of DNA was obtained from the evidence (Item 1). The results support that it is a mixture of (fill in relevant assumption).

5.2.2.4 Example of an inclusion as a contributor to a mixture when the number of contributors is assumed (likelihood ratio scenario)

A mixture of DNA was recovered from the evidence (Item 1). The DNA results are consistent with a mixture of two people. John Doe is included as a possible DNA contributor to this mixture. Assuming two contributors to the DNA mixture, the DNA results suggest (see verbal wording below) support that this represents a mixture of DNA from John Doe and one unknown individual rather than if it is a mixture of DNA from two unknown individuals as expressed in the following table:

The following represents suggested wording to convey the strength of the likelihood ratio calculation

Likelihood Ratio Results	Verbal wording	Support for
1,000,000+	Extremely strong	H_x
100,000	Very strong	
10,000	Strong	
1000	Moderately strong	
100	Moderate	
10	Limited	
1	Inconclusive	
0.1	Limited	H_y
0.01	Moderate	
0.001	Moderately strong	
0.0001	Strong	
0.00001	Very strong	
0.000001	Extremely strong	

Verbal wording suggestions obtained from Dr. Chris Maguire – Northumbria University Center for Forensic Science

5.2.2.5 Example of an inclusion as a contributor to a mixture when an assumption is made regarding the number of contributors and with one or more assumed contributors

A mixture of DNA from at least two people was recovered from the evidence (Item 1). 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.

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The probabilities that a person selected at random would be included as a contributor to the DNA foreign to Jane Victim are:

5.2.2.6 Example of an inclusion in a paternity case

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 observe 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%.

5.2.2.7 Inclusions to single source profiles 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.

The words “match” and “very likely the source” may be used for inclusions to single source or predominant profiles on non-probative samples if a 15 locus match is obtained, given the minimum 15 locus profile frequencies using the Applied Biosystems Identifiler Plus 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 inclusion to a single source sample 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 (see below), 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.

5.2.2.8 Non-probative inclusions to 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 unknown DNA contributor to the mixture. DNA from the evidence will be searched against the databases of the Combined DNA Index System (CODIS).

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5.2.3 Examples of wording when a comparison is inconclusive

A mixture of DNA from at least five people was obtained from the evidence (Item 1). Due to the complexity of the mixture, it is inconclusive whether John Doe can be included as a possible contributor to the DNA mixture.

During DNA testing of the evidence (Item 1), the positive amplification control did not provide the expected results. Therefore no conclusions will be drawn from this item.

Results obtained from the evidence (Item 1) do not meet the laboratory requirements for comparison and are thus inconclusive.

5.2.4 Examples of exclusions in common scenarios

5.2.4.1 Example of an exclusion to a 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.

5.2.4.2 Example of an exclusion to a DNA mixture

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. DNA types from the mixture will be searched against the databases of the Combined DNA Index System (CODIS).

5.2.5 Reporting Staff Matches

When an evidence DNA profile is found to match a staff member of the laboratory the analyst should refer to the **"Reporting DNA results matching laboratory staff"** portion of the **CODIS** section of the Forensic Biology Unit Policy Manual.

5.2.5.1 Example of wording for staff matches

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The DNA profile from Evidence Item X was found to match my DNA profile. No other item in this case contains this profile and therefore interpretations of these items were not affected.

The DNA profile from Evidence Item X was found to match DNA from a laboratory analyst from Unit X. No other item in this case contains this profile and therefore interpretations of these items were not affected.

Minor contributor scenario

...also detected in this sample was DNA that is consistent with my DNA profile. The presence of this DNA in the sample did not affect the overall interpretation for this item. No other item in this case contains this profile and the interpretations of these items were not affected.

5.2.6 Streamlining conclusions

It may be appropriate in some circumstances to streamline the reporting of the conclusions. The following are examples of streamlined conclusions.

5.2.6.1 Single source profile examples

DNA results from evidence Item X will be searched in CODIS.

The DNA obtained from evidence Item X is from an unknown male. The DNA profile will be searched in CODIS.

5.2.6.2 Predominant DNA profiles

DNA results from evidence Item X will be searched in CODIS.

The predominant DNA obtained from evidence Item X is from an unknown male. This profile will be searched in CODIS.

5.2.6.3 Mixtures

DNA types from evidence Item X will be searched in CODIS.

A subset of DNA types from a mixture obtained from evidence Item X will be searched in CODIS.

DNA results from evidence Item X are unsuitable to be searched in CODIS, but can be compared with reference samples.

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The DNA results from evidence Item X are unsuitable to be searched in CODIS, but they can be compared with reference samples if submitted.

5.2.6.4 Unsearchable results

DNA results from evidence Item X are unsuitable to be searched in CODIS or compare with reference samples.

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METHOD: **Y-STR Interpretation Guidelines**

Date: 06/01/2004

Revision Date: 04/01/2011

Approved by: SAM

Introduction

The Scientific Working Group on DNA Analysis Methods (SWGDM) states that:

“The interpretation of DNA typing results for human identification purposes requires professional judgment and expertise. Additionally, laboratories that analyze DNA samples for forensic casework purposes are required by the Quality Assurance Standards for Forensic DNA Testing Laboratories (effective July 1, 2009) to establish and follow documented procedures for the interpretation of DNA typing results and reporting. Due to the multiplicity of forensic sample types and the potential complexity of DNA typing results, it is impractical and infeasible to cover every aspect of DNA interpretation by a preset rule. However, the laboratory should utilize written procedures for interpretation of analytical results with the understanding that specificity in the standard operating protocols will enable greater consistency and accuracy among analysts within a laboratory. It is recommended that standard operating procedures for the interpretation of DNA typing results be sufficiently detailed that other forensic DNA analysts can review, understand in full, and assess the laboratory’s policies and practices. The laboratory’s interpretation guidelines should be based upon validation studies, scientific literature, and experience.”

Here we present a number of general interpretation guidelines for interpreting samples in forensic Y-STR casework. The rationale behind these guidelines is to establish a set of standards to ensure that conclusions are supported by reliable data, derived using a scientific approach, and that interpretation of results is as objective as possible and is consistent from one analyst to another.

The interpretation of Y-STR results should take into account the DNA results obtained, the nature of the sample, the condition of the profile, any forensically valid assumptions made, and should be scientifically justifiable and based on the collected experience and knowledge of the laboratory and the scientific community. These guidelines are designed to provide a general foundation for interpretation.

DNA interpretation should take place in the following order:

- Preliminary Evaluation of Data and Allele Designation (section 1)
- Interpretation of DNA Typing Results (section 2)
- Comparison of DNA Typing Results (section 3)
- Statistical Analysis of DNA Typing Results (section 4)
- Reporting of DNA Typing Results and Conclusions (section 5)

This document will reference the Autosomal STR Interpretation Guidelines document where information applicable to Y-STR interpretation is available.

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Section 1 – Preliminary Evaluation of Data and Allele Designation

Please refer to section 1 of the Autosomal STR Interpretation Guidelines document as all the information therein is relevant to Y-STR interpretation.

Section 2 – Interpretation of DNA Typing Results

In general, interpretation of Y-STR data will be completed prior to comparison of reference samples. All interpretation of samples will be performed using the actual electrophoresis data and not the table of results. DNA interpretation will include the following steps:

- Determine whether a sample is a mixture (section 2.1)
- Estimate the number of contributors to a sample (section 2.2)
- Identify whether a sample is degraded and/or inhibited (section 2.3)
- Determine contribution level in mixed DNA samples (section 2.4)
- Document relevant assumptions (section 2.5)
- Determine which loci and combinations of DNA types are useful for statistical support of inclusions (section 2.6).

2.1 Determination of whether a sample is a mixture

A mixed Y-STR 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 one allele present at a locus other than DYS385 where a gene duplication results in a bi-allelic pattern.
- Significant imbalance in peak height between peaks at a DYS385.
- Generally, evidence of a mixed DNA sample exists at multiple loci.

To determine whether a mixed Y-STR 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 (see section 5).

2.2 Estimation of the number of contributors to a sample

Conclusions with respect to the minimum number of contributors must be included in a report. Generally, an estimate of the minimum number of contributors is based on the locus that exhibits the greatest number of allelic peaks and the fact that each contributor would contribute at most one allele to the locus (except DYS385). While the minimum number of contributors to a mixture can be established with a high probability, an absolute determination of the number of contributors cannot be ascertained.

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2.3 Determination of whether inhibition and/or degradation is present

Please refer to section 2.4 of the Autosomal STR Interpretation Guidelines document for more information on inhibition and degradation. The SDPD does not currently have good examples of degraded or inhibited Y-STR profiles, however, the same basic tenets apply to both autosomal and Y-chromosome analysis.

2.4 Determination of contribution levels in mixed DNA samples

The relative peak intensity differences between detected alleles can be used to identify differences in contribution levels between contributors to relatively simple (i.e. 2-person) mixtures. In general a 3:1 difference in peak intensity will be used to identify major vs. minor alleles. In more complex mixtures it may be difficult to determine major vs. minor alleles and analysts are encouraged to exercise caution when interpreting Y-STR mixtures of more than two individuals.

Alleles designated as likely coming from a minor DNA contributor to a Y-STR mixture will be denoted with brackets (e.g. [16]) in the table of results.

2.5 Documentation of Assumptions

In certain circumstances, ***assumptions regarding DNA profiles*** may be made to assist the analyst in drawing conclusions and to put the evidence in the proper context. All assumptions regarding any interpretation will be presented in the report and should also be in the notes (see section 5).

In certain circumstances ***assumptions regarding the presence of a contributor*** in a DNA mixture is permissible (i.e. intimate samples). In these circumstances the DNA profile from the assumed contributor(s) can be used to assist interpretation of the additional contributor(s) DNA profile/types (section 2.7).

Prior to making comparisons, the profile should be evaluated to ***determine what loci and/or combinations of DNA types are acceptable to support a conclusion statistically***. Since there is technically no stochastic threshold to be concerned with (with the exception of DYS385) much of the evaluation to determine what parts of the DNA results can be used for statistical weight takes into account the possible presence of DNA types below the analytical (or detection). If, based on the totality of the information in the Y-STR result, it is reasonable to assume that all information is accounted for in the results, all DNA types can be used for comparison, but it may useful to further refine the genotypes for comparison if a limited number of combinations are likely.

If an analyst believes that ***filtered stutter peaks*** should be evaluated for comparison, the mean stutter percentage observed for the allele during validation and other studies should be used to assess whether excess stutter is likely. Peaks in stutter positions that are more than 2 standard deviations above the mean observed stutter indicate the presence of excess stutter and that DNA contribution from a source other than stutter is likely. If a peak in a stutter position is deemed to have originated from a separate contributor to the sample, then

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all stutter peaks in the mixture should be evaluated in the same manner and included in the assessment of the mixture. After the evaluation of stutter peaks in the mixture it may be necessary to reassess the minimum number of DNA contributors to the sample.

Section 3 - Comparison of DNA Typing Results

- 3.1 **Inclusion.** If a person of interest has no unexplainable differences from the alleles or allele combinations determined to be useful for statistical support of inclusions, and thus comparison, they are included as a source of the DNA or possible contributor to the DNA mixture.
- 3.2 **Exclusion.** If a person of interest has unexplainable differences from the alleles or allele combinations determined to be useful for comparisons, they are excluded as a source of the DNA or possible contributor to the DNA mixture.
- 3.3 **Inconclusive.** Some mixtures such as those with five or more contributors may not be suitable for comparison, unless a minimal number of major contributors exist. Comparisons to these mixtures will be considered inconclusive.

An analyst may determine that due to a combination of variables including (but not limited to) the number of DNA contributors as well as a lack of information in the results that no comparisons can be made to the profile (or comparison for possible minor contributors).

Section 4 - Statistical Analysis of DNA Typing Results

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.

4.1 Apparent Single Source profiles

For the purposes of expressing the significance of a Y-STR haplotype match all alleles for loci deemed acceptable for comparison will be searched in the database. More DNA profiles in the US YSTR database have a subset of the Yfiler loci than have the full complement of the Yfiler loci. As a result, an analyst may elect to search a subset of the Y-STR results in the database for the purposes of obtaining a more discriminating search.

4.2 Mixed Y-STR DNA profiles

- 4.2.1 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

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applicable alleles from the predominant haplotype will be searched in the database (as in section 4.1). For the purposes of expressing the significance of a Y-STR haplotype match all applicable alleles will be searched in the database.

- 4.2.2 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.

- 4.3 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/pplexxy/

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

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Section 5 - Reporting of DNA Typing Results and Conclusions

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.

5.1 Reporting of DNA typing results

Please refer to the Autosomal STR Interpretation Guidelines document for additional information.

5.2 Reporting of Conclusions

Conclusions for each item should be present in the conclusions section of the report. It is acceptable to group similar conclusions for multiple items together.

- 5.2.1 In general, conclusions should state whether a mixture of DNA was obtained, and if a mixture was obtained, the minimum number of contributors should be stated. The conclusion should indicate if the sample is not suitable for comparison purposes. A brief description of the reason that it is not suitable should be included. In the case of a mixture that is only suitable for comparison to possible major contributors, an indication should be included that the sample is not suitable for comparison to possible minor contributors. It may in some circumstances be relevant to indicate whether male or female DNA is present in the sample.

If the sample is suitable for comparison and if comparisons are made, the conclusion should indicate whether the persons of interest are included or excluded. If the comparison is determined to be inconclusive, a statement giving reasons leading to the inconclusive determination.

If an inclusion is made, and if the inclusion warrants a probability calculation (see section 4), the applicable probabilities should be listed.

Any assumptions made regarding the comparisons and/or the probability calculations will be listed in the conclusion.

If the inclusion of an individual requires a larger number of contributors than the minimum based on allele number and peak height ratios, a statement to that effect should be included.

- 5.2.2 Example of an inclusion to an 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.

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The number of times the Y-STR DNA types from the evidence were observed in the ... Database (website of appropriate database) is:

5.2.3 Example of an inclusion to a Y-STR mixture

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:

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.

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Appendix A: Glossary of terms (adapted from SWGDAM)

Allelic dropout: failure to detect an allele within a sample or failure to amplify an allele during PCR.

Analytical threshold: the minimum height requirement at and above which detected peaks can be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles.

Artifact: a non-allelic product of the amplification process (e.g., stutter, non-templated nucleotide addition, or other non-specific product), an anomaly of the detection process (e.g., pull-up or spike), or a by-product of primer synthesis (e.g., “dye blob”).

Composite profile: a DNA profile generated by combining typing results from different loci obtained from multiple injections of the same amplified sample and/or multiple amplifications of the same DNA extract. When separate extracts from different locations on a given evidentiary item are combined prior to amplification, the resultant DNA profile is not considered a composite profile.

Conditional: an interpretation category that incorporates assumption(s) as to the number of contributors.

CPI: combined probability of inclusion; produced by multiplying the probabilities of inclusion from each locus.

Deconvolution: separation of contributors to a mixed DNA profile based on quantitative peak height information and any underlying assumptions.

Deduced: inference of an unknown contributor's DNA profile after taking into consideration the contribution of a known/assumed contributor's DNA profile based on quantitative peak height information.

Differential Degradation: a DNA typing result in which contributors to a DNA mixture are subject to different levels of degradation (e.g., due to time of deposition), thereby impacting the mixture ratios across the entire profile.

DNA Contributor: A general category of contribution for samples that are indistinguishable with regard to major/minor contributors.

Evidence sample: also known as Questioned sample.

Exclusion: a conclusion that eliminates an individual as a potential contributor of DNA obtained from an evidentiary item based on the comparison of known and questioned DNA profiles (or multiple questioned DNA profiles to each other).

Guidelines: a set of general principles used to provide directions and parameters for decision making.

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Heterozygote: an individual having different alleles at a particular locus; usually manifested as two distinct peaks for a locus in an electropherogram.

Homozygote: an individual having the same (or indistinguishable) alleles at a particular locus; manifested as a single peak for a locus in an electropherogram.

Inclusion: a conclusion for which an individual cannot be excluded as a potential contributor of DNA obtained from an evidentiary item based on the comparison of known and questioned DNA profiles (or multiple questioned DNA profiles to each other).

Inconclusive/uninterpretable: an interpretation or conclusion in which the DNA typing results are insufficient, as defined by the laboratory, for comparison purposes.

Intimate sample: a biological sample from an evidence item that is obtained directly from an individual's body; or one where it is not unexpected to detect that individual's allele(s) in the DNA typing results.

Known sample: biological material for which the identity of the donor is established and used for comparison purposes (referred to as a "K").

Likelihood ratio (LR): the ratio of two probabilities of the same event under different hypotheses; typically the numerator contains the prosecution's hypothesis and the denominator the defense's hypothesis.

Major contributor(s): an individual who appears to contribute a significant portion of the DNA to a mixture.

Masked allele: an allele of the minor contributor that may not be readily distinguishable from the alleles of the major contributor or an artifact.

Minor contributor(s): an individual(s) who can account for the lesser portion of the DNA in a mixed profile.

Mixture: a DNA typing result originating from two or more individuals.

Mixture ratio: the relative ratio of the DNA contributions of multiple individuals to a mixed DNA typing result, as determined by the use of quantitative peak height information; may also be expressed as a percentage.

Noise: background signal detected by a data collection instrument.

No results: no allelic peaks detected above the analytical threshold.

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Obligate allele: an allele in a mixed DNA typing result that is (a) foreign to an assumed contributor, or (b) based on quantitative peak height information, determined to be shared with the assumed contributor.

Partial profile: a DNA profile for which typing results are not obtained at all tested loci due, for example, to DNA degradation, inhibition of amplification and/or low- quantity template.

Peak height ratio (PHR): the relative ratio of two alleles at a given locus, as determined by dividing the peak height of an allele with a lower relative fluorescence unit (RFU) value by the peak height of an allele with a higher RFU value, and then multiplying this value by 100 to express the PHR as a percentage; used as an indication of which alleles may be heterozygous pairs and also in mixture deconvolution.

Predominant DNA contributor: A subcategory in an assumption of a single major DNA contributor when a single source genotype from the single strongest contributor to a mixture is able to be easily deduced from all of the DNA markers tested.

Probability of inclusion (PI): the percentage of the population that can be included as potential contributors to a DNA mixture; also known as Random Man Not Excluded. .

Questioned sample: biological sample recovered from a crime scene or collected from persons or objects associated with a crime (referred to as a “Q”).

Random Match Probability (RMP): the probability of randomly selecting an unrelated individual from the population who could be a potential contributor to an evidentiary profile.

Reference sample: also known as Known sample.

Restricted: referring to a statistical approach conditioned on the number of contributors and with consideration of quantitative peak height information and inference of contributor mixture ratios; used to limit the genotypic combinations of possible contributors.

Signal-to-noise ratio: an assessment used to establish an analytical threshold to distinguish allelic peaks (signal) from background/instrumental noise.

Single-source profile: DNA typing results determined to originate from one individual based on peak height ratio assessments and the number of alleles at given loci.

Stochastic effects: the observation of intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles in low-quantity template samples.

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Stochastic threshold: the peak height value above which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele has not occurred.

Stutter: a minor peak typically observed one repeat unit smaller than a primary STR allele resulting from strand slippage during amplification.

Unrestricted: referring to a statistical approach performed without consideration of quantitative peak height information and inference of contributor mixture ratios; for CPE/CPI this may or may not be conditioned on the number of contributors.

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