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Department of Forensic Science

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

FORENSIC BIOLOGY SECTION PROCEDURES MANUAL

SECTION VIII

CAPILLARY ELECTROPHORETIC DETECTION PCR-BASED STR DNA PROTOCOL: POWERPLEX® 16 SYSTEM

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PREFACE

General

Since the first report of DNA amplification using the process known as polymerase chain reaction (PCR) in 1985, many advances and modifications have been made to the basic method. PCR is an *in vitro* method for the enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA. A repetitive series of cycles involving template denaturation, primer annealing, and extension of the annealed primers by DNA polymerase results in the exponential accumulation of a specific fragment whose termini are defined by the 5' ends of the primers. Because the primer extension products synthesized in one cycle can serve as a template in the next, the number of target DNA copies approximately doubles at every cycle. Thus, 20 cycles of PCR yields about a million-fold (2^{20}) amplification. The original PCR method, invented by Kary Mullis, was used to diagnose prenatal sickle-cell anemia.[1,3] With advances in the polymerase chain reaction process, analyzing forensic casework has been made more efficient. The ability to extract and type DNA from forensic evidentiary samples has revolutionized the field of forensic DNA. Previously, genetic marker typing was limited to the analysis of blood group markers and soluble polymorphic protein markers. Because the number of suitable markers expressed in particular fluids and tissues is relatively small, and because mixtures of fluids cannot be separated for conventional genetic marker typing, a suspect frequently cannot be included or excluded as a fluid donor in a case. However, the development of methods to extract DNA from virtually all biological specimens has greatly expanded the potential for individual identification.[3]

Taq DNA Polymerase

Initially, PCR amplification used the Klenow fragment of *E. coli* DNA polymerase I to extend the annealed primers. This enzyme was inactivated by the high temperatures required to separate the two DNA strands at the outset of each PCR cycle. Fresh enzyme had to be added constantly during every cycle. To make the process more time efficient molecular biologists began to use an enzyme from a thermophilic eubacterial microorganism, *Thermus aquaticus* (*Taq*), capable of growth at 70°C-75°C.[1,2] Although *Taq* DNA polymerase has a very limited ability to synthesize DNA above 90°C, the enzyme is relatively stable and is not denatured irreversibly by exposure to high temperatures. Preliminary results indicate retention of 65% activity after a 50-cycle PCR amplification when the upper limit temperature was 95°C.[1] Under normal reaction conditions, the amount of *Taq* DNA polymerase becomes limiting after 25-30 cycles of amplification.[4] Two forms of *Taq* DNA polymerase that are now available are both a genetically engineered form of the native enzyme synthesized in *E. coli*. One of these DNA polymerases, AmpliTaq Gold™ (Perkin-Elmer), is provided in an inactive state. Heat activates the enzyme. This feature allows flexibility in reaction setup. Both forms of the polymerase carry a 5'→ 3' polymerization-dependent exonuclease activity, but they lack a 3' → 5' exonuclease activity.[4,5]

PCR Primers

In order to amplify a sequence of DNA, oligonucleotide primers flanking the target DNA sequence of interest are used. These oligonucleotide primers typically have different sequences and are complementary to sequences that lie on opposite strands of the template DNA with their 3' ends oriented toward each other. The template DNA is first denatured by heating in the presence of a large molar excess of each of the two oligonucleotide primers and the four dNTPs. The reaction is then cooled to a temperature that allows the oligonucleotide primers to anneal to their target sequences, after which the annealing primers are extended with the enzyme *Thermus aquaticus* (*Taq*) DNA polymerase in an automated series of heating and cooling cycles. With each cycle the DNA will approximately double the number of copies. The primers are responsible for the sequence specificity of the PCR reaction. Typically the length of the primers used in the PCR amplification should be at least 16 nucleotides (preferably 20 to 30 nucleotides) in length and single stranded.[4]

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INTRODUCTION

An important development in the field of forensic science was the introduction of DNA typing systems for the analysis of biological samples. The RFLP-based typing system is highly informative, but requires greater than 50 ng of high molecular weight DNA and involves the use of multiple radioactive/chemiluminescent probes to obtain a result. An alternative approach to forensic DNA typing is based on the powerful polymerase chain reaction (PCR) technology, which does not require the use of radioactivity [1,2]. Specific regions of the genome containing either sequence polymorphisms or length polymorphisms are amplified from minute amounts of DNA. Alleles which have been amplified and contain sequence differences may be typed using the "reverse dot blot technology.[3] Amplified alleles containing length variations, such as short tandem repeats (STRs) and amplified fragment length polymorphisms (AMP-FLPs) may be typed by polyacyrlamide gel or capillary electrophoresis.

STR loci consist of short, repetitive sequence elements of 3 to 7 base pairs in length.[4] These abundant repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers which may be detected using the polymerase chain reaction. The alleles for these loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using radioactive, silver stain, or fluorescence detection following electrophoresis. In the capillary system, fluorescence detection of the amplified product is accomplished during the electrophoresis. The PowerPlex® 16 System provides a rapid, non-isotopic method which can be used to evaluate very small amounts of human DNA. STR typing is more tolerant of the use of degraded DNA templates than other typing methods because the amplification products are less than 500 bp long, much smaller than the material detected with AMP-FLP[5,6] or VNTR[7,8] analysis.

The PowerPlex® 16 System allows the co-amplification and four-color detection of sixteen loci (fifteen STR loci and Amelogenin) and the internal lane standard (ILS 600). The PowerPlex® 16 System contains the loci Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818. In the system, the primer sets specific for Penta E, D18S51, D21S11, TH01, and D3S1358, contain one primer of each pair that is labeled with fluorescein (FL); the primer sets specific for the following loci: FGA, TPOX, D8S1179, vWA, and Amelogenin contain one primer of each pair that is labeled with carboxy-tetramethylrhodamine (TMR); and the primer sets specific for the following loci: Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818 contain one primer of each pair that is labeled with 6-carboxy-4',5'-dichloro 2',7' – dimethoxyfluorescein (JOE). All sixteen loci are amplified simultaneously in a single tube or plate well and analyzed in a single capillary[9].

Capillary electrophoresis involves the use of acrylamide polymers packaged into glass capillaries for separating fragments of DNA of varying length depending on the function for which it is used (i.e., sequencing versus fragment analysis). The acrylamide polymers used are not cross-linked, however, DNA fragments are retarded in proportion to the length of the molecule in a manner similar to conventional gel electrophoresis. The internal diameter of the capillaries used is small, 50 µm.[10] The narrow capillary (thus high surface area) enables the application of high electric fields, and thus faster run times, without overheating problems associated with the high voltages due to the more efficient heat dissipation.

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1 PCR AMPLIFICATION

1.1 Technical Notes

1.1.1 Taq DNA Polymerase, purified from *Thermus aquaticus*, attaches nucleotides in a 5'--->3' direction to extend the DNA chain..

1.1.2 The PowerPlex® 16 System contains 16 sets of primers and allows for the co-amplification and three-color detection of 16 loci (15 STR loci and Amelogenin), which include the FGA, TPOX, D8S1179, vWA, Amelogenin, Penta E, D18S51, D21S11, TH01, D3S1358, Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818 loci.

1.1.3 If a batch of samples will be amplified using the Biomek® Automation Workstation as few as one amplification negative control and amplification positive control may be amplified per Biomek® Automation Workstation run. However, in order to report the typing results for the evidence samples associated with these controls, the controls must work appropriately in accordance with the procedures outlined in Chapter 8, Interpretation of The PowerPlex® 16 System PCR Amplification Results.

1.2 Equipment

1.2.1 Thermal Cycler – 9700

1.2.2 Pipettes - 2 µL, 10 µL, 20 µL, 100 µL and/or 200 µL

1.2.3 8- Channel Pipette – Range 0.5 µL to 10 µL

1.2.4 Biological Safety Hood

1.2.5 Microcentrifuge tube racks

1.3 Materials

1.3.1 Sterile ART pipette tips - 2 µL, 10 µL, 20 µL, 100 µL and/or 200 µL

1.3.2 1.5 mL microcentfuge tubes

1.3.3 Kimwipes

1.3.4 Sterile MicroAmp® tubes (0.2 mL tubes)

1.3.5 Gloves

1.3.6 96 well amplification plates (TempPlate(R) III P/N 1402-9700 or similar) and strip caps or plate sealing film (TempPlate® P/N 2921-000 or similar)

1.3.7 Compression pad (ABI P/N 4312639 or similar)

1.4 Reagents

1.4.1 Powerplex® 16 System Primer Pairs

1.4.2 Sterile Type I Water

1.4.3 Gold ST*R 10X Buffer

1.4.4 Amplification Positive Control

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1.4.5 AmpliTaq™ Gold DNA Polymerase

1.5 PowerPlex® 16 System Amplification Procedure for the 9700 Thermal Cycler

NOTE: A dedicated area, such as a biological hood or a separate room, should be used for preparing PCR amplification reactions. All equipment and supplies used to prepare amplification reactions should be kept in this dedicated "clean" area at all times. Do not use these items to handle amplified DNA or other potential sources of contaminating DNA. Trace amounts of amplified DNA, if carried over into other samples before amplification, can lead to results that can be misinterpreted. DO NOT bring amplified DNA, or equipment and supplies used to handle amplified DNA, into the designated "clean" area. Wear clean disposable laboratory gloves while preparing samples for PCR amplification. Change gloves frequently or whenever there is a chance they have been contaminated with DNA.

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1.5.1 Amplification Parameters for the 9700 Thermal Cyclers:

1.5.1.1 Turn on the 9700 thermal cycler. Program as follows using the step cycle file, change the default setting to:

Set a hold file for 11 minutes at 95^{0}C.
Link this hold file to a separate hold file to denature at 96°C for 1 minute.
Link this preheat program to a separate program as follows:
Ramp 100% to denature at 94°C for 30 seconds
Ramp 29% to anneal at 60°C for 30 seconds
Ramp 23% to extend at 70°C for 45 seconds

Program for 10 cycles
Link this program to a separate program as follows:
Ramp 100% to denature at 90°C for 30 seconds
Ramp 29% to anneal at 60°C for 30 seconds
Ramp 23% to extend at 70°C for 45 seconds

Program for 21 cycles
Link to a 60°C hold file for 30 minutes
Link to a 4°C hold cycle.
This program may be saved as a “User File” for later use.

NOTE: The PowerPlex® 16 Allelic Ladder and Internal Lane Standard 600 supplied in the PowerPlex® 16 System kit do not require amplification and should not be stored in the sample preparation area.

1.5.2 Transfer the PowerPlex® 16 System amplification reagents to the designated "clean" area and allow the Gold ST*R 10X Buffer, amplification positive control and the PowerPlex® 16 10X Primer Pairs to thaw completely at room temperature.

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1.5.3 Place the required number of labeled sterile MicroAmp® reaction tubes (or 96-well plate, incl. one label for entire plate indicating the plate name) in a rack.

1.5.4 Calculate the required volume of each PCR amplification component to prepare a master mix by multiplying the volume by the number of samples. This should include reagent blanks, plate blanks (associated only with samples extracted on the Biomek® Automation Workstation), and positive and negative amplification controls and additional reaction volumes (depending on the number of samples being amplified) to compensate for any pipetting variation. The PCR Master Mix should be prepared in a clean 1.5 mL microcentrifuge tube.

Half Reaction Master Mix:

1.25 µL Gold ST*R 10X Buffer
1.25 µL PowerPlex® 16 10X Primer Pairs
4.60 µL Sterile Type I Water
0.40 µL AmpliTaq™ Gold Enzyme (2 Units)

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1.5.5 Add 7.5 µL of PCR Master Mix to each labeled sterile MicroAmp® tube or well of the 96 well plate using sterile ART tips. Cap each tube and ensure that the solution is at the bottom of the tube by gently tapping each tube on the counter top.

NOTE: Each PowerPlex® 16 System amplification is performed in a final volume of 12.5 µL. Exactly 5 µL has been allocated for the sample DNA. However, when a body identification or reference sample contains a low level of DNA (e.g., 0.05 ng/µL or less DNA) estimated based upon the DNA quantitation, 9.6 µL of sample DNA may be used and the water removed from the Master Mix cocktail.

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No more than one tube should be opened at a time when making the following additions. Use a new sterile ART tip for each addition.

1.5.6 Prepare the sample DNA and controls as follows:

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1.5.6.1 Open the MicroAmp® tube containing the DNA extract and carefully add 5 µL of sample DNA (0.62 to 1.25 ng/5 µL is in general the optimal target concentration, however the full 5 µL of undiluted DNA may be added when the target concentration cannot be reached) to the appropriate labeled sample tube or well in 96-well amplification plate.

1.5.6.1.1 If samples intended solely to collect “touch” or “wearer” DNA do not have more than 0.006 ng (6 pg) /ul of autosomal or Y DNA (using the Plexor quantitation system), these samples will not proceed through the DNA typing step. This is not intended to apply to samples collected for other body fluids that may also contain "touch" DNA (such as a knife handle contaminated with blood). Exceptions will be considered by the Program Manager on a case-by-case basis.

1.5.6.2 Positive Amplification Control: Gently mix by hand the Control DNA followed by a light tapping of the tube on the counter top to remove any liquid from the cap. Open the MicroAmp® tube and carefully add 5 µL (0.62 to 1.25 ng/5 µL) of the Control DNA to the appropriate labeled sample tube or well in 96-well amplification plate.

NOTE: When preparing the stock Control DNA, dilute the sample to a concentration of 0.12 to 0.25 ng/µL with sterile Type I Water.

1.5.6.3 Negative Amplification Control: Open the MicroAmp® tube and carefully add 5 µL of sterile Type I water to the appropriate labeled sample tube or well in 96-well amplification plate. Note: if a volume of 9.6 µL of sample DNA will be used for any samples during the amplification step, 9.6 µL of the Type I water will be used.

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1.5.6.4 Reagent Blank(s) or Plate Blank(s): Open the MicroAmp® tube and carefully add 5 µL of the reagent blank(s)/plate blank(s) to the appropriate labeled sample tube or well in 96-well amplification plate. Note: if a volume of 9.6 µL of sample DNA will be used for any samples during the amplification step, 9.6 µL of the reagent blank(s)/plate blank will be used.

1.5.7 Place the MicroAmp® tubes or the 96-well amplification plate into the thermal cycler. Push the tubes or plate down completely into the block. Add a compression pad (ABI P/N 4312639 or similar) (for amplification in plates only), place the thermal cycler lid over the samples, and tighten.

1.5.8 When the thermal cycler prompts the user for the sample volume, choose 13 µL.

1.5.9 Start the amplification file and verify the cycling parameters by monitoring the first cycle.

NOTE: The PCR amplification program will run for approximately 3.5 hours.

1.5.10 After the amplification process is complete remove the tubes or 96-well amplification plate from the thermal cycler and proceed with all samples to the "Capillary Electrophoresis" chapter of this manual. A product gel may be run (in accordance with Chapter 6 "Product Gel for Determining Amplified Product" of the Commonwealth of Virginia Forensic Biology Section Procedure Manual Section III, Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 BIO System) prior to capillary electrophoresis,

1.5.11 If the sample will not be analyzed within approximately three days, the samples should be stored in the dark at 2 to 8 °C for a few days or at -20 °C for extended periods.

NOTE: Store amplified DNA samples separate from PCR pre-amplification reagents and extracted DNA samples.

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2 CAPILLARY ELECTROPHORESIS

2.1 Technical Notes

2.1.1 The Applied Biosystems 3130*xl* Genetic Analyzer is a fluorescent capillary electrophoresis (CE) instrument with a 16 capillary array which can be used for DNA sequencing and fragment analysis applications. This includes STR (Short Tandem Repeat) analysis as well as DNA sequencing and SNP (Single Nucleotide Polymorphism) analysis.

2.1.2 The capillary length and polymer type will vary based on the individual application. Sample handling and injection is automated by use of the autosampler, which holds two 96- or 384-well plates.

2.1.3 The polymer delivery pump (PDP) automates the replenishment of polymer between injections so that the instrument is capable of fully-automated operation.

2.1.4 Fluorescent detection is accomplished by utilizing an argon-ion multi-line, single mode laser with primary excitation lines at 488 nm and 514.5 nm to excite fluorescent dyes and a CCD camera records the fluorescence emitted from samples within each of the 16 capillaries.

2.1.5 Data analysis, including color separation, is performed semi-automatically through the use of GeneMapper® ID software v3.2.1.

2.1.6 For the purpose of this manual, the following definitions apply:

2.1.6.1 Injection - all samples that were separated via CE at a single time. Thus, for one injection on the 3130*xl*, up to 16 samples can be separated at a single time.

2.1.6.2 Run - all samples that were defined on a single plate and loaded onto the instrument at a single time for separation, regardless of the number of injections utilized to separate each sample contained on the plate. Thus, a run could contain up to 96 samples for separation on a single plate. At least 1 ladder sample is needed per run.

2.2 Equipment

2.2.1 Applied Biosystems 3130*xl* Genetic Analyzer

2.2.2 Heat block, capable of 95 ^{o}C

2.3 Materials

2.3.1 36-cm capillary array (Applied Biosystems, Inc. P/N 4315931 (16-capillary))

2.3.2 MicroAmp® Optical 96-Well Reaction Plate (Applied Biosystems, Inc. P/N N801-0560)

2.3.3 96-well plate septum (Applied Biosystems, Inc. P/N 4315933)

2.3.4 96-well plate retainer and base (Applied Biosystems, Inc. P/N 403081 and N801-0531)

2.3.5 50 mL conical tube

2.3.6 Freezer block

2.3.7 Sterile ART tips for pipettes – 10µL, 20µL, 100µL, 200µL, and 1000 µL

2.4 Reagents

2.4.1 PowerPlex® Matrix Standard 3100/3130 (Promega Corp. P/N DG4650)

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2.4.2 10X Genetic Analyzer Buffer with EDTA (Applied Biosystems, Inc. P/N 402824)

2.4.3 Hi-Di Formamide (Applied Biosystems, Inc. P/N 4311320)

2.4.4 PowerPlex® 16 Allelic Ladder (Promega Corp. P/N DG600B)

2.4.5 Internal Lane Standard (ILS) 600 Size Standard (Promega Corp. P/N DG261A)

2.4.6 POP-4 Polymer (Applied Biosystems, Inc. P/N 4363752 (3.5 mL) or P/N 4352755 (7 mL))

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2.5 Safety Considerations

2.5.1 The Applied Biosystems 3130/3130*xl* Genetic Analyzer uses an Argon-ion laser. Under normal operating conditions, the instrument laser is categorized as a Class I laser. All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the laser is operating, (during service with safety interlocks disabled) you may be exposed to laser emissions in excess of the Class 3B rating. Do not remove safety labels or disable safety interlocks.

2.5.2 Performance Optimized Polymer (POP) is an irritant, always wear gloves when handling or cleaning liquid or dried polymer. It may cause skin, eye, and respiratory tract irritation.

2.5.3 Exposure to Hi-Di formamide causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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2.6 Computer

2.6.1 Turn on the power for the computer.

2.6.2 Turn on the power for the 3130*xl* instrument. Wait for the solid green light on the front of the instrument.

2.6.3 Select the Data Collection icon from the screen to start the Data Collection software. The service console opens, as shown in Figure 1. When all of the applications are running, the Data Collection Viewer window opens and green squares appear, as shown in Figure 1.

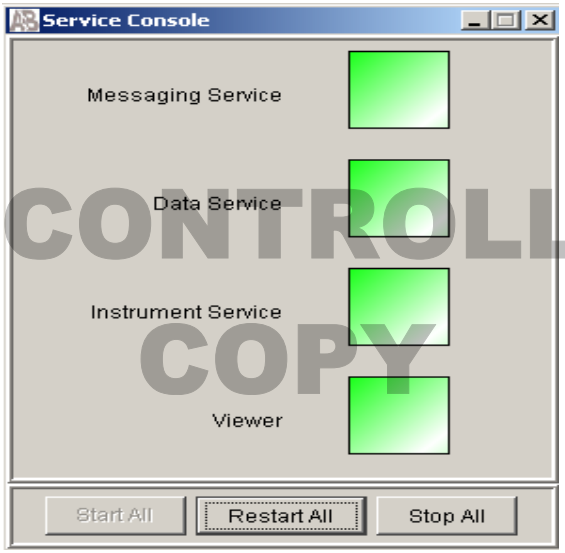

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Figure 1. Data Collection service console.

2.7 Sample and Instrument Preparation

2.7.1 Install the Capillary Array

2.7.1.1 If a capillary array has already been installed on the instrument, proceed to section 6.7.2 for continued preparation of the instrument.

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2.7.1.2 In the Data Collection software, select the "Wizards" menu and choose "Install Array". Follow the on-screen directions for installing a capillary array.

2.7.1.3 If a new capillary array has been installed on the instrument, a spatial calibration must be completed prior to running samples on the instrument. Refer to Appendix F of the Commonwealth of Virginia Forensic Biology Section Procedure Manual Section VIII, Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 System for detailed instructions on running the calibrations.

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2.7.2 Set the oven temperature to 60 °C.

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2.7.2.1 In the Data Collection software, select Manual Control in the tree pane to bring up the manual control dialog box, as shown in Figure 2.

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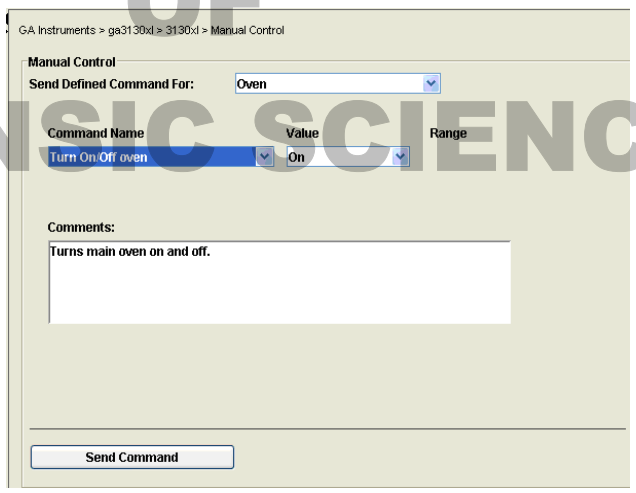

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Figure 2. Manual Control dialog box.

2.7.2.2 Select "Oven" in the drop down menu for "Send Defined Command For:"

2.7.2.3 Select "Set oven temperature" from the drop down menu under "Command Name".

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2.7.2.4 Type 60 for the value and click "Send Command".

2.7.2.4.1 Note: the instrument default oven temperature is set at 50 °C. Therefore, the temperature must always be set to 60 °C for the pre-warming, prior to turning the oven on.

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2.7.3 Turn on the oven. The oven should be warming up as samples are being prepared, or approximately 30-45 minutes.

2.7.3.1 Select "Turn On/Off oven" under command name and "On" from the drop down list under Value.

2.7.3.2 Click "Send Command".

2.7.4 Preparation of the sample plate

NOTE: When loaded onto the same plate, all evidence samples pertaining to a case must be loaded on to the plate prior to loading reference samples for the same case. Samples may not be added to empty wells previously subject to sample injection.

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2.7.4.1 Prepare a 'master mix' of formamide and size standard as follows:

2.7.4.1.1 Thaw the Hi-Di formamide to room temperature, vortex to mix, and pipet 9.5 µL of Hi-Di formamide for each sample (as well as extra samples to account for pipetting variation which will vary depending on the number of samples) to a clean microcentrifuge tube.

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2.7.4.1.2 Vortex the ILS 600 Size Standard (stored at -20 ^{o}C) and pipet 0.5 µL per sample (as well as the same number of extra samples as in 2.7.4.1.1) to the microcentrifuge tube or trough containing the formamide.

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2.7.4.1.3 Vortex this 'master mix' to ensure the contents are thoroughly mixed.

2.7.4.2 Pipet 10 µL of the 'master mix' into each used well of the 96-well plate.

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2.7.4.3 10 µL of Hi-Di formamide, extra 'master mix' or Type I water must be pipetted into unused wells of the plate that will be injected to ensure that air is not injected into some of the capillaries. (That is, if one injection (16 samples total) will be completed on the 16-capillary 3130*xl*, it is necessary to ensure that all sample wells in the two columns contain liquid.)

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2.7.4.4 Pipet PCR product (for samples, positive controls, and negative controls) into the corresponding well of the 96-well plate. Typically, 1 µL of PCR product will be added. However, 0.5 µL or 2 µL of the PCR product may be used at the analyst's discretion. For over-amplified samples, it may also be necessary to dilute the PCR product prior to removing an aliquot to place into the injection cocktail.

NOTE: The same volume of PCR product (or more) used for the samples must also be used for the negative controls and associated blanks.

2.7.4.5 Pipet 1 µL of allelic ladder into at least one well per run.

2.7.4.6 After addition of all samples, the plate septum should be placed on top of the sample wells and the plate heated at 95 ^{o}C for ≥2 minutes.

2.7.4.7 Following heating, the plate should be placed in a freezer block or on ice for at least two minutes prior to loading.

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2.7.5 Complete the following maintenance tasks:

2.7.5.1 Prepare 35 mL of 1X Genetic Analysis Buffer with EDTA by diluting the 10X stock Genetic Analysis Buffer with EDTA. The 1X Genetic Analyzer Buffer may be used for up to 48 hours after its preparation. Ensure adequate levels of buffer are in the respective reservoirs (reservoir 1, as shown in Figure 3, and the anode buffer reservoir, as shown in Figure 5) and the outside of the reservoirs are dry. Remove any buffer droplets from the inside of the reservoirs that may be clinging to the walls.

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2.7.5.2 When replacing the 1X running buffer, the water reservoirs (2 and 4) should also be rinsed and refilled with Type 1 H_2O to the fill line. Figure 3 shows a diagram of the reservoirs, indicating which contain H_2O and buffer.

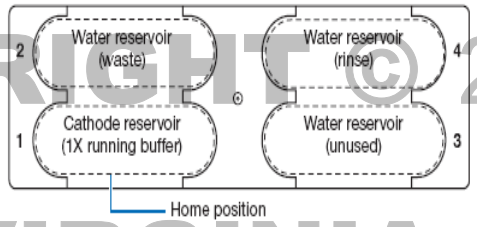

Figure 3. Location of buffer and water reservoirs on the 3130*xl*
Figure reproduced from Applied Biosystems 3130/3130xl Genetic Analyzers (2004) Maintenance and Troubleshooting Guide.

2.7.5.3 Ensure the plate assemblies are properly assembled (as shown in Figure 4) and positioned on the deck properly. Note: if the plate assembly is properly assembled, the gray septa will not be visible when looking down through the holes of the plate retainer. Instead, the wells will appear black. If the plate assembly is not properly assembled, damage to the capillary array is likely to occur.

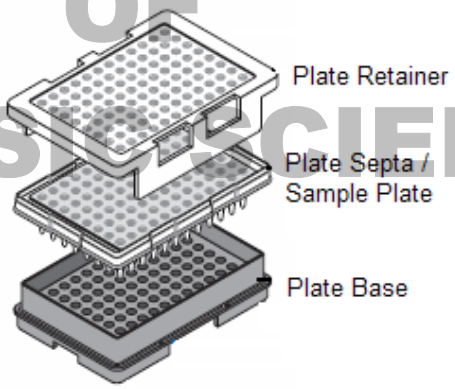

Figure 4. Proper assembly of the plate for loading onto the instrument
Figure reproduced from Applied Biosystems 3130/3130xl Genetic Analyzers (2004) Maintenance and Troubleshooting Guide

2.7.5.4 Check for bubbles in the pump block, lower polymer block, interconnect tube, polymer supply tube, and channels. If bubbles are present, remove all bubbles with the Bubble Remove wizard.

2.7.5.5 Check the loading-end header to ensure the capillary tips are not crushed or damaged.

2.7.5.6 Ensure that sufficient volume of polymer is present in the bottle for run. (Note: approximately 0.5 mL of polymer is sufficient to run approximately a plate of samples without introduction of bubbles into the pump block. Additional polymer may be necessary to clear bubbles, etc.)

2.7.5.7 Ensure that the pump block and lower polymer block are securely fit on the instrument.

2.7.5.8 Ensure that the instrument surfaces are clean; always use deionized water to clean.

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2.7.5.9 Check for leaks around the array knob, interconnecting tube nuts, and check valve, all of which are shown in Figure 5.

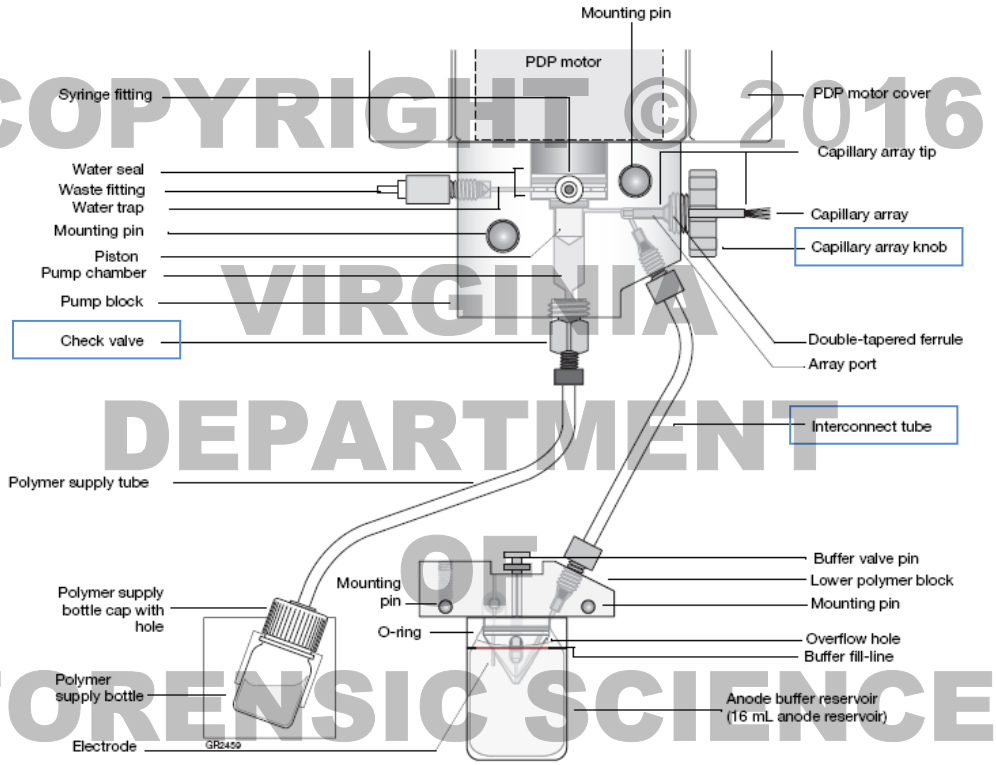

Figure 5. Diagram of the pump block and associated components of the ABI 3130*xl* Genetic Analyzer.
Figure reproduced from Applied Biosystems 3130/3130xl Genetic Analyzers (2004) Maintenance and Troubleshooting Guide

2.8 Instrument Operation

2.8.1 Ensure that the instrument doors are closed and the green light on the front panel of the instrument is on. Open the 3130*xl* data collection software. The data collection software sets up the instrument for operation and data collection and manages the database for every aspect of the capillary electrophoresis. The software has two basic components: Data Management (e.g., ga3130*xl*) and Instrument Control (e.g., 3130*xl*), both of which are found in the tree pane shown in Figure 6.

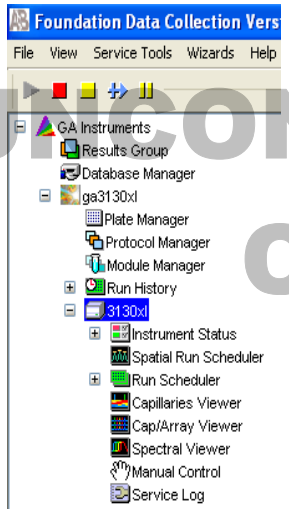

Figure 6. Tree pane of the Data Collection software on the computer associated with the 3130*xl* Genetic Analyzer.

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2.8.2 **Plate Manager** - Sample information is entered into corresponding plate locations along with the individual protocol, results group, and analysis method for each sample loaded on the plate. Sample information can be entered manually within the plate manager or imported from an external worksheet. Each injection group (16 samples corresponding to the 16 capillaries) must have the same instrument protocol as it represents a single injection. Samples should be organized on the plate in order to maximize efficiency considering the capillaries labeled 1-16 correspond to A1-H2, A3-H4, A5-H6, A7-H8, A9-H10, and A11-H12 on the sample plate for each injection. See Figure 7.

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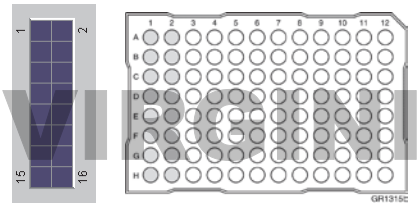

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Figure 7. Schematic of capillary locations (left) and depiction of sample plate (right). Capillaries labeled 1-16 correspond to A1-H2, A3-H4, A5-H6, A7-H8, A9-H10, and A11-H12 on the sample plate for each injection, resulting in a maximum of six injections per 96 sample plate.
Figure reproduced from Applied Biosystems 3130/3130xl Genetic Analyzers (2004) Maintenance and Troubleshooting Guide

2.8.2.1 Click on the Plate Manager from the ga3130*xl* dropdown list.

2.8.2.2 The sample information may be entered in a manual or automated fashion.

2.8.2.3 Manual Sample Input

2.8.2.3.1 Click the “New” button at the bottom of the display to open the New Plate Dialog box.

2.8.2.3.2 Complete the information in the New Plate Dialog: Type a name and description (optional) for the plate.

2.8.2.3.3 Select your fragment analysis application in the “Application” drop-down list. Typically, this will be “3130xl_1”.

2.8.2.3.4 Select 96-well or 384-well in the “Plate Type” drop-down list.

2.8.2.3.5 Type DFS for the owner and a name or initials for the operator.

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2.8.2.3.6 Click “OK” to open the Plate Editor, as shown in Figure 8.

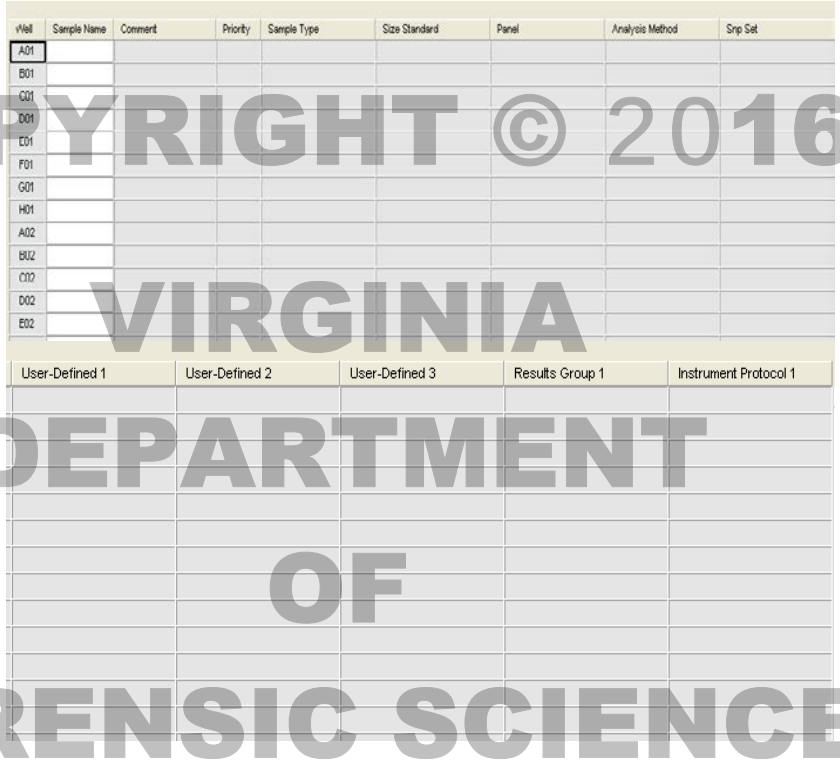

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Figure 8. Plate Editor. The sample name, results group, instrument protocol, dye set, etc. are set for each sample being separated on the 3130*xl*.

2.8.2.3.7 For each sample, fill in the following:

Sample Name

The Priority column will fill in automatically. Lowering the number (100 is default) for each set of 16 samples increases the priority.

Comments column, enter any comments or notations for the sample (optional)

Sample Type – choose either allelic ladder, positive control, negative control, or unknown from the drop down list.

Size Standard – choose ILS600 from the drop down menu

Panel – choose the PowerPlex® 16 panel from the options listed. Typically, this will be “PowerPlex_16_ID3.2.0”.

Analysis Method – choose the appropriate analysis method. Typically, this will be “PowerPlex16advanced”, found under a sub-menu.

Results Group - select a group from the drop-down list or create a new one (as described in Appendix L).

Instrument Protocol – select a protocol from the drop-down list. This will be “PP16_5sec” for the PowerPlex® 16 kit unless otherwise indicated.

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2.8.2.3.8 When finished entering the information in the plate manager, click “OK”.

2.8.2.4 Automated Sample Input

2.8.2.4.1 The plate data must be created from an outside program such as Microsoft Excel and be in a tab-delimited, .txt, format before it can be imported.

2.8.2.4.2 Click on the "Import" button on the bottom of the display to open the "Import Plate" dialog box.

2.8.2.4.3 Select the location and file containing the plate data and click on "OK". The plate will be added to the list of plate records.

2.8.2.4.4 Check the plate record by selecting the plate and clicking "Edit" to ensure the plate samples, results groups, protocols, and analysis methods are correct.

2.8.3 **Run Scheduler** – Two plates may be loaded onto the autosampler at a time in positions A and B. The run scheduler designates which plates are located in which position on the autosampler. When loading the autosampler, ensure the plate is properly positioned in either position A or B. If two plates are loaded into the autosampler, the first plate linked to its plate map will be the first plate that is injected, independent of whether it is loaded into position A or B (unless the priority column of the plate map has been used to re-prioritize the injections). The plate on the left in the autosampler is defined as position A and the plate on the right is defined as position B, as described below.

2.8.3.1 Click "Run Scheduler" in the tree pane of the Data Collection software to open the Run Scheduler window, as shown in Figure 9.

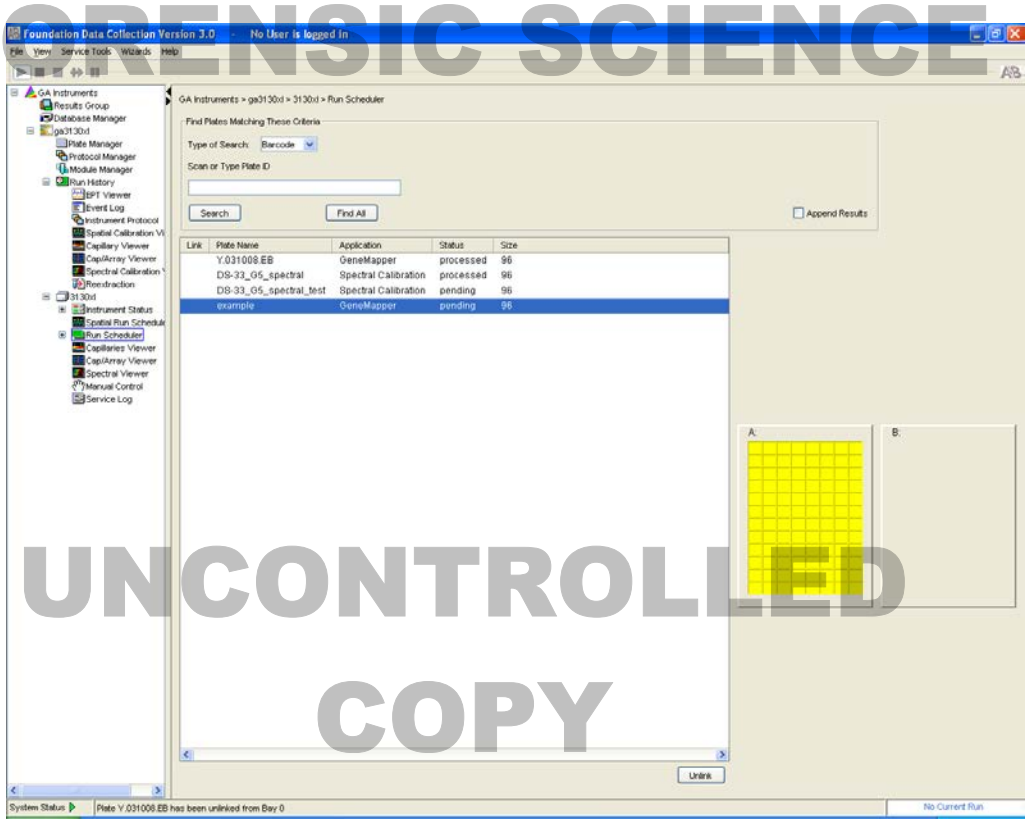

Figure 9. Run Scheduler.

2.8.3.1.1 Repeat 2.8.3 to indicate the location of a second 96-well plate, if necessary.

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2.8.3.2 Once the plates have been linked, the run can be started. Click the green button in the toolbar.

2.8.3.3 The Processing Plates dialog box opens. Click “OK”.

2.8.3.4 Following completion of the run, proceed to the “Analysis of Capillary Electrophoretic Data” chapter of the Commonwealth of Virginia Forensic Biology Section Procedure Manual Section VIII, Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 System for data analysis.

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3 ANALYSIS OF CAPILLARY ELECTROPHORETIC DATA

3.1 Technical Notes

3.1.1 The raw data obtained with the Applied Biosystems 3130*xl* Genetic Analyzer can be analyzed using GeneMapper® ID software v3.2.1.

3.1.2 GeneMapper® ID is an automated genotyping software program for forensic data analysis that supports analysis of electropherogram data obtained from the ABI 310 Genetic Analyzer, ABI 377 DNA Sequencer, ABI 3100 and 3100-*Avant* Genetic Analyzers, and the ABI 3130 and 3130*xl* Genetic Analyzers.

3.1.3 To the extent possible, the evidence samples will be analyzed by the examiner/reviewer prior to analyzing/reviewing the reference sample results.

3.2 Logging On

3.2.1 Open the GeneMapper® ID software v3.2.1 by double-clicking on the software icon.

3.2.2 Select the appropriate user name and type the password.

3.2.3 Click OK.

3.3 Project Window

3.3.1 Upon opening the GeneMapper® ID software, the user is taken to the Project Window, as shown in Figure 1.

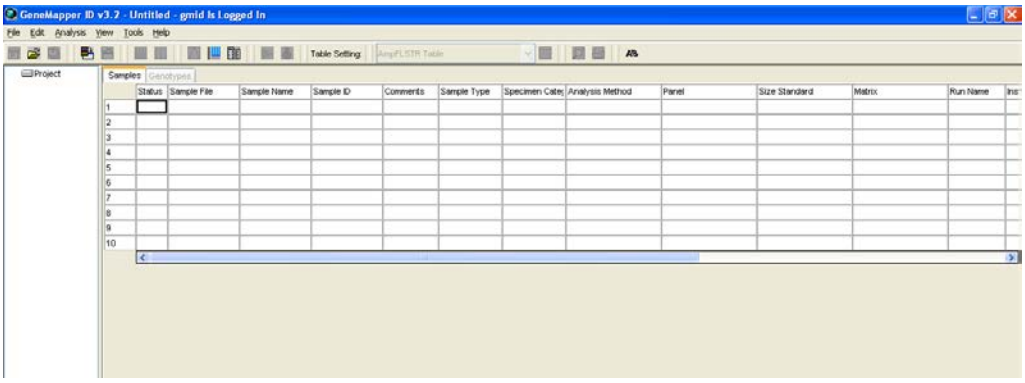

Figure 1. Project Window.

3.3.2 To begin the data analysis, samples must be added to the project to be analyzed.

NOTE: All samples to be analyzed from a single run should be added at once. If not done in this manner, the GeneMapper® ID software may not associate the correct allelic ladders with the given samples.

3.3.2.1 Choose “File” and “Add samples to Project” or, click the icon.

3.3.2.2 Browse to the data files to analyze. You may select the entire folder or pick the files within the folder to analyze.

3.3.2.3 Click “Add to List” (bottom left of screen) then “Add” (bottom right of screen).

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3.3.3 Upon addition of the samples to the project, the "Samples" tab will become populated, as shown in Figure 2. The population of the information is based on that entered in the Plate Map in the ABI 3130*xl* Data Collection Software. Double check to ensure that the correct panel, sample type, and analysis method are selected. If changes need to be made to this information, select the necessary cells and choose the appropriate settings from the drop-down menus. Verify that the information is correct before proceeding.

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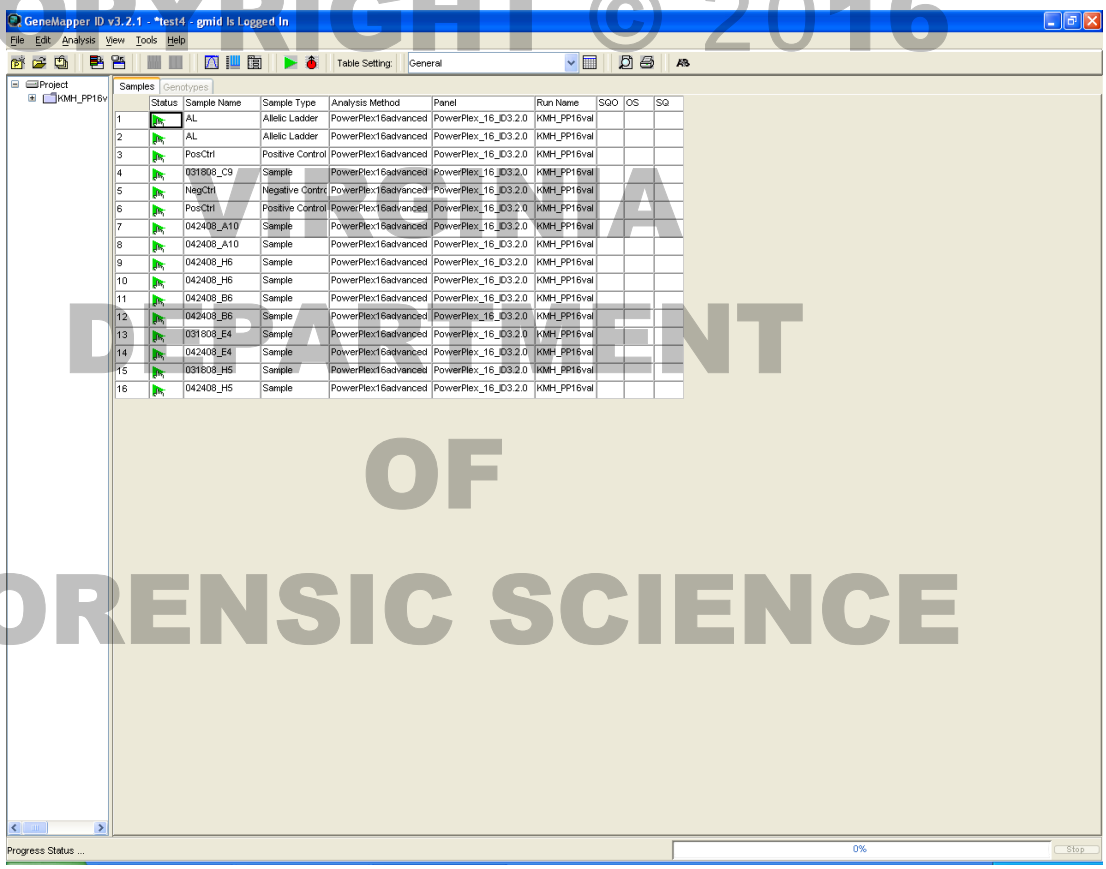

Figure 2. "Samples" tab upon adding samples to the newly created project.

3.3.3.1 Analysis Method = "PP16"

3.3.3.2 Panel = "PowerPlex_16_ID3.2.0"

3.3.4 Each data file has an on in the Status column indicating that it needs to be analyzed.

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3.3.5 In the tree pane view on the left side of the screen, choose the file name of a negative control. Choose View>Raw Data to view the unseparated raw fluorescence data.

3.3.6 The recognizable pattern of the size standard should appear in orange. The raw data may be used to evaluate baseline problems or noise that could result in poor sizing. In addition, the start and stop points for the analysis should be noted. The start and stop points should be such that all size standards are encompassed by the data points analyzed. Typically, this is approximately 3000 – 10000 data points, but may need to be modified if, for example, at the 10,000 end point, the 600 bp size standard is missing. In that example, extending the range beyond 10,000 will include the missing 600 bp fragment. This start and stop point will be selected in the Analysis Method Editor, as shown below.

3.3.7 Exit from the Negative Control raw data, and select Analysis>Analysis Method Editor

3.3.7.1 All of the settings should remain the same with the exception of the starting and stopping points, defined in 3.3.6.

3.3.7.2 Click on the “Peak Detector” tab.

3.3.7.3 The Peak Detector tab is where the values such as dye specific detection thresholds are set (Figure 3).

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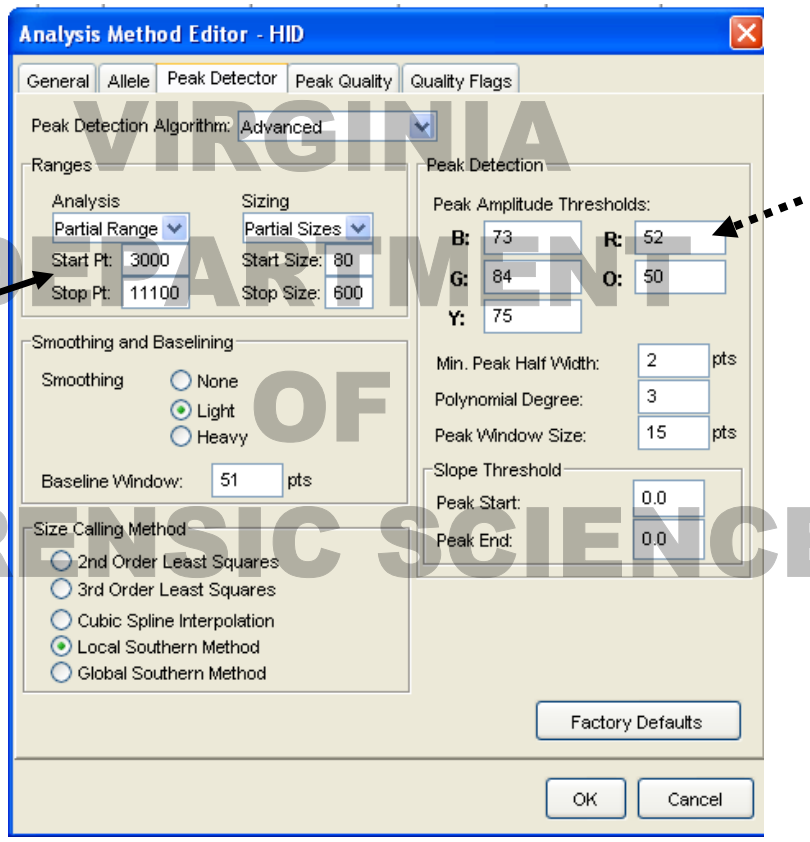

Figure 3. The Peak Detector Tab window. An example of peak amplitude threshold settings is shown. A solid arrow indicates the data analysis range start and stop points. The dashed arrow indicates the peak amplitude threshold for the red dye, which may be altered if needed. No other threshold values may be altered.

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3.3.7.3.1 The values for the peak amplitude thresholds are based upon empirical data derived from the limit of detection analysis performed during the validation work. While the ILS 600 (which uses the red dye) will have its default peak amplitude threshold set based on the LOD analysis, the threshold may be adjusted upward high enough to prevent detection of a known artifact present in the Promega ILS 600 size standard (Figure 4) or reduced when the ILS 600 peaks may fall below the default threshold (this can occasionally occur with the 2 second injection). Only the red dye (for the ILS 600) can have its LOD value modified. If such adjustment is necessary, refer to Appendix D for directions.

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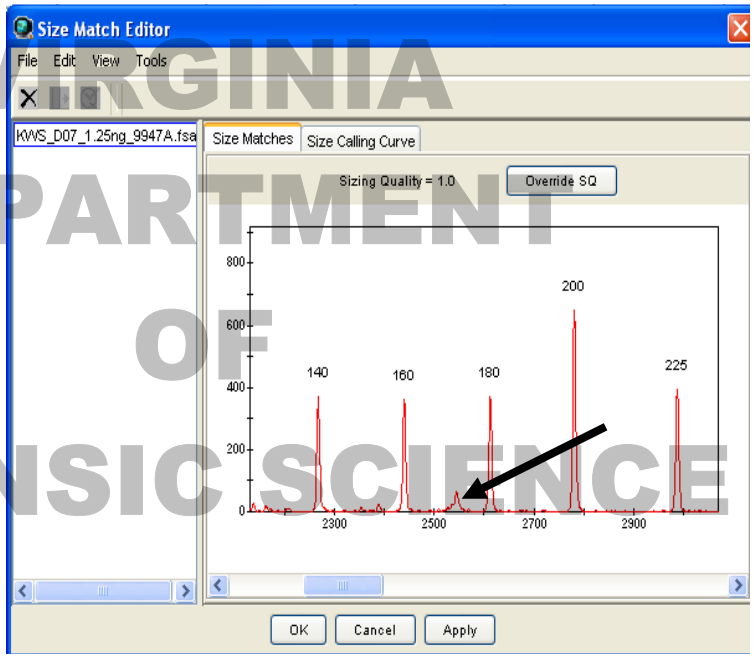

Figure 4. Size Match Editor window showing the ILS 600 size standard. The arrow points to the artifact at approximately 172 bp.

3.3.8 Analyze the samples by clicking the green arrow: (Analyze). Upon prompting, type a name for the project. Click OK.

3.3.9 Examining Analysis Results

NOTE: When loaded on the same plate, all evidence samples pertaining to a case **must** be analyzed prior to opening any reference sample electropherograms pertaining to the same case.

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3.3.9.1 Examine the size standard by examining the flags in the SQ (sizing quality) column. A green square indicates that a sample has passed the sizing criteria. For any samples that do not pass the sizing criteria, indicated by a yellow triangle or red octagon, assess the size standard by highlighting the samples and click on the icon: or selecting Analysis>Size Match Editor.

NOTE: If the majority of the size standards fail, look for a global problem with the run, such as a poorly-chosen data analysis range in the Analysis Method Editor (see 3.3.6).

3.3.9.2 If the sizing standard is observed in the sample, check to see that all peaks are detected and the peaks are labeled correctly. If no sizing standard is observed, the injection may have failed or no ILS and/or sample was added to the well. The peaks should follow the pattern shown in Figure 5. Spikes or other artifacts may be mislabeled by the software, resulting in poor sizing.

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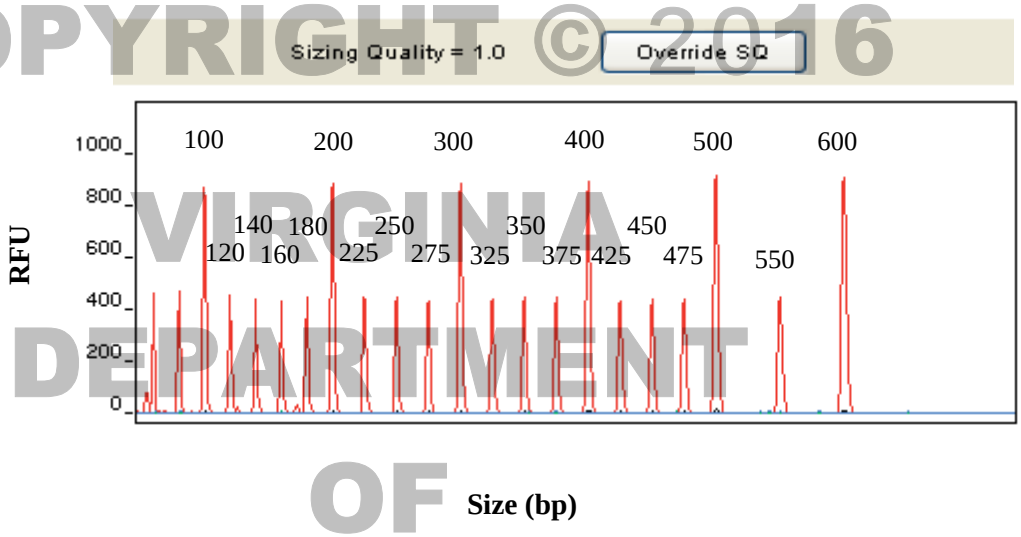

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Figure 5. ILS 600 Size standard. The peaks are labeled with their base pair size. The 60 bp and 80 bp peaks are unlabeled in this figure and may or may not be utilized for sizing purposes.

3.3.9.2.1 If the size standard peak assignments are incorrect for one or more of the samples, adjust the peak assignments within the size match editor.

3.3.9.2.1.1 The sizing quality of the sample is shown directly above the labeled size match electropherogram, as shown in Figure 5. Typically, a low sizing quality value (e.g., 0.5) is a result of a peak shift (the software incorrectly identifies one peak resulting in invalid positions for the other size standard peaks), a missing peak (the software does not identify a peak), or an extra peak (the software identifies a peak that is not a size standard peak, but a spike or pull-up peak).

3.3.9.2.1.2 To edit a particular size standard peak, right-click the peak to be edited to open the editing pop-up window and select “Add”, “Delete” or “Change” the selected peak.

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3.3.9.2.1.2.1 Choose Add or Change, move the cursor to the right to open the Select Size sub-menu. Choose the desired size from the menu.

Note: The 225 bp size standard may be deleted without compromising the sizing accuracy (source: Promega Corp). This may be occasionally necessary since pull-up from the yellow (TMR) channel, particularly from the amplification positive control, can produce a pull-up peak which overlaps with the 225 bp fragment and results in inaccurate sizing and allele calls.

3.3.9.2.2 When the standard peaks are properly designated, check the sizing quality by selecting Tools>Check Sizing Quality. If the peaks are sized correctly, the

quality value will improve. If the peaks are sized correctly but the quality score is still below passing, click the "Override SQ" button, as shown in Figure 5 to set the sizing quality to 1.0. Note that the sizing quality will now read <1.0> and ✕ will appear in the Sample tab under the SQO (Sizing Quality Override) column to indicate the sizing quality has been overridden by the analyst. If the 225 bp standard is deleted due to pull-up as described above, the ✕ will also appear if the sizing quality is improved, even if the "Override SQ" button is not clicked, indicating that the size standard was edited. The same icon will also appear in the electropherogram printout in the header for each dye. Alternatively, the sample may be re-injected at the analyst's discretion.

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3.3.9.3 Review of the Allelic Ladder Data

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3.3.9.3.1 In the "Samples" tab, select each allelic ladder.

3.3.9.3.2 Display the plots by clicking the sample plots icon:

3.3.9.3.3 Verify that the allelic ladder is called correctly for each locus. Figure 6 represents an example of an accurately designated PowerPlex® 16 allelic ladder.

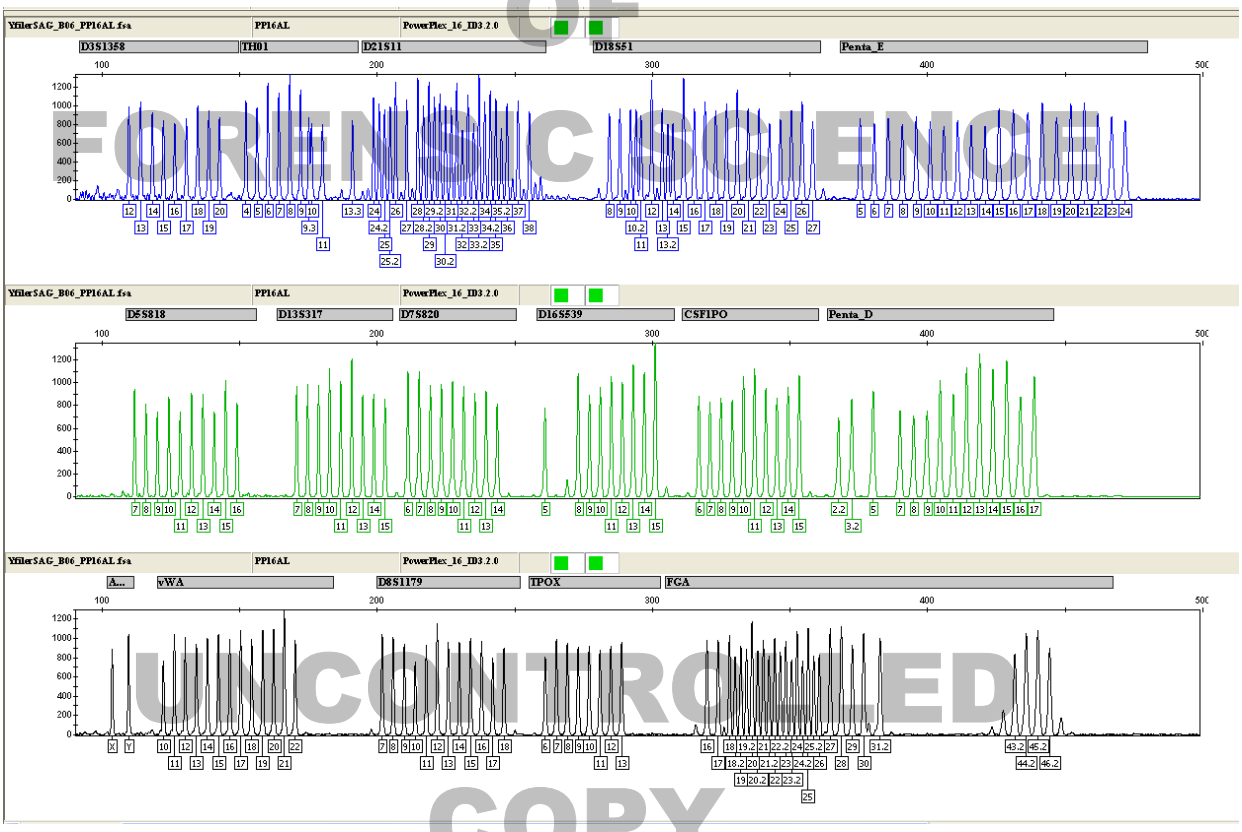

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Figure 6. PowerPlex® 16 Allelic Ladder.

3.3.9.4 Review of sample data

3.3.9.4.1 All sample data must be reviewed carefully, with special attention paid to samples that display a yellow triangle or red octagon, indicating low quality analysis.

3.3.9.4.1.1 A green square indicates that the data passed the initial (automated) analysis by the software in the respective category (such as sizing quality, off-scale data, etc.).

3.3.9.4.1.2 If a flag is yellow or red, it may be helpful to look at the raw data to determine the problem. To look at the raw data from the samples tab, highlight the sample and select View>Raw Data.

3.3.9.4.1.3 If it is determined that the sample should not be considered for interpretation due to problems within the raw data, the sample may be re-injected or run again using a different amplification reaction, more PCR product, less PCR product, or other necessary procedures.

3.3.9.4.2 If the sizing quality for a given sample is low, the sample will not be genotyped by the software.

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3.3.9.4.3 The sample data can be viewed by selecting View>Sample Plot or choosing the icon: The data is displayed as shown in Figure 7.

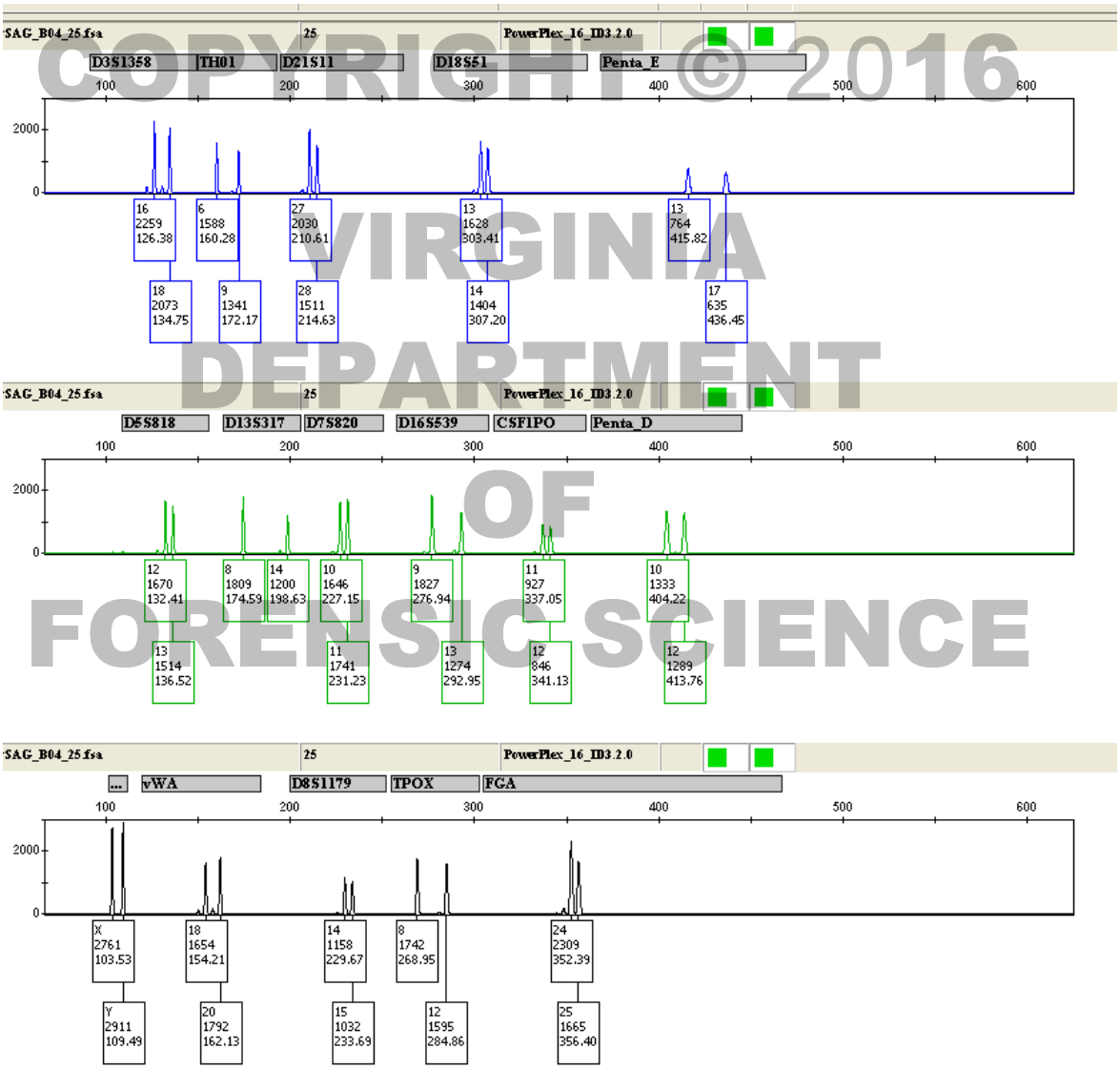

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Figure 7. An example of PowerPlex® 16 sample data. Under each allele, the allele call (repeat number), height (RFUs), and size (bp) are listed. If an edit was made to the allele call, this comment would also be indicated in the box under the peak.

3.3.9.4.4 The negative control(s) should be checked to ensure that no amplification product (called alleles above LOD threshold) was observed. A called allele is a peak which fits the criteria to be designated an allele or OL (off ladder) and is above the allele calling threshold designated in the software and therefore is labeled. Refer to the "Interpretation of PowerPlex® 16 System Results" chapter of the Commonwealth of Virginia Forensic Biology Section Procedure Manual Section VIII, Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 System for interpretation guidelines.

3.3.9.4.5 The positive control(s) should be checked to ensure that injection and electrophoresis performance was satisfactory. Refer to the "Interpretation of PowerPlex® 16 System Results" chapter of the Commonwealth of Virginia Forensic Biology Section Procedure Manual Section VIII, Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 System for interpretation guidelines.

3.3.9.4.5.1 If the positive control was injected poorly, it can be re-injected or if necessary, the positive control sample can be re-prepared and injected. The original sample injections can be analyzed if the correct types for the positive control are documented upon the re-injection/re-run. However, in order to use the data produced in a run, at least one positive control on the run must result in the properly-designated alleles.

3.3.9.4.5.2 Artifacts such as pull-up and spikes may occasionally occur and must be documented as described in the "Interpretation of PowerPlex® 16 System Results" chapter of the Commonwealth of Virginia Forensic Biology Section Procedure Manual Section VIII, Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 System.

3.3.9.4.5.3 If pull-up peaks are due to poor color separation and not excessive peak height of peaks in other channels, then a new spectral calibration may need to be performed and the samples analyzed using the new spectral.

3.3.9.4.6 The Genotypes tab of the project window displays a number of additional "flags" to indicate the quality of the data. These may be used, at the analyst's discretion, to aid in interpretation of the data. Table 1 gives the name and description of the flags and their columns.

3.3.9.4.7 The flags are check marks, green squares, yellow triangles, or red octagons.

3.3.9.4.7.1 A check mark or green square indicates the data passes the analysis for that quality indicator.

3.3.9.4.7.2 A yellow triangle indicates that the data should be reviewed.

3.3.9.4.7.3 A red octagon indicates poor data quality or analysis quality. The sample may not be analyzed by GeneMapper® ID in some instances.

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Table 1. Peak Quality Value Flags on the Genotypes Tab

Column	Description
AE	Allele Edit ♦ The check box is unchecked immediately after analysis. ♦ The check box is checked when the marker allele calls have been edited by the analyst.
ADO	Allele Display Override ♦ The checkbox is checked when the number of the marker calls exceeds the number to display previously set (in Table Settings Editor).
SHP	Sharp Peak ♦ This flag is used for SNP analysis only, not fragment analysis.
AN	Allele Number ♦ A flag is displayed when the number of alleles exceeds the maximum (or no alleles are found). This maximum is set in the analysis method.
OBA	One Basepair Allele ♦ This flag is used for SNP analysis only, not fragment analysis.
SPA	Single Peak Artifact ♦ This flag is used for SNP analysis only, not fragment analysis.
Bin	Out of Bin Allele ♦ A flag is displayed when the called alleles' peak apex is out of the bin boundary. The peak is then labeled "OL".
PHR	Peak Height Ratio ♦ A flag is displayed when there are two alleles present and the ratio between the lower allele height and the higher allele height is below a certain level (default = 50%, set in the Peak Quality tab of the analysis method). The ratio is based on the peak height of the called allele peaks.
LPH	Low Peak Height ♦ A flag is displayed when the alleles are lower than the specified values and do not result in the proper intensity. The default is 200 RFU, set in the Peak Quality tab of the analysis method.
SPU	Spectral Pull-Up ♦ A flag is displayed when the marker signal contains bleed-through peaks ♦ Pull-up is when the peak height of the called peak is less than 10% of the larger peak within ± 1 data point.
BD	Broad Peak ♦ A flag is displayed when the called alleles' peak width is wider than 1.5 basepairs.
SP	Split Peak ♦ A flag is displayed when a split peak is found by the software. ♦ A split peak is defined as overlapping peaks that are < 0.25 basepairs apart.
OS	Offscale ♦ A flag is displayed when there are offscale peaks present within the marker size range.
CC	Control Concordance ♦ A flag is displayed when the designated control sample does not exactly match the defined alleles for this marker in the panel.
OVL	Overlap ♦ A flag is displayed when a peak in the overlapped region is called twice, as it is possible to have two allele size ranges that overlap.
NB	Narrow Bin ♦ This flag is used for SNP analysis only, not fragment analysis.
DP	Double Peak ♦ This flag is used for SNP analysis only, not fragment analysis.
GQ	Genotype Quality ♦ A red octagon is shown when the analysis for a sample is of low quality. ♦ A green square indicates the analysis is of good quality. ♦ A yellow triangle indicates the analysis should be checked. ♦ The weighting of the various factors that are used for calculating the genotype quality are set by the analysis method.

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3.3.10 Printing the results

3.3.10.1 If the project file must be transferred to another, non-networked computer, the GeneMapper® ID project must be exported.

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3.3.10.1.1 Under the Tools drop-down window, select GeneMapper Manager and the following window will open up (Figure 8).

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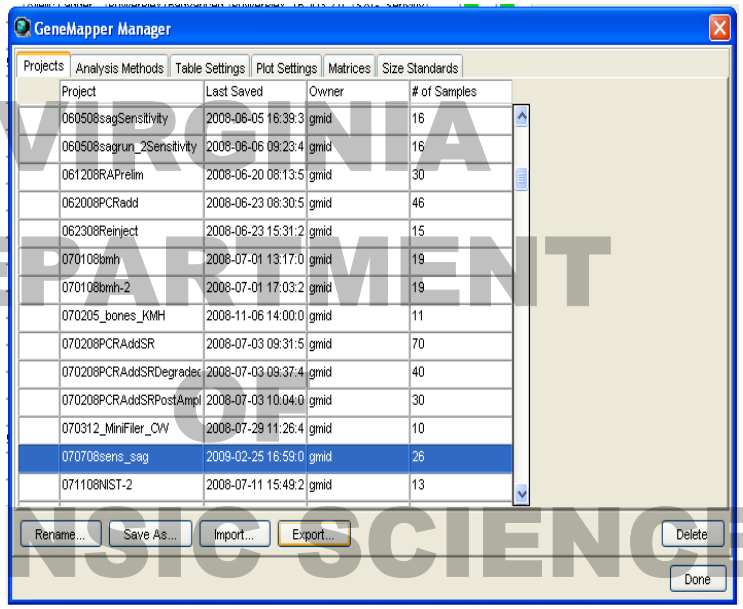

Figure 8. GeneMapper Manager.

3.3.10.1.2 Highlight the project file that is to be exported and select export. The project will be saved to the location and file folder designated. Transfer the project file to a CD or approved flash drive for transfer to another computer workstation.

3.3.10.1.3 To open the project file at another computer workstation, transfer the project file from the CD or flash drive to the desktop or a designated folder. Open GeneMapper® ID and then open GeneMapper Manager as shown in Figure 8. Select import and your project file will imported into the GeneMapper® ID project file list. Select Open file in GeneMapper® ID and find your project in the file list.

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3.3.10.2 The electropherogram of each evidence and reference sample pertaining to a case will be printed for the case file and initialed by the analyst. The electropherogram will display the allele calls, any editing comments, the peak height, and basepair size. Any remaining electropherograms (including blanks, controls, and allelic ladders) will be contained in electronic form or paper form in the case file.

3.3.10.3 After reviewing each allele call in the samples plot, the x- and y-axes should be set accordingly for printing (as shown below). If the axis needs to be changed, right-click on the axis to display the scale settings.

3.3.10.3.1 X-axis = 95bp – 500bp

3.3.10.3.2 Negative Control, Reagent Blank, or samples with no typing results: Y-axis = 0 – 100 RFU

3.3.10.4 Print the electropherogram by File>Print.

3.3.10.5 Should additional documentation be necessary to support an allele designation, other views of the data (such as a zoomed-in view of an electropherogram) may be printed and retained in the case file, at the analyst's discretion.

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4 INTERPRETATION OF POWERPLEX® 16 SYSTEM PCR AMPLIFICATION RESULTS

4.1 Technical Notes

4.1.1 STR alleles are small in size, generally less than 500 bp and contain repeat units ranging from 3 to 7 bases.

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4.1.2 If an allele contains an incomplete repeat, the allele is considered a microvariant and is designated by the number of complete repeats present followed by a decimal point, followed by the number of bases of the incomplete repeat. For example, the FGA 22.2 allele contains 22 tetrameric repeats plus 2 bases. Because of a deletion of two bases the FGA 22.2 allele is two bases shorter than the FGA 23 allele.

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4.1.3 The characteristics of the PowerPlex® 16 System and the allelic ladders are given in the table below[1]:

Table 1. PowerPlex 16® System loci and allelic ladder alleles

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Locus	* Repeat Sequence 5' 3'	Chromosome Location	Size Range of Allelic Ladder (bp)	Alleles present in Allelic Ladder	Fluorescent Label
FGA	TTTC Complex	4q31.3, αfibrinogen, 3rd intron	322-444	16-30, 31.2, 43.2, 44.2, 45.2, 46.2	TMR
TPOX	AATG	2p25.3 thyroid peroxidase, 10th intron	262-290	6-13	TMR
D8S1179	TCTA Complex	8q24.13	203-247	7-18	TMR
vWA	TCTA Complex	12p13.31 von Willebrand Factor, 40th intron	123-171	10-22	TMR
Amelogenin	NA	Xp22.1-22.3 and Y	106 – X 112 – Y	X, Y	TMR
Penta E	AAAGA	15q26.2	379-474	5-24	FL
D18S51	AGAA	18q21.33	290-366	8-10, 10.2, 11-13, 13.2, 14-27	FL
D21S11	TCTA Complex	21q21.1	203-259	24, 24.2, 25, 25.2, 26-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36-38	FL
TH01	AATG	11p15.5 tyrosine hydroxylase 1st intron	156-195	4-9, 9.3, 10-11, 13.3	FL
D3S1358	TCTA Complex	3p21.31	115-147	12-20	FL
Penta D	AAAGA	21q22.3	376-449	2.2, 3.2, 5, 7-17	JOE
CSF1PO	AGAT	5q33.1 c-fms proto-oncogene, 6th intron	321-357	6 – 15	JOE
D16S539	AGAT	16q24.1	264 – 304	5, 8 –15	JOE
D7S820	AGAT	7q21.11	215 –247	6 – 14	JOE
D13S317	AGAT	13q31.1	169 –201	7 – 15	JOE
D5S818	AGAT	5q23.2	119 -155	7 – 16	JOE

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*All repeat sequences are defined using the recommendation of the DNA Commission of the International Society of Forensic Haemogenetics (ISFH): 1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5' nucleotide of the repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used.[2,3]

KEY: TMR = carboxy-tetramethylrhodamine, FL = fluorescein, JOE = 6-carboxy-4’,5’-dichloro-2’,7’-dimethoxyfluorescein.

Applied Biosystem 3130*xl* Genetic Analyzer[1,4]:

Fluorescein is detected at a wavelength of ~530-540 nm.

TMR is detected at a wavelength of ~580-590 nm.

JOE is detected at a wavelength of ~555-565 nm.

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4.1.4 The Fluorescent Internal Lane Standard 600 (CXR – carboxy-X-rhodamine) consists of 22 DNA fragments (60, 80,100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, and 600 bp) and can be detected at a wavelength of ~615-625 nm[1,4] .

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4.1.5 The "Known" Genotypes for the amplification positive control DNA using the PowerPlex® 16 System are given in the table below[1]

Table 2. 2800M PowerPlex® 16 profile

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Locus	Genotype
Penta E	7,14
D18S51	16,18
D21S11	29,31.2
TH01	6,9.3
D3S1358	17,18
FGA	20,23
TPOX	11,11
D8S1179	14,15
VWA	16,19
Amelogenin	X,Y
Penta D	12,13
CSF1PO	12,12
D16S539	9,13
D7S820	8,11
D13S317	9,11
D5S818	12,12

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4.2 Procedure

4.2.1 Verification of the allelic ladder

Figure 1 shows electopherograms of the PowerPlex® 16 allelic ladder. Ensure that alleles in the allelic ladder are properly designated.

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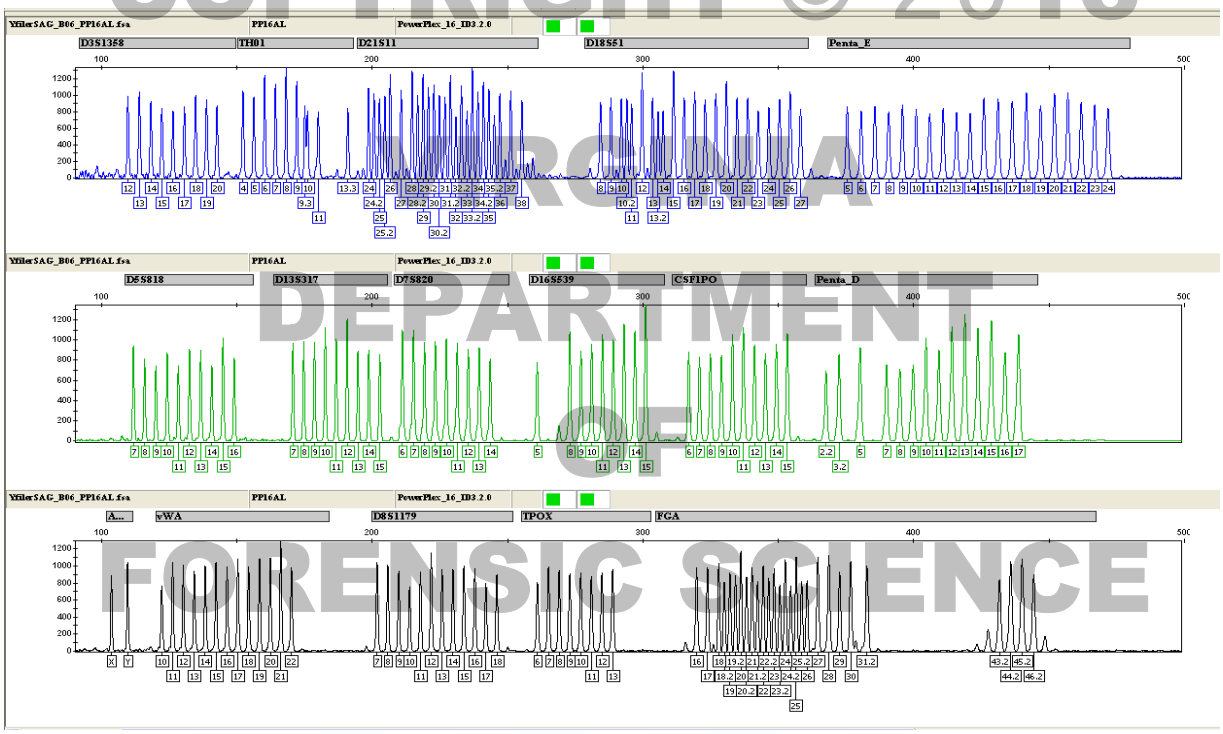

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Figure 1. PowerPlex® 16 allelic ladder

4.2.2 The electropherogram data will be evaluated in order to determine if samples need to be re-injected, injected for a longer or shorter period of time, more/less amplified DNA added to the injection cocktail or if a new spectral must be created due to excessive pull-up.

4.2.3 The source of a DNA sample may be from a single person or more than one person. This may be determined by examination of the number of alleles (peaks) at each locus as well as additional information such as peak height ratios at loci with more than one allele.

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4.2.4 A DNA profile may be considered as having originated from a single individual if no more than two alleles (peaks) are observed at each locus. Figure 2 displays an example of a single source profile. However, there are examples of three allele patterns due to gene duplication or translocation events.[5] All loci should be taken into account when making this determination as well as the peak height ratios when two peaks are present.

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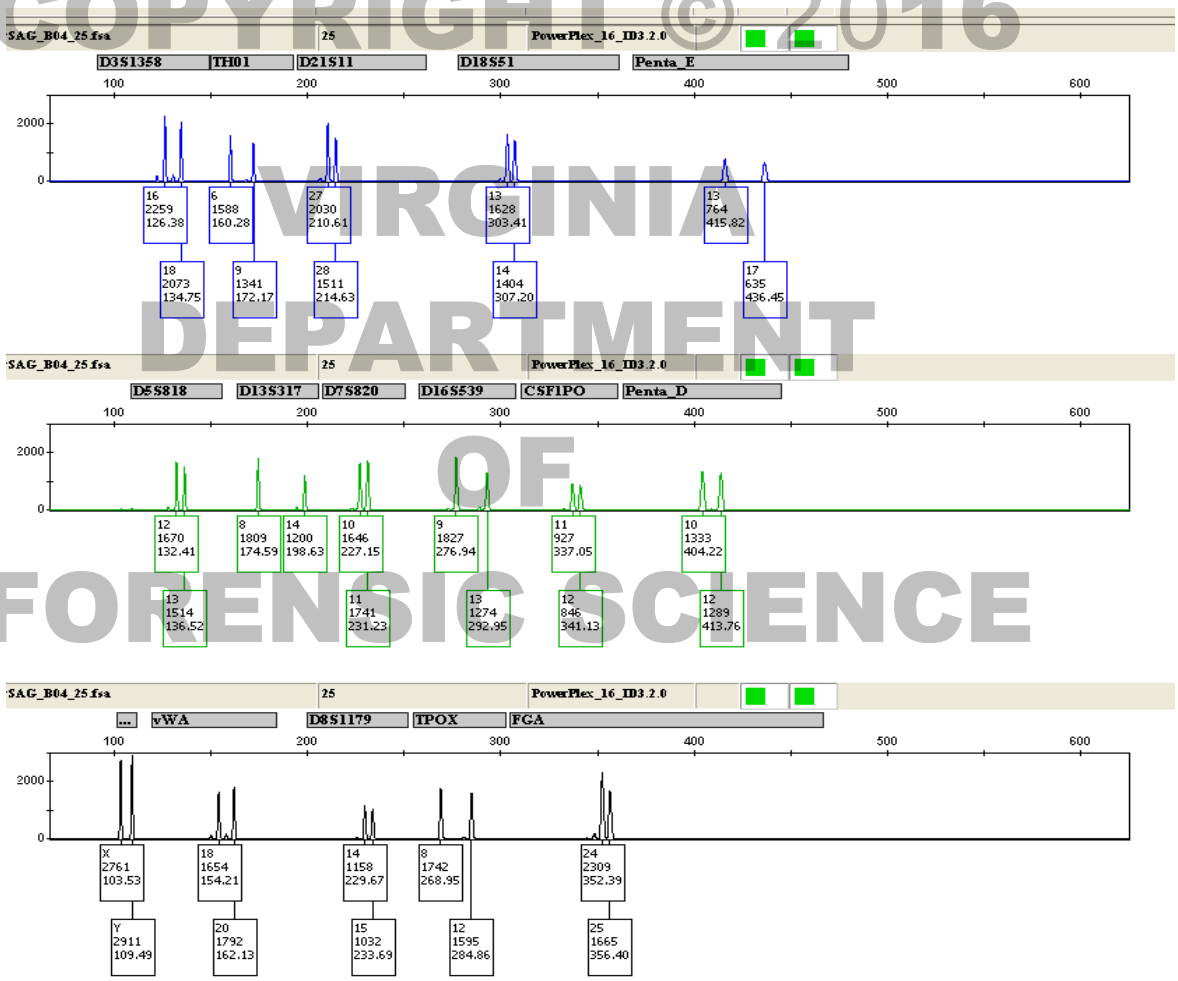

Figure 2. Electopherogram of a single source PowerPlex® 16 profile

NOTE: Although average peak height ratios for a single source sample at a heterozygous locus may be above 0.6 when DNA quantity is above stochastic levels (250 pg and greater), minimum heterozygote balances have been observed below 0.6 for single source samples even at sufficient quantities of input DNA (250 pg and greater,).[6] Table 3 displays the heterozygote peak ratio data reported in the VDFS "Validation of the PowerPlex® 16 Amplification System: Heterozygous Peak Balance" study.

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Table 3. Lower limit heterozygote peak height percentage for each locus in the PowerPlex® 16 System. Data generated from amplifications of 1 ng and 0.5 ng template DNA (eleven purified DNA samples each) during validation testing.

Locus	Lower Limit Difference Between Heterozygous Alleles (minimum observed)	Locus	Lower Limit Difference Between Heterozygous Alleles (minimum observed)
Penta E	33%	VWA	60%
D18S51	61%	Amelogenin	64%
D21S11	72%	Penta D	53%
TH01	53%	CSF1PO	61%
D3S1358	69%	D16S539	51%
FGA	50%	D7S820	59%
TPOX	59%	D13S317	55%
D8S1179	78%	D5S818	75%

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4.2.5 The GeneMapper® ID software provides information on peak quality (height, base pair size, shape, number) as well as designating alleles. The GeneMapper® ID data along with the electropherogram data should be used in combination to determine the potential number of contributors to a DNA sample.

4.2.6 If it is determined that an unknown sample is a mixture, then a determination of whether or not a major profile can be ascertained will be made.following the guidelines described in 4.2.7.7. If a major profile can be determined, then a match probability can be applied, where feasible, as described in the "Statistical Calculations" chapter of this manual.

4.2.7 When an excess of DNA is added to the amplification cocktail, artifacts may occur. Excess DNA added to the PCR can produce an increased amount of PCR product and this can result in the following:

4.2.7.1 Fluorescence intensity that exceeds the linear dynamic range for detection by the instrument ("off-scale" data). Quantitation (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause the corresponding stutter peak to appear higher in relative intensity, thus increasing the calculated percent stutter. These samples should be diluted and/or reinjected for a shorter time period.

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4.2.7.2 Multicomponent analysis of off-scale data can result in poor spectral separation ("pull-up"). Refer to Figure 3 for an example of pull-up at the CSF1PO locus.

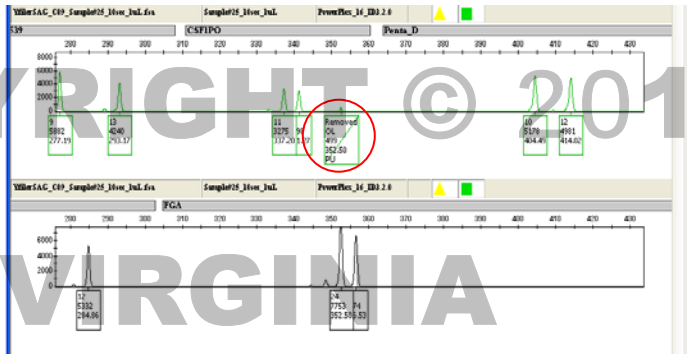

Figure 3. Pull-up from the yellow/black channel (TMR) into the green channel (JOE), indicated by the red circle and shown as an edited off-ladder (OL) peak that has been changed to indicate pull-up (PU). Editing allele calls is described in 4.2.7.6.

4.2.7.3 Incomplete +A nucleotide addition

The PCR process using AmpliTaq Gold® is optimized such that an additional Adenosine nucleotide is added onto the extended fragment. Excessive input DNA makes the Adenosine addition less efficient and thus PCR fragments are shorter by one nucleotide than the true amplicon size (-A).

4.2.7.4 Stutter peaks

Stutter peaks frequently appear during PowerPlex® 16 System profiling. These are most commonly 4 nucleotides smaller than the amplicon size (true peak) for the tetranucleotide repeats and 5 nucleotides smaller for the pentanucleotide repeats. Careful consideration, such as the evaluation of whether or not the sample appears to be degraded and if the peak height ratios fall below 0.6, must be made when differentiating stutter peaks and a possible mixture. Stutter is typically more pronounced when excessive quantities of DNA are placed into the amplification cocktail. Stutter may also appear as multiples of the repeat unit (e.g., 8 nucleotides) or may be larger than the amplicon size (+4 stutter or “up-stutter”).

NOTE: Stutter peaks are believed to be a result of slippage of the *Taq* polymerase during amplification. The GeneMapper® ID software is programmed to detect (i.e., not call as an allele) stutter peaks that are less than or equal to a given stutter ratio (defined in the panel manager based upon VDFS validation data shown in Table 4) for each locus. This will only be applied to the n-1 repeat stutter. Examples of stutter peaks are shown in Figure 4.

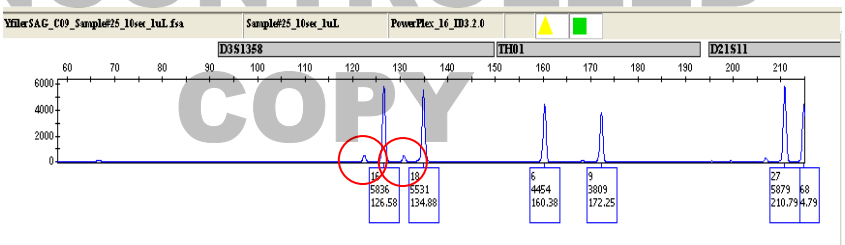

Figure 4. Stutter peaks at the D3S1358 locus, indicated by red circles.

4.2.7.4.1 If the ratio of the peak height for the smaller peak to the larger peak is less than the established stutter value (listed in the Tables 4 and 5 below), the allele will

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be considered to be stutter by the GeneMapper® ID software and no allele will be designated even if it is suspected that the peak is a true allele..

Table 4. Stutter percentages (n-1 repeat) generated at VDFS for the PowerPlex® 16 System loci. The stutter values are averages (plus three standard deviations) of the observed stutter values.

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Locus	% Stutter	Imported into GeneMapper ID	Locus	% Stutter	Imported into GeneMapper ID
D3S135	12.6	13	**D16S539**	11.4	11
TH01	5.4	5.0	**CSF1PO**	10.8	11
D21S11	13.8	14	**Penta D**	4.1	4
D18S51	13.5	14	**vWA**	17.8	18
Penta E	8.2	8	**D8S1179**	10.8	11
D5S818	10.9	11	**TPOX**	5.9	6
D13S31	10.5	11	**FGA**	12.6	13
D7S820	9.8	10	**Amel.**	NA	NA

NA = not applicable.

Table 5. Stutter percentage n+(1 repeat) stutter observed at VDFS for the PowerPlex® 16 System loci.

Locus	N	Ave. n+1 Stutter %	Std Dev (SD) %	Average+ 3 SD	Maimum observed stutter
D3S1358	17	1.6	0.9	4.3	
TH01	0			NA	
D21S11	25	2.6	1.4	6.8	
D18S51	6	2.8	2.3	9.7	
Penta E	1	1.0		*	1.0
D5S818	8	1.5	0.8	3.9	
D13S317	10	1.7	0.7	3.8	
D7S820	12	1.7	1.1	5.0	
D16S539	4	1.7	0.5	*	2.4
CSF1PO	8	2.4	1.4	6.6	
Penta D	1	1.0		*	1.0
vWA	11	2.5	1.9	8.2	
D8S1179	6	1.2	0.3	2.1	
TPOX	0			NA	
FGA	3	1.9	0.4	*	2.4
Amel.	NA	NA	NA	NA	NA

NA = not applicable.

4.2.7.4.2 If the ratio of the peak height for the smaller peak to the larger peak is above the established stutter value (this event is generally observed in samples containing a high concentration of DNA or the DNA is partially degraded), the allele may still be called stutter once all loci have been taken into account and both the examiner/analyst and the technical reviewer are in agreement (based upon knowledge of the system and experience) that the smaller peak is stutter.

Alternatively, the examiner must also evaluate the entire profile for evidence of more than a single contributor (such as more than three peaks at a locus, peak height ratios below 60%) to conclude that the peak is not stutter and the profile is a mixture.

4.2.7.5 For analysis of sample data, all electropherograms must be visually evaluated by the analyst to ensure that artifacts are not mislabeled. If a peak is mislabeled (e.g., spike, pull-up, etc.), the allele call can be edited by the analyst, either electronically or manually (by hand).

4.2.7.6 Indication that a peak may be an artifact can result if a given PowerPlex® 16 System STR profile is known to be single-source or appears to be single source based upon the providence of the sample, but an extra peak(s) is observed. In such cases, the extra peak(s) must be assessed to determine if it is a true allele or an artifact. Similarly, artifacts may be observed in amplification products arising from more than one individual. In all cases, the determination of artifact vs. true allele is made by using one or more of the following as needed: re-injection, re-amplification and review of the data.

4.2.7.6.1 To electronically edit the allele call of a mislabeled peak, select the peak of interest by right-clicking on it.

4.2.7.6.2 If the peak is already labeled, the allele call can be deleted. The user will be prompted to add a comment.

4.2.7.6.2.1 If the deleted label is on a stutter peak, the comment will indicate “stutter” or “ST”.

4.2.7.6.2.2 If the deleted label is on a pull-up peak, the comment will indicate “pull-up” or “PU”.

NOTE: Pull-up, an example of which is shown in Figure 3, refers to peaks that are not true alleles but result from poor color separation of the raw data or off-scale data in one or more channels. Recurrent pull-up due to poor color separation in samples indicates the need to complete a new spectral calibration.

4.2.7.6.2.3 If the deleted label is on a spike, the comment will indicate “spike”.

NOTE: Signal spikes can frequently resemble peaks. Spikes usually occur at the same/similar location in more than one color (dye). Spikes are also characterized by their very narrow width. GeneMapper® ID has an algorithm that discriminates between true peaks and spikes, although, occasionally, the analyst may encounter a spike called as an allele. While the signal spike does not result from an actual DNA fragment, the software cannot always make this distinction and therefore calculates a size in base pairs for the spike.

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4.2.7.6.2.4 If the deleted label is on a peak that appears to be raised baseline, as opposed to a true peak, the comment should indicate “raised baseline” or “RB”. See Figure 5 for an example.

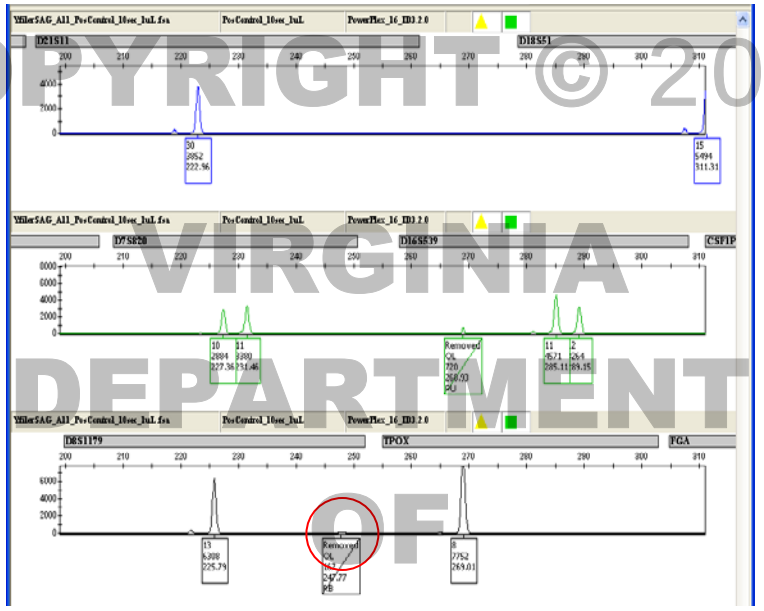

Figure 5. An example of raised baseline at the D8S1179 locus, indicated by the red circle.

4.2.7.6.2.5 If the deleted label is on –A peak, the comment will indicate “-A”.

NOTE The comment “ART” may also be used to indicate any type of artifact in place of the specific comments listed above, however to the extent possible, more descriptive designations will be used. Alternatively, a “?” may be used to indicate that a clear interpretation of the signal (peak) cannot be made.

NOTE: If a sample is believed to be inhibited (due to qPCR and/or STR results), a Microcon® 100 may be utilized in accordance with Chapter 2, DNA Purification, to improve typing.

4.2.7.6.3 Allele edits are displayed in the Genotypes Table (an X is placed in the AE [Allele Edit] column) and on the electropherogram. On the electropherogram, the initial allele call is displayed (with a strike-through) and the comment added to the label, as shown in Figure 6.

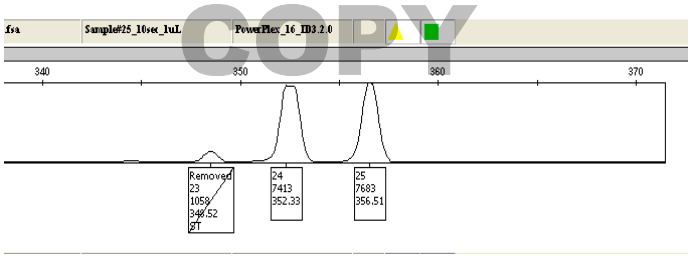

Figure 6. An example of a peak label that has been deleted to indicate it is a result of stutter.

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4.2.7.7 Evidence samples should be evaluated to see if a DNA statistic can be calculated. The traditional statistics evaluated should be the single source statistic, the combined probability of inclusion (CPI), the unrestricted random match probability (uRMP) and the likelihood ratio method that is calculated in POPSTATS.

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4.2.7.7.1 If the case is a current non-person's case and one of the above calculations cannot be calculated, no comparisons will be conducted on that particular item.

4.2.7.7.2 If the case is a current person's case, additional statistical calculations may be applicable such as a restricted RMP using Armed Xpert (AX) or likelihood ratio using True Allele (TA) and comparisons will be made and conclusions will be reported.

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4.2.7.7.3 If the case is a historical case where conclusions have already been provided, the statistical calculations listed in 4.2.7.7 and/or 4.2.7.7.2 will be used.

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4.2.8 A comparison is performed between known samples and questioned samples.

4.2.8.1 If the DNA profiles are distinctly different, it is concluded that the sample originated from a different source than the individual of interest and the individual is excluded.

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4.2.8.2 If the DNA profiles appear consistent, indicating the possibility they may have originated from the same source, that individual in question cannot be eliminated as a possible source. Refer to section 4.3 for further guidance.

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4.2.8.3 A sample may be considered to be a mixture of DNA from two or more individuals if the sample contains 3 or more peaks at one or more loci and/or there is a distinct difference in peak heights at heterozygous loci. However, as described earlier, validation studies conducted at the Virginia Department of Forensic Science have demonstrated that the heterozygote peak balance may fall below 0.6 even for single source samples with 1 ng of DNA in the amplification reaction. All loci should be taken into account when making this determination.

4.2.8.4 In a two contributor mixture, a major profile will be called when the peak height of the minor source of DNA is 33.0% or less than the peak height of the major source of DNA. If one or both the minor and the major contributors is heterozygous, then the 33.0% value applies to the largest of the minor contributor peaks and the smallest of the major contributor peaks. At least one allele from the major contributor shall be above the stochastic threshold. Four or more loci meeting the above requirement of 33.0% or less of the minor to the major peak heights and at least one allele above the stochastic threshold are required to calculate major RMP statistics. Additionally, heterozygous DNA types shall be in peak height ratio for 2 person mixtures. All remaining loci are taken into account to support the conclusion of the major contributor. The major profile can be treated statistically as a single source profile as described in the Statistical Calculations chapter of this manual. In 2 person mixtures the minor contributor may be defined and treated as a single source profile for statistical purposes.

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4.2.8.5 A major profile may still be called when there are multiple minor contributors to the mixture if a clear major profile is observed. The clear major profile must still conform to the rules described in 4.2.7.8.4 with the exception that the heterozygous DNA types should generally be within peak height ratio.

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4.2.8.6 DNA typing results are required at 7 loci or more before DNA comparisons can be made. Otherwise, the partial profile shall be reported as inconclusive.

4.2.8.7 When a probative result is obtained for an evidence sample and an inconclusive result is obtained at more than one locus for a known reference sample, the known reference sample will be re-typed or re-amplified in order to produce a complete DNA profile. To the extent possible, complete DNA typing results will be obtained for reference samples.

4.2.8.8 Generally, statistical frequencies will be generated for DNA profiles (mixture and non-mixture profiles) obtained from evidence where the reference profile cannot be eliminated.

4.2.8.9 Statistical frequencies will not be generated at a locus if the known standard from a reference sample exhibits a 3 or 4 peak pattern.

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4.2.8.10 In criminal paternity/maternity and missing person cases, an individual must be eliminated at three or more loci to account for the possibility of mutations before the individual is eliminated as a parent/offspring.

4.2.8.10.1 In criminal paternity and missing person cases, inconsistencies may be observed without declaring the individual as eliminated as the source of genetic material. However, statistical analysis must be performed to incorporate the possibility that a mutation occurred. Mutations typically result in a full repeat difference, larger or smaller, for the allele.

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4.2.8.10.2 When a couple is evaluated as possible biological parents of a missing person, each possible parent's DNA profile will be evaluated separately to determine if the individual is included or eliminated as a biological parent. Subsequently the profiles from both individuals will be evaluated together to determine if as a couple they could have conceived the missing person.

4.2.8.11 Allele designations for evidence and reference samples will be determined by the examiner/analyst using the GeneMapper® ID software (see the "Analysis of Capillary Electrophoretic Data" chapter of this manual for instructions on utilizing the GeneMapper® ID software).

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4.2.8.12 All evidentiary sample electropherogram data **must** be analyzed prior to the known, reference samples except when references are used as assumed known contributors such as intimate samples.

4.2.8.13 A technical review of the data analysis will be conducted by a second qualified examiner/analyst using the GeneMapper® ID software. The reviewer will review the evidentiary samples prior to the reference samples.

4.2.8.14 If both the examiner/analyst and the technical reviewer are in agreement, the alleles can be called.

4.2.8.15 The technical reviewer must agree with the major/minor designation.

4.2.8.16 The examiner's/analyst's reviewed allele calls are the "official" values entered into CODIS.

4.2.9 Each electropherogram will include the peaks from each locus labeled with the designated allelic number, height, and base pair size.

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4.2.10 If an allele is seen in a region "off ladder" or OL alleles, i.e., larger than the largest allelic ladder peak of the largest MW locus on the electropherogram, smaller than the smallest allelic ladder peak of the smallest MW locus on the electropherogram, or between loci, an allele designation based upon the nomenclature referenced in 4.2.9.1 or 4.2.9.2 will be used. Once the allele designations have been independently generated for both the sample and the standard, the allele assignment and base pair values for the evidence sample and the standard will be compared.

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EXCEPTION: The Penta D "6 allele", which is not in the allelic ladder, will be assigned its allele designation as a "6" allele based upon the base pair size in relation to the base pair size of the Penta D 5 and 7 alleles.

4.2.10.1 If the allele is seen to the right (higher MW) of the largest allelic ladder peak of the largest MW locus, it will be assigned the type of the largest allele of the allelic ladder with a greater than sign (>). If the allele is seen to the left of the smallest allelic ladder peak of the smallest MW locus, the allele will be assigned the type of the smallest allele of the allelic ladder with a less than sign (<). The "OL" value on the electropherogram will be manually changed to reflect the allele designation.

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If an allele is seen between two loci and either the locus to the right OR left of the peak contains two peaks, the allele will be considered to belong with the locus not containing two peaks. The assignment of the allele designation will be based upon the nomenclature described in 4.2.9.2.

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4.2.10.2 If the allele is to the right of the largest allelic ladder peak of the locus not containing two alleles, it will be assigned the type of the largest allele of the allelic ladder with a greater than sign (>). If the allele is smaller than the smallest allelic ladder peak of the locus not containing two peaks, the allele will be assigned the type of the smallest allele of the allelic ladder with a less than sign (<). The "OL" value will be manually changed to reflect the allele designation

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4.2.10.3 If an allele is seen between two loci and neither the higher nor lower molecular weight locus contains two alleles, follow the guidelines outlined in steps 4.2.9.4 through 4.2.9.5. The assignment of the allele designation will be based upon the nomenclature described in 4.2.9.2.

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4.2.10.4 The base pair size for the allele in question will be compared to the base pair values for the largest allelic ladder peak of the lower molecular weight locus and to the smallest allelic ladder peak of the higher molecular weight locus. An evaluation of the physical location of the allele in question with respect to the higher and lower molecular weight loci will be conducted.

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4.2.10.5 The allele in question will be considered to belong to the locus which falls within an appropriate size distance from the locus (i.e., full repeat units from the largest/smallest allelic ladder peak). This OL variant may not always be assigned to the locus which is closest in proximity.

4.2.11 If an allele is between two allelic ladder peaks of the same locus, the GeneMapper® ID program generates a value “off ladder”. The allele will be considered to be a microvariant. The allele will be assigned an allele designation of the lower repeat value followed by the number of bases in the incomplete repeat. Example: an allele that migrates one base pair below the CSF1PO 14 allele will be designated as a CSF1PO 13.3. The "off ladder" value on the electropherogram will be manually changed to reflect the allele designation.

NOTE: To determine a microvariant or OL allele call in the GeneMapper™ ID software, highlight the sample in question and an allelic ladder under the “Samples” tab. Click on “Display plots” and magnify the peak in question heavily. The ladder will also magnify automatically. Show bins, if needed, by clicking on “Bins” under the “View” header. The bin will appear as gray shading behind each peak. Compare the base pair size of the peak in question to the surrounding known ladder allele(s) to determine the size of the peak.

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4.2.12 If an allele falls between the FGA 31.2 and the 43.2 allelic ladder bands, the allele will be assigned an allele designation of >31.2.

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4.2.13 For a typing result to be reported, all controls must give the expected result and have been treated appropriately (i.e., same or longer injection times and/or same or larger volumes loaded into the injection cocktail).

Reagent Blanks

If a signal (peak) is detected above the LOD in a reagent blank at a single locus and the signal is

demonstrated to be part of the control, the test results associated with the reagent blank will be considered inconclusive at that locus or the samples may be re-extracted or re-amplified. If it is imperative that the locus be used, the samples will be re-extracted and/or re-amplified.

If a signal (peak) is detected above the LOD in a reagent blank at multiple loci and the signal is demonstrated to be part of the control, the test results for samples associated with the reagent blank will be considered inconclusive for all loci and all samples associated with the reagent blank, if possible, will be re-extracted and/or re-amplified.

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Positive Amplification Control

The positive control DNA must elicit the "known" genotype for each locus as specified in 4.1.5 and Table 2. If an allele is detected in the positive control DNA at a specific locus that is not consistent with the known genotype or if no result is obtained at a single locus, the test will be considered inconclusive at that locus. If it is imperative that the locus be used, the samples will be re-amplified.

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Negative Amplification Control

If a signal (peak) is detected above the LOD in the negative amplification control at a single locus and the signal is demonstrated to be part of the control, the test results associated with the negative amplification control may be considered inconclusive at that locus. If it is imperative that the locus be used, the samples will be re-amplified.

If a signal (peak) is detected above the LOD in the negative amplification control at multiple loci and the signal is demonstrated to be part of the control, the test results for samples associated with the control will be considered inconclusive for all loci and all samples, if possible, will be re-amplified.

4.2.14 If it is determined that a sample contains stutter peaks at a majority of the loci and other artifactual peaks are visible throughout the electropherogram due to overloading of the sample and/or amplifying too much sample DNA, the sample may be re-injected using a reduced injection time or using less amplified DNA in the injection cocktail following the guidelines described in the “Capillary Electrophoresis” chapter of this manual. Samples may also be re-amplified with less template DNA and then re-typed. If however, the accurate profile can be determined from the original sample profile by both the examiner and the technical reviewer, then the data may be used.

4.2.15 To indicate the gender of the contributor of a particular biological sample the Amelogenin locus may be used. A biological sample exhibiting a single peak at approximately 106 bp (X allele) will generally be considered to have originated from a female individual. A biological sample exhibiting a peak at approximately 106 bp (X allele) and a peak at approximately 112 bp (Y allele) will generally be considered to have originated from a male individual. If only a Y allele is obtained or only an X allele is obtained from a known male sample, the results at the Amelogenin locus will be reported as inconclusive.

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4.3 General Interpretation of Single Source Samples

- 1 or 2 alleles are detected per locus.
- DNA types should be detected at 7 or more loci before comparisons are conducted.
- Restricted genotypes types should be deconvoluted at 4 or more loci.
- For low level or partial profiles, heterozygote alleles do not need to be in peak height ratio to be used in statistical calculations.
- Possible allelic drop-out can occur for genetic data that is detected below the stochastic threshold.
  - A maximum of 3 instances of allelic dropout at 3 loci can occur for data below the stochastic threshold when compared to a reference sample for a conclusion of cannot be eliminated to be reached.
- Allelic drop-out is not counted at loci where no DNA typing results are obtained.
- Allelic drop-out for genetic data above the stochastic threshold is less likely to occur.

- For genetic data above the stochastic threshold, the DNA types from the evidence should match the DNA types from the reference sample for a conclusion of cannot be eliminated to be reached.
- Statistics are calculated at all loci where DNA typing results are obtained.

4.4 General Interpretations of 2 Person Mixtures

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- 1-4 alleles are detected per locus.
- DNA types should be detected at 7 or more loci before conclusions can be reached.
- Major/minor can be determined for some 2 person mixtures.
  - Deconvoluted major alleles shall be within peak height ratio.
  - Deconvoluted minor alleles are generally within peak height ratio.
  - Minor alleles are deconvoluted at loci where major alleles are deconvoluted.
  - Minor alleles may be masked by major alleles and are not deconvoluted at these loci.
  - Allelic drop-out is not counted at loci where the major alleles mask the minor alleles and no minor types are deconvoluted.
  - 4 loci or more are required for statistics to be calculated for major or minor contributors
- For intimate samples, DNA types from a known contributor can be subtracted or dosage considered to determine which types are foreign at a locus.
  - Foreign alleles are generally within peak height ratio.
  - 4 loci or more are required for statistics to be calculated.
  - Allelic drop-out is not counted at loci where the owner alleles mask the foreign alleles and no foreign types are deconvoluted.
- Unresolved 2 person mixture (no major/minor determinations)
  - Determine if a CPI or uRMP statistic can be conducted.
    - CPI will be calculated only with mixture profiles in which all the data is above the stochastic threshold and assumptions can be made about the number of contributors; exceptions will be considered on a case by case basis.
    - uRMP will be calculated only where breakout has occurred (3 allele or 4 alleles per locus) and the number of DNA contributors can be reported.
      - uRMP is calculated at minimum of 1 locus.
  - If a CPI or uRMP cannot be calculated for non-person cases, no comparisons will be conducted for that sample.
  - Person cases are forwarded to the True Allele (TA) examiners if the TA analysis is required on a probative piece of evidence.
- Allelic drop-out considerations are the same as listed in the single source section.

4.5 General Interpretations of 3 Person Mixtures

- 1-6 alleles are detected per locus and/or PHR imbalance is evident.
- DNA types should be detected at 7 or more loci before conclusions can be reached.
- Major contributor can be determined for some 3 person mixtures.
  - Major alleles are generally within peak height ratio.
  - 4 loci or more are required for statistics to be calculated for a major contributor.
- Unresolved 3 person mixture
  - Determine if a CPI or uRMP statistic can be conducted.
    - CPI with all data above the stochastic threshold and assumptions can be made for the number of contributors, exceptions considered on a case by case basis.
    - uRMP where breakout has occurred (5 alleles or 6 alleles per locus) if the number of DNA contributors can be reported.
      - uRMP is calculated at minimum of 1 locus.
  - If a CPI or uRMP cannot be calculated for non-person cases, no comparisons will be conducted for that sample.
  - Person cases are forwarded to the True Allele (TA) examiners if TA analysis is required on a probative piece of evidence.
- Allelic drop-out considerations are the same as listed in the single source section.

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4.6 General Interpretations of 4 Person Mixtures

- These mixtures are typically not interpreted

Exceptions to 4.3 - 4.6 will be considered by the Program Manager or designee on a case-by-case basis.

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4.7 Interpretation of a Foreign Profile from a Mixture Searched in CODIS (Combined DNA Index System):

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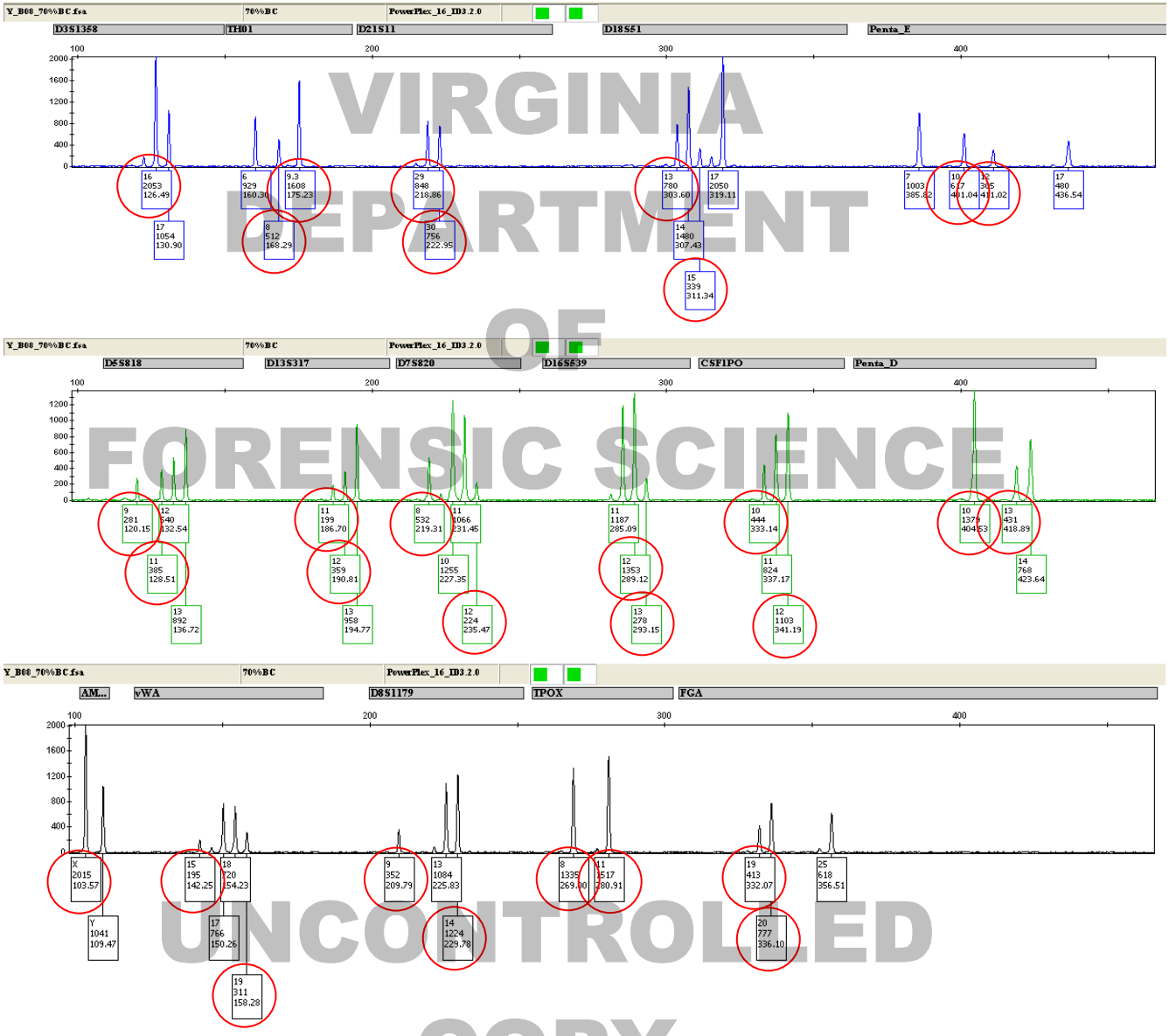

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Figure 7. Example of a female:male mixture. Female contributor alleles are circle in red.

In the above example, the victim (V) has the following profile: D3S1358 16, TH01 8.9.3, D21S11 29,30, D18S51 13,15, Penta E 10,12, D5S818 9,11, D13S317 11,12, D7S820 8,12, D16S539 12,13, CSF1PO 10,12, Penta D 10,13, vWA 15,19, D8S1179 9,14, TPOX 8,11 and FGA 19,20. Therefore, the minimum known foreign DNA profile that would be searched in CODIS would be:

D3S1358 17

TH01	6, 9.3	The 9.3 peak is 3X the height of the 8 peak which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 9.3 peak may not be solely from the victim. The 6 peak is foreign to the victim.
D18S51	14,17	
Penta E	7, 17	
D5S818	12, 13	
D13S317	13	
D7S820	10,11	
D16S539	11,12	The 12 peak is >4X the height of the 13 peak which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 12 peak may not be not solely from the victim. The 11 peak is foreign to the victim.
CSF1PO	11,12	The 12 peak is >2X the height of the 10 peak which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 12 peak may not be solely from the victim. The 11 peak is foreign to the victim.
Penta D	10,14	The 10 peak is >3X the height of the 13 peak which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 10 peak may not be solely from the victim. The 14 peak is foreign to the victim.
vWA	17,18	
D8S1179	13,14	The 14 peak is 4X the height of the 9 peak which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 14 peak may not be solely from the victim. The 13 peak is foreign to the victim.
FGA	25	

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5 STATISTICAL CALCULATIONS

Before a statistical calculation is performed, the DNA profile will be evaluated to determine both suitability of loci for interpretation, statistical calculations, and to determine if the individual of interest is included or eliminated as a possible source of the genetic material. Refer to the "Interpretation of Powerplex® 16 System Results" chapter of this manual, for guidelines on interpreting DNA profiles. Only if the individual of interest cannot be eliminated as a possible source of the genetic material will a statistical calculation be performed. The statistical approach that is applied will depend on the circumstances of the case and the criterion addressed in the remainder of this chapter.

The stochastic threshold (STH) can be used as a predictor for allelic drop-out. The average RFU of the detected allele of a heterozygous pair demonstrating allelic drop-out was used to generate a STH for each injection time (2 sec, 5 sec, 10 sec). Two standard deviations of this average is listed below.

The stochastic thresholds are as follows:

2 sec injection – 210 RFU
5 sec injection – 320 RFU
10 sec injection – 460 RFU

Alleles detected below these thresholds may be missing their sister allele. If the alleles are above this threshold, generally both sister alleles can be presumed to be present; however, if the majority of other loci have alleles below this threshold, it can be an indication that DNA from the low-level donor has dropped out completely.

The STH range will be evaluated for any profile determined to be a single source profile (or a deduced major profile) for which a random match probability will be calculated and for any profile determined to be a mixture suitable for combined probability of inclusion calculations.

Conclusions for positive associations (not eliminated) and the supporting statistics for the item will be addressed in the same Certificate of Analysis.

5.1 The frequency of occurrence of allele fragments reported as being consistent is determined for each polymorphic locus within a racial/ethnic population group. Using the allele designations specified in the "Interpretation of Powerplex® 16 System Results" chapter of this manual, determine the frequency for each allele from the appropriate population database.

5.2 The genotype frequency associated with a particular pattern of alleles from a sample is based upon principles of Hardy-Weinberg equilibrium.

5.2.1 If a single source sample under analysis demonstrates two different alleles, the genotypic frequency at a particular locus is determined by the equation 2pq, where p and q represent the frequencies of allele #1 and #2[1].

5.2.2 If a single source sample under analysis consists of the same allele, the genotypic frequency at a particular locus is determined by the equation $p^2 + p(1-p)\theta$, where $\theta = 0.01$ and p represents the frequency of the allele [1].

5.2.3 If a known sample consists of more than two alleles at a particular locus, no frequency data will be generated for that locus.

5.3 A random match probability will be determined for single source samples using the product rule. The product rule may also be applied for a mixture sample when the complete major contributor profile can be determined or a foreign contributor profile can be determined by subtracting the contribution of the known donor from an intimate mixture.

5.3.1 For a single source sample (or a deduced major profile in a mixture), all homozygous loci will be evaluated using the STH values. If a single homozygous allele detected at a locus falls below the STH for the respective injection time, the formula $2p-p^2$ may be applied.

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5.4 Traditional likelihood ratios will be used to calculate the match probability for the DNA profile from a sample when a mixture of DNA profiles from TWO individuals is observed and the entire foreign DNA profile cannot be determined by subtracting the contribution of the known donor from the mixture profile which would otherwise be a RMP calculation. This situation is further defined as a 2 person mixture where one of the contributors is a "known" for both the prosecution proposition and the defense proposition listed below. The "Likelihood Ratio" frequency (L) is determined by the equation [1, 2, 9, 10]:

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$$LR = \frac{P(E|C_X)}{P(E|C_Y)}$$

The LR compares two hypotheses or scenarios – informally they are 1) what is the probability of the DNA evidence if the prosecution proposition is true? and 2) what is the probability of the DNA evidence if the defense proposition is true? The LR provides a measure, based upon the DNA evidence, of how many times more likely hypothesis (1) is versus hypothesis (2).

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Where: C_X is the first explanation (the alleles of the mixed DNA profile attributed to unknown contributors under explanation C_X), where X is the number of unknown contributors under explanation C_X.

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C_Y is the second explanation (the alleles of the mixed DNA profile attributed to unknown contributors under explanation C_y), where Y is the number of unknown contributors under explanation C_y.

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$P(E|C_X)$ is the probability of the DNA profile (E) using explanation C_X

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$P(E|C_Y)$ is the probability of the DNA profile (E) using explanation C_Y

Refer to Appendix I of the Commonwealth of Virginia Forensic Biology Section Procedure Manual Section III, Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 BIO System for specific formulas used for calculating likelihood ratio frequencies.

NOTE: Because the likelihood ratio calculation takes into account all possible contributors involved in the mixture and therefore provides a conservative probability, θ is not used in the calculation.

5.4.1 When the victim and suspect share the same two alleles (i.e., 12,13), they may be accounted for as both unknown or knowns in the likelihood ratio calculation. An example when both of the alleles would be counted as unknowns is if the victim's contribution to the mixture is the minor component throughout the mixture profile and the type that is shared by the victim and suspect is consistent with the major contributor (based upon the peak heights of the alleles in relationship to the rest of the DNA profile) the alleles will be considered to have originated from the foreign contributor and will be used in the calculation.

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Example: Victim - 12,13 (minor contributor)
Suspect - 12,13 (major contributor)
Evidence - 12,13 [both alleles will be considered as unknowns]

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5.4.2 If four alleles are observed in a mixture, the alleles that are foreign to the victim will be used in the calculation.

Example: Victim - 5,8
Suspect - 7,9
Evidence - 5,8,7,9 [the 7 and 9 alleles should be considered as unknowns]

5.4.3 Once the profile has been evaluated and it has been determined that the individual of interest cannot be eliminated, if any of the alleles for the individual of interest is missing at a particular locus, the locus will

not be used in the calculation. However, the results at that locus may be used in the overall decision of an inclusion or exclusion.

Example: Victim - 10,11
Suspect - 6,8
Evidence - 10,11,6 [this locus will not be used in the overall calculation of the likelihood ratio frequency]

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5.5 Combined probability of inclusion (CPI) may be used to calculate the match probability when a mixture of DNA profiles from MORE THAN TWO individuals is obtained and no major contributor can be determined or a mixture of two individuals is obtained and a likelihood ratio cannot be calculated as outlined above.

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NOTE: The CPI calculation assumes that all alleles from all individual(s) of interest to the mixture are present at the loci for which the statistic is being calculated. Therefore, a locus at which drop-out is presumed (with stochastic thresholds) or known to have occurred (based on comparisons to known samples) cannot be used in the calculation of this statistic.

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The following combined probability of inclusion calculation will be used to establish the frequency of individuals who would be included as possible contributors to the mixture.

Probability of exclusion (PE) = Probability representing all genotypes **not** contributing to the mixture.

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$PE = Q^2 + 2Q(1-Q)$ Where, Q = (1-P), frequency of alleles associated with genotypes at each locus **not** contributing to the mixture and P represents the sum of allele frequencies in the mixture.

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$CPE = 1 - [(1-PE_1)(1-PE_2)....(1-PE_n)]$

The combined probability of inclusion (CPI) can then be calculated by the equation:

CPI = 1 – CPE

5.5.1 When an evidence profile is determined to be a mixture that requires a CPI calculation, all loci will be evaluated using the STH values (for the injection time of that sample) prior to any comparisons to known samples.

5.5.1.1 Known samples from donors of intimate samples or owners of items are the exception. Their profiles may be used to determine the number of contributors to a mixture profile and whether it is of value.

5.5.2 If one or more alleles at a locus falls below the STH for the injection time of the sample, the CPI calculation will not be used unless an exception is granted.

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5.5.3 Once the profile has been evaluated using the STH values and determined to be suitable for a CPI calculation, the stutter peaks at all loci determined to be suitable for CPI will be compared to the 'true' allele peaks. If, at any locus, the largest stutter peak is larger or equal to the smallest 'true' allele peak, then all of the stutter alleles at that locus will be used in the CPI calculation.

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5.5.3.1 The profile may be re-analyzed in GeneMapper with the stutter cutoff filter turned off to assist with this evaluation.

5.6 An unrestricted modified random match probability (uRMP) can be calculated for mixtures following the procedures in mRMP manual. Report writing for this application is also addressed in the mRMP manual section 1.5.

5.7 If it is suspected that a relative of the suspect may have left the genetic material at the crime scene, the DNA profile from the relative should be obtained whenever feasible. However if a suspected relative cannot be profiled,

the following formulas may be used to determine the conditional probability that the relative has a particular genotype consistent with that of the suspect [1]:

Genotype of the suspect	Probability of the same genotype in a relative
Homozygote: A_iA_i	$p_i^2 + 4p_i(1-p_i)F$
Heterozygote: A_iA_j	$2p_ip_j + 2(p_i + p_j - 4p_ip_j)F$

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F represents the kinship coefficient and p_i and p_j represent the frequency of the alleles for the race of the relative in question.

For parents and offspring, F = 1/4; for half-siblings F= 1/8; for uncle or nephew F = 1/8; for first cousins F= 1/16.

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For full siblings the following formulas will be used:

Homozygote: A_iA_i	$(1 + 2p_i + p_i^2)/4$
Heterozygote: A_iA_j	$(1 + p_i + p_j + 2p_ip_j)/4$

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5.8 The following are the formulas which may be used when calculating Paternity/Relationship type calculations:

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5.8.1 Random Man Not Excluded (RMNE): the frequency with which men selected at random from the same racial background as the Alleged Father would not be excluded as the Biological Father in a given test which includes the Mother-Child Combination.

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Mother	P	R
Child	P	**Q**
Alleged Father	Q	N

i. RMNE = $2q - q^2$ (Where q equals the obligatory allele frequency)

Mother	P	Q
Child	P	Q
Alleged Father	Q	N

ii. RMNE = $2(p + q) - (p + q)^2$ (Where p and q equal the obligate allele frequency)

5.8.2 Paternity Index (PI): This is the ratio of the chance that the mother (M) and a man of the Alleged Father's (AF) phenotype produced the child (passed the obligate allele) compared to the chance that the mother and a random man produced the child (passed the obligate allele).

H_0 = Alleged father is the biological father
H_1 = Alleged father is not the biological father

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$P(R|H_0)$ = Probability of the child (with R genetic information of M and AF) given H_0.
$P(R|H_1)$ = Probability of the child (with R genetic information of M and AF) given H_1.

When the numerator and denominator are divided by $P(R|H_0)$

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$P(H_0|R) = P(R|H_0) / P(R|H_0) + P(R|H_1)$

Let $P(R|H_0) = X$
$P(R|H_1) = Y$

$P(H_0|R) = 1 / 1 + Y/X$

PI = X/Y or 1 / Y/X, also known as a likelihood ratio (L)[2,4]

PI compares two hypotheses or scenarios – informally they are 1.) paternity, and 2) non-paternity. PI provides a measure of how many times more characteristic of (1) the genetic evidence is than of (2).

Example: Mother (M) = 10, 11
Child (C) = 10, 11
Alleged Father (AF) = 10, 12

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$$\text{Paternity Index} = \frac{(M_{10})(AF_{11}) + (M_{11})(AF_{10})}{(M_{10})(RM_{11}) + (M_{11})(RM_{10})}$$

$$= \frac{(0) + (AF_{10})}{(RM_{11}) + (RM_{10})}$$

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$$= (AF_{10})/(RM_{11}) + (RM_{10})$$

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Child	Mother	Alleged Father	Paternity Index
AA	AA	AA	$1/P_A$
AA	AB	AA	$1/P_A$
AB	AA	AA	$1/P_A$
AB	BB	AA	$1/P_A$
AB	BC	AA	$1/P_A$
AA	AB	AB	$1/2P_A$
AA	AA	AB	$1/2P_A$
AB	BB	AB	$1/2P_A$
AB	BB	AC	$1/2P_A$
AB	BC	AD	$1/2P_A$
AB	BC	AB	$1/2P_A$
AA	AB	AC	$1/2P_A$
AB	AA	BB	$1/P_B$
AB	AC	BB	$1/P_B$
AB	AA	AB	$1/2P_B$
AB	AA	BC	$1/2P_B$
AB	AC	BD	$1/2P_B$
AB	AA	BC	$1/2P_B$
AB	AC	AB	$1/2P_B$
AB	AB	AA	$1/P_A + P_B$
AB	AB	AB	$1/P_A + P_B$
AB	AB	BB	$1/P_A + P_B$
AB	AB	BC	$½(P_A + P_B)$
AB	AB	AC	$½(P_A + P_B)$

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5.8.3 Combined Paternity Index (CPI): The combined paternity index is calculated by multiplying together each individual paternity index.

$$CPI = PI_1 \text{ x } PI_2 \text{ x } PI3 \ldots\ldots\ldots\ldots PI_n$$

5.8.4 Probability of Paternity: The probability of paternity is expressed as a frequency (or percentage), incorporating the combined paternity index and a prior probability (which is most typically set at 0.5) which compares the likelihood that the tested man may pass the required genes to the likelihood that an untested, unrelated random man of the same race may pass these genes.

Probability of Paternity = (CPI)(Pr) / (CPI)(Pr) + (1-Pr)
Pr = Prior Probability and CPI = Combined Paternity Index
With a Pr = 0.5, the formula reduces to:
Probability of Paternity[2,3,4] = CPI / CPI + 1

Refer to Appendix L of the Commonwealth of Virginia Forensic Biology Section Procedure Manual Section III, Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 BIO System for acceptable values of Probability of Paternity.

5.9 The following are the formulas used when calculating Paternity/Maternity/Relationship Type calculations from a single parent [5]:

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Where:

p = frequency in the population
q = frequency in the population
AF_p = chance of passing p
AF_q = chance of passing q

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5.9.1 $PI = (p)(AF_q) + (q)(AF_p) / 2pq = (p)(0.5) + (q)(0) / 2pq = 1/4q$

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Person		
Child	P	Q
Alleged Father	Q	R

5.9.2 $PI = (p)(AF_q) + (q)(AF_p) / 2pq = (p)(1) + (q)(0) / 2pq = 1/2q$

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Person		
Child	P	Q
Alleged Father	Q	Q

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5.9.3 $PI = (p)(AF_q) + (q)(AF_p) / 2pq = (p)(0.5) + (q)(0.5) / 2pq = (p + q) / 4pq$

Person		
Child	P	Q
Alleged Father	P	Q

5.9.4 $PI = (q)(AF_p) / q^2 = (q)(0.5) / q^2 = 1 / 2q$

Person		
Child	Q	Q
Alleged Father	Q	R

5.9.5 $PI = (q)(AF_p) / q^2 = (q)(1) / q^2 = 1 / q$

Person		
Child	Q	Q
Alleged Father	Q	Q

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5.10 The following are the formulas used when calculating Paternity/Maternity/Relationship Type Calculations from a single grandparent [5,6]:

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Child	Grandmother	Grandfather	Paternity Index
AA	AA	Unknown	(1 + a) / 2a
AA	AB	Unknown	(1 + 2a) / 4a
AA	BC	Unknown	1 / 2
AA	BB	Unknown	1 / 2
AB	AA	Unknown	(1 + 2a) / 4a
AB	AB	Unknown	(a + b + 4ab) / (8ab)
AB	BC	Unknown	(1 + 4b) / (8b)
AB	BB	Unknown	(1 + 2b) / (4b)

AB	CC	Unknown	1 / 2
AB	CD	Unknown	1 / 2

5.11 The following are the formulas used when calculating Missing Person Calculations (where mother's and father's genotypes are known) [5]:

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Prob(EIM, F, Q) is the probability that the evidence would be observed given that the mother and the father were the parents of the evidence sample (Q).

Prob(EIM, F, U) is the probability that the evidence would be observed given that a random member of the population was the questioned sample (U).

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LR is the ratio of the two probabilities = Prob(EIM, F, Q) / Prob(EIM, F, U)
$P_{A,}$ etc. is the estimated frequency of the "A" allele in the population

Mother	Question	Father	Prob(EIM, F, Q)	Prob(EIM, F, U)	LR
AA	AA	AA	$P_A^2 \times P_A^2$	$P_A^2 \times P_A^2 \times P_A^2$	$1 / P_A^2$
AA	AA	AB	$P_A^2 \times 2P_AP_B \times ½$	$P_A^2 \times 2P_AP_B \times P_A^2$	$1 / 2P_A^2$
AA	AB	BB	$P_A^2 \times P_B^2$	$P_A^2 \times P_B^2 \times 2P_AP_B$	$1 / 2P_AP_B$
AA	AB	AB	$P_A^2 \times 2P_AP_B \times ½$	$P_A^2 \times 2P_AP_B \times 2P_AP_B$	$1 / 4P_AP_B$
AA	AB	BC	$P_A^2 \times 2P_BP_C \times ½$	$P_A^2 \times 2P_BP_C \times 2P_AP_B$	$1 / 4P_AP_B$
AA	AB	BB	$2P_AP_B \times P_B^2 \times ½$	$2P_AP_B \times P_B^2 \times 2P_AP_B$	$1 / 4P_AP_B$
AB	AB	AB	$2P_AP_B \times 2P_AP_B \times (¼ + ¼)$	$2P_AP_B \times 2P_AP_B \times 2P_AP_B$	$1 / 4P_AP_B$
AB	AB	AC	$2P_AP_B \times 2P_AP_C \times ½ \times ½$	$2P_AP_B \times 2P_AP_C \times 2P_AP_B$	$1 / 8P_AP_B$
AB	AA	AA	$2P_AP_B \times P_A^2 \times ½$	$2P_AP_B \times P_A^2 \times P_A^2$	$1 / 2P_A^2$
AB	AA	AB	$2P_AP_B \times 2P_AP_B \times ½ \times ½$	$2P_AP_B \times 2P_AP_B \times P_A^2$	$1 / 4P_A^2$
AB	AA	AC	$2P_AP_B \times 2P_AP_C \times ½ \times ½$	$2P_AP_B \times 2P_AP_C \times P_A^2$	$1 / 4P_A^2$
AB	AC	CC	$2P_AP_B \times P_C^2 \times ½$	$2P_AP_B \times Pc^2 \times 2P_AP_C$	$1 / 4P_AP_C$
AB	AC	BC	$2P_AP_B \times 2P_BP_C \times ½ \times ½$	$2P_AP_B \times 2P_BP_C \times 2P_AP_C$	$1 / 8P_AP_C$
AB	AC	AC	$2P_AP_B \times 2P_AP_C \times ½ \times ½$	$2P_AP_B \times 2P_AP_C \times 2P_AP_C$	$1 / 8P_AP_C$
AB	AC	CD	$2P_AP_B \times 2P_CP_D \times ½ \times ½$	$2P_AP_B \times 2P_CP_D \times 2P_AP_C$	$1 / 8P_AP_C$

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5.12 When performing a Missing Person or Paternity Index calculation on a partial DNA profile it is possible that an individual or an evidence sample may be identified that cannot be eliminated as a possible offspring/parent or have originated from the offspring/parent. However the individual's DNA profile or the evidence sample is missing an allele at a particular locus that is observed in the mother/father/child's DNA profile. The allele may be missing as a result of allelic dropout or a mutation. Therefore to account for both of these situations two probabilities will be provided in the Certificate of Analysis and will be calculated in the following manner:

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5.12.1 To account for the possibility of allele dropout, the locus will not be used in the overall calculation.

5.12.2 To account for the possibility of a mutation, the formula addressed below will be used for the locus and factored into the overall calculation.

5.13 When applying the formulas for Missing Person or Paternity Index calculations, if a partial profile is obtained and the individual cannot be eliminated, however an allele is missing at a particular locus the probability for the locus will be calculated and reported two ways:

5.13.1 To account for the possibility of allele dropout, the locus will not be used in the overall calculation.

5.13.2 To account for the possibility of a mutation, the formula addressed below will be used.

5.14 The following formula is used when calculating Paternity/Maternity type calculations involving a potential mutation [7, 8]:

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$PI = M_{SR} / 2P_A$ M_{SR} refers to the specific mutation rate for changing allele S to R

$M_{SR} \cong \mu$ μ represents the average mutation rate reported by the American Association of Blood Banks (AABB)

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$PI = \mu / 2P_A$ $P_{A,}$ etc. is the estimated frequency of the "A" allele in the population

Average Mutation Rates Established by the AABB[11]

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Locus	Paternal	Maternal
FGA	0.0029	0.0005
TPOX	0.0002	<0.0001
D8S1179	0.002	0.0003
VWA	0.0032	0.0003
Penta E	0.0012	0.0002
D18S51	0.002	0.0006
D21S11	0.0015	0.0011
TH01	0.0001	0.0001
D3S1358	0.0015	0.0003
Penta D[12]	0.00066	0.00064
CSF1PO	0.0014	0.0002
D16S539	0.0011	0.0002
D7S820	0.0013	0.0002
D13S317	0.0015	0.0005
D5S818	0.0014	0.0002

5.15 The following formulas are for use when it is suspected that two individuals are siblings [6,12,13]:

Sibling Genotype		Probability of S2 genotype if two persons are:		
S1	**S2**	**Truly Related**	**Are Unrelated**	**Sibling Index**
AA	AA	$(1+a)^2/4$	a^2	$(1+a)^2/4\ a^2$
AA	AB	(b+ab)/2	2ab	(1+a)/4a
AA	BB	$b^2/4$	b^2	¼
AA	BC	bc/2	2bc	¼
AB	AB	(1+a+b+2ab)/4	2ab	(1+a+b+2ab)/8ab
AB	AA	$(a+a^2)/4$	a^2	(1+a)/4a
AB	AC	(c+2ac)/4	2ac	(1+2a)/8a
AB	CD	cd/2	2cd	¼

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5.16 A sibling index threshold of 33 is designated based upon the literature, at and above which, it will be concluded that the statistical data support a sibling relationship and below which the evidence in support of a biological relationship is deemed inconclusive [13,14].

5.17 The following formulas are for use when it is suspected that two individuals are half-siblings (also grandparent-child or uncle-niece/nephew)[6]:

Half-Sibling Genotype	Probability of S2 genotype if two persons are:	

S1	S2	Truly Related	Are Unrelated	Half-Sibling Index
AA	AA	$(a+a)^2/2$	a^2	(1+a)/2a
AA	AB	(b+2ab)/2	2ab	(1+2a)/4a
AA	BB	$b^2/2$	b^2	½
AA	BC	bc	2bc	½
AB	AB	(a+b+4ab)/4	2ab	(a+b+4ab)/8ab
AB	AA	$(a+2a^2)/4$	a^2	(1+2a)/4a
AB	AC	(c+4ac)/4	2ac	(1+4a)/8a
AB	CD	cd	2cd	½

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5.18 Procedure for rounding frequencies:

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5.18.1 Allele and genotype frequencies will be carried out to 3 digits. If the fourth digit is 4 or less the third digit will remain the same. If the fourth digit is 5 or greater then the third digit will be rounded up.

Example: 0.346**4** would be truncated to 0.346
0.346**7** would be rounded to 0.347

5.18.2 Mutation rate frequencies will be carried out to the number of digits in the actual mutation rate.

Example: 0.0002 will be carried out to 4 digits
0.0020 will be carried out to 3 digits

5.18.3 The frequency for the overall DNA pattern, termed a DNA profile, can be determined by multiplying together the genotype frequency obtained from each locus. The overall match probability/Likelihood Ratio/CPI (combined probability of inclusion/combined paternity index) will be truncated to two significant figures.

Example: $8.169738341 \times 10^{-11}$ = 1 in 12,240,294,100

Reported match probability: 1 in 12 billion

Refer to Chapter 10, Report Writing, for the specific wording used when reporting match probabilities/likelihood ratios/combined probability of inclusion/probability of paternity.

5.19 Procedure for calculating allele and genotype frequencies:

The following represents an example of data collected for a STR database and the procedures used to determine the allele and genotype frequencies. Refer to Appendix H of the Commonwealth of Virginia Forensic Biology Section Procedure Manual Section III, Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 BIO System for the Virginia Department of Forensic Science population databases.

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Example: TH01 locus in Caucasian population (n = 209)

Allele Frequency:

Frequency of allele = Number of times the allele was observed out of all possible alleles for a particular locus/2n.

NOTE: Alleles that contain fewer than 5 events are defaulted to 5 events in order to provide a more conservative frequency.

Examples:

Allele 5 was observed 3 times out of 418 alleles. Therefore, the allele 5 will default to a total of 5 events (5/418) = 0.012

Allele 6 was observed 100 times out of 418 alleles (100/418) = 0.239

Allele 7 was observed 59 times out of 418 alleles (59/418) = 0.141

Allele 8 was observed 50 times out of 418 alleles (50/418) = 0.120

Allele 9 was observed 64 times out of 418 alleles (64/418) = 0.153

Allele 9.3 was observed 68 times out of 418 alleles (68/418) = 0.163

Allele 10 was observed 74 times out of 418 alleles (74/418) = 0.177

Allele 11 was observed 0 times out of 418 alleles. Therefore, the allele 5 will default to a total of 5 events (5/418) = 0.012

The sum of the individual allele frequencies should equal approximately 1.000. However, because events less than 5 are defaulted to 5, the total frequencies may not total to exactly 1.000:

(0.012) + (0.239) + (0.141) + (0.120) + (0.153) + (0.163) + (0.177) + (0.012) = 1.017

Expected Genotype Frequency:

Based on the assumption that the TH01 genetic locus is in Hardy-Weinberg equilibrium, the expected genotype frequencies are calculated from the allele frequencies, as in the following examples:

TH01 Genotype 7, 7:

(Frequency of the 7 allele)^2 + Frequency of the 7 allele(1-Frequency of the 7 allele)θ
=
$(0.141)^2 + 0.141(1-0.141)0.01 = 0.021$

OR

TH01 Genotype 7, 9.3:

2(Frequency of the 7 allele)(Frequency of the 9.3 allele)
=
2(0.141)(0.340) = 0.096

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6 REPORT WRITING

6.1 Report Requirements

In accordance with the FBI's "Quality Assurance Standards for Forensic DNA Testing Laboratories", all Forensic Biology Certificates of Analysis in which DNA analysis was conducted will contain at a minimum the following information:

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6.1.1 Case identifier

6.1.2 Description of evidence examined

6.1.3 A description of the methodology

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6.1.4 Polymorphic loci examined or amplification system

6.1.5 A quantitative or qualitative interpretative statement

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6.1.6 Statistical frequencies of inclusion

6.1.7 Date issued

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6.1.8 Disposition of evidence

6.1.9 A signature and title, or equivalent identification of the person accepting responsibility for the content of the report

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6.2 Categories of Results

The results will fall within one of the following categories:

6.2.1 **Usable DNA Profiles**

6.2.1.1 No DNA profiles foreign/different to individual "A" were developed.

6.2.1.2 The DNA profiles developed for samples under comparison are consistent (match).

6.2.1.3 The DNA profiles developed for samples under comparison are consistent with a mixture.

6.2.1.4 The DNA profiles developed for samples under comparison are different (do not match) or are from non-subject cases.

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6.2.1.4.1 The DNA profiles for "unknown" samples (samples from non-subject cases or samples not attributable to any corresponding known samples) were searched in the Virginia DNA Data Bank.

6.2.1.4.1.1 The DNA Data Bank search resulted in a match.

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6.2.1.4.1.2 The DNA Data Bank search resulted in no matches.

6.2.1.4.1.2.1 The DNA profile for an unknown sample will be submitted to the National DNA Data Bank (if suitable).

6.2.1.4.2 The DNA profile for an "unknown" sample was not suitable for searching in the Virginia DNA Data Bank or submission to the National DNA Data Bank; however, it may be suitable for direct comparison purposes.

6.2.2 **Potentially Usable DNA Profiles** (preferential amplification, drop-out, insufficient quantity, and/or sample degradation)

6.2.2.1 Based on a review of the data, considering the type of sample and DNA yield, a partial DNA profile may be considered a "usable" profile.

6.2.2.2 No conclusive DNA profiles were developed (after cautious and conservative review, no comparison or interpretation can be offered).

6.2.2.3 A partial DNA profile may not be suitable for searching in the DNA Data Bank; however, it may be suitable for direct comparison purposes.

6.2.2.4 A complex mixture profile of 4 or more contributors (see 4.3 for definition) will be determined to be either of no value or limited value for elimination purposes only.

6.2.2.4.1 Mixture profiles that fall below the defined criteria can still be determined to be complex and treated in the same manner.

6.2.3 **No DNA Profiles** (insufficient quantity and/or sample degradation).

6.3 General Reporting Format

The Certificate of Analysis will contain a METHODS section (as appropriate) and RESULTS section, and may contain a CONCLUSIONS section. Depending on the case scenario and what questions the analyses are trying to answer as part of the investigation, a report may need to be written using several of the guidelines listed below.

6.3.1 The **METHODS SECTION** (as outlined below) will be placed prior to the RESULTS section in the Certificate of Analysis and will include the description of methodology, the PCR kit used, and polymorphic loci tested.

DNA ANALYSIS METHODS:

- The method of deoxyribonucleic acid (DNA) analysis used was the Polymerase Chain Reaction (PCR).
- The PCR amplification kit used was the PowerPlex® 16 system.
- The PowerPlex® 16 system contains 16 genetic loci (FGA, TPOX, D8S1179, vWA, Penta E, D18S51, D21S11, TH01, D3S1358, Penta D, CSF1PO, D16S539, D7S820, D13S317, D5S818 and Amelogenin, a gender determining locus which is not used for statistical purposes).

NOTE**:** The METHODS section is not included in Supplemental Reports (i.e., subsequent hit reports) when no additional DNA analysis has been performed.

6.3.2 For cases which were terminated after extraction due to low DNA yields, the following METHODS SECTION will be used:

DNA ANALYSIS METHODS:

- The method of deoxyribonucleic acid (DNA) analysis used is the Polymerase Chain Reaction (PCR).
- The PCR amplification kit, PowerPlex® 16, contains 16 genetic loci (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, FGA and Amelogenin, a gender determining locus which is not used for statistical purposes).

6.3.3 The **RESULTS SECTION** will contain the results of all analyses including comparisons, Data Bank searches, conclusions and statistics and will follow the METHODS section in the Certificate of Analysis. The results section may list results in a bulleted format or a narrative format.

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6.3.3.1 **Known Reference Samples**: For known reference samples, the results of the DNA analysis will be reported in the following manner:

"A DNA profile was developed from the blood sample (or buccal swabs) from VICTIM/SUSPECT/ELIMINATION SAMPLE."

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6.3.3.1.1 **Referencing Previously Analyzed Known Sample/Evidence**: When performing a comparison between a known sample and a previously analyzed evidence sample or vice versa the previously analyzed sample will be reported in the following manner:

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Examples: Evidence and known samples from the same case

A DNA profile was developed from the buccal swabs from SUSPECT (Item 3). SUSPECT cannot be eliminated as a contributor of the foreign DNA profile previously developed from the vaginal sample (Item 2) and reported in the Certificate of Analysis dated December 12, 2004.

or

A DNA profile foreign to VICTIM was developed from the vaginal sample from VICTIM (Item 2). SUSPECT (Item 3, previously reported in the Certificate of Analysis dated December 12, 2004) cannot be eliminated/is eliminated as a contributor of the foreign DNA profile developed from the vaginal sample.

Example: Evidence and known samples from different cases used for comparison. The report is generated by the examiner who analyzed the unknown (evidence) samples, referencing the report with the known samples.

A DNA profile foreign to VICTIM was developed from the vaginal sample (Item 2). SUSPECT (Item 3, submitted under FS Lab # C04-XXXX and the subject of the Certificate of Analysis dated _____ and addressed to NAME OF INVESTIGATOR, NAME OF AGENCY, and Agency Case # 2004-XXXX) cannot be eliminated/is eliminated as a contributor of this foreign DNA profile.

NOTE: If the evidence in the above example was previously analyzed and reported, then that Certificate of Analysis needs to be referenced in the statement above.

or

DNA profiles were previously developed from the buccal swabs from Suspects 1, 2, and 3 (Items 100, 101, and 102 submitted under FS Lab # ---- and the subject of the Certificate of Analysis dated ---- addressed to ---, Agency, and Agency Case # ---).

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A DNA profile foreign to VICTIM was developed from the vaginal sample (Item 2). Suspect 1 cannot be eliminated as a contributor of the foreign DNA profile developed from the vaginal sample. Suspects 2 and 3 are eliminated as contributors.

If the intercomparison of samples involves an analysis conducted by an outside laboratory, it will be clear in the report that those profiles were based on a report dated ---- issued by OUTSIDE LABORATORY, their case #----)

6.3.3.2 **Alternate Knowns:** When an alternate known sample (cigarette butt, can, bottle, shoes, etc.) is submitted in place of the traditional known sample (blood or buccal swabs) the results for this sample will be reported in the following manner:

"A DNA profile was developed from the ALTERNATE KNOWN from VICTIM/SUSPECT."

When drawing a conclusion using an alternate known, this will be reported in the following manner:

"If the DNA profile developed from the ALTERNATE KNOWN is from VICTIM/SUSPECT, then VICTIM/SUSPECT cannot be eliminated (or is eliminated) as a contributor of the DNA profile developed from the EVIDENCE."

NOTE: If a complete, single source profile is developed from the alternate known, no request will be made in the Certificate of Analysis for another, traditional known sample. If a partial, single source profile is developed from the alternate known, it may be necessary to request a traditional known sample from the victim/suspect. If a mixture profile is developed from the alternate known and no additional information supports the decision to use the major/minor portion of the mixture, the alternate known will not be considered as a known sample, but rather as an additional evidentiary item, and a traditional known sample (or known samples from parents/offspring) will be requested in the Certificate of Analysis.

6.3.3.3 **Request for Known Samples:** When a suspect, other person of interest, victim or elimination known sample has not been submitted and/or no suspect has been identified, and a profile has been obtained in the case for which the source is unknown, a request will be made in the Certificate of Analysis for a known sample to be submitted using the following statement:

"DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect (or SUSPECT/VICTIM/ELIMINATION SAMPLE/ other person of interest) or other person of interest to the Laboratory."

For deceased individuals or body ID cases:

"DNA comparisons can be conducted following the submission of an alternate known sample (such as a toothbrush or cigarette butts) from VICTIM or two buccal (cheek) swabs from the biological parents/offspring of VICTIM to the laboratory."

NOTES: In certain case scenarios, a profile with an unknown source may be developed; however, this profile is not probative to the case. It is not necessary to request a known sample for comparison in this situation.

If a known sample is not available, an alternate known may be used in place of the known sample for comparison.

6.3.3.4 **Reporting of Clippings and Swabbings:** Clippings and swabbings of a sample (techniques used during the examination of an item to collect a DNA sample) are considered part of the examination process and will not be reported in the Certificate of Analysis.

EXAMPLE: A swab is used to swab the mouth area of a bottle and DNA analysis is conducted on the swab. The report will be worded in the following manner: "A DNA profile was developed from the mouth area of the bottle."

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6.3.3.5 **Sampling:** Certificates of Analysis should reflect the sampling method

Examples

- A sample collected from several different areas

"No DNA amplification results were obtained from a combined sample collected from the grips, trigger, slide release and magazine release of the pistol."

or

"A DNA profile was developed from combined stains from the center of the mattress."

- A single stain tested

"A DNA profile was developed from a stain (selected stain) found on the cuff of the right sleeve of the shirt."

6.3.3.6 **Reporting of Differentially Extracted Samples**: Routinely, sperm and non-sperm fraction results will not be reported separately in the results section for samples that are differentially extracted. Instead, the probative results will be reported for the sample (i.e., vaginal/cervical sample rather than sperm fraction of the vaginal/cervical swabs). However, the statistical statement associated with the results statement will make reference to the probative fraction (i.e., sperm and/or non-sperm fraction) for samples that are differentially extracted. The table included as an appendix to the Certificate of Analysis will list the sperm and non-sperm fractions for the evidence sample when the sample as a whole is considered probative.

6.3.3.7 **Inconclusive Locus or No Result:** Inconclusive results and/or no results developed at a particular locus or loci (e.g., a partial profile) will not be addressed in the report.

Inconclusive DNA Profile: An entire profile is deemed inconclusive when the associated quality control measures fail. Profiles that are deemed inconclusive will be reported as follows:

"No reportable DNA results were obtained because the quality control standard was not achieved."

6.3.3.8 **Data Bank Searches:** When a DNA Data Bank search is conducted the loci used for searching will not be reported.

6.3.3.9 **Samples added to the DNA Data Bank:** When samples are added to the DNA Data Bank they will be addressed in the certificate of analysis.

6.3.3.9.1 For DNA profiles from solved or unsolved cases entered into the forensic index

State only
"This DNA profile will be submitted to the Virginia DNA Data Bank."

State and National
"This profile will be submitted to the Virginia and National DNA Data Banks."

6.3.3.10 **No Typing Results:** If no STR typing results are obtained for a sample, either due to no peaks observed or no peaks observed above threshold, this will be reported in the following manner:

"No DNA typing results were obtained from the EVIDENCE."

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6.3.3.10.1 There may be rare extenuating circumstances in which the detection of human DNA, in the absence of amplification results, is critical to the case and may supply investigative information. This will be reported in the following manner:

"Human DNA was isolated from the EVIDENCE; however, no DNA typing results were obtained."

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6.3.3.11 **"Touch" and "Wearer" DNA samples**: If samples collected solely for "touch" or "wearer" DNA do not have more than 0.006 ng/ul of autosomal or Y DNA (using the Plexor quantitation system), these samples will not proceed through the DNA typing step and will be reported in the following manner:

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"Due to the limited quantity of nuclear DNA obtained from the EVIDENCE, it is not suitable for PCR analysis using the PowerPlex® 16 system and no further testing was conducted on this sample."

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6.3.3.12 **Profile vs. Types:** The term 'profile' will routinely be used when referring to the typing results as a whole developed from an item. Typically, 'profile' describes typing results for which conclusions can be drawn and/or entered into the Data Bank for a meaningful search. However, it may also be used to describe typing results which have limited value, no value, and/or not suitable for a meaningful Data Bank search. The term 'type' or 'types' is described as components of a profile (i.e., results at one or a few loci). These terms may be used instead of 'profile' when referring to typing results of limited value, no value and/or not suitable for a meaningful Data Bank search in a report. Examiner discretion will be used in deciding whether to use 'profile' or 'type/types' in the report.

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6.3.3.13 **Complex Mixture Profiles:** Complex mixture profiles indicative of 4 or more contributors (as defined in 4.3) will be determined to be of **no value** or **limited value** and reported as such:

"Due to the unknown number of potential contributors and complex nature of this mixture profile, it is not suitable for comparison, searching against the Virginia DNA Data Bank, or submission to the National DNA Data Bank." (The Data Bank statement is used as appropriate).

Or the profile may be determined to be of **limited value and used for elimination purposes** only and reported as:

"Due to the unknown number of potential contributors and complex nature of this mixture profile, it is only suitable for elimination purposes. It is not suitable to searching against the Virginia DNA Data Bank, or submission to the National DNA Data Bank." (The Data Bank statement is used as appropriate).

6.3.3.14 **Use of the term "Foreign":** The term 'foreign' will routinely be used when the profile developed from an intimate item of evidence contains a DNA profile which is different than the owner/source of the evidence (i.e., vaginal/cervical swabs containing a profile that could not have come from the victim herself). DNA profile attribution statement(s) regarding the owner/source are used as necessary.

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6.3.3.15 **Use of the term "Different":** The term "different" may be used in situations where the probative finding would be a profile different than the source (i.e., cigarette butts recovered from the victim's house, steering wheel of victim's car, victim's wallet recovered from his house, etc.). This situation is typically encountered in cases when investigators are looking for investigative leads (ie, profiles different than the victim). It is necessary to note that analysis of these items is useless without the proper known samples. DNA profile attribution statement(s) regarding the source are used as necessary.

6.3.3.16 All positive associations must be clearly and properly qualified in the Certificate of Analysis. This can be done by either:

1 - A statistical statement

2 – A qualitative statement. A qualitative statement (not based on a statistical calculation) will be limited to situations in which the presence of an individual's DNA on an item is reasonably expected limited to the following:

1 – Intimate samples (e.g., samples and items collected or removed from an individual's body). A qualitative statement may also be used for profiles developed from consensual sexual partners on intimate samples.

2 – Items owned or worn by an individual where that individual's DNA is expected to be found (e.g., swabs from cars, homes, clothing, etc).

3 – Crime scene evidence where profiles different from the victim are being sought (e.g., blood samples from the scene of a homicide where DNA profiles are consistent with the victim).

The qualitative statement will indicate the number of loci at which all the owner's alleles are present. See below for reporting wording examples:

Intimate sample examples (no minimum number of loci requirement)

Vaginal / cervical sample (carryover of NSP into SP)

- A DNA profile foreign to VICTIM was developed. SUSPECT cannot be eliminated as a contributor.
  - Statistical statement for sperm fraction foreign
- A DNA profile/DNA types attributable to the VICTIM was also present in this sample (at 15 loci in the non-sperm fraction and 4 loci in the sperm fraction)

Vaginal/cervical sample (profile consistent with elimination and victim)

- No DNA profile foreign to VICTIM and ELIM was developed. SUSPECT is eliminated as a contributor. A DNA profile attributable to the VICTIM is present in this sample (at 15 loci in the non-sperm fraction).
- A DNA profile attributable to ELIM is also present in this sample (at 15 loci in the sperm fraction)

OR

- DNA profiles attributable to VICTIM and ELIM were present in this sample at 15 loci in the non-sperm fraction and 15 loci in the sperm fraction, respectively.

Ownership sample examples (must be 4 or more loci)

Steering wheel swabs, door handle swabs, clothing, etc. (ownership-type items)

- A DNA profile foreign to OWNER was developed.
  - SUSPECT cannot be eliminated as a contributor of this foreign profile.
    - Statistical statement for different profile.
  - A DNA profile attributable to OWNER is also present in this sample at 8 loci.

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Crime scene sample examples (must be 4 or more loci)

Five stained swabs tested from homicide of the victim, she is found in her home:

Item 1 – Stained swab
Item 2 – Stained swab
Item 3 – Stained swab
Item 4 – Stained swab

- No DNA profile foreign to VICTIM was developed. DNA profile(s) attributable to her were developed at 15 loci (or list # of loci separately, respectively).
- Suspect is eliminated as a contributor.

Item 5 – Stained swab

- A DNA profile was developed.
  - VICTIM is eliminated as a contributor.
  - SUSPECT cannot be eliminated as a contributor.
    - Statistical statement

6.3.3.17 **Probative Evidence:** Evidence through testing that demonstrates the proposition that a biological fluid may or may not have been deposited by a specific "individual of interest" that is believed to be associated with the evidence in question.

6.3.3.18 **Table/Appendices:** A table of the typing results will be included in the Certificate of Analysis as an appendix based on the following criteria.

6.3.3.18.1 Tables will only be included in cases where there is a positive association (i.e., inclusion) to a probative item of evidence.

6.3.3.18.2 In cases where a table is included, all DNA evidence profiles suitable for comparison will be included in the table, regardless of what conclusion is reached for the profile (inclusion, elimination, insufficient information, elimination only).

6.3.3.18.3 In cases where a table is included, DNA reference profiles for individuals for which a conclusion of cannot be eliminated or insufficient information was reached will be included in the table. DNA reference profiles will NOT be included in the table if the individual is eliminated from all charted evidence.

6.3.3.18.4 Any DNA profile determined to be of no value for comparison (for any reason) will not be included in the chart. This includes DNA mixture and single source profiles of no value, intimate DNA profiles with no foreign/different types (note exception below), and intimate DNA profiles containing foreign/different types of no value.

6.3.3.18.5 If a sperm or non-sperm fraction is suitable for comparison and is included in the table, the other fraction MUST be included in the table as well, even if that profile is of no value, no types foreign or no typing results are obtained. This may affect which reference samples are charted. In the example of no foreign types to the victim are detected in the non-sperm fraction of a sample from her, the victim reference sample should also be charted.

6.3.3.18.6 For cases (paternity/maternity, body ID, TrueAllele) where conclusions and statistics are to be the subject of a supplemental report and/or the profiles are not suitable for traditional statistics, tables will be included with the original Certificate of Analysis.

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6.3.3.18.7 In cases where the original analysis did not result in a table being included in the Certificate of Analysis and where the subsequent submission of new evidence or known standards results in positive associations to probative item(s) of evidence, a table will be included in a new Certificate of Analysis including all profiles developed in the case *following the guidelines above*.

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6.3.3.18.8 In cases where the original analysis did result in a table being included in the Certificate of Analysis and where the subsequent submission of new evidence or known standards results in additional inclusions to probative item(s) of evidence, only the newly developed profiles need to be included in the table. Previously charted profiles are not required to be included in any subsequent tables.

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6.3.3.19 **Termination During Examinations:** If DNA analysis has begun in the case, and a request for terminating the case has been received, this will be reported as follows: "The DNA analysis on the EVIDENCE was discontinued at the request of INVESTIGATOR/ COMMONWEALTH'S ATTORNEY on DATE."

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6.3.4 A **CONCLUSIONS SECTION** may be included in the Certificate of Analysis and will follow the RESULTS section. This section is reserved for reports containing multiple items tested and hence more results to be reported. It may be incorporated into the report to assist in streamlining results, thus creating a more reader friendly report. When incorporated, it will contain results of comparisons, Data Bank searches, conclusions and statistics, if applicable. Refer to the below examples for when a CONCLUSIONS section might be incorporated into the report.

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EXAMPLE: A reconstruction case involving 2 victims and 2 suspects and 21 items was submitted for analysis. DNA profiles were developed from 17 of the 21 items; the results of comparison to the known samples may be summarized in a CONCLUSIONS section in the Certificate of Analysis. The RESULTS section would address whether DNA profiles were developed or not.

EXAMPLE: A homicide case involving 1 victim, 5 suspects, and multiple submissions with numerous items for examination was submitted for analysis. Grouping of evidence results for reporting purposes is minimal due to the varying results obtained (full profiles, no foreign profiles, mixtures suitable for searching, limited foreign profiles, etc.). A CONCLUSIONS section may be used to report the results of the comparisons and Data Bank searches. The RESULTS section of this report would address whether DNA profiles were developed or not.

6.4 Reporting Format for *Subject Cases*

The results of the analysis for a subject case will be reported in the Certificate of Analysis in the following manner.

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6.4.1 **Intimate Evidence Samples** (i.e., Victim or Suspect PERK samples)

NOTES: The following section is written as if the evidence is from the victim. If the evidence is from the suspect, substitute "SUSPECT" for "VICTIM" and "VICTIM" for "SUSPECT", as appropriate.

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The VICTIM/SUSPECT EVIDENCE SAMPLE(S) listed below refers to the sample as a whole (i.e., vaginal swabs, pubic area swabs, etc.). The sperm and non-sperm fractions will not routinely be reported separately in the "Results" section.

6.4.1.1 **Single Source Foreign Donor**

6.4.1.1.1 **INCLUSION – Victim's Type NOT Present**

"A DNA profile foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). SUSPECT cannot be eliminated as a contributor of this foreign DNA profile."

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6.4.1.1.2 **INCLUSION – Victim's Type Present**

"A DNA profile foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). SUSPECT cannot be eliminated as a contributor of this foreign DNA profile. A DNA profile attributable to VICTIM is also present in this sample."

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6.4.1.1.3 **EXCLUSION**

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"A DNA profile foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). SUSPECT is eliminated as a contributor of this foreign DNA profile."

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NOTE: If the victim's DNA profile is present add the statement, "A DNA profile attributable to the VICTIM is also present in the sample." *(Refer to the appropriate non-subject case subsection for reporting results of Data Bank searches, if appropriate.)*

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6.4.1.2 **Multiple Foreign Donors**

6.4.1.2.1 **INCLUSION – Victim's Type NOT Present**

"A DNA mixture profile foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). SUSPECT 1 and SUSPECT 2 cannot be eliminated as contributors to this foreign DNA mixture profile.

If any portion of mixture profile remains unaccounted for, it needs to be addressed in the Certificate of Analysis.

6.4.1.2.2 **INCLUSION – Victim's Type Present**

"A DNA mixture profile foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). SUSPECT 1 and SUSPECT 2 cannot be eliminated as contributors to this foreign DNA mixture profile. A DNA profile attributable to VICTIM is also present in this sample."

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If any portion of mixture profile remains unaccounted for, it needs to be addressed in the Certificate of Analysis.

6.4.1.2.3 **EXCLUSION**

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"A DNA mixture profile foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). SUSPECT is eliminated as a contributor of this foreign DNA mixture profile."

NOTE: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample." *(Refer to the appropriate non-subject case subsection for reporting results of Data Bank searches, if appropriate.)*

NOTE: If one of the donors to the foreign mixture profile is an elimination sample (i.e., boyfriend or husband), the results can alternatively be reported as follows:

"A DNA profile foreign to VICTIM and different from ELIMINATION was developed from the VICTIM EVIDENCE SAMPLE(S). SUSPECT cannot be eliminated (or is eliminated) as a contributor of this foreign DNA profile."

NOTE: If the victim or elimination DNA profile is present add the statement, "DNA profile(s) attributable to VICTIM and/or ELIMINATION are also present in this sample."

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6.4.1.3 **No Foreign Profiles**

"No DNA profile foreign to VICTIM (and/or different from ELIMINATION) was developed from the VICTIM EVIDENCE SAMPLE(S).

6.4.1.4 **Foreign Profiles of Limited Value (Single Source or Mixture Profiles)**

6.4.1.4.1 **NO CONCLUSIONS REGARDING KNOWN SAMPLE**

If a DNA profile foreign to the victim is developed, and no conclusions relative to the suspect can be made due to the limited information obtained, this will be reported in the following manner:

"A DNA (mixture) profile foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). Insufficient information exists to draw a conclusion regarding SUSPECT as a contributor of (to) this foreign DNA (mixture) profile."

NOTE: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample."

6.4.1.4.2 **EXCLUSION (COMPARISON ONLY/ELIMINATION ONLY)**

6.4.1.4.2.1 **Comparison Only:** If a DNA profile foreign to the victim is deemed suitable for comparison purposes, but not suitable for searching against the Data Bank, this will be reported in the following manner:

"A DNA (mixture) profile of limited value and foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). SUSPECT is eliminated as a contributor of (to) this foreign DNA (mixture) profile. This profile may be suitable for comparison. Due to the limited information obtained, it is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

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NOTE: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample."

or

Complex mixture

A DNA mixture profile of limited value and foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). SUSPECT is eliminated as a contributor to this foreign DNA (mixture) profile. This profile may be suitable for comparison. Due to the unknown number of potential contributors and the complex nature of this profile. it is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank.

NOTE: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample."

"DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect (or other person of interest) to the Laboratory."

NOTE: If the evidence is from the suspect or the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

6.4.1.4.2.2 **Elimination Only** : If a DNA profile foreign to the victim is deemed of limited value for comparison purposes and will only be used for elimination purposes, and the profile is not suitable for searching against the Data Bank, this will be reported in the following manner:

"A DNA (mixture) profile of limited value and foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). SUSPECT is eliminated as a contributor of (to) this foreign DNA (mixture) profile. This profile is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank. Due to the limited information obtained, it may only be used for elimination purposes. "

NOTE: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample."

"DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect (or other person of interest) to the Laboratory."

NOTE: If the evidence is from the suspect or the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

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6.4.1.5 **Foreign Profiles of No Value (Single source or mixture profiles)**

When a DNA profile foreign to the victim is developed from the victim evidence, and this profile is determined to be of no value for comparison or Data Bank searching, report this in the following manner:

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"A DNA (mixture) profile of no value and foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). Due to the limited information obtained, this profile is not suitable for comparison, searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

NOTE: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample."

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OR

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Complex mixture

"A DNA mixture profile of no value and foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). Due to the unknown number of potential contributors and the complex nature of this mixture profile, it is not suitable for comparison, searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

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NOTES: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample."

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If the evidence is from the suspect or the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

6.4.2 **Non-Intimate Evidence Samples** (i.e., typically items from the scene such as a knife, T-shirt, cigarette butts, etc.)

6.4.2.1 **Single Source Profiles**

6.4.2.1.1 **INCLUSION**

"A DNA profile was developed from the EVIDENCE. SUSPECT (or VICTIM) cannot be eliminated as a contributor of this DNA profile."

6.4.2.1.2 **EXCLUSION**

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"A DNA profile was developed from the EVIDENCE. SUSPECT (or VICTIM) is eliminated as a contributor of this DNA profile." *(Refer to the appropriate non-subject case subsection for reporting results of Data Bank searches, if appropriate.)*

6.4.2.2 **Mixture Profiles with Discernible Major/Minor Contributors**

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NOTE: Both portions of the mixture profile (major and minor) must be addressed in the "Results" section of the Certificate of Analysis.

6.4.2.2.1 **INCLUSION**

Two Contributor Mixtures

"A DNA mixture profile was developed from the EVIDENCE. Assuming this

DNA profile is a mixture of one major contributor and one minor contributor, SUSPECT (and/or VICTIM) cannot be eliminated as a major (or minor) contributor to this DNA mixture profile." *(Refer to the appropriate non-subject case subsection for reporting results of a Data Bank search, if appropriate, on the remaining portion of the mixture).*

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NOTE: Conclusions to both major and minor profiles should be reported

Three Contributor Mixtures

"A DNA mixture profile was developed from the EVIDENCE. Assuming this DNA profile is a mixture of one major contributor and minor contributor(s), SUSPECT (and/or VICTIM) cannot be eliminated as a major (or minor) contributor to this DNA mixture profile." *(Refer to the appropriate non-subject case subsection for reporting results of a Data Bank search, if appropriate, on the remaining portion of the mixture).*

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6.4.2.2.2 **EXCLUSION**

"A DNA mixture profile was developed from the EVIDENCE. SUSPECT (and/or VICTIM) is eliminated as a contributor to this DNA mixture profile." *(Refer to the appropriate non-subject case subsection for reporting results of a Data Bank search, if appropriate).*

NOTE: Depending on the case scenario and the results, it may be necessary to report the exclusion relative to a major or minor contributor. However, if the individual is eliminated from the DNA mixture profile entirely, use the format listed above.

6.4.2.3 **Mixture Profiles with NO Discernible Major/Minor Contributors**

NOTE: All portions of the mixture profile must be addressed in the "Results" section of the Certificate of Analysis.

6.4.2.3.1 **INCLUSION**

"A DNA mixture profile was developed from the EVIDENCE. SUSPECT (and/or VICTIM) cannot be eliminated as a contributor to this DNA mixture profile." *(Refer to the appropriate non-subject case subsection for reporting the results of a Data Bank search, if appropriate, on the remaining portion of the mixture).*

6.4.2.3.2 **EXCLUSION**

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"A DNA mixture profile was developed from the EVIDENCE. SUSPECT (and/or VICTIM) is eliminated as a contributor to this DNA mixture profile." *(Refer to the appropriate non-subject case subsection for reporting results of Data Bank searches, if appropriate.)*

6.4.2.4 **Profiles of Limited Value (Single Source or Mixture Profiles)**

6.4.2.4.1 **NO CONCLUSIONS REGARDING KNOWN SAMPLE**

If a DNA profile is developed, and no conclusions relative to the victim or suspect can be made due to the limited information obtained, this will be reported in the following manner:

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"A DNA (mixture) profile was developed from the EVIDENCE. Insufficient information exists to draw a conclusion regarding SUSPECT (or VICTIM) as a contributor of (to) this DNA (mixture) profile."

6.4.2.4.2 **EXCLUSION (COMPARISON ONLY/ELIMINATION ONLY)**

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6.4.2.4.2.1 **COMPARISON ONLY:** If a DNA profile is deemed suitable for comparison purposes, but not suitable for searching against the Data Bank, this will be reported in the following manner:

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"A DNA (mixture) profile of limited value was developed from the EVIDENCE. SUSPECT (and/or VICTIM) is eliminated as a contributor of (to) this DNA (mixture) profile. This profile may be suitable for comparison. Due to the limited information obtained, it is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

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OR

COMPLEX MIXTURES

"A DNA mixture profile of limited value was developed from the EVIDENCE. SUSPECT (and/or VICTIM) is eliminated as a contributor to this DNA (mixture) profile. This profile may be suitable for comparison. Due to the unknown number of potential contributors and the complex nature of this mixture profile, it is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

"DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect (or other person of interest) to the Laboratory."

NOTE: If the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

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6.4.2.4.2.2 **ELIMINATION ONLY:** If a DNA profile is deemed of limited value for comparison purposes and will only be used for elimination purposes, and the profile is not suitable for searching against the Data Bank, this will be reported in the following manner:

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"A DNA (mixture) profile of limited value was developed from the EVIDENCE. SUSPECT (and/or VICTIM) is eliminated as a contributor of (to) this DNA (mixture) profile. This profile is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank. Due to the limited

information obtained, it may only be used for elimination purposes. ”

“DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect (or other person of interest) to the Laboratory.”

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NOTE: If the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

6.4.2.5 **Profiles of No Value (Single Source or Mixture Profiles)**

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When a DNA profile is determined to be of no value for comparison purposes or Data Bank searching, this will be reported in the following manner:
“A DNA (mixture) profile of no value was developed from the EVIDENCE. Due to the limited information obtained, this profile is not suitable for comparison, searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank.”

OR

Complex Mixture

A DNA mixture profile of no value was developed from the EVIDENCE. Due to the unknown number of potential contributors and the complex nature of this mixture profile it is not suitable for comparison, searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank.

NOTE: If the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

6.4.3 **Formatting Conclusions**

Depending on the number of samples tested and the complexity of the results, samples with similar conclusions can be listed in bullet/list format. Alternatively, the conclusions may be reported in a chart format which includes the item number, item description and names of individuals tested in the header row, and the results (i.e., eliminated, not eliminated) within the appropriate chart cells. Refer to the examples below.

6.4.3.1 **Bullet/List Format**

VICTIM cannot be eliminated, and SUSPECT is eliminated, as a contributor of the DNA profile developed from the following samples:

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- Stained swabs (Items 1, 2 and 4)
- Stains A and C on T-shirt (Item 3)
- Stains A, B and C on pants (Item 5)
- Knife blade (Item 7)

SUSPECT cannot be eliminated, and VICTIM is eliminated, as a contributor of the DNA profile developed from the following samples:

- Stained swabs (Item 6)
- Stain B on T-shirt (Item 3)
- Knife handle (Item 7)

6.4.3.2 **Chart Format**

ITEM	ITEM DESCRIPTION	VICTIM	SUSPECT
1	Stained swabs	Not Eliminated	Eliminated
2	Stained swabs	Not Eliminated	Eliminated
3	T-shirt – Stains A and C	Not Eliminated	Eliminated
3	T-shirt – Stain B	Eliminated	Not Eliminated
4	Stained swabs	Not Eliminated	Eliminated
5	Pants – Stains A, B and C	Not Eliminated	Eliminated
6	Stained swabs	Eliminated	Not Eliminated
7	Knife – Blade	Not Eliminated	Eliminated
7	Knife – Handle	Eliminated	Not Eliminated

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6.5 Report Format for *Non-Subject Cases*

The results of the analysis of a non-subject case will be reported in the following manner:

6.5.1 **Intimate Evidence Samples** (i.e., Victim PERK samples)

NOTES: The following section is written as if the evidence is from the victim. If the evidence is from the suspect, substitute the "SUSPECT" for "VICTIM" and "VICTIM" for "SUSPECT", as appropriate.

The VICTIM/SUSPECT EVIDENCE SAMPLE(S) listed below refers to the sample as a whole (i.e., vaginal swabs, pubic area swabs, etc.). The sperm and non-sperm fractions will not routinely be reported separately in the "Results" section.

6.5.1.1 **Single Source Foreign Donor**

6.5.1.1.1 **Victim's Type NOT Present**

"A DNA profile foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S)." *(Refer to the appropriate subsection under non-intimate evidence samples for reporting results of Data Bank searches.)*

6.5.1.1.2 **Victim's Type Present**

"A DNA profile foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). A DNA profile attributable to VICTIM is also present in this sample." *(Refer to the appropriate subsection under non-intimate evidence samples for reporting results of Data Bank searches.)*

6.5.1.2 **Multiple Foreign Donors**

6.5.1.2.1 **Victim's Type NOT Present**

"A DNA mixture profile foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). *(Refer to the appropriate subsection under non-intimate evidence samples for reporting results of Data Bank searches.)*

NOTE: If one of the donors to the foreign mixture profile is an elimination sample, the results can alternatively be reported as follows:

"A DNA profile foreign to VICTIM and different from ELIMINATION was developed from the VICTIM EVIDENCE SAMPLE(S).

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NOTE: If the victim or elimination DNA profile is present add the statement, "DNA profile(s) attributable to VICTIM and/or ELIMINATION are also present in this sample."

6.5.1.3 **NO Foreign Profiles**

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"No DNA profile foreign to VICTIM (and/or different from ELIMINATION) was developed from the VICTIM EVIDENCE SAMPLE(S)."

6.5.1.4 **Foreign Profiles of Limited Value (Single Source or Mixture Profiles)**

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6.5.1.4.1 **COMPARISON ONLY:** If a DNA profile foreign to the victim is deemed suitable for comparison purposes, but not suitable for searching against the Data Bank, this will be reported in the following manner:

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"A DNA (mixture) profile of limited value and foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). This profile may be suitable for comparison. Due to the limited information obtained, it is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

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NOTE: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample."

OR

COMPLEX MIXTURE:

"A DNA mixture profile of limited value and foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). This profile may be suitable for comparison. Due to the unknown number of potential contributors and the complex nature of this mixture profile, it is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank.

NOTE: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample."

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"DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect (or other person of interest) to the Laboratory."

NOTE: If the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

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6.5.1.4.2 **ELIMINATION ONLY:** If a DNA profile foreign to the victim is deemed of limited value for comparison and will only be used for elimination purposes, and this profile is not suitable for searching against the Data Bank, this will be reported in the following manner:

"A DNA (mixture) profile of limited value and foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). This profile is not suitable for

searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank. Due to the limited information obtained, it may only be used for elimination purposes."

NOTE: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample."

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"DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect to the Laboratory."

NOTE: If the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

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6.5.1.5 **Foreign Profiles of NO Value (Single Source or Mixture Profiles)**

DEPARTMENT

When a DNA profile foreign to the victim is developed from the victim evidence, and this profile is determined to be of no value for comparison or Data Bank searching, report this in the following manner:

"A DNA (mixture) profile of no value and foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). Due to the limited information obtained, this profile is not suitable for comparison, searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

OF

FORENSIC SCIENCE

NOTE: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample."

OR

COMPLEX MIXTURE:

"A DNA mixture profile of no value and foreign to VICTIM was developed from the VICTIM EVIDENCE SAMPLE(S). Due to the unknown number of potential contributors and the complex nature of this mixture profile, it is not suitable for comparison, searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

NOTES: If the victim's DNA profile is present add the statement, "A DNA profile attributable to VICTIM is also present in the sample."

If the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

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6.5.2 **Non-Intimate Evidence Samples** (i.e., typically items from the scene such as a knife, T-shirt, cigarette butts, etc.)

6.5.2.1 **Single Source Profiles**

COPY

6.5.2.1.1 **No Match in Data Bank**

"A DNA profile was developed from the EVIDENCE. This profile was searched against the Virginia DNA Data Bank and no DNA profile consistent with this profile was found. Future searches will be conducted on a periodic basis. This profile is indicative of a male (or female) contributor and will be submitted (or is not suitable for submission) to the National DNA Data Bank.

DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect to the Laboratory"

NOTE: If the Amelogenin results are inconclusive or no Amelogenin results are obtained, report this finding in the Certificate of Analysis as "No conclusions can be made regarding the gender of the contributor of this sample."

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6.5.2.1.2 **Match Obtained in Data Bank to an INDIVIDUAL**

"A DNA profile was developed from the EVIDENCE. This profile was searched against the Virginia DNA Data Bank and found to be consistent with the following individual (in the "LISTED STATE" DNA Data Bank; use when a NDIS hit occurs):

VIRGINIA

Name:
SSN:
DOC #
DCN #
SID #
Date of Birth:
Race:
Gender:

DEPARTMENT OF

This information is provided only as an investigative lead, and any possible connection or involvement of this individual to the case must be determined through further investigation.

FORENSIC SCIENCE

In order to complete the direct DNA comparison, two buccal (cheek) swabs from INDIVIDUAL must be submitted to the Laboratory."

NOTES: When a NDIS hit occurs and the information provided by another state is more comprehensive than the categories listed above, the additional identifying information (i.e., FBI number) will be provided in the Certificate of Analysis. No information regarding height, weight, hair color, eye color, etc. will be included in the Certificate of Analysis if this information is provided by the other state.

If the match is made to the **listed victim**, the two paragraphs following the hit information will be omitted from the Certificate of Analysis since this type of hit is not probative.

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If the match is made to the **listed suspect**, the paragraph referencing an investigative lead will be omitted from the Certificate of Analysis.

If the match made is from a sample in a **solved case** and the match is not probative, the two paragraphs following the hit information will be omitted and the following statement will be included if the match is a subsequent search, "The DNA profile from EVIDENCE has been removed from the Virginia DNA Data Bank."

COPY

If the match was the result of a **subsequent search** and a new report will be written, substitute the following sentence for the beginning statements above: "A subsequent search of the DNA profile previously developed from EVIDENCE and addressed in the Certificate of Analysis dated _____ against the Virginia DNA Data Bank found it to be consistent with the following individual:"

If the match was obtained at **NDIS**, substitute "National DNA Data Bank" for "Virginia DNA Data Bank" listed above. Refer to the Commonwealth of Virginia Department of Forensic Science CODIS Operating Policies and Procedures Manual for further instructions.

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If a match is made to specimens in the **convicted offender/arrestee and the forensic indices**, etc., the information associated with only the convicted offender/arrestee index will be reported in the Certificate of Analysis.

If a match to an **individual occurs after previous matches** have been made to other unsolved cases, a separate report will be issued for each linked case and contain the information of the individual.

VIRGINIA

6.5.2.1.3 **Match Obtained in Data Bank to Another CASE (Solved or Unsolved)**

DEPARTMENT

"A DNA profile was developed from the EVIDENCE. This profile was searched against the Virginia DNA Data Bank and found to be consistent with the DNA profile developed from the EVIDENCE submitted under FS LAB # and the subject of the Certificate of Analysis dated _____ and addressed to NAME OF INVESTIGATOR, NAME OF AGENCY, AGENCY CASE NUMBER. These results indicate that the DNA profile developed from both of these samples could have been deposited by the same individual.

OF

FORENSIC SCIENCE

Future searches will be conducted on a periodic basis. The DNA profile developed from the EVIDENCE FROM THE CASE WHICH IS BEING REPORTED is indicative of a male (or female) contributor and will be submitted (or is not suitable for submission) to the National DNA Data Bank.

This information is provided only as an investigative lead, and any possible connection between these cases must be determined through further investigation.

DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect or other person of interest to the Laboratory."

NOTES: The "Future searches…" paragraph will not be used if the candidate case is a **solved** case. If the target mixture profile hits on a **candidate mixture profile**, the above statement will be modified to reflect the appropriate situation (i.e., "This mixture profile was searched against the Virginia DNA Data Bank. As a result of this search, a contributor (or the major contributor/the minor contributor) to the DNA mixture profile developed from the EVIDENCE submitted under FS LAB # and the subject of the Certificate of Analysis dated _____ addressed to NAME OF INVESTIGATOR, NAME OF AGENCY, AGENCY CASE NUMBER cannot be eliminated as a contributor to this mixture profile."

UNCONTROLLED

In the **absence of a simultaneous offender/arrestee hit**, the examiner assigned to the target profile case will issue a Certificate of Analysis and send a copy of that report to the investigator of the candidate profile case regardless of whether that case is solved or unsolved. No report is issued by the examiner of the candidate profile case.

COPY

If the match was obtained at **NDIS**, substitute "National DNA Data Bank" for "Virginia DNA Data Bank" listed above. Generally, reports will be issued for all case to case matches made at NDIS. If cases have been previously linked as a result of matches made in the Virginia DNA Data Bank and a subsequent case to case match is made to these linked

cases at NDIS, a report can be issued on one of the linked Virginia cases with a copy sent to the investigator of the other linked cases. Refer to the Commonwealth of Virginia Department of Forensic Science CODIS Operating Policies and Procedures Manual for further instructions.

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If a match is made to specimens in the **convicted offender/arrestee and the forensic** indices, etc., the information associated with only the convicted offender/arrestee index will be reported in the Certificate of Analysis.

6.5.2.2 **Mixture Profiles with Discernible Major/Minor Contributors**

VIRGINIA

NOTE: Both portions of the mixture profile (major and minor) must be addressed in the "Results" section of the Certificate of Analysis.

6.5.2.2.1 **NO Match in Data Bank**

DEPARTMENT

"A DNA mixture profile was developed from the EVIDENCE. The major (or minor) profile from this mixture was searched against the Virginia DNA Data Bank and no DNA profile(s) consistent with this profile was found. Future searches will be conducted on a periodic basis. This profile will be submitted (or is not suitable for submission) to the National DNA Data Bank.

OF

DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect to the Laboratory."

FORENSIC SCIENCE

NOTE: Depending on the mixture profile obtained and the case scenario, the gender of the major/minor contributor may be able to be determined. If so, gender will be reported similar to the statement under SINGLE SOURCE PROFILES, NO MATCH IN DATA BANK.

6.5.2.2.2 **Match Obtained in Data Bank to an INDIVIDUAL**

"A DNA mixture profile was developed from the EVIDENCE. The major (or minor) profile developed from this mixture was searched against the Virginia DNA Data Bank and found to be consistent with the following individual (in the "LISTED STATE" DNA Data Bank; use when a NDIS hit occurs):

Name:
SSN:
DOC #
DCN #
SID #:
Date of Birth:
Race:
Gender:

UNCONTROLLED

COPY

This information is provided only as an investigative lead, and any possible connection or involvement of this individual to the case must be determined through further investigation.

In order to complete the direct DNA comparison, two buccal (cheek) swabs from INDIVIDUAL must be submitted to the Laboratory."

NOTES: When a NDIS hit occurs and the information provided by another state is more comprehensive than the categories listed above, the additional identifying information (i.e., FBI number) will be provided

in the Certificate of Analysis. No information regarding height, weight, hair color, eye color, etc. will be included in the Certificate of Analysis if this information is provided by the other state.

If the match is made to the **listed victim**, the two paragraphs following the hit information will be omitted from the Certificate of Analysis since this type of hit is not probative.

If the match is made to the **listed suspect**, the paragraph referencing an investigative lead will be omitted from the Certificate of Analysis.

If the match made is from a sample in a **solved case** and the match is not probative, the two paragraphs following the hit information will be omitted and the following statement will be included if the match is a subsequent search, "The DNA profile from EVIDENCE has been removed from the Virginia DNA Data Bank."

If the match was the result of a **subsequent search** and a new report will be written, substitute the following sentence for the beginning statements above: "A subsequent search of the DNA profile previously developed from EVIDENCE and addressed in the Certificate of Analysis dated _____ against the Virginia DNA Data Bank, found it to be consistent with the following individual:"

If the match was obtained at **NDIS**, substitute "National DNA Data Bank" for "Virginia DNA Data Bank" listed above. Refer to the Commonwealth of Virginia of Department Forensic Science CODIS Operating Policies and Procedures Manual for further instructions.
If a match is made to specimens in the **convicted offender/arrestee and the forensic indices**, etc., the information associated with only the convicted offender/ arrestee index will be reported in the Certificate of Analysis.

If a match to an **individual occurs after previous matches** have been made to other unsolved cases, a separate report will be issued for each linked case and contain the information of the individual.

6.5.2.2.3 **Match Obtained in Data Bank to Another CASE (Solved or Unsolved)**

"A DNA mixture profile was developed from the EVIDENCE. The major (or minor) profile developed from this mixture was searched against the Virginia DNA Data Bank. As a result of this search, the contributor of the DNA profile developed from the EVIDENCE submitted under FS LAB # and the subject of the Certificate of Analysis dated ____ addressed to NAME OF INVESTIGATOR, NAME OF AGENCY, AGENCY CASE NUMBER, cannot be eliminated as a contributor to this mixture profile.

Future searches will be conducted on a periodic basis. The DNA profile developed from the EVIDENCE FROM THE CASE WHICH IS BEING REPORTED will be submitted (or is not suitable for submission) to the National DNA Data Bank.

This information is provided only as an investigative lead, and any possible connection between these cases must be determined through further investigation.

DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect to the Laboratory."

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NOTES: The "Future searches…" paragraph will not be used if the candidate case is a **solved** case.

Depending on the results and case scenario, the gender of the major/minor contributor may be able to be determined. If so, the gender will be reported similar to the statement under SINGLE SOURCE PROFILES, NO MATCH IN DATA BANK.

In the **absence of a simultaneous offender or arrestee hit**, the examiner assigned to the target profile case will issue a Certificate of Analysis report and send a copy of that report to the investigator of the candidate profile case regardless of whether that case is solved or unsolved. No report is issued by the examiner of the candidate profile case.

If the match was obtained at **NDIS**, substitute "National DNA Data Bank" for "Virginia DNA Data Bank" listed above. Generally, reports will be issued for all case to case matches made at NDIS. If cases have been previously linked as a result of matches made in the Virginia DNA Data Bank and a subsequent case to case match is made to these linked cases at NDIS, a report can be issued on one of the linked Virginia cases with a copy sent to the investigator of the other linked cases. Refer to the Commonwealth of Virginia Department of Forensic Science CODIS Operating Policies and Procedures Manual for further instructions.

If a match is made to specimens in the **convicted offender/arrestee and the forensic indices**, etc., the information associated with only the convicted offender/arrestee index will be reported in the Certificate of Analysis.

6.5.2.3 Mixture Profiles with No Discernible Major/Minor Contributor

6.5.2.3.1 NO Match in Data Bank

"A DNA mixture profile was developed from the EVIDENCE. This mixture profile was searched against the Virginia DNA Data Bank and no DNA profiles consistent with this profile were found. Future searches will be conducted on a periodic basis. This mixture profile will be submitted (or is not suitable for submission) to the National DNA Data Bank.

DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect to the Laboratory.

NOTE: No information regarding gender will be reported for mixture profiles when no discernible major/minor contributor is obtained.

6.5.2.3.2 Match Obtained in Data Bank to an INDIVIDUAL

"A DNA mixture profile was developed from the EVIDENCE. This mixture profile was searched against the Virginia DNA Data Bank. As a result of this search, the following individual (in the "LISTED STATE" DNA Data Bank; use when a NDIS hit occurs) has a DNA profile consistent with a possible contributor to this mixture:

Name:
SSN:

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DOC #:
DCN #:
SID #:
Date of Birth:
Race:
Gender:

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This information is provided only as an investigative lead, and any possible connection or involvement of this individual to the case must be determined through further investigation.

In order to complete the direct DNA comparison, two buccal (cheek) swabs from INDIVIDUAL must be submitted to the Laboratory.

This profile will submitted to the Virginia (and National) DNA Data Bank(s)."

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NOTES: When a NDIS hit occurs and the information provided by another state is more comprehensive than the categories listed above, the additional identifying information (i.e., FBI number) will be provided in the Certificate of Analysis. No information regarding height, weight, hair color, eye color, etc. will be included in the C of A if this information is provided by the other state.

When a **NDIS hit** occurs and the information provided by another state is more comprehensive than the categories listed above, the additional identifying information (i.e., FBI number) will be provided in the Certificate of Analysis. No information regarding height, weight, hair color, eye color, etc. will be included in the C of A if this information is provided by the other state.

If the match is made to the **listed victim**, the two paragraphs following the hit information will be omitted from the Certificate of Analysis since this type of hit is not probative.

If the match is made to the **listed suspect**, the paragraph referencing an investigative lead will be omitted from the Certificate of Analysis.

If the match made is from a sample in a **solved case** and the match is not probative, the two paragraphs following the hit information will be omitted and the following statement will be included if the match is a subsequent search, "The DNA profile from EVIDENCE has been removed from the Virginia DNA Data Bank."

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If the match was the result of a **subsequent search** and a new report will be written, substitute the following sentences for the beginning statements above: "A subsequent search of the DNA mixture profile previously developed from EVIDENCE and addressed in Certificate of Analysis dated _____ against the Virginia DNA Data Bank was conducted. As a result of this search, the following individual (in the "LISTED STATE" DNA Data Bank; use when a NDIS hit occurs) cannot be eliminated as a contributor to this mixture profile:"

If the match was obtained at **NDIS**, substitute "National DNA Data Bank" for "Virginia DNA Data Bank" listed above and refer to the Commonwealth of Virginia Department of Forensic Science CODIS Operating Policies and Procedures Manual for further instructions.

If a match is made to specimens in the **convicted offender/arrestee and the forensic indices**, etc., the information associated with only the convicted offender/arrestee index will be reported in the Certificate of Analysis.

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If a match to an individual occurs **after previous matches** have been made to other unsolved cases, a separate report will be issued for each linked case and contain the information of the individual.

6.5.2.3.3 **Match Obtained in Data Bank to Another CASE (Solved or Unsolved)**

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

"A DNA mixture profile was developed from the EVIDENCE. This mixture profile was searched against the Virginia DNA Data Bank. As a result of this search, the contributor of the DNA profile developed from the EVIDENCE submitted under FS LAB # and the subject of the Certificate of Analysis dated _____ addressed to NAME OF INVESTIGATOR, NAME OF AGENCY, AGENCY CASE NUMBER cannot be eliminated as a contributor to this mixture profile.

Future searches will be conducted on a periodic basis. The DNA mixture profile developed from the EVIDENCE FROM THE CASE WHICH IS BEING REPORTED will be submitted (or is not suitable for submission) to the National DNA Data Bank.

This information is provided only as an investigative lead, and any possible connection between these cases must be determined through further investigation.

DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect to the Laboratory."

NOTES: The "Future searches…" paragraph will not be used if the candidate case is a **solved** case.

No information regarding gender will be reported for mixture profiles when no discernible major/minor profile is obtained.

If the target mixture profile hits on a **candidate mixture profile**, the above statement will be modified to reflect the appropriate situation (i.e., "This mixture profile was searched against the Virginia DNA Data Bank. As a result of this search, a contributor (or the major contributor/the minor contributor) to the DNA mixture profile developed from the EVIDENCE submitted under FS LAB # and the subject of the Certificate of Analysis dated _____ addressed to NAME OF INVESTIGATOR, NAME OF AGENCY, AGENCY CASE NUMBER cannot be eliminated as a contributor to this mixture profile.)"

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In the absence of a **simultaneous arrestee or offender hit**, the examiner assigned to the target profile case will issue a Certificate of Analysis and send a copy of that report to the investigator of the candidate profile case regardless of whether that case is solved or unsolved. No report is issued by the examiner of the candidate profile case.

If the match was obtained at **NDIS**, substitute "National DNA Data Bank" for "Virginia DNA Data Bank" listed above. Generally, reports will be issued for all case to case matches made at NDIS. If cases have been previously linked as a result of matches made in the Virginia DNA Data Bank and a subsequent case to case match is made to these linked

cases at NDIS, a report can be issued on one of the linked Virginia cases with a copy sent to the investigator of the other linked cases. Refer to the Commonwealth of Virginia Department of Forensic Science CODIS Operating Policies and Procedures Manual for further instructions.

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If a match is made to specimens in the **convicted offender/arrestee and the forensic indices**, etc., the information associated with only the convicted offender/ arrestee index will be reported in the Certificate of Analysis.

6.5.2.4 **Profiles of Limited Value (Single Source or Mixture Profiles)**

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

6.5.2.4.1 **COMPARISON ONLY:** If a profile is deemed suitable for comparison purposes, but not suitable for searching against the Data Bank, this will be reported in the following manner:

"A DNA (mixture) profile of limited value was developed from the EVIDENCE. This mixture profile may be suitable for comparison. Due to the limited information obtained, it is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

OR

"Due to the limited information obtained, the minor profile (or remaining portion / DNA types different than…) developed from this mixture may be suitable for comparison, but is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

OR

COMPLEX MIXTURE

"A DNA mixture profile of limited value was developed from the EVIDENCE. This profile may be suitable for comparison. Due to the unknown number of potential contributors and the complex nature of this mixture profile, it is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

"DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect (or other person of interest) to the Laboratory."

NOTE: If the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

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6.5.2.4.2 **ELIMINATION ONLY:** If a DNA profile is deemed of limited value for comparison purposes and will only be used for elimination purposes and this profile is not suitable for searching against the Data Bank, this will be reported in the following manner:

"A DNA (mixture) profile of limited value was developed from the EVIDENCE. This profile is not suitable for searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank. Due to the limited information obtained it may only be used for elimination purposes."

DNA comparisons can be conducted following the submission of two buccal (cheek) swabs from a suspect (or other person of interest) to the Laboratory."

NOTE: If the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

6.5.2.5 **Profiles of NO Value (Single Source or Mixture Profiles)**

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When a profile is deemed to be of no value for comparison purposes or Data Bank searching, this will be reported in the following manner:

"A DNA (mixture) profile of no value was developed from the EVIDENCE. Due to the limited information obtained, this profile is not suitable for comparison, searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

VIRGINIA

OR

"Due to the limited information obtained, the minor profile (or remaining portion / DNA types different than…) developed from this mixture DNA profile is not suitable for comparison, searching against the Virginia DNA Data Bank, or submission to the National DNA Data Bank."

DEPARTMENT

OR

OF

COMPLEX MIXTURE

"A DNA mixture profile of no value was developed from the EVIDENCE . Due to the unknown number of potential contributors and the complex nature of this mixture profile, it is not suitable for comparison, searching against the Virginia DNA Data Bank or submission to the National DNA Data Bank."

FORENSIC SCIENCE

NOTE: If the case is not a CODIS case, omit the latter part of the statement above regarding searching of the Data Bank and submission to the National DNA Data Bank.

6.5.3 **Multiples Samples Tested within a Case (Single Source or Mixture Profiles)**

When multiple samples from a non-subject case are analyzed (i.e., 4 cigarette butts, 2 bottles and 1 can), the report should address as follows how many different profiles were obtained from these items and what the Data Bank searching results were.

"DNA profiles were developed from the EVIDENCE SAMPLES. Of these TOTAL NUMBER DNA profiles, NUMBER OF DIFFERENT PROFILES different profiles, indicative of NUMBER OF CONTRIBUTORS were developed." *(Refer to the appropriate subsection under non-intimate evidence samples for reporting results of Data Bank searches.)*

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EXAMPLE: All items submitted were recovered from a trash can outside of a convenience store which was burglarized.

"DNA profiles were developed from the four cigarette butts, and the mouth openings of the two bottles and one can. Of these seven DNA profiles, four different profiles, indicative of four different contributors, were developed. Three of these profiles were searched against the Virginia DNA Data Bank and no profile consistent with these profiles was found. These profiles are each indicative of a male contributor and will be submitted to the National DNA Data Bank.

COPY

The fourth DNA profile (Item 7) was searched against the Virginia DNA Data Bank and found to be consistent with the following individual (in the "LISTED STATE" DNA Data Bank; use when a NDIS hit occurs):

Name:
SSN:
Etc."

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When multiple samples from a non-subject case are analyzed and the profiles, although indicative of originating from the same contributor, are not identical, they will be reported in the following manner:

"Similar DNA profiles were developed from the EVIDENCE SAMPLES. The DNA profile from the SPECIFIC EVIDENCE SAMPLE was selected for searching against the Virginia DNA Data Bank and no profile consistent with this profile was found. This profile is indicative of a male contributor and will be submitted to the National DNA Data Bank."

VIRGINIA

or

DEPARTMENT

"DNA profiles were developed from the EVIDENCE SAMPLES. These DNA profiles are consistent with originating from a common contributor. The DNA profile from the SPECIFIC EVIDENCE SAMPLE was selected for searching against the Virginia DNA Data Bank and no profile consistent with this profile was found. This profile is indicative of a male contributor and will be submitted to the National DNA Data Bank."

OF

When multiple samples from a non-subject case are analyzed resulting in mixture profiles from which a common contributor can be identified, they can be reported in the following manner:

FORENSIC SCIENCE

"DNA mixture profiles were developed from the EVIDENCE. A common contributor to these profiles was identified/observed and was searched (or DNA types common to both mixtures were searched) against the Virginia DNA Data Bank and no profile consistent with this profile was found. This profile is indicative of a male contributor and will be submitted to the National DNA Data Bank."

NOTES: Intercomparisons of multiple samples within a non-subject case or intercomparisons of samples from two or more non-subject cases should be conducted with only single source profiles or discernible major contributors to mixture profiles. "No intercomparisons will be conducted with the remaining DNA profiles" may be used as appropriate.

Judgment should be used when grouping items from multiple locations within the report. It may be important to note that all profiles in one location are the same versus different profiles obtained from other locations.

A statement will be included in the Certificate of Analysis to indicate that the results from the intercomparison of the samples indicate that the contributor of the DNA profile developed from EVIDENCE 1 cannot be eliminated as the contributor of the profile from EVIDENCE 2.

UNCONTROLLED

6.6 Reporting Format for *Paternity, Missing Person and Body Identification Cases*

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When a comparison is made to a biological relative of an individual, rather than directly to the individual, the results will be reported in the following manner:

6.6.1 Paternity Cases

DNA profiles were developed from the CHILD'S KNOWN SAMPLE/EVIDENCE SAMPLE and the blood (or buccal) sample from the MOTHER and ALLEGED FATHER.

NOTE: A table listing the probative typing results will be included as an appendix to the Certificate of Analysis. The statement, "Refer to Appendix 1 for the PowerPlex® 16 typing results and

Appendix 2 for the Abbreviations", will be listed just prior to the disposition statement in the Certificate of Analysis.

If conclusions and statistics will be issued in a separate report by another examiner, include the following statement or if included in the same report use the bottom statement:

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"DNA conclusions and statistical calculations regarding the above analysis will be the subject of a separate report."

Or

"The ALLEGED FATHER cannot be eliminated (or is eliminated) as the biological parent (or father) of the CHILD/EVIDENCE SAMPLE."

VIRGINIA

NOTES: If the mother is the alleged parent, the wording will be changed to reflect the appropriate conclusion.

DEPARTMENT

If the mother and father are both alleged, the wording will be changed to reflect the appropriate conclusions.

6.6.2 **Missing Person Cases**

OF

"DNA profiles were developed from the EVIDENCE and from the blood (or buccal) samples from RELATIVE.

FORENSIC SCIENCE

NOTE: A table listing the probative typing results will be included as an appendix to the Certificate of Analysis. The statement, "Refer to Appendix 1 for the PowerPlex® 16 typing results and Appendix 2 for the Abbreviations", will be listed just prior to the disposition statement in the Certificate of Analysis.

If conclusions and statistics will be issued in a separate report by another examiner, include the following statement or if included in the same report use the bottom statement:

"DNA conclusions and statistical calculations regarding the above analysis will be the subject of a separate report."

Or

"RELATIVE cannot be eliminated (or is eliminated) as a biological parent/offspring of the contributor of the DNA profile developed from EVIDENCE."

6.6.3 **Body Identification Cases**

UNCONTROLLED

6.6.3.1 **Comparison to Personal Effects**

COPY

"DNA profiles were developed from the SAMPLE FROM DECEDENT and from the PERSONAL EFFECTS SAMPLE (i.e., toothbrush, razor,). If the DNA profile developed from the PERSONAL EFFECTS SAMPLE is from DECEDENT, then DECEDENT cannot be eliminated (or is eliminated) as a contributor of the DNA profile developed from the SAMPLE FROM DECEDENT.

Investigators are advised to evaluate all associated case information in addition to the provided genetic results before declaring the identity of the remains."

NOTE: If a mixture profile is developed from a personal effects sample, this can be used for comparison purposes as long as the major contributor to this mixture profile can be

discerned. No reference will be made in the C of A regarding the remaining portion of the mixture in the personal effects sample.

6.6.3.2 **Comparison to Biological Relatives**

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"A DNA profile was developed from the SAMPLE FROM DECEDENT and from the blood (or buccal) samples from RELATIVE."

If conclusions and statistics will be issued in a separate report by another examiner, include the following statement or if included in the same report use the bottom statement:

"DNA conclusions and statistical calculations regarding the above analysis will be the subject of a separate report."

VIRGINIA

Or

DEPARTMENT

RELATIVE cannot be eliminated (or is eliminated) as a biological parent/offspring of the contributor of the DNA profile developed from EVIDENCE."

6.7 Reporting Format for *Statistical Calculations*

OF

Statistical calculations will be conducted and reported for the most probative profile(s). The types of statistical calculations reported by DFS include random match probability, modified random match probability, likelihood ratio, combined probability of exclusion/inclusion, paternity index and probability of paternity, and missing person. Routinely, statistical calculations will be reported for the Caucasian, Black and Hispanic populations in accordance with The Evaluation of Forensic DNA Evidence, National Academy Press, Washington D.C., 1996 (i.e., National Research Council II).

FORENSIC SCIENCE

If statistics are appropriate and will be issued in a separate report by another examiner, include the following statement:

"DNA conclusions and statistical calculations regarding the above analysis will be the subject of a separate report."

6.7.1 Random Match Probability

The loci that are used for calculating the random match probability will be listed in the report. This can be done by listing the loci individually, stating "the PowerPlex® 16 loci" or stating "the PowerPlex® 16 loci with the exception of LOCUS/LOCI". A random match probability will be reported in the following manner:

6.7.1.1 When all population groups exceed 7.2 billion (approximate world population):

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"The probability of randomly selecting an unrelated individual with a DNA profile matching that developed from the EVIDENCE, SPERM OR NON-SPERM FRACTION OF EVIDENCE, MAJOR PROFILE OF THE EVIDENCE, ETC. at the LOCI USED FOR CALCULATION loci is 1 in greater than 7.2 billion (which is approximately the world population) in the Caucasian, Black and Hispanic populations."

COPY

6.7.1.2 When only one or two population groups exceed 7.2 billion (approximate world population):

"The probability of randomly selecting an unrelated individual with a DNA profile matching that developed from the EVIDENCE, SPERM OR NON-SPERM FRACTION OF EVIDENCE, MAJOR PROFILE OF THE EVIDENCE, ETC. at the LOCI USED FOR CALCULATION loci is approximately 1 in _____ in the Black population, 1 in _____ in the Hispanic population, and 1 in greater than 7.2 billion (which is approximately the world population) in the Caucasian population."

6.7.1.3 When none of the population groups exceed 7.2 billion (approximate world population):

"The probability of randomly selecting an unrelated individual with a DNA profile matching that developed from the EVIDENCE, SPERM OR NON-SPERM FRACTION OF EVIDENCE, MAJOR PROFILE OF THE EVIDENCE, ETC. at the LOCI USED FOR CALCULATION loci is approximately 1 in _____ in the Caucasian population, 1 in _____ in the Black population, and 1 in _____ in the Hispanic population."

6.7.1.4 If it is suspected that a relative of the suspect may have left the DNA profile at the crime scene, the following wording will be used:

"The probability of the RELATIVE having a DNA profile consistent with that of SUSPECT is approximately 1 in _____ in the Caucasian population, 1 in _____ in the Black population, and 1 in _____ in the Hispanic population."

NOTE: Based upon the question that is being asked above, the frequency provided will not be limited to 1 in greater than 7.2 billion (which is approximately the world population), but instead will be reported as the actual truncated probability that is obtained using the formulas in Section 10, STATISTICAL CALCULATIONS. The reported value for the random match probability will be truncated to 2 significant figures.

6.7.2 **Likelihood Ratio**

The loci that are used for calculating the likelihood ratio will be listed in the report. This can be done by listing the loci individually, stating "the PowerPlex® 16 loci" or stating "the PowerPlex® 16 loci with the exception of LOCUS/LOCI". A likelihood ratio will be reported in the following manner:

"The DNA profile developed from the EVIDENCE, SPERM OR NON-SPERM FRACTION OF EVIDENCE, ETC. at the LOCI USED FOR CALCULATION loci is:
times more likely to be observed if it originated from the SUSPECT and VICTIM than if it originated from the VICTIM and an unknown individual in the Caucasian population.
times more likely to be observed if it originated from the SUSPECT and VICTIM than if it originated from the VICTIM and an unknown individual in the Black population.
times more likely to be observed if it originated from the SUSPECT and VICTIM than if it originated from the VICTIM and an unknown individual in the Hispanic population."

NOTES: Depending on the assumptions made during the likelihood ratio calculation, there may be other individuals (i.e., ELIMINATION SAMPLE, UNKNOWN INDIVIDUAL) that need to be included in the likelihood ratio statement.

The reported value for the likelihood ratio probability will be truncated to 2 significant figures.

6.7.3 **Combined Probability of Exclusion/Inclusion (CPE/CPI)**

The loci that are used for calculating the CPE/CPI will be listed in the report. This can be done by listing the loci individually, stating "the PowerPlex® 16 loci" or stating "the PowerPlex® 16 loci with the exception of LOCUS/LOCI". The combined probability of exclusion/combined probability of inclusion will be reported in the following manner:

6.7.3.1 The Methods statement will contain following statements when applicable.

STATISTICAL METHODS

"The DNA statistics calculated herein used the population allele frequencies generated by the Virginia Department of Forensic Science."

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"The [name] statistical method addressed below has been applied in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) 2010 Interpretation Guidelines and Departmental procedures."

6.7.3.2 When all population groups exceed 7.2 billion (approximate world population):

"The probability of randomly selecting an unrelated individual who would be included as a contributor to the DNA mixture profile developed from the EVIDENCE, SPERM OR NON-SPERM FRACTION OF EVIDENCE, ETC. at the LOCI USED FOR CALCULATION loci is 1 in greater than 7.2 billion (which is approximately the world population) in the Caucasian, Black and Hispanic populations."

6.7.3.3 When only one or two population groups exceed 7.2 billion (approximate world population):

"The probability of randomly selecting an unrelated individual who would be included as a contributor to the DNA mixture profile developed from the EVIDENCE, SPERM OR NON-SPERM FRACTION OF EVIDENCE, ETC. at the LOCI USED FOR CALCULATION loci is approximately 1 in _____ in the Black population, 1 in _____ in the Hispanic population, and 1 in greater than 7.2 billion (which is approximately the world population) in the Caucasian population."

6.7.3.4 When none of the population groups exceed 7.2 billion (approximate world population):

"The probability of randomly selecting an unrelated individual who would be included as a contributor to the DNA mixture profile developed from the EVIDENCE, SPERM OR NON-SPERM FRACTION OF EVIDENCE, ETC. at the LOCI USED IN THE CALCULATION loci is approximately 1 in _____ in the Caucasian population, 1 in_____ in the Black population, and 1 in _____ in the Hispanic population.

NOTE: The reported value for the combined probability of inclusion will be truncated to 2 significant figures.

6.7.3.5 In some instances a CPI, uRMP or major/minor statistic cannot be calculated. In current cases involving non-person's type crimes (stolen car, gun possession) the following wording will be used.

"The significance of this DNA mixture profile cannot be determined using traditional statistical methods. Accordingly, no comparisons were conducted and no conclusions are being offered regarding possible contributors."

Or

"The significance of this DNA mixture profile cannot be determined using traditional statistical methods. Accordingly, no conclusions are being offered regarding possible contributors."

6.7.4 Paternity Index and Probability of Paternity

The loci that are used for calculating the paternity index and probability of paternity need to be listed in the report. This can be done by listing the loci individually, stating "the PowerPlex® 16 loci" or stating "the PowerPlex® 16 loci with the exception of LOCUS/LOCI". Paternity Index and Probability of Paternity will be reported in the following manner:

"Based on a review of the typing results for the CHILD/EVIDENCE and the VICTIM and ALLEGED FATHER previously analyzed at the PowerPlex® 16 loci and addressed in the Certificate of Analysis dated _____, the following Combined Paternity Index (CPI) and Probability of Paternity, using a

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prior probability of 0.5, were calculated for the following populations at the LOCI USED IN THE CALCULATION:

Caucasian	CPI = _____	Probability of Paternity = _____%
Black	CPI = _____	Probability of Paternity = _____%
Hispanic	CPI = _____	Probability of Paternity = _____%

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The Combined Paternity Index is a likelihood ratio that expresses the odds that the ALLEGED FATHER is the biological parent (father) of the CHILD/EVIDENCE SAMPLE (S) rather than another unrelated random man. Therefore, the odds are ____ times more likely in the Caucasian population, _____ times more likely in the Black population, and _____ times more likely in the Hispanic population that these alleles would be observed if ALLEGED FATHER is the biological parent (father) of the CHILD/EVIDENCE SAMPLE (S) rather than another unrelated random man."

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NOTES: If one report is issued containing the PowerPlex® 16 typing results and the paternity index and probability of paternity statistics, the first sentence above will be replaced with the following: "The following Combined Paternity Index (CPI) and Probability of Paternity, using a prior probability of 0.5, were calculated for the following populations at the LOCI USED IN THE CALCULATION:"

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If a paternity calculation is being conducted in relation to a Body Identification case, the following statement will be added to the Certificate of Analysis:

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"Investigators are advised to evaluate all associated case information in addition to the provided genetic results before declaring the identity of the remains."

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The reported value for the combined paternity index will be truncated to 2 significant figures.

Refer to Appendix L of the Commonwealth of Virginia Forensic Biology Section Procedure Manual Section III, Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 BIO System for acceptable values for the Probability of Paternity. The Probability of Paternity percentage (%) will be carried out to 4 places after the decimal point.

"Based on a review of the typing results for the REFERENCE SAMPLE and for the SUSPECTED SIBLING/HALF-SIBLING, previously analyzed at the PowerPlex® 16 loci and addressed in the Certificate of Analysis dated _____, the following Combined Full Sibling (CSI) and Combined Half-Sibling (CHSI) indices were calculated for the following populations at the PowerPlex® 16 loci:

Caucasian	CSI =	CHSI =
Black	CSI =	CHSI =
Hispanic	CSI =	CHSI =

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The Combined Full Sibling Index is a likelihood ratio that expresses the odds that the REFERENCE SAMPLE and the SUSPECTED SIBLING share common biological parents, rather than unrelated. Therefore, the odds are _____times more likely in the Caucasian population, _____times more likely in the Black population and _____times more likely in the Hispanic population that these alleles would be observed if the REFERNCE SAMPLE and the SUSPECTED SIBLING share common biological parents, rather than unrelated.

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The Combined Half Sibling Index is a likelihood ratio that expresses the odds that the REFERENCE SAMPLE and the SUSPECTED HALF-SIBLING share one common biological parent, rather than unrelated. Therefore, the odds are _____times more likely in the Caucasian population, _____times more likely in the Black population and _____times more likely in the Hispanic population that these alleles would be observed if the REFERENCE SAMPLE and the SUSPECTED HALF-SIBLING share a common biological parent, rather than unrelated.

These findings support the conclusion that the REFERENCE SAMPLE and the SUSPECTED SIBLING/HALF-SIBLING are full siblings.

[Or INC: The values reported do not meet the minimum value required in all population groups for an inclusion as siblings. Therefore, these results support a finding of inconclusive regarding the question of a sibling relationship between the donor of the EVIDENCE from the unidentified remains and REFERENCE KNOWN.]

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Investigators are advised to evaluate all associated case information in addition to the provided genetic results before declaring the identity of the remains."

6.7.5 Missing Person

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The loci that are used for calculating the missing person statistics need to be listed in the report. This can be done by listing the loci individually, stating "the PowerPlex® 16 loci" or stating "the PowerPlex® 16 loci with the exception of LOCUS/LOCI". Missing person statistics will be reported in the following manner:

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6.7.5.1 BOTH Parents Available for Testing

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"Based on a review of the typing results for the EVIDENCE and MOTHER and FATHER previously analyzed at the PowerPlex® 16 loci and addressed in the Certificate of Analysis dated_____:

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It is ____ times more likely to observe the DNA profile developed from the EVIDENCE at the LOCI USED IN THE CALCULATION if it were from a biological child of MOTHER and FATHER than if it were from a random couple of the Caucasian population.

It is _____ times more likely to observe the DNA profile developed from the EVIDENCE at the LOCI USED IN THE CALCULATION if it were from a biological child of MOTHER and FATHER than if it were from a random couple of the Black population.

It is _____ times more likely to observe the DNA profile developed from the EVIDENCE at the LOCI USED IN THE CALCULATION if it were from a biological child of MOTHER and FATHER than if it were from a random couple of the Hispanic population."

"Investigators are advised to evaluate all associated case information in addition to the provided genetic results before declaring the identity of the remains."

NOTE: If one report is issued containing the PowerPlex® 16 typing results and the missing person statistics, the first sentence above will be replaced with the following: "Given the DNA typing results developed from the EVIDENCE and the reference samples from the MOTHER and FATHER:"

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The reported value for the likelihood ratio probability will be truncated to 2 significant figures.

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6.7.5.2 Only ONE Parent/ Grandparent Available for Testing

"Based on a review of the typing results for the EVIDENCE and the PARENT/GRANDPARENT previously analyzed at the PowerPlex® 16 loci and addressed in the Certificate of Analysis dated_____ , the following Combined Paternity/Maternity/Grandparent Index (CPI/CMI/CGI) and Probability of Paternity/Maternity/Grandparentage, using a prior probability of 0.5, were calculated for the following populations at the LOCI USED IN THE CALCULATION:

Caucasian CPI* = _____ Probability of Paternity* = _____%

Black	CPI* = _____	Probability of Paternity* = _____%
Hispanic	CPI* = _____	Probability of Paternity* = _____%”

*Replace with CMI or CGI as appropriate

“Investigators are advised to evaluate all associated case information in addition to the provided genetic results before declaring the identity of the remains.”

NOTE: The Probability of Paternity/Maternity/Grandparentage percentage (%) will be carried out to 4 places after the decimal point.

“The Combined Paternity/Maternity/Grandparent Index is a likelihood ratio that expresses the odds that the PARENT/GRANDPARENT is the biological parent/grandparent (mother/father/grandparent) of the person whose DNA was found on the EVIDENCE SAMPLE(S) rather than another unrelated random man/woman. Therefore, the odds are ____ times more likely in the Caucasian population, ____ times more likely in the Black population, and ____ times more likely in the Hispanic population that these alleles would be observed if PARENT/GRANDPARENT is the biological parent/grandparent (mother/father/grandparent) of the person whose DNA was found on the EVIDENCE than another unrelated random man/woman.”

NOTE: If one report is issued containing the PowerPlex® 16 typing results and the missing person statistics, the first sentence above will be replaced with the following:

“The following Combined Paternity/Maternity/Grandparent Index (CPI/CMI/CGI) and Probability of Paternity/Maternity/Grandparentage, using a prior probability of 0.5, were calculated for the following populations:”

The reported value for the combined paternity/maternity/grandparent Index will be truncated to 2 significant figures.

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Appendix A - Capillary Electrophoresis Reagents

1. This appendix describes the preparation of reagents necessary for the DNA analysis. For each reagent listed, the company and catalog number is included. As a reagent is prepared, it will be labeled to include the following information:

   Identity
   Concentration
   Lot number
   Date of preparation
   Initials of preparer
   Date of expiration

   and if appropriate:

   Date of autoclaving
   Storage requirements

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2. All reagents will be prepared with Type I water, unless otherwise stated.

3. All chemicals and reagents will be stored according to the manufacturers' specifications. All chemicals containing biologicals will be disposed of in biohazard bags. Waste organic reagents and Ethidium Bromide will be placed in designated waste containers in a hood and removed from the laboratory by an appropriate means of disposal. All other reagents can be disposed of in the laboratory sink.

4. Any changes in chemical supply companies will be carefully checked by the Section Supervisor to ensure the chemical being provided meets the specifications necessary for the reagent. Any changes in chemical supply companies will be brought to the Forensic Biology Program Manager's attention so the list can be updated as necessary.

5. Concentrations preceding reagent components reflect the final concentrations of that specific component in the resulting mixture.

6. When a reagent is diluted and used by a number of examiners and retained, a lot number must be created and the reagent must be traceable to the reagent log. The container with the diluted reagent must contain the reagent stock lot number, the date the dilution was prepared, and the initials of the individual preparing the dilution. If a stock solution is diluted and used once and discarded or the dilution is used only by the examiner preparing the dilution, a new lot number does not need to be assigned or recorded in the reagent log. The Gold ST*R 10X Buffer and the Positive Control DNA are examples of such reagents where this applies. The original reagent stock lot number will be used.

AMPLITAQ™ GOLD DNA POLYMERASE
Expiration date: Manufacturer's expiration date
Purchased from Applied BioSystems, Foster City, CA., Catalog number N808-0243 (6 pack) or N808-0244 (12 pack).

GENETIC ANALYZER BUFFER WITH EDTA (10x)
Expiration date: Manufacturer's expiration date
Purchased from Applied Biosystems, Foster City, CA. PN 402824, 25 mL each.

HI-DI™ FORMAMIDE
Expiration date: Manufacturer's expiration date
Purchased from Applied Biosystems, Inc., Foster City, CA, PN 4311230, 25 mL each.
Aliquot into 250 µL aliquots and store at -20°C.

PERFORMANCE OPTIMIZED POLYMER – 4 (POP-4)

Expiration date: Manufacturer's expiration date

Purchased from Applied Biosystems, Foster City, CA. PN 4363752 (3.5 mL) OR PN 4352755, (7 mL)

POWERPLEX® 16 SYSTEM KIT

Expiration date: Manufacturer's expiration date

Purchased from Promega, Madison, WI., Catalog Number DC6531 for 100 reaction kit or DC6530 for 400 reaction kit.

Kit components included (expiration date same as kit unless specified):

- POWERPLEX® 16 PRIMER PAIRS
- POWERPLEX® 16 ALLELIC LADDER
- GOLD ST*R 10X BUFFER - Purchased from Promega, Madison, WI. Catalog Number DM2411, 1.2 mL tube
- FLUORESCENT INTERNAL LANE STANDARD 600
- CONTROL DNA (2800M) - Purchased from Promega Corporation, Madison, WI., 250 ng tube, 10 ng/µL, Catalog number DD1001.

POWERPLEX® MATRIX STANDARDS 3100/3130

Expiration date: Manufacturer's expiration date

Purchase from Promega, Madison, WI. Catalog Number DG4650.

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Appendix B - Maintenance of the ABI 3130*xl* Genetic Analyzer

1. General Maintenance Schedule for the 3130*xl*

Table 1. Maintenance Tasks

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Task	Frequency
Ensure adequate levels of buffer and water in reservoirs	Before each run
Ensure the level of buffer in the buffer jar reaches the red line. The overflow hole should not be occluded.	Before each run
Replace the water and 1X running buffer in the reservoirs and make sure the outside of the reservoirs are dry.	Every 48 hours or before each run, whichever comes last
Check for bubbles in the pump block, lower polymer block, interconnect tube, polymer supply tube, and channels. Remove all bubbles with the Bubble Remove wizard.	Before each run
Check the loading-end header to ensure the capillary tips are not crushed or damaged.	Before each run
Ensure that sufficient volume of polymer is present in the bottle for run.	Before each run
Ensure the pump block and lower polymer block are securely fit on the instrument.	Before each run or daily, whichever comes last
Clean the instrument surfaces.	As needed
Check for leaks around the array knob, interconnecting tube nuts, and check valve.	Before each run or as needed
Replace the polymer using the Replenish Polymer Wizard	Every two weeks or as needed.
Check the storage conditions of used arrays, if applicable.	Weekly
Restart the computer and instrument	Weekly
Run the Water wash wizard. Flush the array port during this wizard, whether or not bubbles are present in the array port.	Monthly or as needed
Flush the water trap.	Monthly or as needed
Defragment the PC's hard drive (**only** C and E).	Monthly or as needed
Clean the drip tray.	As needed
Change the array.	As needed
Remove any dried polymer from the capillary tips. Use a lint-free wipe moistened with deionized water.	As needed

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2. Wizards

Wizards are automated instrument processes which allow the user to perform a variety of tasks. Each wizard has been designed with specific instructions to achieve the purpose of the wizard. The wizards are accessed by selecting the desired wizard from the "Wizards" menu and activated when the instrument name is selected in the tree pane. The wizards include:

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- Install Array- used to install or replace capillary arrays
- Change Polymer Type- change to a different polymer type (ex. POP-4 to POP-6)
- Replenish Polymer – replace the polymer in the PDP with polymer with the same or different lot number.
- Bubble Remove – remove bubbles (>0.2mm) in the PDP chamber, channels, tubing, as well as the array port
- Water Wash – rinse the PDP chamber, lower polymer block, channels and tubing
- Instrument Shutdown – prepares the instrument for long term storage and removes the capillary array
- Autosampler Calibration – calibrates the positions on the Autosampler
- Update Cap Array Info – correct or update capillary array information

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3 Replacing the Polymer using the Replenish Polymer Wizard

3.1 NOTE: Only use the replenish polymer wizard if the polymer type is not changing. If a different polymer type is desired, select the Change Polymer Type Wizard.

3.2 In the Data Collection Software 3.0, select Wizards> Replenish Polymer Wizard

3.3 Follow the directions given in the wizard to load fresh polymer on the instrument.

3.4 Record the date, lot number, expiration date, and polymer type in the 3130*xl* Instrument Log.

4 Flushing the Water Trap

4.1 The water trap in the polymer delivery pump creates a water barrier between the pump chamber seal and the actual pump. The water trap should be flushed with Type I H_2O on a monthly basis or as needed to wash out any polymer and to clear bubbles. The trap should be left filled with type I H_2O.

4.1.1 Fill a 20 mL luer lock syringe with type 1 H_2O. Remove any bubbles from the syringe.

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4.1.2 Attach the syringe to the forward facing luer fitting at the top of the pump block, as shown in Figure 1. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.

4.1.3 Open the luer fitting by grasping the body of the fitting and turning it and the attached syringe approx. one-half turn counterclockwise.

4.1.4 Open the exit fitting at the top left side of the pump block by turning it approximately one-half turn counterclockwise.

4.1.5 Hold an empty beaker or tube under the exit fitting to catch approximately 5 mL of waste. Flush the trap by pushing steadily on the syringe plunger. Take approximately 30 seconds to flush 5 mL of type 1 H_2O through the trap.

4.1.6 Close the luer fitting then the exit fitting by turning each clockwise until the fittings seal against the block. Do not over-tighten the fittings.

4.1.7 Remove the syringe from the luer fitting. Hold the fitting with one hand while turning the syringes counterclockwise with the other hand.

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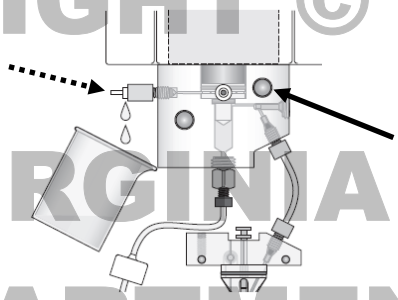

Figure 1. Diagram of the pump block for flushing the water trap. The solid arrow points to the luer fitting described in 4.1.2 above. The dashed arrow points to the exit fitting described in 4.1.4. *Figure reproduced from Applied Biosystems 3130/3130xl Genetic Analyzers (2004) Maintenance and Troubleshooting Guide.*

5 Rinsing and Filling Buffer and Water Reservoirs

5.1 Ensure the instrument doors are closed.

5.2 Press the TRAY button on the outside of the instrument to bring the autosampler to the forward position.

5.3 Wait until the autosampler has stopped moving and then open the instrument doors.

5.4 Remove the anode buffer reservoir by gently pulling down and twisting slightly.

5.5 Remove the cathode buffer reservoir and H_2O reservoirs.

5.6 Discard the solutions into the sink and rinse out the reservoirs with ddH_2O or Type 1 H_2O.

5.7 Dry with a lint-free wipe the cathode and anode reservoirs and then fill the reservoir to the fill line with 1X Genetic Analyzer buffer.

5.8 Fill the water reservoirs to the fill line with ddH_2O or Type 1 H_2O(approximately 17 mL).

5.9 Place a clean septa strip on each reservoir and dry the outside of the reservoirs using a lint-free wipe. Be sure that the septa are dry and fitted flush on the tops of the reservoirs in order to prevent damaging the capillary tips.

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5.10 Place the reservoirs into position on the autosampler as shown in Figure 2.

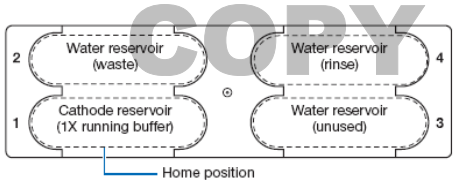

Figure 2. Position of water and buffer reservoirs on the 3130*xl*. *Figure reproduced from Applied Biosystems 3130/3130xl Genetic Analyzers (2004) Maintenance and Troubleshooting Guide.*

6 Changing the Capillary Array

6.1 The capillary array should last approximately 100 runs.

6.2 The capillary array may need to be replaced if poor sizing precision or allele calling results, or if poor resolution and/or decreased signal intensity is demonstrated.

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6.3 Close the oven and instrument doors, and then press the “Tray” button.

6.4 In the Data Collection 3.0 software, select Wizards>Install Array Wizard.

6.5 Open the instrument and oven doors

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6.6 Follow the directions given in the wizard to install or replace an array.

6.7 Click Finish when done.

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6.8 Close and lock the oven door.

6.9 Note: if you have replaced the array with one of a different length, a new spectral calibration must be completed for the dye set and array length combination.

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6.10 Use the Update Capillary Array Info wizard (Wizard>Update Capillary Array Info) to identify the capillary array length and serial number information. The capillary serial number and date must also be added to the 3130xl instrument log.

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6.11 A new spatial calibration must be completed. See 7 below for directions.

7 Performing a Spatial Calibration

7.1 A spatial calibration is used by the instrument to correlate the signal from each capillary with the signal detected by the CCD camera.

7.2 A spatial calibration must be performed when the capillary array is installed or replaced, any time the capillary array is removed from the detection block, and when the instrument is moved.

7.3 Creation of the spatial calibration file

7.3.1 On the left side of the Data Collection Software, select GA Instruments>ga3130xl>3130xl>Spatial Run Scheduler, as shown in Figure 3.

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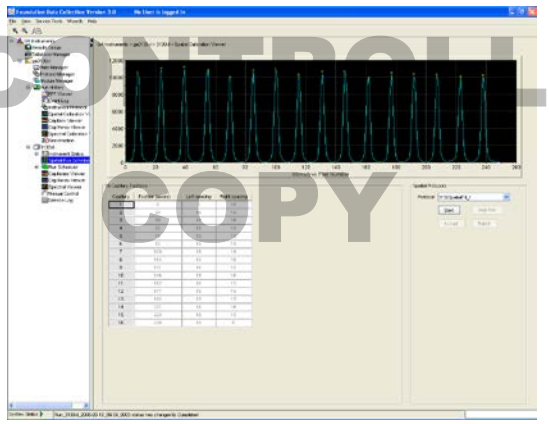

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Figure 3. Spatial Run Scheduler

7.3.2 In the spatial protocols section on the right side of the screen, select either:

7.3.2.1 Protocol>3130 SpatialFill_1, if the capillaries do not contain fresh polymer, or

7.3.2.2 Protocol>3130 SpatialNoFill_1, if the capillaries do contain fresh polymer.

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7.3.3 Click Start. The calibration run lasts approximately 2 min (6 min when filling the capillaries as well.)

7.4 Evaluation of the spatial calibration file

7.4.1 The spatial calibration profile should be evaluated according to:

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- Peak Height – Similar peak heights should be observed for all peaks.
- Orange Crosses – One orange cross should mark the top of each peak.
- Peak Shape – Each peak should be sharp. Small shoulders are acceptable.
- Spacing – The difference between adjacent peaks should be approximately 15 pixels.

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7.4.2 See Figure 4 for an example of an acceptable spatial calibration profile.

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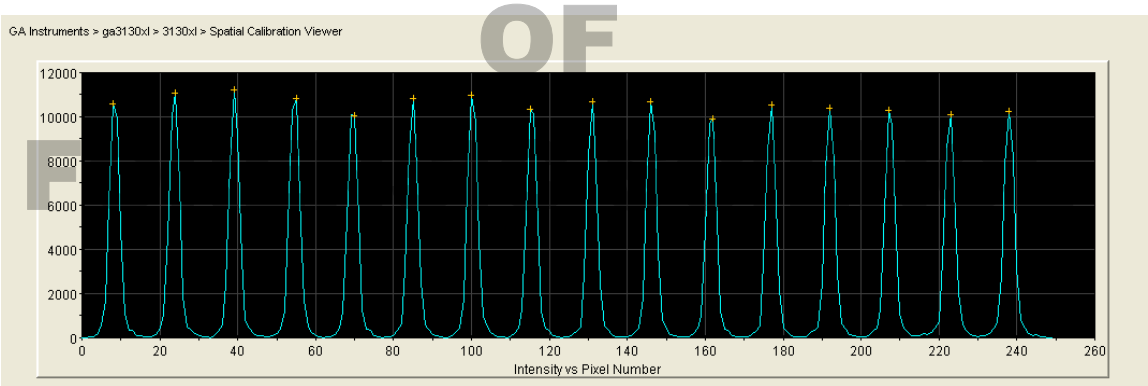

Figure 4. An example of an acceptable spatial calibration profile.

7.4.3 In the Capillary Positions table, ensure that the values in both the Left spacing and Right spacing columns range between 13 and 16 pixels.

7.4.3.1 To move the cross, type in a new value in the Positions box for the capillary of interest and press Enter.

7.4.4 If the calibration passed, click Accept to write the calibration data to the database and the *.ini file. If the calibration failed, click Reject and repeat the spatial calibration.

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7.4.4.1 Poor spatial calibration is most often due to a dirty detection window or one or more broken capillaries. The capillary array detection cell should be removed from the instrument and the mirrored side cleaned with methanol (air dry) to clean the window. While the detection cell is off the instrument, ensure that the capillaries are not broken near the detection cell.

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8 Performing a Spectral Calibration

8.1 The spectral calibration creates a matrix that is used during a run to reduce the raw (collected) data from the instrument to the 4-dye or 5-dye data stored in the sample files.

8.2 The spectral calibration is specific to each dye set.

8.3 The spectral calibration needs to be performed when using a new dye set on the instrument, when the capillary array length or polymer type is changed, after the laser or CCD camera have been serviced/replaced, or if you see extensive pull-up or pull-down peaks in the data.

Note: For each dye set, a spectral calibration for each capillary array length and polymer type combination must be created.

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Note: A new spectral cannot be associated with previously-collected data. For example, if a new kit (with a new dye set) is used on the instrument, it is necessary to perform a spectral calibration before running the samples with that new kit.

8.4 For Fragment analysis, two common dye sets are:

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- Dye Set F – used with PowerPlex® 16, AmpFlSTR® COfiler® Kit, AmpFlSTR® Profiler Plus® Kit, etc.
- Dye Set G5 – used with AmpFlSTR® Yfiler™ Kit, AmpFlSTR® Identifiler® Kit, AmpFlSTR® Minifiler® Kit, etc.

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8.5 For Dye Set F in conjunction with the PowerPlex® 16 kit, the Promega PowerPlex® Matrix 3100/3130 should be used. (For the AmpFlSTR® COfiler® Kit or AmpFlSTR® Profiler Plus® Kit, the DS-32 Matrix Standards should be used.)

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8.6 For Dye Set G5, the DS-33 Matrix Standards should be used.

8.7 Preparing the matrix standard for PowerPlex® 16

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8.7.1 For PowerPlex® 16, remove the matrix standards (P/N DG4650, Promega Corp.) from the refrigerator, vortex to mix thoroughly, but do NOT centrifuge.

8.7.2 Dilute each of the concentrated dyes 1:10 with nuclease-free water (ex. 5 µL concentrated dye + 45 µL nuclease-free water). Vortex 5-10 seconds to mix. Using these dilutions, then mix 5 µL of each diluted dye with 480 µL Hi-Di™ formamide for a total volume of 500 µL.

8.7.3 Vortex the formamide/matrix standard mixture thoroughly. Do not centrifuge the mixture.

8.8 Loading the samples

8.8.1 Add 25 µL of the formamide/dye mix to each well of two columns of a 96-well plate.

8.8.2 Seal the plate by laying the septum flat on the plate.

8.8.3 Inspect the plate and septum to ensure that the septum fits snugly and flush on the plate.

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8.8.4 Denature the samples at 95 °C for 3 minutes then immediately chill the plate in a freezer block for 3 minutes.

8.8.5 Inspect the plate to ensure no bubbles are located at the bottom of a well. If so, either briefly centrifuge the plate or use a pipet tip to remove the bubbles.

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8.8.6 Leave the plate on ice until ready to prepare the plate assembly and place the assembly on the autosampler.

8.8.7 Assemble the plate assembly by placing the sample plate into the black plate base. Snap the white plate retainer onto the plate and plate base. Note: Verify that the holes of the plate retainer and the septum are aligned. If not, reassemble the plate assembly.

8.9 Creating a Spectral Instrument protocol

8.9.1 Before creating the protocol, go into the Module Manager and select “New”. Select “Spectral” in the “Type” drop-down list and select “Spec36_POP4” in the “Template” drop-down list. Confirm or change the following settings:

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Inj. kV:	3
Inj. Secs:	5
Data Delay Time:	100
Run time:	800 seconds

8.9.2 On the left side of the Data Collection software window, choose GA Instruments>ga3130xl>Protocol Manager to open the Protocol Manager, as shown in Figure 5.

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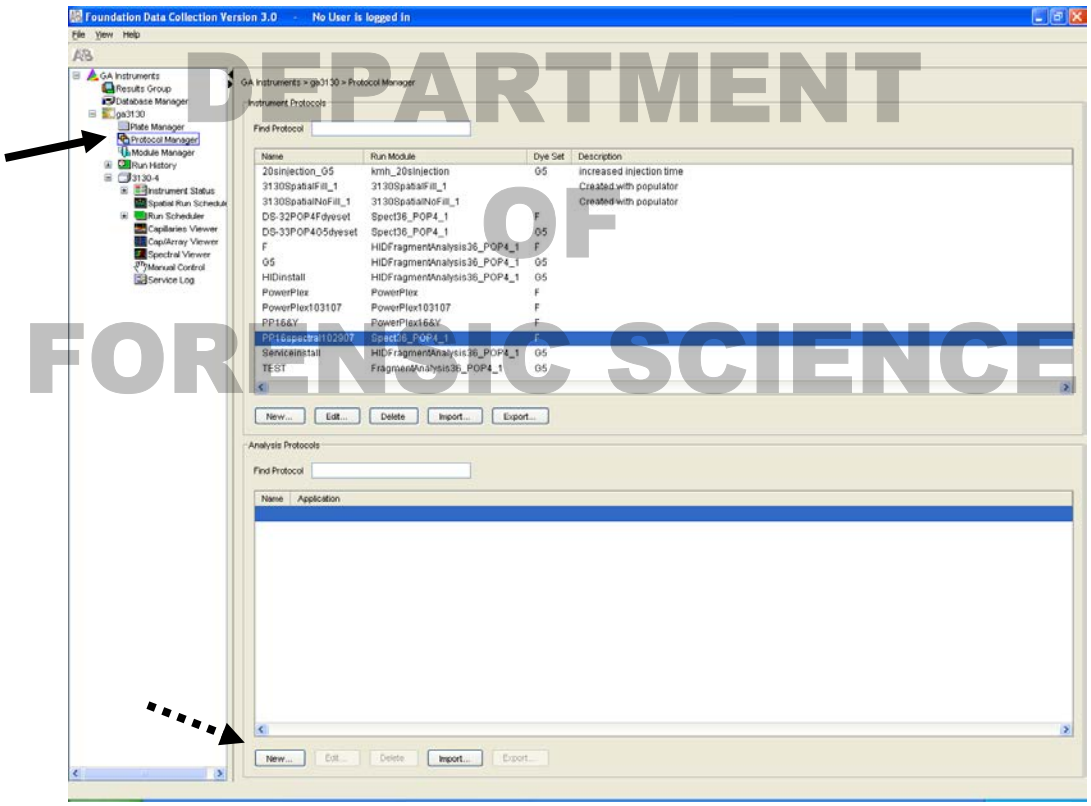

Figure 5. Creation of a protocol for a spectral calibration. The solid arrow points to the appropriate selection on the menu at the left side of the screen. The dashed arrow (near bottom) points to the “New” button for creating a new instrument protocol, as described in 8.9.2 below.

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8.9.3 In the Instrument Protocols box, click “New…”, which opens the Protocol Editor dialog box.

8.9.4 Complete the Protocol editor dialog box:

- Type a name for the protocol, typically including “Spectral” and the dye set utilized in the title.
- Select Spectral in the Type drop-down list.
- Select the correct dye set (F) for your run.
- Select the polymer and array length from the drop-down lists
- Select your chemistry file (Matrix Standard or Sequencing Standard) in the Chemistry drop-down list.
- In the Run Module drop-down list, select the run module you created in step 8.9.1.

- These modules are named with the array length and polymer type in the title. For example, Spect36_POP4 is the name of the run module that should be used when the array length is 36 cm and the polymer type used is POP-4.
- Select "Edit Parameters". Change the lower condition bound to 4.0 and change the upper condition bound to 12.0. Select "OK" in the "Edit Parameters" window and select "OK" in the "Protocol Manager".

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8.10 Performing the Spectral Calibration Plate Record

8.10.1 On the left side of the Data Collection software window, choose GA Instruments>ga3130xl> 3130xl>Plate Manager to open the Plate Manager, as shown in Figure 6.

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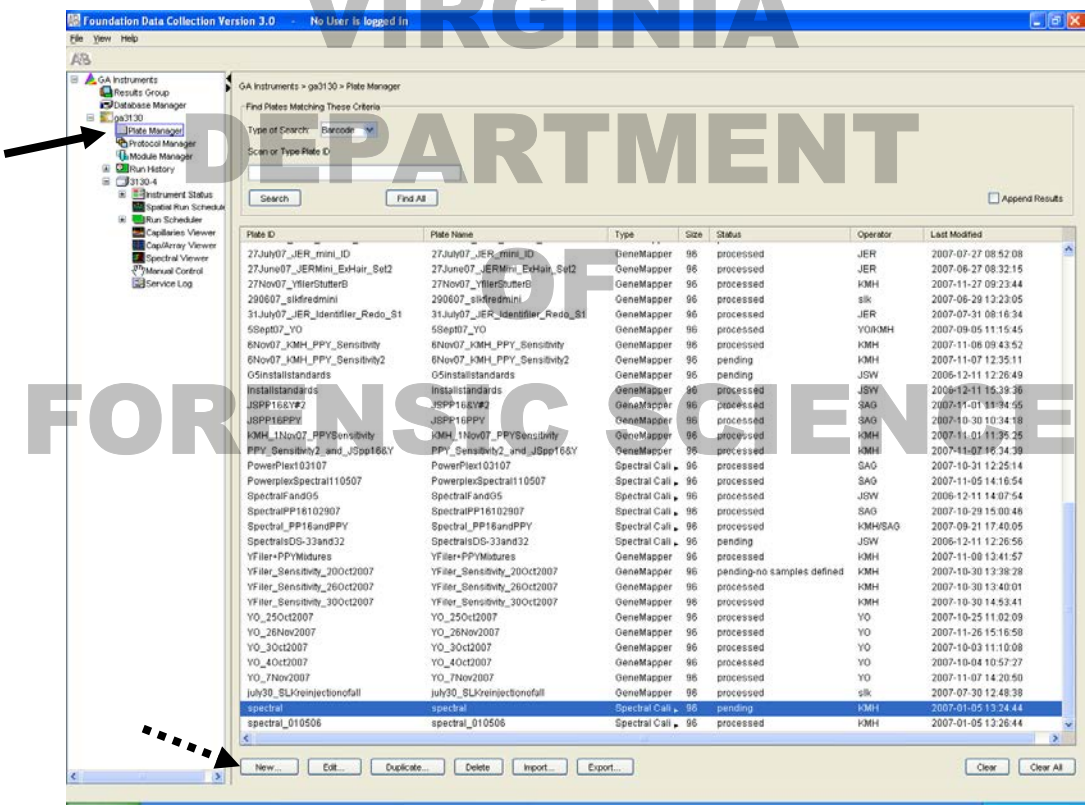

Figure 6. Plate Manager for creation of plate for spectral calibration. The solid arrow points to the appropriate selection on the menu at the left side of the screen. The dashed arrow (near bottom) points to the "New" button for creating a new plate record, as described in 8.10.2 below.

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8.10.2 Click "New" to open the New Plate dialog box and fill out as indicated:

8.10.2.1 Type a name for the plate – typically a combination of the date, "Spectral", and kit type.

8.10.2.2 In the Application field, select "Spectral Calibration"

8.10.2.3 In the Plate Type field, select "96-well"

8.10.2.4 Enter "DFS" for the Owner Name field.

8.10.2.5 Enter your initials in the Operator Name field.

8.10.2.6 Click OK.

8.10.3 The Spectral Calibration Plate Editor dialog box will appear, as shown in Figure 7.

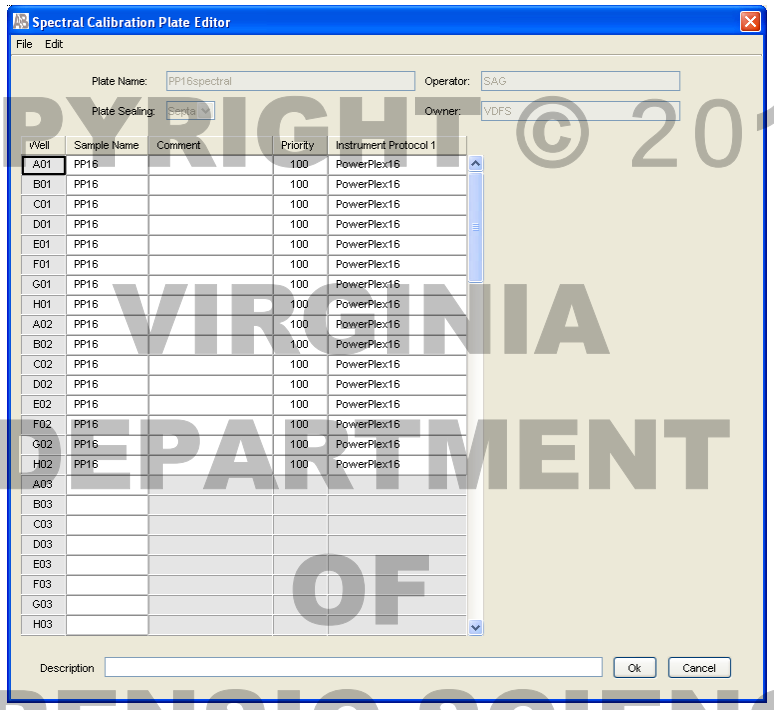

Figure 7. Spectral Calibration Plate Editor

8.10.4 In the row corresponding to the location of your first sample, select:

8.10.4.1 A sample name in the “Sample Name” column. The value “100” automatically appears in the Priority column. There is no need to change this value.

8.10.4.2 Comments may be entered in the “Comment” column, but are not necessary.

8.10.4.3 In the “Instrument Protocol 1” select the protocol name created in 8.9, above.

8.10.5 Select the entire row by clicking on the sample well number, such as A01. Select Edit>Fill Down Special. This will populate the rows corresponding to your run.

8.10.6 Click “OK”. The Plate record is now complete and the spectral calibration can be completed after loading the plate onto the instrument and final preparation of the instrument.

8.11 Running the Spectral Calibration Plate

8.11.1 The plate assembly should be placed in the instrument.

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8.11.2 On the left side of the Data Collection software window, choose GA Instruments>ga3130*xl*> 3130xl>Run scheduler>Plate View to see the listing of prepared plates, as shown in Figure 8.

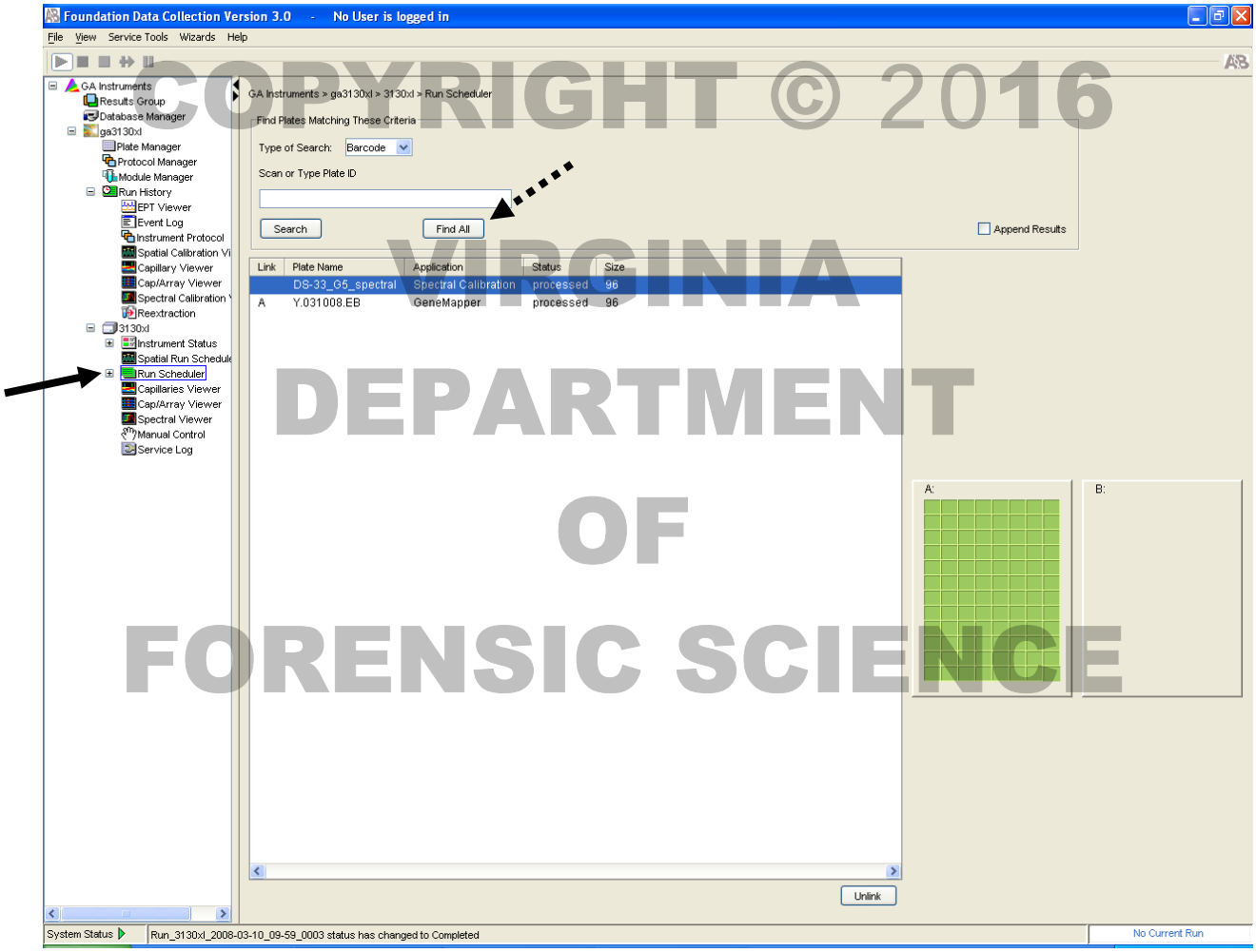

Figure 8. Run scheduler for spectral calibration plate. The solid arrow points to the appropriate selection on the menu at the left side of the screen. The dashed arrow points to the "Find All" button for finding the plate record created in 8.10.

8.11.3 Choose "Find All" to see a listing of all plate records. Alternatively, an advanced search can be completed. The plate name from 8.10.2 can be used to find the spectral plate of interest. Select "Search" and "Advanced" in the "Type of Search" drop-down list.

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8.11.4 Once the plate of interest is found, select the plate record by clicking on the plate name. Click on the plate position indicator (grid) which corresponds to the location in which the plate resides, as completed in 8.11.1 above, shown in Figure 9. The plate map color will change from yellow to green to indicate that the plate is successfully linked. In the toolbar or the Data Collection software, click the green arrow near the top of the screen to begin the run. Click OK when the dialog box appears ("You are about to start processing plates…").

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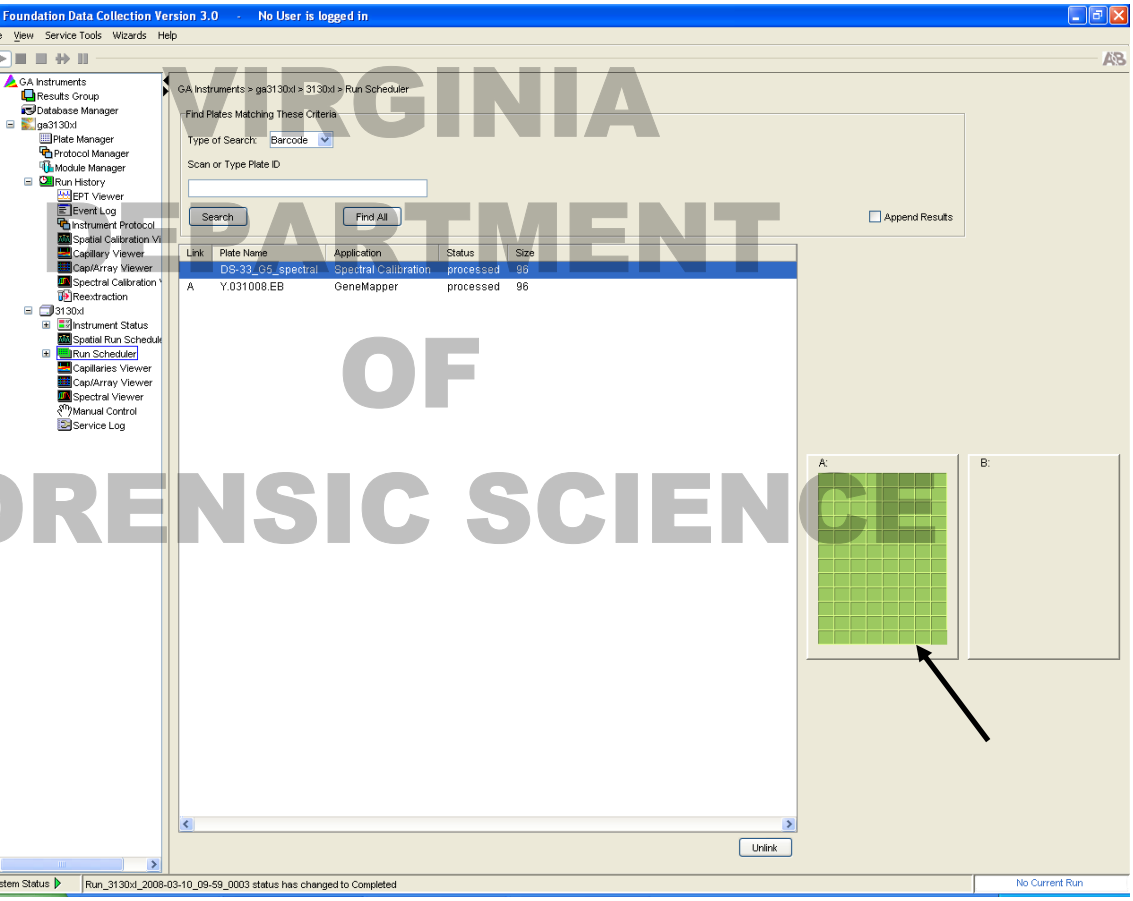

Figure 9. Plate Editor. The arrow points to the plate position indicator grid.

8.12 Evaluating the Spectral Calibration Data

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8.12.1 Viewing the Pass/Fail Status after the run

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8.12.1.1 On the left side of the Data Collection software window, choose GA Instruments>ga3130*xl*> 3130xl>Instrument Status>Event Log to see the event messages.

8.12.1.2 In the "Event Messages" section of the window, view the pass/fail status of each capillary. Each capillary (1-16, for the 3130*xl*) should have a Q-value (q) and a condition number (c) in the range shown in Table 1.

Table 1. Acceptable condition number range and Q-values for analysis of spectral calibration data.

Dye Set	Acceptable Condition Number Range (c)	Acceptable Q-value (q)
Fragment Analysis		
D	4 – 8.5	above 0.95
F	6 – 12	above 0.95
E5	2.5 – 4	above 0.95
G5	8.5 – 14.5	above 0.95
Any other 4- or 5-dye set	1 – 20	above 0.80

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8.12.2 Assessment of the Spectral Profiles and Raw Data

8.12.2.1 After assessing the pass/fail status of the capillaries in 8.12.1, the spectral calibration profile for each capillary must be assessed.

8.12.2.2 On the left side of the Data Collection software window, choose GA Instruments>ga3130*xl*> 3130xl>Spectral Viewer to see the spectral data, as shown in Figure 10.

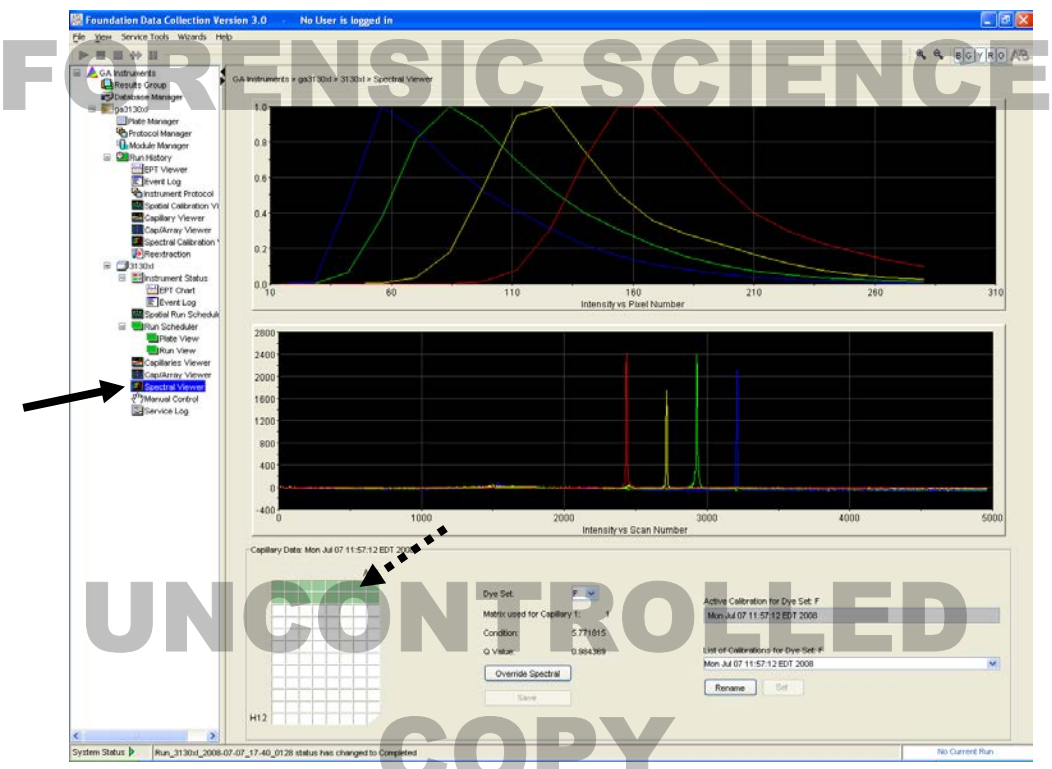

Figure 10. Spectral Viewer. The solid arrow points to the appropriate selection on the menu at the left side of the screen. The dashed arrow (near bottom) points to passing wells (green) indicated on the plate diagram.

8.12.2.3 In the "Dye Set" drop down list, choose the dye set utilized.

8.12.2.4 In the "List of calibrations for Dye Set ___", choose the spectral just created by ensuring the date and time correspond to that run. Do not click "set" until the data has been verified as acceptable.

8.12.2.5 Each well containing spectral sample of the 96-well plate is colored green (if passing) in the plate diagram, as shown in Figure 10. Each well can be clicked to verify the spectral profile and raw data for each independently. A failing capillary is colored tan (or yellow when selected).

8.12.2.6 Verify that the spectral and raw data profiles for each well are acceptable by meeting the following criteria:

8.12.2.6.1 For 4-dye chemistry, the order of peaks in the spectral (top) profile should be: blue-green-yellow-red (left to right). For 5-dye chemistry, the order of the peaks in the spectral (top) profile should be blue-green-yellow-red-orange (left to right). If the peaks are not in the correct order, the calibration run has failed.

8.12.2.6.2 The order of peaks in the raw data (bottom) profile should be:

8.12.2.6.2.1 Sequencing (4-dye): red-yellow-blue-green (left to right)

8.12.2.6.2.2 Fragment Analysis (e.g., STRs) – 4-dye: red-yellow-green-blue and 5-dye: orange, red-yellow-green-blue.

8.12.2.6.2.3 If the peaks are in the wrong order or extraneous peaks are affecting the spectral profile, the calibration run has failed.

8.12.2.6.3 The peaks in the spectral profile should not contain "gross overlaps, dips or other irregularities". If so, the calibration has failed. A proper looking spectral profile is shown in Figure 12.

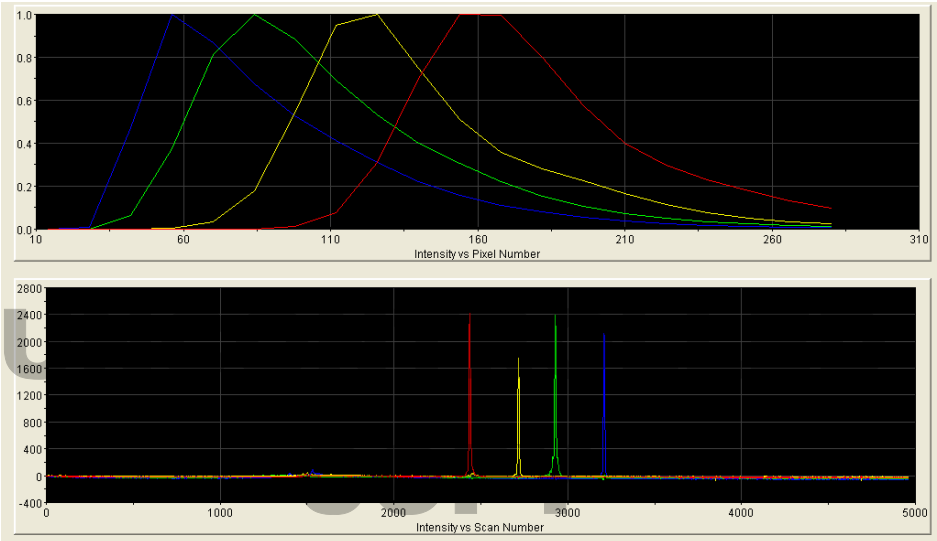

Figure 12. An example of a proper spectral calibration profile for PowerPlex® 16.

8.12.2.7 If the spectral profile passes, the file name can be changed to reflect the date, dye set, array length, and polymer type by clicking "Rename".

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8.12.3 Activating a Spectral Calibration File

8.12.3.1 Note: every new spectral calibration is automatically the active one for that dye set, unless manually changed. For example, if a spectral calibration were repeated but the first spectral was optimal, one may wish to choose the first spectral as the active spectral.

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8.12.3.2 On the left side of the Data Collection software window, choose GA Instruments>ga3130xl> 3130-4DFS>Spectral Viewer to see the spectral data, as shown in Figure 12.

8.12.3.3 In the "Dye Set" drop down list, select the dye set of interest.

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8.12.3.4 In the "List of Calibrations for Dye Set: ___", select the spectral calibration you wish to activate. The spectral profile and raw data are then displayed. If the spectral calibration is acceptable as described in 8.12.2.6, click "Set".

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9 Long Periods of Inactivity

9.1 If the ABI 3130*xl* Genetic Analyzer remains unused for an extended period of time (approximately 2 weeks or longer), it is recommended that either a blank run (consisting of only formamide and size standard) be completed *or* the capillary array removed and the capillary ends stored in Type I H_2O until further use.

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Appendix C – Storage of Electronic Data

Saving Digital Images

Sample files and project files generated using the Applied Biosystems, Inc. 3130*xl* Genetic Analyzer and analyzed using the GeneMapper® ID software will be saved onto a storage medium and stored according to the following procedures:

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After the data has been technically reviewed and found to be acceptable, the electronic sample files and project files will be transferred (via flash drive or other electronic means) by the analyst to the supervisor. The supervisor will be responsible for establishing a monthly deadline for the data to be transferred by the analyst. The collected data files from all the databank analysts will be provided to the CODIS Administrator, who will transfer the data onto a medium for permanent storage. The storage medium will be labeled appropriately to indicate the data files are PowerPlex® 16 samples and with the Month(s) and Year(s) which is included. After the data has been stored, the folders may be deleted from the CODIS server

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The storage medium will then be stored securely in a specified location within the laboratory for easy retrieval in accordance with the laboratory's Administrative Operation Procedures.

If a file(s) needs to be added to the storage medium for a given month, the analyst will make arrangements with his/her supervisor to have the storage medium retrieved from storage and have the file(s) added to the appropriate storage medium.

If electropherogram data must be retrieved and a copy made, the CODIS Administrator shall be notified to retrieve the data from the appropriate storage medium.

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Appendix D - Initial Setup of GeneMapper® ID and Data Collection Software

After installation of a new instrument and installation of the GeneMapper™ ID software on a new computer, a number of customizations must be made. These include defining: panels and bin sets, the size standard, plot settings, and table settings. If the new computer will also be used to control the instrument, appropriate protocols, modules, and results groups must also be created. This appendix describes how to make all of these settings modifications.

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1 Creation of new run modules

1.1 Instrument procedures are controlled by the module manager. The control of operations such as injection time, oven temperature, voltage during electrophoresis, data collection interval and z-axis (not adjustable by user) can be modified. Modules must be created or modified from the templates provided.

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1.2 While setting up a new instrument computer, it will be necessary to create a new module. For use with the PowerPlex® 16 system, the module "PP16_5sec" will be used for a 5s injection. For a 2s injection, "PP16_2sec" will be used, and "PP16_10sec" will be used for the 10s injection.

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1.3 To create a new module, navigate to Instruments>ga3130xl>Module Manager in the tree pane view on the left side of the screen (in the Data Collection software) to open the Module Manager window as shown in Figure 1. Click on "New" and a Run Module Editor window appears.

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1.3.1 A previously made module can be exported from another computer and imported into the Module Manager rather than creating a new module, if desired. The import and export buttons are shown at the bottom of Figure 1.

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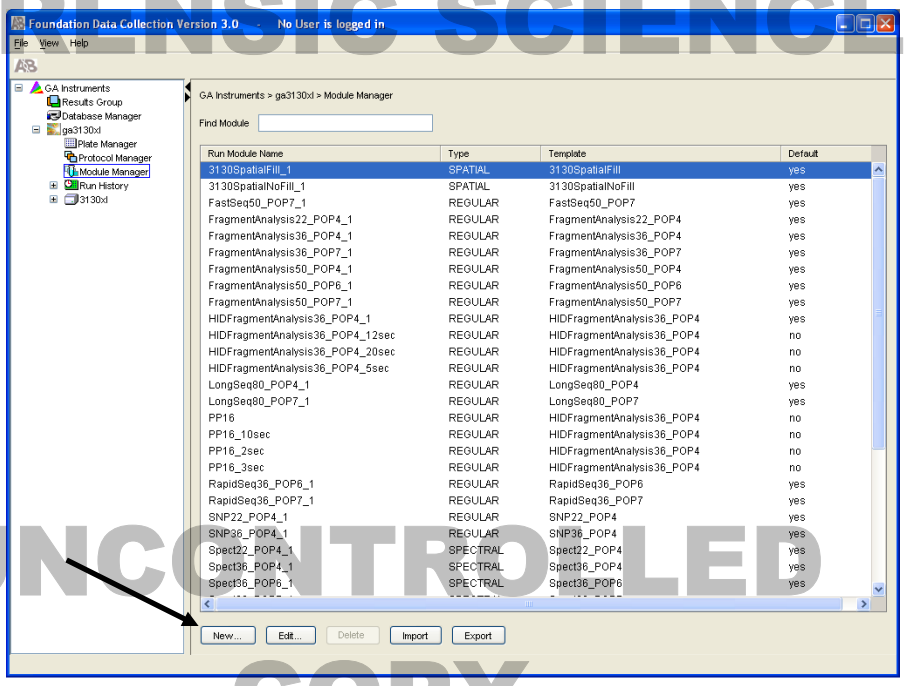

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Figure 1. Module Manager.

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1.4 Fill in the name and select the type of module (Regular) as well as a template (HIDFragmentAnalysis36_POP4) from the drop-down list. Make modifications to the run conditions as necessary to satisfy the run requirements. Figure 2 shows the conditions for the PP16_5sec run module.

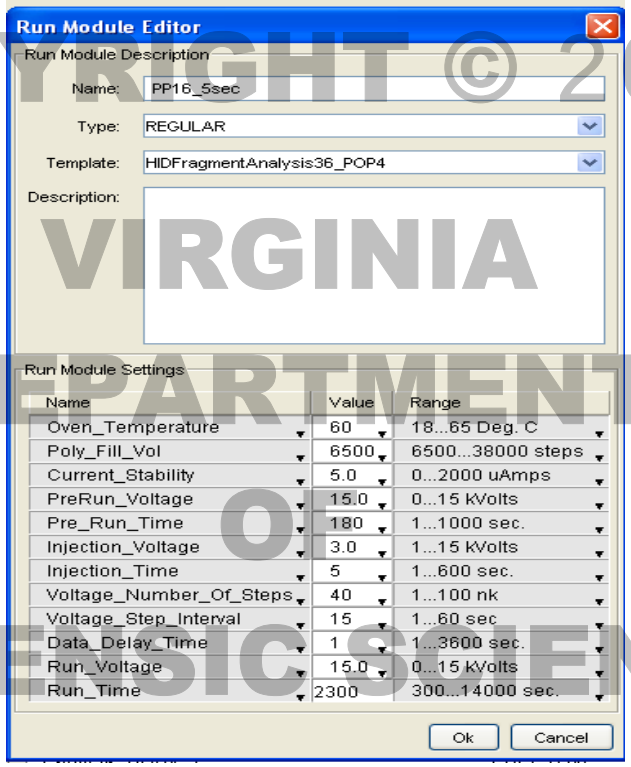

Figure 2. Run Module Editor.

1.4.1 After the settings have been verified, save the new module by clicking “OK”.

1.4.2 Similarly, a run module must be created for the 2 sec and 10 sec injections. To do so, click new at the “Module Manager” and repeat the above steps to create the modules entitled “PP16_2sec” and “PP16_10sec”. All settings should be as indicated in Figure y, except the injection time which should be 2 and 10, respectively.

2 <u>Creation of New Protocols</u>

2.1 The instrument protocol is used to tell the instrument all of the necessary run conditions for the sample, including the type of run, the run module, and the dye set. Typically, once a protocol is defined for a particular STR multiplex, it will be used repeatedly and it will not be necessary to create a new one. If a new protocol needs to be generated, proceed for directions.

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2.2 On the left side of the Data Collection Software, select GA Instruments>ga3130xl>Protocol Manager, as shown in Figure 3.

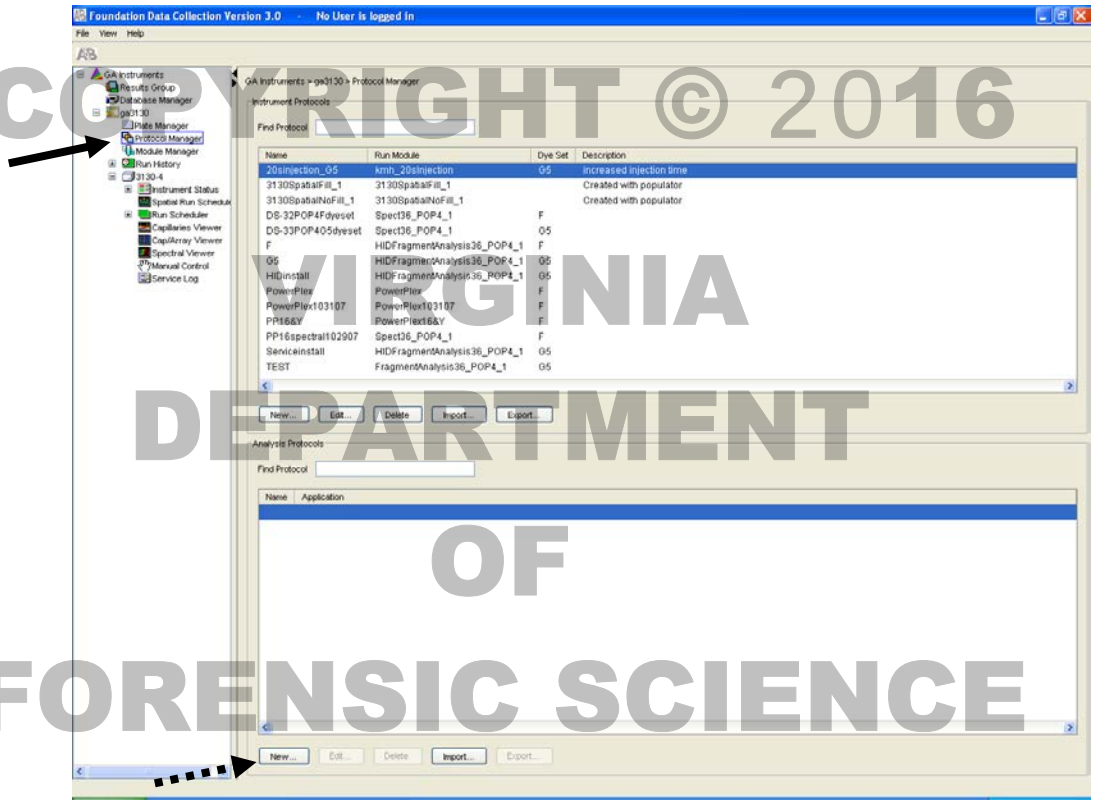

Figure 3. Protocol Manager. The solid arrow points to the appropriate selection on the menu at the left side of the screen. The dashed arrow (near middle) points to the "New" button for creating a new instrument protocol.

2.3 In the Instrument Protocols section, click "New" to open the Protocol Editor and create a new protocol.

2.3.1 A previously-made instrument protocol can be exported from another computer and imported into the Protocol Manager rather than creating a new protocol, if desired. The import and export buttons are shown at the bottom of Figure 3.

2.4 Complete the Protocol Editor, as shown in Figure 4.

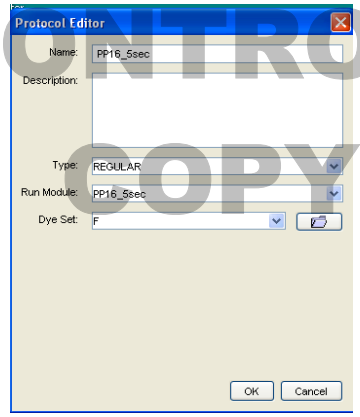

Figure 4. Protocol Editor.

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2.4.1 Type a name for the protocol. Typically, this names the amplification kit or dye set utilized for the samples of interest.

2.4.2 Writing a description is optional.

2.4.3 Select "Regular" in the "Type" drop-down list.

2.4.4 Select the run module for your run. Typically, this will be: "PP16_5sec", as this is the default run module for PowerPlex® 16. A custom run module may be desired (e.g., varying injection time, run time, etc.).

2.4.5 Select the Dye Set for your samples. Typically, this will be "F", which is the dye set for PowerPlex® 16, PowerPlex® Y, Profiler Plus®, and COfiler®. 5-dye chemistries such as Yfiler™, Identifiler™, and MiniFiler™ typically utilize dye set G5.

2.4.6 Click OK.

2.4.7 Repeat the above steps to create an instrument protocol for each injection time (ex. PP16_2sec and PP16_10sec).

3 New Results Groups

3.1 The results group defines the file type, file name, analysis parameters, and file save location and run folder name preferences for the samples separated on the instrument.

3.2 Prior to utilizing the Results Group Editor, make a folder, with the examiners initials for whom the Results Group folder will be created, in the appropriate folder on the E drive (shown in Figure 5).

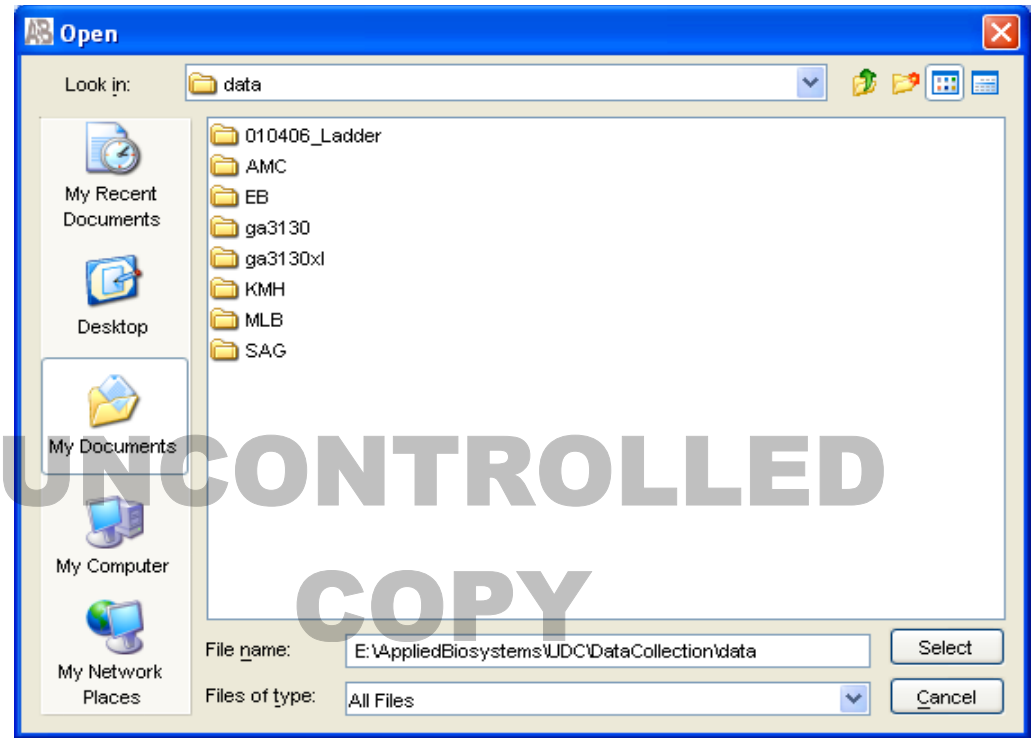

Figure 5. Making a folder for the Results Group.

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3.3 Create a results group by clicking on “Results Group” in the Tree Pane and click on the “New” button, as shown in Figure 6. Alternatively, select a previously created results group to modify and click “Duplicate” and rename the results group. The results group editor, as shown in Figure 7, opens.

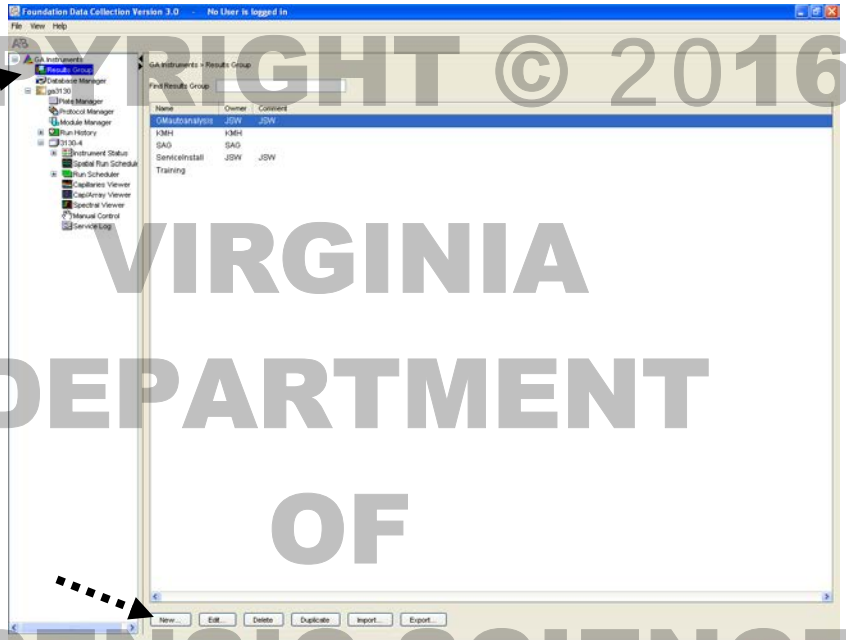

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Figure 6. Results Group listing. The solid arrow points to the appropriate selection on the menu at the left side of the screen. The dashed arrow (near bottom) points to the “New” button for creating a new results group, as described below.

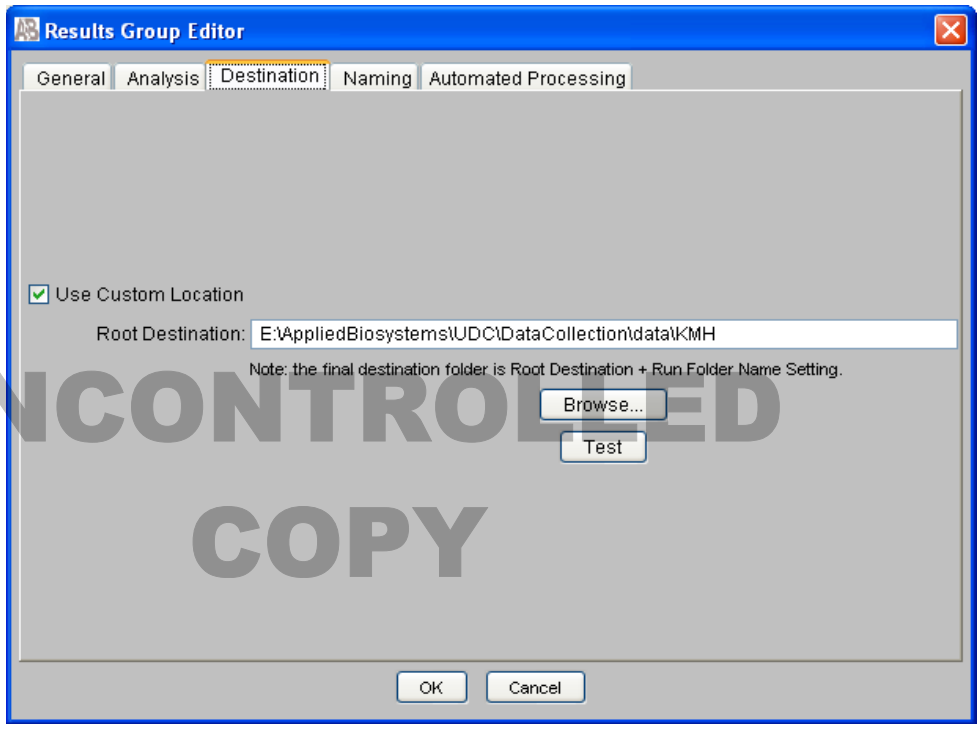

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Figure 7. Defining the destination for the Results Group folder.

3.3.1 A previously made results group can be exported from another computer and imported rather than creating a new results group, if desired. The import and export buttons are shown at the bottom of Figure 6.

3.3.1.1 Complete the general tab by entering a name for the results group and owner; the comment is not required

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3.3.1.2 Select the Analysis tab, choose “GeneMapper-3130xl_1” (or the name the instrument was assigned) as the analysis type, and ensure that Autoanalysis is not selected.

3.3.1.3 Select the Destination tab, and choose a destination folder for the results. The default location is: E:\AppliedBiosystems\udc\datacollection\Data\Folder Name. Click “Use Custom Location” and browse to a location by clicking the “Browse” button (Figure 7).

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3.3.1.4 Select the Naming tab to customize sample file and run folder names.

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3.3.1.4.1 Sample file names shall be formatted as: <WellPosition>_<SampleName>_<Plate Name>. Figure 8 shows how one would select this option in the Results Group Editor. A potential filename produced by the selection shown is: A01_AllelicLadder_2009-000.fsa

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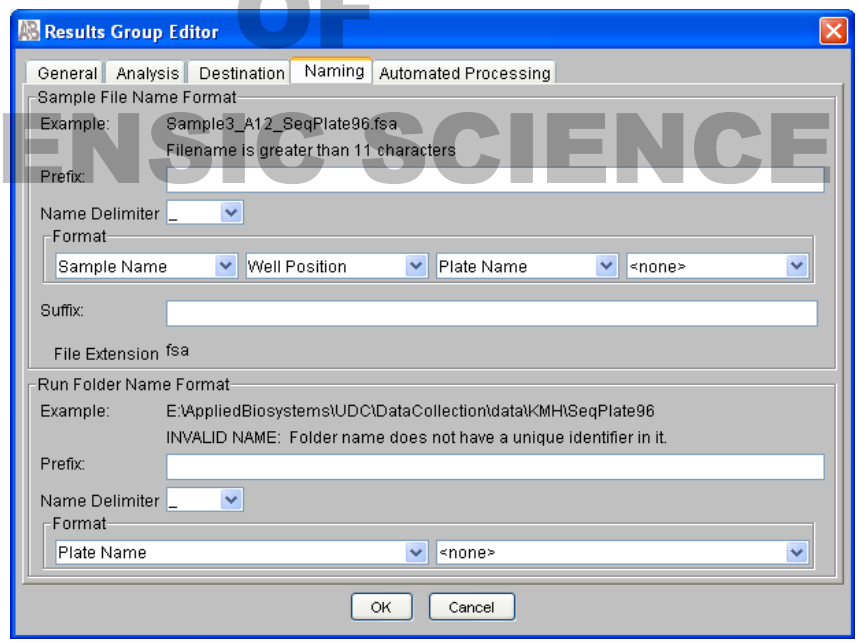

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Figure 8. Results Group Editor. An example of how run files and folders might be named is shown.

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3.3.1.4.2 In a similar manner, the run folder name format shall be formatted as: <Plate Name>.fsa. Figure 8 shows how one would select this option in the Results Group Editor. An example of a run folder name is: 2009-000.fsa

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3.3.1.5 Select the Automated Processing tab and confirm that “only when the results group is complete” is selected.

3.3.1.6 Click on “OK” to save the Results Group. This results group will be used during the sample data input in the Plate Manager.

4 New Size Standard

4.1 In the GeneMapper™ ID software, choose “GeneMapper Manager” in the “Tools” pull-down menu. The GeneMapper Manager dialog box appears.

4.2 Choose the “Size Standards” tab, as shown in Figure 9.

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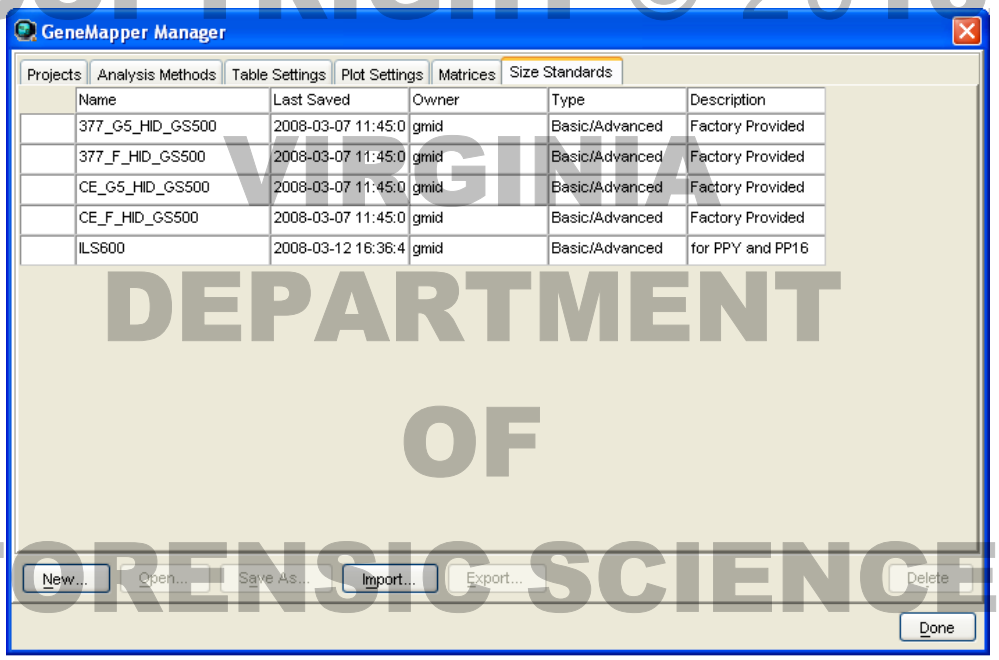

Figure 9. GeneMapper Manager. Size Standards tab for creating new or editing an already existing size standard.

4.3 Choose “New…” (near bottom of dialog box) to create a new size standard.

4.3.1 Alternatively, a previously made size standard can be imported by choosing “Import…”

4.4 In the dialog box that opens, choose “Basic” or “Advanced” and click OK.

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4.5 In the Size Standard Editor dialog box that opens, name the new size standard "ILS600" and enter the size standard dye (Red) and fragment sizes as shown in Figure 10.

Note: The 60 and 80 bp fragments may be included or excluded from the fragments listed. Either choice will not affect the accurate sizing of the Amelogenin alleles since both the X and Y are larger than the 100 bp fragment.

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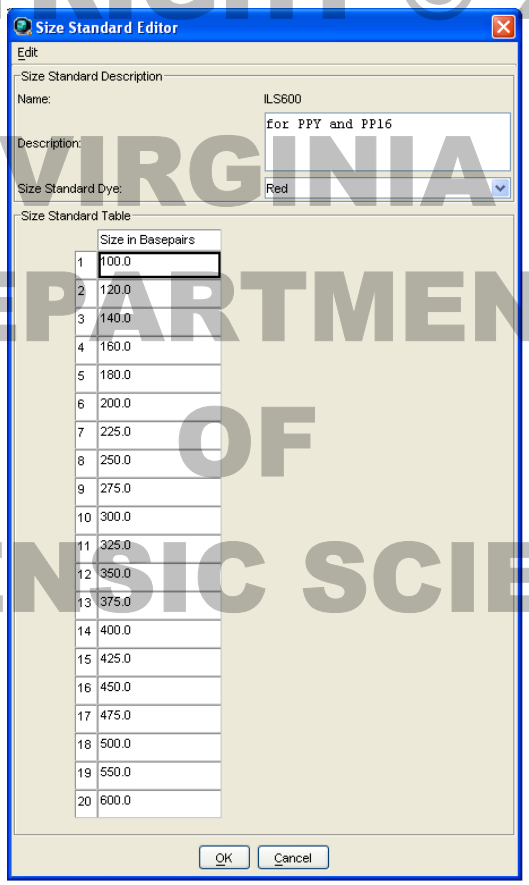

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Figure 10. Size Standard Editor. Settings shown are those utilized for the ILS600.

5 Analysis Method Editor

5.1 In the GeneMapper Manager, select the "Analysis Methods" tab and click "New". In the dialog box that opens, choose "HD" and click OK. Give it the name, "PowerPlex16advanced". In the GeneMapper™ ID software, select Analysis>Analysis Method Editor.

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5.2 Click on the "Peak Detector" tab.

5.3 Ensure that all selections are properly designated, as indicated in Figure 11. The size calling method used for the PowerPlex® 16 STR kit is the Local Southern method.

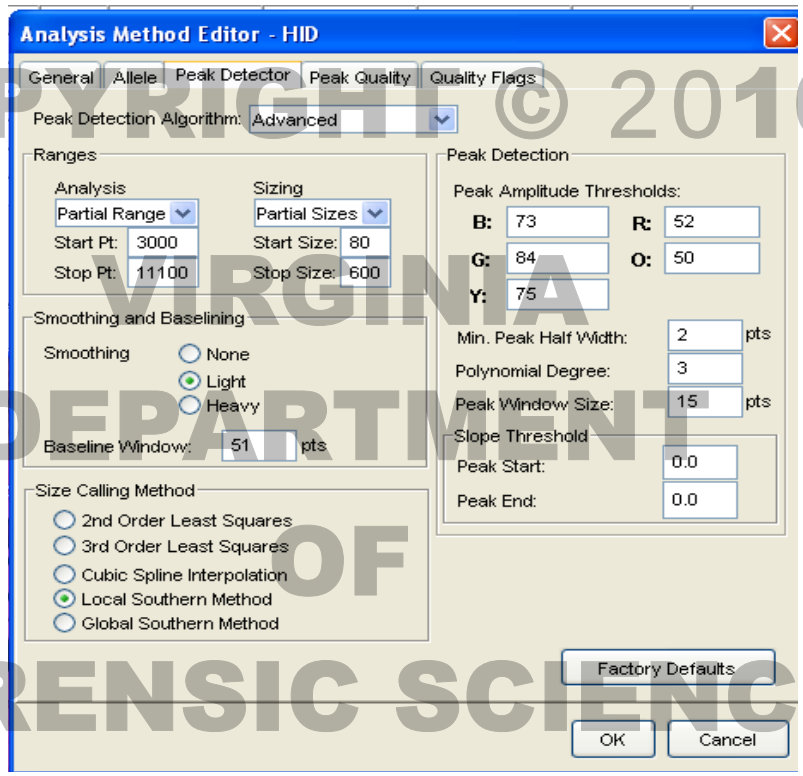

Figure 11. Peak detector tab of the Analysis Method Editor. Only the analysis range and threshold for the red channel may be altered during data analysis.

5.3.1 The analysis range starting and stopping points may be altered on a run-by-run basis to accommodate migration times that may vary between injections. Typically, a starting point of 3000 and stopping point of 10,000 is appropriate.

5.3.2 The values for the peak amplitude thresholds are based upon empirical data derived from the limit of detection analysis performed during the validation work and specific for each 3130*xl* instrument. While the ILS 600 (which uses the red dye) will have its default peak amplitude threshold set based on the LOD analysis, the threshold may be adusted upward, high enough to prevent detection of a known artifact present in the Promega ILS 600 size standard (Figure 4), or reduced when the ILS 600 peaks may fall below the default threshold (this can occasionally occur with the 2 second injection). It is important to note that ONLY the Red dye (ILS 600 size standard) may be adjusted if such conditions are present.

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5.4 The settings under the "Allele" tab should remain as shown in Figure 12. The Bin Set is defined as "Promega_Bins_ID3.2.0" and the marker specific stutter ratio is used.

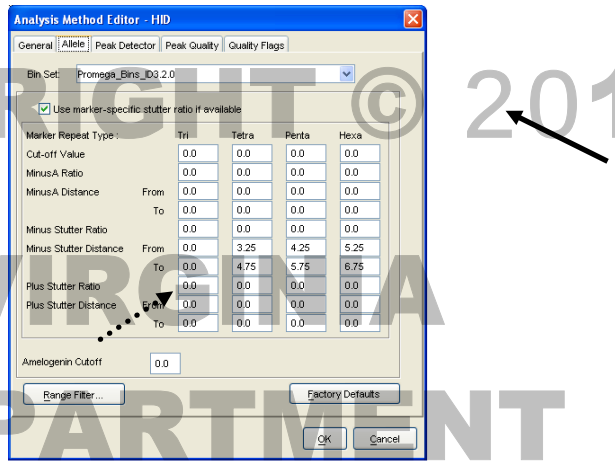

Figure 12. Analysis Method Editor – Allele Tab. The solid arrow points to where the bin set is defined. The dashed arrow points to where the stutter distances are defined.

5.5 The default settings for the Peak Quality and Quality Flags tabs are used, as shown in Figures 13 and 14.

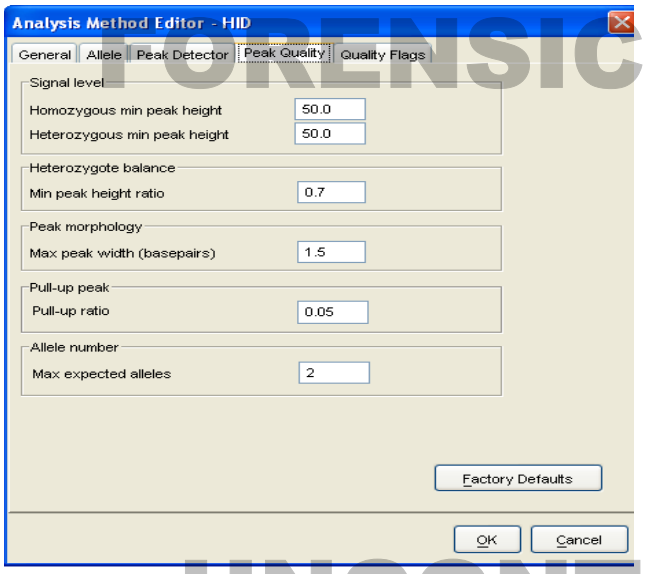

Figure 13. Analysis Method Editor – Peak Quality Tab

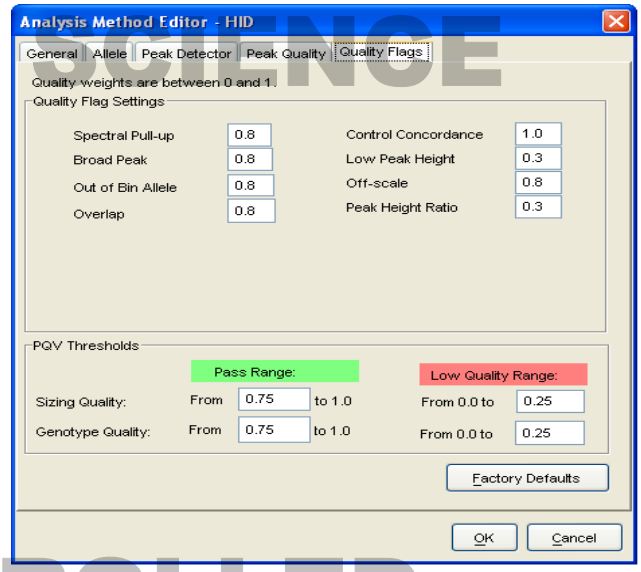

Figure 14. Analysis Method Editor –Quality Flags Tab

5.5.1 Verify that the settings are the same as those shown in Figures 11 and 12, then click OK.

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6 Panels and Bins

6.1 Panels and bins must be created for allele calling of PowerPlex 16 data. In the GeneMapper® ID software, navigate to “Tools” > “Panel Manager”. In the tree pane view on the left side of the dialog box (shown in Figure 15), choose “Panel Manager”.

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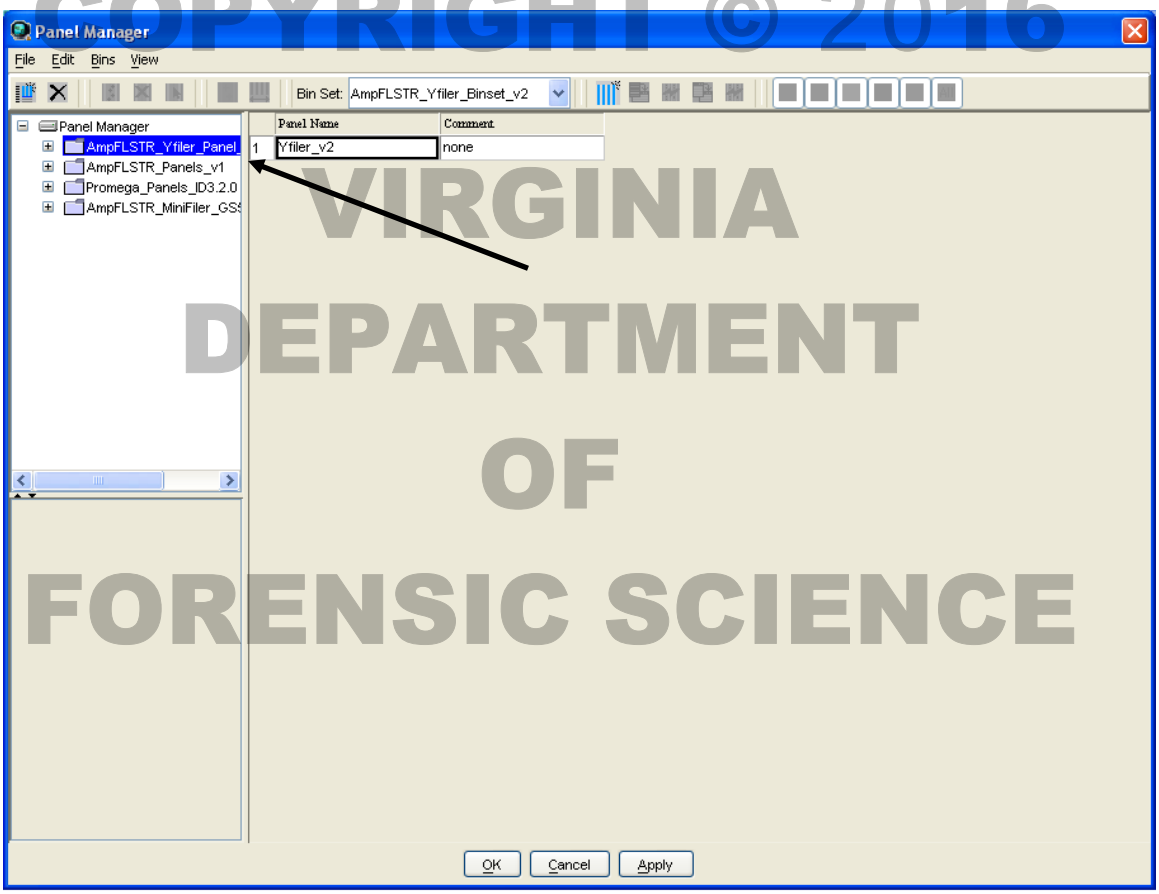

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Figure 15. Panel Manager.

6.2 Choose “File”>”Import Panels” and browse to the file on CD containing the panels for the PowerPlex 16 kit. Note: the panels need to be obtained from Promega, as they are not installed on the computer by Applied Biosystems.

6.3 Navigate to “Promega_Panels_ID3.2.0” of the treepane view (at the left side of the screen), choose “File”>”Import Bin Set” and browse to the Promega bins file, typically entitled “Promega_Bins_ID3.2.0”.

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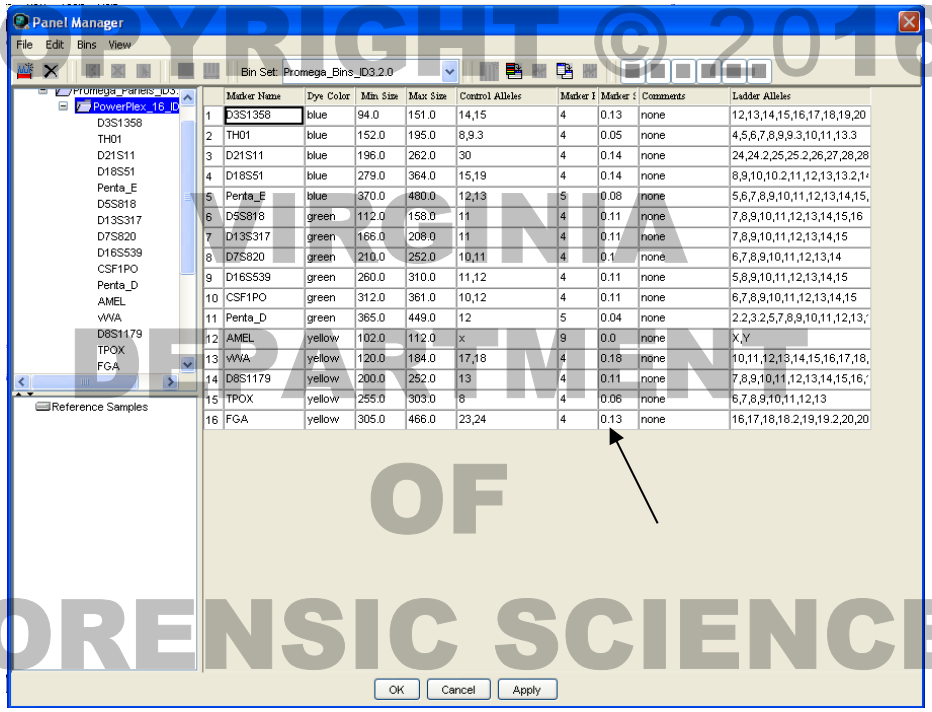
6.4 Navigate to “Promega_Panels_IS3.2.0”> “PowerPlex_16_ID3.2.0” of the treepane view (at the left side of the screen). The resulting PowerPlex® 16 panel/bin set is shown in Figure 16. The marker specific stutter ratios must be updated to reflect the validation data obtained at VDFS. The new stutter ratios should be edited to reflect those shown in Figure 16. Click OK after completing.



	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker I	Marker S	Comments	Ladder Alleles
1	D3S1358	blue	94.0	151.0	14,15	4	0.13	none	12,13,14,15,16,17,18,19,20
2	TH01	blue	152.0	195.0	8,9.3	4	0.05	none	4,5,6,7,8,9,9.3,10,11,13.3
3	D21S11	blue	196.0	262.0	30	4	0.14	none	24,24.2,25,25.2,26,27,28,28
4	D18S51	blue	279.0	364.0	15,19	4	0.14	none	8,9,10,10.2,11,12,13,13.2,1
5	Penta_E	blue	370.0	480.0	12,13	5	0.08	none	5,6,7,8,9,10,11,12,13,14,15,
6	D5S818	green	112.0	158.0	11	4	0.11	none	7,8,9,10,11,12,13,14,15,16
7	D13S317	green	166.0	208.0	11	4	0.11	none	7,8,9,10,11,12,13,14,15
8	D7S820	green	210.0	252.0	10,11	4	0.1	none	6,7,8,9,10,11,12,13,14
9	D16S539	green	260.0	310.0	11,12	4	0.11	none	5,8,9,10,11,12,13,14,15
10	CSF1PO	green	312.0	361.0	10,12	4	0.11	none	6,7,8,9,10,11,12,13,14,15
11	Penta_D	green	365.0	449.0	12	5	0.04	none	2.2,3.2,5,7,8,9,10,11,12,13,
12	AMEL	yellow	102.0	112.0	x	9	0.0	none	X,Y
13	vWA	yellow	120.0	184.0	17,18	4	0.18	none	10,11,12,13,14,15,16,17,18,
14	D8S1179	yellow	200.0	252.0	13	4	0.11	none	7,8,9,10,11,12,13,14,15,16,
15	TPOX	yellow	255.0	303.0	8	4	0.06	none	6,7,8,9,10,11,12,13
16	FGA	yellow	305.0	466.0	23,24	4	0.13	none	16,17,18,18.2,19,19.2,20,20

Figure 16. Panel/bin set for PowerPlex® 16. An arrow indicated the column of marker specific stutter ratios that must be edited to reflect the validation data obtained at VDFS. The marker-specific stutter ratios shown here are the values obtained at VDFS.

7 Plot Settings

7.1 In GeneMapper® ID, navigate to “Tools”>”GeneMapper Manager”. In the tabs near the top of the GeneMapper Manager dialog box, choose “Plot Settings” to create custom plot settings. Two different custom plot settings will be created: “Samples” and “Allelic Ladders”.

7.2 Create a new plot setting by clicking “New” and the “Plot Settings Editor” will appear. In the “General” tab, type a name for the plot settings. In this instance, choose “Samples”.

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7.3 Figures 17 – 21 show the various tabs of the plot settings editor. Verify that the plot settings match those in the Figures below.

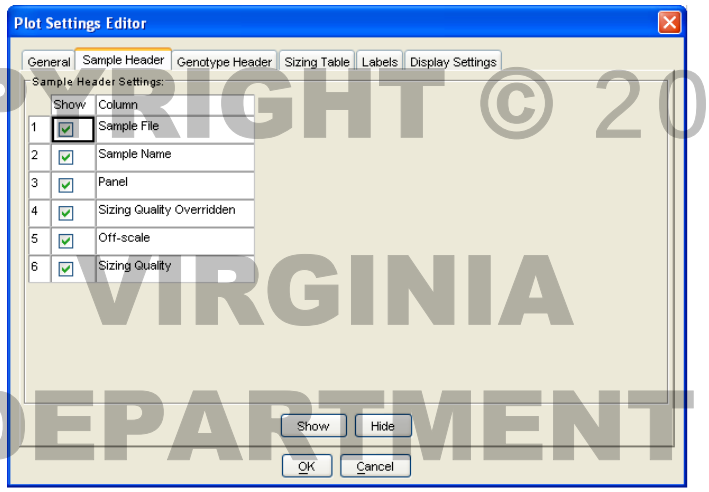
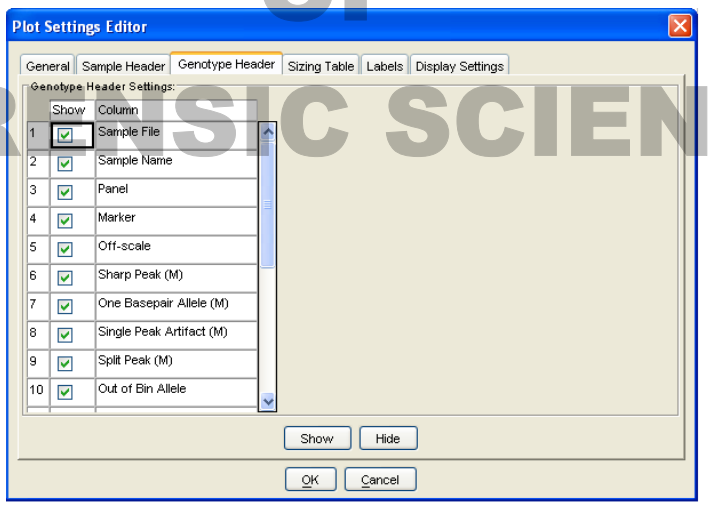


Figure 17. Plot Settings Editor: Sample Header.

A



B

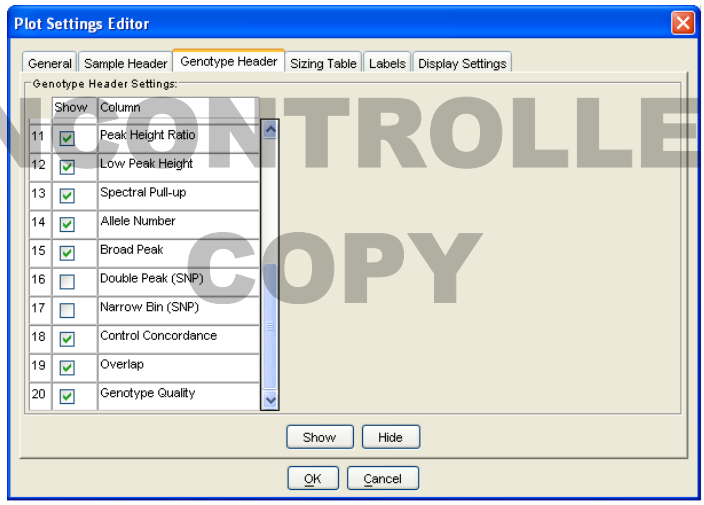


Figure 18. Plot Settings Editor: Genotype Header.

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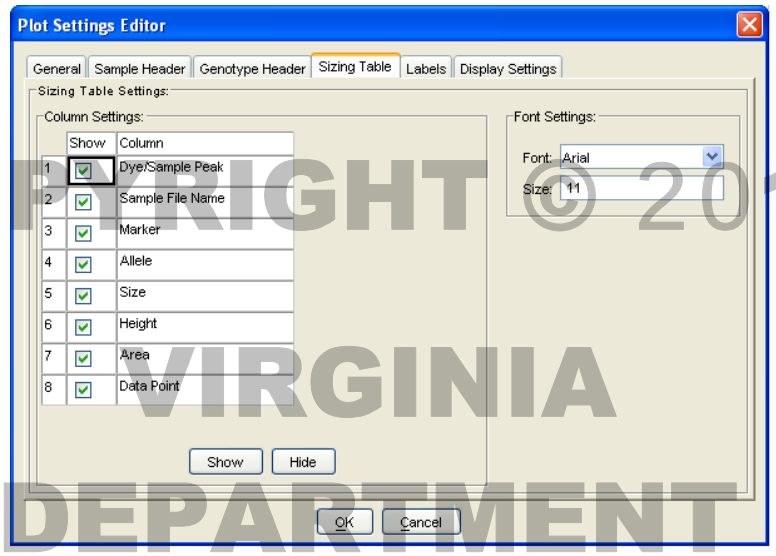

Figure 19. Plot Settings Editor: Sizing Table.

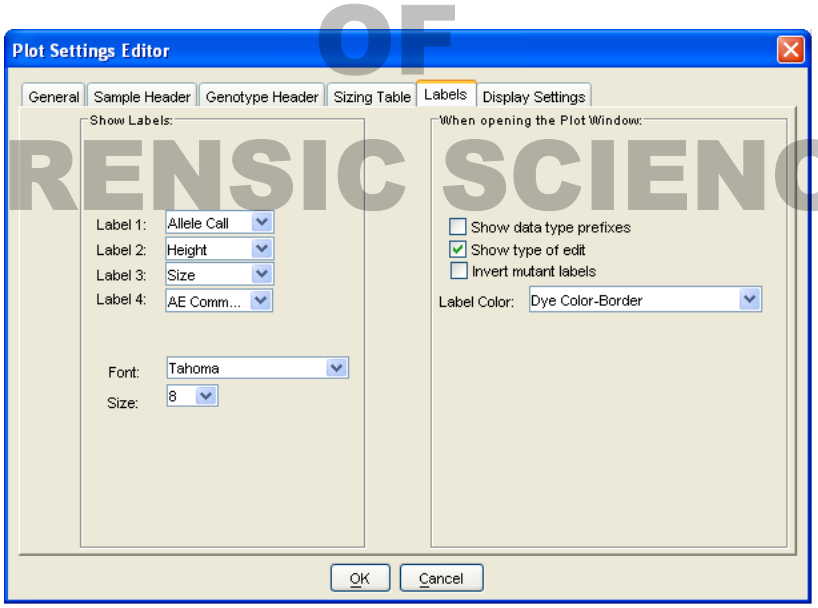

Figure 20. Plot Settings Editor: Labels. Note: these label settings are specific to the "Samples" plot settings. A second plot settings file will be created for allelic ladders in which the allele labels are different.

NOTE: Labels may be adjusted by an examiner based on his or her preferences.

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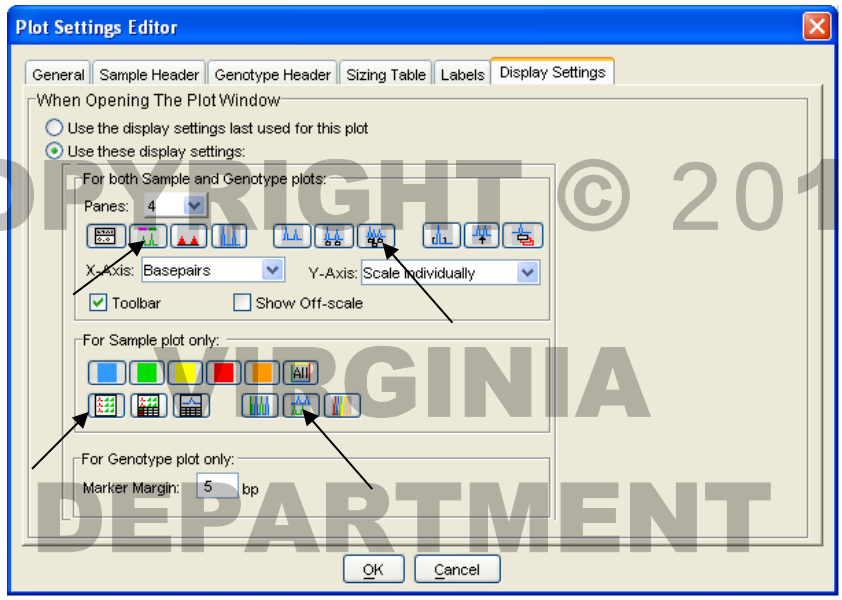

Figure 21. Plot Settings Editor: Display Settings. The arrows indicate the icons that must be clicked.

7.3.1 Note that many of the settings in Figure 21 are difficult to discern from the figure because they are toggle switches. The icons to which an arrow is pointing must be clicked (once) for the appropriate settings. The number of panes may be set at the analyst's discretion, but this is typically 2.

7.4 Click OK.

7.5 A second Plot Setting shall be created for ease of printing allelic ladders. Create a new plot setting by clicking "New" and the "Plot Settings Editor" will appear. In the "General" tab, type a name for the plot settings. In this instance, choose "Allelic Ladders".

7.6 Figures 17 – 21 show the various tabs of the plot settings editor. Verify that the plot settings match those in the Figures. The only difference between the allelic ladder and samples plot settings is the allele label. For allelic ladders, only the allele call is used, as indicated in Figure 22.

7.7 Choose OK to close and save the plot settings and Done to close GeneMapper Manager.

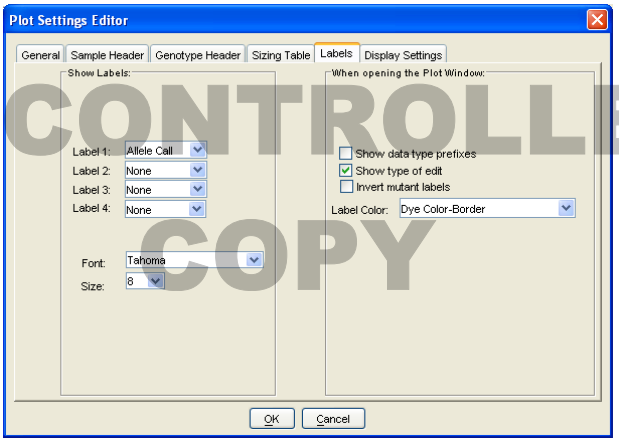

Figure 22. Plot Settings Editor: Labels. Note that these label settings are specific to the "Allelic Ladders" plot settings.

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