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Department of Forensic Science

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

FORENSIC BIOLOGY SECTION PROCEDURES MANUAL

SECTION III

FLUORESCENT DETECTION PCR-BASED STR DNA PROTOCOL: POWERPLEX® 16 BIO 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 theses 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 [4, 7]. 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 [8]. Amplified alleles containing length variations, such as short tandem repeats (STR's) and amplified fragment length polymorphisms (AMP-FLPs) may be typed by gel electrophoresis.

STR loci consist of short, repetitive sequence elements of 3 to 7 base pairs in length [3]. 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. The PowerPlex® 16 BIO System provide 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[1,5] or VNTR [2,6] analysis.

The PowerPlex® 16 BIO System allows the co-amplification and three-color detection of sixteen loci (fifteen STR loci and Amelogenin). The PowerPlex® 16 BIO 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, one primer specific for Penta E, D18S51, D21S11, TH01, and D3S1358 is labeled with fluorescein (FL); one primer specific for FGA, TPOX, D8S1179, vWA, and Amelogenin is labeled with Rhodamine Red™ – X (RRX); and one primer is specific for Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818 is labeled with 6-carboxy-4',5'-dichloro 2',7' – dimethoxyfluorescein (JOE). All sixteen loci are amplified simultaneously in a single tube and analyzed in a single gel lane [9].

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9. Promega PowerPlex® 16 BIO System Manual, Version 9/01

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1 ISOLATION OF DNA

When the DNA examiner has completed his/her analysis, all remaining evidence samples, including extracted DNA which has been dried down using a DNA concentrator/evaporator (Refer to Appendix J, Procedure For Drying Down And Resolubilizing Extracted DNA) or other suitable methods, will be returned to the submitting agency. However, amplified DNA samples WILL NOT be returned to the submitting agency, but instead will be discarded in the designated area to prevent possible transfer of amplified DNA to the remaining evidence samples. If the biological sample deposited on the evidence is consumed during the analysis then the extracted DNA sample and the cutting (in separate tubes) will be returned to the submitting agency with the evidence. Proper case file documentation and chain of custody documentation for these samples must be maintained.

DNA may be extracted from bloodstains, sperm cells, buccal cells, hair, tissue, bone, and other samples. Slightly different extraction procedures are required for each type of specimen and therefore are outlined in this chapter.

It is important to handle all samples aseptically to prevent contamination by extraneous DNA. It is also important to prepare evidence samples at a separate time and/or space from reference samples to prevent possible cross-contamination.

> **NOTE**: For tracking purposes the samples will be listed on the worksheet in the order in which they were processed/handled. The samples will be maintained in the same order throughout the rest of the analysis until the typing gel step. All samples will be processed in accordance with the procedures and policies outlined in the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section I, General Documentation and Evidence Handling Requirements, Chapter 3, Contamination Prevention and Detection Procedures, and Section VI, Quality Assurance Program DNA Typing of Biological Materials, Chapter 6, Dedicated PCR Facilities.

Special Precautions:

- Manual DNA extraction and manual PCR setup of evidence samples will be performed at a separate time or space from the manual DNA extraction and manual PCR setup of reference samples. This helps to prevent potential cross-contamination between evidence samples and reference samples.

- Manual DNA extraction from samples containing high levels of DNA (for example, tissue) will be performed after samples expected to contain low levels of DNA (single hairs, small bloodstains, etc.) to minimize the potential for sample-to-sample contamination.

- Disposable gloves will be used at all times. Gloves will be changed frequently to avoid sample-to-sample contamination with DNA and whenever moving between work areas. Gloves will be changed if suspected direct contamination has occurred from the sample DNA.

- To minimize transferring DNA to the disposable gloves a clean Kimwipe will be used to open each microcentrifuge/amplification tube. If the evidence (i.e., stained area or the liquid from the cap of the tube) comes in contact with the disposable glove, change gloves before proceeding to the next stained area, item of evidence, or sample tube.

- Scissors and tweezers will be thoroughly cleaned with a 10% solution of bleach or a solution that will remove/degrade the DNA after cutting each item/stain. Subsequently use isopropanol to remove the residue left by the chemicals, using special care to remove all residue left on surfaces. A fresh scalpel blade may also be used to cut each item/stain.

- A clean cutting surface will be used for each piece of evidence.

- Disposable plugged pipette tips and microcentrifuge tubes will be used.

- Pipette tips will be changed between samples.

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- Reagents will be stored in small quantities to reduce the risk of possible contamination to the stock solution.
- To avoid splashing and minimize aerosols, all liquid will be centrifuged to the bottom of the closed tube before the tube is opened.
- Reagent blank controls will be included with each set of DNA extractions to check for the presence of contaminating DNA in the reagents.
- All work surfaces will be thoroughly cleaned with a 10% bleach solution or a solution that will remove/degrade the DNA. Subsequently use isopropanol to remove the residue left by the chemicals, using special care to remove all residue left on surfaces before setting up the DNA Extraction Work Area. Disposable bench paper will be used to prevent the accumulation of human DNA on permanent work surfaces.
- The quantity of samples handled during a single analysis will be limited to a manageable number. This precaution reduces the risk of sample mix-up and the potential for sample-to-sample contamination.
- A dedicated lab coat will be worn for pre-amplification sample handling when working in the DNA Extraction Work Area.
- A dedicated disposable lab coat will be worn when working with amplified DNA in the PCR Post Amplification Work Area. NOTE: the disposable lab coats are discarded in the post amplification room as necessary.
- Once the DNA has been purified, it will be stored in a refrigerator or freezer for long term storage.

1.1 Technical Notes

1.1.1 Stain extraction buffer lyses the red blood cells (erythrocytes) and helps to remove the DNA from other cellular components.

1.1.2 Sodium dodecyl sulfate (SDS) serves to rupture the white blood cell (leukocyte) nuclear membrane to expose the nucleic acids. It also assists in the denaturation of the nuclear proteins which are attached to the DNA.

1.1.3 Proteinase K (Pro K) is a proteolytic enzyme that reduces proteins to their constituent amino acids. In particular, Pro K removes the histone groups that keep the DNA tightly bound within the cell. The enzymatic activity of Pro K lasts for approximately 2 hours and eventually, it will self-digest.

1.1.4 EDTA is a component of the reagents used in the lysis process which inhibits nuclease activity.

1.1.5 Dithiothreitol (DTT) reduces disulfide bonds that maintain the integrity of the sperm head. Sperm heads do not readily lyse in the absence of heat (56^0C) or DTT.

1.1.6 A differential lysis is included in the procedure for the analysis of mixed stains containing semen and other biological fluid. It includes what is referred to as a sperm and a non-sperm fraction. The non-sperm fraction is contained in the aqueous portion remaining after a gentle lysis treatment of the stain. A more rigorous treatment is conducted for the pelleted material (generally sperm); this is referred to as the sperm fraction.

1.1.7 The DNA IQ™ System is designed to rapidly purify small quantities of DNA, approximately 100 ng or less, and becomes more efficient with samples containing less then 50 ng of DNA.

1.1.8 The DNA IQ™ Lysis buffer contains Guanidine Thiocyanate (GTC), which is a chaotropic agent used to attach the DNA to a silica-coated bead.

1.1.9 The QIAamp® extraction procedure uses spin columns to extract/purify DNA from buccal samples and dried blood stains. The QIAGEN® AL lysis buffer, included in the QIAamp® extraction kit, is a

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guanidine-based buffer. The guanidine helps to set up the binding conditions needed for the DNA to adhere to the spin column membrane. The QIAGEN® AL lysis buffer also contains a detergent to rupture leukocyte nuclear membranes which exposes the nucleic acids.

1.1.10 The QIAGEN® protease is similar to, but less stringent than, the above mentioned ProK, and serves the same purpose for the breakdown of proteins into their constituent amino acids. The DNA yield reaches a maximum after lysis for 10 min at 56^0 C. Longer incubation times have no effect on the overall yield of the purified DNA.

1.1.11 The QIAGEN® AW1 wash buffer is an ethanol-based stringent wash solution containing a low concentration of guanidine. This wash step removes any non-specific binding to the spin column membrane.

1.1.12 The QIAGEN® AW2 wash buffer is a Tris-based solution containing ethanol which will wash away any salts that are present.

1.1.13 The QIAGEN® AE elution buffer is a Tris-EDTA solution which elutes the DNA attached to the membrane and serves as a stable storage medium.

1.1.14 A random sample will be run with each set of convicted offender and arrestee sample extractions to serve as a verification that the samples are successfully being entered into Combined DNA Index System (CODIS) and the search algorithm is working properly. This sample serves as an internal laboratory control since the DNA profile is not known to the Data Bank analyst and must be verified by the Forensic Biology Program Manager or designee prior to the sizing data being considered acceptable. If a sample must be re-extracted a new random sample must be extracted along with the sample.

1.1.15 Routinely the organic or DNA IQ™ extraction methods are used to isolate DNA from blood/buccal samples obtained in criminal cases and the QIAGEN® BioRobot™ 9604 is used to isolate DNA from convicted offenders blood/buccal or arrestee buccal samples in accordance with the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Manual, Section V - QIAGEN® BioRobot™ 9604 Procedure Manual. However, when necessary convicted offender blood/buccal and arrestee buccal samples may also be extracted using the Organic Extraction Method for Bloodstains and Tissue, the DNA IQ™ Extraction Method For Buccal Cell Type Samples and Bloodstains, the QIAamp® Extraction Procedure, or the QIAamp® 96 Well Extraction Procedure.

1.1.16 The organic, QIAGEN®, and DNA IQ™ Extraction methods will isolate DNA from both human and non-human DNA. However, only the higher primate specific DNA will be quantitated and amplified during the subsequent processing steps.

1.2 Equipment

1.2.1 Heat block or incubator, 37^0 C

1.2.2 Heat block or incubator, 56^0 C

1.2.3 Microcentrifuge

1.2.4 Vortex mixer

1.2.5 Tweezers

1.2.6 Microcentrifuge tube rack

1.2.7 Pipettes - 10 µL, 20 µL, 100 µL, 200 µL, 1000 µL

1.2.8 8 – Channel Pipette – Range 0.5 µL to 10 µL (for QIAamp® extraction procedure)

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1.2.9 Refrigerator/Freezer

1.2.10 Scalpel and blades (for DNA extraction from swabs or hair)

1.2.11 Chisel and hammer (for DNA extraction from bone)

1.2.12 Stereo microscope (for DNA extraction from hair)

1.2.13 Biological Safety Hood

1.2.14 Beaker, 50 mL (for DNA extraction from hair)

1.2.15 Mortar and Pestle (for DNA extraction from bone and teeth)

1.2.16 Incubator, 70^0C (for QIAamp® extraction procedure)

1.2.17 Hole punch (for QIAamp® extraction procedure from dried blood stains)

1.2.18 Centrifuge, equipped with a microplate rotor (for QIAamp® extraction procedure)

1.2.19 Plate orientation adapter for centrifuge (for QIAamp® extraction procedure)

1.2.20 Acrylic block

1.2.21 High Speed Electric Drill (bone procedure)

1.2.22 Rotary shaft tool (tooth procedure - maintained in the firearms section)

1.2.23 1/8” or 9/64” drill bit (bone procedure)

1.3 Materials

1.3.1 Microcentrifuge tubes, 1.5 mL

1.3.2 Transfer pipettes

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

1.3.4 Microcentrifuge tube lids

1.3.5 Kimwipes

1.3.6 Conical tubes, 15 mL and 50 mL (for DNA extraction from bone)

1.3.7 Spin-Ease basket (Optional)

1.3.8 White/black paper (for DNA extraction from hair)

1.3.9 Weigh boats (for DNA extraction from bone)

1.3.10 Microscope slides (Optional)

1.3.11 Plastic ziploc bags

1.3.12 Gloves

1.3.13 QAamp® spin columns

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1.3.14 QIAamp® 2-mL collection tubes

1.3.15 Parafilm

1.3.16 Round well block (supplied with QIAamp 96 DNA Blood Kit)

1.3.17 8-strip caps for use with the round well block (supplied with QIAamp 96 DNA Blood Kit)

1.3.18 Microplate adhesive tape (supplied with QIAamp 96 DNA Blood Kit)

1.3.19 Airpore tape (supplied with QIAamp 96 DNA Blood Kit)

1.3.20 QIAamp 96 plate (supplied with QIAamp 96 DNA Blood Kit)

1.3.21 S-block (supplied with QIAamp 96 DNA Blood Kit)

1.3.22 13/16" diamond mini cutting disc ("Diamond Life" brand – tooth procedure)

1.3.23 Collection tube rack (supplied with QIAamp 96 DNA Blood Kit)

1.4 Reagents

1.4.1 Stain extraction buffer

1.4.2 Proteinase K - 20 mg/mL (Keep on ice.)

1.4.3 TNE

1.4.4 20% Sarkosyl

1.4.5 0.39*M* Dithiothreitol (DTT)

1.4.6 Sterile Type I Water

1.4.7 PCR Digestion Buffer

1.4.8 10% Household bleach (for DNA extraction from bone)

1.4.9 70% Ethanol (for DNA extraction from bone)

1.4.10 Isopropyl Alcohol

1.4.11 95% Ethanol/Reagent Grade (for DNA extraction from bone and use with the QIAamp® blood extraction procedure)

1.4.12 Liquid Nitrogen (for DNA extraction from bone and teeth)

1.4.13 1X phosphate-buffered saline (PBS), pH 7.2 (for DNA extraction from blood/buccal samples)

1.4.14 QIAGEN® protease (for DNA extraction from blood/buccal samples)

1.4.15 QIAGEN® AL lysis buffer (for DNA extraction from blood/buccal samples)

1.4.16 QIAGEN® AW1 wash buffer (for DNA extraction from blood/buccal samples)

1.4.17 QIAGEN® AW2 wash buffer (for DNA extraction from blood/buccal samples)

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1.4.18 QAGEN® AE elution buffer (for DNA extraction from blood/buccal samples)

1.4.19 DNA IQ™ Lysis Buffer

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1.5 Organic Extraction Method for Bloodstains and Tissue Samples

1.5.1 Cut an approximate 3 mm^2 bloodstain/buccal sample and place into a labeled 1.5 mL microcentrifuge tube with a depression in the lid. If a stain is smeared over a large area of the fabric, more than one microcentrifuge tube may be needed to extract the stain. If the evidential sample consists of tissue, cut a very thin slice of tissue (i.e. 1 mm thick slice of muscle) and place the sample into a 1.5 mL labeled microcentrifuge tube. Alternatively, cellular material may be collected from the tissue by swabbing the sample and placing a portion of the swab into a labeled 1.5 mL microcentrifuge tube. If the evidential sample is a blood flake place sample directly into a 1.5 mL labeled microcentrifuge tube.

NOTE: Process a reagent blank along with each set of samples.

1.5.2 Add 400 µL of stain extraction buffer and 10 µL of Proteinase K to saturate the cutting. If a larger cutting is used, add proportional amounts of stain extraction buffer and Proteinase K until the cutting is saturated.

1.5.3 Mix by hand or light vortexing and pulse spin to force the cutting into the liquid.

1.5.4 Place the tube into a 56^0 C incubator or heat block for a minimum of 2 hours.

NOTE: If the sample has been preserved in Formaldehyde or Formalin add a second 10 µL aliquot of Proteinase K after the 2 hour incubation, then place the tube into a 56 ^oC incubator or heat block for an additional 2 hours.

1.5.5 Pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. Alternatively, a Spin-Ease basket may be used instead of placing the cutting in the lid of the tube. If the cutting is small in size and contains a concentrated blood stain, the cutting may be left in the tube.

1.5.6 Remove the lid containing the cutting and discard. (If the entire biological sample deposited on the evidence was consumed the cutting will be returned with the evidence). Place a new lid on the tube containing the liquid.

1.5.7 Proceed to Section 2.5 Microcon® Purification Procedure

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1.6 Organic Extraction Method for Hair

1.6.1 Using clean tweezers, place the hair on a clean piece of white or black paper, whichever is appropriate, and examine under a stereo microscope for the presence of sheath material. Note the presence of any body fluids on the hair.

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1.6.2 Wash the hair to reduce surface dirt and contaminants by immersing the hair in sterile Type I Water in a clean 50 mL beaker. If the hair contains a biological fluid that is important to the investigation DO NOT wash the hair.

1.6.3 Return the hair to the stereo microscope. Use a clean scalpel blade to cut a 0.5 to 1 cm portion from the root end of the hair and then place the hair root into a 1.5 mL microcentrifuge tube.

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1.6.4 Add 400 µL of stain extraction buffer and 10 µL of Proteinase K to saturate the hair sample.

1.6.5 Mix by hand or lightly vortex, then pulse spin the microcentrifuge tube to force the hair sample into the liquid.

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1.6.6 Place the tube into a 56ºC incubator or heat block overnight (18-24 hours).

1.6.7 Pulse spin the tube in a microcentrifuge for 10 seconds to force the condensate to the bottom of the tube.

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1.6.8 Proceed to Section 2.5 Microcon® Purification Procedure.

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1.7 Organic/DNA IQ™ Extraction Method for Mixed Body Fluid Stains (Differential Procedure)

OPTION: Mixed body fluid samples may also be extracted using the DNA IQ™ extraction method for mixed body fluid stains outlined in the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedures Manual, Section IV - BioMek® 2000 Automation Workstation Procedures Manual.

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1.7.1 Cut an approximate 3 mm^2 stain or a portion of a cotton swab proportional to the number of sperm identified. Place the stain into a labeled 1.5 mL microcentrifuge tube with a depression in the lid. If the stain is smeared over a large area on the fabric or the body fluids on a swab are weak and more than one swab must be used, more than one microcentrifuge tube may be needed to extract the stain.

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1.7.1.1 If a sample contains a weak smear over a large surface area the sample should be placed in several 1.5 mL microcentrifuge tubes and the entire sample condensed into one sample tube during the purification step, Section 2.5 Microcon® Purification Procedure or Section 2.6 DNA IQ™ Purification Procedure.

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NOTE: Process a reagent blank along with each set of samples.

1.7.2 Add: 400 µL TNE
25 µL 20% Sarkosyl
75 µL Sterile Type I Water
5 µL Proteinase K
in proportional amounts to saturate the cutting.

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1.7.3 Mix by hand or light vortexing then pulse spin to force the cutting into the liquid

1.7.4 Place the tube into a 37^0C incubator or heat block for a minimum of 2 hours.

1.7.5 Pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. Alternatively, a Spin-Ease basket may be used instead of placing the cutting in the lid of the tube.

1.7.6 Using a pipette, carefully transfer all but approximately 50 µL of the supernatant into a new 1.5 mL labeled tube with a lid. Be careful not to dislodge or disturb the pellet on the bottom of the tube. The supernatant removed from the pellet is the NON-SPERM FRACTION.

1.7.7 At this stage set the non-sperm fraction tube aside and wait until the sperm fraction is ready, then proceed to Section 2.5, Microcon® Purification Procedure or Section 2.6, DNA IQ™ Purification Procedure.

NOTE: If necessary the sample(s) may be capped and left at room temperature overnight before proceeding.

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1.7.8 Remove and discard the old lid and cutting from the original tube containing the pellet. (If the entire biological sample deposited on the evidence was consumed the cutting will be returned with the evidence). Place a new colored lid on the tube. This tube contains the SPERM FRACTION.

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1.7.9 Wash the pellet as follows: Resuspend the pellet in 500 µL of PCR digestion buffer by vortexing briefly. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm. Using a pipette with a sterile 1 mL pipette tip, remove all but 50 µL of the supernatant and discard.

1.7.10 Repeat the wash in step 1.7.9 an additional 2 times. If a low sperm count has been determined, the sperm pellet may be washed up to 5 times. If the SPERM FRACTION will be purified using the DNA IQ™ isolation method, proceed to Section 2.6, DNA IQ™ Purification Procedure with both the sperm and non-sperm fractions, otherwise proceed to step 1.7.11.

NOTE: If necessary the sample(s) once capped can remain at room temperature overnight before proceeding.

OPTION: If a sperm search has not previously been conducted, remove 3 µL of the supernatant and spot the sample onto a glass microscope slide for examination.

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1.7.11 Add to each sperm fraction or reagent blank tube:

150 µL TNE
50 µL 20% Sarkosyl
40 µL 0.39*M* DTT
150 µL Sterile Type I Water
10 µL Proteinase K

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1.7.12 Mix by hand or light vortexing then place the tube into a 56^0 C incubator or heat block for 2-3 hours.

1.7.13 Pulse spin the tube.

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1.7.14 Proceed to Section 2.5, Microcon® Purification Procedure, with both the sperm and non-sperm fractions.

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1.8 Organic/DNA IQ Extraction Method for Isolating DNA from Heat Fixed and Permounted Slides/Smears (Differential Procedure)

NOTE: If the slide/smear has not been heat fix and permounted, skip to step 1.8.5.

1.8.1 Place the stained, fixed and permounted smear/slide into a clean glass Petri dish with a cover.

1.8.2 Pour a sufficient volume of xylene over the smear/slide until completely submerged.

1.8.3 Incubate the smear/slide overnight at room temperature. If the coverslip does not float off easily, continue to soak the smear/slide in xylene until the coverslip can be removed easily. NOTE: smears/slides that have been mounted with an excess amount of permount may need to be incubated in the xylene solution of as much as 5 days.

1.8.4 Once the coverslip floats off, remove the smear/slide from the Petri dish and air dry for a minimum of 5 minutes. DO NOT DISCARD THE COVERSLIP.

1.8.5 Scrape the cellular material off the smear/slide with a clean, unused scalpel or razor blade and place it into a labeled 1.5 mL microcentrifuge tube.

1.8.6 Remove half the cotton from a sterile cotton tipped swab. Wet the remaining portion of the swab attached to the stick slightly with sterile water and then use the stick and swab to scrape smear/slide to remove any remaining cellular material.

1.8.7 Remove the cotton from the stick and place it into the 1.5 mL microcentrifuge tube with the respective scrapings from the smear/slide or break off the portion of the stick with the swab attached and place into the 1.5 mL tube.

1.8.8 Retain the smear/slide along with the coverslip until it is certain that DNA has successfully been obtained. Cells can stick to both the slide and the coverslip.

NOTE: Process a reagent blank along with each set of samples

1.8.9 To the 1.5 mL tube add:

400 µL TNE
25 µL 20% Sarkosyl
75 µL Sterile Type I Water
5 µL Proteinase K

in proportional amounts to saturate the cutting.

1.8.10 Mix by hand or light vortexing then pulse spin to force the cutting into the liquid.

1.8.11 Place the tube into a 37^0C incubator or heat block for a minimum of 2 hours.

1.8.12 Pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. Alternatively, a Spin-Ease basket may be used instead of placing the cutting in the lid of the tube.

1.8.13 Using a pipette, carefully transfer all but approximately 50 µL of the supernatant into a new 1.5 mL labeled tube with a lid. Be careful not to dislodge or disturb the pellet on the bottom of the tube. The supernatant removed from the pellet is the NON-SPERM FRACTION.

1.8.14 At this stage set the non-sperm fraction tube aside and wait until the sperm fraction is ready, then proceed to Section 2.5, Microcon® Purification Procedure or Section 2.6, DNA IQ™ Purification Procedure.

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NOTE: If necessary the sample(s) may be capped and left at room temperature overnight before proceeding.

1.8.15 Remove and discard the old lid and cutting from the original tube containing the pellet. (If the entire biological sample deposited on the evidence was consumed the cutting will be returned with the evidence). Place a new colored lid on the tube. This tube contains the SPERM FRACTION.

1.8.16 Wash the pellet as follows: Resuspend the pellet in 500 µL of PCR digestion buffer by vortexing briefly. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm. Using a pipette with a sterile 1 mL pipette tip, remove all but 50 µL of the supernatant and discard.

1.8.17 Repeat the wash in step 1.8.16 an additional 2 times. If a low sperm count has been determined, the sperm pellet may be washed up to 5 times. If the SPERM FRACTION will be purified using the DNA IQ™ isolation method, proceed to Section 2.6, DNA IQ™ Purification Procedure with both the sperm and non-sperm fractions, otherwise proceed to step 1.8.18.

NOTE: If necessary the sample(s) once capped can remain at room temperature overnight before proceeding.

OPTION: If a sperm search has not previously been conducted, remove 3 µL of the supernatant and spot the sample onto a glass microscope slide for examination.

1.8.18 Add to each sperm fraction or reagent blank tube:

150 µL TNE
50 µL 20% Sarkosyl
40 µL 0.39*M* DTT
150 µL Sterile Type I Water
10 µL Proteinase K

1.8.19 Mix by hand or light vortexing then place the tube into a 56 °C incubator or heat block for 2-3 hours

1.8.20 Pulse spin the tube

1.8.21 Proceed to Section 2.5, Microcon® Purification Procedure, with both the sperm and non-sperm fractions.

References:

1. Dimo-Simonin, N., Grange, F., Brandt-Casadevall, C., PCR-based forensic testing of DNA from stained cytological smears. J. Forensic Sci. 1997:42(3):506-509.

2. Gall, K., Pavicic, D., Pavelic, J., Audy-Jurkovic, S., Pavelic, K., PCR amplification of DNA from stained cytological smears. J. Clin. Pathol. 1993;46:378-379.

3. Pavelic, J., Gall-Troselj, K., Bosnar, M.H., Kardum, M.M., and Pavelic, K., PCR amplification of DNA from archival specimens, a methological approach. Neoplasma, 1996;43(2):75-81.

4. Sweet, D., Hildebrand, D., Phillips, D., Identification of a skeleton using DNA from teeth and a PAP smear. J. Forensic Sci. 1999;44(3):630-633.

5. Poljak, M., Barlic, J., Rapid, simple method of extracting DNA from archival Papanicolaou-stained cervical smears. Acta Cytol. 1996;40:374-375.

6. Part 1: Protocol For Recovery of DNA From Biological Evidence Material, Section C: Collection of Cellular Material From Microscope Slides, Oakland Police Department Criminalistics Laboratory DNA Methods Manual, Page 1-2, June 20, 2005.

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1.9 Organic Extraction Method for Bone

1.9.1 Using a Kimwipe and 95% ethanol vigorously clean the outer surface of the bone sample. Repeat this step 2 –3 more time to remove any surface dirt or contaminates.

1.9.2 Place bone into a weigh boat or lay on a piece of clean butcher paper inside of a biological safety hood to dry.

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1.9.3 The bone sample (i.e., small in size) may be pulverized using liquid nitrogen (1.9.3.1) or an electric drill and bit (1.9.3.2) following the procedures listed below:

1.9.3.1 Pulverizing the bone sample using liquid nitrogen:

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1.9.3.1.1 Place the bone fragment(s) into a clean mortar then pour a small volume of liquid nitrogen over the top of the bone. Ensure there is a sufficient amount of liquid nitrogen in the mortar to cover the bone fragment(s).

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1.9.3.1.2 Allow the bone to sit in the liquid nitrogen for 30 to 60 seconds, then pour off the excess liquid nitrogen into a separate container. Using a pestle crush the bone fragments. Place a piece of parafilm over the top of the mortar and pestle to prevent the pulverized bone fragments from flying out of the mortar.

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1.9.3.1.3 Transfer a small portion of the pulverized bone to a 1.5 mL microcentrofuge tube. Proceed to step 1.9.4.

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1.9.3.2 Pulverizing the bone sample using a drill and bit(s):

1.9.3.2.1 Initially clean a 1/8” or 9/64” drill bit with 10% bleach and a Kimwipe followed by 95% ethanol.

1.9.3.2.2 Place the bone sample in a hood on a clean sheet of butcher paper.

1.9.3.2.3 While holding the bone sample firmly use an electric drill and either the cleaned 1/8” or 9/64” drill bit and drill a hole approximately 1.0 mm deep.

1.9.3.2.4 Tap the bone gently on the butcher paper to dislodge the surface bone powder. Discard the sheet of butcher paper and replace with a new sheet of butcher paper.

1.9.3.2.5 Clean the 1/8” or 9/64” drill bit with a Kimwipe and 95% ethanol.

1.9.3.2.6 Place the drill bit back into the hole in the bone and drill approximately 3-5 mm further into the bone.

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1.9.3.2.7 Tap the bone gently on the butcher paper to dislodge the bone powder onto the butcher paper.

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1.9.3.2.8 Transfer a sample of bone powder at minimum the size of a PEA to a clean 1.5 mL microcentrifuge tube. Proceed to step 1.9.4.

1.9.4 Add 1 mL of stain extraction buffer and 35 µL of Proteinase K to the pulverized/powder bone sample in proportional amounts to saturate the sample.

NOTE: Process a reagent blank along with each set of samples.

1.9.5 Place the tube into 56 °C heat block or incubator. After one hour of incubation, mix thoroughly and re-secure the cap of the tube. Continue to incubate the bone sample in the 56 °C heat block overnight.

1.9.6 Proceed to Section 2.5, Microcon® Purification Procedure

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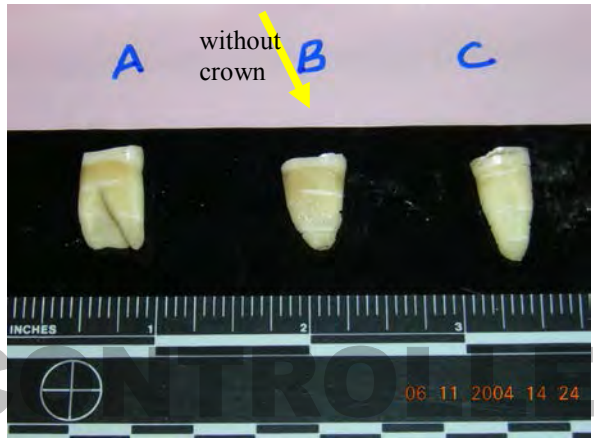
1.10 Organic Extraction Method for Teeth[1]

NOTE: Molars are the tooth of choice for DNA recovery.

1.10.1 Clean the outer surface of the tooth with a Kimwipe and 10% bleach (avoid introducing the bleach solution into any cracks in the tooth). Subsequently removed the 10% bleach residue using a Kimwipe and sterile water followed by isopropanol.

1.10.2 While working in a hood, using a sterile diamond saw blade and a rotary shaft tool cleaned with bleach and isopropanol, cut/grind away the upper crown portion of the tooth until the pulp chamber becomes visible. In addition, make small nicks in the sides of the tooth to facilitate crushing the lower portion of the tooth. Refer to the diagrams below.

NOTE: It will take between 5 and 10 minutes to remove the crown of the tooth.



1.10.3 To gain access to the pulp, place the tooth minus the crown into a small sterile ziploc plastic bag. Insert the ziploc plastic bag into a second ziploc bag. Ensure the ziploc bags are sealed.

1.10.4 Lay the ziploc plastic bag on a hard surface. Using a hammer pulverize the tooth sample inside of the ziploc plastic bag. Be careful not to puncture the ziploc plastic bag.

1.10.5 While holding the plastic bag with the ziploc portion of the plastic bag up, shake the sample to transfer the pulverized sample to the corner of the ziploc plastic bag. Open the ziploc plastic bags and transfer the pulverized/crushed tooth to a 1.5 mL microcentrifuge tube.

NOTE: Process a reagent blank along with each set of samples.

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1.10.6 Add 400 µL of stain extraction buffer and 10 µL of Proteinase K proportionally to the pulverized tooth sample.

1.10.7 Mix by hand or light vortexing and pulse spin to force the pulverized tooth sample into the liquid.

1.10.8 Place the tube into a 56°C incubator or heat block for a minimum of 2 hours.

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1.10.9 Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to force the pulverized tooth sample to the bottom of the tube.

1.10.10 Proceed to Section 2.5, Microcon® Purification Procedure

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Reference:

1. Gaytmenn, et.al. "Quantification of Forensic DNA from Various Regions of Human Teeth, JFS, Vol.48, No. 3, pp.622-625.

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1.11 DNA IQ™ Extraction Method for Buccal Cell Type Samples and Bloodstains

OPTION: Non-differential extractions may also be conducted using the DNA IQ™ extraction procedure outlined in the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedures Manual, Section IV - BioMek® 2000 Automation Workstation Procedures Manual.

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The portion size of the swab or bloodstain removed for DNA extraction should be judged based on a number of criteria, such as whether the stain appears dilute (for bloodstains) or if the sample may be heavily soiled or possibly degraded. Examine the bloodstain or buccal swab and remove a reasonable portion for DNA extraction. For example, only a 3 mm^2 section may be all that is necessary to remove from a heavily bloodstained item.

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NOTE: Process a reagent blank along with each set of samples.

1.11.1 Cut an approximate 3 mm^2 blood stain or other biological stain and place into a labeled 1.5 mL microcentrifuge tube with a depression in the lid. If a stain is smeared over a large area of fabric, more than one microcentrifuge tube may be needed to extract the stain. If the sample is a buccal swab, remove a small portion of the swab and place into a labeled microcentrifuge tube.

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NOTE: Prior to the addition of the DNA IQ™ Lysis buffer, DTT at the concentration described in the reagent preparation section (Appendix B) MUST be added.

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1.11.2 Add 250 uL of DNA IQ™ Lysis buffer. If the biological sample is dispersed over a large area of the substrate, add up to 325 uL of DNA IQ™ Lysis buffer to the microcentrifuge tube.

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1.11.3 Vortex vigorously for 20-30 seconds, then pulse spin to force the cutting into liquid.

1.11.4 Place the microcentrifuge tube into a 56ºC heat block for a minimum of 30 minutes. If the sample is deposited on FTA paper, place the microcentrifuge tube into a 95° C heat block for a minimum of 30 minutes.

1.11.5 Vortex vigorously for 20-30 seconds, then pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. Alternatively, a Spin-Ease basket may be used instead of placing the cutting in the lid of the tube. If the entire biological sample deposited on the evidence was consumed the cutting will be returned with the evidence.

NOTE: Samples can be left at room temperature in DNA IQ™ Lysis buffer for up to 24 hours after heating and centrifugation before proceeding to the BioMek® 2000 Automation Workstation. If the samples are stored in a refrigerator before proceeding to Section 2, DNA Purification, place the samples in a 56ºC heat block for 5 minutes to resolubilize the samples.

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1.11.6 Proceed to Section 2.6, DNA IQ™ Purification Procedure

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1.12 DNA IQ™ Extraction Method for Hair, Highly Concentrated Bloodstains, and Low Level Samples

NOTE: This procedure may also be used for samples that are believed to have a low concentration of DNA (i.e., envelopes, stamps, cigarette butts) or highly concentrated bloodstain (i.e., dried blood flakes).

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1.12.1 Follow the steps outlined below for the extraction of hairs. Proceed to step 1.12.2 for all other sample types:

1.12.1.1 Using clean tweezers, place the hair on a clean piece of white or black paper, whichever is appropriate, and examine under a stereo microscope for the presence of sheath material. Note the presence of any body fluids on the hair.

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1.12.1.2 Wash the hair to reduce surface dirt and contaminants by immersing the hair in sterile Type I Water in a clean 50 mL beaker. If the hair contains a biological fluid that is important to the investigation DO NOT wash the hair.

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1.12.1.3 Return the hair to the stereo microscope. Use a clean scalpel blade to cut a 0.5 to 1 cm portion from the root end of the hair and then place the hair root into a 1.5 mL microcentrifuge tube and proceed to step 1.12.3.

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NOTE: Process a reagent blank along with each set of samples.

1.12.2 Cut an approximate 3 mm^2 blood stain or other biological stain and place into a labeled 1.5 mL microcentrifuge tube with a depression in the lid

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1.12.3 Add to the 1.5 mL microcentrifuge tube:

37.5 µL TNE
12.5 µL 20% Sarkosyl
10.0 µL 0.39*M* DTT
32.5 µL Sterile Type I Water
10.0 µL Proteinase K

in proportional amounts to saturate the cutting.

1.12.4 Mix by hand or lightly vortex, then pulse spin the microcentrifuge tube to force the sample into the liquid.

1.12.5 Place the tube into a 56ºC incubator or heat block for minimum of 1 hour.

1.12.6 Pulse spin the tube in a microcentrifuge for 10 seconds to force the condensate to the bottom of the tube.

1.12.7 Proceed to Section 2.6, DNA IQ™ Purification Procedure

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1.13 DNA IQ™ Extraction Method for Tissue Samples

1.13.1 Cut a very thin slice of tissue (i.e. 1 mm thick slice of muscle) and place the sample into a labeled 1.5 mL microcentrifuge tube. If the tissue sample is degraded a slightly larger portion of the sample may be used. Alternatively, cellular material may be collected from the tissue by swabbing the sample and placing a portion of the swab into a labeled 1.5 mL microcentrifuge tube.

NOTE: Process a reagent blank along with each set of samples.

1.13.2 Add 90 µL of 1X $CaCl_2$ buffer and 10 µL of Proteinase K to saturate the sample.

1.13.3 Mix by hand or light vortexing and pulse spin to force the sample into the liquid.

1.13.4 Place the tube into a 56ºC incubator or heat block for a minimum of 2 hours.

NOTE: If the sample has been preserved in Formaldehyde or Formalin add a second 10 µL aliquot of Proteinase K after the 2 hour incubation, then place the tube into a 56° C incubator or heat block for an additional 2 hours.

1.13.5 Spin the 1.5 mL microcentrifuge tube at ~12,000 rpm for 5 minutes to pellet any undigested debris.

1.13.6 Proceed to Section 2.6, DNA IQ™ Purification Procedure.

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1.14 QIAamp® Extraction Procedure – Arrestee and Offender Samples

NOTE: Process a reagent blank and random sample along with each set of samples.

1.14.1 Dried blood stains: Cut a 3 - 5 mm^2 blood stain or using a hole puncher, punch one hole from the dried blood stain and transfer to a labeled 1.5 mL microcentrifuge tube using tweezers. If a hole punch is used, the hole punch should be cleaned between each punch using a Kimwipe and isopropanol. Add 180 µL PBS buffer to each tube and then pulse spin to force the bloodstain into the buffer. Incubate at 56 °C for one hour, then proceed to step 1.14.2.

1.14.2 Add 20 µL of the QIAGEN® protease to each tube and vortex for 15 seconds to thoroughly mix the sample.

WARNING!

The QIAGEN® AL lysis contains Guanidine Hydrochloride. This chemical will produce chlorine gas when mixed with bleach. When cleaning the counter tops after using the QIAGEN® extraction method wash the counter top first with water, then isopropanol, followed by a 10% bleach solution to disinfect the area.

1.14.3 Mix the QIAGEN® AL lysis buffer by shaking thoroughly before use. If a precipitate is observed, incubate at 70°C until dissolved. Add 200 µL of the QIAGEN® AL lysis buffer to each tube. Vortex the tube for 15 seconds to thoroughly mix the sample. **The QIAGEN® AL lysis buffer is light sensitive and should not be left in the light longer than is necessary**.

1.14.4 Incubate the tube in a 56°C heat block for 10 minutes.

1.14.5 Place the tube into a microcentrifuge and pulse spin the sample to remove condensation from lid.

1.14.6 Add 200 µL 95% ethanol to each tube. Vortex the tube for 15 seconds to thoroughly mix the sample.

1.14.7 Place the tube into a microcentrifuge and pulse spin the sample to remove condensation from lid.

1.14.8 Transfer the supernatant from the tube to a labeled QIAamp® spin column that is inside of a 2.0 mL collection tube. Centrifuge the sample for 1 minute at 8,000 rpm. Be careful not to apply the sample to the rim of the spin column. Sample that has been deposited on the rim during the centrifugation will be transferred from the tube to the inside of the microcentrifuge.

1.14.9 Discard the 2.0 mL collection tube containing the filtrate and place the QIAamp® spin column into a clean 2.0 mL collection tube.

1.14.10 Add 500 µL of the QIAGEN® AW1 wash buffer and centrifuge for 1 minute at 8,000 rpm.

1.14.11 Discard the 2.0 mL collection tube containing the filtrate and place the QIAamp® spin column into a clean 2.0 mL collection tube.

1.14.12 Add 500 µL of the QIAGEN® AW2 wash buffer and centrifuge for 3.5 minutes at 13,200 rpm.

1.14.13 Discard the 2.0 mL collection tube containing the filtrate and place the QIAamp® spin column into a clean, labeled 1.5 mL microcentrifuge tube.

NOTE: Use capless tubes or remove the caps from the tubes before centrifugation.

1.14.14 Add 50 µL of the QIAGEN® AE Elution Buffer or Type 1 water to each QIAamp® spin column.

1.14.15 Incubate the sample at room temperature for 1 minute.

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1.14.16 Place the 1.5 mL microcentrifuge tube containing the QIAamp® spin column into a microcentrifuge and spin for 1 minute at 8,000 rpm.

1.14.17 Discard the QIAamp® spin column and cap the 1.5 mL microcentrifuge tube containing the extracted DNA sample.

1.14.18 Proceed to Section 5, PCR Amplification.

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1.15 QIAamp® 96 WELL EXTRACTION PROCEDURE – ARRESTEE AND OFFENDER SAMPLES

NOTE: Process a reagent blank and random sample along with each set of samples.

1.15.1 **Dried blood stains and/or buccal samples:** Using a 6 mm^2 hole puncher, punch one hole from the dried blood stain onto a Kimwipe. Using tweezers carefully fold the punch in half and then transfer to the appropriate position in the 96 well round well block. **If a hole punch is used, the hole punch and tweezers should be cleaned between each punch using a Kimwipe and isopropanol**. Add 195 µL PBS buffer to each of the wells, then cap the wells with an 8-strip cap. Pulse spin the 96 well round well block to force the punch into the buffer. Incubate at 70°C in an oven for one hour. Place an acrylic block on top of the plate to prevent the caps from popping off. Once incubation is complete, pulse spin the 96 well round well block to remove condensation from the caps, then proceed to step 1.15.2.

NOTE: When the procedure makes reference to pulse spinning the sample(s), allow the centrifuge to reach approximately 3,000 rpm and then turn off the centrifuge and allow the rotor to come to rest.

1.15.2 Add 20 µL of the QIAGEN® protease to each well. Cap all wells and shake the 96 well round well block using two hands to thoroughly mix the samples. Pulse spin the 96 well round well block to remove condensation from the caps.

WARNING!

The QIAGEN® AL lysis buffer contains Guanidine Hydrochloride. This chemical will produce chlorine gas when mixed with bleach. When cleaning the counter tops after using the QIAGEN® extraction method wash the counter top first with water, then isopropanol, followed by a 10% bleach solution to disinfect the area.

1.15.3 Mix the QIAGEN® AL lysis buffer by shaking thoroughly before use. If a precipitate is observed, incubate at 70°C until dissolved. Add 200 µL of the QIAGEN® AL lysis buffer to each well. Cap each well, then using two hands shake the 96 well round well block for 15 seconds to thoroughly mix the samples. **The QIAGEN® AL lysis buffer is light sensitive and should not be left in the light longer than is necessary.**

1.15.4 Incubate the 96 well round well block in a 70°C incubator for 10 minutes. Place an acrylic block on top of the round well block to prevent the caps from opening.

1.15.5 Place the 96 well round well block into a centrifuge with a plate rotor and pulse spin the samples to remove condensation from lid.

1.15.6 Add 200 µL 95% ethanol to each well. Seal the wells using new caps for the round well blocks. Shake using two hands the 96 well round well block for 15 seconds to thoroughly mix the samples.

1.15.7 Place the 96 well round well block into a centrifuge with a plate rotor and pulse spin the samples to remove condensation from cap.

1.15.8 Place a QIAamp 96 well plate on top of an S-block. Mark the plate on the side for identification.

1.15.9 Transfer the supernatant from the round well block to the corresponding well of the QIAamp® 96 well plate. Be careful not to apply the sample to the rim of QIAamp® 96 well plate. Sample that has been deposited on the rim may cross contaminate other samples.

1.15.10 Seal the QIAamp 96 well plate with an airpore tape sheet. Load the S-block and QIAamp 96 well plate into the centrifuge carrier, then place the carrier into the rotor basket. Centrifuge the samples for 4 minutes at approximately 6,000 rpm.

1.15.11 Remove the airpore tape sheet and add 500 µL of the QIAGEN® AW1 wash buffer to each well.

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1.15.12 Seal the QIAamp 96 well plate with an airpore tape sheet. Load the S-block and the QIAamp 96 well plate into the centrifuge carrier, then place the carrier into the rotor basket. Centrifuge the samples for 2 minutes at approximately 6,000 rpm.

1.15.13 Remove the airpore tape sheet and add 500 µL of the QIAGEN® AW2 wash buffer to each well.

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1.15.14 Seal the QIAamp 96 well plate with an airpore tape sheet. Load the S-block and the QIAamp 96 well plate into the centrifuge carrier, then place the carrier into the rotor basket. Centrifuge the samples for 15 minutes at approximately 6,000 rpm.

NOTE: The heat generated during centrifugation allows for evaporation of any residual ethanol on the membrane.

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1.15.15 Place the white orientation adapter ring on top of a rack of collection tubes, then place the QIAamp 96 well plate on top of the adapter. Ensure the pieces fit snugly together. **IF THE PIECES DO NOT FIT SNUGLY CHECK THE ORIENTATION OF THE ADAPTER**.

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1.15.16 To elute the DNA, add 50 µL of the QIAGEN® AE Buffer or Type 1 water to each well. Seal the QIAamp 96 well plate with an airpore tape sheet and incubate for 1 minute at room temperature.

1.15.17 Load the plate combination into the centrifuge carrier, then place the carrier into the rotor bucket. Centrifuge the samples for 4 minutes at approximately 6,000 rpm.

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NOTE: If a partial plate was processed, seal the used wells with adhesive tape and label "used".

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1.15.18 Seal the wells of the collection tubes using the 8-strip caps. Label tubes appropriately.

1.15.19 Proceed to Section 5, PCR Amplification.

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2 DNA PURIFICATION

2.1 Technical Notes

2.1.1 Organic extractions denature and remove the proteins that were released by the proteolytic activity of the Proteinase K. The denatured proteins will collect in the organic phase of the extraction.

2.1.2 The interphase may contain some DNA and therefore a minimal amount should be brought along in subsequent organic extractions.

2.1.3 The anisotropic, hydrophilic membrane in the Microcon® 50 concentrator is designed to retain molecules that have molecular weights above 50,000 and for the Microcon® 100 concentrator molecules that have molecular weights above 100,000.

2.1.4 The Microcon® 100 concentrator is designed to remove low molecular weight contaminants (i.e., peptides and lipids) and inhibitors that may affect the amplification process. Therefore, the Microcon® 100 concentrator is used to purify the sample DNA where the Microcon® 50 concentrator is used for minute samples to concentrate the sample down to a lower volume. If a sample is believed to contain PCR inhibitors, a Microcon® 100 may be utilized to improve typing results.

2.1.5 The DNA IQ™ System is designed to rapidly purify small quantities of DNA, approximately 100 ng or less, and becomes more efficient with samples containing less then 50 ng of DNA.

2.1.6 The DNA IQ™ Lysis buffer is a proprietary detergent mixture containing Guanidine Thiocyanate (GTC). The GTC is a chaotropic agent, necessary for the DNA to stick to the silica-coated, paramagnetic resin.

2.1.7 The DNA sticks to the resin through hydrophobic interactions, however the exact mechanism is unknown.

2.1.8 The DNA IQ™ Wash Buffer is a low salt buffer, which is 50% alcohol (50% isopropyl: 50% ethanol), and 50 % NaAcetate. The alcohol in the wash keeps the DNA stuck to the resin and the low salt buffer helps to remove excess salt (Guanidine Thiocyanate) from the DNA stuck to the resin. If the Guanidine Thiocyanate is not removed this could interfere with the PCR amplification.

2.1.9 The DNA is removed from the resin with DNA IQ™ Elution Buffer using heat (56 ºC). When the DNA is initially eluted from the DNA IQ™ paramagnetic resin some of it is single stranded and therefore cannot be quantitated on an agarose gel.

2.2 Equipment

2.2.1 Pipettes - 20 µL, 100 µL and 1000 µL

2.2.2 Microcentrifuge

2.2.3 Microcentrifuge tube rack

2.2.4 DNA concentrator/evaporator

2.2.5 Freezer, -20° C

2.2.6 Vortex mixer

2.2.7 Heat block or incubator, 56 °C

2.2.8 Magnet Sphere Stand (Promega Catalog Number Z5342)

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2.3 Materials

2.3.1 Transfer pipettes

2.3.2 Microcentrifuge tubes, 1.5 mL

2.3.3 Sterile ART tips for pipettes, 20 µL, 100 µL and 1000 µL

2.3.4 Microcon® 50 Concentrator Assembly (for concentrating samples)

2.3.5 Microcon® 100 Concentrator Assembly (for purifying samples)

2.3.6 Kimwipes

2.3.7 Gloves

2.4 Reagents

2.4.1 Phenol-chloroform-isoamyl alcohol, prewarmed to room temperature

2.4.2 1X TE^{-4}

2.4.3 Sterile Type 1 Water

2.4.4 DNA IQ™ Lysis Buffer

2.4.5 DNA IQ™ Wash Buffer

2.4.6 DNA IQ™ Elution Buffer

2.5 MICROCON® Purification Procedure

The Microcon® purification procedure will be used when the biological stain extracted was deposited on a substrate known to cause inhibition during amplification (i.e., denim or velvet), the substrate released an excessive amount of dye during the extraction process, or the biological stain/material was minute in size (e.g., hair root).

2.5.1 Add 500 µL phenol-chloroform-isoamyl alcohol, prewarmed to room temperature, to each tube.

2.5.2 Cap the tube **tightly** and mix thoroughly by hand or light vortexing for 2-3 seconds (until solution has a milky appearance).

2.5.3 Spin the tube for 3 minutes in a microcentrifuge at approximately 10,000 rpm to separate the two phases.

2.5.4 Insert a labeled Microcon® 100 concentrator (or Microcon® 50 concentrator for minute biological stains/material) into a labeled filtrate vial ((microcentrifuge tube provided with the Microcon® assembly); then add 100 µL of sterile Type 1 Water to the concentrator. Using a transfer pipette, transfer the aqueous phase (top layer containing DNA in step 2.5.3) to the Microcon® concentrator. Avoid pipetting organic solvent from the tube into the concentrator. Place the cap from the filtrate vial on the concentrator.

2.5.5 Place the Microcon® assembly in a microcentrifuge and spin for 10 to 40 minutes at approximately 5,000 rpm until the volume is reduced.

2.5.6 Carefully remove the concentrator unit from the Microcon® assembly and discard the fluid from the filtrate vial. Return the concentrator to the top of the filtrate vial.

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2.5.7 Add 200 µL of sterile Type 1 Water. Replace the cap and spin the Microcon® assembly in a microcentrifuge at 5,000 rpm for 10-30 minutes until the volume is reduced.

NOTE: If the concentrator filter becomes stained due to the dyes released from the substrate, step 2.5.7 may be repeated additional times to reduce the potential of the dye inhibiting the PCR amplification.

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2.5.8 Remove the cap from the concentrator and add 30 µL 1X TE^{-4} buffer. Remove the concentrator from the filtrate vial and discard the vial. Carefully invert the concentrator and place into a new labeled retentate vial (same type of tube as the filtrate vial).

2.5.9 Place the Microcon® assembly into a microcentrifuge (the retentate cup end first) and spin at 5,000 rpm for 5 minutes.

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2.5.10 Discard the concentrator unit; place the cap on the retentate cup.

2.5.11 Proceed to Section 4, DNA Quantitation.

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2.5.12 Refer to Appendix J for the procedure for drying down and resolubilizing extracted DNA samples.

2.6 DNA IQ™ Manual Purification Procedure

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2.6.1 Depending on the type of sample being purified, add the following volumes of DNA IQ™ Lysis Buffer to the sample:

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NOTE: On the day of use add fresh DTT to the DNA IQ™ Lysis buffer at the concentration described in the reagent preparation section (Appendix B).

2.6.1.1 Non-sperm fractions and tissue – Remove 100 µL of the nonsperm fraction/tissue lysate and place it into a new labeled 1.5 mL microcentrifuge then add 220 µL of DNA IQ™ Lysis Buffer. Note: if more that 100 µL of the non-sperm fraction is used add proportional volumes of DNA IQ™ Lysis Buffer.

2.6.1.2 Sperm pellet – add 220 µL of DNA IQ™ Lysis Buffer

2.6.1.3 Hair samples (optional: envelopes, stamps, cigarette butts, flakes of blood, and other low level samples) – add 220 µL of DNA IQ™ Lysis Buffer. Note: this can be increased proportionally depending on the volume of ProK based digestion buffer used.

NOTE: The hair sample/fragment will be dissolved in solution at the completion of the DNA IQ™ isolation procedure.

2.6.1.4 Blood Stains and buccal cell type samples – add 100 µL of DNA IQ™ Lysis Buffer

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2.6.2 Vigorously vortex the bottle of DNA IQ™ Resin for 30 seconds prior to dispensing. Then add 8 µL of the DNA IQ™ Resin to each tube.

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NOTE: If the stock bottle of DNA IQ™ Resin has set for a prolonged period of time between samples re-vortex before further use.

2.6.3 After adding resin vigorously vortex each sample for several seconds

2.6.4 Place the tubes in a microcentrifuge rack and allow the samples to set at room temperature for a minimum of 5 minutes to adhere the DNA to the resin. After incubation a brief pulse spin in a microcentrifuge.

2.6.5 Transfer the sample tubes to a magnet sphere stand. Open the caps and without disturbing the resin pellet remove the liquid in each tube using a P1000 pipette and discard.

2.6.6 Add 100 µL of Lysis buffer into each tube, then remove the tubes from the magnetic stand and vortex vigorously for several seconds. Place the tube in a microcentrifuge and briefly pulse spin.

2.6.7 Place tubes back into the magnet sphere stand and without disturbing the resin pellet remove the liquid in each tube using a P100 pipette and discard.

2.6.8 Add 100 µL of 1X DNA IQ™ Wash buffer to each tube and vigorously vortex for several seconds, followed by a brief pulse spin in a microcentrifuge.

2.6.9 Place the tubes into the magnet sphere stand and remove the wash buffer using a P100 pipette and discard.

2.6.10 Repeat steps 2.6.8 and 2.6.9 two additional times.

2.6.11 After the last wash step, open the cap on each tube and allow the samples to completely air-dry. Note: This will take approximately 5 minutes depending on the volume of liquid remaining in the tube.

2.6.12 Add 40 µL of the DNA IQ™ Elution buffer to each tube to remove the DNA from the resin. Vortex each tube vigorously for 5 seconds.

2.6.13 Place the tubes in 56 °C heat block for 5 minutes, then in a microcentrifuge and briefly pulse spin.

2.6.14 Place the tubes into the magnet sphere stand and remove the supernatant using a P100 pipette. **DO NOT DISCARD. THE SUPERNATANT CONTAINS THE ISOLATED DNA SAMPLE. IF DNA IQ™ RESIN IS REMOVED WITH THE SUPERNATANT THIS MAY CAUSE INHIBITION AND PREVENT THE SAMPLE FROM AMPLIFYING.**

2.6.15 Place the supernatant into a clean, labeled 1.5 mL microcentrifuge tube.

2.6.16 Proceed to Section 4, DNA Quantitation.

2.6.17 Refer to Appendix J for the procedure for drying down and resolubilizing extracted DNA samples.

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3 YIELD GEL FOR ASSESSING QUALITY AND QUANTITY OF ISOLATED DNA

3.1 Technical Notes

3.1.1 Yield gels are best interpreted from the photograph because of excess fluorescence and background from the transilluminator. This also reduces the danger of overexposure to UV light.

3.1.2 Results serve as a quantitative tool for estimating the amount of DNA as well as a qualitative indication of sample integrity.

3.1.3 Non-human DNA present in a sample may produce results on the gel. These results may be indistinguishable from human DNA results.

3.1.4 Quantitation standards contain K562 of known concentrations. A range of standards between 15 and 500 ng is generally used.

3.1.5 Ethidium bromide (EtBr) is used to detect DNA by staining the yield gel. The ethidium bromide intercalates into the DNA molecule and fluoresces under UV light.

3.1.6 A UV transilluminator, at a wavelength of 302 nm, is used to visualize the fluorescent reaction between the EtBr and the DNA. At this wavelength, the saturation point of the DNA is 250-500 ng with a lower threshold of 5 ng. Therefore, samples exceeding this range should be diluted and requantitated.

3.1.7 The visual marker, used to monitor migration, contains known concentrations of lambda DNA digested with the restriction enzyme Hind III. Eight bands with the following molecular weights are present in this digest:

23,130 base pairs (bp)
9,416 bp
6,557 bp
4,361 bp
2,322 bp
2,027 bp
564 bp (may not be detected)
125 bp (not usually detected)

3.2 Equipment

3.2.1 Pipettes - 10 µL and 20 µL

3.2.2 Microcentrifuge

3.2.3 Gel tank, cover, and electrodes

3.2.4 Power supply

3.2.5 Heat block, 56 °C

3.2.6 Vortex mixer

3.2.7 Microcentrifuge tube rack

3.2.8 Voltmeter

3.3 Materials

3.3.1 Agarose gel, 16 or 20 cm (Refer to Appendix C.)

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3.3.2 Microtiter plate

3.3.3 Sterile ART tips for pipettes - 10 µL and 20 µL

3.3.4 Gloves

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3.4 Reagents

3.4.1 5X Loading buffer

3.4.2 Quantitation standatds (K562)

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3.4.3 Visual Marker – Lambda Hind III (Use is optional.)

3.4.4 Ethidium bromide – 5 mg/ml (Optional – refer to Appendix C.)

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3.4.5 1X TAE buffer

3.5 Procedure

NOTE: The yield gel procedure for assessing the quality and quantity of isolated DNA should not be used for casework samples in place of the AluQuant® Human Quantitation procedure for assessing the quantity of isolated DNA addressed in Section 4. However, the yield gel procedure may be used to evaluate and dilute casework samples believed to contain a high concentration of DNA prior to the AluQuant® Human Quantitation procedure and to quantitate known reference samples.

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3.5.1 Following the procedure outlined in Appendix C, prepare a 1% yield gel using DNA typing grade agarose in 1X TAE buffer.

3.5.2 Remove the quantitation standards and the visual marker from the freezer and bring to room temperature (or thaw at 56° C for 5 minutes). Vortex the tubes and then microcentrifuge briefly, to mix the contents.

An example of suggested quantitation standards follows:

500 ng/6 µL
250 ng/6 µL
125 ng/6 µL
63 ng/6 µL
31 ng/6 µL
15 ng/6 µL

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3.5.3 After the DNA has resolubilized, spin the tube for 5 seconds in a microcentrifuge to remove any condensation that may be on the lid.

3.5.4 Add 2 µL of 5X loading buffer to the appropriate number of wells of a microtiter plate.

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3.5.5 Aliquot 4 µL of each sample into a different well in the microtiter plate.

3.5.6 Load the yield gel into the tank and add sufficient 1X TAE Buffer to cover the gel.

3.5.7 Load 6 µL of visual marker into the first well of the gel. When loading the wells, take great care that DNA does not trail up out of the wells. Make sure there are no air bubbles at the end of the pipette tip. Remove the pipette slowly and carefully after gently voiding.

3.5.8 Load the quantitation standards in order of decreasing concentration into the yield gel, such as:

2nd well	6 µL 500 ng control
3rd well	6 µL 250 ng control
4th well	6 µL 125 ng control

... and so forth until 6 µL of each quantitation standard has been loaded in wells 2 through 7 of the yield gel.

3.5.9 Load samples from the microtiter plate into the remaining wells of the yield gel.

3.5.10 Place the cover on the gel tank so that the red (positive) electrode is farthest from the loading wells.

3.5.11 Plug the red (positive) electrode into the positive plug of the power supply. Plug the black (negative) electrode into the negative plug of the power supply.

3.5.12 Turn the power supply on and set the voltage to the equivalent of 200 volts (power supply reading). Electrophorese for 30 minutes or until the loading buffer moves 2-3 cm from the origin.

NOTE: If ethidium bromide was not incorporated into the yield gel, the gel must be stained with ehidium bromide after electrophoresis. Soak the gel in 200 mL of 1X TAE and 40 µL of EtBr for 20-30 minutes.

WARNING!

ETHIDIUM BROMIDE IS MUTAGENIC. ALWAYS WEAR GLOVES AND A LABORATORY COAT WHEN HANDLING EtBr. IF USING EtBr SOLUTION TO STAIN THE GEL AFTER ELECTROPHORESIS, NEUTRALIZE THE SOLUTION WITH DOWEX 2 BEADS BEFORE DISCARDING.

3.5.13 View the yield gel on a UV transilluminator or in a UV viewing cabinet. Following the steps outlined in Appendix D, Steps 3.1 or 3.2, as appropriate, photograph the gel.

3.5.14 Determine the concentration of the DNA sample by comparing its intensity to the quantitation standards (K562). Divide the concentration by 4 µL, the volume of the sample added to the yield gel. This calculation will give the DNA concentration in ng/µL.

3.5.15 If the extracted DNA is below the detection level, and therefore cannot be visualized on the yield gel, the sample will be carried through the amplification process.

3.5.16 Continue with Section 5, PCR Amplification.

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4 DNA QUANTITATION

4.1 Plexor HY® System for Human DNA Quantitation

4.1.1 Technical Notes

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The Plexor® HY System is a real-time quantitative PCR assay for the simultaneous quantitation of total autosomal and male DNA. If the use of the AluQuant Human DNA Quantation System is desired, go to step 4.2.

4.1.1.1 The Plexor® HY System is a quantitative PCR assay for the simultaneous quantitation of human genomic DNA and human male DNA. The assay utilizes two primers – one with a modified base (iso-dC) and a fluorescent label at the 5' end. The second PCR primer is unlabeled. During amplification, the modified base, iso-dC, base pairs specifically with iso-dGTP, included in the reaction mixture, as it is incorporated into the complementary strand. The iso-dGTP is modified to include a quencher, dabcyl. Thus, upon extension and incorporation of the iso-dG base, quenching of the fluorescent signal is observed.

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4.1.1.2 A human DNA sample can be accurately quantitated in the range of ~8 pg/µL to 50 ng/µL. Quantitation values <8 pg/µL should be considered as indistinguishable from background, although values below 8 pg/µL have yielded weak partial profiles.

4.1.1.3 The Plexor® HY System uses primers specific to a 99 bp multicopy target on chromosome 17 to quantitate the total amount of human DNA in a sample. A second pair of primers specific to a 133 bp multicopy target on the Y-chromosome is used to quantitate the total amount of male DNA in a sample.

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4.1.1.4 Because the lengths of the amplicons are relatively short (as opposed to the conventional STR amplicons), the Plexor® HY System is relatively unaffected by DNA degradation, except in extreme cases.

4.1.1.5 The Plexor® HY System also simultaneously amplifies an internal positive control (IPC) which can be used to assess the qPCR reaction. A synthetic DNA template is added to the PCR master mix and, thus, is present in every reaction at approximately the same quantity. As a result, a similar C_T value (± 1 C_T) is expected for the IPC in each well. If one of the samples is not within 1 C_T value of that exhibited by the standards, then that is suggestive of the presence of a PCR inhibitor in the sample. However, lack of an indication of a PCR inhibitor by the IPC is does not rule out the possibility of inhibition in subsequent amplifications or the possibility that the inhibitor is present in a concentration that is undetected by the qPCR assay, but still affects the STR amplification.

4.1.1.6 The Plexor® HY System relies upon the specific interaction between the two modified bases for quantitation. The two modified bases, isoguanine (iso-dG) and 5'-methylisocytosine (iso-dC), base pair, whereas iso-dG and the unmodified cytosine or, alternatively, iso-dC and the unmodified guanine, do not base pair. During amplification, the fluorescently-labeled primer anneals and extends, becoming template for subsequent cycles. During subsequent cycles of amplification, the iso-dC base on the fluorescently-labeled primer is paired with the iso-dG (modified with an attached quencher) in the reaction mixture. The proximity of the fluorescent label and quencher result in a decrease in fluorescent signal detected by the instrument. This process is illustrated in Figure 1.

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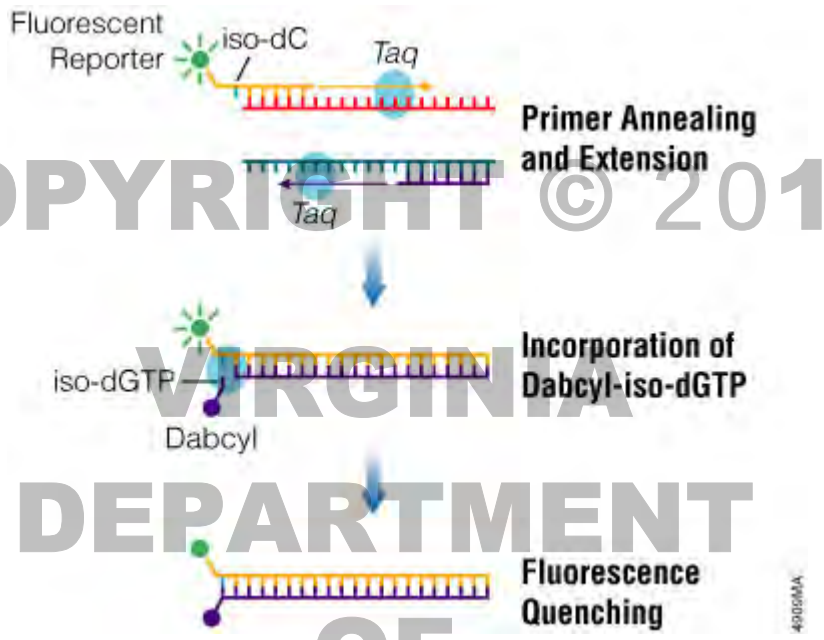

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Figure 1. PCR amplification of a target sequence using the Plexor® technology**.** The first step involves the primer annealing and extension. Note that one primer contains a 5' modified base (iso-dC) and fluorescent label. Upon extension of the DNA in subsequent cycles, a second modified base (dabcyl iso-dG) is incorporated. The proximity of the fluorescent label and quencher (dabcyl) results in a decrease in fluorescent signal, indicating accumulation of product. (Figure reproduced from the Plexor® HY System Technical Manual.)

4.1.1.7 Gloves must be worn at all times when performing the Plexor® HY System reactions because the introduction of nucleases that could occur from un-gloved handling would interfere with the reaction**.**

4.1.1.8 The Plexor® Analysis Software is used in conjunction with the Plexor® HY System and the Stratagene Mx3005P™ Quantitative PCR instrument. The qPCR assay can be setup manually or robotically using the appropriate methods on the Biomek® 2000 Automation Workstation.

4.1.2 Equipment

4.1.2.1 Stratagene Mx3005P™ Quantitative PCR instrument

4.1.3 Materials

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4.1.3.1 96-well qPCR Plate - Greiner P/N 652260

4.1.3.2 Optically Clear Strip Caps – Phenix Research P/N 101100-082 or Greiner P/N 373250

4.1.3.3 Optional: Optically clear film – VWR Cat#82050-994

4.1.3.4 Micro Amp strip tubes (in strips of 8 tubes)– ABI Cat# N801-0580

4.1.3.5 Black PCR support base (96 well) – ABI Cat# N801-0531

4.1.3.6 P20 Tips – aerosol resistant – Beckman Catalog # 609043

4.1.3.7 Quarter module reservoir – Beckman Cat# 372788

4.1.4 Reagents

4.1.4.1 Plexor® HY System kit (Promega Cat# DC1000 = 800 determinations or Promega Cat# DC1001 = 200 determinations) – (stored at -20°C) which contains:

4.1.4.1.1 Plexor® HY 2X Master Mix

Type I water is considered nuclease free and may be used instead of the amplification grade water that comes with the Plexor® HY kit

4.1.4.1.2 Nuclease free or Type I water

Type I water is considered nuclease free and may be used instead of the amplification grade water that comes with the Plexor® HY kit

4.1.4.2 Plexor® HY Genomic DNA Standard (50 ng/µL)

NOTE: The genomic standard **must** be stored at 4 °C after thawing. Freezing and thawing can affect accuracy of the standard

4.1.5 Starting the Stratagene MX3005P™ Quantitative PCR Instrument

4.1.5.1 Turn on the Stratagene Mx3005P™ Quantitative PCR instrument by using the power button located on the back left side of the unit.

4.1.5.2 Turn on the computer.

4.1.5.3 Open the Mx3005P™ software "MxPro" and select "SYBR Green (with Dissociation Curve)" from the New Experiment Options window. In the same window, check the box "Turn lamp on for warm-up?" Select "OK". The lamp requires 20 minutes to warm up before running the assay. The software can be configured and the plate set up on the BioMek® 2000 Automation Workstation while the lamp is warming up.

4.1.5.4 The software will ask "Do you wish to use SYBR Green Plate Setup from the active set "plexor"?" Click "yes" to load the Plexor® HY assay default plate setup.

4.1.5.5 The software will ask "Do you wish to use SYBR Green Thermal Profile Setup from the active set "plexor"?" Click "yes" to load the Plexor® HY assay default thermal profile.

4.1.6 Programming the Stratagene Mx3005P™ for Thermocycling

4.1.6.1 Select the Plate Setup tab.

4.1.6.2 Select the wells that will NOT be used and choose "blank" as the well type.

NOTE: All wells to be used will be designated "Unknown". Designation of wells as standards, sample names, and standard DNA quantities will be entered into the Plexor Analysis software, **not** the Stratagene Data Collection software. The FAM, CO560 and CR610 boxes under "Collect fluorescence data" will be selected for each well. IC5 (a.k.a. CY5) will be designated as the reference dye. Refer to Figure 2 to ensure the proper selections were made.

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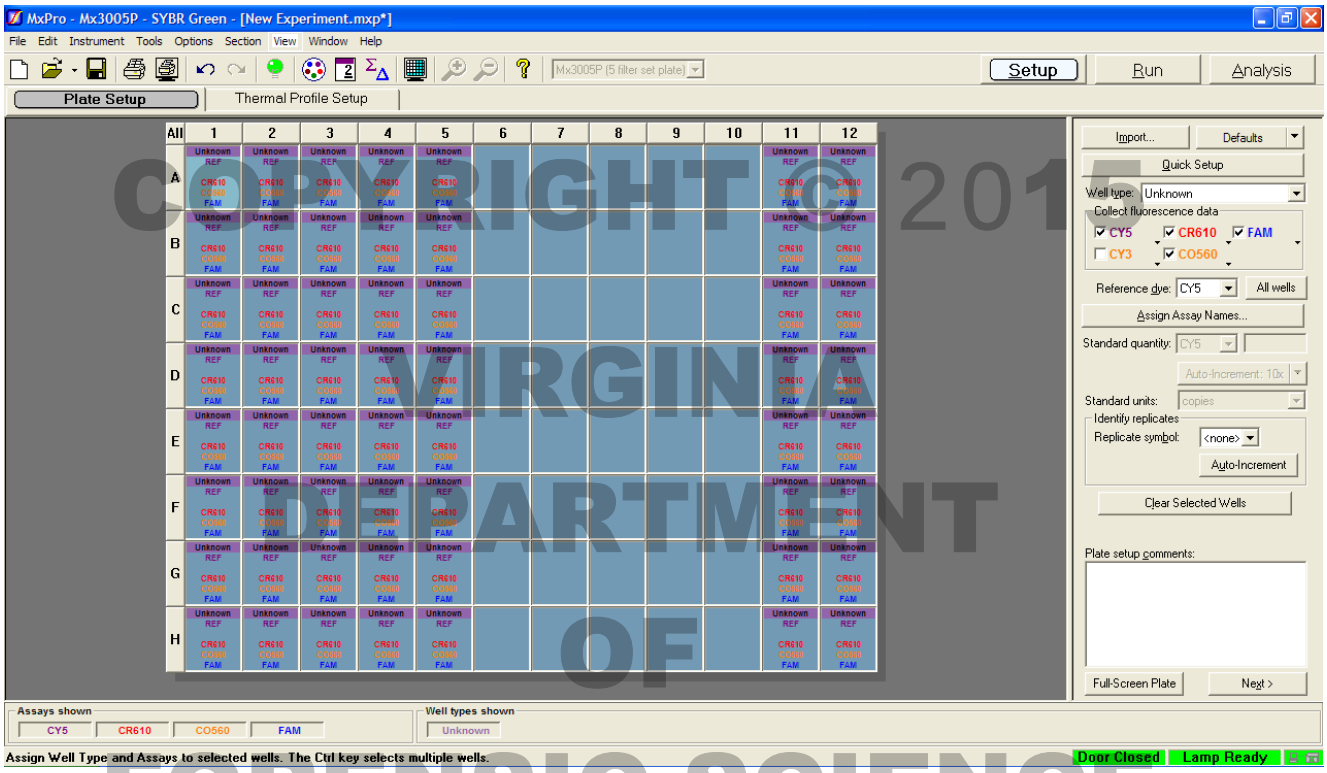

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Figure 2. The Plate Setup tab**.** Note that the wells selected will vary from plate to plate. Standards will be located in the last two columns, columns 11 and 12.

4.1.6.3 Select the Thermal Profile Setup tab. The default thermocycling conditions should be programmed for the Plexor® HY assay as shown below in Figures 3 and 4.

Step	Temperature	Time	Number of Cycles
Initial denaturation:	95°C	2 minutes	1 cycle
Denaturation:	95°C	5 seconds	38 cycles
Annealing and extension:	60°C	40 seconds	
Melt temperature curve:	65°C initial temperature with 0.6°C increase each cycle, 40-second hold each cycle		48 cycles

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Figure 3. Thermocycling parameters**.** (Figure obtained from the Plexor® HY System Technical Manual.)

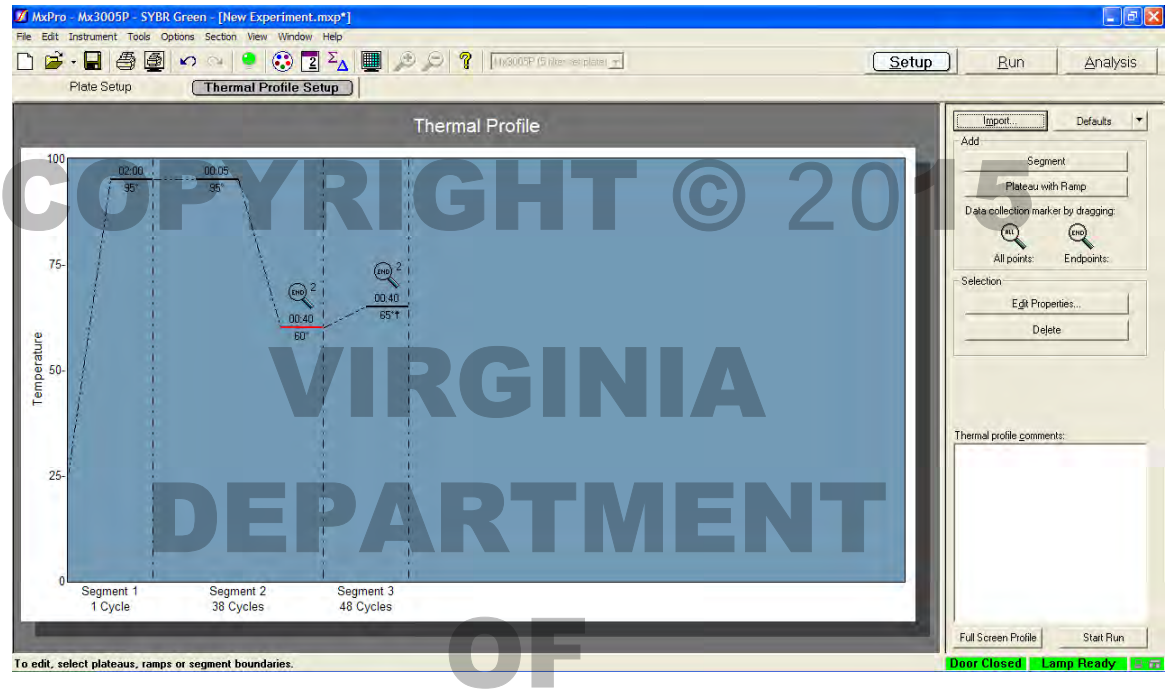

Figure 4. Thermal Profile Setup for the Plexor® HY System assay.

4.1.6.4 If you plan to use film to seal the plate, select "Instrument" and "Set Filter Gain Settings". In the dialog box that opens, the filter setting should be changed to: 1X for each dye except FAM, which should be 2X.

Note: The default filter gain settings are configured for the caps (1X for each dye except 2X for CO560 and 4X for FAM)

4.1.6.5 The instrument is now ready for the running the Plexor® HY System assay once the reactions are prepared in the qPCR amplification plate.

4.1.7 Preparation of the Standard Curve

4.1.7.1 After the initial use, the Plexor® human genomic DNA Standard (50 ng/µL) **must be** stored at 4 °C to prevent deleterious effects on the DNA due to freeze-thawing.

4.1.7.2 A 5x serial dilution of the DNA standard will be prepared as follows.

4.1.7.2.1 Label seven microcentrifuge tubes B through H.

4.1.7.2.2 Transfer 40 µL of Type I H_2O or the amplification quality water which comes with the Plexor kit to tubes B-H.

4.1.7.2.3 Vortex the Plexor® DNA Standard (50 ng/µL) to mix it thoroughly.

4.1.7.2.4 Transfer the appropriate amount of DNA to tube B, as shown in Table 1, close the lid, and vortex to mix thoroughly.

4.1.7.2.5 Repeat the DNA transfer, as shown in Table 1, followed by vortexing, for each standard C-G.

4.1.7.2.6 Undiluted genomic standard will be used for standard A, added directly to the Plexor cocktail in the PCR plate.

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4.1.7.2.7 The no template control, sample H, will be made with either Type I H_2O or the amplification quality water that comes with the Plexor kit.

Table 1. Preparation of Plexor® HY standard curve.

DNA Standard	Concentration (ng/µL)	Volume of Type I H_2O to add to tube	Volume of DNA to add to tube
A	50 ng/µL	-	-
B	10 ng/µL	40 µL	10 µL from 50 ng/µL Plexor® DNA stock
C	2 ng/µL	40 µL	10 µL from Standard B
D	0.4 ng/µL	40 µL	10 µL from Standard C
E	0.08 ng/µL	40 µL	10 µL from Standard D
F	0.016 ng/µL	40 µL	10 µL from Standard E
G	0.0032 ng/µL	40 µL	10 µL from Standard F
H	0 ng/µL	40 µL	-

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4.1.8 Preparation of Plexor® qPCR Reactions

Reagents used for the Plexor® HY System come prepared for use. It is critical for optimal performance that the reagents thaw completely and are well mixed prior to use. Unused reagents may be re-frozen at -20ºC, except the genomic DNA standard, which **MUST** be stored at 4 °C between uses

4.1.8.1 Remove the Plexor® HY System reagents from the -20 ºC freezer and allow them to thaw prior to use. All reagents should be thawed and well mixed prior to use.

NOTE: A maximum of 80 unknown samples at a time in a 96 well thermocycle plate can be quantitated with the Plexor® assay since two columns **must** be reserved for the standards

4.1.8.2 Prepare the Plexor master mix in a 1.5 mL tube as described below.

- Plexor 2X Master Mix – 10 µL per reaction
- Nuclease-free (or Type I) H_2O – 7 µL per reaction
- 20X Primer/IPC Mix – 1 µL per reaction

Prepare sufficient reaction mix for the desired number of reactions, making certain that the two columns (16) standards are included as well as an appropriate number of excess reactions (approx. 3 for small runs or 6 for larger runs).

Note: When manually pipetting the genomic DNA and master mix into the 96-well plate, it is imperative to visually inspect the tubes to ensure that no bubbles were introduced. If bubbles are observed, use a clean, unused pipette tip to carefully remove the bubble.

4.1.8.3 Transfer 18 µL of the Plexor Master Mix prepared in 4.1.8.2. into each well of the 96 well plate to contain sample or the standard curve. The standard curve will be placed in the last two columns (11 and 12) of the 96-well plate.

NOTE - GREAT CARE MUST BE TAKEN TO ENSURE THAT BUBBLES ARE NOT INTRODUCED WHEN TRANSFERRING THE DNA TO THE 96-WELL PLATE!

4.1.8.4 Transfer 2 µL of each sample or standard curve sample into the 96 well plate, according to the plate map. The DNA standards should be placed, in duplicate, in columns 11 and 12. Two microliters of standard A (the undiluted Plexor human genomic DNA standard) should be transferred to wells A11 and A12. Two microliters of standard B should be transferred to wells B11 and B12, etc. Sample wells H11 and H12 contain standard H, which have no DNA and, therefore, act as no-template controls.

4.1.8.5 Once the entire plate is loaded with reaction mix and samples, visually examine the wells to ensure that no bubbles are present. If a bubble is observed, dislodge it with a sterile pipette tip and seal the plate with optically clear strip caps or film. If using the optically clear film, ensure that a secure seal is made with the plate such that no evaporation of liquid will occur. In addition, if you are using optically clear film, be sure that the filter gain settings have been adjusted in the MxPro data collection software as described in 4.1.6.4.

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4.1.8.6 Cover the samples containing the isolated DNA to prevent evaporation of the samples.

4.1.9 Stratagene Mx3005P™ Operating Procedure

4.1.9.1 Place the 96 well qPCR plate in the Stratagene Mx3005P thermocycler. Note that the plate holder in the thermocycler opens like a waffle maker. The plate should be under the black top.

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4.1.9.2 The run can be initiated by selecting "Start Run". The run should be launched immediately after the qPCR plate is placed into the instrument. The computer will prompt the user to designate a filename in which the data will be saved.

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4.1.9.3 Once the run is over, remove the PCR plate containing the Plexor® reactions from the thermocycler. It should be discarded into the biohazard waste.

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4.1.10 Data Analysis

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4.1.10.1 To analyze the data, prior to import into the Plexor software, in the Stratagene data collection program, click "File"and "Export Instrument Data". The export function provides a drop-down window, from that window select, "Export Instrument Data to text file" and "Type 3 – Grouped by wells". A dialog box will appear asking for a filename to save the file. Choose the same name as was used for the Stratagene file, which is typically the date followed by the operator's initials. This will not overwrite the Stratagene data collection file (raw data) as this will be a *.txt file.

4.1.10.2 Open the Plexor® Analysis Desktop program. Choose "File", "Import New Run". Type "Plexor HY" in the Assay Name box. The parameters should be as shown below in Figure 5. Click "Next>".

(NOTE: if the setup does not look like that shown below, click "Import" in the upper right hand corner of this box and browse to the assay setup template called "plexor.atp"

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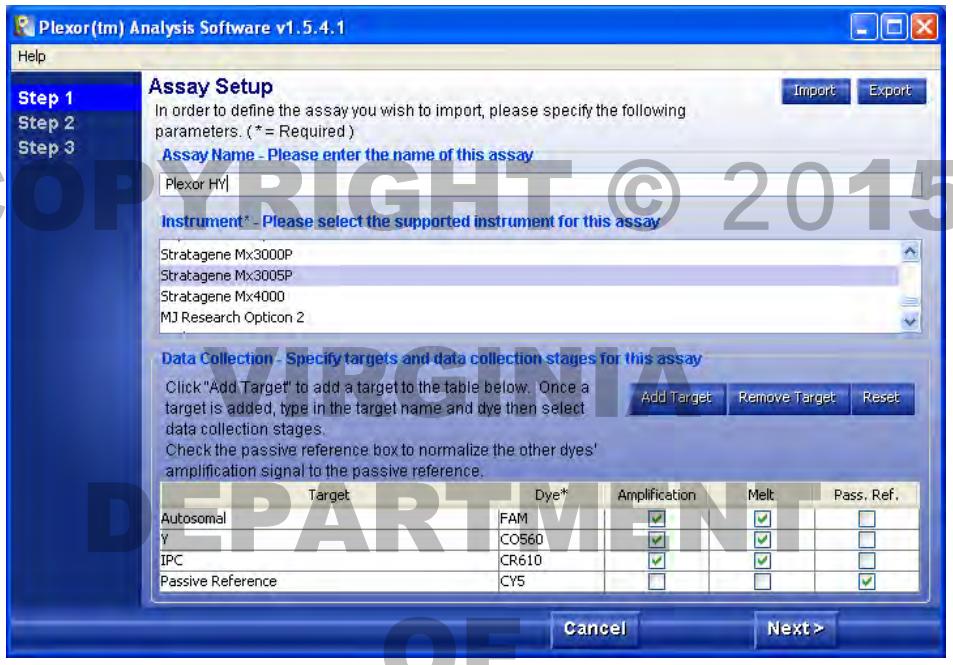

Figure 5. Import parameters. The instrument and data collection should be selected as shown for the Plexor® HY system amplification. Note: the order of the dyes shown in the "Data Collection" box is not important, although the boxes selected for each dye are important.

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4.1.10.3 At the "Run Info" dialog box, fill in the Experiment Title with the robot run name. Also fill in the operator name. The date should be set correctly, if the computer's date is correct. Notebook ID may be left blank. The Plexor® HY System lot number should be entered into the "Reagent ID" box. Click "Next".

4.1.10.4 At the File Import screen, click "Browse" and find the *.txt file that was generated in which the raw data was saved. Click "Run Template". In the "Run Template" dialog box, shown in Figure 6, click on the "Plate Setup" tab and choose "Import" and select "Plexor_run template.rtp". Click "OK" in the bottom right hand corner of the dialog box.

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Figure 6. Run Template screen for analysis of Plexor® HY System data. The standard curves and no template controls (columns 11 and 12) are indicated by the circles and diamonds, respectively.

4.1.10.5 The Plexor® Analysis settings can be imported from the file "Plexor_analysistemplate.ntp". At the File Import screen, click "Analysis Template". In the "Analysis Template" dialog box, choose "Import" and select "Plexor_analysistemplate.ntp". Click "Open".

4.1.10.6 The defaults are used from all settings, as shown in Figure 7, except the expected melt temperature. For autosomal, T_m = 81.5°C, Y = 82.7°C, and IPC = 82°C. These melt temperatures are imported when you select the Plexor® analysis template. Click "OK".

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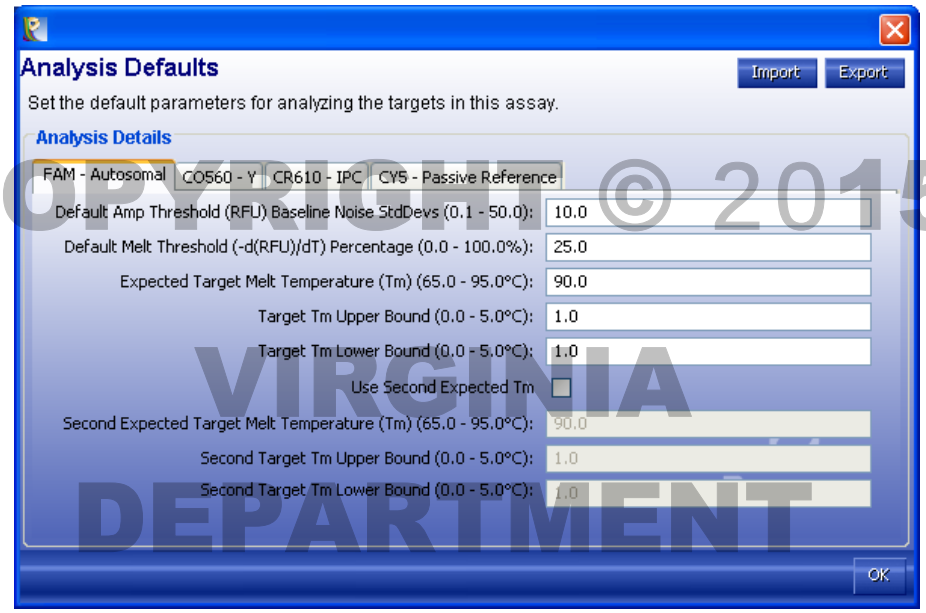

Figure 7. Analysis Template Window. After importing the analysis template, the expected melt temperature for the autosomal amplicon should be approximately 81.5°C.

4.1.10.7 Click "Finish" to see the analyzed data.

4.1.10.8 The sample IDs can now be imported from the Biomek populatable worksheet (Excel spreadsheet, Form 210-F500). Open the Populatable Worksheet and select the "Raw Data" Tab. Copy the sample names from the "sample names" grid (the bottom-most grid) by selecting the appropriate wells and pressing <Ctrl-C>. Do not copy column or row headers (i.e. A, B, C...or 1, 2, 3...). In the Plexor Analysis software, click on the Sample IDs tab, and paste the sample IDs from Template by pressing <Ctrl-T>.

4.1.10.9 The current version of the software may not import the standards properly from the template, so they must be defined. Define the standard curves by highlighting the wells of the two columns of standards, but not the No Template Control wells (Figure 8). Click on the standard series definition button, indicated by the arrow in Figure 8 and the inset window shown in Figure 8 will appear. Be certain that the most concentrated standard is defined as 25 ng/µL and that the series is a vertical series, decreasing by a factor of five. Click "Apply".

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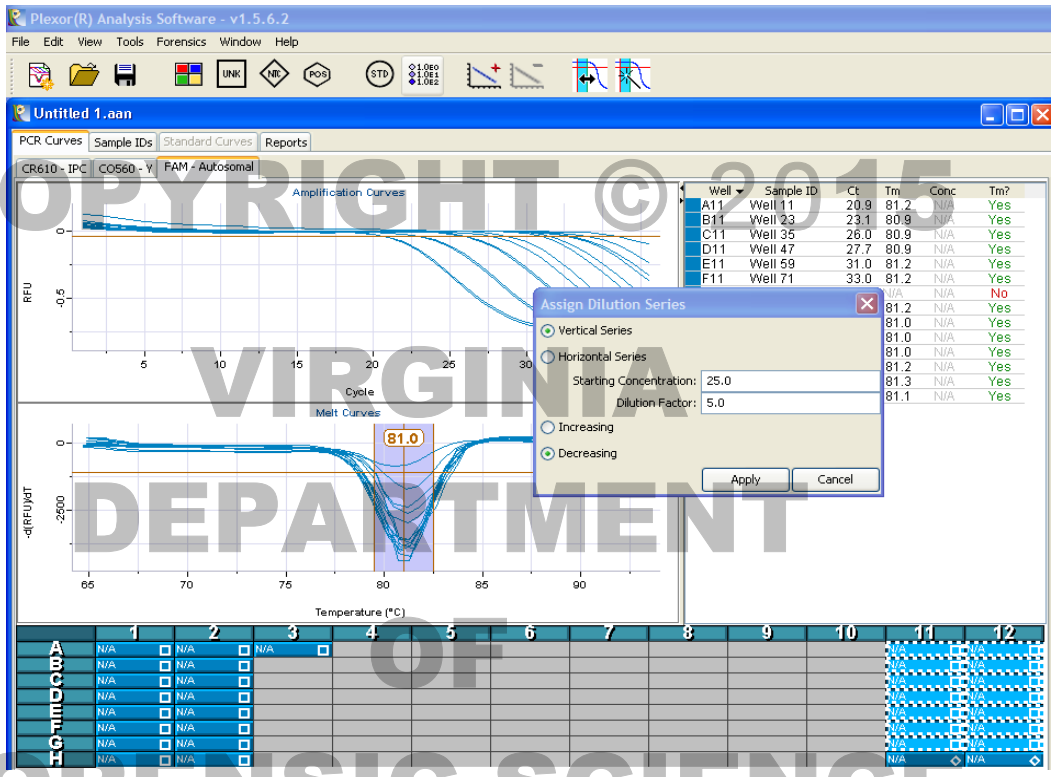

Figure 8. Defining the standard dilution series.

4.1.10.10 In the "PCR curves" tab, click on the box in the upper left hand corner of the plate map. This will highlight the entire plate. Be sure you are in the FAM tab, for the autosomal quantitation data. Choose "Edit", "Add Standard Curve" or click on the corresponding icon in the toolbar, as indicated in Figure 9. This creates a standard curve for the dye selected and quantitates the unknowns for that dye as well. Click on the tab for CO560 – Y and repeat to create a standard curve for the Y amplicon.
NOTE: No standard curve is necessary for the IPC.

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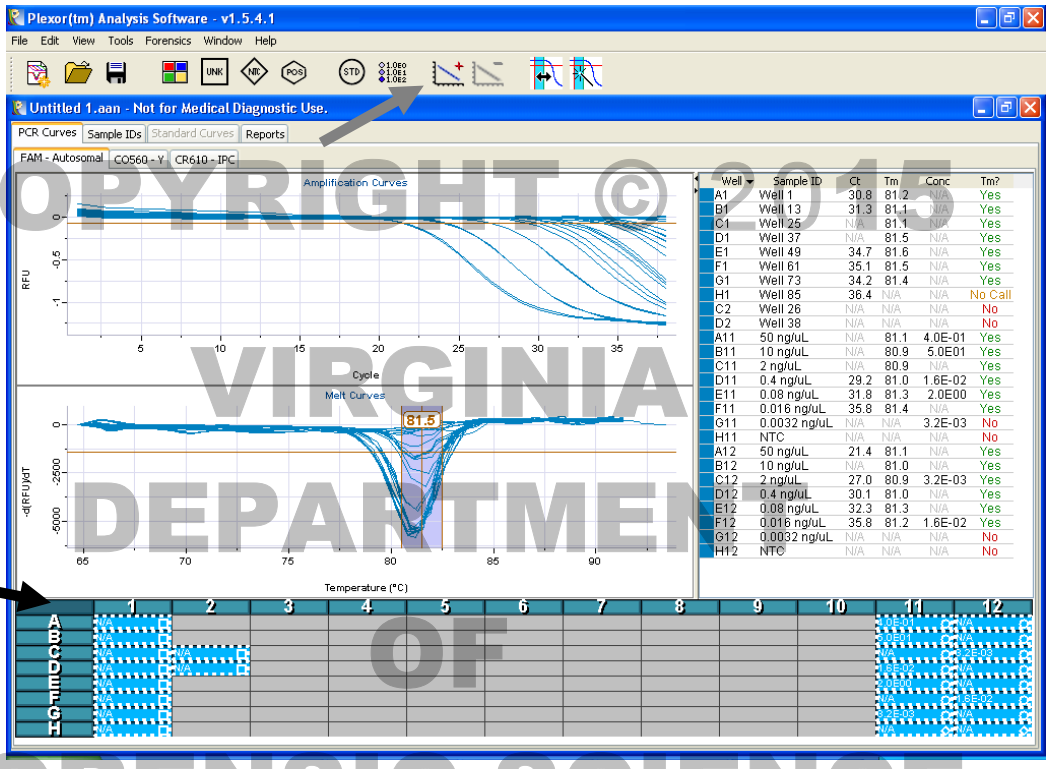

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Figure 9. Making the Standard Curves**.** After selecting the entire plate of samples by clicking in the box indicated by the black arrow, a standard curve can be created by clicking the appropriate icon as indicated by the second (gray) arrow.

4.1.10.11 Evaluate the standard curves by selecting the Standard Curves tab. You will need to assess the curves for the autosomal and Y DNA quantitation separately by selecting each of the tabs separately (FAM – Autosomal and CO560 – Y). The linearity (r^2) and efficiency (eff) of both curves should be assessed. Each standard curve should have an r^2 value of 0.98 or above to be acceptable. Evaluate the curve generated to determine if any data points need to be de-selected to improve the quality of the extrapolated curve generated. Up to 3 data points can be de-selected per standard dilution series (for a total of 6 data points) when necessary to produce a linear standard curve.

NOTE: this includes data points in the standard curves (A11-G11 and A12-G12) that are not detected. The software does not include any undetected data points when constructing the standard curve. The efficiency is usually 100% ± 15%. Refer to Appendix of the Biomek manual, Troubleshooting chapter for directions on de-selecting data points to improve the standard curve.

NOTE: The baseline regions are computed and selected automatically. Should the baseline of any specific amplification plot be improperly estimated, it can be adjusted manually. Each well can be highlighted individually to inspect the amplification plot and adjust the baseline. Refer to Appendix Aof the Biomek manual for directions on baseline adjustment: when it is needed and how to perform it.

4.1.10.12 A copy of either the "Forensics Report" (prepared in 4.1.10.13.1) and the "Sample Details Report" (as described in 4.1.10.13.2) or the quantitation report (Form 210-F504, prepared and printed in 4.1.10.14) should be printed for each examiner with samples in the Plexor® run. If the report does not indicate the r^2 value for the two standard curves, this should be added by hand and the notation initialed.

NOTE: There may be apparent discrepancies between the quantitation reports and the normalization wizard. These apparent discrepancies will be minor (usually 0.01 ng/µL or less) and originate from the rounding and truncating that each software program performs.

4.1.10.13 Generation of the Forensics Report and Sample Details Report.

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NOTE: If the Forensics Report and Samples Details Report will not be used, skip to 4.1.10.14 to generate the quantitation report (Form 210-F504).

4.1.10.13.1 Generation of the Forensics Report. To generate a report ("Forensics report") of the quantitation data, click "Forensics" and "Set Normalization and IPC parameters". A check mark should appear near the top of the dialog box so that the report is limited to the concentration data and C_T only. The default parameters are correct and should read that the autosomal dye is FAM and the Y-STR dye is CO560. The IPC dye is CR610 and the default to flag an improper (inhibited) amplification for the IPC is 2 C_Ts.

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4.1.10.13.1.1 The forensics report should contain the following columns: Sample Name, Location, Sample Type, [Auto], [Y], and [Auto]/[Y]. To change the columns shown, right-click on the table (not on the column headings) and choose "Change columns shown" from the drop-down menu. The columns shown are arranged by dye in the subsequent window. To rearrange the order of the columns shown on the Sample Details report, click and drag the column header horizontally to the desired location.

4.1.10.13.1.2 To print this report, select the entire table and click on the printer icon in the upper right hand corner of the window. Printing from the "File" drop down menu will only print a screenshot. If the Forensics Report does not indicate the r^2 value for the two standard curves, this should be added by hand and the notation initialed.

4.1.10.13.1.3 The Forensics report indicates the sample concentrations (in ng/uL) as well as the ratio of autosomal to Y DNA in a sample. This ratio of autosomal to Y DNA may be utilized by an examiner, along with other pertinent case information, to recommend Y STR typing. NOTE: A result of "N/A" in the concentration column indicates that no DNA (either autosomal, Y, or both, depending on the location of the N/A result) was detected. "N/A" may be reported for a sample's ratio of autosomal/male DNA concentration if no Y DNA is detected in the sample.

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4.1.10.13.2 Generation of the Sample Details Report

4.1.10.13.2.1 In the Plexor Analysis software, navigate to the Sample Details tab. Make sure that only the correct columns are showing and in the correct order (Location, Sample ID, CR610 Ct, CO560 Conc, CO560 NS Call, FAM Conc, FAM NS Call). To change the columns shown, right-click on the table (not on the column headings) and choose "Change columns shown" from the drop-down menu. The columns shown are arranged by dye in the subsequent window. To rearrange the order of the columns shown on the Sample

Details report, click and drag the column header horizontally to the desired location.

4.1.10.13.2.2 Sort the samples into column order (if desired) by clicking on the top of the "Location" column.

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4.1.10.13.2.3 Highlight all of the samples and standards. Click on the printer icon in the upper right corner to print the report.

4.1.10.14 Generation of the Quantitation Report (Form 210-F504).

4.1.10.14.1 If the quantitation report (Form 210-504) will not be printed (see 4.1.10.12), skip to step 4.1.10.15.

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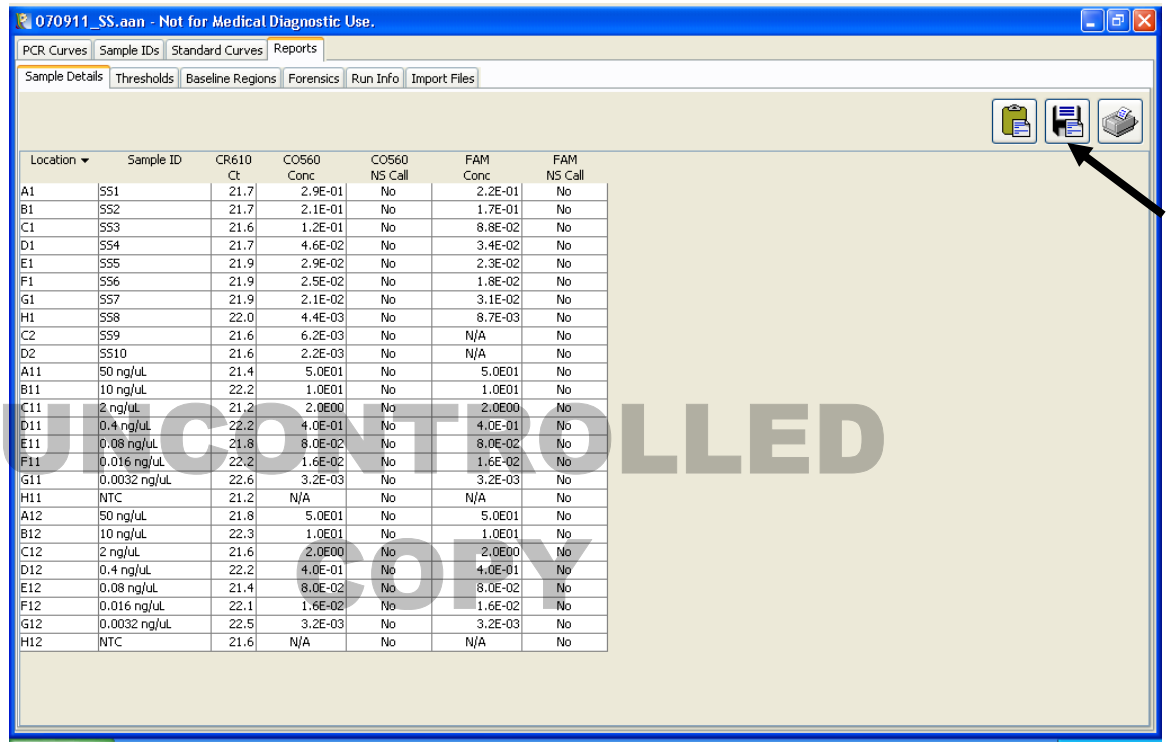
4.1.10.14.2 In the Plexor Analysis software, navigate to the Sample Details tab. Make sure that only the correct columns are showing and in the correct order (Location, Sample ID, CR610 Ct, CO560 Conc, CO560 NS Call, FAM Conc, FAM NS Call). To change the columns shown, right-click on the table (not on the column headings) and choose "Change columns shown" from the drop-down menu. The columns shown are arranged by dye in the subsequent window. To rearrange the order of the columns shown on the Sample Details report, click and drag the column header horizontally to the desired location.

4.1.10.14.3 Sort the samples into column order (if desired) by clicking on the top of the "Location" column.

4.1.10.14.4 Click on the icon in the upper right corner that is called the "Export Selected" icon as indicated in Figure 10.



Location	Sample ID	CR610 Ct	CO560 Conc	CO560 NS Call	FAM Conc	FAM NS Call
A1	SS1	21.7	2.9E-01	No	2.2E-01	No
B1	SS2	21.7	2.1E-01	No	1.7E-01	No
C1	SS3	21.6	1.2E-01	No	8.8E-02	No
D1	SS4	21.7	4.6E-02	No	3.4E-02	No
E1	SS5	21.9	2.9E-02	No	2.3E-02	No
F1	SS6	21.9	2.5E-02	No	1.8E-02	No
G1	SS7	21.9	2.1E-02	No	3.1E-02	No
H1	SS8	22.0	4.4E-03	No	8.7E-03	No
C2	SS9	21.6	6.2E-03	No	N/A	No
D2	SS10	21.6	2.2E-03	No	N/A	No
A11	50 ng/uL	21.4	5.0E01	No	5.0E01	No
B11	10 ng/uL	22.2	1.0E01	No	1.0E01	No
C11	2 ng/uL	21.2	2.0E00	No	2.0E00	No
D11	0.4 ng/uL	22.2	4.0E-01	No	4.0E-01	No
E11	0.08 ng/uL	21.8	8.0E-02	No	8.0E-02	No
F11	0.016 ng/uL	22.2	1.6E-02	No	1.6E-02	No
G11	0.0032 ng/uL	22.6	3.2E-03	No	3.2E-03	No
H11	NTC	21.2	N/A	No	N/A	No
A12	50 ng/uL	21.8	5.0E01	No	5.0E01	No
B12	10 ng/uL	22.3	1.0E01	No	1.0E01	No
C12	2 ng/uL	21.6	2.0E00	No	2.0E00	No
D12	0.4 ng/uL	22.2	4.0E-01	No	4.0E-01	No
E12	0.08 ng/uL	21.4	8.0E-02	No	8.0E-02	No
F12	0.016 ng/uL	22.1	1.6E-02	No	1.6E-02	No
G12	0.0032 ng/uL	22.5	3.2E-03	No	3.2E-03	No
H12	NTC	21.6	N/A	No	N/A	No

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Figure 10. Sample details tab of the Plexor HY reports. The arrow points to the "Export Selected" icon for exporting Plexor HY data to a tab-delimited format for preparing the report for the casefile.

4.1.10.14.5 Save the file when prompted.

4.1.10.14.6 Open Microsoft Excel. Choose File>Open and browse to the file saved in a designated folder to import the tab-delimited data into Excel. (Note: it may be necessary to change the "Files of Type:" to "All Files" for the exported data file to be observed).

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4.1.10.14.7 At the prompt, choose "Finish" as shown in Figure 11.

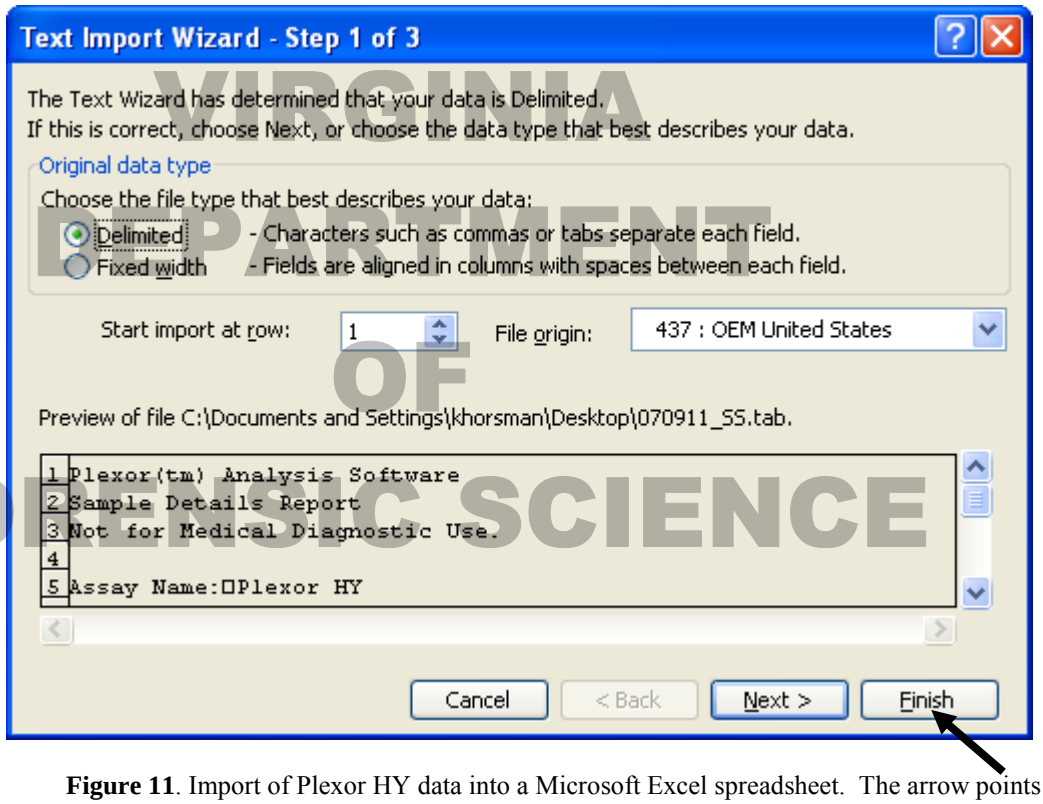

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Figure 11. Import of Plexor HY data into a Microsoft Excel spreadsheet. The arrow points to the "Finish" button to complete the data import.

4.1.10.14.8 On the spreadsheet, click <CTRL-p> to generate the Plexor HY report for the casefile.

4.1.10.14.9 The report may be printed via File>Print. The print settings may be altered using File>Page Setup, if desired.

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4.1.10.15 The "CR610, Ct" column is a flag for whether inhibition of a sample may be observed via the IPC, as determined by whether the cycle at which the IPC crossed the threshold was within the expected result. The IPC should cross the threshold at a similar cycle number in each sample with a degree of tolerance between samples. If a given sample's IPC C_T is not within 2 cycles of those observed in the standard curve, this may be an indication of inhibition by the sample/sample matrix. This range is typically 21.5 ± 2 cycles. Routinely, these samples are amplified for autosomal STRs regardless of whether inhibition is indicated. However, given this information, the examiner may choose to amplify a dilution of the sample to overcome the potential inhibition. In addition, if a sample is believed to contain PCR inhibitors, a Microcon® 100 may be utilized to improve typing results.

4.1.10.16 The "NS Call" column is an indication of whether non-specific amplification is potentially observed in the melt curve for the sample. Any melt observed outside of the expected melt window (80.2 ± 2.0°C for autosomal and 81.7 ± 2.0°C for Y) and of sufficient magnitude to

cross the threshold will result in a "YES" in the "NS Call" column. Routinely, these samples are amplified for autosomal STRs regardless of whether non-specific amplification is indicated. If a "YES" is observed in several samples (greater than 5 that have concentrations of greater than 50 pg/µL), this could be an indication of poor reagents or thermocycling conditions. If this occurs, QC measures should be taken to ensure the instrument and reagents are functioning properly.

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4.1.10.17 Saving the Plexor Analysis File. Under File, select Save As and give the Plexor® Analysis file the same filename as used for the qPCR setup and *.txt export. This will not overwrite the previous files, as it has a *.aan extension. This file should be saved to a designated folder in the computer for future reference or use. If the Normalization Wizard will be used, the quantitation data can be exported to a file (as described in 4.1.10.18) that can be imported into the Normalization Wizard.

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4.1.10.18 Export of quantitation data to a file for import into the Normalization Wizard.

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4.1.10.18.1 Under the "Forensics" drop-down window, select "IPC and Normalization Wizard Parameters"and make certain that the correct dyes are selected for each of the targets (e.g.FAM for autosomal) and then click OK. In the Plexor® Analysis Software, choose "Forensics" and "Export Concentrations in 96 Well Plate Format" and "Export FAM – Autosomal Concentrations". (If amplification setup based on the Y chromosome quantitation is desired, choose "Export CO560 – Y Chromosome Concentrations"). A dialog box will appear asking for a filename. Save this file with an appropriate filename on the network or if the Plexor computer is directly linked to the Biomek computer, to the appropriate designated folder on the C-drive of the Biomek robot. This data can then be accessed from the Biomek Workstation computers for the Normalization Wizard.

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4.1.10.18.2 Once the data has been exported to a format recognizable by the Normalization Wizard, proceed to step 5.5 in Chapter 5 of the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section IV, BioMek® 2000 Automation Workstation Procedures Manual which directs the user how to import the quantitation data file into the Normalization Wizard.

4.1.10.19 In the Stratagene data collection software, ensure that the lamp is turned off by looking at the indicator in the bottom right hand corner of the screen. Close out the Plexor® Analysis Software and Stratagene data collection software. If prompted to save the changes, click "YES". The instrument can then be turned off.

4.1.10.20 Proceed to section 4.1.11 once the quantitation data are returned to the examiners for additional testing for Y-STR typing.

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4.1.11 Using Plexor® Quantitation Data for Y-STR Typing Decisions

4.1.11.1 Examine the Plexor® HY Quantiation System data during or after PowerPlex® 16 BIO typing of casework samples.

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4.1.11.2 Use the Decision Tree, shown in Figure 12 to determine if Y-STR typing should be performed.

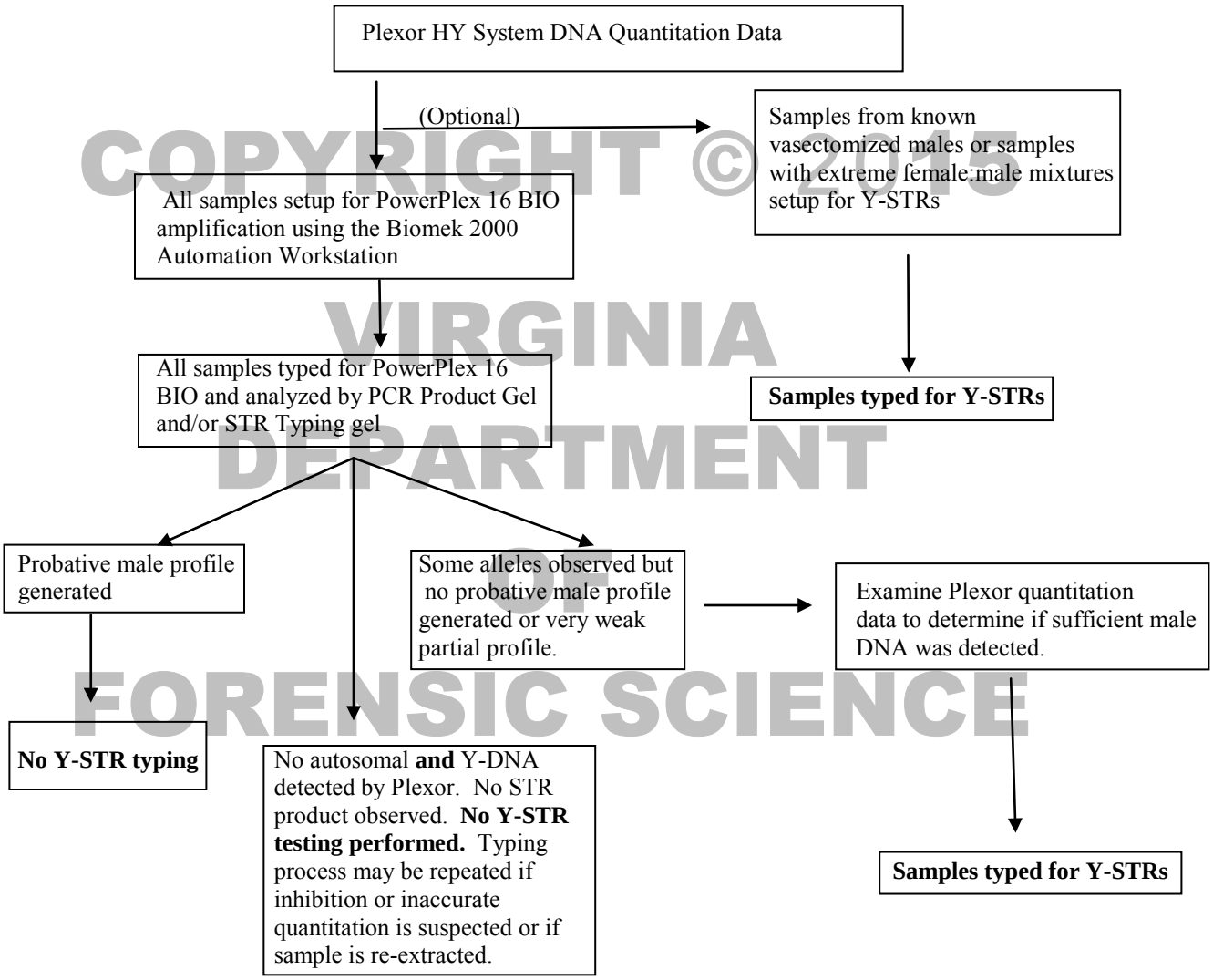

Figure 12. Decision Tree For Y-STR Typing

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4.2 AluQuant Human DNA Quantitation System

4.2.1 Technical Notes

4.2.1.1 The AluQuant® System utilizes a solution hybridization approach instead of hybridization to immobilized DNA on a membrane. Light is generated (luciferase reaction) proportional to the quantity of human DNA present that can be quantitated using a luminometer. Human DNA can be accurately quantitated in the range of 20 pg/µL to 4 ng/µL in the DNA sample being analyzed.

4.2.1.2 The AluQuant® Human Quantitation System uses a probe specific to human repetitive sequence elements to distinguish human from non-human DNA.

4.2.1.3 Because the target sequence and probe length is small and the hybridization of the AluQuant® probe to the target DNA takes place in solution and not on a solid support, the AluQuant®Human Quantitation System is relatively unaffected by DNA degradation.

4.2.1.4 Purified DNA is chemically denatured at high pH to allow access of the AluQuant® probe to its complementary target sequence. Following denaturation, the samples are neutralized. The AluQuant® Probe Mix (a buffered solution containing the AluQuant® probe) and the AluQuant® Enzyme Solution are added, and the samples are incubated at the 55°C annealing/reaction temperature. The AluQuant® Enzyme Solution contains two enzymes that carry out a coupled enzymatic reaction using the annealed AluQuant®probe as a substrate to generate ATP. The first enzyme (READase™ Polymerase) is a DNA polymerase that catalyzes a reverse polymerization reaction on the 3´ end of the perfectly annealed AluQuant® probe in the presence of excess inorganic pyrophosphate to generate free dNTP (Figure 13, Section A). This process is also known as pyrophosphorylation. The second enzyme (READase™ Kinase) transfers the gamma phosphate from the liberated dNTPs to ADP, which is also present in the enzyme cocktail, to generate ATP (Figure 13, Section B).

Because the pyrophosphorylation reaction carried out by the READase™ Polymerase requires a perfect match at the 3´ end of the AluQuant® probe/target DNA hybrid, dNTPs are only generated when this probe is annealed to human DNA, or a very close higher primate. Nonhuman DNA will not anneal to the AluQuant® probe or will have mismatches near the 3´ end of the probe/target hybrid, which preclude the probe's use as a substrate in the pyrophosphorylation reaction, resulting in the generation of very little dNTP and ultimately ATP. In the presence of excess luciferin, firefly luciferase uses the ATP generated in the coupled AluQuant® reaction to generate light (measured in Relative Light Units, RLU). Refer to Figure 13. As ATP is limiting in this reaction, the amount of light generated is proportional to the amount of ATP present and consequently the amount of human DNA present. The amount of human DNA present in the purified DNA sample is then determined by comparing the net light signal from the unknown to a standard curve with known amounts of human DNA.

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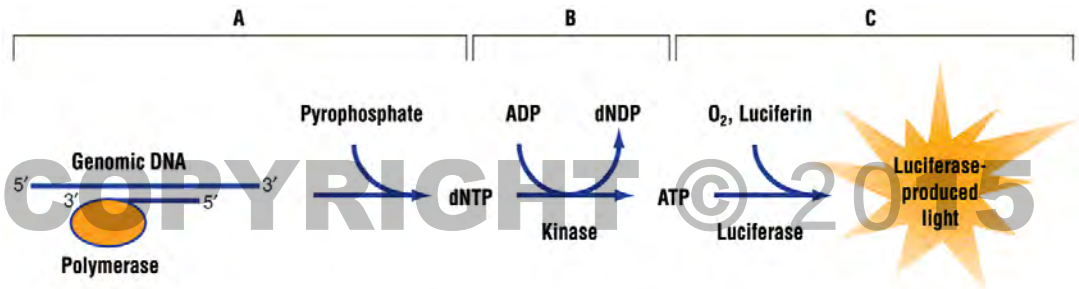

Figure 13. **Chemical pathway of the AluQuant® Human DNA Quantitation System.** Section A illustrates the pyrophosphorylation reaction. Section B illustrates the transfer of the terminal phosphate group from the released dNTP to ADP by the READase™ Kinase to form ATP. Section C illustrates the production of light by luciferase and ATP.

4.2.1.5 Gloves must be worn at all times when performing the AluQuant® Human DNA Quantitation reactions because nucleases may be shed from the fingertips and these nucleases would interfere with the reaction.

4.2.1.6 The AluQuant® Calculator (refer to Figure 14) is used in conjunction with the AluQuant® Human Quantitation System and the BioMek® 2000 Automation Workstation to estimate the concentration of DNA isolated.

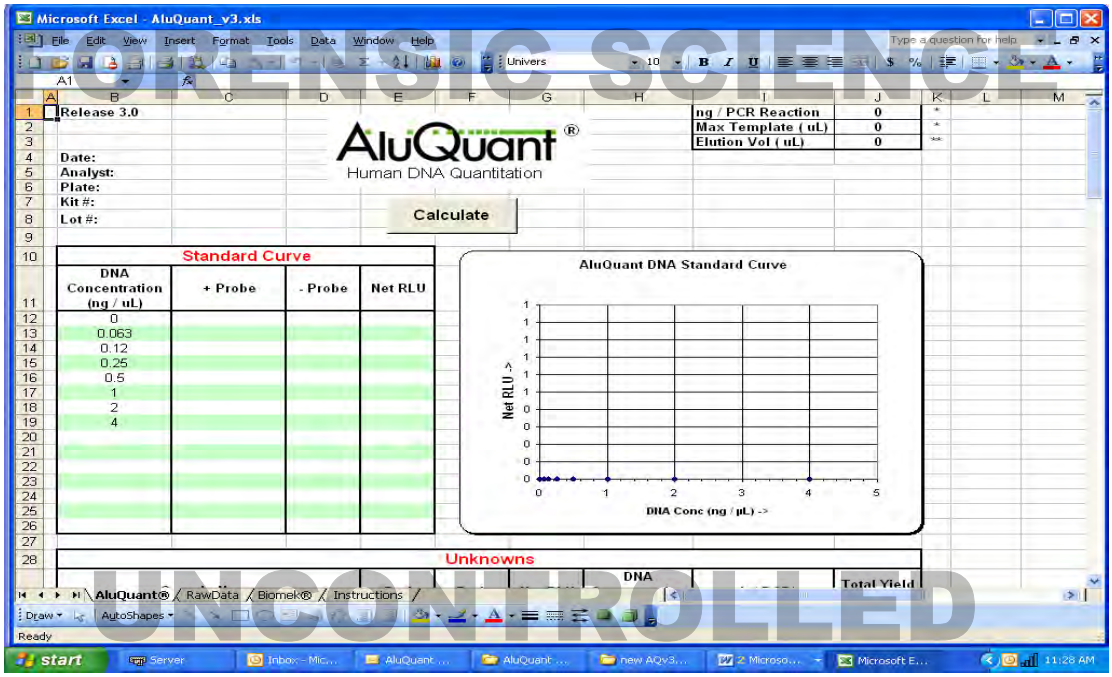

Figure 14. **Calculation of DNA Concentrations with the AluQuant® Calculator.** The RLU value is the signal as determined by the AluQuant® Calculator for both the standard curve and the unknown DNA samples. After pressing the "Calculate" button, the AluQuant® Calculator calculates a nonlinear curve of best fit for the DNA standards and then uses this to determine the DNA concentration of the unknown DNA samples. If the desired mass of template DNA per amplification reaction and elution volume of the DNA sample are specified, the AluQuant® Calculator will also determine the volume of each DNA sample required per PCR as well as the total yield of DNA.

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4.2.2 Equipment

4.2.2.1 96 well Heat block, water bath or dry incubator/oven

4.2.2.2 Luminoskan luminometer

4.2.2.3 8 well multichannel pipettes (0.5-10 µL, 10-50 µL, 50-300 µL)

4.2.3 Materials

4.2.3.1 Thin wall 96 well PCR plates – Robbins Scientific Cat# 1055-00-0

4.2.3.2 Gray labware holder and black tip box holder

4.2.3.3 Black PCR support base (96 well) – ABI Cat# N801-0531

4.2.3.4 White luminometer plates – Promega Cat # Z3291

4.2.3.5 RT-L200F (aerosol resistant) pipette tips

4.2.3.6 RT-L10F (aerosol resistant) pipette tips

4.2.3.7 Clear plastic reservoir holder – Beckman Cat# P2-11-15

4.2.3.8 Sticky foil plate cover

4.2.3.9 Applied Biosystems Micro Amp Strip Tubes – N801-0580

4.2.3.10 Metal strip tube holders – Promega Cat # Z3341

4.2.3.11 Gloves

4.2.4 Reagents

4.2.4.1 AluQuant® Human DNA Quantitation kit

4.2.4.2 AluQuant® Enzyme Solution

4.2.4.3 Denaturation Solution

4.2.4.4 AluQuant® Neutralization Solution

4.2.4.5 AluQuant® Probe Mix

4.2.4.6 Nuclease-Free Water

Type I water is considered nuclease free and may be used if the nuclease free water that comes with the AluQuant® kit is consumed before the remaining reagents in the kit.

4.2.4.7 Hydrochloric Acid (250 mM)

4.2.4.8 Human Genomic DNA Standard (20 ng/µL)

4.2.4.9 ENLITEN® Luciferase/Luciferin (L/L) Reagent

4.2.4.10 ENLITEN® Luciferase/Luciferin (L/L) Reconstitution Buffer

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4.2.5 AluQuant® Human Quantitation Procedure

Reagents used for the AluQuant® Human Quantitation System come prepared for use except for the ENLITEN® Luciferase/Luciferin (L/L) reagent and the HCl. It is critical for optimal performance that both the L/L reagent and the ENLITEN® Reconstitution buffer equilibrate to room temperature prior to use. Since the reagents are stored frozen, this takes approximately 1.5 hours. The remaining reagents are simply thawed before use. Unused reconstituted L/L reagent may be frozen at -20 ºC either in the brown bottle or dispensed into another container and protected from light. Frozen reconstituted L/L reagent will show a decrease in RLU (relative light units) values.

It has been demonstrated that the HCl reagent provides improved performance if it is diluted from the 250 mM concentration to 200 mM.

4.2.5.1 Remove the AluQuant® Human Quantitation System reagents from the -20 ºC and allow them to thaw prior to use.

4.2.5.1.1 It is imperative that the ENLITEN® Luciferase/Luciferin (L/L) reagents are protected from light and are allowed to equilibrate to room temperature prior to use. The L/L reagent comes dried in a brown bottle. The Reconstitution buffer is added to the L/L reagent in the brown bottle and the bottle inverted several times to mix.

4.2.5.1.2 Once the AluQuant® Enzyme Solution has thawed, place it on ice prior to dispensation into the appropriate strip tubes on the robot deck.

4.2.5.1.3 The HCl reagent is diluted prior to use. Refer to Appendix B, Reagents, for directions on the dilution of the HCl reagent.

4.2.5.1.4 Follow the procedure outlined in Appendix B for preparing a serial dilution of Human Genomic DNA (20 ng/µL) for use as standards. Refer to the table below for the concentration of DNA used for each Genomic DNA Standard.

NOTE: The Human Genomic DNA Standards should be prepared fresh each day of use.

Genomic DNA Standards

Concentration (ng/µL)	DNA (ng/5 µL)
4	20
2	10
1	5
0.5	2.5
0.25	1.25
0.125	0.625
0.062	0.31

4.2.5.2 Using the table below, determine the appropriate amount of reagent to pipette into the strip tubes given the number of columns of DNA samples that will be quantitated.

Note: volumes include reagents for the standards.

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Reagent	2 columns	3 columns	4 columns	5 columns	6 columns
Probe	25 µL	30 µL	35 µL	40 µL	45 µL
Neutralization solution	35 µL	40 µL	45 µL	50 µL	55 µL
HCl	40 µL	46 µL	52 µL	60 µL	66 µL
Denaturation solution	35 µL	35 µL	40 µL	40 µL	40 µL
Enzyme solution	65 µL	70 µL	80 µL	90 µL	100 µL

Reagent	7 columns	8 columns	9 columns	10 columns	11 columns
Probe	50 µL	55 µL	60 µL	65 µL	70 µL
Neutralization solution	60 µL	65 µL	70 µL	75 µL	80 µL
HCl	72 µL	78 µL	84 µL	90 µL	100 µL
Denaturation solution	40 µL	50 µL	50 µL	60 µL	60 µL
Enzyme solution	110 µL	120 µL	130 µL	140 µL	150 µL

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4.2.5.3 Using clean gloves obtain 5 columns of the Micro Amp strip tubes and push the tubes firmly into the metal strip tube holders. The strip tube holder will fit into notches in the plastic reservoir holder. Two strips, one on the left and the other on the right will be placed into the first strip tube holder which sits in the position of the first reservoir. The second reservoir position will have only one strip of tubes, pushed into the column on the right. The third reservoir position will have two strips, one on the left and the other on the right, pushed into the strip tube holder. Tubes must be pushed all the way in and all of the tubes should be even with each other. Each tube will contain the volume indicated above for the reagent specified for that column of tubes, given the number of columns of DNA samples to quantitate. Probe solution will be pipetted into the first column, Neutralization solution will be pipetted into the second column. The third column is unused. HCl is pipetted into the forth column. Denaturation solution is pipetted into the fifth column and Enzyme solution is pipetted into the sixth column. See Figure 15 below:

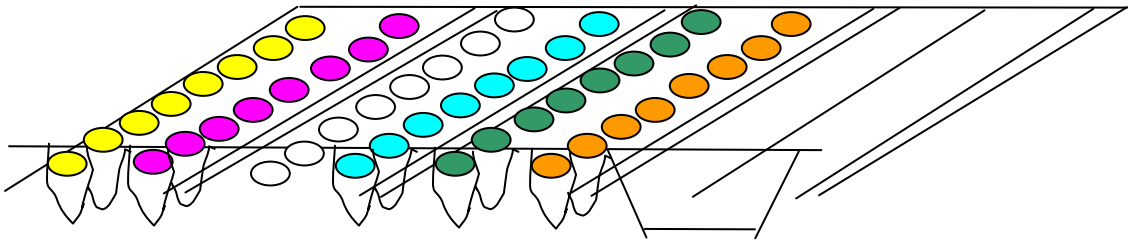

Figure 15. Arrangement of strip tubes in the strip tube holders.

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4.2.5.4 Transfer 40 µL of each DNA standard into a clean column of strip tubes immediately adjacent to the last column of DNA samples located in the PCR base at position A3. Load the DNA standards starting with the most concentrated DNA sample in position H, to the least concentrated DNA sample in position B. A TE^{-4} or nuclease free water blank is placed into position A.

4.2.5.5 Place a clean Robbins 96 well PCR plate into positions B2 and B3 on the BioMek® 2000 Automation Workstation as is indicated in the diagram below (Figure 16).

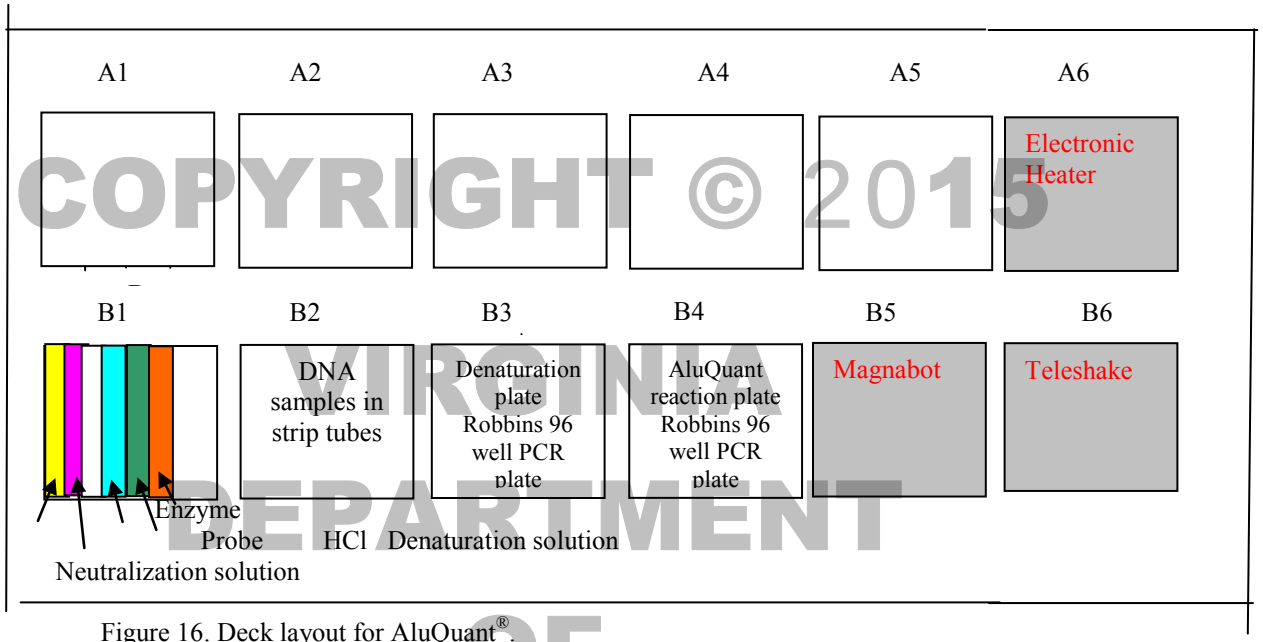

Figure 16. Deck layout for AluQuant®.

4.2.5.6 Based upon the number of samples and the column of DNA standards that will be quantitated, add 3 µL of AluQuant® Denaturation Solution using a multchannel pipettor into the appropriate number of columns of the Robbins 96 well PCR plate located at position B2. The last column of DNA samples will contain the Human DNA standards.

4.2.5.7 Using a multichannel pipettor, transfer 6 µL of isolated DNA (at position B2) and Human Genomic DNA Standards in the last column to the corresponding positions in the Robbins PCR plate at position B3 containing the AluQuant® Denaturation solution. Change tips after each column of DNA samples have been transferred. Take care to maintain the positions of the DNA samples. For example, a DNA sample loaded into a strip tube in the rack at B2 in row C in column 3 should be in well C3 in the Robbins PCR plate.

4.2.5.8 Incubate the mixture for 10 minutes at room temperature.

4.2.5.9 Using a multichannel pipettor, pipette 6 µL of HCL to each of the denaturation tube reactions in the Robbins PCR plate in position B3 after the 10 minute incubation. Pipette up and down 6 times after the transfer to mix. Change tips with each transfer/mix of the HCL.

4.2.5.10 Using a multichannel pipettor, transfer 10 µL of the denatured DNA samples in the Robbins PCR plate in position B3 into the same column positions in the new Robbins 96 well plate located in position B4 (refer to Figure 16). Change tips after each column is transferred.

4.2.5.11 Using a multichannel pipettor, pipette the appropriate volume of Neutralization solution into the strip tubes containing the Probe solution in the first column of strip tubes in the strip tube holder in position B1. Pipette up and down several times to mix. Change tips and pipette the appropriate volume of Enzyme solution into the strip tubes containing the Probe/Neutralization mixture. See table below for the appropriate volumes.

Reagent	**2 columns**	**3 columns**	**4 columns**	**5 columns**	**6 columns**
Neutralization solution	25 µL	30 µL	35 µL	40 µL	45 µL
Enzyme solution	50 µL	60 µL	70 µL	80 µL	90 µL

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Reagent	7 columns	8 columns	9 columns	10 columns	11 columns
Neutralization solution	50 µL	55 µL	60 µL	65 µL	70 µL
Enzyme solution	100 µL	110 µL	120 µL	130 µL	140 µL

4.2.5.12 Using an 8 well multichannel pipette transfer 20 µL per well of the AluQuant® Enzyme/Probe master mix located in the Probe strip tubes in position B1 to each of the denatured DNA samples located in the Robbins PCR plate at position B4. Using the 8 well multichannel pipette, briefly pipette up and down to mix the solution. Change tips between each column of denatured DNA sample.

4.2.5.13 Check for bubbles in PCR plate prior to covering. Carefully remove bubbles with a clean, unused pipette tip if observed.

4.2.5.14 Cover the 96 well PCR plate with a sticky foil plate cover and then place the plate into a 55ºC heat block, incubator or water bath (taking care that the water does not reach the top of the plate) for 1 hour.

4.2.5.15 Prepare the luminometer for use near the end of the 1 hour incubation period as follows. Refer to the diagram of the luminometer below (Figure 17):

4.2.5.16 Open the Ascent software program which will either be a desktop icon or will be located in the AluQuant® desktop folder on the BioMek® 2000 Automation Workstation computer.

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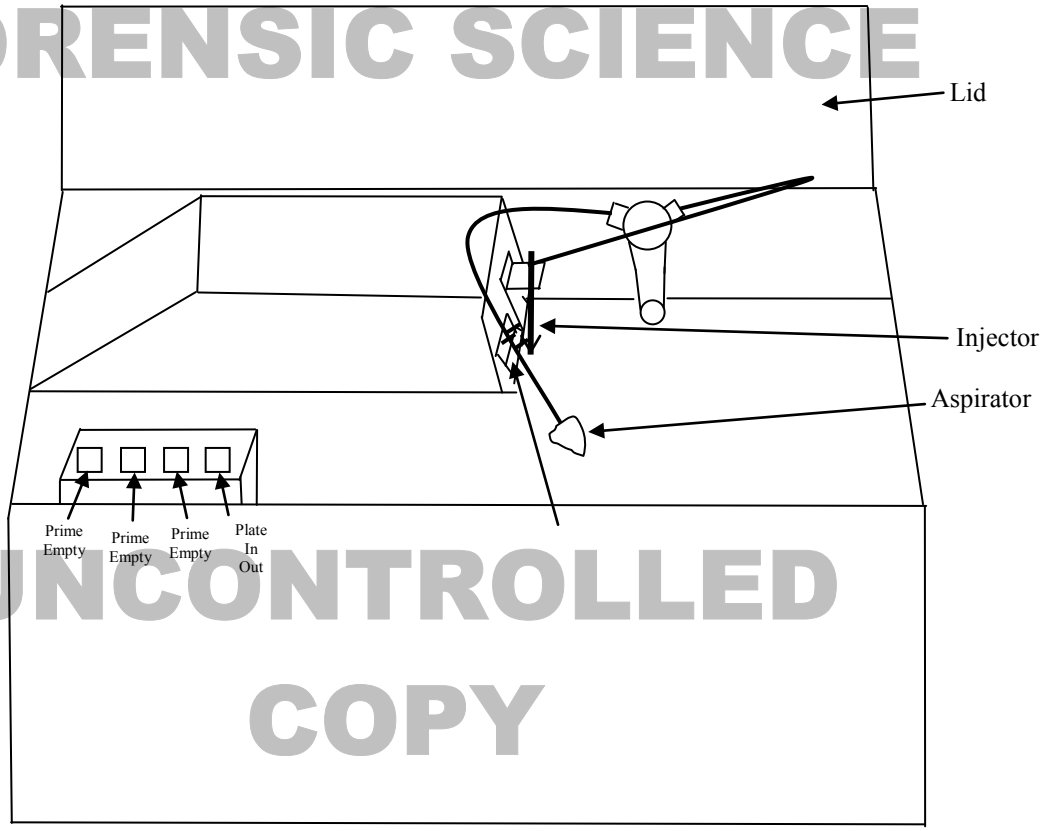

Figure 17. View of Luminoskan luminometer with lid open.

4.2.5.16.1 The following window (Figure 18) will appear which will have a variety of functions listed at the top left.

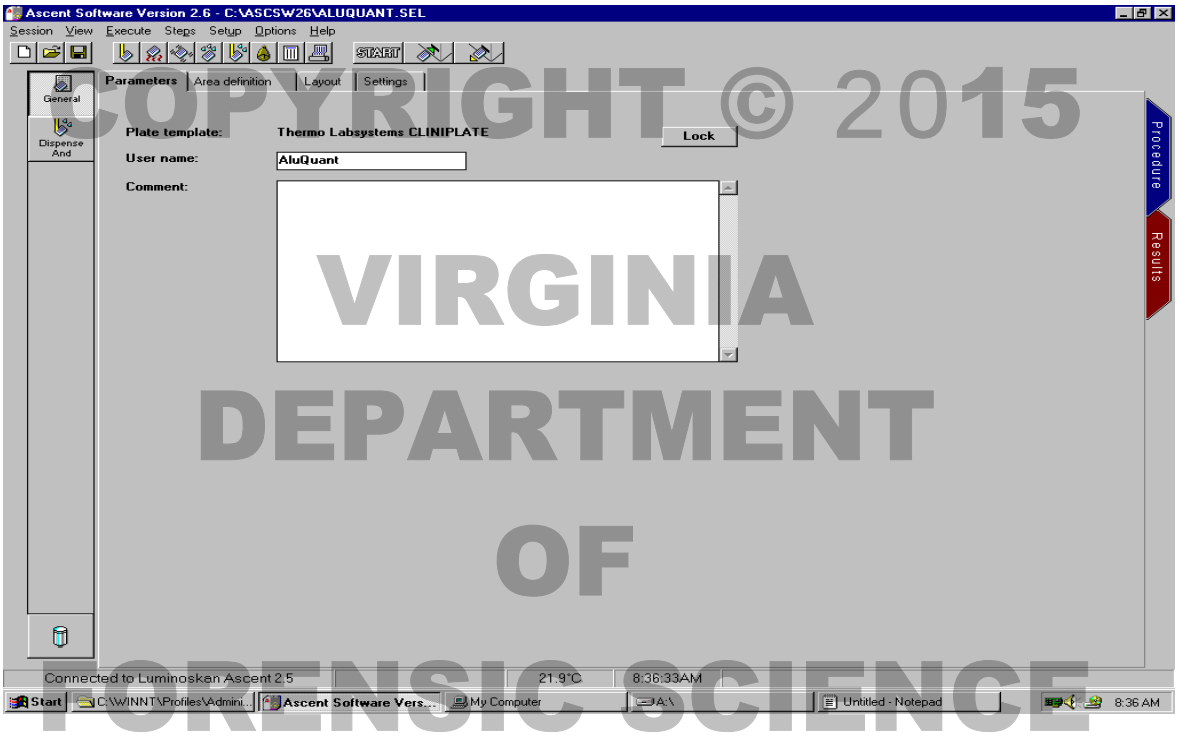

Figure 18. Ascent Session window.

4.2.5.16.2 Using the Session pull-down window, select Open.

4.2.5.16.3 Double click on "AluQuant® Session". A window will open that looks nearly identical to the original Ascent window, except it will have saved settings appropriate for the AluQuant® method.

4.2.5.16.4 Under the Execute pull-down window, select Prime. Another window will open for the priming function.

4.2.5.16.5 Use the prime dispenser #1 and select 2000 µL. Before priming, make certain that a container (eg., the lid from a box of tips) is carefully placed under the injector to catch the liquid. Refer to the diagram of the luminometer above as needed. Take care not to damage the plastic injector tip. The injector can be primed with the injector in the stand or it can be manually held over the container.

4.2.5.16.6 Place the plunger into a bottle of sterile water to rinse out the injector tubing. Click on the prime button for dispenser #1. A window will open saying "please ensure that dispensing heads to be primed are located in external waste vessel or appropriate priming vessel is located in the plate carrier. Continue?" Click "Yes". This message will come up each time the prime function is used.

4.2.5.16.7 Prime once with the L/L reagent using 1200 µL prime volume.

4.2.5.16.8 Remove the injector from its stand and hold it over the L/L reagent bottle. Change the prime volume to 2000 µL and click on dispenser #1. This will remove the air from the lines while not consuming additional L/L reagent since it will be circulated back into the L/L bottle.

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4.2.5.16.9 Carefully slide the dispensing tip into the "M" position for injection. Do not force the injector into the slot. If there is resistance, turn it slightly and continue sliding it in. If there is a black pin in the slot, remove it and slide in the injector, then close the lid. The AluQuant® session may be kept open by minimizing the Ascent session window or the software can be closed out. If the software is closed out, then the AluQuant® session must be re-opened. The luminometer is now ready for use.

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Note: It is best not to prepare the luminometer for use too much in advance of actual usage since air can leak into the tubing and create problems with accurate dispensing of the L/L reagent.

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4.2.6 Once the 1 hour incubation period is over, remove the 96 well PCR plate containing the AluQuant® reactions, peel off the foil seal and place it in biohazard waste.

4.2.6.1 Place a white luminometer plate in position B1 in a gray labware holder on the deck of the BioMek® 2000 Automation Workstation. The white luminometer plate is necessary for optimal detection of the luminescent signal. Using an 8 well multichannel pipette, transfer 25 µL of the samples in exactly the same position from the 96 well PCR plate to a luminometer plate.

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4.2.6.2 Open the lid of the luminometer and push the Plate Out manual button in order to place the white luminometer plate containing the AluQuant® reactions into the luminometer tray. It can also be accomplished using the Ascent software. Maximize the Ascent Session window or if the Ascent program was closed out, open the Ascent software and under the Session drop down window, select Open and then select AluQuant® Session. Once in the AluQuant® Session, click the Plate Out button (upper right).

4.2.6.3 Place the white luminometer plate into the tray and either manually or using the Ascent software (using the Plate in button), command the luminometer to retract the tray containing the luminometer plate.

4.2.6.4 In the AluQuant® session window there well be tabs labeled "Parameters", "Area definition", "Layout", and "Settings" in the upper left (refer to Figure 19). Select the tab labeled "Area Definition" and a window will open depicting the 96 well luminometer plate as diagrammed below. Wells which are highlighted in yellow will be injected with L/L reagent and the light measured.

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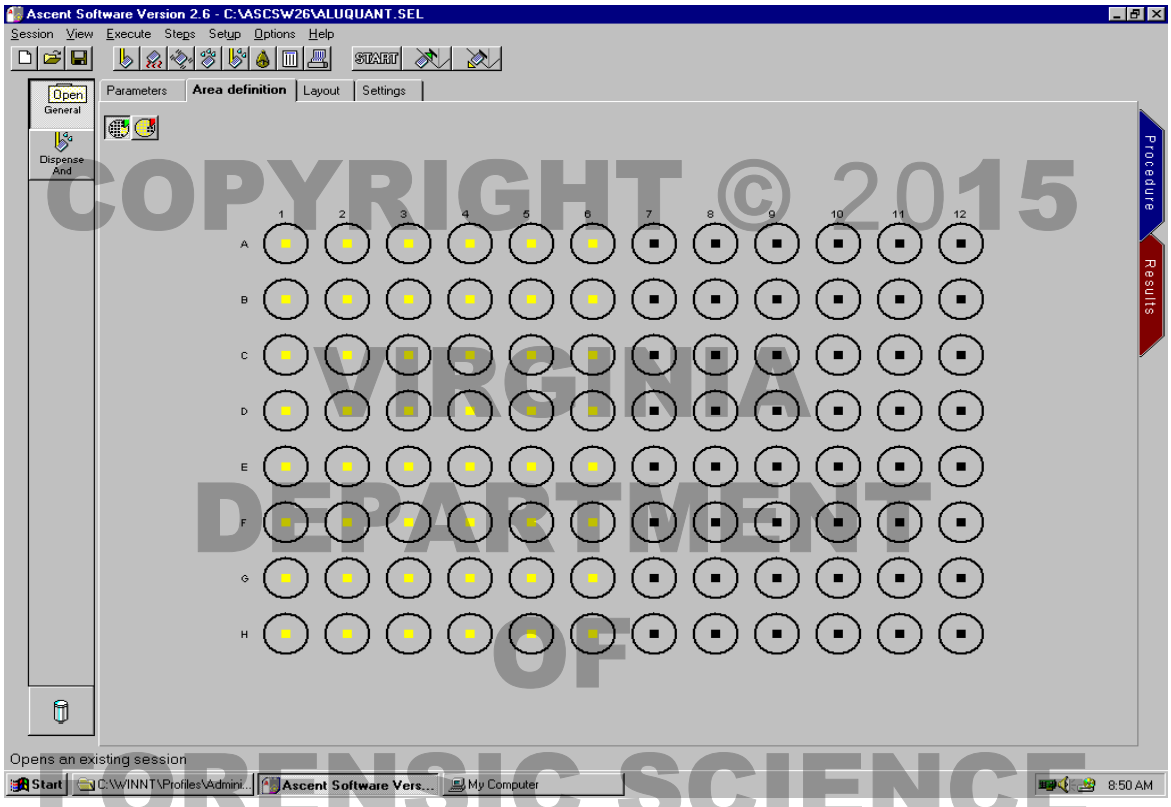

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Figure 19. Defining the wells to inject.

4.2.6.4.1 If a well should be injected, the pie diagram in black with a yellow wedge should be clicked in order to highlight the well yellow.

4.2.6.4.2 If a well should not be injected, the pie diagram in yellow with the black wedge should be clicked to un-highlight the well.

NOTE: Wells can be highlighted by clicking on the individual wells. To highlight many wells or columns, box an area by depressing the mouse while dragging a box around the area to be highlighted (or un-highlighted).

4.2.6.5 Once the appropriate wells have been selected, click on the Start button. Dispense and Measure will begin for the session and a status window will be displayed which shows the wells that have been tested and what percent of all the testing has been completed. The entire process takes several minutes.

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4.2.6.6 Once the session is finished, a window will automatically open displaying the data in a table format. Highlight all the data and then under Edit, select Copy.

4.2.6.7 Minimize the session window.

4.2.6.8 Open the AluQuant® Calculator v3.0 Excel macro by double clicking on the AluQuant.xls program found in the AluQuant® folder or as a desktop icon. Click on the “Enable Macros” button when the window appears. The AluQuant® Calculator will open.

4.2.6.9 Select the tab at the bottom of the screen that reads Raw Data. Another table will open with 12 columns. The odd numbered columns have a “+” over them and the even numbered columns have a “-“ over them. Paste the Ascent software luminometer table data into the Raw Data sheet of AluQuant Calculator v3.0.

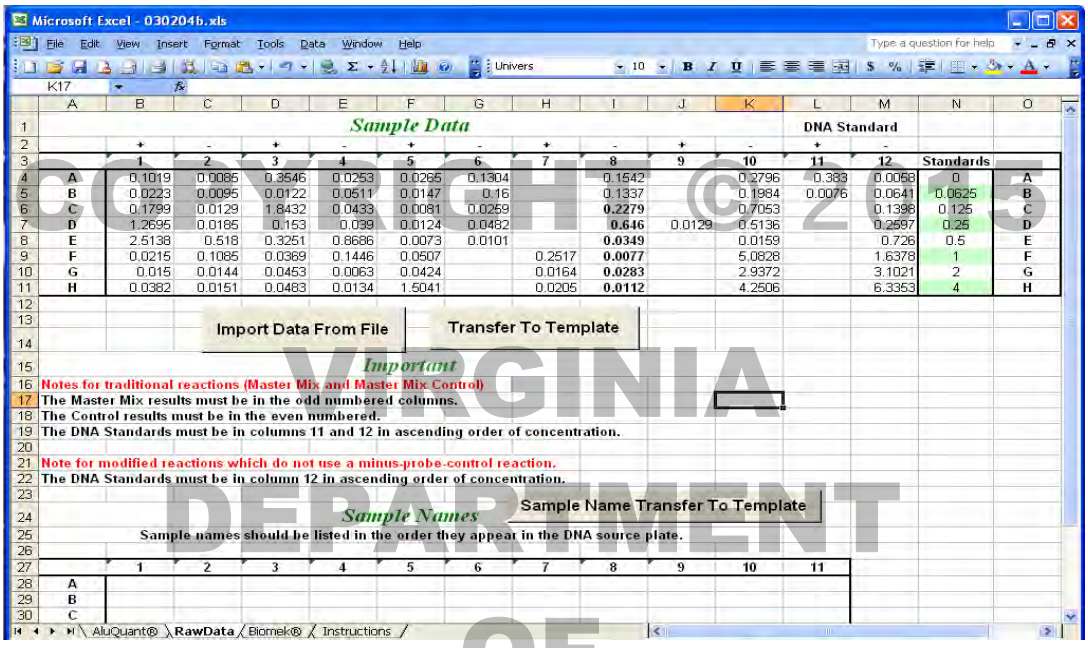

Figure 20. AluQuant® Calculator v3.0.

4.2.6.10 Follow the directions in red shown in Figure 20 that read "Note for modified reactions which do not use a minus-probe-control reaction". Make certain that the standards are pasted into column 12.

4.2.6.11 Click on the "Transfer to Template" button.

4.2.6.12 Another window will pop up shown in Figure 21.

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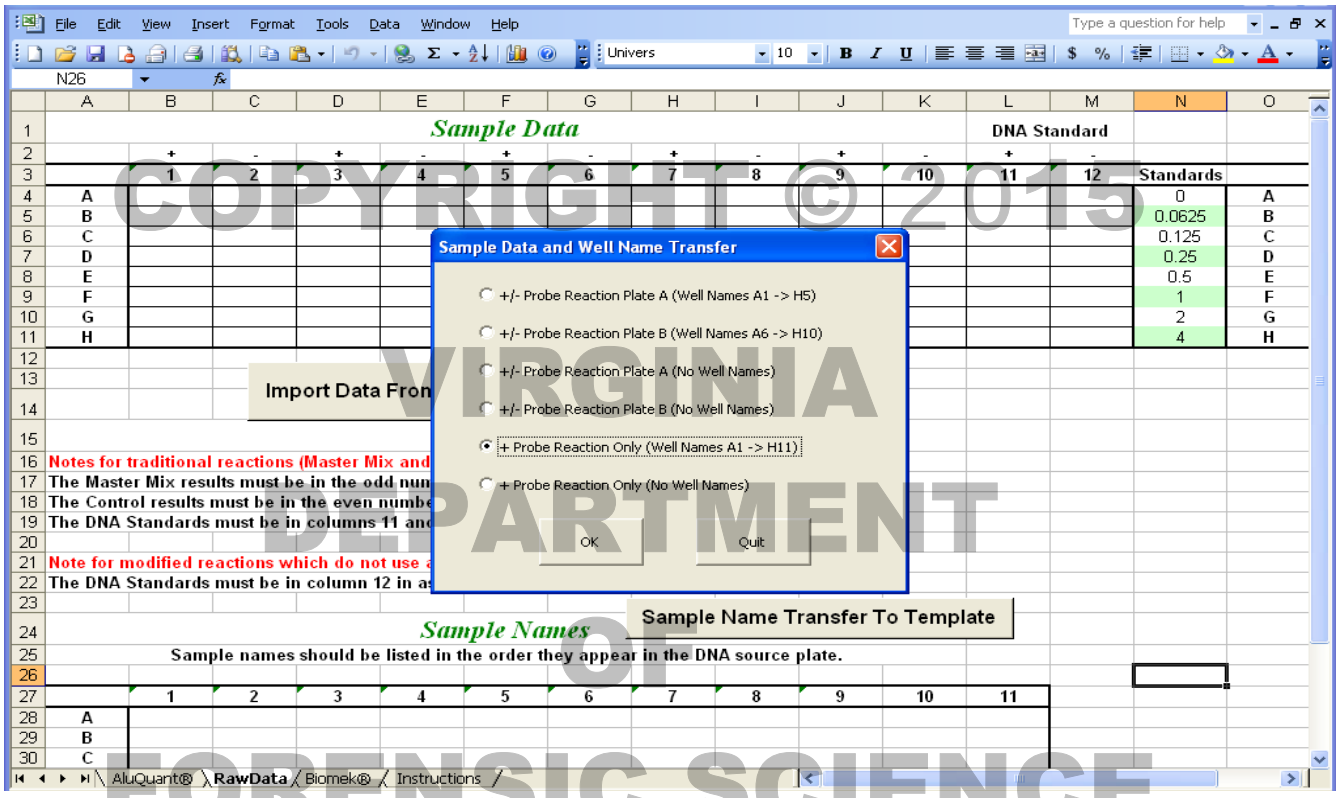

Figure 21. Transferring data to the template.

4.2.6.13 Select the choice indicated in Figure 21; the + Probe Reaction Only (Well Names A1→H11) and click "OK".

4.2.6.14 Select the AluQuant® tab to open the AluQuant® Calculator window.

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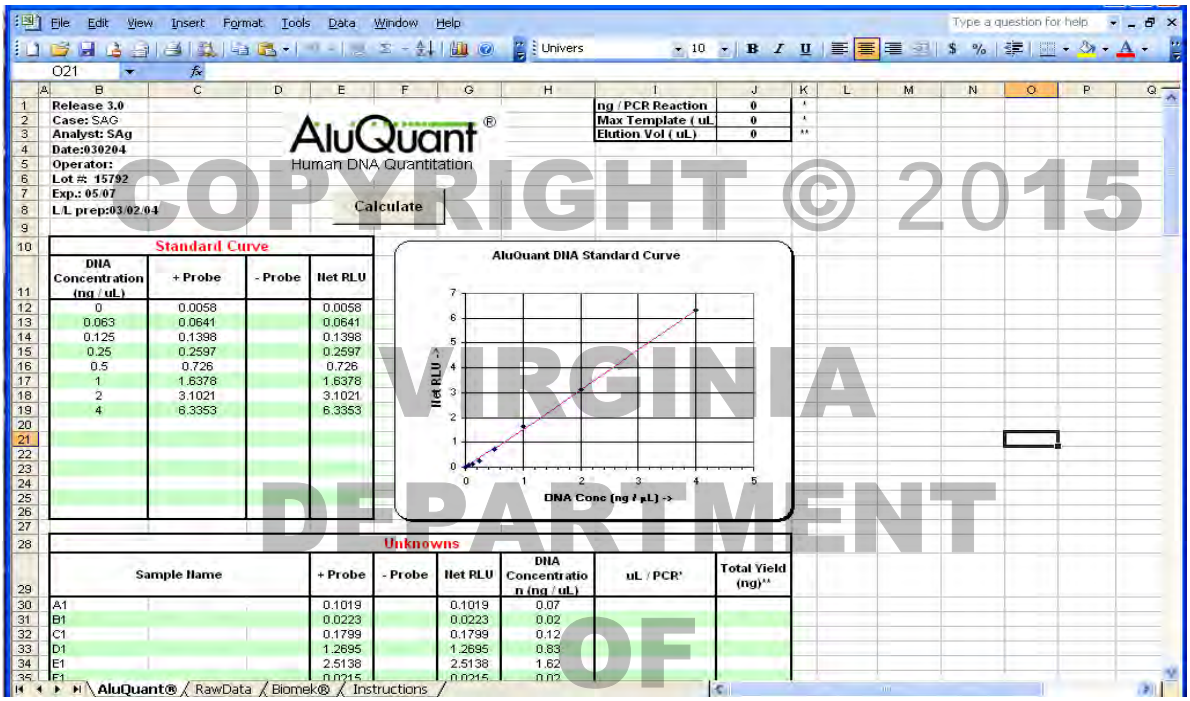

Figure 22. AluQuant® Calculator v3.0

4.2.6.15 Click on the "Calculate" button in the Calculator window.

4.2.6.16 A pink line will appear creating the AluQuant® standard curve in the standard curve box. AluQuant® Calculator v3.0 uses a quadratic formula to generate the standard curve.

NOTE: Unlike AluQuant® Calculator v3.0 will not flag the user with error messages when a standard curve appears problematic since the quadratic formula can fit any curve. The user **MUST** examine the standard curve to determine if any data points should be deleted. An example is provided in the Troubleshooting section for guidance. See a Project Coordinator or Supervisor if data point deletion is necessary.

4.2.6.17 Evaluate the quantitation data making sure that the data make sense, i.e. the reagent blanks display no or very low values, e.g. 0.02 ng/µL. Evaluate the curve generated to determine if any data points need to be deleted to improve the quality of the extrapolated curve generated.Even though the quadratic formula can fit any curve, the same general rules apply to deleting bad data points as for AluQuant® Calculator v2.0. Up to 3 data points can be deleted when necessary to produce a linear standard curve which is at an approximate 45º angle.

4.2.6.18 A copy of the spreadsheet should be printed for each examiner with samples in the AluQuant® run or if batching, print out a single copy.

4.2.6.19 Under File, select Save As and give the AluQuant® Calculator file a new name so as not to overwrite the AluQuant® Calculator spreadsheet. This file should be saved to a designated folder in the computer for future reference or use. At this point or during the use of the Normalization Wizard method outlined in Chapter 5, if the Normalization Wizard will be used, the quantitation data can be transferred to the BioMek® sheet.

4.2.6.20 Tab to the BioMek® sheet by using the tab at the bottom of the spreadsheet (Figure 23).

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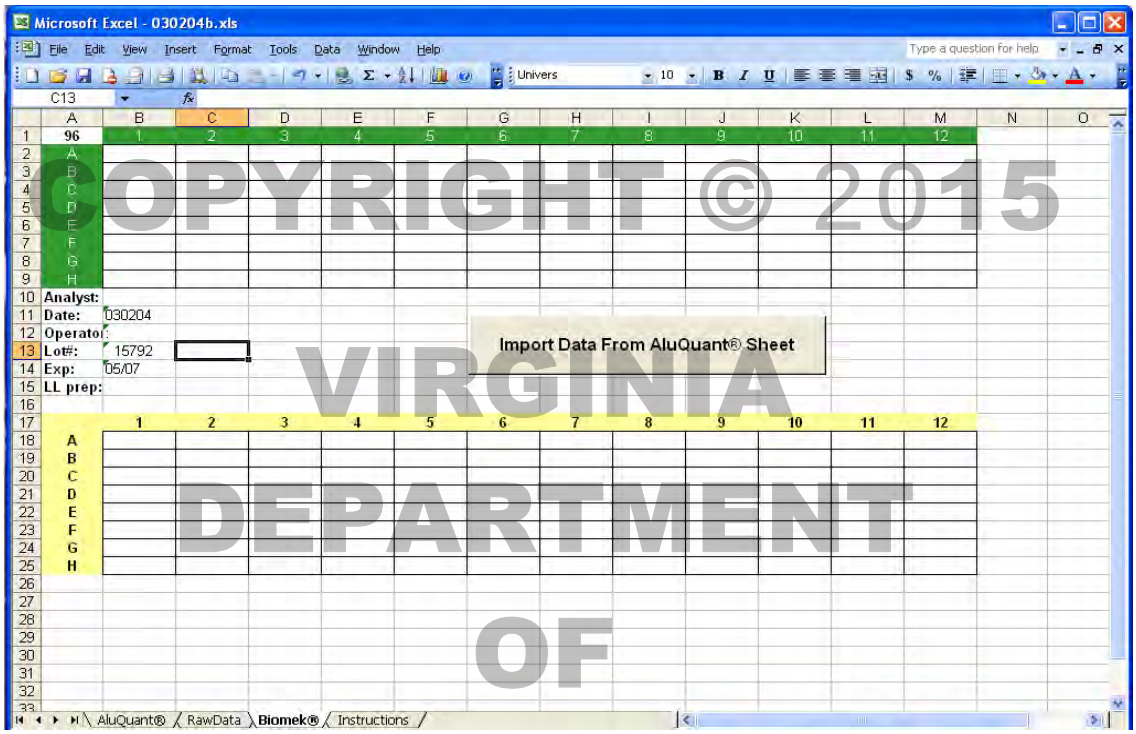

Figure 23. Transferring quantitation data to the BioMek® sheets

4.2.6.21 Click on "Import Data From AluQuant® Sheet" box. The window shown in Figure 24 will pop up. Select the full plate (wells A1→ H11), then select OK. The quantitation data will transfer to the BioMek spreadsheet (refer to Figure 25).

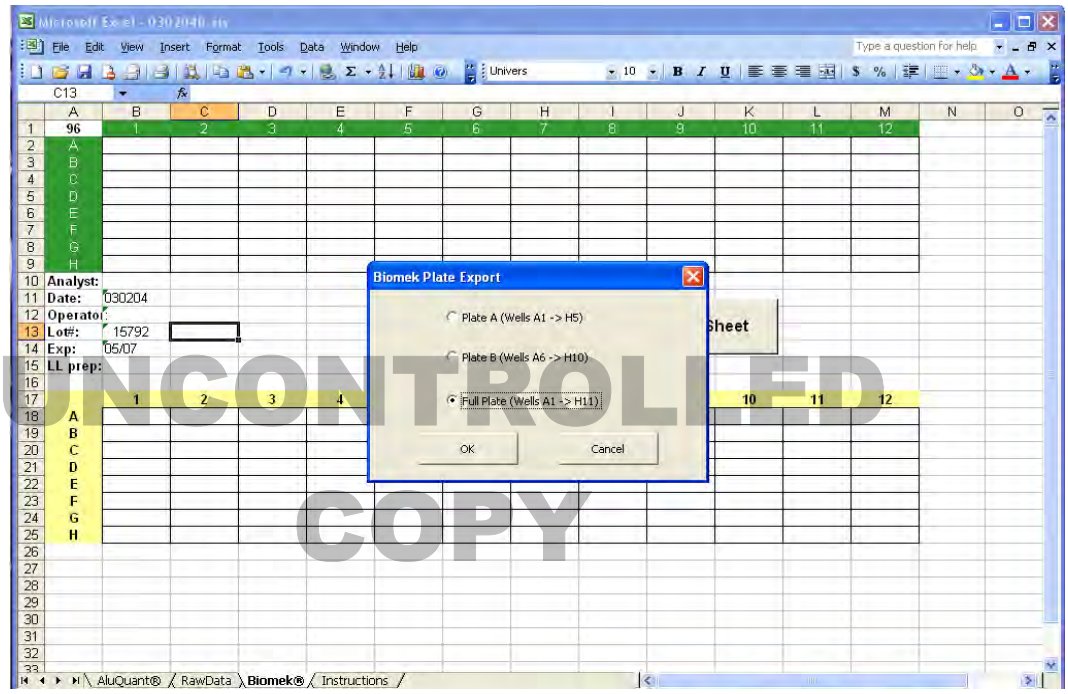

Figure 24. Selecting the plate format for data transfer

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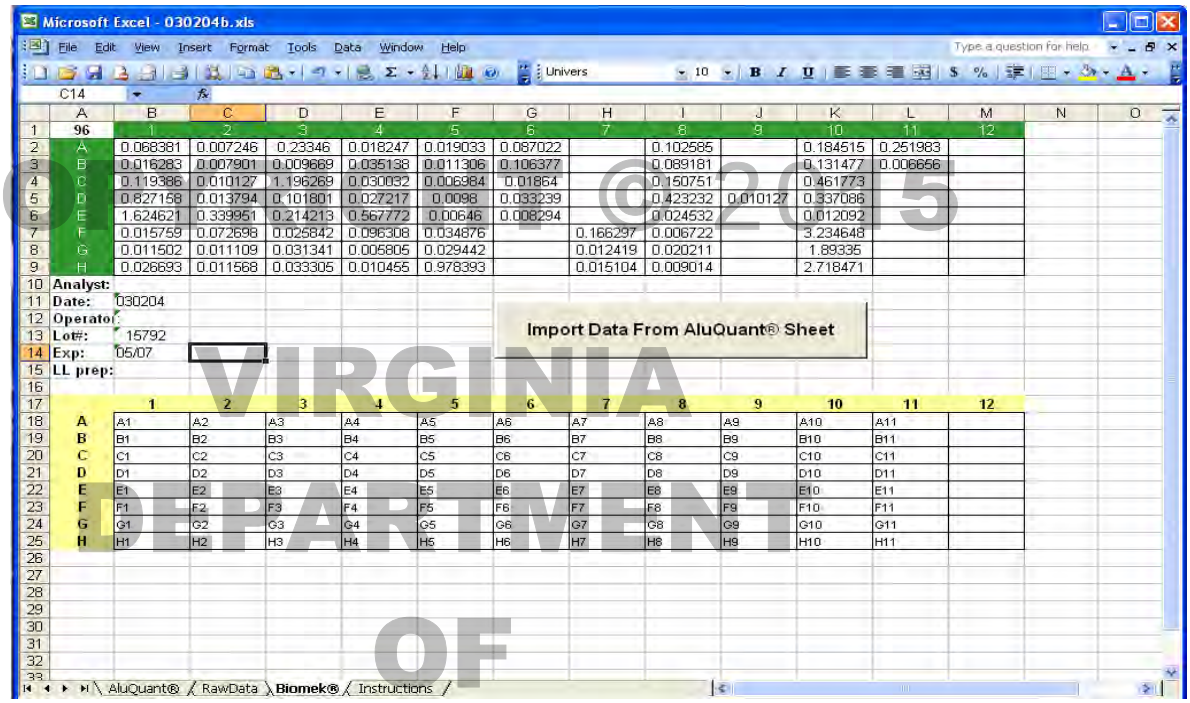

Figure 25. Quantitation data transferred to the BioMek® sheet

4.2.6.22 Save the AluQuant® file using the name designated for the AluQuant® file data and the "Save As" function. The quantitation data can be accessed by the Normalization Wizard directly from the BioMek® sheet of the AluQuant® file once that data has been transferred to the BioMek® sheet.

4.2.6.23 Close out the AluQuant® Calculator spreadsheet. Click "No", when the window pops up asking if changes to the AluQuant® Calculator spreadsheet should be saved.

4.2.6.24 Visually inspect the luminometer plate at the completion of the Dispense and Measure process. Occasionally, the luminometer will fail to inject into a well and the well may need to be re-injected. Also, occasionally the standard curve improves upon re-injection of the entire plate.

4.2.6.24.1 The re-injection should be performed after the AluQuant® Calculator analysis of the data has been performed and the file saved.

4.2.6.24.2 To re-inject a single well (or more than one), the Ascent software must be closed without saving the settings. Re-open the Ascent software and open the AluQuant® Session and repeat the previous steps for injection the wells except that only the wells which failed to be injected the first time need to be injected. There is no need to re-prime the luminometer since it was already primed.

4.2.6.24.3 Select the individual well(s) and start the program.

4.2.6.24.4 Once the table opens with the RLU values, copy and paste into the appropriate position in the previously saved data file and re-calculate that file. Save the new file as described above after filling out the pertinent information.

4.2.6.25 Before closing out the Ascent AluQuant® Session, under Execute, select prime and choose the 2000 µL volume. Carefully remove the injector from the M slot, place it in its stand and place a BioMek tip lid or another disposable container underneath the injector to catch the waste.

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Put the plunger in sterile Type I water and prime 2 times. Next prime one time with Methanol.

4.2.6.26 Close out the Ascent AluQuant® Session. Again, do not save the changes or it will overwrite the data from that run onto the Session.

NOTE: The luminometer must be cleaned thoroughly as described above to prevent microorganisms from growing in the lines.

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5 PCR AMPLIFICATION

5.2 Technical Notes

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

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5.2.2 STRs differ from VNTRs and Dinucleotide repeats in that the repeat unit length for these markers range from 3 to 7 bases.

5.2.3 The PowerPlex® 16 BIO 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 Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818 loci.

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5.2.4 A random sample will be run with each set of convicted offender and arrestee sample amplifications to serve as a verification that the samples are successfully being entered into Combined DNA Index System (CODIS) and the search algorithm is working properly.

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5.2.5 If a sample must be re-amplified, providing that the random sample has amplified, the random sample does not need to be re-amplified. However, the Control DNA (GM9947A Cell Line – positive amplification control) and negative amplification control must be amplified with the re-amplified sample.

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5.2.6 If a batch of samples will be amplified using the BioMek® 2000 Automation Workstation as few as one amplification negative control and amplification positive (GM9947A) may be amplified per BioMek® 2000 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 9, Interpretation of The PowerPlex® 16 BIO System PCR Amplification Results.

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5.3 Equipment

5.3.1 Thermal Cycler – 9600 or 9700

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

5.3.3 8- Channel Pipette – Range 0.5 µL to 10 µL

5.3.4 Biological Safety Hood

5.3.5 Microcentrifuge tube racks

5.4 Materials

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

5.4.2 1.5 mL microcentfuge tubes

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5.4.3 Kimwipes

5.4.4 Sterile MicroAmp® tubes (0.2 mL tubes)

5.4.5 Gloves

5.4.6 96 well amplification plates and strip caps

5.5 Reagents

5.5.1 Powerplex® 16 BIO System Primer Pairs

5.5.2 Sterile Type I Water

5.5.3 Gold ST*R 10X Buffer

5.5.4 Control DNA (GM9947A Cell Line)

5.5.5 AmpliTaq™ Gold DNA Polymerase

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5.6 PowerPlex® 16 BIO System Amplification Procedure for the 9600 and 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 are not interpretable. 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.

5.6.1 Amplification Parameters for the 9600 and 9700 Thermal Cyclers:

5.6.1.1 Turn on the 9600 thermal cycler. Program as follows using the step cycle file, change the default setting to:

Set a hold file for 11 minutes at 95 °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 0 seconds to denature at 94°C for 30 seconds
Ramp 68 seconds to anneal at 60°C for 30 seconds
Ramp 50 seconds to extend at 70°C for 45 seconds

Program for 10 cycles

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Link this program to a separate program as follows:

Ramp 0 seconds to denature at 90°C for 30 seconds
Ramp 60 seconds to anneal at 60°C for 30 seconds
Ramp 50 seconds 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.

5.6.1.2 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°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 BIO Allelic Ladder, Fluorescent Internal Lane Standard 600 BIO, Matrix 16 BIO and Bromophenol Blue Loading Buffer, and Tracking Dye supplied in the PowerPlex® 16 BIO System kit do not require amplification and should not be stored in the sample preparation area.

5.6.2 Transfer the PowerPlex® 16 BIO System amplification reagents to the designated "clean" area and allow the Gold ST*R 10X Buffer, GM9947A Cell Line and the PowerPlex® 16 BIO 10X Primer Pairs to thaw completely at room temperature.

5.6.3 Place the required number of labeled sterile MicroAmp® reaction tubes in a rack.

5.6.4 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 analyzed on the BioMek® 2000 Automation Workstation), a random sample (associated only with the analysis of offender and arrestee samples), and positive and negative amplification controls and two to three 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 BIO 10X Primer Pairs
4.60 µL Sterile Type I Water
0.40 µL AmpliTaq™ Gold Enzyme (2 Units)

5.6.5 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 BIO 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 sample contains a low level of DNA (e.g., 0.05 ng/µL or less DNA) estimated based

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upon the AluQuant® Human Quantitation System, 9.6 µL of sample DNA may be used and the water removed from the Master Mix cocktail.
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.

5.6.6 Prepare the sample DNA and controls as follows:

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For convicted offender or arrestee samples extracted using the QIAamp® manual or robotic extraction procedure, proceed to step 5.5.6.1.1.

5.6.6.1 Sample DNA (Evidence Samples): Open the MicroAmp® tube and carefully add 5 µL of DNA (0.75 to 1.25 ng/5 µL) to the appropriate labeled sample tube. Discard the pipette tip and recap the tube tightly before proceeding to the next sample. **DO NOT** vortex, mix or spin. If the DNA concentration is greater than 0.2 ng/µL, dilute a portion of it with sterile Type I Water so that no more than 1.0 ng of DNA will be added to the PCR reaction in a volume of 5 µL. If the sample DNA concentration is less than 0.10 ng/µL add 5 µL of the sample DNA to the PCR reaction mix. However, if the sample contains low level DNA (e.g., 0.05 ng/µL or less), 9.6 µL of sample DNA may be used during the amplification step.

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NOTE: If a **STRONG** signal was observed using the AluQuant™ Human Quantitation System, but **NO** amplified DNA was observed on the product gel a higher concentration (i.e., 1.5 ng) of the isolated DNA may be used to enhance the possibility of obtaining amplified product from a highly degraded sample.

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

5.6.6.1.1 **Dried blood stains/buccal cells (Offender and Arrestee Samples ONLY):** Add 2.0 µL (blood - robotically), 1.5 µL (blood – manually), 1.0 µL (buccal - robotically), or 1.0 µL (buccal – manually) of extracted DNA to the appropriate labeled sample tube. Bring the total volume up to 5 µL by adding sterile Type 1 Water to the tube. NOTE: weaker/difficult samples may require a higher volume than is specified to obtain a complete DNA profile.

Reference Samples and Random Sample (Convicted Offender and Arrestee Samples): Open the MicroAmp® tube and carefully add 5 µL of DNA for half reaction (0.50 to 1.0 ng/5 µL) to the appropriate labeled sample tube. Discard the pipette tip and recap the tube tightly before proceeding to the next sample. **DO NOT** vortex, mix or spin. If the DNA concentration is greater than 0.2 ng/µL, dilute a portion of it with sterile Type I Water so that no more than 1.0 ng of DNA will be added to the PCR reaction in a volume of 5 µL. If the sample DNA concentration is less than 0.10 ng/µL add 5µL of the sample DNA to the PCR reaction mix. However, if the sample contains low level DNA (e.g., 0.05 ng/µL or less), 9.6 µL of sample DNA may be used during the amplification step.

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5.6.6.2 Positive Amplification Control: Gently mix by hand the Control DNA (GM9947A Cell Line) 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 of DNA (0.75 to 1.0 ng/5 µL) of the Control DNA to the appropriate labeled sample tube.

NOTE: When preparing the stock Control DNA (GM9947A Cell Line), dilute the sample to a concentration of 0.15 to 0.20 ng/µL with sterile Type I Water.

5.6.6.3 Negative Amplification Control: Open the MicroAmp® tube and carefully add 5 µL of sterile Type I Water to the appropriate labeled sample tube. 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 Negative Amplification Control will be used.

5.6.6.4 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. 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/plate blank will be used.

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5.6.7 Place the MicroAmp® tubes into the thermal cycler. Push the tubes down completely into the block. Place the thermal cycler lid over the samples and tighten.

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

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5.6.9 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.

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5.6.10 After the amplification process is complete remove the tubes from the thermal cycler and proceed with all samples to Section 6, Product Gel For Determining Amplified Product. **EXCEPTION: It is not necessary to evaluate convicted offender or arrestee samples that have been isolated using the QIAamp® robotic extraction procedure on a product gel. Therefore, proceed with all samples to Section 7, Vertical Gel Electrophoresis.**

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5.6.11 If the sample will not be loaded into the product gel within a short time period, the samples should be stored in the dark at 2 ° to 8 °C for a few hours or at -20 °C for extended periods.

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

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6 PRODUCT GEL FOR DETERMINING AMPLIFIED PRODUCT

6.2 Technical Notes

6.2.1 The product gel is used to determine the success of the amplification process and for assessing the concentration of amplified DNA that should be loaded into the typing gel.

6.2.2 The 123 base pair ladder consists of 34 repeats of a 123 bp DNA fragment ranging in length from 123 to 4,182 bp.

6.2.3 Ethidium bromide (EtBr) is used to detect DNA by staining. It intercalates into the DNA molecule and fluoresces under UV light.

6.2.4 A UV transilluminator, at a wavelength of 302 nm, is used to visualize the fluorescent reaction between the EtBr and the DNA.

6.2.5 Primer-dimer bands and unincorporated primers may appear as broad bands in the lower molecular weight region of the gel.

6.3 Equipment

6.3.1 Pipette - 10 µL

6.3.2 Gel tank, cover and electrodes

6.3.3 Voltmeter

6.3.4 Power supply

6.3.5 Freezer, -20 ^{o}C

6.3.6 Microcentrifuge tube rack

6.4 Materials

6.4.1 Agarose gel, (Refer to Appendix C)

6.4.2 Sterile ART tips - 10 µL

6.4.3 Microtiter plate

6.4.4 Gloves

6.5 Reagents

6.5.1 5X Loading buffer

6.5.2 123 bp Ladder

6.5.3 Ethidium bromide - 5 mg/mL (Optional - refer to Appendix C)

6.5.4 0.5X TBE buffer

6.6 Procedure

6.6.1 Following the procedure outlined in Appendix C, prepare a product gel using 3.0% NuSieve agarose in 0.5X TBE buffer.

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6.6.2 Load the product gel into the tank and add a sufficient volume of 0.5X TBE buffer to the tank to cover the product gel.

6.6.3 Add 2.5 µL of 5X loading buffer into a sufficient number of wells of the microtiter plate to correspond to the number of amplified samples that will be loaded into the product gel. Add 5 µL of the amplified sample to the appropriate well containing 5X loading buffer. Store the remainder of the sample at -20°C.

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6.6.4 Load 1 µg of the 123 bp ladder plus 5X loading buffer into at least one well of the product gel (the 123 bp ladder and 5X loading buffer may be pre-mixed ahead of time). A total volume of 5 µL, which includes the 123 bp ladder and 5X loading buffer, should be loaded into the product gel. Actual volume of ladder must be determined based on concentration of stock 123 bp ladder.

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6.6.5 Load the entire amount of sample from the microtiter plate into the designated well of the gel. Continue until all of the samples have been loaded.

6.6.6 Place the cover on the gel tank. Ensure that the gel tank is situated so that the red (positive) electrode is farthest from the loading wells.

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6.6.7 Plug the red (positive) electrode into the positive plug of the power supply. Plug the black (negative) electrode into the negative plug of the power supply.

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6.6.8 Turn on the power supply and set the voltage to the equivalent of 115 volts (using a voltmeter reading) for a minimum of 30 minutes, or until the loading buffer moves approximately one-fourth of the way across the gel.

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NOTE: If ethidium bromide was not incorporated into the gel, the gel must be stained with ethidium bromide after electrophoresis. Soak the gel in 200 mL of 1X TBE and 40 µL EtBr for 20-30 minutes.

WARNING:

ETHIDIUM BROMIDE IS MUTAGENIC. ALWAYS WEAR GLOVES WHEN HANDLING EtBr.

6.6.9 When electrophoresis is complete, view the gel on a UV transilluminator or in a UV viewing cabinet. Following the steps outlined in Appendix D, Section 3.1 or 3.2, as appropriate, photograph the gel.

Options: The FMBIO II Fluorescent Image Analysis System may also be used to view and capture the product gel image. Refer to Appendix F, Fluorescent Detection of the Electrophoresis Gel-FMBIO II, for instructions.

A digital camera may also be used (Appendix D).

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6.6.10 Label the picture(s) with the FS Laboratory number and the examiner's initials and determine if the amplification of the sample(s) is complete by the visual appearance of bands in the gel. Under optimum conditions the amplified DNA for the PowerPlex® 16 BIO Systems should appear between the 123 bp and the 492 bp bands of the 123 bp ladder.

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6.6.10.1 Based on the DNA quantation if a **WEAK** signal is observed (i.e., less than or equal to 0.05 ng/µL) and **NO** amplified DNA is observed on the product gel, the sample may be reamplified with more input DNA.

6.6.10.2 Based on the DNA quantation if a **STRONG** signal is observed, but **NO** amplified DNA is observed on the product gel a dilution (i.e., 1:5) may be made of the original volume of the extracted DNA in order to reduce the level of any possible inhibitors. In addition, a higher concentration (i.e., 1.5 ng) of the isolated DNA may be used to enhance the possibility of

obtaining amplified product from a highly degraded sample. Amplify these samples following the procedure outlined in Section 5, PCR Amplification, then repeat steps 6.5.1 through 6.5.9.

6.6.10.3 If **NO** DNA is detected and **NO** amplified DNA is observed on the product gel, no further analysis will be conducted on this sample.

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6.6.11 If amplified product has been observed proceed to Chapter 7, Vertical Gel Electrophoresis.

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7 VERTICAL GEL ELECTROPHORESIS

7.2 Technical Notes

7.2.1 The PowerPlex® 16 BIO System allelic ladder consists of amplified alleles for use in allele identification of the amplification product at the Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818 loci.

7.2.2 The Fluorescent Internal Lane Standard 600 BIO consists of 21 DNA fragments (80,100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, and 600 bp). Seventeen of these fragments (100 – 475 bp) are used during sizing.

7.2.3 The amplified known Control DNA (GM9947A Cell Line) is used to assess the migration of the DNA through the typing gel and therefore must be loaded on every typing gel. If it is necessary to re-type a sample and the control associated with the sample is no longer available, a different amplified known Control DNA may be used on the typing gels as long as the original amplified known Control DNA was typed and determined to be acceptable.

7.2.4 The amplification negative control(s), the reagent blanks, the amplified known control DNA and the plate controls associated with a plate of evidence samples analyzed using the BioMek® 2000 Automation Workstation may be run on a single typing gel. However, in order to report the typing results for the evidence samples associated with these controls and run on other typing gels, the controls must work appropriately in accordance with the procedures outlined in Chapter 9, Interpretation of The PowerPlex® 16 BIO System PCR Amplification Results.

7.2.5 Evidence samples and reference samples will be run on separate typing gels.

7.2.6 At least one random sample is required to be run on each gel containing convicted offender or arrestee samples. The random sample that is used on the typing gel should have been extracted and amplified with the samples associated with the typing gel, unless the sample was re-amplified then the same or a different previously verified random sample may be used as a migration control.

7.3 Equipment

7.3.1 SA-43 vertical electrophoresis tank

7.3.2 Power supply

7.3.3 Electrophoresis glass plate set, 19 cm X 43 cm

7.3.4 Delrin spacer sets, 0.4 mm thick

7.3.5 Delrin 30 or 38 well comb (for offender and arrestee samples)

7.3.6 Binder clips, 1 inch

7.3.7 Autopipet

7.3.8 Pipettes - 2 µL, 10 µL, 20 µL, 200 µL, and 1000 µL

7.3.9 8 –Channel Pipette – Range 0.5 µL to 10 µL

7.3.10 Microcentrifuge

7.3.11 Ice Bucket/Pack

7.3.12 Heat Block, 95 ^{o}C

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7.3.13 Fume Hood

7.3.14 Analytical Balance

7.3.15 Hot Water Bath, 50 ^{o}C

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7.4 Materials

7.4.1 Flat gel loading capillary pipette tips, 10 µL

7.4.2 Kimwipes

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7.4.3 Disposable 60 cc syringe and needle

7.4.4 Disposable, large bore transfer pipettes

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7.4.5 Disposable plastic pipettes, 50 mL

7.4.6 Microcentrifuge tubes, 0.5mL and 1.5 mL

7.4.7 Sterile ART tips for pipettes, 10 µL, 20 µL, 200 µL and 1000 µL

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7.4.8 Plastic wrap

7.4.9 Conical tubes, 50 mL

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7.4.10 Paper towels

7.4.11 Nitrile Gloves

7.5 Reagents

7.5.1 Ethanol, 95%

7.5.2 Type I Water

7.5.3 10X and 1X TBE buffer

7.5.4 10% Ammonium Persulfate

7.5.5 TEMED

7.5.6 Urea

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7.5.7 6.0% Gene PAGE Plus™ Gel Mix

7.5.8 40% PAGE Plus™ Gel Mix

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7.5.9 Bind Silane

7.5.10 Acetic Acid, 0.5% in 95% Ethanol

7.5.11 Bromophenol Blue Loading Buffer

7.5.12 STR 2X Loading Buffer (Tracking Dye)

7.5.13 PowerPlex® 16 BIO System allelic ladder

7.5.14 Fluorescent Internal Lane Standard 600 BIO

7.5.15 Matrix 16 BIO

7.5.16 Gold ST*R 1X Buffer

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7.6 PowerPlex® 16 BIO System Vertical Gel Electrophoresis Procedure

CAUTION

Acrylamide is a neurotoxin, avoid inhalation and contact with skin. Always wear nitrile gloves and safety glasses when working with acrylamide solutions.

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NOTE: Low-fluorescent glass plates should only be used to prepare the typing gel and extreme care should be taken to prevent scratching the glass plates. Scratches on the surface of the glass in the area of the scanned image will reflect the laser light and appear as heavy dark lines on the scanned image.

New plates that have not been previously used should be soaked in 2N NaOH for 30 minutes prior to use.

7.6.1 If the glass plates are being used for the first time, etch each glass plate on one side in one corner with a diamond pencil to identify the treated sides of the glass plates. If plates have been used before, proceed to step 7.5.2.

7.6.2 Thoroughly clean the short and long plates twice with 95% ethanol and a Kimwipe.

7.6.3 Prepare a binding solution by adding 1.0 mL of 0.5% acetic acid in 95% ethanol to a 1.5 mL microcentrifuge tube. Then add 3.0 µL of Bind Silane and mix thoroughly.

7.6.4 In a well ventilated area, place the short glass plate on a flat, horizontal surface. Using a 1000 µL pipette, add the binding solution to the etched side of the glass plate in the area where the comb will be placed. Using a Kimwipe, wipe the binding solution across the entire top portion of the short glass plate in the area where the comb will be placed.

7.6.5 Wait 5 minutes for the binding solution to dry. Then wipe the short glass plate 3 or 4 times with a Kimwipe containing 95% ethanol to remove the excess binding solution.

7.6.6 Place the long glass plate on a flat horizontal surface. Place the 0.4 mm thick spacers along the right and left edges of the plate. Carefully place the short plate on top of the spacers. Align the sides and the bottom edges of the short and long plate without disturbing the placement of the spacers. The foam blocks on the spacers should be snug against the top edge of the short plate. Tightly clamp the top and bottom plates together along the sides using binder clips. **In order to prevent well malformation ensure the 30 or 38 well comb fits tightly between the glass plates before pouring the gel.**

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7.6.7 Prepare a 6.0% Gene PAGE Plus™ gel mix as follows:

7.6.7.1 Add 50 mL of 6.0% Gene PAGE Plus™ gel solution to a 50 mL conical tube container.

7.6.7.2 In a well ventilated area, add 300 µL of 10% ammonium persulfate and 30 µL of TEMED to the PAGE Plus™ gel solution.

NOTE: 10% ammonium persulfate should be prepared the day of use.

7.6.7.3 Using a 50 mL disposable pipette and an autopipet, pipette the gel mix between the glass plates. Pipette the gel mixture slowly and maintain a continuous flow. Avoid creating bubbles. Carefully tap the plates to dislodge any air bubbles.

7.6.7.4 Insert the 30 or 38 well comb at the top of the gel. Be careful not to form bubbles around the comb teeth.

7.6.7.5 Allow the gel to polymerize in a horizontal position for at least 1 hour prior to setting up for electrophoresis.

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NOTE: The polymerized gel can be stored in the dark at room temperature by wrapping the gel in plastic wrap or a large Ziploc™ bag. Do not store the gel in the refrigerator once it has polymerized. To prevent the gel from drying, add a small amount of 1X TBE buffer or water to several paper towels and then lay the paper towel on the comb, and both sides and the bottom of the glass plates before wrapping with plastic wrap or a large Ziploc™ bag.

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OPTION: Alternatively, a 40% PAGE Plus™ gel matrix solution may be used to prepare the typing gel. This procedure is outlined in Appendix K, 6% PAGE Plus™

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7.6.8 Place the gel into the SA-43 vertical gel electrophoresis tank, the long plate 'facing out', and then turn the clamps to secure the gel in place. Add 1X TBE buffer to the upper reservoir of the gel tank. Gently remove the comb and carefully flush the wells with tank buffer using a syringe or a large bore transfer pipette. If necessary, straighten the sides of the wells with a gel loading tip or a needle.

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7.6.9 Fill the lower reservoir of the gel tank with 1X TBE buffer. Remove any air bubbles that are trapped between the bottom of the gel and the buffer in the lower reservoir.

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7.6.10 Connect the leads from the power supply so that the red positive (+) electrode is attached to the bottom and the black negative (-) electrode is attached to the top of the electrophoresis apparatus.

7.6.11 Pre-run the gel for 20 to 30 minutes at 60 Watts; use this time to prepare the samples for loading.

7.6.12 Casework Samples:

Prepare the samples to be typed by mixing 3.0 µL of Bromophenol Blue Loading Buffer, 0.25 (when 0.75 µL ILS is used) to 0.50 µL of 1X Gold ST*R Buffer and 0.50 to 0.75 µL of Fluorescent Internal Lane Standard (ILS) 600 BIO with 2.0 µL of each sample, including all the controls (i.e., GM9947A Cell Line/positive amplification control, negative amplification control, reagent blanks and plate blanks). If less than 2.0 µL of sample is used based on the intensity of the sample determined from the product gel, bring the sample volume up to 2.0 µL with Gold ST*R 1X Buffer. For weak sample 3.0 µL of sample may be used. However 3.0 µL of the negative amplification control, reagent blank, and plate blank must be loaded. **Note: Evidence samples should be prepared at a different time and/or space than the reference samples.**

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For microvariant or off-ladder variant samples refer to the section below.

- Microvariant/Off-Ladder Variant Alleles: To confirm an allele equal to or greater than 300 bp in size is a microvariant, the sample will either be loaded into a new typing gel and run for a sufficiently longer period of time or the original typing gel will be placed back into the electrophoresis tank and run for a longer period of time to separate the alleles sufficiently. This will provide better separation of the allelic ladder bands. The sample(s) will be analyzed against the PowerPlex® 16 BIO allelic ladder in conjunction with a positive amplification control.

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Convicted Offender or Arrestee Samples:

Prepare the samples to be typed by mixing 1.5 µL of Bromophenol Blue Loading Buffer, 0.85 µL of 1X Gold ST*R Buffer and 0.25 µL of Fluorescent Internal Lane Standard (ILS) 600 BIO with 0.50 µL of each sample, including all the controls (i.e., GM9947A Cell

Line/positive amplification control, negative amplification control, reagent blanks, and random sample). For weak samples, the volume of the sample may be increased and the volume of 1X Gold ST*R Buffer adjusted to total sample/1X Gold ST*R Buffer volume of 1.25 µL.

7.6.13 Casework Samples:

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Prepare the PowerPlex® 16 BIO System Allelic Ladder by gently mixing the tube by hand, then briefly spinning in a microcentrifuge. For each ladder lane, mix 3.0 µL of Bromophenol Blue Loading Buffer, 0.50 to 0.75 µL of Fluorescent Internal Lane Standard (ILS) 600 BIO with to 0.65 µL to 0.75 µL of PowerPlex® 16 BIO System Allelic ladder. Bring the total volume up to 6.0 µL by adding 1X Gold ST*R Buffer to the tube.

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Convicted Offender or Arrestee Samples:

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Prepare the PowerPlex® 16 BIO System Allelic Ladder by gently mixing the tube by hand, then briefly spinning in a microcentrifuge. For each ladder lane, mix 1.5 µL of Bromophenol Blue Loading Buffer, 0.25 to 0.375 µL of Fluorescent Internal Lane Standard (ILS) 600 BIO with to 0.50 µL to 1.0 µL of PowerPlex® 16 BIO System Allelic ladder. Bring the total volume up to 3.0 µL by adding 1X Gold ST*R Buffer to the tube.

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7.6.14 Prepare either a Matrix 16 BIO sample/Tracking Dye cocktail or a Matrix 16 BIO sample cocktail separately as follows:

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7.6.14.1 Matrix 16 BIO Sample/Tracking Dye Cocktail: Mix 1.5 µL of Matrix 16 BIO, 1.5 µL Tracking Dye, and 3.0 µL of 1X Gold ST*R Buffer.

7.6.14.2 Matrix 16 BIO Sample Cocktail: Mix 1.5 µL of Matrix 16 BIO, 1.5 µL Bromophenol Blue Loading Buffer, and 3.0 µL of 1X Gold ST*R Buffer.

NOTE: At a minimum, two allelic ladders shall be loaded on each typing gel.

7.6.15 Just prior to loading the samples, allelic ladders, and the Matrix 16 BIO sample/Tracking Dye or the Matrix 16 BIO sample onto the gel, denature all of these samples by heating to 95 °C for 3 minutes and then immediately chill on ice. Samples will re-anneal if denatured and allowed to remain on ice for too long before loading into the typing gel.

7.6.16 After the power supply has been disconnected, flush the wells one additional time with tank buffer. Then carefully load 2.0 µL of the Matrix 16 BIO sample/Tracking Dye cocktail or the Tracking Dye separately from the Matrix 16 BIO sample using gel-loading capillary pipette tips in an even layer at the bottom of the outer well on at least one side of the typing gel. Be careful not to introduce bubbles into the wells. The Matrix 16 BIO/Tracking Dye or Tracking Dye should be a minimum of one well from the first or last sample lane in order to determine the distance the samples have migrated.

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7.6.17 To reduce the effect of bowing of the samples across the gel, 2.0 µL of STR 2X Loading Buffer may be loaded into the outer wells of each side of the gel flanking the samples.

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7.6.18 Load 3.0 µL (or 2.5 µL if a 38 well comb is used) of the allelic ladder, the samples, the Matrix 16 BIO sample (if not already loaded as part of the Matrix 16 BIO/Tracking Dye cocktail) and the controls (i.e., GM9947A Cell Line/positive amplification control, negative amplification control, reagent blanks, and plate blanks) using gel-loading capillary pipette tips in an even layer at the bottom of each well. Be careful not to introduce bubbles into the well or cause carry over of the samples between wells.

7.6.19 Connect the electrophoresis apparatus to the power supply such that the red positive (+) electrode is attached to the bottom and the black negative (-) electrode is attached to the top of the electrophoresis apparatus. Turn the power supply on and begin electrophoresis at a setting of 60 Watts for approximately 2 hours or until the xylene cyanol dye front has migrated approximately 2.0 cm below the bottom clips

holding the gel. The Bromophenol Blue dye front will run off of the gel under these conditions. **NOTE:** Because the speed of migration can vary from lot to lot of gel mix, the distance will be further defined during the quality control testing of the lot of gel mix.

7.6.20 After the typing gel is finished running, disconnect the power supply, drain the upper reservoir tank buffer and remove the gel plate setup from the electrophoresis apparatus.

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7.6.21 Proceed to Chapter 8, Fluorescent Detection of the Electrophoresis Gel

NOTE: **If the polyacrylamide gel will be reused after it has been scanned, refer to Appendix N, Reuse of Polyacrylamide Electrophoresis Gel, for the procedure.**

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8 FLUORESCENT DETECTION OF THE ELECTROPHORESIS GEL

NOTE: Review the FMBIO manual before proceeding. Refer to this manual for further details on the following procedures.

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8.2 Equipment

8.2.1 FMBIO II or III Plus Fluorescent Imaging Analysis System (Hitachi/MiraiBio)

8.2.2 Power Mac or Mac Clone Computer

8.2.3 Laser Printer

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8.3 Materials

8.3.1 Kimwipes

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8.4 Reagents

8.4.1 Type 1 Water

8.4.2 70% Ethanol

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8.5 Fluorescent Detection for PowerPlex® System Gels

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8.5.1 The FMBIO and Macintosh/PC computer should be turned on at least 30 minutes before scanning a typing gel.

8.5.2 Clean the glass plate setup by placing it under running water and removing all the acrylamide and urea from the outside.

8.5.3 Rinse the glass plate setup with Type I water and dry with a Kimwipe. Remove any smudges using 70% Ethanol and a Kimwipe.

8.5.4 Refer to Appendix F (FMBIO II) or Appendix G (FMBIO III Plus) for procedure for operating the instrument.

NOTE: **If the polyacrylamide gel will be reused after it has been scanned, refer to Appendix N, Reuse of Polyacrylamide Electrophoresis Gel, for the procedure.**

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9 INTERPRETATION OF THE POWERPLEX® 16 BIO SYSTEM PCR AMPLIFICATION RESULTS

Note: To the extent possible the evidence sample typing gel(s) will be sized by the examiner/analyst, independently sized by a second qualified individual and the calls between them confirmed prior to reviewing the sizing of the reference sample typing gel(s).

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9.2 Technical Notes

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

9.2.2 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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9.2.3 The characteristics of the PowerPlex® 16 BIO System and the allelic ladders are given in the table below[1]:

Locus	* Repeat Sequence 5' 3'	Chromosome Location	Size Range of Allelic Ladder (bp)	Alleles present in Allelic Ladder	Fluorescent Label
FGA	TTTC Complex	4q28	322-444	16-30, 31.2, 43.2, 44.2, 45.2, 46.2	Rhodamine Red™ –X
TPOX	AATG	2p23-2pter	262-290	6-13	Rhodamine Red™ –X
D8S1179	TCTA Complex	8q	203-247	7-18	Rhodamine Red™ –X
vWA	TCTA Complex	12p12-pter	123-171	10-22	Rhodamine Red™ –X
Amelogenin	NA	Xp22.1-22.3 and Y	106 – X 112 – Y	X, Y	Rhodamine Red™ –X
Penta E	AAAGA	15q	379-474	5-24	Fluorescein
D18S51	AGAA	18q21.3	290-366	8-10, 10.2, 11-13, 13.2, 14-27	Fluorescein
D21S11	TCTA Complex	21q11-21q21	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	Fluorescein
TH01	AATG	11p15.5	156-195	4-9, 9.3, 10-11, 13.3	Fluorescein
D3S1358	TCTA Complex	3p	115-147	12-20	Fluorescein

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NOTE: table continued below

Locus	*Repeat Sequence 5' 3'	Chromosome Location	Size Range of Allelic Ladder (bp)	Alleles present in Allelic Ladder	Fluorescent Label
Penta D	AAAGA	21q	376-449	2.2, 3.2, 5, 7-17	JOE
CSF1PO	AGAT	5q33.3-34	321-357	6 – 15	JOE
D16S539	AGAT	16q24-qter	264 – 304	5, 8 –15	JOE
D7S820	AGAT	7q11.21-22	215 –247	6 – 14	JOE
D13S317	AGAT	13q22-q31	169 –201	7 – 15	JOE
D5S818	AGAT	5q23.3-32	119 -155	7 – 16	JOE

* 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]

<u>FMBIO II and FMBIO III Plus Fluorescent Image Analysis Systems:</u>

Fluorescein is detected at a wavelength of 505 nm (FMBIO II) or 520 nm (FMBIO III Plus) - Green

Rhodamine Red™ –X is detected at a wavelength of 598 nm - Red

JOE = 6-carboxy-4',5'-dichloro 2',7' – dimethoxyfluorescein is detected at a wavelength of 577 nm - Yellow

9.2.4 The Fluorescent Internal Lane Standard 600 BIO (Texas Red®-X) consists of 21 DNA fragments (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 665 nm (FMBIO II) or 650 nm (FMBIO III Plus) – Blue.[1]

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9.2.5 The "Known" Genotypes for the Control DNA (GM9947A Cell Line) using the PowerPlex® 16 BIO System are given in the table below[1]

Locus	Genotype GM9947A
Penta E	12,13
D18S51	15,19
D21S11	30,30
TH01	8,9.3
D3S1358	14,15
FGA	23,24
TPOX	8,8
D8S1179	13,13
VWA	17,18
Amelogenin	X,X
Penta D	12,12
CSF1PO	10,12
D16S539	11,12
D7S820	10,11
D13S317	11,11
D5S818	11,11

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9.3 Procedure

9.3.1 The gel image is visually inspected to determine if the number, position, and intensity of the alleles for the allelic ladder, controls and samples are suitable for interpretation.

9.3.1.1 If the overall quality of the gel image is unsuitable for interpretation, no further comparisons are conducted.

9.3.1.2 If the overall quality of the gel image is suitable for interpretation, a visual comparison is performed.

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9.3.2 The source of a DNA sample may be from a single person or more than one person. This can be determined by examination of the number of alleles at each locus, optical densities and/or band intensities.

9.3.2.1 A DNA profile may be considered to have originated from a single individual if the expected number of alleles (i.e., 1 or 2) is observed at each locus and the intensity of the alleles within a locus is approximately the same. All loci should be taken into account when making this determination.

9.3.2.2 A sample may be considered to be a mixture of DNA from two or more individuals if the sample contains 3 or more bands at one or more loci and/or there is a distinct difference in signal intensity. All loci should be taken into account when making this determination.

9.3.3 To the extent possible the DNA profiles of the evidence samples are determined first and then compared to the reference samples.

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9.3.3.1 If the banding patterns of samples under comparison are distinctly different in position, it is concluded that the samples originated from different sources than the individual of interest and the individual is excluded.

9.3.3.2 If the banding patterns of samples under comparison appear visually consistent in position, the possibility that both samples may have originated from the same source cannot be eliminated. Therefore the individual of interest is included as a possible source.

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9.3.3.2.1 Generally statistical frequencies will be generated for DNA profiles (mixture and non-mixture profiles) obtained from evidence of a probative value where the individual(s) of interest cannot be eliminated and the reference standard of the individual(s) of interest contains a 1 or 2 banded patterns at each locus.

9.3.3.2.2 Statistical frequencies will not be generated at a locus if the reference standard from the individual of interest exhibits a 3 or 4 banded pattern.

9.3.3.3 If evidence samples under comparison contain a partial profile (i.e., allele dropout) or an incomplete profile (i.e., locus dropout) due to degradation, inhibition or limited DNA, the DNA profile may or may not be interpretable.

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9.3.3.3.1 All loci will be taken into account when making this determination using knowledge of the system and experience.

9.3.3.3.2 If allele/locus dropout is observed at a majority of the loci, in order to include an individual, at least four callable loci from the evidence must match the known standard (this includes loci where masking has occurred). Otherwise the partial profile will be reported as inconclusive or may be used only for elimination purposes. However, additional information such as the presence of a rare allele observed only in a small portion of the population will be taken into consideration in consultation with a supervisor and/or the Program Manager when reaching a conclusion.

9.3.3.4 To the extent possible complete DNA typing results will be obtained for reference samples.

9.3.3.5 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.

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9.3.3.5.1 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.

9.3.4 Following a visual examination of the gel image, the amplified alleles are designated by noting which allelic ladder band(s) lines up with the sample band(s), as demonstrated in the examples in section 9.3.

9.3.5 Allele designations for evidence and reference samples will be determined by the examiner/analyst using the FMBIO Fluorescent Imaging System STaRCall software. Refer to Appendix F (FMBIO II) or

Appendix G (FMBIO III Plus) for the FMBIO Fluorescent Imaging Analysis System procedure and instructions on assigning allele designations using the STaRCall software.

9.3.6 A blind independent calling of the alleles for each sample will be conducted by a second qualified individual using the FMBIO Fluorescent Imaging Analysis System.

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9.3.7 The DNA types for the evidence samples will be determined to the extent possible first and second sized prior to comparing these results to the reference samples.

9.3.7.1 If the examiner/analyst and the independent sizer's allele designations are in agreement the alleles can be called.

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9.3.7.2 If the examiner/analyst and the independent sizer's allele designations are different and no agreement can be reached, the results for the allele(s) in question will be called inconclusive. If possible, the sample(s) will be re-analyzed.

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9.3.7.3 If upon reviewing the DNA types of the reference sample it is determined that the DNA the types of the evidence sample need to be edited this action is noted and explained in the casefile.

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9.3.7.4 The examiner/analyst will place lighter alleles in parentheses.

NOTE: It is not necessary to size or independently size the typing gel that contains only the amplification negative control(s), reagent blanks, and plate controls when the expected alleles for the positive control are observed and no alleles are observed for the additional controls.

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

9.3.9 If an allele is seen in a region "off- ladder", i.e., above the top allelic ladder band of the upper most locus on the typing gel, below the bottom allelic ladder band of the bottom most locus on the typing gel, or between loci, an allele designation based upon the nomenclature referenced in step 9.2.9.1 will be used. However, to ensure that the evidence and standard samples contain the same allele, the base pair values for the evidence sample and the standard will be compared to the lookup table to verify that both samples would be assigned the same repeat unit value (i.e., vWA 8).

EXCEPTION: If the evidence sample is from a non-subject case, the sample will be typed and assigned an allele designation based upon the nomenclature referenced in step 9.2.9.1. When the suspect's standard is subsequently analyzed, assuming there is an off-ladder allele, the same nomenclature referenced in step 9.2.9.1 will be used. The base pair values for the evidence and suspect's standard samples will be compared to the lookup table associated with the analysis of the sample to assign the repeat unit value (i.e., vWA 8) to ensure both alleles are the same.

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9.3.9.1 If the two bands are determined to be the same and the allele is seen above the top allelic ladder band of the upper most locus on the typing gel, it will be assigned the type of the top allele of the allelic ladder with a greater than sign (>). If the allele is seen below the bottom allelic ladder band of the bottom most locus on the typing gel, the allele will be assigned the type of the bottom allele of the allelic ladder with a less than sign (<). The "out of range" value on the STaRCall spreadsheet will be manually changed to reflect the allele designation.

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9.3.10 If an allele is seen between two loci and either the locus above **OR** below the band contains two bands the allele will be considered to belong with the locus containing the single band. The assignment of the allele designation will be based upon the nomenclature referenced in step 9.2.10.1. However, to ensure that the evidence and standard samples contain the same allele, the base pair values for the evidence sample and the standard will be compared to the lookup table to verify that both samples would be assigned the same repeat unit value (i.e., D5S818 17).

EXCEPTION: If the evidence sample is from a non-subject case, the sample will be typed and assigned an allele designation based upon the nomenclature referenced in step 9.2.10.1. When the suspect's standard is subsequently analyzed, assuming there is an off-ladder allele, the same nomenclature referenced in step 9.2.10.1 will be used. The base pair values for the evidence and suspect's standard samples will be compared to the lookup table associated with the analysis of the sample to assign the repeat unit value (i.e., D5S818 17) to ensure both alleles are the same.

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9.3.10.1 If the two bands are determined to be the same and the allele is above the top allelic ladder band of the locus containing the single band, it will be assigned the type of the top allele of the allelic ladder with a greater than sign (>). If the allele is below the bottom allelic ladder band of the locus containing the single band, the allele will be assigned the type of the bottom allele of the allelic ladder with a less than sign (<). The "out of range" value on the STaRCall spreadsheet will be manually changed to reflect the allele designation.

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9.3.11 If an allele is seen between two loci and the locus above **AND** below the band contains a single band, follow the guidelines outlined in steps 9.2.11.1 through 9.2.11.3.

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EXCEPTION: If the evidence sample is from a non-subject case, the sample will be typed and assigned an allele designation based upon the procedure outlined in steps 9.2.11.1 through 9.2.11.3. When the suspect's standard is analyzed, assuming there is an off-ladder allele, the same procedure outlined in steps 9.2.11.1 through 9.2.11.3 will be used. The base pair values for the evidence and suspect's standard samples will be compared to the lookup table associated with the analysis of the sample to assign the repeat unit value (i.e., D5S818 17) to ensure both alleles are the same.

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9.3.11.1 The base pair size for the allele in question will be compared to the base pair values for the top allelic ladder band of the lower molecular weight locus and to the bottom allelic ladder band of the higher molecular weight locus. In addition, the optical density for the allele in question will be compared to the optical density of the single allele in higher and lower molecular weight loci, and an evaluation of the physical location of the allele in question to the higher and lower molecular weight loci will be conducted.

9.3.11.2 The allele in question will be considered to belong to the locus which is closest in proximity, contains an allele of approximately the same optical density and falls within an appropriate size distance from the locus (i.e., one or two repeat units from the top/bottom allelic ladder band).

9.3.11.3 Once it has been determined to which locus the allele belongs, if the allele is above the top allelic ladder band it will be assigned the type of the top allele of the allelic ladder with a greater than sign (>). If an allele is below the bottom allelic ladder band, the allele will be assigned the type of the bottom allele of the allelic ladder with a less than sign (<). The "out of range" value on the STaRCall spreadsheet will be manually changed to reflect the allele designation. To ensure that the evidence and the standard samples contain the same allele, the base pair values for the evidence sample and the standard will be compared to the lookup table to verify that both samples would be assigned the same repeat unit value (i.e., D5S818 17).

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9.3.12 If an allele, in relationship to the allelic ladder, is less than 300 bp in size, is visually between two allelic ladder bands, the STaRCall program generates a value "out of range" and the base pair value is +/ - 1.0 base pair or greater from that of the "Known" repeat base pair value, 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 which migrates one base pair below the TH01 9 allele will be designated as a TH01 8.3. The "out of range" value on the STaRCall spreadsheet will be manually changed to reflect the allele designation.

NOTE: The allele designation can be determined manually or using the procedure addressed in Step 5.11 of Appendix F (FMBIO II) or Appendix G (FMBIO III Plus) Fluorescent Detection of The Electrophoresis Gel.

9.3.13 If an allele/band is equal to or greater than 300 bp in size, is visually between two allelic ladder bands (i.e., the allele is not present in the allelic ladder) and/or the STaRCall program generates a value out of range and the base pair value is +/ - 1.0 base pair or greater from that of the "Known" repeat base pair value, the allele/band will be considered to be a microvariant. In order to confirm the allele is a microvariant the typing gel may be placed back into the electrophoresis tank and run for a sufficiently longer period of time or the evidence sample(s) and standard may be re-loaded onto a second typing gel. The typing gel will be run for a sufficiently longer period of time to provide better separation of the allelic ladder bands, thereby attaining better precision in measurement for assigning the allele designation.

EXCEPTION: The Penta D “6 allele”, which is not in the allelic ladder, will not be viewed as a microvariant. The assignment of the allele designation for the Penta D “6 allele” will be based upon the base pair size in relation to the base pair size of the Penta D 5 and 7 alleles.

NOTE: If the typing gel will not be run for a sufficiently longer period of time the allele will be reported as an “M”. For example a sample that appears to be a 12, 15.3 at FGA and the 15.3 allele is not confirmed as described in 9.2.14 the reported alleles will be (12, M). The legend for the Certificate of Analysis chart will reflect the M stands for a variant allele.

To confirm a microvariant the typing gel must contain a positive amplification control and have been independently sized.

9.3.13.1 Only the allele(s) for the locus in question will be sized.

9.3.13.2 Based upon the base pair value obtained, the allele/band will be assigned an allele designation of the lower repeat value followed by the number of bases in the incomplete repeat (Example: an allele which migrates one base below the Penta E 10 allele will be designated as a Penta E 9.4). The "out of range" value on the STaRCall spreadsheet will be manually changed to reflect the allele designation.

9.3.13.3 The allele designations generated from this typing gel will be the official allele designations.

NOTE: The allele designation can be determined manually or using the procedure addressed in Step 5.11 of Appendix F (FMBIO II) or Appendix G (FMBIO III Plus) Fluorescent Detection of The Electrophoresis Gel.

9.3.14 If a single band above 300 bp, based upon the band morphology/intensity, appears to be the coalescing of two closely spaced bands (i.e., one base pair apart, such as FGA 22 and 22.1), or the evidence sample and standard generated allele designations that are a single base pair different and the allele is relevant to the case, the typing gel may be placed back into the electrophoresis tank and run for a sufficiently longer period of time or the evidence sample(s) and standard may be re-loaded onto a second typing gel. The typing gel will be run for a sufficiently longer period of time, then re-scanned at the locus in question to resolve the possible coalesced band(s) or the difference between the evidence sample and standard.

9.3.14.1 Only the allele(s) for the locus in question will be sized.

9.3.14.2 The allele designations generated from this typing gel will be the “official” allele designations.

NOTE: If the typing gel will not be run for a sufficiently longer period of time to resolve the possible coalesced band(s), the locus must be reported as inconclusive.

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9.3.15 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.

9.3.16 If an allele (i.e., microvariant, off-ladder allele or rare allele) has been observed 5 times or less in the Department of Forensic Science population database, an allele frequency of 5/n will be assigned to the allele.

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9.3.17 To indicate the gender of the contributor of a particular biological sample the Amelogenin locus may be used. A biological sample exhibiting a single band at approximately 106 bp (X allele) will generally be considered to have originated from a female individual. A biological sample exhibiting a band at approximately 106 bp (X allele) and a band 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. Refer to Technical Notes 9.1.3 and 9.1.5 for additional information about the Amelogenin locus.

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9.3.18 For a typing result to be reported all controls must work appropriately.

9.3.18.1 Reagent Blanks

9.3.18.1.1 If a weak signal is detected 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. If it is imperative that the locus be used, the samples will be re-extracted and/or re-amplified.

9.3.18.1.2 If a weak signal is detected in a reagent blank at multiple loci, the test results for all loci will be considered inconclusive and all samples associated with the reagent blank, if possible, will be re-extracted and/or re-amplified.

9.3.18.1.3 If a strong signal is detected in a reagent blank at a single locus or at multiple loci, the test results for all loci will be considered inconclusive and all samples associated with the reagent blank, if possible, will be re-extracted and/or re-amplified.

9.3.18.2 For convicted offender and arrestee sample analysis, the Random Sample profile when searched in CODIS must elicit the correct result. If the DNA sample number identified as a result of the search is different from the DNA sample number on the master list (maintained by the Forensic Biology Program Manager or designee) all samples associated with the Random Sample will be re-extracted and/or re-amplified if possible.

9.3.18.3 The Control DNA (GM9947A Cell Line) must elicit the "Known" genotype for each locus as specified in Technical Note 9.1.5. If an allele is detected in the Control DNA at a specific locus that is not consistent with the known genotype, 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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9.3.18.4 Negative Amplification Control

9.3.18.4.1 If a weak signal is detected 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 will be considered inconclusive at that locus. If it is imperative that the locus be used, the samples will be re-amplified.

9.3.18.4.2 If a weak signal is detected in the negative amplification control at multiple loci, the test results for all loci will be considered inconclusive and all samples, if possible, will be re-amplified.

9.3.18.4.3 If a strong signal is detected in the negative amplification control at a single locus or at multiple loci, the test results for all loci will be considered inconclusive and all samples, if possible, will be re-amplified.

9.3.18.5 If no typing result is observed for the Random Sample (for convicted offender and arrestee sample analysis) or the Control DNA (GM9947A Cell Line) at a particular locus all samples at that locus will be considered inconclusive. If it is deemed necessary, such as the locus provides exculpatory information, the sample(s) associated with the Random Sample or Control DNA will be re-amplified and typed.

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9.3.19 When a band stronger in intensity is accompanied by a band weaker in intensity that has migrated one allele position (n-4) farther than the more intense band, this may be a stutter band.

NOTE: Stutter may also be seen at an n+4 position to that of the more intense band. In addition, if the sample is overloaded, a high concentration of DNA was amplified, or the sample is degraded, artifactual bands may also be seen at n-1, n-2, n-3 and n-8 positions to the intense band or the stutter band may have an elevated optical density values [4].

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9.3.19.1 Both the strong and weak bands will be sized using the STaRCall allele calling software. Refer to Appendix F (FMBIO II) or Appendix G (FMBIO III Plus) for the FMBIO Fluorescent Imaging Analysis System procedure.

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9.3.19.2 All loci must be taken into account when determining if a sample is a mixture of biological material from more than one source. In order to determine if a weak band in an n-4 position is the result of normal stutter within a locus or a mixture of two or more sources of biological material, the analyst will use experience and/or the percent stutter values to make an informed decision. If the ratio of the OD (optical density) for the strong band to the weak band is less than the established values (listed below) the band may be considered to be stutter. The following percent stutter values will serve as a guide:

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Locus	% Stutter (FMBIO II)	% Stutter (FMBIO III Plus)	Locus	% Stutter (FMBIO II)	% Stutter (FMBIO III Plus)
FGA	11.0	13.0	D3S1358	12.0	16.0
TPOX	8.0	11.0	Penta D	2.0**	2.0**
D8S1179	10.0	12.0	CSF1PO	11.0	13.0
VWA	16.0	16.0	D16S539	12.0	13.0
Penta E	2.0**	2.0**	D7S820	11.0	12.0
D18S51	13.0	13.0	D13S317	10.0	11.0
D21S11	15.0	15.0	D5S818	13.0	13.0
TH01	5.0	5.0			

** No stutter was observed during the validation studies. Therefore, the percent stutter specified is based upon recommendations of the manufacturer reported in the PowerPlex® 2.1 System Technical Manual.

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9.3.19.3 If the ratio of the OD (optical density) for the strong band to the weak band is less than the established stutter value (listed above), the allele will be considered to be stutter and will not be called even if it is believed that the band is a true allele.

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9.3.19.4 If the ratio of the OD (optical density) for the strong band to the weak band 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 independent sizer are in agreement (based upon knowledge of the system and experience) that the less intense band is stutter. The examiner will type “ST” in the percentage column of the STaRCall spreadsheet to signify that what was observed was considered to be stutter.

9.3.20 When a strong band in intensity and a weak band in intensity are observed within a single locus and the bands are separated by greater than one repeat unit, the difference in intensity could be the result of a null

allele or the inability of the primer to bind to the template fully in the flanking region of one of the alleles. In order to determine if a DNA profile containing bands with a difference in intensity is a result of a null allele, primer mis-pairing or a mixture of biological material from more than one source, the analyst must take into account all of the loci, use experience and/or the heterozygous percent intensity values to make an informed decision. If the percentage values obtained from the ratio of the OD (optical density) for the stronger band to the weaker band is equal to or greater than the established values (listed below), both bands within the locus may be considered to have originated from a single donor. The following heterozygous percent intensity values will serve as a guide:

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Locus	Lower Limit Difference Between Two Heterozygous Alleles (3 STD below the mean)	Locus	Lower Limit Difference Between Two Heterozygous Alleles (3 STD below the mean)
Penta E	45%	VWA	65%
D18S51	54%	Amelogenin	N/A
D21S11	63%	Penta D	No data available at this time
TH01	70%	CSF1PO	62%
D3S1358	67%	D16S539	64%
FGA	57%	D7S820	63%
TPOX	63%	D13S317	66%
D8S1179	63%	D5S818	68%

Note: Data generated from internal validation conducted by the Virginia Department of Forensic Science

9.3.21 If it is determined that a sample contains stutter bands at a majority of the loci and other artifactual bands are visible throughout the sample lane due to overloading of the sample and/or amplifying too much sample DNA, the sample will be re-amplified with less template DNA and then re-typed. A review of the product gel results may be needed to ensure that overloading of the sample has not occurred.

9.3.21.1 If it is determined that a band of lesser intensity in relationship to a more intense band is a true allele, the allele(s) will be reported on the landscape spreadsheet inside parentheses ().

9.3.22 When comparing the bands on the gel image to the allele calls generated by the STaRCall program, if the band visually is too weak to call or it is unclear whether the area that was marked by the computer program is actually a band, the examiner will manually type in a double question mark (??) in the percentage column of the STaRCall spreadsheet to signify that something was observed; however no conclusions could be made. If an artifactual band (i.e., n-1, n-2, n-3 or n-8) is observed due to overloading of the sample, the examiner will manually type "ART" in the percentage column of the STaRCall spreadsheet. If a band is believed to have originated from another channel and therefore is a result of bleed through, the examiner will manually type "BT" in the percentage column of the STaRCall spreadsheet.

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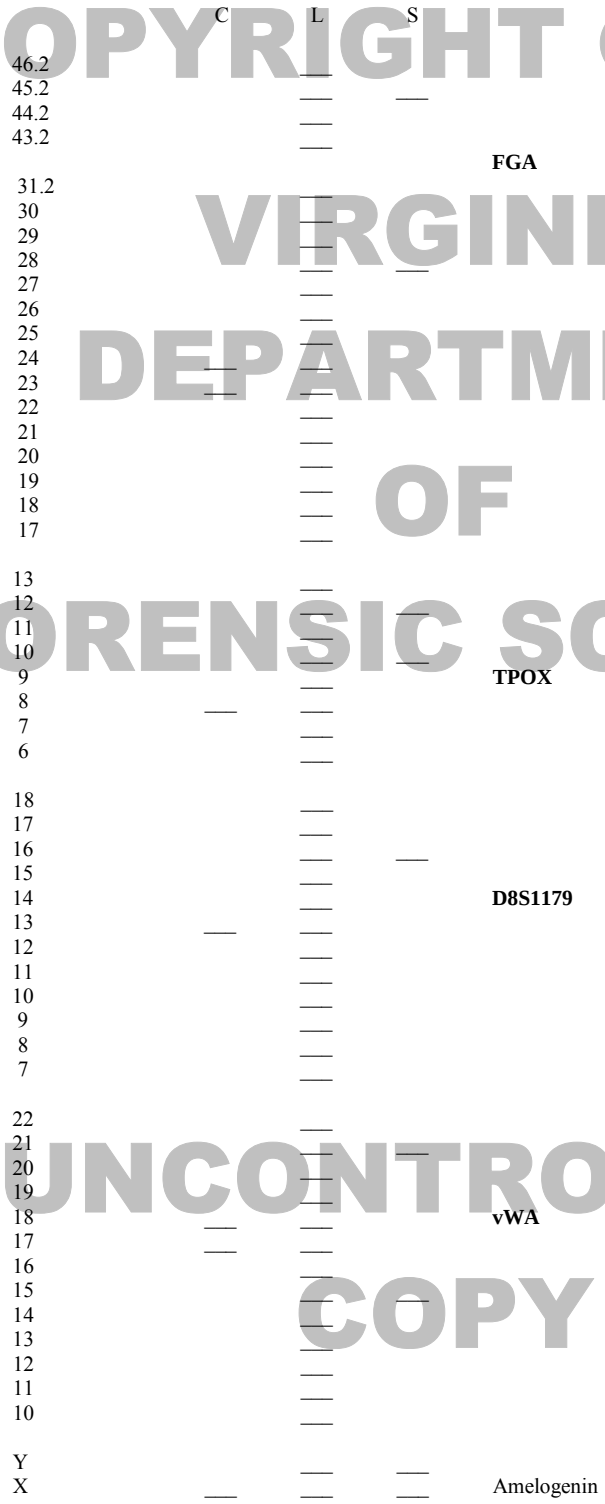
9.3.22.1 As a result of the out of range allele(s)/artifact(s) being listed out of sequence on the STaRCall speadsheet, the examiner will record on the STaRCall spreadsheet to which locus the band/artifact is associated.

9.4 Interpretation of PowerPlex® 16 BIO System Alleles

Amplified PowerPlex® 16 BIO System alleles are typed by noting which allelic ladder band(s) lines up with the test sample band(s), as demonstrated in the following examples:



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In this example, the FGA alleles for the sample lane (S) line up with alleles 28 and 45.2 of the allelic ladder (L), the TPOX alleles line up with alleles 10 and 12 of the allelic ladder, the D8S1179 allele lines up with allele 16 of the allelic ladder, the vWA alleles line up with alleles 15 and 21 of the allelic ladder, and the Amelogenin alleles line up with the X and Y alleles of the allelic ladder. Therefore, this sample has a genotype of FGA - 28, 45.2; TPOX - 10,12; D8S1179 – 16; vWA - 15,21; and Amelogenin X,Y. The Control DNA (9947A Cell Line designated as C) which is run on every gel has a genotype of FGA -23,24; TPOX – 8; D8S1179 – 13; vWA - 17,18; and Amelogenin X,X.

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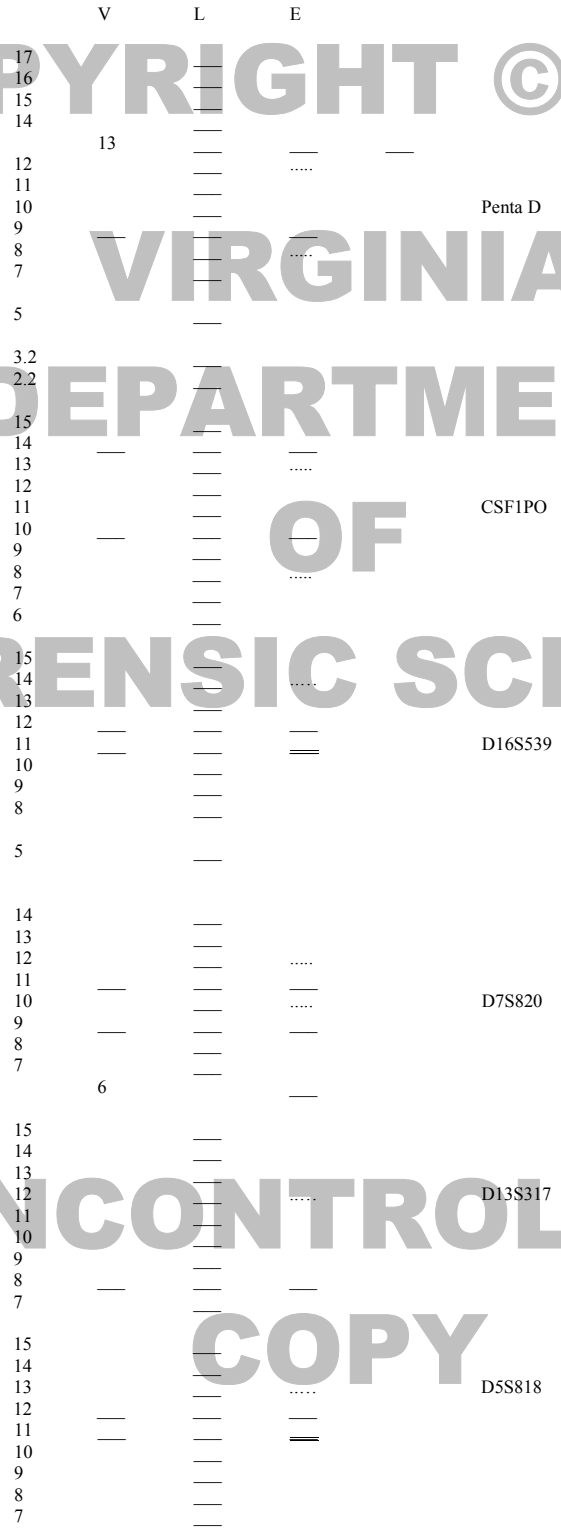
9.5 Interpretation of a Foreign Profile from a Mixture Searched in CODIS (Combined DNA Index System):

Example: Channel 4 – JOE



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Note: ····· Indicates an allele of weaker intensity ═ Indicates an allele of stronger intensity

In the above example, the victim (V) has the following profile: Penta D 9, 13; CSF1PO 10, 14; D16S539 11,12; D7S820 9,11; D13S317 8; and D5S818 11,12. Therefore, the minimum known foreign DNA profile that would be searched in CODIS would be:

Penta D	8, 12	(weaker intensity alleles)
CSF1P0	8, 13	(weaker intensity alleles)
D16S539	11, 14	The 11 allele is twice as intense as the 12 allele which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 11 allele based on the intensity is not solely from the victim. The 14 allele is foreign to the victim.
D7S820	10, 12	(weaker intensity alleles)
D13S317	12	(weaker intensity allele)
D5S818	11, 13	The 11 allele is twice as intense as the 12 allele which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 11 allele based on the intensity is not solely from the victim. The 13 allele is foreign to the victim.

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10 STATISTICAL CALCULATIONS

NOTE: Before a statistical calculation is applied the DNA profile will be evaluated to determine if the individual of interest is included or eliminated as a possible source of the genetic material. Refer to Chapter 9, Interpretation Of Powerplex® 16 BIO System PCR Amplification Results, 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.

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10.2 The frequency of occurrence of allele fragments reported as being consistent is determined for each polymorphic locus within a racial group. Using the allele designations specified in Chapter 9, Interpretation Of Powerplex® 16 BIO System PCR Amplification Results, determine the frequency for each allele from the appropriate population database.

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

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10.3.1 If a single source sample under analysis demonstrates two 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].

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10.3.2 If a single source sample under analysis consists of a single 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].

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

10.4 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 by subtracting the contribution of the known donor from the mixture. Exception: If the complete major contributor profile can be determined at all but one locus, where there is an overlapping pattern between the victim and suspect, a random match probability can be applied by considering the locus inconclusive for statistical purposes. Otherwise a "Likelihood Ratio or a "Combined Probability of Inclusion" discussed below must be applied.

10.5 Likelihood ratios will be used to calculate the match probability for the foreign DNA profile from an intimate sample (i.e., a biological sample that is known to have originated from one of the individuals involved, such as a vaginal swab collected from the victim) 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. The "Likelihood Ratio" frequency (L) is determined by the equation [1, 2, 9, 10]:

$$L = \frac{P(E/C_X)}{P(E/C_Y)}$$

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L 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? L provides a measure of how many times more characteristic of (1) the genetic evidence than of (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.

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.

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

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

Refer to Appendix I for specific formulas used for calculating likelihood ratio frequencies.

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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.

10.5.1 When the victim and suspect share the same two alleles (i.e., 12,13), they may be accounted as both unknowns or both knowns in the popstats 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 intensities 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
Suspect - 12,13
Evidence - 12,13 [both alleles will be considered as unknowns]

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

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

* Alleles in parentheses ( ) are lesser in intensity than the other alleles.

10.5.3 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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10.6 Combined probability of inclusion (CPI) will be used to calculate the match probability when a complex 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 from a NON-INTIMATE SAMPLE (i.e., biological samples where the source of origin is uncertain, such as a knife containing blood found in a wooded area). 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.

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

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

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

CPI = 1 – CPE

NOTE: All individuals of interest must be present in the profile at a particular locus in order to use that locus for CPE calculations (e.g., a three person mixture consisting of the victim and two suspects, all three individuals must be present).

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

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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	$2p_ip_j + 2(p_i + p_j - 4p_ip_j)F$

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.

For full siblings the following formulas will be used:

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

10.8 The following are the formulas used when calculating Paternity/Relationship type calculations:

10.8.1 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 give testing in the Mother-Child Combination.

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

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

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

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

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

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

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]

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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).

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

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

$$= \frac{(1/2)(0) + (1/2)(AF_{10})}{(1/2)(RM_{11}) + (1/2)(RM_{10})}$$

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

$$CPI = PI_1 \times PI_2 \times PI3 \ldots\ldots\ldots\ldots PI_n$$

10.8.4 Probability of Paternity: The probability of paternity is expressed as a frequency (or percentage), incorporating the paternity index and a prior probability (i.e., 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.

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

When the equation is divided by $P(R/H_1)$[2,3,4]

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Let $P(R/H_0) = X$ $PI = X/Y$
$P(R/H_1) = Y$

Probability of Paternity = (X/Y) / (X/Y) + 1

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OR

Probability of Paternity = (CPI)(Pr) / (CPI)(Pr) + (1-Pr), with Pr = 0.5

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Where: CPI = Combined Paternity Index
Pr = Prior Probability, Pr is 0.5

Refer to Appendix L for acceptable values of Probability of Paternity.

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10.9 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

10.9.1 $PI = (p)(AF_q) + (q)(AF_p) / 2pq = (p)(0.5) + (q)(0) / 2pq = 1/4q$

Person		
Child	P	Q
Alleged Father	Q	R

10.9.2 $PI = (p)(AF_q) + (q)(AF_p) / 2pq = (p)(1) + (q)(0) / 2pq = 1/2q$

Person		
Child	P	Q
Alleged Father	Q	Q

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10.9.3 $PI = (p)(AF_q) + (q)(AF_p) / 2pq = (p)(0.5) + (q)(0.5) / 2pq = (p + q) / 4pq$

Person		
Child	P	Q
Alleged Father	P	Q

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10.9.4 $PI = (q)(AF_p) / q^2 = (q)(0.5) / q^2 = 1 / 2q$

Person		
Child	Q	Q
Alleged Father	Q	R

10.9.5 $PI = (q)(AF_p) / q^2 = (q)(1) / q^2 = 1 / q$

Person		
Child	Q	Q
Alleged Father	Q	Q

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

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

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

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).

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	$2P_AP_B \times 2P_CP_D \times ½ \times$	$2P_AP_B \times 2P_CP_D \times$	$1 / 8P_AP_C$

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			½	$2P_AP_C$	

10.12 When performing a Missing Person (10.11) or Paternity Index (10.7.2) 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:

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

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

10.13 When applying the formulas for Missing Person (10.11) or Paternity Index (10.7.2) 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:

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

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

10.14 The following formula is used when calculating Paternity/Maternity type Calculations involving a potential mutation [7, 8].

$PI = M_{SR} / 2P_A$ — 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)

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

Average Mutation Rates Established by the AABB[11]

Locus	Paternal	Maternal
FGA	0.00312	0.00056
TPOX	0.00013	0.00005
D8S1179	0.00150	0.00023
VWA	0.00140	0.00033
Penta E	0.00131	0.00056
D18S51	0.00224	0.00064
D21S11	0.00148	0.00110
TH01	0.00008	0.00012
D3S1358	0.00128	0.00015
Penta D	0.00066	0.00064
CSF1PO	0.00144	0.00039
D16S539	0.00110	0.00026
D7S820	0.00119	0.00013
D13S317	0.00140	0.00041
D5S818	0.00115	0.00027

Note: When μ is used in the CPI

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10.15 Procedure for rounding frequencies:

10.15.1 Allele and genotype frequencies will be carried out to 3 digits. If the forth 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

10.15.2 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

10.15.3 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 Section 11, Report Writing, for the specific wording used when reporting match probabilities/likelihood ratios/combined probability of inclusion/probability of paternity.

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10.16 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 for the Virginia Division of Forensic Science population databases.

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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9. Gill, P. *et al*. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA, Forensic Science International 112, 17-40.

10. Curran, J.M., *et al*. (2005) Interpretation of repeat measurement DNA evidence allowing for multiple contributors and population substructure, Forensic Science International 148, 47-53.

11. 2003 AABB Annual Report

11 REPORT WRITING

11.2 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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11.2.1 Case identifier

11.2.2 Description of evidence examined

11.2.3 A description of the methodology

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11.2.4 Polymorphic loci examined or amplification system

11.2.5 A quantitative or qualitative interpretative statement

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11.2.6 Statistical frequencies of inclusion

11.2.7 Date issued

OF

11.2.8 Disposition of evidence

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

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11.3 Categories of Results

The results will fall within one of the following categories:

11.3.1 **Usable DNA Profiles**

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

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

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

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

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11.3.1.4.1 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.

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11.3.1.4.1.1 The DNA Data Bank search resulted in a match.

11.3.1.4.1.2 The DNA Data Bank search resulted in no matches.

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

11.3.1.4.2 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.

11.3.2 **Partial DNA Profiles** (preferential amplification, drop-out, insufficient quantity, and/or sample degradation)

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

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

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

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

11.4 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.

11.4.1 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.

METHODS:

- The method of deoxyribonucleic acid (DNA) analysis used was the Polymerase Chain Reaction (PCR).
- The PCR amplification kit used was the PowerPlex® 16 BIO system.
- The PowerPlex® 16 BIO 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.

11.4.2 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.

11.4.2.1 **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."

11.4.2.1.1 **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:

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

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(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 # ---).

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 #----)

11.4.2.2 **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."

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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.

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11.4.2.3 **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.

11.4.2.4 **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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11.4.2.5 **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."

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11.4.2.6 **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.

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11.4.2.7 **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."

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

11.4.2.9 **No Amplification:** If no DNA amplification results are obtained for a sample, this will be reported in the following manner:

No DNA amplification results were obtained from the EVIDENCE."

11.4.2.9.1 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 amplification results were obtained."

11.4.2.10 **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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11.4.2.11 **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.

11.4.2.12 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.

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11.4.2.13 **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.

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11.4.2.14 **Table/Appendices:** A table of the typing results will be included in the Certificate of Analysis as an Appendix. Both sperm and non-sperm fractions will be reported in the table for differentially extracted samples. The statement "Refer to Appendix 1 for the PowerPlex 16 BIO® typing results and Appendix 2 for the Abbreviations" will be listed just prior to the disposition statement in the Certificate of Analysis. If the suspect(s) is eliminated from all the analyzed evidence, the Certificate of Analysis will not contain Appendices. The table in the unedited format is not intended for use as the final court exhibit, but may be used as a court exhibit once edited and peer reviewed.

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11.4.2.15 **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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11.4.3 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 and conclusions. Refer to the below examples for when a CONCLUSIONS section might be incorporated into the report.

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.

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11.5 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.

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

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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.

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.

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11.5.1.1 **Single Source Foreign Donor**

11.5.1.1.1 **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."

11.5.1.1.2 **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."

11.5.1.1.3 **EXCLUSION**

"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."

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.)*

11.5.1.2 **Multiple Foreign Donors**

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11.5.1.2.1 **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.

11.5.1.2.2 **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."

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

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11.5.1.2.3 **EXCLUSION**

"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.)*

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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."

11.5.1.3 **No Foreign Profiles**

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

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

11.5.1.4.1 **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."

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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."

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

11.5.1.4.2.1 **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."

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.

11.5.1.4.2.2 **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

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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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11.5.1.5 **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:

“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.”

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.”

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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.”

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.

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

11.5.2.1 **Single Source Profiles**

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11.5.2.1.1 **INCLUSION**

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

11.5.2.1.2 **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.)*

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

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

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11.5.2.2.1 **INCLUSION**

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"A DNA mixture profile was developed from the EVIDENCE. 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).*

11.5.2.2.2 **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.

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11.5.2.3 **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.

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11.5.2.3.1 **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).*

11.5.2.3.2 **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 Data Bank searches, if appropriate.)*

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

11.5.2.4.1 **NO CONCLUSIONS REGARDING KNOWN SAMPLE**

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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."

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

11.5.2.4.2.1 **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."

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.

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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."

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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.

11.5.2.4.2.2 **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:

"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."

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

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

<u>Complex Mixture</u>

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.

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11.5.3 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.

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11.5.3.1 Bullet/List Format

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

- 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)

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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)

11.5.3.2 Chart Format

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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

11.6 Report Format for Non-Subject Cases

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

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11.6.1 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.

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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.

11.6.1.1 **Single Source Foreign Donor**

11.6.1.1.1 **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.)*

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11.6.1.1.2 **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.)*

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11.6.1.2 **Multiple Foreign Donors**

11.6.1.2.1 **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).

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."

11.6.1.3 **NO Foreign Profiles**

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

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

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11.6.1.4.1 **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:

"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."

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."

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

11.6.1.4.2 **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."

"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.

11.6.1.5 **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:

"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

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.

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

11.6.2.1 **Single Source Profiles**

11.6.2.1.1 **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."

11.6.2.1.2 **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):

Name:
SSN:
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."

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.

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 to a **decedent,** the paragraph requesting the direct comparison sample 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 C of A 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.

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. A Memorandum of Record will be generated by the examiner who made the hit (target profile) and forwarded to the examiner whose case the hit was made against (candidate profile) for placement in the case file. The memorandum of record should state that a hit was obtained between the specimen id # _____ and specimen id # _____. In addition, the examiner who made the hit will print out a copy of the Match Details Report and include this with the memorandum of record in the case file.

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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.

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

"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.

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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.

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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.

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."

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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.

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. A Memorandum of Record will be generated by the examiner who made the hit (target profile) and forwarded to the examiner whose case the hit was made against (candidate profile) for placement in the case file. The memorandum of record should state that a hit was obtained between the specimen id # _____ and specimen id # _____. In addition, the examiner who made the hit will print out a copy of the Match Details Report and include this with the memorandum of record in the case file.

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

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

11.6.2.2.1 **NO Match in Data Bank**

"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.

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

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.

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11.6.2.2.2 **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:

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 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 to a **decedent**, the paragraph requesting the direct comparison sample 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. A Memorandum of Record will be generated by the examiner who made the hit (target profile) and forwarded to the examiner whose case the hit was made against (candidate profile) for placement in the case file. The memorandum of record should state that a hit was obtained between the specimen id # _____ to specimen id #

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_____. In addition, the examiner who made the hit will print out a copy of the Match Details Report and include this with the memorandum of record in the case file.

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.

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

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"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."

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.

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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. A Memorandum of Record will be generated by the examiner who made the hit (target profile) and forwarded to the examiner whose case the hit was made against (candidate profile) for placement in the case file. The memorandum of record should state that a hit was obtained between the specimen id # _____ to specimen id # _____. In addition, the examiner who made the hit will print out a copy of the Match Details Report and include this with the memorandum of record in the case file.

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11.6.2.3 **Mixture Profiles with No Discernible Major/Minor Contributor**

11.6.2.3.1 **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 was 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.

11.6.2.3.2 **Match Obtained in Data Bank to and 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) cannot be eliminated as a contributor to this mixture profile:

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

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 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 to a **decedent**, the paragraph requesting the direct comparison sample 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 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. A Memorandum of Record will be generated by the examiner who made the hit (target profile) and

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forwarded to the examiner whose case the hit was made against (candidate profile) for placement in the case file. The memorandum of record should state that a hit was obtained between the specimen id # _____ and specimen id # _____. In addition, the examiner who made the hit will print out a copy of the Match Details Report and include this with the memorandum of record in the case file.

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.

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

"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.

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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.)"

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.

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. A Memorandum of Record will be generated by the examiner who made the hit (target profile) and forwarded to the examiner whose case the hit was made against (candidate profile) for placement in the case file. The memorandum of record should state that a hit was obtained between the specimen id # _____ and specimen id # _____. In addition, the examiner who made the hit will print out a copy of the Match Details Report and include this with the memorandum of record in the case file.

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

11.6.2.4.1 **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

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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.

11.6.2.4.2 **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.

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

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."

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."

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."

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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.

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

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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.

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."

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."

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or

"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."

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:

"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.

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11.7 Reporting Format for Reconstruction Cases

When DNA results are used to assist in crime scene reconstruction, the identity of the individual(s) involved is typically not in question; however, the question is which donors deposited each sample. Therefore, it is generally not necessary to report each donor who is eliminated from a sample, but rather which donor cannot be eliminated as the contributor of the various samples tested throughout the crime scene. Results for reconstruction cases will be reported in the following manner:

11.7.1 Single Source Profiles

"DNA profiles were developed from the EVIDENCE. VICTIM 1 cannot be eliminated as a contributor of the DNA profiles developed from EVIDENCE. VICTIM 2 cannot be eliminated as a contributor of the DNA profiles developed from EVIDENCE."

11.7.2 Mixture Profiles

"A DNA mixture profile was developed from the EVIDENCE. VICTIM 1 and VICTIM 2 cannot be eliminated as contributors to the DNA mixture profile developed from EVIDENCE."

NOTE: No mention of major/minor contributors should be made for mixture profiles obtained in reconstruction cases.

11.7.3 Depending on the complexity of the results and the number of items tested, the final conclusions for a reconstruction case may alternatively be reported in a chart format which includes the item number, item description and contributor.

11.7.3.1 Chart Format

"DNA profiles were developed from the EVIDENCE. DNA profiles consistent with mixtures were developed from the EVIDENCE. Refer to the chart below for a summary of the conclusions.

ITEM	ITEM DESCRIPTION	CONTRIBUTOR
1	Swab from front stoop	John Doe
2	Swab from front door knob	Mary Jane Doe
5	Swab from sidewalk	John Doe and Mary Jane Doe
7	Swab from bathroom sink	John Doe and Mary Jane Doe
10	Swab from bathroom light switch	John Doe*

* DNA types different from John Doe were developed from this sample. Insufficient information exists to draw a conclusion regarding Mary Jane Doe as a contributor to these DNA types.

NOTES: It may be important in some cases to indicate that no foreign/different profiles to the individuals involved were developed. If so, use the following wording: "No DNA profiles foreign to VICTIM(S) were developed from EVIDENCE" or "No DNA profiles different from VICTIM(S) were developed from EVIDENCE".

If a DNA profile foreign/different to the individuals involved is developed, refer to the appropriate subsection under REPORTING FORMAT FOR SUBJECT CASES.

11.8 Reporting Format for Paternity, Missing Person and Body Identification Cases

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:

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

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

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

"Paternity calculations regarding the above analysis will be the subject of a separate report."

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

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

11.8.2 Missing Person Cases

"DNA profiles were developed from the EVIDENCE and from the blood (or buccal) samples from RELATIVE. RELATIVE cannot be eliminated as a biological parent/offspring of the contributor of the DNA profile developed from EVIDENCE."

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 BIO typing results and

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

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

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"Missing person calculations regarding the above analysis will be the subject of a separate report."

11.8.3 Body Identification Cases

11.8.3.1 Comparison to Personal Effects

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"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."

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.

11.8.3.2 Comparison to Biological Relatives

"A DNA profile was developed from the SAMPLE FROM DECEDENT and from the blood (or buccal) samples from RELATIVE. RELATIVE cannot be eliminated as a biological parent/offspring of the contributor of the DNA profile developed from EVIDENCE."

NOTE: Generally, statistical calculations are not performed for body identification cases nor are the typing results reported in a table.

11.9 Reporting Format for Statistical Calculations

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, 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).

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11.9.1 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 BIO loci" or stating "the PowerPlex® 16 BIO loci with the exception of LOCUS/LOCI". A random match probability will be reported in the following manner:

11.9.1.1 When all population groups exceed 6.5 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 1 in greater than 6.5 billion (which

is approximately the world population) in the Caucasian, Black and Hispanic populations."

11.9.1.2 When only one or two population groups exceed 6.5 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 6.5 billion (which is approximately the world population) in the Caucasian population."

11.9.1.3 When none of the population groups exceed 6.5 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."

11.9.1.4 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 6.5 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.

11.9.2 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 BIO loci" or stating "the PowerPlex® 16 BIO 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.

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The reported value for the likelihood ratio probability will be truncated to 2 significant figures.

11.9.3 Combined Probability of Exclusion/Inclusion (CPE/CPI)

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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 BIO loci" or stating "the PowerPlex® 16 BIO loci with the exception of LOCUS/LOCI". The combined probability of exclusion/combined probability of inclusion will be reported in the following manner:

11.9.3.1 When all population groups exceed 6.5 billion (approximate world population):

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"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 6.5 billion (which is approximately the world population) in the Caucasian, Black and Hispanic populations."

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11.9.3.2 When only one or two population groups exceed 6.5 billion (approximate world population):

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"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 6.5 billion (which is approximately the world population) in the Caucasian population."

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11.9.3.3 When none of the population groups exceed 6.5 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.

11.9.4 Paternity Index and Probability of Paternity

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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 BIO loci" or stating "the PowerPlex® 16 BIO loci with the exception of LOCUS/LOCI". Paternity Index and Probability of Paternity will be reported in the following manner:

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"Based on a review of the typing results for the CHILD/EVIDENCE and the VICTIM and ALLEGED FATHER previously analyzed at the PowerPlex® 16 BIO loci and addressed in the Certificate of Analysis dated _____, 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:

Caucasian	CPI = _____	Probability of Paternity = _____%
Black	CPI = _____	Probability of Paternity = _____%
Hispanic	CPI = _____	Probability of Paternity = _____%

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."

NOTES: If one report is issued containing the PowerPlex® 16 BIO 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:"

The reported value for the combined paternity index will be truncated to 2 significant figures.

Refer to Appendix L for acceptable values for the Probability of Paternity. The Probability of Paternity percentage (%) will be carried out to 4 places after the decimal point.

11.9.5 Missing Person

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 BIO loci" or stating "the PowerPlex® 16 BIO loci with the exception of LOCUS/LOCI". Missing person statistics will be reported in the following manner:

11.9.5.1 BOTH Parents Available for Testing

"Based on a review of the typing results for the EVIDENCE and MOTHER and FATHER previously analyzed at the PowerPlex® 16 BIO loci and addressed in the Certificate of Analysis dated_____:

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."

NOTE: If one report is issued containing the PowerPlex® 16 BIO 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:"

The reported value for the likelihood ratio probability will be truncated to 2 significant figures.

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11.9.5.2 **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 BIO 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

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 BIO 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 – Conversion Factors and Other Useful Information

1 Conversions

One microgram = 1 µg = 1 x 10^{-6} g = 1000 ng

One nanogram = 1 ng = 1 x 10^{-9} g = 1000 pg

One picogram = 1 pg = 1 x 10^{-12} g = 1 x 10^{-3} ng

One liter = 1 L = 1000 mL

One milliliter = 1 mL = 1 x 10^{-3} (0.001) L = 1000 µL

One microliter = 1 µL = 1 x 10^{-3} mL = 0.001 mL

2 Concentrations

2.1 Concentration can be expressed several ways:

2.1.1 **Weight percent = (mass A / total mass of solution) x 100**
or
simplified : $Wt\%_A = (g_A/100mL \text{ of solution}) \times 100$

2.1.2 **Volume percent = (volume A/total volume of solution) x 100**
or
simplified: $Volume\ \%_A = (volume_A/100 \text{ mL of solution}) \times 100$

2.1.3 **Molarity (*M*): *M* = no. moles solute A/no. liters solution**

= molecular weight of solute A in 1000 mL solution

where 1 mole of A = 1 gram formula weight of A

2.1.4 **Normality *(N)* = no. MW/no. liters solution**

where in acid-base reactions:

MW acid = weight of acid which reacts with 1 mole of OH^-
MW base = weight of base which reacts with 1 mole of H^+

The normality of a given reagent depends on the reaction in which it participates. (Example: 1 L of 1*M* H_3PO_4 which can have *N* = 1, 2 or 3 depending upon the reaction in which it is involved.) Because of ambiguities, the concept of normality is to be used carefully.

2.2 Examples:

2.2.1 **Prepare 50% solution of polyethylene glycol (PEG):**

Place 50 g of PEG in a flask and bring to a volume of 100 mL with H_2O.

Wt % PEG = (50 g/100 mL) x 100 = 50%

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2.2.2 **Prepare 100 mL of 2*M* HCl from a stock solution of 12*M* HCl. (The question is how much stock solution of 12*M* HCl is needed?)**

Conc of Stock Soln = C_s = 12*M* Conc of Final Soln = C_f = 2*M*

Volume of Stock Soln = V_s = ? Volume of Final Soln = V_f = 0.1 L

$C_s \cdot V_s = C_f \cdot V_f$

$V_s = (C_f \cdot V_f)/C_s = 2(0.1)/12 = 0.2/12 = 0.0167$ L

$V_s = 0.0167$ L = 16.7 mL

Answer: Take 16.7 mL of 12*M* HCl and dilute to final volume of 100 mL to give 2*M* HCl.

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Appendix B- 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:

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Identity
Concentration
Lot number
Date of preparation
Initials of preparer
Date of expiration

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and if appropriate:

Date of autoclaving
Storage requirements

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2. All reagents will be prepared with Type I water, unless other wise 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.

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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 Control DNA (9947A) are examples of such reagents where this applies. The original reagent stock lot number will be used.

ACETIC ACID - GLACIAL

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Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date

Purchased from Fisher Scientific, Fair Lawn, NJ., Catalog number A507-212.

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ACETIC ACID, 0.5% IN 95% ETHANOL

Expiration date: Twelve months from date of preparation

	100 mL	200 mL
Glacial acetic acid	500 µL	1 mL
95% ethanol	99.5 mL	199 mL

Add the glacial acetic acid to 95% ethanol and mix well.

AGAROSE - DNA TYPING GRADE

Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date

Purchased from Invitrogen, Carlsbad, CA, Catalog number 14610-018, 500 g bottle.

Also purchased from Bio-Rad, Melville, NY., Catalog number 162-034, 500 g bottle.

AGAROSE - 3:1 NUSIEVE

Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date

Purchased from BMA, Rockland, ME., Catalog number 50090, 125 g bottle.

ALUQUANT™ HUMAN DNA QUANTITATION KIT

Expiration date: Manufacturer's expiration date.

Purchased from Promega Corporation, Madison, WI., Catalog Number DC1011 for 400 reaction kit.

Store all AluQuant™ Human Quantitation System reagents at -20ºC.

Kit components included (expiration date same as kit unless specified):

- ALUQUANT™ ENZYME SOLUTION
- ALUQUANT™ DENATURATION SOLUTION
- ALUQUANT™ NEUTRALIZATION SOLUTION
- ALUQUANT™ PROBE MIX
- ENLITEN® LUCIFERASE/LUCIFERIN (L/L) REAGENT

  Expiration date: AluQuant™ Human Quantitation System kit expiration date when in lyophilized state. Once reconstituted discard after two weeks.

  Equilibrate to room temperature prior to use. Add the ENLITEN® Luciferase/Luciferin (L/L) Reconstitution Buffer to the ENLITEN® Luciferase/Luciferin Reagent in the brown bottle and then invert the bottle several times to mix.

  Store unused portions of Enliten® Luciferase/Luciferin (L/L) Reagent in a -20ºC up to two weeks.

- ENLITEN® LUCIFERASE/LUCIFERIN (L/L) RECONSTITUTION BUFFER - Equilibrate to room temperature prior to use.

- HUMAN GENOMIC DNA STANDARD (20 ng/µL) -Prepare dilution of the Human Genomic DNA Standard as follows:

  NOTE: Dilution series should be made up fresh each use.

  1. Label seven 0.5 mL autoclaved tubes (4 ng/µL, 2 ng/µL, 1 ng/µL, 0.5 ng/µL, 0.25 ng/µL, 0.125 ng/µL, and 0.062 ng/µL)

  2. Vortex the Human Genomic DNA Standard (20 ng/µL) to mix it thoroughly.

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3. Pipette into the tube labeled 4 ng/µL (tube 1) 60 µL of TE^{-4} Buffer and 15 µL of Human Genomic DNA Standard; vortex well.

4. Pipette into the tube labeled 2 ng/µL (tube 2) 40 µL of TE^{-4} Buffer and 40 µL of sample from tube 1. Vortex well

5. Pipette into the tube labeled 1 ng/µL (tube 3) 40 µL of TE^{-4} Buffer and 40 µL of sample from tube 2.
Vortex well

6. Pipette into the tube labeled 0.5 ng/µL (tube 4) 40 µL of TE^{-4} Buffer and 40 µL of sample from tube 3.
Vortex well

7. Pipette into the tube labeled 0.25 ng/µL (tube 5) 40 µL of TE^{-4} Buffer and 40 µL of sample from tube 4.
Vortex well

8. Pipette into the tube labeled 0.125 ng/µL (tube 6) 40 µL of TE^{-4} Buffer and 40 µL of sample from tube 5. Vortex well

9. Pipette into the tube labeled 0.062 ng/µL (tube 7) 40 µL of TE^{-4} Buffer and 40 µL of sample from tube 6. Vortex well

10. Pipette in the tube labeled control (tube 8) 40 µL of TE^{-4} Buffer.

- HYDROCHLORIC ACID (250 mM)

  Prepare a 200 mM HCl solution by briefly vortexing the 250 mM HCl solution and then placing 12 mL of the solution into a 15 mL conical tube. Subsequently add 3 mL of sterile Type I water and mix by inverting several times. The HCl solution can be stored at room temperature.

- NUCLEASE-FREE WATER

AMMONIUM PERSULFATE

Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date

NOTE: Prepare on the day of use

Based on the volume needed, add 0.1g increments of ammonium persulfate to a 15 mL conical vial. Add 1 mL Type I Water per 0.1 g of ammonium persulfate.

Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number A6761, 250 g bottle.

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).

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BIND SILANE

Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date

Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number M-6514, 25 mL bottle.

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CALCUM CHLORIDE, 1M

Expiration date: Twelve months from the date of preparation

	MW	100 mL
$CaCl_2$	110.9	11.09 g
Type I Water		90 mL

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Dissolve the appropriate amount of $CaCl_2$ into Type I Water. Bring up to final volume

$CaCl_2$ - Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number C1016, 100 g bottle.

CALCUM CHLORIDE BUFFER, 1X

Expiration date: Twelve months from date of preparation

	MW	100 mL
1M Tris	121.1	5 mL
1 M $CaCl_2$	110.9	1 mL
Type I water		94 mL

Mix well. May be stored at room temperature.

DITHIOTHREITOL, 0.39 M (DTT)

Expiration date: Twelve months from date of preparation

	MW	25 mL
DTT	154.2	1.50 g
Type I Water		15 mL

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Add DTT to Sterile Type I Water and mix well. When DTT is completely dissolved, bring up to final volume with Sterile Type I Water. Filter sterilize and divide into 500 µL aliquots in sterile microcentrifuge tubes and store at -20 C.

DTT (Molecular Biology Grade) - Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number D0632, 25 g bottle.

DNA IQ™ SYSTEM

Expiration date: Manufacturer's expiration date

Purchased from Promega Corporation, Madison, WI., Catalog Number DC6700 for 400 reaction kit.

Store all DNA IQ™ Isolation System reagents at room temperature.

Kit components included (expiration date same as kit unless specified):

- DNA IQ™ LYSIS BUFFER

  On the day of use add 2.5 µL of 0.39 M DTT for every 100 µL of DNA IQ™ Lysis Buffer that is prepared.

- DNA IQ™ RESIN
- DNA IQ™ WASH BUFFER

  Add 35 mL of 95% ethanol and 35 mL of Isopropyl Alcohol to 2X Wash Buffer. Be certain to accurately measure the alcohol volumes since this could negatively impact the performance of the extraction. Mix contents and store at room temperature in a tightly capped container.

- DNA IQ™ ELUTION BUFFER

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ETHANOL, 95%

Expiration date: Dispose of when necessary

Purchased as Reagent Alcohol (190 Proof) from VWR, Bridgeport, NJ, Catalog number 6590-1, 4 L bottle.

ETHIDIUM BROMIDE (EtBr), 5 mg/mL

WARNING!

ETHIDIUM BROMIDE IS MUTAGENIC. ALWAYS WEAR GLOVES WHEN HANDLING EtBr.

Expiration date: Twelve months from the date of preparation

	25 mL	50 mL	100 mL
Ethidium Bromide	0.125 g	0.25 g	0.5 g
Type I Water	20 mL	40 mL	80 mL

Add EtBr to Type I Water. When EtBr is in solution, bring up to final volume. (Caution: EtBr is light sensitive. Be careful not to expose it to light for any extended period of time.) Store in a brown bottle or foil wrapped container at room temperature.

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Ethidium Bromide - Purchased from VWR, Bridgeport, NJ., Catalog number 7738, 100 g bottle.

Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number E8751, 5 g bottle.

NOTE: Ethidium Bromide may also be made using ethidium bromide solution (10 mg/mL) in a 1:2 dilution with Type I Water (10 mL EtBr + 10 mL Type I Water = 20 mL EtBr solution, 5 mg/mL).

Ethidium Bromide Solution (10 mg/mL) - purchased from GIBCO BRL, Grand Island, NY Catalog number 5585UA, 10 mL bottle.

ETHYLENEDIAMINETETRAACETIC ACID (EDTA), 0.5 M

Expiration date: Twelve months from date of preparation

	MW	500 mL	1L	2 L
EDTA	372.2	93.05 g	186.1 g	372.2 g
Type I Water		375 mL	750 mL	1.5 L

Add EDTA to Type I Water. Mix well and pH to 8.0 with 10 N NaOH (EDTA will not go into solution unless pH = 8.0). When totally dissolved, bring up to final volume with Type I Water and recheck pH. Dispense into appropriate container and autoclave at 215 F at 20 lb for 20 minutes.

EDTA (Molecular Biology Grade) - Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number E 5134, 500 g bottle.

EDTA (Disodium Salt) purchased from Invitrogen, Carlsbad, CA , Catalog number 15575-028, 500 g bottle.

GENE PAGE PLUS™ GEL SOLUTION, 6.0%

Expiration date: Manufacturer's expiration date

Purchased from Amresco Branded Product Group, Solon, OH., Catalog number E568, 500 mL bottle (800-892-2805).

GLYCEROL

Expiration date: Manufacturer’s expiration date if listed, otherwise no expiration date

Purchased from Invitrogen, Carlsbad, CA. Catalog Number 15514-011, 500 mL bottle.

8-HYDROXYQUINOLINE

Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date

Purchased from VWR, Bridgeport, NJ., Catalog number 4969-125*NY, 125 gram bottle.

LADDER, 123 BASE PAIR

Expiration date: Twelve months from the date of receipt when stored at -20^{0}C

Purchased from Invitrogen, Carlsbad, CA, Catalog number 15613-011, 100 ug vial.

LOADING BUFFER, 5X

Expiration date: No expiration date. Discard as needed.

Stock	20 mL	50 mL
Bromophenol Blue	0.02 g	0.05 g
Glycerol	10 mL	25 mL

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0.5 M EDTA	4 mL	10 mL
Sterile 1X TE-4 Buffer	6 mL	15 mL

Combine components and mix well. Divide into 1 mL aliquots and store at -20 C. One aliquot may be left at room temperature for daily use.

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Bromophenol Blue - Purchased from Sigma Chemical Co., St. Louis, MO, Catalog Number B5525, 10 g bottle.

For Glycerol, EDTA and TE-4 Buffer see this list.

PAGE PLUS™ GEL SOLUTION, 40%

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Expiration date: Manufacturer's expiration date

Purchased from Amresco Branded Product Group, Solon, OH., Catalog number E562, 100 or 500 mL bottle (800-892-2805).

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PCR DIGESTION BUFFER, 1.0%

OF

Expiration date: Twelve months from date of preparation

Stock	100 mL
1 M Tris	1 mL
0.5 M EDTA	2 mL
NaCl	0.29 g
20% SDS	5 mL
Type I Water	91mL

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Mix all reagents together and pH to 7.5 using dilute HCL. Adjust to final volume with Type I Water and store at room temperature.

PHENOL-CHLOROFORM-ISOAMYL ALCOHOL, 25:24:1

Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date

To aid visualization add approximately 0.07125 g of 8-Hydroxyquinoline/100 mL of phenol-chloroform-isoamyl alcohol solution until the phenol turns slightly yellow in color. CAUTION: Measure 8-Hydroxyquinoline under hood. Store at 2-5 C.

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Purchased from Sigma Chemical Co., St. Louis, MO Catalog number P 3803, 100 and 400 mL bottle.

Purchased from Invitrogen, Carlsbad, CA, Catalog number 5593UB, 400 mL bottle.

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PHOSPHATE-BUFFERED SALINE (PBS), 1X, pH 7.2

Expiration date: Manufacturer’s expiration date if listed, otherwise no expiration date

Purchased from Cambrex, Walkersville, MD., Catalog # 17-516Q 1L bottle.

PLEXOR® HY SYSTEM

Expiration date: Manufacturer's expiration date.

Purchased from Promega Corporation, Madison, WI., Catalog Number DC1001 for 200 reaction kit (or DC1000 for 800 reaction kit).

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Store all Plexor® HY System reagents at -20 ºC upon receipt. After first use, the DNA standard must be stored at 4 ºC, and all other kit components stored at -20 ºC.

Kit components included (expiration date same as kit unless specified):

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- PLEXOR® HY 2X MASTER MIX
- PLEXOR® HY 20X PRIMER/IPC MIX
- PLEXOR® HY MALE GENOMIC DNA STANDARD, 50 ng/µL
  - Diluted DNA standard should be freshly prepared each day of use.
- H_2O, AMPLIFICATION GRADE

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POWERPLEX® 16 BIO SYSTEM KIT

OF

Expiration date: Manufacturer's expiration date

Purchased from Promega, Madison, WI., Catalog Number DC6541 for 100 reaction kit or DC6540 for 400 reaction kit.

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Kit components included (expiration date same as kit unless specified):

- POWERPLEX® 16 BIO PRIMER PAIRS
- POWERPLEX® 16 BIO ALLELIC LADDER
- STR LOADING BUFFER, 2X (TRACKING DYE)
- MATRIX 16 BIO
- GOLD ST*R 10X BUFFER - Purchased from Promega, Madison, WI. Catalog Number DM2411, 1.2 mL tube
- FLUORESCENT INTERNAL LANE STANDARD 600 BIO
- CONTROL DNA (GM9947A CELL LINE) - Purchased from Promega Corporation, Madison, WI., 250 ng tube, 10 ng/µL, Catalog number DD1001.
- BROMOPHENOL BLUE LOADING BUFFER

PROTEINASE K ENZYME (20 mg/mL)

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Expiration date: Twelve months from date of preparation

Stock	5 mL	15 mL	25 mL
Proteinase K	100 mg	300 mg	500 mg
Sterile Type I Water	5 mL	15 mL	25 mL

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Add lyophilized Proteinase K to appropriate amounts of sterile Type I Water. When completely reconstituted, divide into 250 µL aliquots in microcentrifuge tubes and store at -20 C. Thaw at room temperature prior to use and keep on ice once thawed.

Purchased from Invitrogen, Carlsbad, CA., Catalog number 25530-031, 1.0 g bottle. Store dry at 2-5 C.

Purchased from Sigma Chemical CO., St. Louis, MO, Catalog number P 2308, 100 mg bottle.

QIAamp® DNA BLOOD MINI KIT

Expiration date: Refer to individual kit component expiration dates.

Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 51104 for 50 sample kit, 51106 for 250 sample kit, or 51108 for 1000 sample kit.

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Kit components included:

- QIAGEN® AE ELUTION BUFFER - Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 19077.

  Expiration date: Twelve months from date of receipt into the laboratory

- QIAGEN® AL LYSIS BUFFER - Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 19075.

  Expiration date: Twelve months from date of receipt into the laboratory

- QIAGEN® AW1 WASH BUFFER (concentrate) - Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 19081.

  Expiration date: Twelve months after dilution with ethanol

  Before initial use, dilute concentrate with 95% ethanol according to the following table:

Kit Size	AW1 concentrate	Ethanol	Final volume
50	19 mL	25 mL	44 mL
250	95 mL	125 mL	220 mL
1000	175 mL	230 mL	405 mL

  Once the QIAGEN® AW1 wash buffer has been diluted with ethanol, store the buffer at room temperature.

- QIAGEN® AW2 WASH BUFFER (concentrate) - Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 19072.

  Expiration date: Twelve months after dilution with ethanol

  Before initial use, dilute concentrate with 95% ethanol according to the following table:

Kit Size	AW2 concentrate	Ethanol	Final volume
50	13 mL	30 mL	43 mL
250	66 mL	160 mL	226 mL
1000	127 mL	300 mL	427 mL

  Once the QIAGEN® AW2 wash buffer has been diluted with ethanol, store the buffer at room temperature.

- QIAGEN® PROTEASE - Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 19155 (125 mg) or 19157 (4 x 125 mg).

  Expiration date: 6 months after sample is reconstituted if stored at –20°C.
  2 months after sample is reconstituted if stored at 4°C.

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Reconstitute with supplied QIAGEN® protease solvent before initial use according to the table below:

Kit Size	QIAGEN® Protease	Protease solvent
50	24 mg	1.2 mL
250	110 mg	5.5 mL
1000	4 x 110 mg	4 x 5.5 mL

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- QIAGEN® PROTEASE SOLVENT

Expiration date: Twelve months from date of receipt into the laboratory

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QIAamp® 96 DNA BLOOD KIT

Expiration date: Refer to individual kit component expiration dates as specified under QIAamp® DNA BLOOD MINI KIT

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Purchased from QIAGEN, Inc., Valencia, CA, Catalog # 51161 for 4 plate kit, or #51162 for 12 plate kit.

Kit components included:

OF

- QIAGEN® AE ELUTION BUFFER
- QIAGEN® AL LYSIS BUFFER
- QIAGEN® AW1 WASH BUFFER (concentrate)
- QIAGEN® AW2 WASH BUFFER (concentrate)
- QIAGEN® PROTEASE
- QIAGEN® PROTEASE SOLVENT

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QUANTITATION STANDARDS - K562 DNA, UNCUT

Expiration date: Manufacturer's expiration date

Purchased from Invitrogen, Carlsbad, CA., Catalog number 4420SA, 300 µL each. Standards include 500 ng, 250 ng, 125 ng, 63 ng, 31 ng and 15 ng.

SARKOSYL, 20%

Expiration date: Twelve months from date of preparation

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	MW	250 mL	500 mL	2 L
N-Lauroylsarcisine	293.4	50 g	100 g	400 g
Type I Water		200 mL	400 mL	1600 mL

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Add the appropriate amount of N-Lauroylsarcisine to Type I Water and mix until completely dissolved and the solution is clear. Bring up to volume with Type I Water, filter sterilize and store in sterile bottles at room temperature.

N-Lauroylsarcisine, Sodium Salt - Purchased from Sigma Chemical Co., St, Louis, MO., Catalog number L 5125, 500 g bottle.

SDS (SODIUM DODECYL SULFATE), 0.1%

Expiration date: Twelve months from date of preparation

Add 5 mL of 20% SDS solution to 995 mL of Type 1 Water.

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SDS (SODIUM DODECYL SULFATE), 20%

Expiration date: Twelve months from date of preparation

	MW	1 L	2 L
20% SDS	288.3	200 g	400 g
Type I Water		750 mL	1500 mL

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CAUTION: AN AEROSOL MASK OR FUME HOOD SHOULD BE USED WHEN MAKING THIS SOLUTION.

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Add the appropriate amount of SDS to Type I Water and mix until SDS is completely into solution and solution is clear, not cloudy. Adjust to final volume with Type I Water and store at room temperature.

OF

SDS - Purchased from VWR, Bridgeport, NJ., Catalog number 7718, 100 g bottle.

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SODIUM CHLORIDE (NaCl), 5 M

Expiration date: Dispose of when necessary

	MW	500 mL	1 L	4 L	16 L
NaCl	58.44	146.1 g	292.2 g	1168.8 g	4675.2 g
Type I Water		500 mL	1 L	4 L	16 L

Begin with 60-75% of the total volume of Type I Water in a beaker on a stir plate and add the NaCl slowly. Next add almost all of the required volume of Type I Water because it will be needed in order for the NaCl to go into solution. When the NaCl is dissolved, bring up to final volume by adding Type I Water. Larger volumes (4 L) that are to be used for denaturation solutions need not be autoclaved. NaCl for all other uses must be dispensed into appropriate containers and autoclaved at 215 F for 20 minutes at 20 lb.

NaCl - Purchased from VWR, Bridgeport, NJ., Catalog number 7532, 12 kg box.

NaCl, 5 M - Purchased from Invitrogen, Carlsbad, CA., Catalog number 4740UB, 10 liter bottle.

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SODIUM HYDROXIDE (NaOH), 2 N

COPY

Expiration date: Twelve months from date of preparation.

	MW	2 L	20 L
10 N NaOH	40.0	400 mL	4 L
Type I Water		1600 mL	16 L

Purchased from VWR, Bridgeport, NJ., Catalog number H385, 4 liter bottle.

Combine components and mix well. Store at room temperature.

SODIUM HYDROXIDE (NaOH), 5 N

Expiration date: Dispose of when necessary

	MW	1 L
10 N NaOH	40.0	500 mL
Type I Water		500 mL

Begin with 60-75% of the total volume of Type I Water in a beaker on a stir plate and add the NaOH slowly. Bring up to final volume by adding Type I Water.

Purchased from VWR, Bridgeport, NJ., Catalog number H385, 4 liter bottle.

SODIUM PHOSPHATE, MONOBASIC, MONOHYDRATE (NaH2PO4-H2O)

Expiration date: Manufacturer's expiration date

Purchased from VWR, Bridgeport, NJ., Catalog number 7892, 500 g bottle.

SSPE, 25 X

Expiration date: Twelve months from date of preparation

	MW	1 L	2 L
4.5 M NaCl	58.44	262.98 g	438.3 g
250 mM NaH2PO4-H2O	138.0	34.5 g	69.0 g
25 mM EDTA	372.2	9.3 g	18.61 g
Type I Water		450 mL	900 mL

Add the EDTA to Type I Water, and let it dissolve completely. (It is necessary to pH to 8.0 with 10 N NaOH in order for the EDTA to go into solution.) Once dissolved, add the NaH2PO4-H2O first and the NaCl second. Add Type I Water until 95% of the total volume is reached. When all solids are dissolved, pH to 7.4 with NaOH (will take about 20-25 mL NaOH/liter). Bring the solution up to total volume with Type I Water. The SSPE should be dispensed into bottles and autoclaved. Store at room temperature.

For NaCl, EDTA and NaOH see this list.

STAIN EXTRACTION BUFFER

Expiration date: Twelve months from date of preparation

	MW	1 L	2 L
Tris	121.1	1.21 g	2.42 g
EDTA	372.2	3.72 g	7.44 g
NaCl	58.44	5.84 g	11.68 g
Type I Water		600 mL	1200 mL

Adjust pH to 8.0 with NaOH

20% SDS	288.3	100 mL	200 mL

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Adjust to final volume with Type I Water and store at room temperature.

For Tris, NaCl and EDTA see this list.

TAE, 1X

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1:19 dilution of 20X TAE

Expiration date: Twelve months from date of preparation

	2 L	4 L
20X TAE	100 mL	200 mL
Type I water	1900 mL	3800 mL

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1:50 dilution of 50X TAE

Expiration date: Twelve months from date of preparation

	2 L	20 L
50X TAE	40 mL	400 mL
Type I water	1960 mL	19.6 L

TAE, 20X

Expiration date: Twelve months from date of preparation

	MW	2 L	4 L
Tris	121.1	193.2 g	386.4 g
Glacial Acetic Acid		45.6 mL	91.2 mL
0.5 M EDTA, pH 8.0		80.0 mL	160.0 mL
Type I Water		1874.4 mL	3748.8 mL

Begin with 60-75% of the total volume of Type I Water and add the appropriate amounts of the above chemicals; mix well. When dissolved, pH solution to 7.9 with glacial acetic acid. Bring up to final volume and store in bulk. Autoclave for 20 minutes.

Glacial Acetic Acid - Purchased from Fisher Scientific, Fair Lawn, NJ Catalog number A-38, 500 mL bottle.

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For EDTA and Tris see this list.

TAE, 50X

Expiration date: Twelve months from date of preparation.

Stock	MW	1 L	2 L
Tris	121.1	241.5 g	483 g
Glacial Acetic Acid		57 mL	114 mL
0.5 M EDTA, pH 8.0		100 mL	200 mL
Type I Water		500 mL	1 L

Add components to Type I Water and adjust to pH 7.9 with additional acetic acid. Adjust to final volume with Type I Water. Autoclave at 215 C and 20 lbs pressure for 20 minutes. Store at room temperature.

Expiration date: Twelve months for date of receipt

Purchased from Invitrogen, Carlsbad, CA, Catalog number 4710UA, 1 liter bottle.

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TBE, 0.5X

1:40 dilution of 20X TBE

Expiration date: Twelve months from date preparation

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	2L	20 L
20X TBE	50 mL	500 mL
Type I water	1950 mL	19.5 L

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TBE, 1X

OF

1:10 dilution of 10X TBE

Expiration date: Twelve months from date preparation

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	2 L	20 L
10X TBE	200 mL	2 L
Type I water	1800 mL	18 L

1:20 dilution of 20X TBE

Expiration date: Twelve months from date preparation

	2L	4L
20X TBE	100 mL	200 mL
Type I water	1900 mL	3800 mL

TBE, 10X

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Expiration date: Manufacturer's expiration date

Purchased from Invitrogen, Carlsbad, CA., Catalog Number 15581-028, 10 liter container/box.

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TBE, 20X

Expiration date: Twelve months from date of preparation

	MW	2 L
Tris	121.1	432 g
Boric Acid	61.83	220 g

0.5 M EDTA	372.2	160 mL
Type I Water		1000 mL

Bring up to final volume and store at room temperature.

For Tris and $Na_2EDTA\text{-}2H_2O$ see this list.

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Boric Acid - Purchased from Sigma Chemical Co., St. Louis, MO. Catalog number B-0394.

TEMED

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Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date

Purchased from Sigma Chemical Co., St. Louis, MO. Catalog number T-8133, 100 mL bottle.

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TNE

Expiration date: Twelve months from date of preparation

OF

	MW	500 mL	1 L
Tris	121.1	0.605 g	1.21 g
NaCl	58.44	2.92 g	5.84 g
EDTA	372.2	0.185 g	0.37 g
Type I Water		450 mL	900 mL

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Combine components and mix until completely dissolved. Bring up to final volume with Type I Water and store at room temperature.

For Tris, NaCl and EDTA see this list.

TRIS, 1 M, pH 7.5

Expiration date: Twelve months from the date of preparation

	MW	500 mL	1 L	2 L
Tris 1 M	121.1	60.55 g	121.10 g	242.2 g
Tris 2 M	121.1	121.10 g	242.20 g	484.4 g
Type I Water		300 mL	600 mL	1200 mL

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Dissolve the appropriate amount of Tris base into Type I Water. Mix well and adjust to pH 7.5 with 37.1% HCL. Bring up to final volume and recheck pH. Dispense into bottles and autoclave at 215 F for 20 minutes. Store at room temperature.

COPY

Tris - Purchased from VWR, Bridgeport, NJ., Catalog number 7732, 2.5 kg box.

For HCl see this list.

TRIS-EDTA (TE^{-4} Buffer), 1X, pH 8.0

Expiration date: Twelve months from date of preparation

	Stock	200 mL	500 mL
10 mM Tris	1.0 M	2 mL	5 mL
1mM EDTA	0.5 M	40 µL	100 µL
Type I Water		160 mL	400 mL

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Add Tris to Type I Water and adjust the pH 8.0 using 37.1% HCl. Add EDTA, recheck pH and bring to appropriate final volume. Autoclave for 20 minutes and store at room temperature.

For HCL, Tris, and EDTA see this list.

TRIS-EDTA (TE^{-4} Buffer), 100X

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Expiration date: Twelve months from date of preparation

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	MW	1 L	2 L	4 L	8 L
0.1 M EDTA	372.1	37.2 g	74.4 g	148.8 g	297.6 g
1 M Tris	121.1	121.1 g	242.2 g	484.4 g	968.8 g
Type I Water		1L	2L	4L	8L

OF

Dissolve Tris in 800 mL of Type I Water and adjust the pH to 7.5 with 37.1% HCl. Add EDTA, recheck pH and bring solution to final volume. Autoclave for 20 minutes. (It may be necessary to add 10 N NaOH to adjust pH to 7.5.)

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For HCL, NaOH , Tris and EDTA see this list.

UREA

Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date

Purchased from Sigma, St. Louis, Mo. Catalog Number U-5378, 500 gram bottle.
Purchased from Invitrogen, Carlsbad, CA., Catalog Number 15505-050, 2 Kg container.

VISUAL MARKER - LAMBDA Hind III (DNA/Hind III)

Expiration date: Manufacturer's expiration date

Add the appropriate volumes of lambda Hind III and sterile 1X TE-4 Buffer to 250 µL 5X loading buffer (total volume 1250 µL) to achieve a final concentration of 200 ng/10 µL. (The concentration of commercially supplied lambda Hind III may vary from lot to lot.) Mix well and divide into 100 µL aliquots in sterile microcentrifuge tubes. Store at -20 C.

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NOTE: Lambda Hind III needs to be heated at 65 C for approximately 20 minutes prior to making visual marker.

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Lambda Hind III - Purchased from Invitrogen, Carlsbad, CA., Catalog number 5612SA, in an approximate concentration of 733 ng/µL, in a total volume of 682 µL.

For TE^{-4} Buffer and loading buffer see this list.

XYLENE

Expiration date: Manufacturer's expiration date

Purchased from Fisher Scientific Catalog Number X5-500, 500 ml amber bottle.

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Appendix C - Preparation of Yield and Product Gels

1 Equipment

1.1 Graduated Cylinder

1.2 Erlenmeyer or round bottle flask

1.3 Microwave oven

1.4 Gel beds, Lifecodes (12.0 cm x 16.0 cm) or BRL (11.0 cm x 20.0 cm)

1.5 Gel lane combs, Lifecodes (25 lane) or BRL (20 lane)

1.6 Leveler

1.7 Pipette - 10 or 20 µL

1.8 Orbital platform shaker

1.9 Water bath or oven, 65°C

1.10 Balance, top loading

2 Materials

2.1 Tape

2.2 Large bore transfer pipette

2.3 Weighing paper

2.4 Sterile tips for pipettes

2.5 Plastic wrap

2.6 Ziploc bag

3 Reagents

3.1 1X TAE

3.2 0.5X TAE

3.3 Nusieve Agarose

3.4 DNA Typing Grade Agarose

3.5 Ethidium bromide

3.6 Type I Water

4 Procedure

NOTE: The Yield Gel procedure uses 1X TAE and 1.0% DNA Typing Grade Agarose and the Product Gel procedure uses 0.5X TBE and 3.0% NuSieve Agarose.

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4.1 Calculate the amount of agarose and 1X TAE or 0.5X TBE needed. Use 100 mL of 1X TAE or 0.5X TBE for a 12.0 cm x 16.0 cm gel and 115 mL for a 11.0 cm x 20.0 cm gel. Multiply the desired percentage of the gel by the volume of TAE or TBE to obtain the correct amount of agarose required.

EXAMPLE: 1.0% 16.0 cm gel

(1.0%)(100 mL) ~ 1.0 g of agarose

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4.2 Weigh the agarose and pour it into an Erlenmeyer flask. Measure the 1X TAE or 0.5X TBE in a graduated cylinder and pour into the Erlenmeyer flask. The capacity of the flask should be at least 2 times the volume of the buffer to avoid boiling over in the microwave oven. Loosely cover the flask top with plastic wrap and swirl. Heat in a microwave oven to dissolve. Heating time will depend upon the volume and number of flasks, as well as the characteristics of the microwave oven.

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EXAMPLE: For a 100 mL volume: Heat 1-1.5 minutes in a microwave oven to reach boiling. Remove the flask from the heat source, determine the volume and bring back to original volume with Type I Water. Place on an orbital shaker, and rotate for 5 minutes at 90 rpm. If necessary, return the flask to the heat source and heat for 30-45 seconds to completely dissolve the agarose.

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4.3 If the gel is to be poured immediately, remove the flask from the heat source, place it on the orbital shaker and rotate for 10 minutes at 90 rpm (until agarose cools). If the gel is not to be poured immediately, molten agarose may be kept in a 65°C water bath or oven until it is poured. Reconfirm the original volume at this time. When the flask is cool enough to handle (approximately 15 minutes at room temperature or 30 minutes at 65°C), the agarose can be poured into prepared gel beds. Make sure it is not too hot as it will melt the adhesive tape and cause leakage.

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NOTE: Following boiling and rotating for 5 minutes at 90 rpm, the flask may also be cooled down by swirling under running tap water.

4.4 Prepare gel beds by taping the ends closed, being careful to seal edges. Level the gel bed.

4.5 For yield gel and/or product gel, 10 µL of 5 mg/mL ethidium bromide stock solution per 100 mL of agarose may be added. If ethidium bromide is not added to the agarose, the gel may be stained with the same concentration after electrophoresis is complete.

CAUTION: ETHIDIUM BROMIDE IS A MUTAGEN. AVOID DIRECT CONTACT. ALWAYS WEAR GLOVES WHEN HANDLING ETHIDIUM BROMIDE AND ETHIDIUM BROMIDE GELS.

4.6 Pour molten agarose into center of gel bed. Remove any bubbles with the tip of a transfer pipette. Place the comb in position. Allow the gel to solidify for approximately 30 minutes at room temperature.

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4.7 Place the gel, while still on the gel bed with the comb in place, into a plastic Ziploc bag along with a moistened Kimwipe and refrigerate for a minimum of 15 additional minutes prior to removing the comb.

4.8 If the gel will not be used immediately, carefully remove the comb and store the gel in the Ziploc bag with a moistened Kimwipe in a refrigerator until used.

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Appendix D – Photographing a Gel

1 Equipment

1.1 Transilluminator, UV

1.2 MP4 Polaroid camera with stand and filter (Wratten 8 or 9)

1.3 Ultraviolet Viewing Cabinet and Camera

1.4 FMBIO II Fluorescent Image Analysis System

1.5 Digital Camera

2 Materials

2.1 Black and white Polaroid film, Type 667 or similar product

3 Procedure

CAUTION: ETHIDIUM BROMIDE IS A MUTAGEN. AVOID DIRECT CONTACT ALWAYS WEAR GLOVES WHEN HANDLING ETHIDIUM BROMIDE AND ETHIDIUM BROMIDE GELS.

ALWAYS WEAR UV PROTECTIVE EYE WEAR WHEN USING THE TRANSILLUMINATOR.

3.1 Photographing a gel with an MP4 Camera

3.1.1 Place gel (out of its tray) on the UV transilluminator.

3.1.2 Position the camera and select camera settings according to gel size. The following are suggested, initial settings:

1 sec, f/11

Make sure the gel is in the field of view. If not, adjust the fine and/or coarse settings accordingly.

3.1.3 Position filter over lens area.

3.1.4 Slide camera to the left and take the picture by pressing the shutter release cable.

3.1.5 Slide camera to the right. Turn off the transilluminator. Turn on the lights. Pull out the white tab, then pull out the film tab with a slow, steady motion to remove the photograph. Allow the picture to develop for 30 seconds and then peel the picture from its backing. Carefully discard the backing while avoiding the caustic chemical developers.

3.1.6 Label the picture with the case number and initials.

3.1.7 Always clean off the roller with a wet paper towel containing isopropanol when a new roll of film is loaded.

3.2 Photographing a gel using an Ultraviolet Viewing Cabinet and Camera

3.2.1 Place gel (in its tray) into the Ultraviolet Viewing Cabinet.

3.2.2 Using the eyepiece on the Ultraviolet Viewing Cabinet, position the gel so that it is in the field of view of the camera.

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3.2.1 Remove the eyepiece and attach the camera.

The following are suggested, initial settings:

1 sec, f/11

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3.2.3 Position filter over lens area.

3.2.4 Take the picture by pressing the shutter release button on the handle of camera.

3.2.5 Pull out the white tab, then pull out the film tab with a slow, steady motion to remove the photograph. Allow the picture to develop for 30 seconds and then peel the picture from its backing. Carefully discard the backing while avoiding the caustic chemical developers.

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3.2.6 Label the picture with the case number and initials, as well as at least one well number so the gel may be properly oriented.

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3.2.7 Always clean off the roller with a wet paper towel containing isopropanol when a new roll of film is loaded.

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3.3 Scanning a gel using the FMBIO II Fluorescent Image Analysis System – Optional

3.3.1 Follow the procedure outline in Appendix F, Fluorescent Detection of the Electrophoresis Gel-FMBIO II for scanning a product gel.

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3.4 Photographing a gel using a digital camera

3.4.1 Digital camera settings will be determined in the lab where the camera is used.

3.4.2 Pictures will be labeled appropriately and retained in the case file.

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Appendix E – Troubleshooting DNA Quantitation

AluQuant Data Analysis

1.1 Deleting Outlier Points from the AluQuant Human DNA Quantitation System Standard Curve.

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A minimum of 5 data points, including the water blank, are necessary for the standard curve. Occasionally, data points from the standard need to be deleted to reduce elevated background and/or to produce a more linear standard curve. The data points may be deleted, then the other standards and their RLU values must be copied and pasted into the cells so that the standard table is continuous. See Figures 1A and 1 B for an example. The Calculate button must be pressed again in order to generate a new extrapolated curve and to correct the DNA sample concentrations that may be off. Only the Project Coordinator or a supervisor may approve the deletion of data points. The deletion of data points is noted on the worksheet and initialed.

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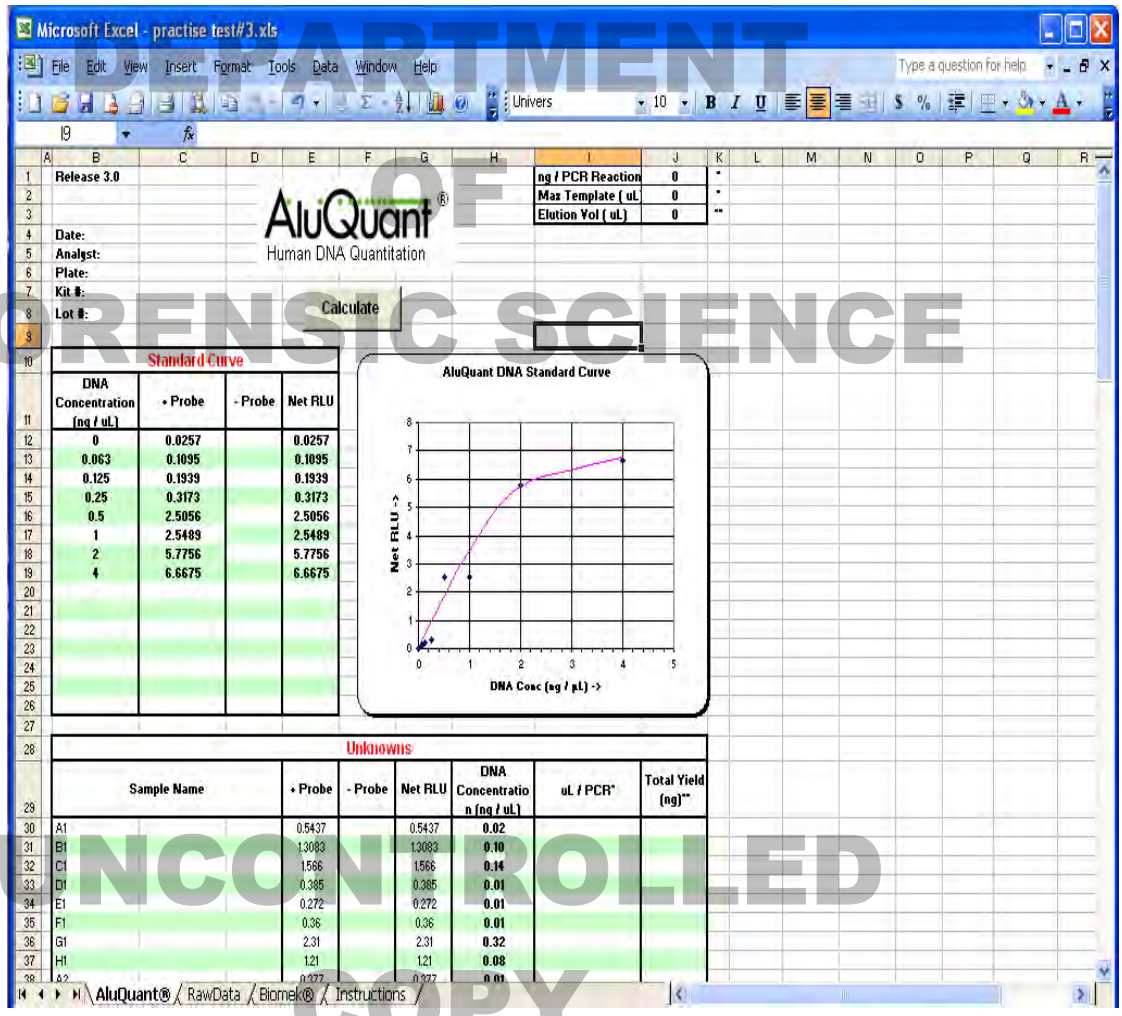

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Figure 1A. Standard curve which may need data points deleted

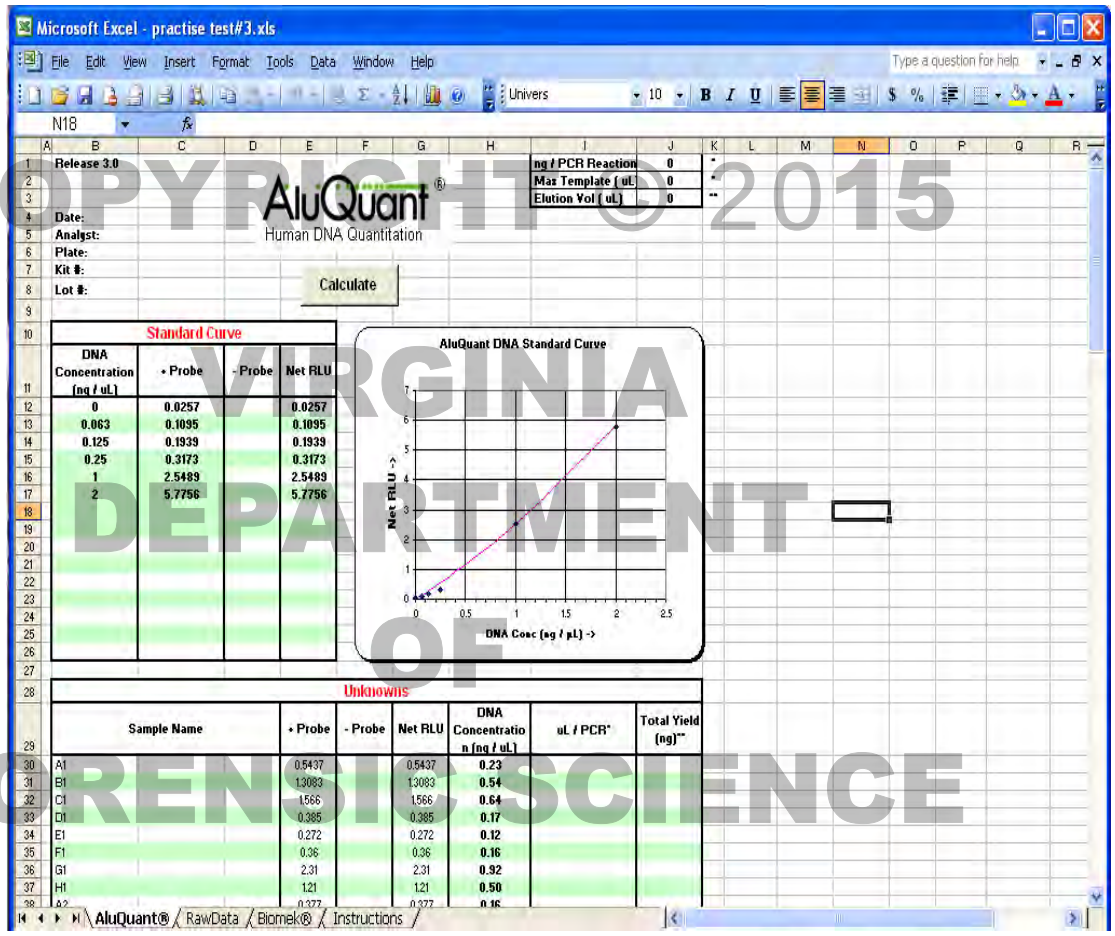

Figure 1B. Deletion of data points from standard curve

NOTE: Figures 1A and 1B illustrate the large difference in estimated DNA concentration for the samples when data points are deleted from the standard curve. Although AluQuant® Calculator v3.0 doesn't flag the user as to irregularities with the standard curve because the quadratic formula is capable of fitting any curve, it is imperative that the user evaluate the slope and character of the extrapolated (pink) curve to ensure the most accurate DNA concentration estimation. A large dip or even a flattening out in the RLU value for the 4 ng/µL standard will usually require that it be deleted since it can throw off the estimates for the most c oncentrated samples. The desired slope for the standard curve is approximately 45°.

1.2 Extremely Low RLU Values

It is normal for the RLU values to vary somewhat from run to run and they will usually halve when the L/L reagent is used that has been reconstituted and frozen. If the RLU values are not greater than 1.0-2.0 RLUs for the 4 ng/µL standard, even if the standards reduce in value proportionately with the lower concentrations as shown in Figure 2, it is likely that the luminometer lens is obscured. This can occur when the injector tip becomes clogged and mis-injects into the luminometer plate, hitting the top of the plate instead of into the wells. Another symptom of an obscured lens is the occasional large over estimate of a sample. This is due to the fact that the lens is usually not uniformly obscured so that most of the signal may be muted, but an occasional sample signal may be detected at full strength and is thus vastly out of proportion with the muted standards.

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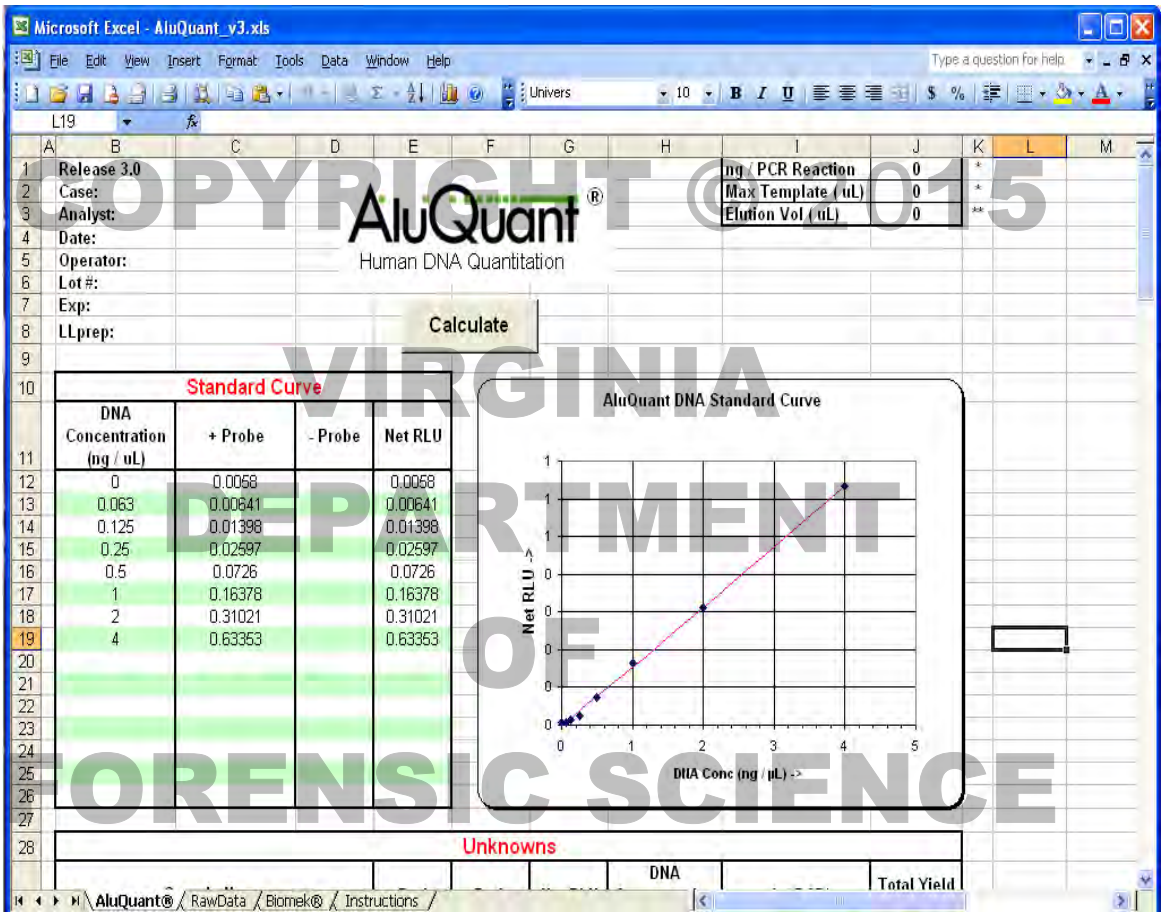

Figure 2. Abnormally low RLU values.

1.2.1 If extremely low RLU values are observed, the lens must be cleaned as described in Appendix E.

1.3 Inconsistent Quantitation Data.

1.3.1 Quantitation will become inconsistent if blockage of the injection tip occurs. Sometimes the data will become inconsistent before it is apparent that the injector tip is blocked. If inconsistent data is observed, then change the injection tip and test the instrument to determine if consistency has improved.

1.3.2 Quantitation will become inconsistent if the injector tubing becomes kinked. If replacing the injector tip fails to restore consistency to the quantitation data, then the injector tubing should be checked. The tubing is thin and can be easily bent, which can possibly impede the injection of the full 50 µL aliquot of L/L reagent. New tubing should be used to replace the injector tubing by following the directions in the luminometer manual for replacing the injector tubing.

1.3.3 The Teflon seal of the plunger can also fail over time. Inconsistent data can also be a symptom that the plunger needs replacement. The plunger generally should be replaced every 3-5 years depending on usage. If replacing the tip and the tubing fails to rectify the issues with inconsistency, then the plunger may need replacement.

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2 Plexor Data Analysis

2.1 Removing outlier data points from a standard curve

2.1.1 Evaluate the standard curves by selecting the Standard Curves tab. You will need to assess the curves for the autosomal and Y DNA quantitation separately by selecting each of the tabs (FAM – Autosomal and CO560 – Y). Each standard curve should have an r^2 value of 0.98 or above to be acceptable. Evaluate the curve generated to determine if any outlier data points need to be removed to improve the quality of the extrapolated curve generated. Up to 4 data points can be deselected when necessary to produce a linear standard curve. The de-selection of data points from either standard curve must be indicated on the report. Only a Project Coordinator may approve the de-selection of data points.

2.1.2 De-selection of data points from the standard curve does not result in deletion of the data. The data for those wells are still reported on the printed reports. De-selection of the wells simply results in not using that data for the generation of the standard curve, from which concentrations of the unknowns are estimated.

2.1.3 Standard Samples can be selected/de-selected for the generation of each standard curve, the autosomal and the Y, independently. That is, if a data point is an obvious outlier in the standard curve for the autosomal target, this well may be deselected for the generation of the autosomal standard curve. If the sample is not an outlier for the Y standard curve, it may be included in the generation of the Y standard curve.

2.1.4 In the generation of a standard curve, all samples and standards are selected by clicking on the box indicated by the gray arrow, as shown in Figure 3. Note that this selection is specific to the dye selected via the tab above the data, as indicated by the black arrow.

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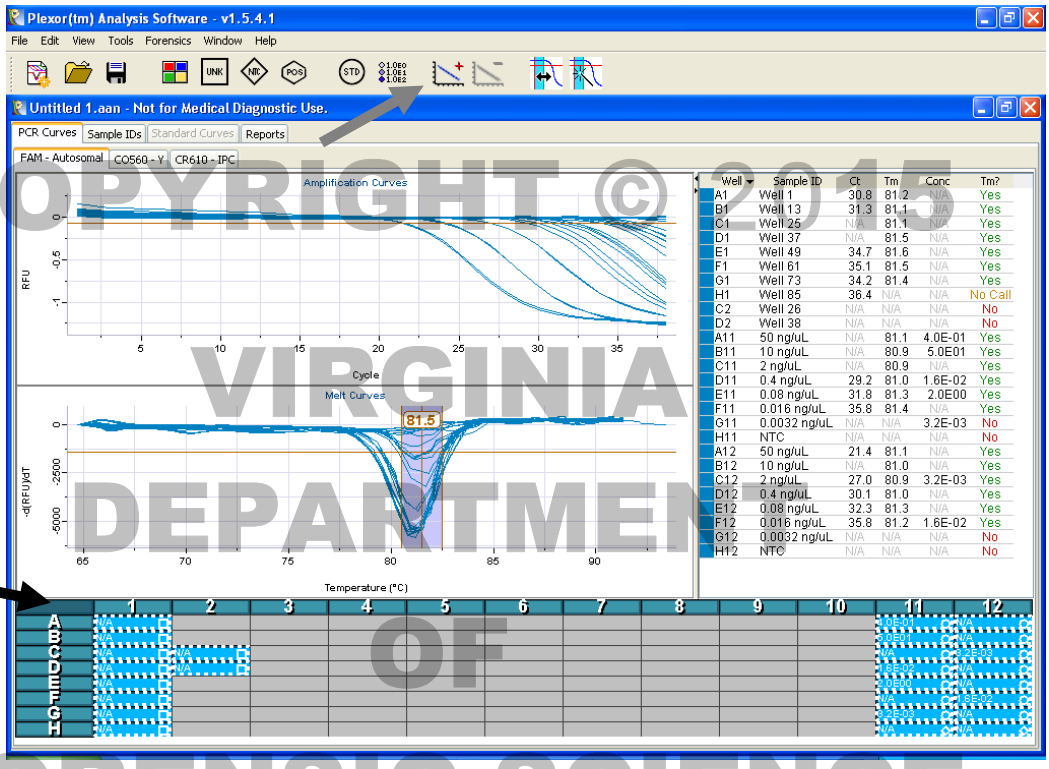

Figure 3. Making the Standard Curves. After selecting the entire plate of samples by clicking in the box indicated by the black arrow, a standard curve can be created by clicking the appropriate icon as indicated by the second (gray) arrow.

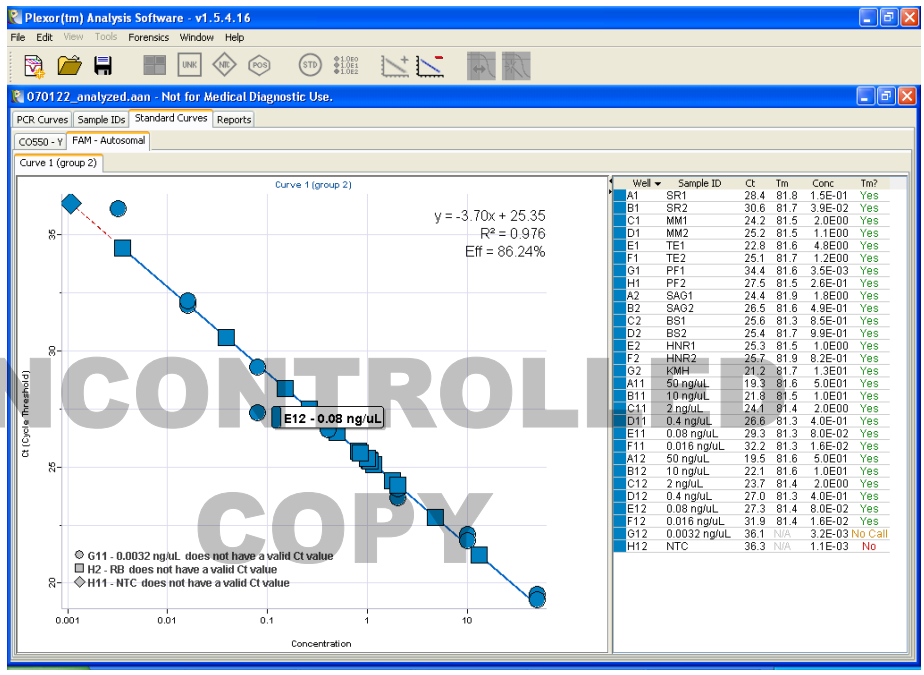

Figure 4. Standard Curve. The well location of the outlier data point is displayed (as shown) by placing the mouse (arrow) over the data point in question.

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2.1.5 If after reviewing the standard curve and the r^2 value is lower than is acceptable, an examination of the curve may reveal what or which data points significantly deviate from the extrapolated curve. These data points can be identified by moving the mouse (arrow) over the outlier data point, which then displays the well location of the data point, as shown in Figure 4. This or these data point(s) are the points which should be de-selected to improve the standard curve.

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2.1.6 Samples are deselected by holding down the <Ctrl> key on the keyboard while using the mouse to click on the sample of interest. This sample is then no longer highlighted.

2.1.7 The standard curve can then be generated in the usual manner, by clicking the icon shown below:

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2.1.8 If a standard curve was already generated for that dye, the software will prompt the user that the generation of a new standard curve will replace the existing standard curve. If this is desired, click "OK".

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2.1.9 If de-selection of data points on the other standard curve is necessary, be sure to select the other tab (as shown in Figure 3) and repeat steps 1.3 – 2.1.8, above.

2.1.10 The de-selection of data points must be indicated on the Plexor report(s) and initialed.

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2.2 Adjusting a Sample's Baseline to More Optimal Values

2.2.1 The baseline regions are computed and selected automatically by the Plexor Analysis Software. Should the baseline of any specific amplification plot be improperly estimated, it can be adjusted manually. Each well can be highlighted individually to inspect the amplification plot and adjust the baseline. The need to manually adjust the baseline for a sample or standard should arise rarely if ever.

2.2.2 The baseline of individual samples should be adjusted, if needed. First, view the amplification plots for the entire plate, be sure you have selected the "PCR Curves" tab, then click on the empty box at the upper left hand corner of the sample plate. Choose "Edit", "Display and manually adjust baseline regions" and click "OK" in the dialog box that appears.

2.2.3 The baseline should be set in a flat region of the amplification curve before the decrease in signal, which is indicative of accumulation of PCR product. Manual adjustment of the baseline region for each sample is possible, although rarely necessary. You can alternate between each dye to view the various amplification plots for each well. An improperly set baseline region can result in a skewed amplification plot, such as that shown in Figure 5.

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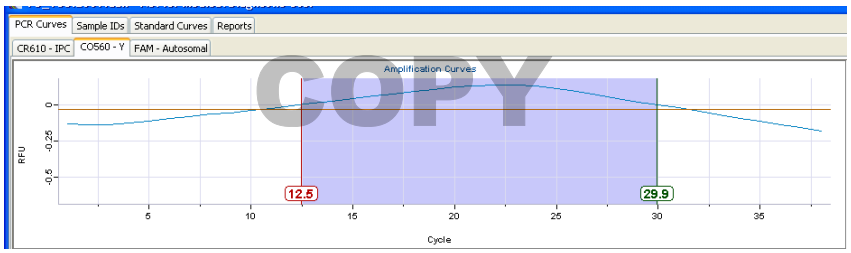

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Figure 5. Improperly-set baseline region for an individual sample.

2.2.4 To correct the baseline region of a sample, if needed, each well can be highlighted individually. Choose "Edit", "Display and manually adjust baseline regions" and click "OK" in the dialog box that appears. The baseline can then be adjusted by dragging the lower and upper limits. The upper limit should be approximately 5 cycles before the decrease in fluorescent signal in an area where the signal is flat. The lower limit is usually set to a region that creates a flat baseline, resulting in a baseline region of approximately 6 cycles or more. Figure 6 shows an amplification plot with an appropriately-selected baseline region. Note that the software automatically recalculates the estimated DNA concentrations and standard curves affected by the altered baseline region.

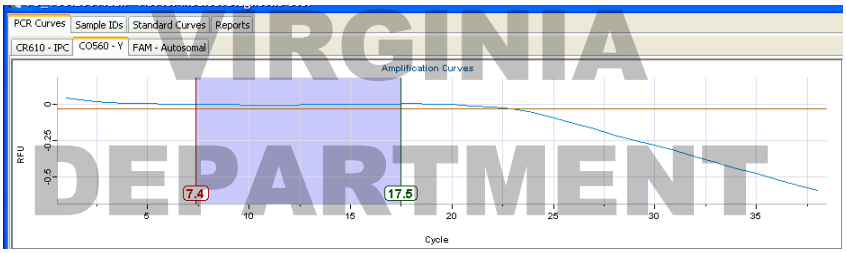

Figure 6. Amplification plot from same sample as in Figure 5, but showing a properly-selected baseline region.

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Appendix F - Fluorescent Detection of the Electrophoresis Gel-FMBIO II

1 Technical Notes

1.1 The FMBIO II utilizes a solid state green laser (532 ηm) to instigate the excitation of Fluorophores. The resulting fluorescent light signals emitted are then collected by an optical fiber array and converted to electrical signals by photo multiplier tubes. Finally, the analog-to-digital converters transform the electrical data into an image for interpretation and analysis by the computer.

1.2 The 577ηm filter will detect wavelengths of light emitted from the JOE fluorescent dye.

1.3 The 505 ηm filter will detect wavelengths of light emitted from the Fluorescein fluorescent dye.

1.4 The 598 ηm filter is used to auto focus the FMBIO laser and will detect wavelengths of light emitted from the Rhodamine Red™ – X fluorescent dye.

1.5 The 665 ηm filter will detect wavelengths of light emitted from the Texas Red® – X (internal lane standard for PowerPlex® 16 BIO) fluorescent dye.

2 Equipment

2.1 FMBIO II Fluorescent Image Analysis System (Hitachi)

2.2 Power Mac or Mac Clone Computer

2.3 Laser Printer

2.4 Optical Disk/ ZIP Disk drive (optional)

3 Procedure for Using the FMBIO Fluorescent Image Analysis System

CAUTION

The FMBIO II Fluorescent Image Analysis System is a laser excited optical scanner, which contains high-energy electrical components that could cause serious injury to the operator if not operated properly. It is imperative that the laser is not radiating when the scanning well cover is opened. Ensure that all external panels and protective utility covers are properly positioned, undamaged and tightly shut, so that no leakage of the laser beam is possible. Shut the instrument down at the first sign of trouble. If the unit emits an unusual smell, smoke, noise, or excessive heat, immediately turn off the power and disconnect the power cord.

3.1 Turn the Power Mac or Mac clone computer on by pressing the power button located at the top right portion of the keyboard. (A tone will sound when the key is pushed).

3.2 Turn the FMBIO II scanning portion of the imaging system on and let the instrument perform the self diagnostic. The green ready light will stop flashing when the unit is ready.

3.3 Place the electrophoresis gel, origin to the right, into the FMBIO unit and close the lid until latched. Ensure the proper orientation is selected depending on whether the short plate is up or down. If short plate up, then select **Rotate 180**. If short plate down, then select **Flip Vertical**.

NOTE: If the lid is not closed completely, an error message will appear at the beginning of the scanning process.

3.4 Click on the PowerPlex® 16 BIO icon located at the right side of the desktop to open the "ReadImage" dialog box.

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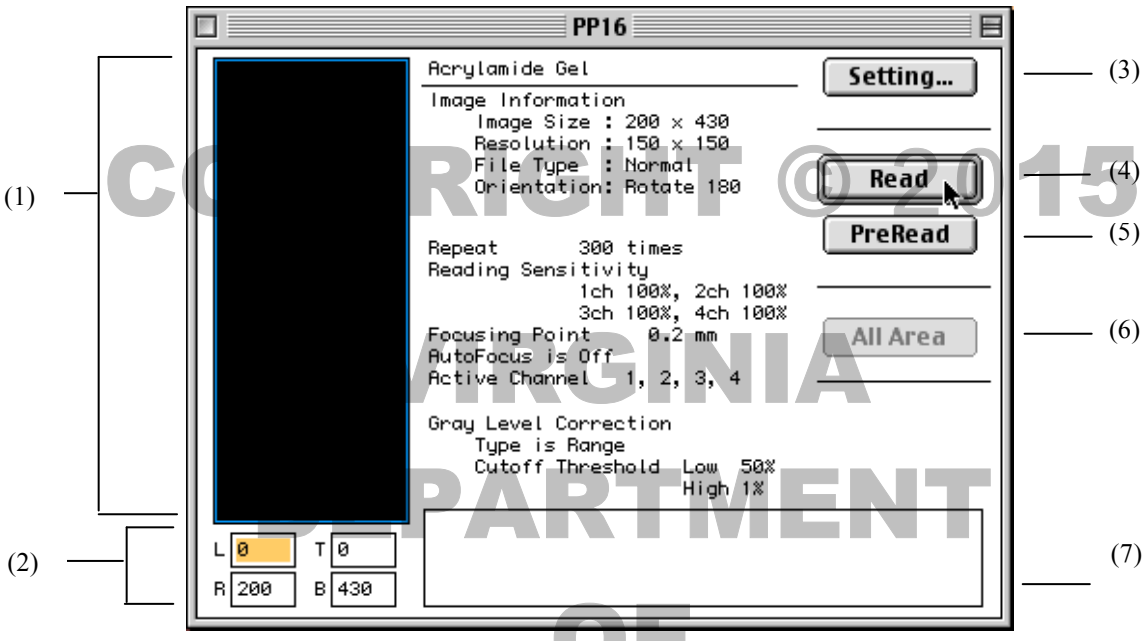

Number	Feature Name	Description
1	Total scan area	Displays available scan area and a pre-defined scan area.
2	Scan area coordinates	Four values that define the scan area.
3	Setting button	Opens the FMBIO Parameters window
4	Read button	Begins an image scan. Changes to Pause during scan. Changes to resume during a Pause.
5	Pre Read button	Begins a pre scan. Changes to Cancel during a scan.
6	All Area button	Sets the scan area to the total scan area.
7	Comment field	Stores your comments.

3.5 Ensure that the following default parameters have been set prior to scanning the gel (these are selected within the 'Command' menu under 'Set Parameter' and 'Set Gray Level'):

- ✓ Material Type: Acrylamide (Agarose for Product Gels)
- ✓ Image Size: 200 x 430 (variable)
- ✓ Resolution: 150 x 150
- ✓ File Type: Normal
- ✓ Orientation: Rotate 180° (short plate up), Flip Vertical (short plate down)
- ✓ Repeat: 200 - 300 times
- ✓ Reading Sensitivity:
- ✓ Recommended Settings PowerPlex® 16 BIO
  1CH (598ηm) 100%, 2CH (665ηm) 100%
  3CH (505ηm) 100%, 4CH (577ηm) 100%
- ✓ Recommended Settings for Product Gels
  1CH (598ηm) 50% (only channel active)

NOTE: These parameters can be varied between 50 to 100% depending on the quality and type of gel.

- ✓ Focusing Point for PowerPlex® 16 BIO: -2.0 to 3.0 mm
  (Variable based on instrument's optimized values)

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- ✓ Focusing Point for Product Gels: 2.4-2.8 mm
- ✓ Auto Focus:
  OFF (Automatic during first FMBIO run following the machine being turned on).
- ✓ Active Channel:
  Select the channels that contain the filters to collect the desired dye's wavelength.
- ✓ Gray Level Correction:
  Type is Range
  Cutoff Threshold: Low 50%
  High 1%

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3.6 If additional comments are required, place the cursor in the comment section with the mouse and type the comment or gel number and any other important information. This information is retained in a small text file associated with the image file and may be printed along with the image as needed.

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3.7 Click the "All Area" button.

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3.8 Click on the "Pre-Read" button. The laser will quickly scan the gel. This pre-read will allow the examiner to designate the area for the laser to scan.

3.9 Once the designated area has been determined, using the mouse, define the area in the image rectangle that contains the area of the gel that is to be scanned. Position the cursor at the top left portion of the area to be included in the scan, then drag down to the right bottom corner. Avoid including areas beyond the region of the gel where there is no DNA. The rectangle should not include the spacers as these will interfere with the laser's detection level.

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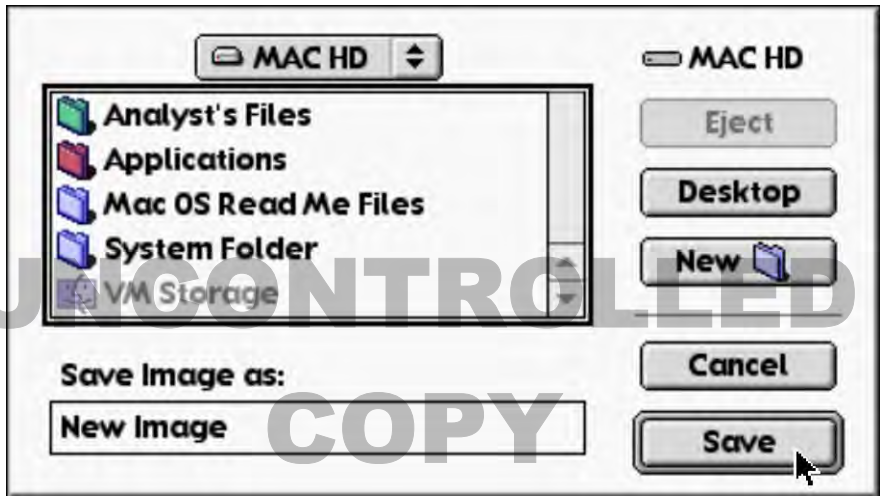
NOTE: Ensure that the entire gel is within the scan area to allow for off ladder variants as well as upper and lower allelic ladder bands.

3.10 Click "Read" to begin the scan. The software will prompt the examiner to save the image. Name the image and save it in a designated file on the desk top or hard drive.

NOTE: The file name given to the image should not be more than 16 characters in length. This includes spaces.



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3.11 The "ReadImage" Dialog Box will reappear. If this is the first scan of the day, the FMBIO II's laser will automatically auto-focus using the 598 ηm filter once the "Read" button is clicked. This function finds the best optical alignment of the rotating mirror and other signal tracking and processing elements. **Once the instrument has been auto-focused, providing the FMBIO unit is not turned off, it is not necessary to auto-focus each time an electrophoresis gel is scanned.**

3.12 Once the gel has been scanned, choose the "Open Analysis with Image(s)" function that is found under "Command" menu located at the top of the screen on the menu bar. Review the images to ensure that all the necessary information has been captured by the scan. When the gel images have been determined to be acceptable, the gel plates can be removed from the FMBIO II unit and the glass plates washed.

3.13 Close the image of the gel by clicking on the button at the upper left corner of the title bar of the image. Transfer the saved image folder from the desk top to the network server, optical/ZIP disk, or hard drive.

3.14 Quit FMBIO Analysis by selecting "Quit" under the "File" menu. Then quit the ReadImage program by selecting "Quit" under the "File" menu, if prompted to save changes to ReadImage program, select don't save. Proceed to Section 4, ANALYSIS OF THE GEL IMAGE.

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4 Analysis of the Gel Image

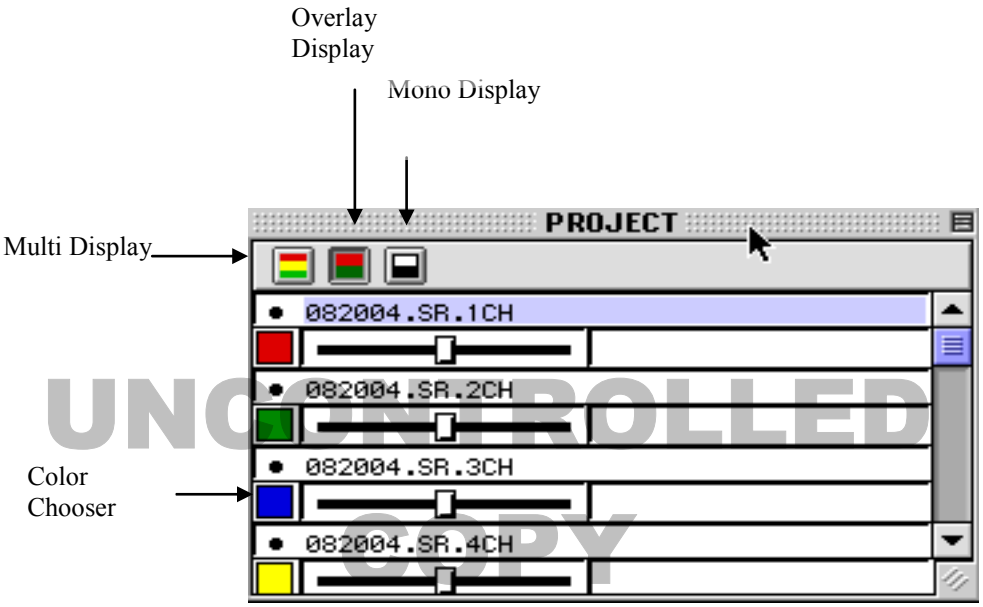
4.1 To begin analysis of the image, Click twice on the "FMBIO Analysis" icon, located on the desktop.

4.2 Under the "File" menu, at the top of the screen click on "NEW". Using the pull down menu locate the folder created when the gel was scanned. Open this folder.

NOTE: All of the channels in the opened folder will appear (e.g. filename.2CH, filename.3CH, etc.)

4.3 Highlight any one of the channels, then click "Open". In the box that specifies "Save New Project As:" give the image a name, and then save the image. The file name should not be greater than 16 characters long, including spaces.

NOTE: After the file has been saved, the colored image of the gel will appear on the screen along with the PROJECT BOX shown below. This will overlay all the scanned images; thus, allowing these images to be analyzed simultaneously and all channels will be displayed at one time.



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NOTE: In order to view a particular channel the small black dot in the upper left corner of the channel must be turned on by a single click in the left corner of the box. If no black dot exists, the channel has been turned off. Several channels can be viewed simultaneously by clicking on the black dot for each channel.

4.4 Click on the "Overlay" box on the Project Menu box.

NOTE: To view the 4th color in the project box, enlarge the project box by dragging the lower right corner to enlarge.

4.5 Click on the "Color Chooser" button for each channel and change channel 1 to RED, channel 2 to BLUE, channel 3 to GREEN, and channel 4 to YELLOW.

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4.6 The scanned images must be printed before any gray level or color separation adjustments are made. Under the "Multi" menu. Click on "Display Mode" and then "Mono" or alternatively single clicking on the "Mono Display" button on the project box and highlighting each individual channel in the Project Box.

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4.6.1 The highlighted channel will appear in black and white. Under the "File" menu, select "Print Option" and ensure that the "Information" box is checked along with "Image Date", "Size", "Resolution", "Reading Parameters", and "Gray Level Correction" boxes.

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4.6.2 The font size can be changed by clicking on the "Font" button located next to the "Information" box. Select desired font and change size to 9 or less.

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4.6.3 The image can be scaled so that it fits onto one page by adjusting the vertical image adjust. The numbers displayed are in fractions. Fractions less than 1/1 will shrink the image; fractions greater than 1/1 will stretch the image.

4.6.4 Use "Print Preview" under the "File" menu to ensure the printout is appropriately sized.

4.6.5 Print each image by highlighting it in the "Project" box and selecting "Print" from the "File" menu.

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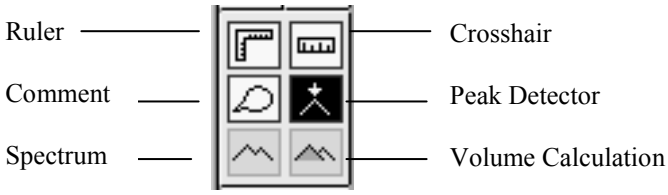
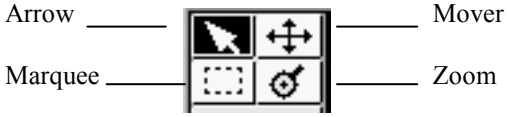
4.7 Adjust the gray level for each of the images by selecting the mono image for each individual channel, click on the "Gray level Adjustment" icon located at the bottom left corner of the screen. (Refer to the Size Tools diagram)

CURSOR AND IMAGE ICONS

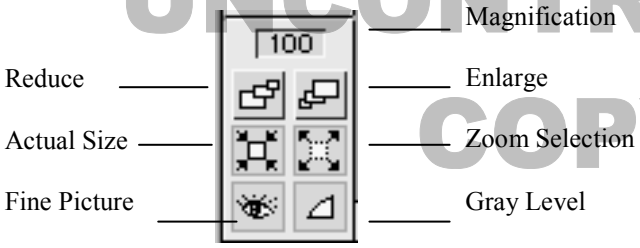
Image Tools



Cursor Tools



Size Tools



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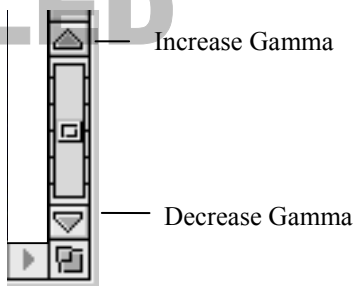
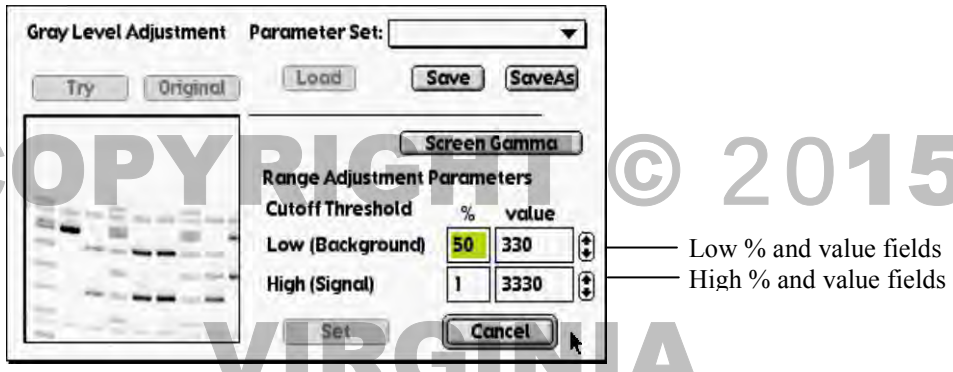
Gamma



4.7.1 The "Gray Level Adjustment" dialog box shown below will appear.



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4.7.2 Position the "Gray Level Adjustment" dialog box on any area of the gel by dragging the dialog box with the mouse.

4.7.3 Highlight the "Low (Background) Value Box". Next, position the cursor in the viewing window. Notice that the cursor becomes a crosshair when in this window. Using the crosshair, drag out a light region to designate the background. All regions of the image with pixel intensity values, which are below this selected area, will display as white.

NOTE: An area must be dragged out; a point will not register as background. To assist in this, the image may be enlarged before gray level adjustment (see Size Tools diagram)

4.7.4 Highlight the "High (Signal) Value Box". Select a dark region to designate as signal. All regions of the image with pixel intensities above those in the selected area will display as black. To return to the uncorrected image, click "Original".

NOTE: The background and signal levels can be manually changed by highlighting the % or values box, then typing in the desired levels. Click "Try" and the changes in the background or signal levels will be reflected in the viewing window. Increasing the difference in values between the background and signal will increase the resolution of the image by increasing the number of shades of gray displayed.

4.7.5 When the signal and background levels are at the desired intensities in the viewing window, click "Set". The entire image will be recalculated with the values selected in the high and low limit levels.

4.8 Repeat steps 4.7 through 4.7.5 for the remaining channels.

NOTE: The ideal balance of gray levels can be observed best in the matrix lane where each colored band is primarily the color it is designated to be (as seen in the overlay display).

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4.9 When a PowerPlex® gel is being analyzed the color must be corrected for carryover or bleed through of one channel into another. For analyzing a PowerPlex® 16 BIO System gel, under the "Multi" menu click on "Display Mode" and then "Over" or alternatively by single clicking on the “Overlay Display” button on the project box.

4.10 Next, click on "Color Separation" under the "Multi Menu". The Color Separator Box will appear. There are two basic ways to obtain the color separation parameter values. Step 4.11 describes the manual method; step 4.12- describes the automated, multi-band method. Both methods may be used for PowerPlex® 16 BIO System gels.

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4.11 To correct for bleed through on a PowerPlex® 16 BIO System gel:

4.11.1 In the color separation box, use the pull down arrow on the “Basis Image” diagram and highlight filename.1CH (Example: 022900ASR.1CH). This will display a red box next to the “Basis Image” diagram. Use the Marquee Tool, select a matrix band, drag a box around a “Red” matrix band, including some of the black background. Then click on “Set”.

4.11.2 Using the pull down arrow on the “Basis Image” diagram, highlight on the next image file name (filename.2CH, filename.3CH, or filename.4CH), which will display a blue, green or yellow box next to the “Basis Image” diagram depending on the channel. From the blended image, drag a box around a “Blue” matrix band for filename.2CH, a Green matrix band for filename.3CH, or a “Yellow” matrix band for finename.4Ch, including some of the black background. Then click on “Set”. Repeat this step until all filename.CHs under the “Basis Image Diagrams” have been adjusted.

NOTE: If the matrix fails, sample bands may be used for color separation. However, it is imperative to ensure that there are no overlapping dyes for the bands selected.

4.11.3 While filename.1CH or filename.2CH is displayed containing a red or blue box next to the “Basis Image” diagram, using the pull down arrow on the “Target Image” diagram, highlight filename.3CH, a green box will be displayed. Type “0” (zero) into the “Difference Rate Percent” box. Then click, “Try”. Repeat this step until filename.1CH and filename.2CH under the “Basis Image” diagram have been adjusted.

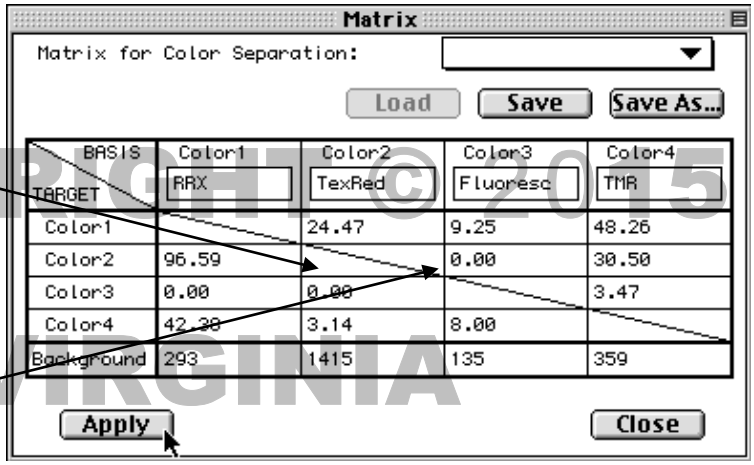
4.11.4 Once the color correction values (background and difference rate) have been set for each channel, click "OK". When the screen display, "Do you want to execute Color Separation" appears, click “OK”.

NOTE: IF BLEED THROUGH IS OBSERVED**,** under the “Multi” menu select “View Separate Image”, then proceed to step 4.11.5. Otherwise proceed to step 4.14.

4.11.5 Click on "Color Separation" under the "Multi Menu". The Color Separator Box will appear. While filename.2CH or filename.4CH is displayed containing a blue box or yellow box next to the “Basis Image” diagram, using the pull down arrow on the “Target Image” diagram, highlight filename.1CH, a red box will be displayed. Increase the value in the “Difference Rate Percent” box by approximately 30 to 40% (e.g., if the value is 20, change the value to 26 or 28). Repeat this step until filename.2CH and filename.4CH under the “Basis Image” diagram have been adjusted.

Zero out value for Color1 (RRX) into Color3 (FL)

Zero out value for Color2 (Texas Red) into Color3 (FL)



NOTE: Difference rates can also be adjusted by clicking on the 'Matrix' button in the Color Separator Box, make changes (as depicted above) and click 'Apply'.

4.12 Color separation values can also be obtained by using a standard lane on the gel (like the matrix sample). This is achieved by assigning bands to a portion of the lane, which represents all of the colors to be separated. The analysis software then determines the separation values based on the lane banded. To do this, create a lane for the selected sample and auto band the selection.

4.12.1 Open "1D-Gel" located under the "Tools" menu. When the "Marker Mode Display" appears select "layer", then check the pull down menu to ensure that the channel that contains the internal lane standard has been checked (Channel 2 for PowerPlex® 16 BIO System gels), then click "OK". Use the single lane tool to draw out a portion or the entire lane to be referenced (ex. Matrix lane). See 1D-Gel Tools diagram and refer to 4.20.3.

4.12.2 Click on "Auto band" to create the bands to be referenced for color separation.

NOTE: Any lane or partial lane created for this method of color separation may need to be deleted prior to creation of the analysis lanes (4.20).

4.12.3 Click on the "Mono Display" button in the "Project" box and remove any of the created bands that do not correspond to actual bands in each of the channel images (4.32.2).

4.12.4 Click on the "Overlay" button (for PowerPlex 16 BIO System gels) in the "Project" box and then activate the selected lane by clicking on either end of the lane with the arrow tool.

4.12.5 Choose the "Color Separation" from the "Multi" menu. Now single click on the Color Separator Box to activate it and select "Multi". The Multi Band Color Separator box will appear (if using the matrix lane, should only have 12 bands). Click on "copy" to use the bands. When the "Copy selected lane information to Color use?" appears select "OK". Check the "use" column boxes to ensure the bands represent the proper color represented on the "overlay" version of the gel (for example the order of the matrix lane is red, green, blue, yellow repeated three times). Bands can be assigned different colors to represent their appropriate channel. Click on the colored box in the "use" column to change colors. Bands can also be excluded from use by not being check marked

4.12.6 Once the appropriate bands have been designated colors, select "Apply" to apply the color separation values to the Color Separation Matrix. When the "Apply to color separation parameter?" appears, select "OK". The appropriate color correction values can then be zeroed out as described in 4.11.3.

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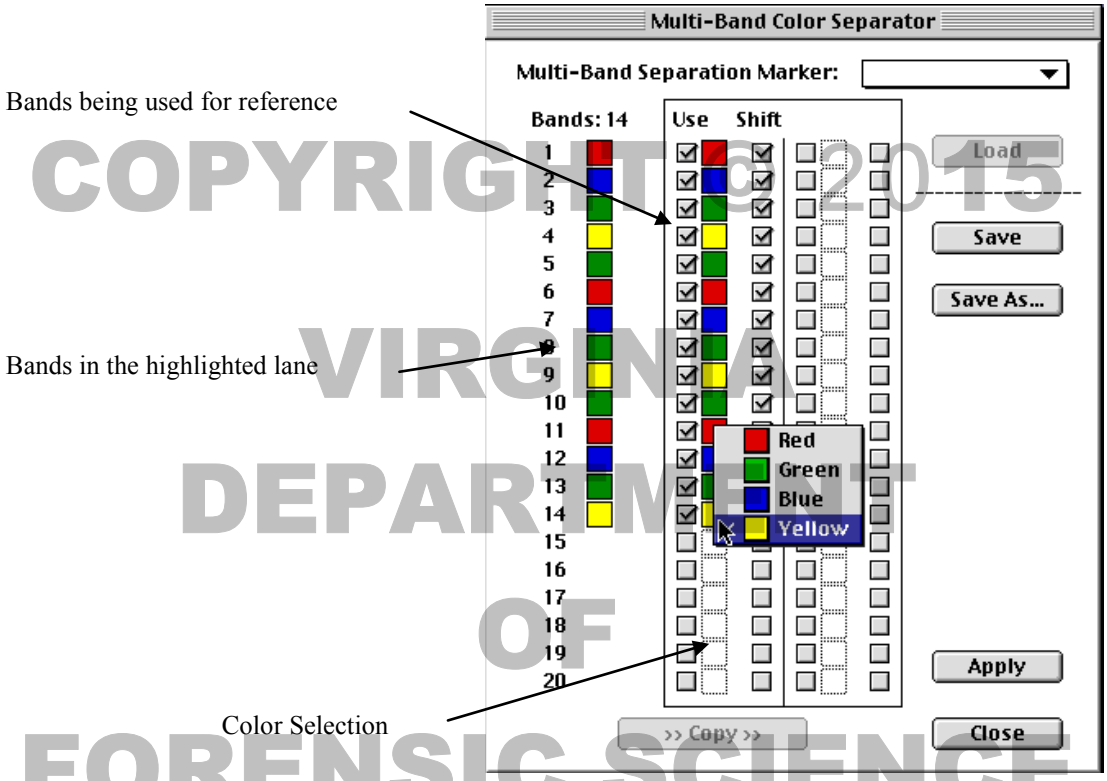

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4.13 Once the color correction values (background and difference rate) have been set for each channel, click "OK". When the screen display, "Do you want to execute Color Separation" appears, click "OK".

NOTE: Based upon the quality of the gel image(s) it may be necessary to repeat some or all of the steps addressed in steps 4.11 or 4.12 or it may be necessary to increase the percentage values in the "Difference Rate Percent" box addressed in step 4.11.5

4.14 The separated images must be printed to reflect the allele calls that are being made. Under the "Multi" menu. Click on "Display Mode" and then "Mono" or alternatively single clicking on the "Mono Display" button on the project box and highlighting each individual channel in the Project Box. Follow instructions to print the separated images as described in 4.6.

4.15 For images that are being analyzed by the primary analyst, once color separation is complete, Quit FMBIO Analysis by selecting "Quit" from the "File" menu.

NOTE: When reviewing the printed color separated images, if a band is observed as a result of bleed through, the bleed through band should be identified as a bleed through band from another channel on the image. The documentation should identify the channel that contains the original band.

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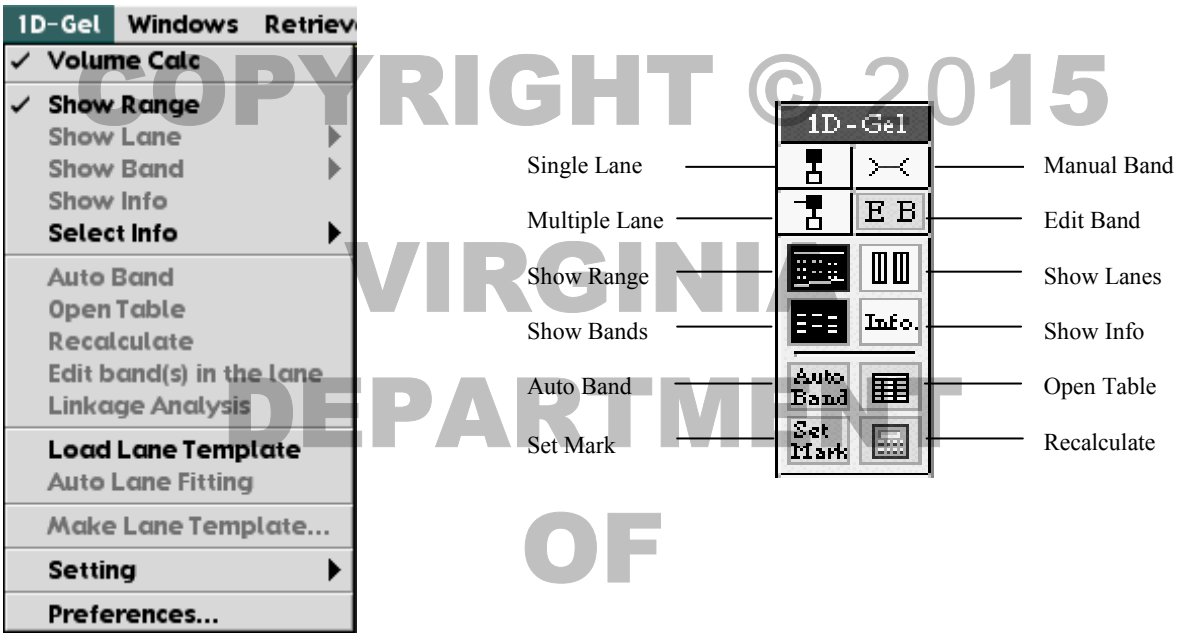
4.16 To create a file for an independent analysis, copy the folder containing the scanned, separated, and colored image icons to the designated location for independent sizing**. Both analyst and independent analyst proceed with step 4.18.**

4.17 Ensure the colored "multi-image" file of the desired gel is open. To assign lanes or bands open "1D-Gel" located under the "Tools" menu. When the "Marker Mode Display" appears select "layer", then check the pull down menu to ensure that the channel that contains the internal lane standard has been checked (Channel 2 for PowerPlex® 16 BIO System gels), then click "OK". This window may not appear if the marker mode has already been set. The "1D-Gel Image" menu item appears and the "1D- gel" icon cluster is displayed on the left side of the gel display.

1D-Gel Menu and Tools



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4.18 Set the upper and lower migration lines. These lines will be seen at the top and bottom of the scanned image. Notice the enlarged boxes or handles at each end of the migration limit lines, the left box moves the migration limit line up and down perpendicular to the gel. The right box changes the angle of the migration limit line with respect to the migration direction of the axis of the gel; use only for gels that are misaligned. With the "Arrow" icon move the migration lines to define the migration area of the gel. Position the upper migration line just above the largest (top most) fragment, and the lower limit line just below the smallest (bottom most) fragment. Adjust for angle deviation as required.

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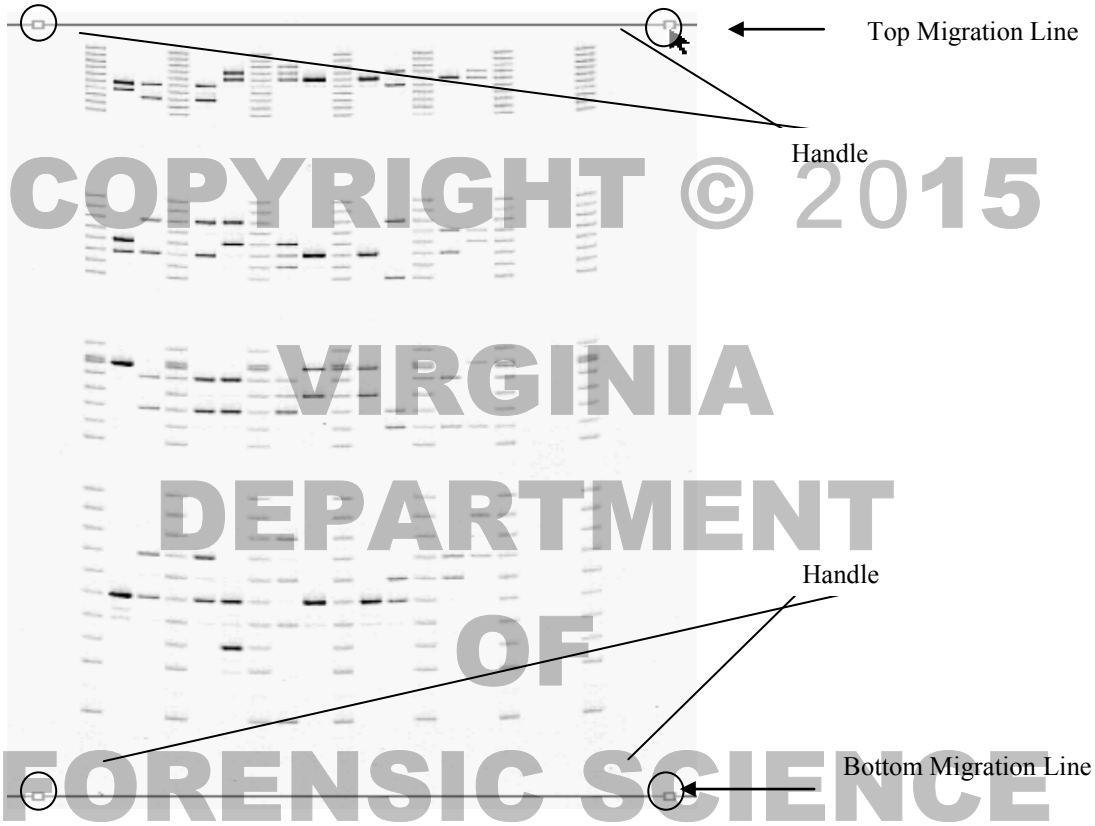

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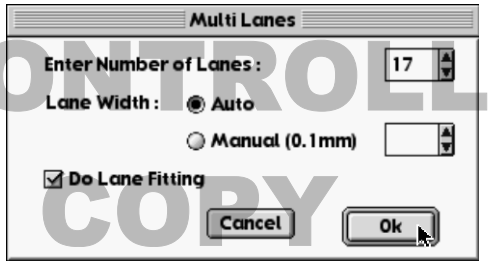
4.19 Define the lanes by clicking on the "Multiple Lane" icon. Position the "Multiple Lane" icon above the upper migration limit over the center of the first lane of the image. Click and hold the mouse button down and move horizontally to the right until the cursor is above the center of the last lane of the gel image. Release the mouse button. Move the mouse down through the center of the first and last lanes on the image to below the bottom migration line. Click the mouse button and "Multi Lanes" dialog box will appear. Define the number of lanes on the image from 2 to 40. Select “Do Lane Fitting” to make minor lane adjustments automatically. Click "OK". The number of lanes entered will be automatically within the selected area.

NOTE: Lanes that will be analyzed must be included in the box. Lanes that are not within the defined box will be dropped from the analysis. “Lane Fitting” only applies to the first time multiple lanes are assigned. If making lanes over skipped lanes, define all lanes of gel with “Lane Fitting”, and then delete any skipped lanes.



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4.19.1 If lane adjustments are necessary, click on the "Show Lane" icon until only one line appears for each lane. This line should run through the center of the bands located in each lane.

NOTE: If the line is not in or near the middle of each lane, it should be changed.

4.19.2 To change the placement of a lane, first select the lane to be changed with the “Arrow” cursor. Activate the lane by clicking the cursor on the desired lane. The lane should look like

"dancing ants". Position the arrow at the top handle of the activated line and drag right or left to the center of the lane. Position the arrow at the bottom handle of the activated line and drag right or left to the desired degree of inclination. The arrow cursor must be placed directly on the handle in order to allow the lane to be moved. To deactivate the lane line, click in an area away from the "dancing ants".

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4.19.3 To create a single lane, click and hold the mouse button over the center of the lane to be drawn and move vertically down until the cursor is at the center of the bottom of the lane. Release the mouse button. Move the mouse horizontally to draw the desired width of the lane. Click the mouse button again to set the lane width.

NOTE: Band recognition in the FMBIO system depends on values derived from calculation of the linear differential coefficient of the spectrum curve. Whether a band will be recognized as standing out from the background depends on four parameters: gradient start, gradient end, duration, and noise level.

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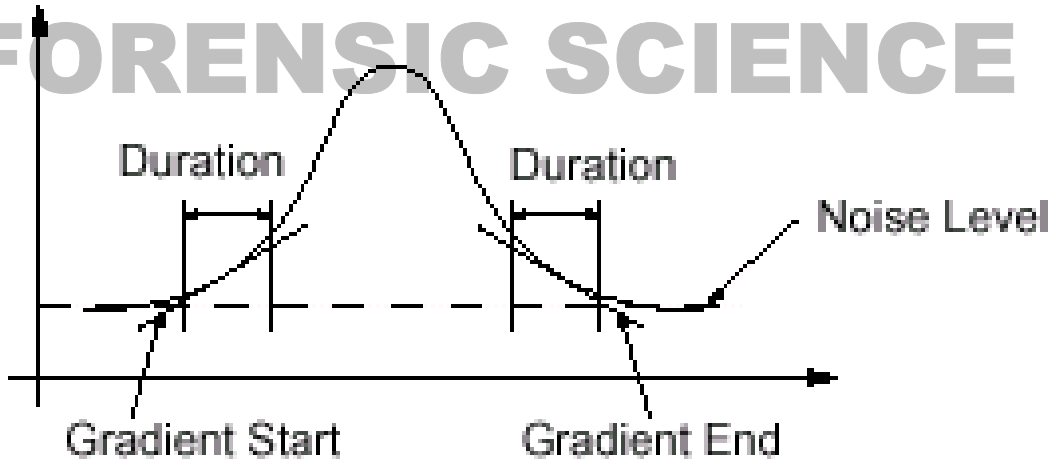
4.20 Under the "1D-Gel" menu, click on "Setting" and select "Auto Band". A "Set Auto Band" dialog box shown below will appear. Set the parameters for gradient start, end, duration, and noise level to reflect the quality of the gel. Heavily loaded and smeared lanes will require more gradual gradients, longer duration and higher background settings. Gels with faint or partially hidden bands will require more stringent settings for these parameters.



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GRADIENT START: The gradient start determines the signal intensity in the "leading edge" of the band. The parameter is the vertical component of the leading edge, which together with the duration parameter defines the slope of the function. Choose a value, in tenths of a millimeter. Start with a setting of 2.0.

END: The gradient end determines the intensity in the "trailing edge" of the band. The parameter is the vertical component of the function, which together with the duration parameter defines the slope of the function. Choose a value, in tenths of a millimeter. Start with a setting of 2.0.

DURATION: The duration defines the length of smear in the leading and trailing edges of the band. Choose a value, in tenths of a millimeter. Start with a setting of 0.1.

NOISE LEVEL: The noise level defines baseline signal intensity value. Noise level must be exceeded by gradient and duration parameters if the signal is to be recognized as a band. Choose a value

(in bits) between 0 and 255. Choose higher values for gels with “dirty” or “noisy” background. Choose lower values for “clean” or “quite” gels. Evaluate the noise level of your gel by trying different Gray Scale Correction values. Start with a setting of 10 to 20.

NOTE: The preset auto-band parameter values for PowerPlex® 16 BIO can be selected. Select the “1D-Gel Set Auto Band” preset parameter from the pull down menu and click on “Load”.

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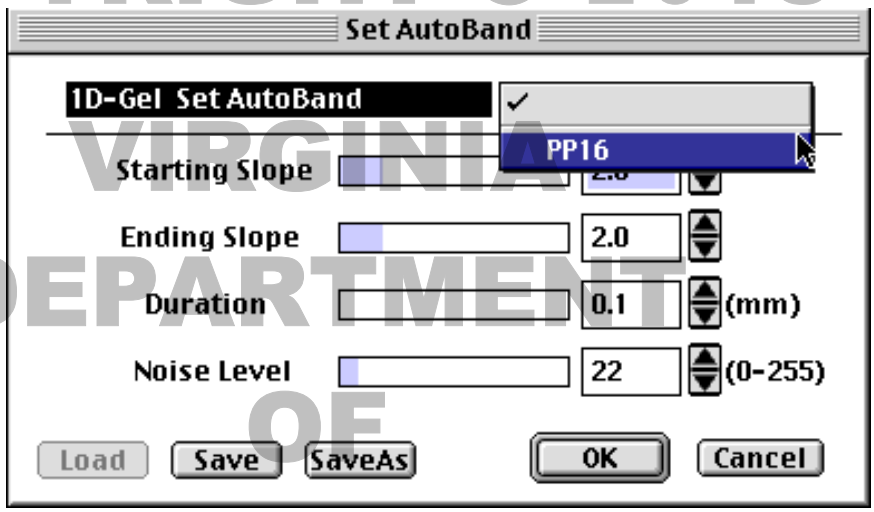

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4.20.1 Click "OK" when all the parameters have been set for auto banding.

4.20.2 While still under the "Setting" menu, select "Calculation". Ensure the "type 4" and "OD calc with background" selections are check marked. Then select only OD and bp parameters under "Table Info" under the "Settings" menu by de-selecting mm, Rf, IOD, and IOD%.

4.20.3 Activate all the lane lines by pressing the **“APPLE” and “A”** key simultaneously. All lane lines must be activated in order for the FMBIO software to recognize the bands in each of the combined images.

4.20.4 Initiate auto band recognition by clicking on the "Auto Band" icon. Bands will be placed according to the parameters set in 4.21.

4.20.5 Once the bands have been marked, turn off the vertical lines by clicking on the "Show Lane" icon until the lines disappear.

4.21 Under the "Multi" menu, choose "Display Mode" and click on "Mono". Or click on the “mono” display button in the project box.

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4.22 To determine if all bands have been selected for a particular image, in the PROJECT BOX, click on Filename.CH for the desired gel image. To ensure that all bands are present for each of the gels of the combined image, each Filename.CH must be checked separately.

NOTE: The volume calculation bands (yellow bands) should be displayed above and below each band. If these bands are not located in the proximity of the allelic band, delete all the bands by pressing the **“APPLE”** and **“A”** keys together, then the **“Delete”** key. The process must be conducted for each channel. Once the bands have been cleared, adjust the gradient start and end as described in step 4.21. Activate the lane line again by pressing the **“APPLE”** and **“A”** key then press the “Auto Band” icon to re-autoband the gel.

4.22.1 Bands that are faint, partially obscured, or highly smeared may not be recognized by the "Auto Band" function. To register such bands, click the "Manual Band" icon and the "Peak

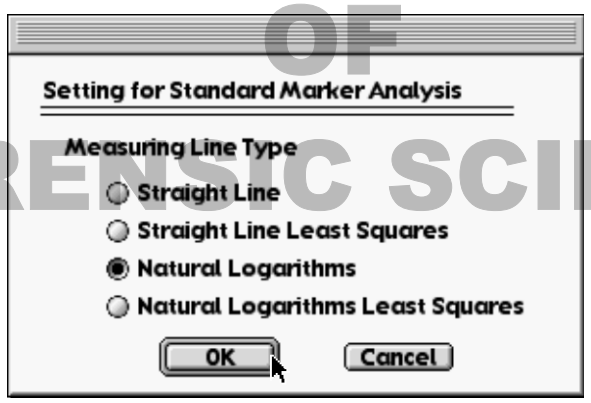
Detector" icon. Position the cursor at the desired position within the defined lane area, and click the mouse. A band will appear near that position where the signal intensity is the greatest.

4.22.2 To remove a band on the image, position the "Manual Band" cursor over the band and click. The band will turn blue then press the large delete key on the keyboard. Edit multiple bands by first clicking on "Marquee (Rectangle)" icon. Use the "Marquee Cursor" to draw a box around the band(s) to be deleted. This function can be used to delete incorrectly recognized bands, such as spots or marks on the image.

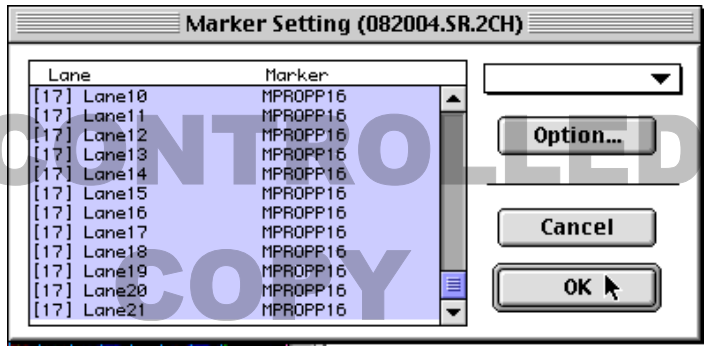
4.22.3 Once it has been determined that all bands are present for each image, under the "Multi Menu" choose "Display Mode" and click on "Over". Or click on the "overlay" button in the project box.

4.23 Make sure the internal lane standard marker (Channel 2) is selected in Project box, and then register the markers or ladder lanes by selecting the "Set Mark" icon.

4.23.1 A "Setting for Standard Marker Analysis" dialog box appears. Choose Natural Logarithms and click "OK".



4.23.2 Next, the "Marker Settings" dialog box appears containing the file name of the marker channel in parentheses (mark sure this is channel 2). Using the mouse, highlight all the marker lanes by holding down the shift key and clicking the mouse button on each lane.



4.23.3 Under the pull down menu screen click on the "(17) MPROPP16" marker for PowerPlex® 16 BIO (the boundary should include 17 ILS bands). A "MPROPP16" should be seen next to each lane. Ensure all the lanes have been appropriately marked. Then close this screen by clicking "OK".

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NOTE: If the typing gel is run too far and the 100 bp internal lane standard band has run off the bottom of the gel, the default marker settings listed above [i.e., (17) MPROPP16] should still be used to size the gel.

4.24 Click the "Recalculate" icon or select "Recalculate" located under the "1D-gel" menu. The marked lanes will be automatically compared to the internal lane standard and the base pair size will be assigned to each band.

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4.25 To display the quantitative values for the base pairs (bp) derived from the migration data for each separate gel image, click on that images respective channel in the project box. Then click the "Open Table" icon, or select "Open Table" option under the "1D gel" menu at the top of the screen.

4.26 Under the "File" menu, select "Save" to save all records. To save the spreadsheet as a table use the "Save Analysis" command under the "File" menu. Once the "Save Data As:" window appears give the file a name (022900ASR.2CH.DAT), the default name assigned.

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IMPORTANT: Name each gel image spreadsheet separately (Example: 022900ASR. 2CH.DAT and 022900ASR.3CH.DAT).

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4.27 Once the spreadsheets for each of the images of the combined image file have been assigned a name and saved, the spreadsheet data can be opened in the Excel software program for allele designation. Close the image and save the newly created file. Then quit FMBIO analysis by selecting "Quit" under File menu. Proceed to Section 5, DETERMINATION OF ALLELE DESIGNATIONS USING THE MICROSOFT EXCEL SOFTWARE PROGRAM.

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5 Determination of Allele Designation Using the Microsoft Excel Software Program

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5.1 Open the EXCEL program using the desktop icon.

5.2 If the STR OD/IOD CUTOFF TABLE does not contain the following values for the appropriate STR system, change the cutoff values to those specified in the appropriate table.

5.2.1 This can be checked by selecting “STR Lookup Table…” from the “Star Call” menu.

5.2.2 Select the appropriate table and click “edit”.

5.2.3 Make the changes in the correct location and select “Save” from the “File” menu.

PowerPlex® 16 BIO

Locus	Cutoff (%)		Locus	Cutoff (%)		Locus	Cutoff (%)	
	From	To		From	To		From	To
FGA	0	11	Penta E	0	2	Penta D	0	2
TPOX	0	8	D18S51	0	13	CSF1PO	0	11
D8S1179	0	10	D21S11	0	15	D16S539	0	12
vWA	0	16	TH01	0	5	D7S820	0	11
			D3S1358	0	12	D13S317	0	10
						D5S818	0	13

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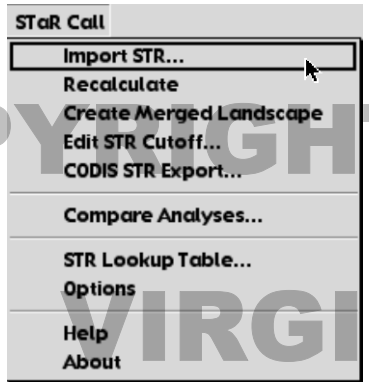
5.3 Under the "STaRCall" menu choose " Import STR…"



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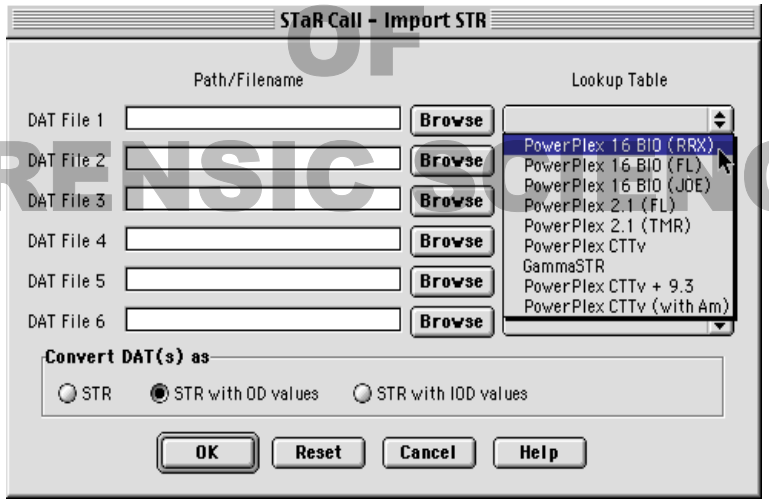
5.4 Select the desired files by clicking on "Browse". Find each of the related data files and select "Open". (Example: 022900ASR.P1.2CH.DAT and 022900ASR.P1.3CH.DAT). Also, select the appropriate look-up tables, which will be used to evaluate the data in these files. Ensure that the "STR with OD values" button is selected and then click on "OK".



5.5 The allelic ladder lanes of the first data file should be highlighted, as in the following diagram. These lanes will be used as allelic ladders in all subsequent data files selected in the Import STR window. Select "OK". If the allelic ladder lanes are not highlighted click the "Auto Select" button, then select "OK".

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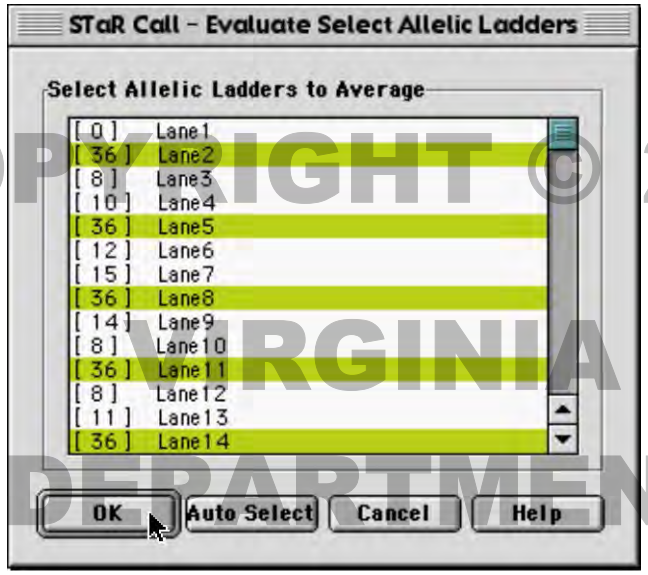
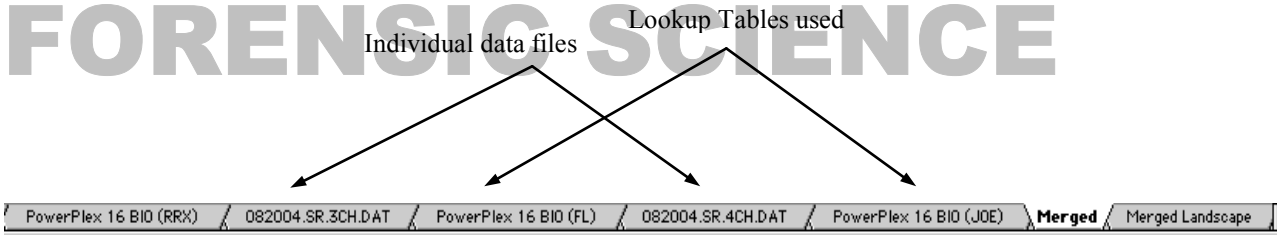


5.6 The STaRCall program creates a workbook, which details the evaluation of the data files. It contains an Analysis Summary sheet, a Merged sheet, a Landscape sheet, as well as the converted individual data files and the associated lookup tables. This workbook must be saved.



5.7 Under the "File" menu, select "Save". Save the workbook as a "Microsoft Excel Workbook" in the appropriate folder either on the file server, hard drive or on an optical/ ZIP disk. For example, 022900ASR.P16.dat includes all the data needed in this analysis.

5.8 Proceed to step 5.9 for instruction on how to label the individual lanes (this step is conducted by the examiner/analyst). IF AN INDEPENDENT ANALYST IS CONDUCTING THE STaRCall ANALYSIS, PROCEED TO 5.10.

5.9 Label the specimen information for export into CODIS, under the "STaRCall" menu; select "CODIS STR Export…"

5.9.1 A screen will appear labeled "STaRCall - CODIS STR Header Information".

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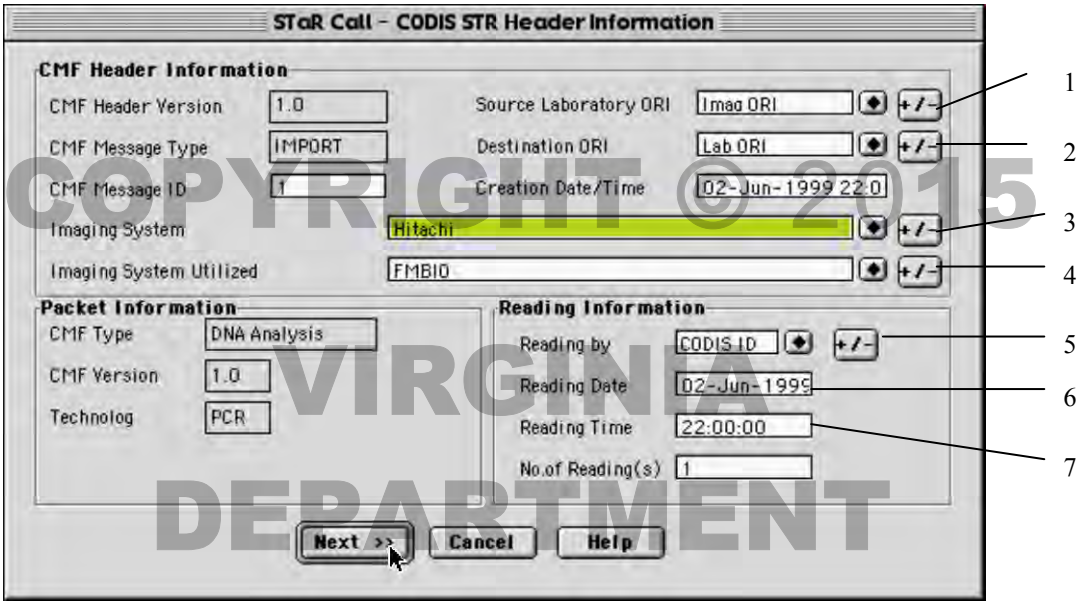

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Number	Feature Name	Description
1	Source Laboratory ORI	Macintosh workstation/location used for export
2	Destination ORI	Each regional laboratory has it's own unique ORI
3	Imaging System	Manufacturer of system (Hitachi)
4	System Utilized	Type of scanning instrument (FMBIO)
5	Reading by	Analyst's CODIS user identification
6	Reading Date	Date of gel scan
7	Reading Time	Time gel was scanned
**	+ / - Button	Creates or deletes items from the pull down menu

5.9.2 Click on the "Next" button and the "STaRCall-CODIS STR Specimen Information" screen will appear.

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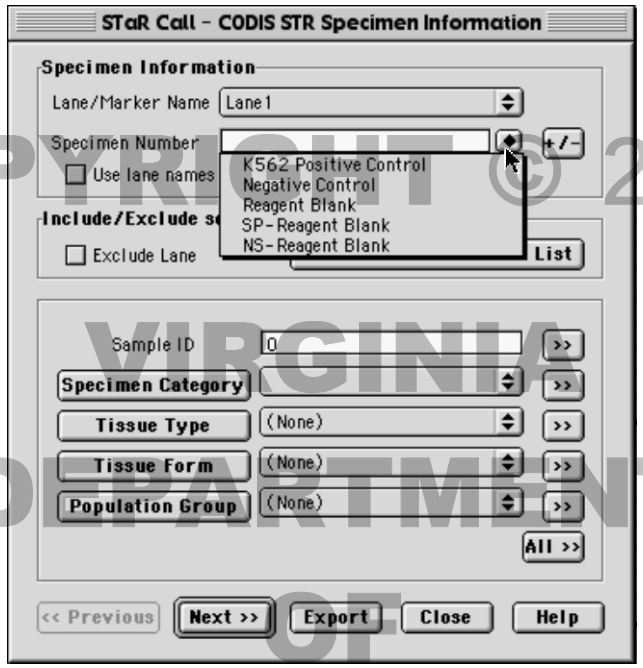

5.9.3 Enter the sample identifier (Example: W98-12345V, 98-12345, or Victim/Suspect, etc. into the "Specimen Number" field. For commonly used lane identifiers, such as controls, the pull down menu may be used.

NOTE: If importing the specimen into CODIS, the specimen identification should be the same nomenclature discussed in the CODIS OPERATING POLICIES AND PROCEDURES MANUAL). If the specimen will be used in an Appendix of Typing Results Chart, the specimen must have enough distinguishing information to identify it in the chart.

5.9.4 Using the pull down menus next to the Specimen Category, highlight the appropriate category. . If the sample identifier is a control sample, a blank lane, or Random Sample, use "OTHER" as the specimen category and skip to 5.9.6

5.9.5 Using the pull down menus next to the Tissue Type, Tissue Form, and Population Group, highlight the appropriate category.

5.9.6 After all categories have been identified, click "Next". The STaRCall - CODIS STR Specimen Information screen will move to the next sample lane. Repeat steps 5.9.3 and 5.9.5 until all sample lanes have been provided with specimen identification.

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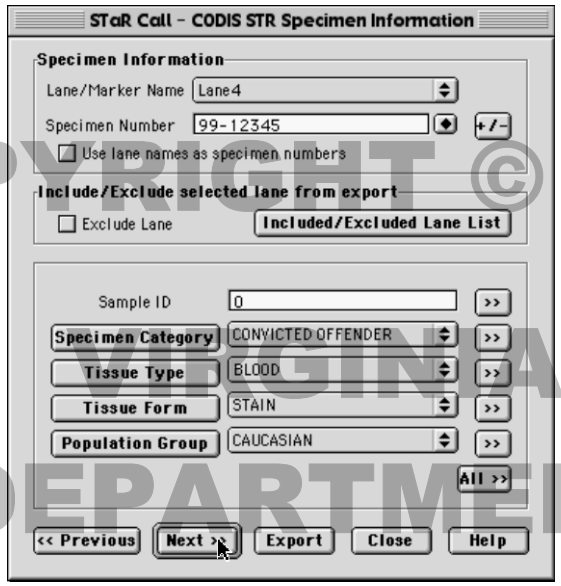
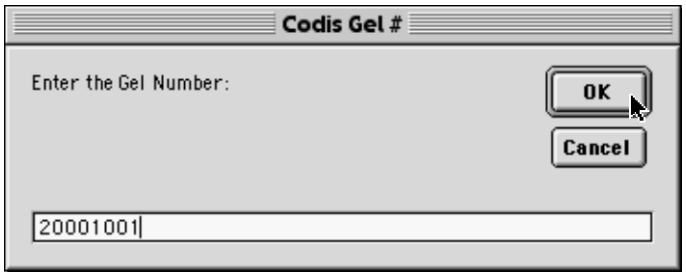


NOTE: If the specimen has already been analyzed by another system and the specimen information already exists in CODIS, the specimen information must be identical.

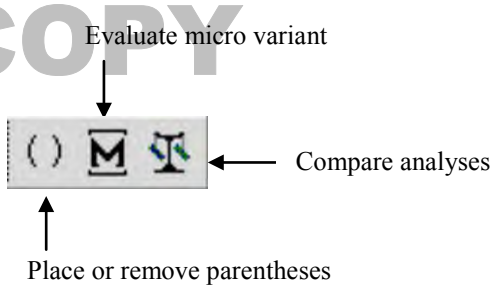
5.9.7 Once all sample lanes have been assigned specimen identification, close the STaRCall – CODIS STR Specimen Information screen by clicking on "Close".

5.10 If a gel number needs to be assigned to the specimens being exported to CODIS, under the "STR Setup" menu, select "Gel Number". When prompted, enter the gel number and click "OK".



5.11 If a microvariant is detected during analysis, an allele may be called as "Out of Range" by the STaRCall software. To see if this allele is a microvariant, select the base pair value for it in the individual data worksheets. Then single click on the M icon located on the toolbar. This will evaluate the base pair to see if it is a microvariant, assign cut off values for adjacent microvariants and report those values in the appropriate look up table for each microvariant reported.

NOTE: The M icon will not work when allelic ladder bands are greater then 4 bps apart (i.e., Penta D and E)



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5.12 To place or remove parentheses on the alleles, select the “Merged Landscape” worksheet, highlight the allele and single click on the () icon diagramed above.

5.13 After the analyst has checked all data for accuracy, the entire analysis can be printed. Under the “STR Setup” menu, select “Print Analysis”.

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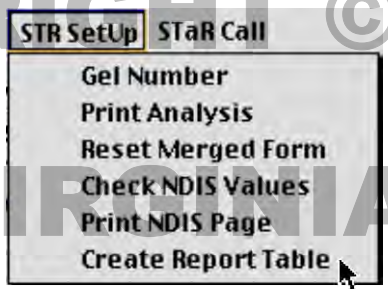

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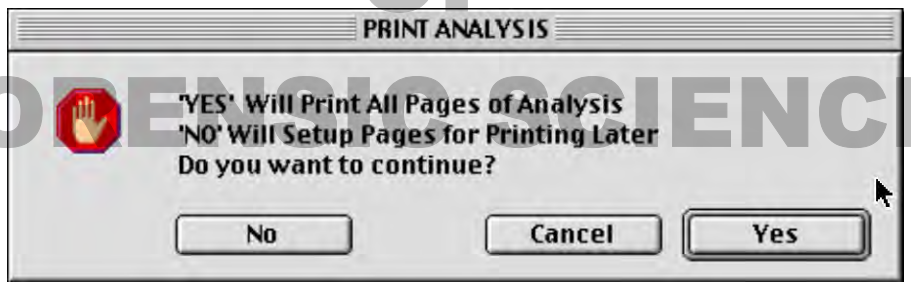
5.14 When prompted enter the analyst's initials, the gel number/case number associated with the data, and the date analysis was conducted-, then click "OK".

5.15 When the Custom Print Box asks, "Do you want to continue?" Click "Yes" if all information entered is correct.



NOTE: Two separate landscape sheets may be printed for PowerPlex 16 BIO data. Choose “Yes” when prompted. Choosing “No” will print the single landscape page.

5.16 If reprinting is required, (i.e. changes are made due to microvariants, stutter, questions marks, etc), repeat steps 5.13-5.15 and select “No” to format the workbook without printing. If reprinting of individual worksheets of the analysis is required, select the desired worksheet from the tab bar at the bottom of the workbook. Then under the file menu, select “Print…”

5.17 Once the analysis has been completed (including microvariant calls) and all pages printed, select “Check NDIS Values” from the “STR SetUp” menu. All data values will be corrected for uploading to NDIS. Also, an “Archived Merged Landscape” sheet will be created which has all of the actual/original allele values.

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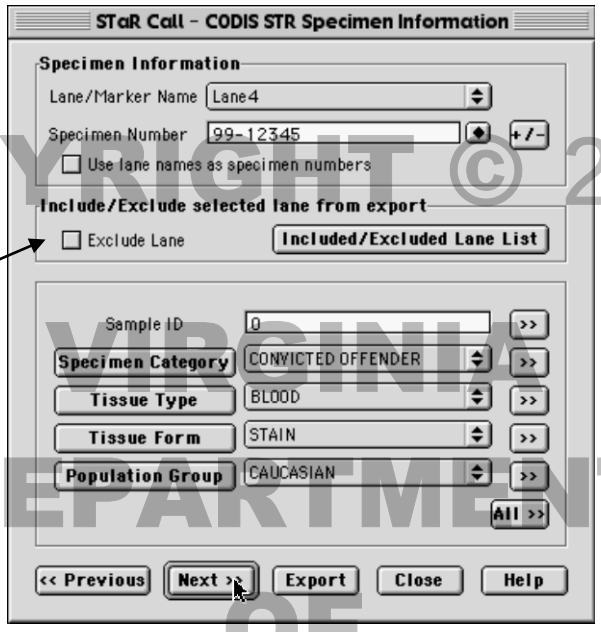
5.18 The NDIS corrected landscape can be printed by selecting “Print NDIS Landscape” from the “STR SetUp” menu.

5.19 If the analyst will be exporting data to CODIS, all controls, blank lanes, and failed sample lanes, need to be excluded. Under the “STaRCall" menu, select "CODIS STR Export…”

5.19.1 A screen will appear labeled "STaRCall - CODIS STR Header Information". Click on the "Next" button and the "STaRCall-CODIS STR Specimen Information" screen will appear.



5.19.2 If sample identifier is a control sample blank lane, or failed sample, click on the "Exclude" box to prevent this lane from being exported into CODIS.

5.19.3 Click "Next" and repeat 5.18.2 for each lane of the gel that is to be excluded. Alternatively click on "Included/Excluded Lane List" and highlight the lanes to be excluded.

5.19.4 When finished, click "Close" to close the STaRCall - CODIS STR Specimen Information screen.

5.20 If no further analysis will be performed, under the "File" menu, select "Save", then close the merged filename.dat file.

5.21 Under the "File" menu, select "Quit", to quit using Microsoft Excel.

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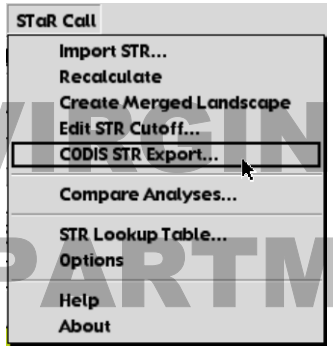
6 Exporting STARCall Data Into CODIS

6.1 Open the EXCEL program using the desktop icon.

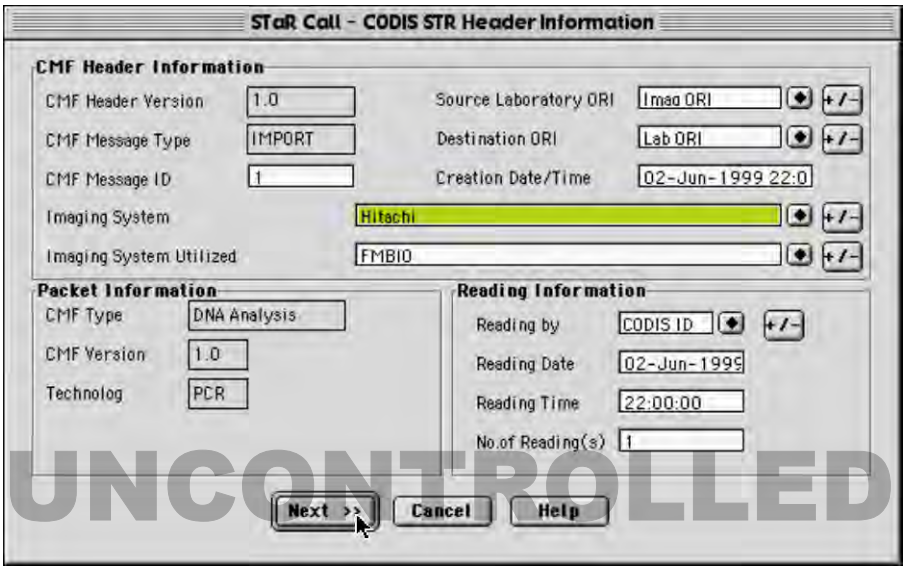
6.2 Under the "File" menu, select "Open". Choose the file name that you wish to work with and click Open.

6.3 Under the "STaRCall" menu, select "CODIS STR Export…"



6.4 A screen will appear labeled "STaRCall - CODIS STR Header Information".

6.5 Ensure that the "Source Laboratory ORI" and "Destination ORI", have the appropriate workstation/location and laboratory identifications, and that the "Reading By" has the analyst's CODIS user identification. Change the "Read Date" to reflect the date the data is being exported to CODIS. Use the pull down menus to make any appropriate change.



6.6 Click on the "Next" button and the STaRCall-CODIS STR Specimen Information screen will appear.

6.7 Ensure that all the proper specimens have been excluded. (See 5.17 for excluding sample lanes)

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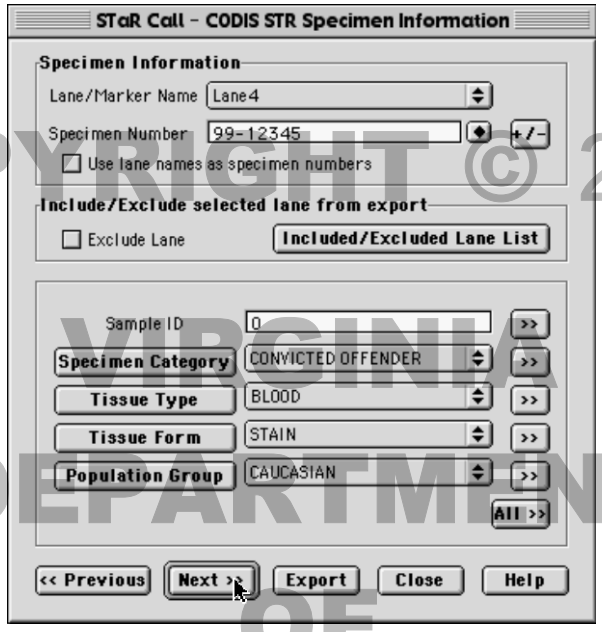
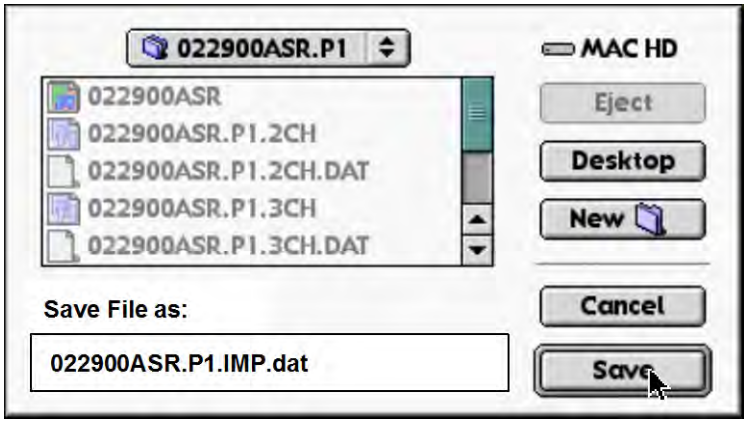


6.8 To create the export file containing the STaRCall data for transfer to CODIS, click on "Export".

6.9 In the "Save As" field, enter the name of the file (Example: FilenameP1.IMP.dat, or FilenameP2.IMP.dat) which will be exported to CODIS. Make sure the file is saved into the appropriate file folder on the file server (i.e. macvol) or transferable media.



6.10 If no further export files will be created, under the "File" menu, select "Save" to include added file information. Then under the "File" menu, select "Close" to close the workbook file. Repeat 6.2-6.10 to create additional export files.

6.11 Under the "File" menu, select "Quit", to quit using.

7 Creating Appendix of DNA Typing Results for Certificate of Analysis

7.1 Once the landscape spreadsheet file has been saved as outlined in step 5.20, delete from the landscape spreadsheet the DNA profiles that do not pertain to the case file of interest.

7.2 Select "Save As" from the file menu and save this modified workbook using the case number in the filename to distinguish it from the original gel's data".

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7.3 Ensure that the Specimen identification is complete enough to properly describe the evidence. Do not exceed 24 characters, including spaces. See section 8 for example of Abbreviations Appendix.

7.4 In the ”STR SetUp” menu choose “Create Report Table”

NOTE: Once the report table has been created it cannot be edited and both the Appendix of DNA Typing Results and the Appendix of Abbreviations are automatically printed together.

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7.5 Continue this process for all of the cases on the landscape spreadsheet

8 Abbreviations for Appendix of DNA Typing Results for Certificate of Analysis

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ANO	Anorectal Sample
BLD	Blood Stain
ELIM	Elimation
ENV	Envelope
FNC	Finger Nail Clippings
FNS	Finger Nail Scrapings
K	Known
L	Left
LLA	Lips/Lip Area Sample
MAT	Material
NSP	Non-Sperm Fraction
OR	Oral Rinse Sample
PA	Pubic Area Sample
PB	Perianal/Buttocks Sample
PH	Pubic Hair
Q	Questioned
R	Right
SC	Stain Card
SMPL	Sample
SP	Sperm Fraction
STN	Stain
STN'D	Stained
SUS	Suspect
SWB	Swab
TEG	Thighs/External Genitalia Sample
UPS	Underpants
VC	Vaginal/Cervical sample
VIC	Victim

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Appendix G - Fluorescent Detection of the Electrophoresis Gel FMBIO® III PLUS™

1 Technical Notes

1.1 The FMBIO® II utilizes a solid state green laser (532 ηm) to instigate the excitation of Fluorophores. The resulting fluorescent light signals emitted are then collected by an optical fiber array and converted to electrical signals by photo multiplier tubes. Finally, the analog-to-digital converters transform the electrical data into an image for interpretation and analysis by the computer.

1.1.1 The 577ηm filter will detect wavelengths of light emitted from the JOE fluorescent dye.

1.1.2 The 505 ηm filter will detect wavelengths of light emitted from the Fluorescein fluorescent dye.

1.1.3 The 598 ηm filter is used to auto focus the FMBIO® laser and will detect wavelengths of light emitted from the Rhodamine Red™ – X (RRX) fluorescent dye.

1.1.4 The 665 ηm filter will detect wavelengths of light emitted from the Texas Red® – X (Tex Red) fluorescent dye (internal lane standard for PowerPlex® 16 BIO).

1.2 The FMBIO® III Plus has the capability to utilize three lasers, a blue laser (488ηm), a green laser (532 ηm), and a red laser (635ηm) to instigate the excitation of Fluorophores. The resulting fluorescent light signals emitted are then collected by an optical fiber array which then passes the light through specific long pass (blocking) as well as band pass filters through photo multiplier tubes. Finally, analog-to-digital converters transform the electrical data into an image for interpretation and analysis by the computer.

1.2.1 The 598ηm filter is used to auto focus the FMBIO® laser and will detect wavelengths of light emitted from Rhodamine Red™ – X (RRX) fluorescent dye. The 570 ηm long pass blocking filter installed with it allows for easier color separation and reduces background from other dyes.

1.2.2 The 577ηm filter will detect wavelengths of light emitted from JOE fluorescent dye. The 550 ηm long pass blocking filter installed with it allows for easier color separation, reduces background, and blocks reflected laser light from the glass plates.

1.2.3 The 520 ηm filter will detect wavelengths of light emitted from Fluorescein fluorescent dye. It also has a 515 ηm long pass blocking filter installed with it to allow for easier color separation and reduces background, and blocks reflected laser light from the glass plates.

1.2.4 The 650 ηm filter will detect wavelengths of light emitted from Texas Red® – X (Tex Red) fluorescent dye (internal lane standard for PowerPlex® 16 BIO).

2 Equipment

2.1 FMBIO®FMBIO II / FMBIO®FMBIO III Plus Fluorescent Image Analysis System (Hitachi)

2.2 IBM PC or compatible computer (1 GHz or faster CPU with 10 Gb hard drive)

2.3 Laser Printer

CAUTION

The FMBIO® Fluorescent Image Analysis System is a laser excited optical scanner, which contains high-energy electrical components that could cause serious injury to the operator if not operated properly. It is imperative that the laser is not radiating when the scanning well cover is opened. Ensure that all external panels and protective utility covers are properly positioned, undamaged and tightly shut, so that no leakage of the laser

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beam is possible. Shut the instrument down at the first sign of trouble. If the unit emits an unusual smell, smoke, noise, or excessive heat, immediately turn off the power and disconnect the power cord.

3 Procedure for Using the FMBIO® II Fluorescent Image Analysis System

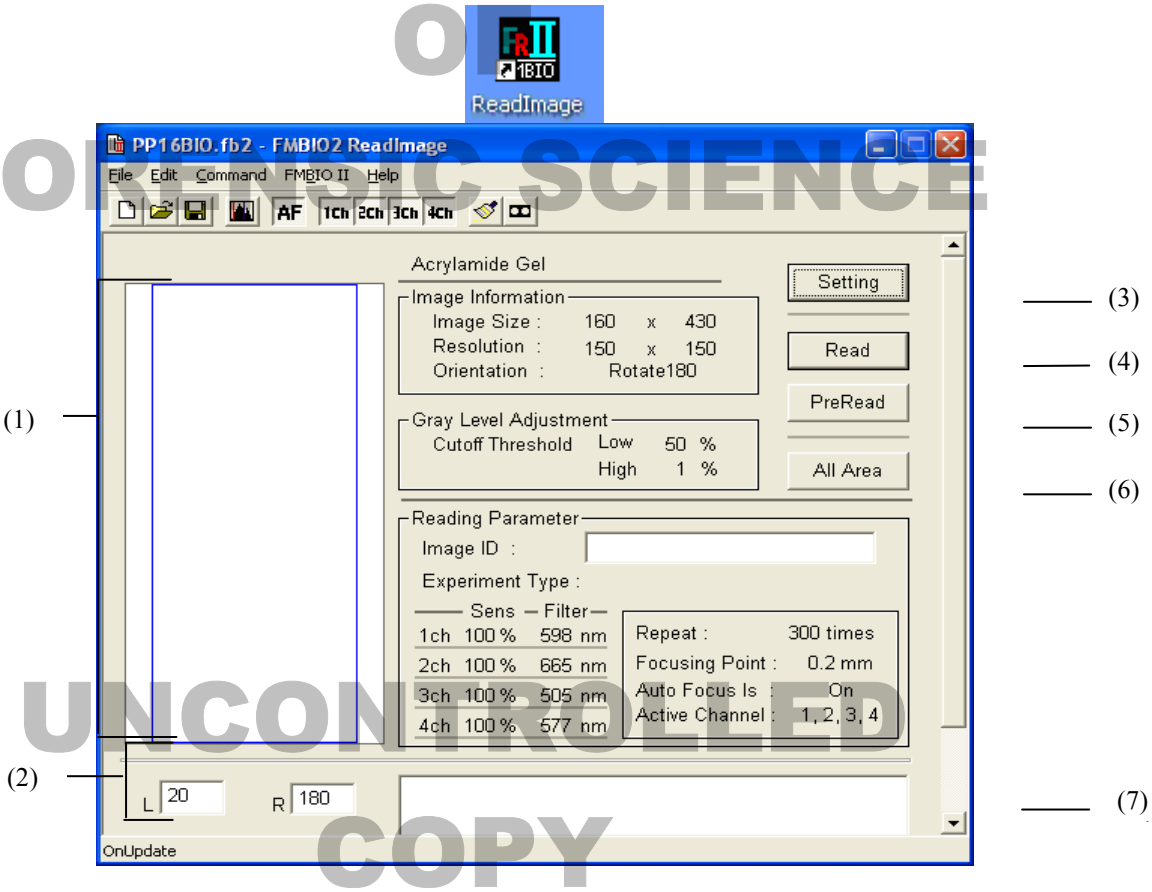
3.1 Turn the FMBIO® II scanning portion of the imaging system on and let the instrument perform the self diagnostic. The green ready light will stop flashing when the unit is ready.

3.2 Turn the PC computer on by pressing the power button.

3.3 Place the electrophoresis gel, origin to the right, into the FMBIO® unit and close the lid until latched. Ensure the proper orientation is selected depending on whether the short plate is up or down. Short plate up, select **Rotate 180**. Short plate down, select **Flip Vertical**.

NOTE: If the lid is not closed completely, an error message will appear at the beginning of the scanning process.

3.4 Click on the Read Image icon located at the right side of the desktop to open the "Read Image" dialog box.



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Number	Feature Name	Description
1	Total scan area	Displays available scan area and a pre-defined scan area.
2	Scan area coordinates	Four values that define the scan area.
3	Setting button	Opens the FMBIO® Parameters window
4	Read button	Begins an image scan. Changes to Pause during scan. Changes to resume during a Pause.
5	Pre Read button	Begins a pre scan. Changes to Cancel during a scan.
6	All Area button	Sets the scan area to the total scan area.
7	Comment field	Stores your comments.

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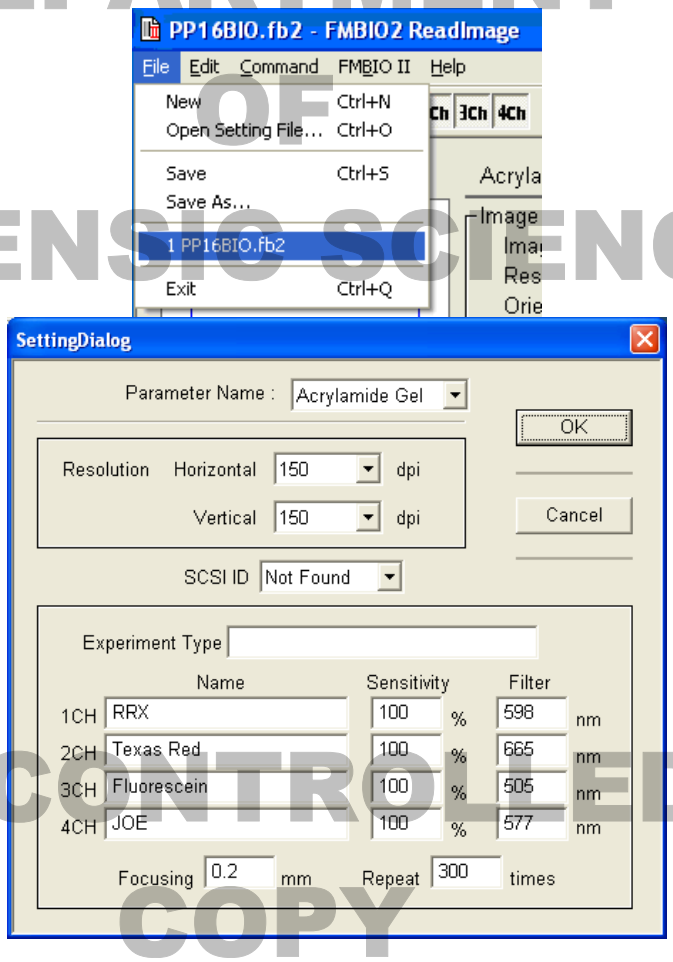
3.5 Select "PP16BIO.fb2" from the file menu to load the pre-set scanning parameters



3.6 Ensure that the following default parameters have been set prior to scanning the gel (these are selected within the 'Command' menu under 'Set Parameter' and 'Set Gray Level'):

- ✓ Material Type: Acrylamide (Agarose for Product Gels)
- ✓ Image Size: 200 x 430 (variable)
- ✓ Resolution: 150 x 150
- ✓ File Type: Normal
- ✓ Orientation: Rotate 180° (short plate up), Flip Vertical (short plate down)

- ✓ Repeat: 200 - 300 times
- ✓ Reading Sensitivity:
- ✓ Recommended Settings PowerPlex® 16 BIO
  1CH (598ηm) 100%, 2CH (665ηm) 100%
  3CH (505ηm) 100%, 4CH (577ηm) 100%
- ✓ Recommended Settings for Product Gels
  1CH (598ηm) 50% (only channel active)

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NOTE: These parameters can be varied between 50 to 100% depending on the quality and type of gel.

- ✓ Focusing Point for PowerPlex® 16 BIO: -2.0 to 3.0 mm
  (Variable based on instrument's optimized values)
- ✓ Focusing Point for Product Gels: 2.4-2.8 mm
- ✓ Auto Focus:
  OFF (Automatic during first FMBIO® run following the machine being turned on).
- ✓ Active Channel:
  Select the channels that contain the filters to collect the desired dye's wavelength.
- ✓ Gray Level Correction:
  Type is Range
  Cutoff Threshold: Low 1%
  High 1%

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3.7 If additional comments are required, place the cursor in the comment section with the mouse and type the comment or gel number and any other important information. This information is retained in a small text file associated with the image file and may be printed along with the image as needed.

3.8 Click the "All Area" button.

3.9 Click on the "Pre-Read" button. The laser will quickly scan the gel. This pre-read will allow the examiner to designate the area for the laser to scan.

3.10 Once the designated area has been determined, using the mouse, define the area in the image rectangle that contains the area of the gel that is to be scanned. Position the cursor at the top left portion of the area to be included in the scan, then drag down to the right bottom corner. Avoid including areas beyond the region of the gel where there is no DNA. The rectangle should not include the spacers as these will interfere with the laser's detection level.

NOTE: Ensure that the entire gel is within the scan area to allow for off ladder variants as well as upper and lower allelic ladder bands.

3.11 Click "Read" to begin the scan. The software will prompt the examiner to save the image. Name the image and save it in a designated file on the desk top or hard drive.

NOTE: The file name given to the image should not be more than 16 characters in length. This includes spaces.

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3.12 The "ReadImage" Dialog Box will reappear. If this is the first scan of the day, the FMBIO® II's laser will automatically auto-focus using the 598 ηm filter once the "Read" button is clicked. This function finds the best optical alignment of the rotating mirror and other signal tracking and processing elements. **Once the instrument has been auto-focused, providing the FMBIO® unit is not turned off, it is not necessary to auto-focus each time an electrophoresis gel is scanned.**

3.13 Once the gel has been scanned, choose the "Open Analysis with Image(s)" function that is found under "Command" menu located at the top of the screen on the menu bar. Review the images to ensure that all the necessary information has been captured by the scan. When the gel images have been determined to be acceptable, the gel plates can be removed from the FMBIO® II unit and the glass plates washed.

3.14 Close the image of the gel by clicking on the button at the upper right corner of the title bar of the image. Transfer the saved image folder from the desk top to the network server or hard drive.

3.15 Quit Image Analysis by selecting "Exit" under the "File" menu. Then quit the Read Image program by selecting "Exit" under the "File" menu, if prompted to save changes to Read Image program, select don't save. Proceed to Section 5, ANALYSIS OF THE GEL IMAGE.

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4 Procedure for using the FMBIO® III Plus Fluorescent Laser Scanner

4.1 Turn on the FMBIO® III Plus scanner and let the instrument perform the self diagnostic. ***The "Ready" LED light will blink for about 3 minutes indicating the warm up status of the software. The scanner cannot be operated during this time period.*** When this process is complete, the green ready light will stop flashing.

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4.2 Turn on the PC.

4.3 Open the FMBIO® III Plus scanner lid and place the electrophoresis gel, short plate up and wells to either the left or the right, into the FMBIO® unit and close the lid until latched.

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NOTE: If the lid is not closed completely, an error message will appear at the beginning of the scanning process.

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4.4 Log onto the computer.

4.5 Double click on the FMBIO® III Plus icon to launch the scanner software.

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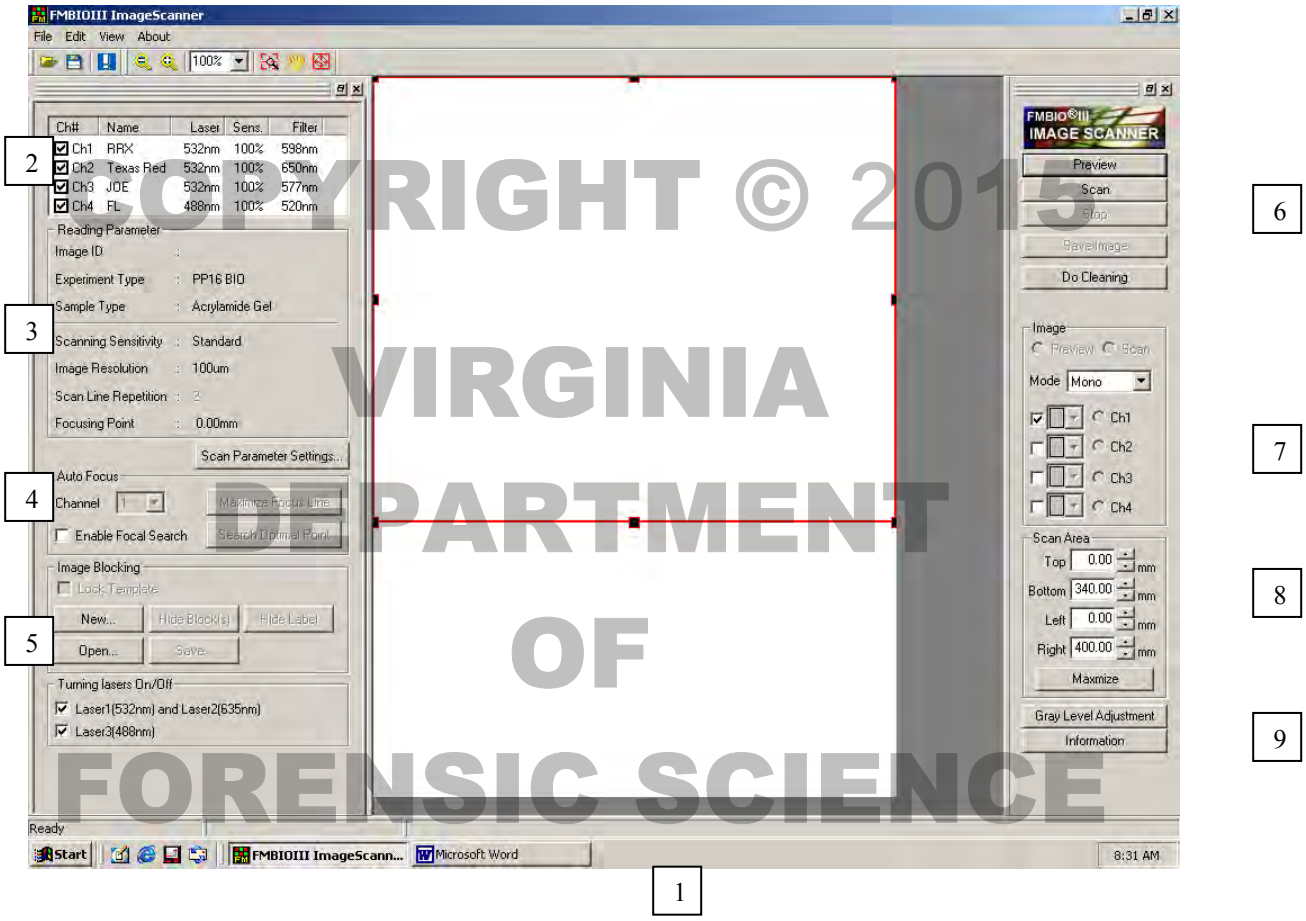

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Number	Panel Name	Description
1	Total Scan Area	Displays available scan area and selected scan area *(boxed in red)*
2	Active Channels	Displays channels which are being scanned (highlighted in blue) a check box selects which channels to scan
3	Reading Parameters	Displays current Reading Parameters
4	Scan Parameters Setting	Opens the FMBIO® Parameters window
5	Image Blocking	Features allow creation and retrieval of pre-defined blocks for scanning and saving scans
6	Scanner Controls	Buttons control the scanner
7	Image Controls	Controls the way the previewed image appears while scanning
8	Scan Area Coordinates	Four number values (mm) that define the scan area
9	Gray Level Adjustment	Opens Gray Level Adjustment window to allow gray level adjustments for the selected image while scanning

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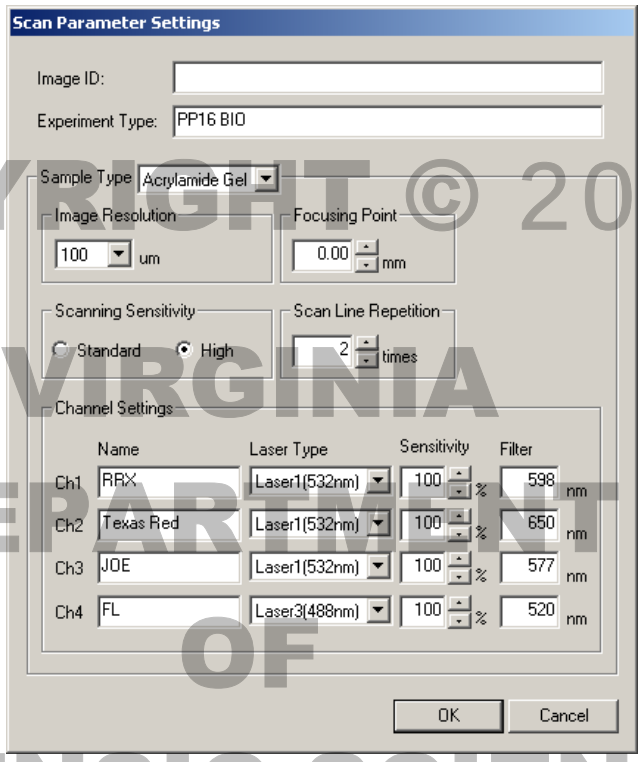
4.6 Ensure that the following default parameters have been set prior to scanning the gel (they may be viewed in the Reading Parameters window).

4.6.1 These parameters may be changed by clicking on the “Scan Parameters Settings” button.



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NOTE: The Sensitivity parameters can be varied between 60 to 100% depending on the gel. The focusing point may be changed in 0.05mm increments to find the optimal point

4.7 The scan area is displayed as a red rectangle. Set the scan area for the scan by using either the mouse or the keyboard.

4.7.1 To use the mouse: Position the mouse on one of the handles located on the perimeter of the rectangle, press down on the left mouse button, and drag the pointer across the image display area. Release the mouse button when the desired position is reached. Repeat with the other three sides of the rectangle to modify the scan area to the user's preference.

4.7.2 To use the keyboard: Go to the "Scan Area Coordinates" panel and change the values in 0.01 mm increments. The red rectangle moves as these values are changed to a maximum of top-bottom (0-550) and left-right (0-400).

4.7.3 To set the full scan area, click the "Maximize" button in the Scan Area Coordinates Panel.

4.8 The Preview function can be used to check the gel position on the stage in order to set the scan range. Previewed images cannot be saved.

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4.8.1 To preview the gel, click the "Preview" button in the Scanner Control panel. The scanned image is displayed in the Image Display Area.

4.8.2 To stop a Preview before completion, click on the "Stop" button in the Scanner Control panel. A dialogue box will appear to verify the request to stop a preview. Clicking the "OK" button will stop the preview function. Clicking the "Cancel" button resumes the preview.

NOTE: Make sure unit has started scanning before clicking "Stop".

4.8.3 Adjust the scan area as necessary.

NOTE: Ensure that the entire gel is within the scan area to allow for off ladder variants as well as upper and lower allelic ladder bands.

4.9 The Image Blocking option allows the user to scan multiple gels at the same time and save each separately as a split image. This is accomplished through the creation of a template which is defined by blocks. The template can be created before or after scanning.

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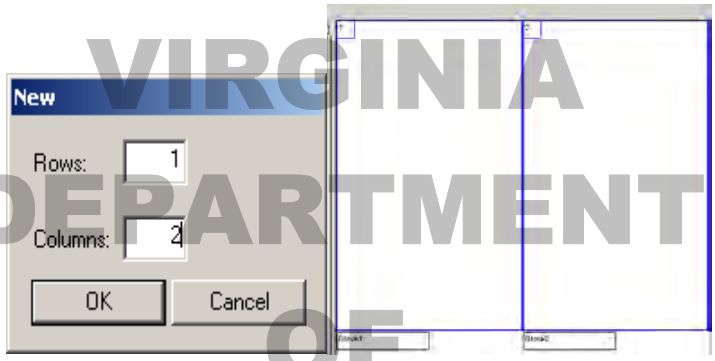
4.9.1 To create the blocks within the template, click the "New" button in the Image Blocking Panel and enter the number of rows and columns.



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4.9.2 The number of gels scanned is entered in "Columns", and enter "1" for the row number.

4.9.3 Position the mouse on one of the handles located on the perimeter of the blue rectangle, press down on the left mouse button, and drag the pointer across the block area. Release the button to set the perimeter. Repeat this action if needed on the three remaining sides.

4.9.4 Double-click the block name of each block to enter the name/number for the gel. Refer to the labeling on the scanner unit to determine the position (Block 1 or Block 2) of each gel.

4.9.5 Blocks and labels may be hidden or viewed by clicking the respective buttons in the Image Blocking Panel.

4.10 Templates can be used to save and retrieve previous settings.

4.10.1 To save a template, click the "Export…" button in the Image Blocking Panel. Specify the save destination file in the dialog box that appears.

4.10.2 To retrieve a previously saved template, click the "Import…" button in the Image Blocking Panel. Specify the file and open the template.

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4.11 To begin a scan, click the "Scan" button in the Scanner Control Panel. The Scan function allows scanning under the currently set parameters to create an image file. The scanner takes an average of 80 minutes to complete a scan using this configuration.

4.12 The scan may be paused or canceled by the same procedure outlined under the preview (above).

4.13 During the scan and any time after each channel is scanned, the operator may make adjustments to the gray scale values. This is accomplished by clicking on the "Gray Level Adjustment" button located at the bottom right of the scan window.

4.14 A window opens with a histogram dialogue box. The red and blue lines represent the low and high cut off values for the gray scale correction. The histogram is updated in real time during preview and scan operations. The red (lower) bar may be moved by clicking and dragging it left or right along the histogram to set the background cut off. The blue (upper) bar may also be moved the same way to set the signal cut off point. By clicking on "Apply" the scanned image is updated with the new gray scale values.

Once adequate gray level adjustments have been made, the dialogue box may be closed by clicking on "close".

4.15 The image display mode may also be changed during or after a scan. To change the display mode, select the mode from the mode menu in the Image Controls Panel. There are five display modes: Blend, Over, Mono, Thermal, and Saturation. Select the mode according to the usage.

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4.15.1 Blend: Overlaps an activated channel image for display in color. Displays an overlapped image in mixed color.

4.15.2 Over: Overlaps an activated channel image for display in color. Displays an overlapped image in color at a higher intensity.

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4.15.3 Mono: Displays only the specified channel in grey scale.

4.15.4 Thermal: Changes the color according to the intensity of the image displayed in gray scale and displays the image in multiple colors.

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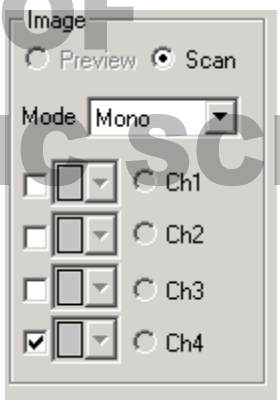
4.15.5 Saturation: Displays the saturated pixels (65,535) in red. Displays other pixels in the same way as in Mono.

OF



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4.16 To switch the channel being displayed, select the channel from the image control selection check box. Each scanned image channel can be viewed in the "Image Display Area".

4.17 A "Save Image Setting" box appears once the scan is complete:

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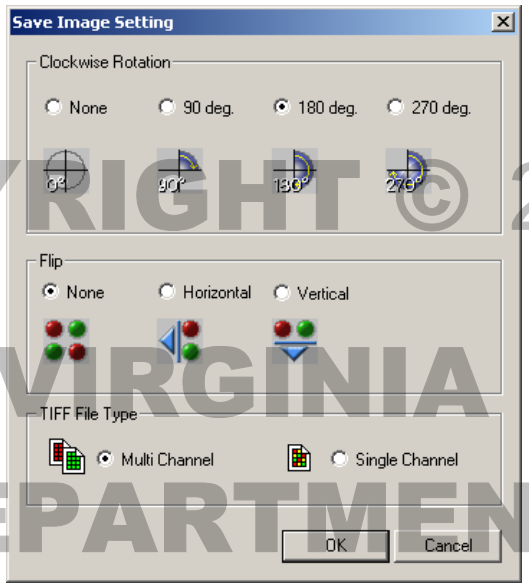

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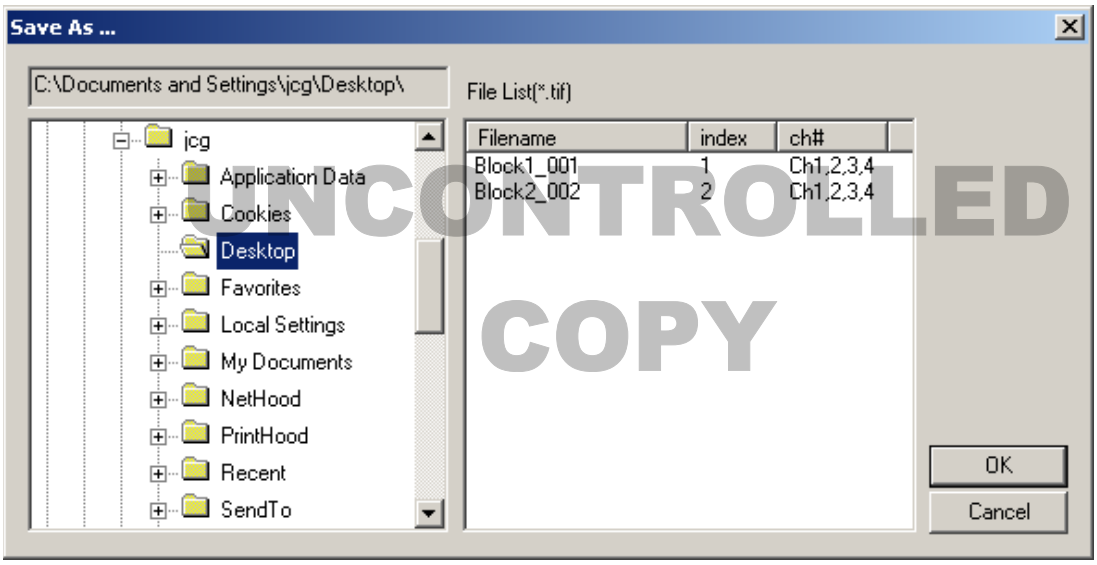
NOTE: Ensure that the Clockwise Rotation of 180 deg. is selected as depicted above if the gels wells were oriented to the right, otherwise no rotation is necessary.

4.18 The user has the option of saving the entire scanned image or saving an image that has been separated into user defined segments. Only the most recently scanned image can be saved.

4.19 If the image blocking and gel naming was done prior to the scan, ensure that the parameters are set according to the box above. Then click "OK".

4.19.1 If Image Blocking and gel naming needs to be completed refer to 4.10 for instructions, then click "Save Image As".

4.19.2 Next, a "Save As" box will appear. Clicking the "OK" button saves the image under a specified name at a specified location. If Image Blocking has not been set, specify name and location for a saved image, and enter the appropriate title in the "File list" box. Then click on "OK".



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4.19.3 The individual blocks are saved according to the block names designated previously. The "001" and "002" suffixes on the filename are added designations by the scanner to indicate the block position of that gel in the scanner. The file names may also be edited from this window by clicking on the name and making the changes.

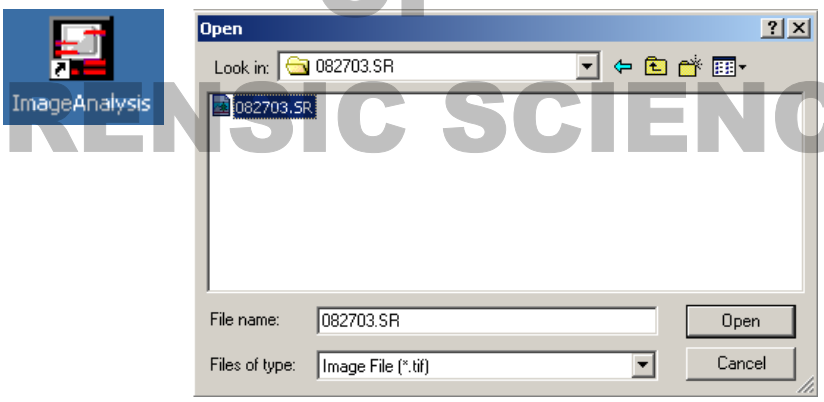
4.19.4 Click "OK" to save. The saving of the .tiff files may take up to 4 minutes depending on the orientation selected. All gels should be saved to the desktop and NOT directly to the server. This is important to note because a network disruption could cause loss of the scan while saving.

4.20 Once the gel is saved to the desktop, create a new folder in the appropriate file on the server. The folder should be given the gel name. Copy the gel file from the desktop to the new folder on the server. Once the transfer is made, the gel file should be deleted from the desktop of the scanning workstation.

4.21 When the gel image has been determined to be acceptable, the gel(s) can be removed from the FMBIO® III Plus unit. Proceed to Section 5, ANALYSIS OF THE GEL IMAGE

5 Analysis of the Gel Image

5.1 To begin analysis of the image, copy the gel file to be analyzed to the desktop. Click twice on the "Image Analysis" icon, located on the desktop.



5.2 Under the "Project" menu, at the top of the screen click on "New 1-D Gel Experiment" and select the TIFF gel image to be analyzed from the open file dialog box and click 'Open'. This creates a project file that stores all color separation and data analysis information.

5.3 Save the new project file by selecting 'Save Project' from the 'File' menu. The file name should not be greater than 16 characters long, including spaces.

5.4 Click on the image window to activate. The Image Setting window is used to select/deselect the channels being viewed with the check mark box. It is also used to switch between the colored (Over and Blend) and black and white (Mono) modes. Individual channel signals may be increased or decreased with the gamma adjustment. Analysis information for each channel can be viewed by clicking on the black dot.

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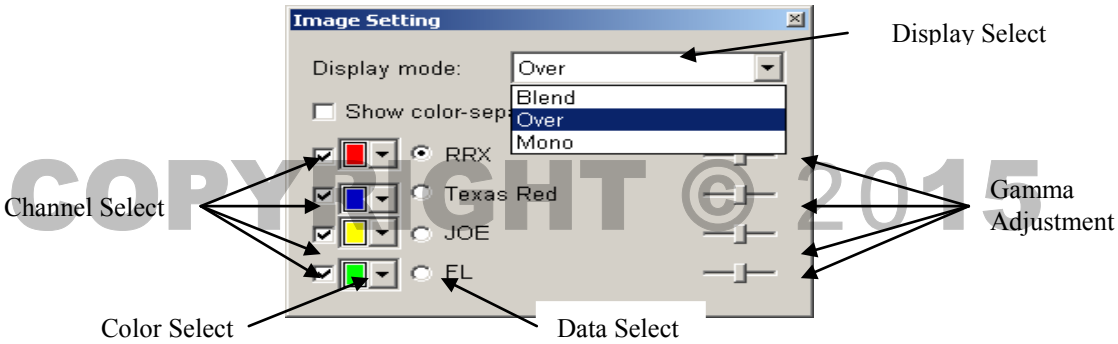
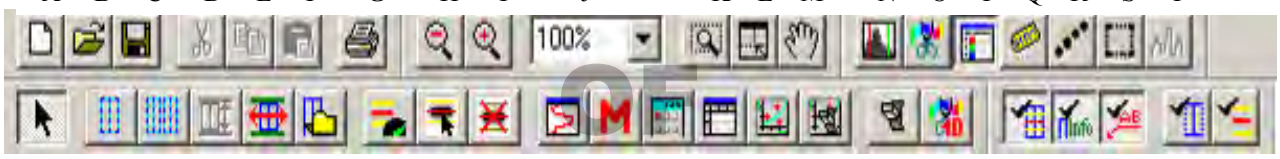


5.5 With display mode in 'Over', click on the "Color Select" drop down button for each channel and change channel 2 (Tex Red) to BLUE, channel 3 (JOE) to YELLOW, channel 4 (FL) to GREEN. Channel 1(RRX) should remain on RED.

A B C D E F G H I J K L M N O P Q R S T



U V W X Y Z AA BB CC DD EE FF GG HH II JJ KK LL MM NN OO PP

A	New project	O	Color separation	CC	Delete band
B	Open project	P	Show image setting dialog	DD	New overlay trace
C	Save	Q	Image information	EE	Set marker
D	Cut	R	Select a line to draw	FF	Volume calculation
E	Copy	S	Select an area to draw	GG	Open spreadsheet
F	Paste	T	Spectrum view	HH	Open BP curve
G	Print	U	Cursor tool	II	Quantification setting
H	Zoom out	V	Lane selection tool	JJ	Preferences
I	Zoom in	W	Multiple lane selection tool	KK	Multi-band color separation
J	Magnification Input	X	Lane alignment	LL	Show/hide lanes
K	Zooming tool	Y	Automatic lane fitting	MM	Show/hide band information
L	Fit window	Z	Lane template	NN	Show/hide comment
M	Moving tool	AA	Automatic band detection	OO	Lane style
N	Gray level adjustment	BB	Band edit	PP	Band style

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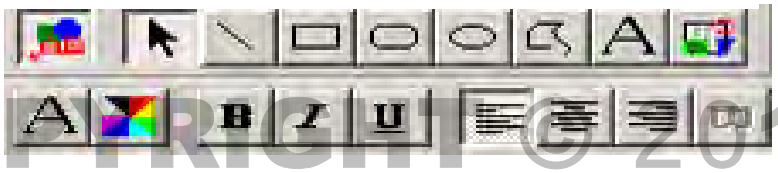

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1	Drawing tool	7	Polygon tool	13	Italics
2	Select tool	8	Text tool	14	Underline
3	Line tool	9	Paper position	15	Left justified
4	Rectangle tool	10	Font	16	Center
5	Ellipse tool	11	Text color	17	Right justified
6	Oval tool	12	Bold	18	Rotate text

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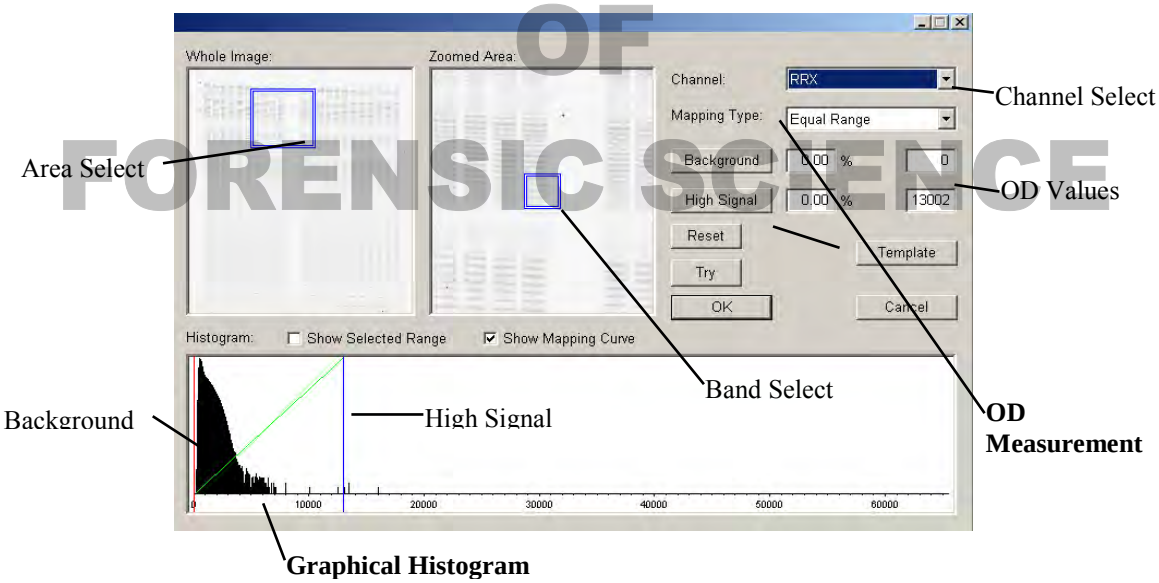
5.6 Adjust the gray level for each of the images by selecting the mono image for each individual channel, click on the "Gray level Adjustment" icon or “Gray Level Adjustment…” from the “Image” menu.



5.7 There are three different ways to adjust the gray scale values. The OD Measurement or the Graphical Histogram may be used, or the OD Values may be manually adjusted.

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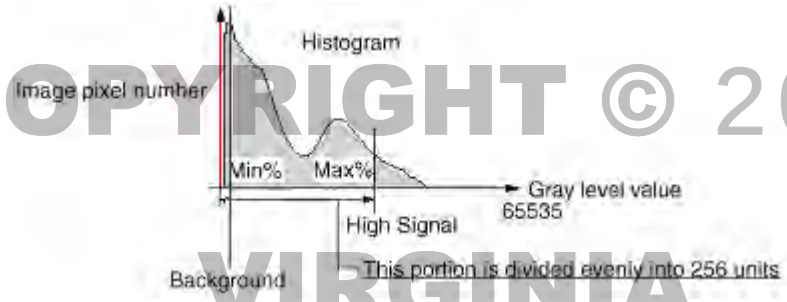
5.7.1 To make adjustments by using the OD Measurement, (the blue box in the zoomed area actually takes the OD measurement) highlight the “Background OD Value” box. The blue box in the Whole Image view window is used to reduce or enlarge the Zoomed Area view window. Use this box to control the size of the area zoomed by moving the borders of the blue box with the mouse, the area can also be moved by clicking and dragging the center of the box to a different area of the gel. Next, position the blue box in the Zoomed Area view window over a light region to designate background and click the “Background OD Measurement” button. All regions of the image with pixel intensity values below the value of this selected area will display as white. Now position the blue box in the Zoomed Area view window over a band to designate the signal and click the “High Signal OD Measurement” button.

5.7.2 To make adjustments using the Graphical Histogram, click and drag the red “Background Signal Slide Bar” to set the Background Signal OD Value. It is usually set at the peak of the

histogram. Now click and drag the blue "High Signal Slide Bar" to set the High Signal OD Values. It is usually set at a region where the slope of the signal on the histogram tapers off.



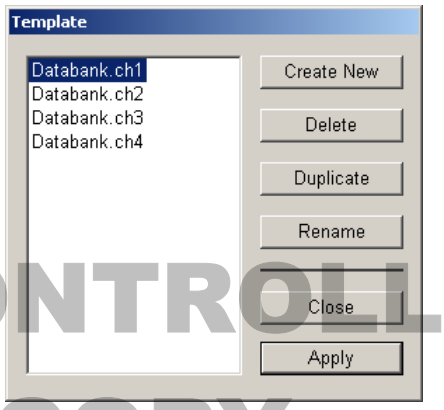
5.7.3 Manual changes to the Background and High Signal OD Values can also be made by clicking on the OD Value boxes and making the numeric adjustment.

5.8 Each channel that needs adjustment must be selected from the "Channel Select" drop down button. The previewed images, histogram and OD values are updated within the window for each channel. Once the desired gray levels have been selected, record the gray level values in the format as depicted in section 9 of this chapter.

NOTE: Click "Try" and the changes in the background or signal levels will be reflected in the main image window. Increasing the difference in values between the background and signal will I ncrease the resolution of the image by increasing the number of shades of gray displayed.

5.9 When the background and signal levels are set at the desired intensities in the viewing window, click "OK". The entire image will be recalculated with the values selected in the high and low limit levels. All four colors of the matrix sample should be clearly visible in the "Over" colored image.

NOTE: Preset gray level values can be used to set 'typical' gray levels for gel images. Click the "template" button and either create a new template or retrieve a previously saved template. To use the template, highlight the desired file and click "apply".



5.10 These un-separated images must be printed before color separation adjustments are made. Click on the "Display Select" drop down menu and select "Mono" in the Image Setting box. The check-marked channel will appear in black and white.

5.11 Before printing, text can be added to the images. Click on the "drawing tool" in the toolbar to activate the text functions. Then click on the "text tool" and use the mouse to draw a box where the text is to be positioned. Click inside the box to begin typing.

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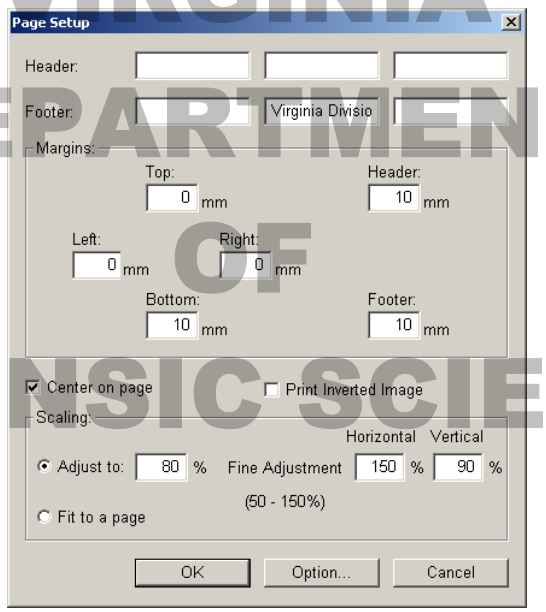
NOTE: The "drawing tool" button acts as a toggle switch. If the "drawing tool" button is activated, then none of the "1D gel" functions can be accessed and vice versa

5.12 The font can be adjusted by using the "font tool". The "font tool" is available on the "text attribute toolbar". If this toolbar is not showing, go under "view" and select it. The box should also be made transparent so it does not obscure the image behind it. To do this, highlight the entire box, then go under "Draw", then "paint" then select "transparency". To delete the lines around the text box, go under "Draw", then "line", then "type", and select "null".

5.13 To print the scanned images, under the "File" menu, select "Print Setup…" The scaling, margins, header and footer settings can be changed to accommodate a variety of image sizes. The recommended settings are depicted in the following image.



5.14 Use "Print Preview" under the "File" menu to ensure the printout is appropriately sized. Print each image by selecting it with a check mark in the "Image Setting" box and selecting "Print" from the "File" menu.

5.15 Select "Save Project" from the "file" menu and save the project in the same folder that the multi-layer TIFF file is located.

5.16 Color separation is required to remove/reduce the effects of signal bleed-through that is inherent with 4-color fluorescent image detection. There are two different ways to achieve color separation of the multi-layer TIFF image. They are Multi Band and Manual Measured color separation.

5.17 The multi band method requires first that an analysis lane be auto-banded to select the bands for measurement. This is an effective use of the matrix sample, although other lanes of the gel may be used as well.

NOTE: When the matrix sample is used, it is best that the gray scale values have been adjusted such that all 4 colors are represented when viewed in the 'over' mode.

5.17.1 To create a lane, Click on the "Lane Selection Tool" icon, and place the cursor on the upper left-hand corner of one lane, click and drag the cursor to the bottom right-hand corner of the lane and release the mouse button to finish the selection.

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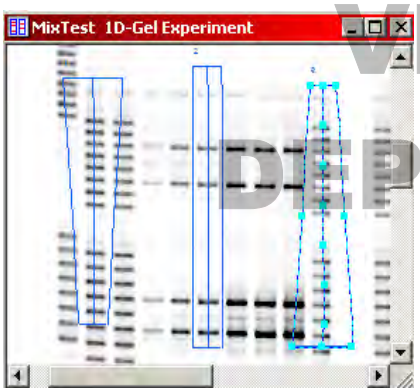
5.17.2 Click on the cursor icon to select and make adjustments to the lane. The small "Move Lane" icon is displayed when the cursor is placed on the lane. This moves the entire lane in place. The enlarged "Move Lane" icon is used to move the lane at the curve points or handles (small boxes located along the lane lines). The width of the lane may also be adjusted by clicking on the handles on the outer lane line borders.



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5.17.3 Now click on the "Automatic Band Detection" icon and allow the software to automatically find and mark the bands. Refer to 5.28 to adjust the Automatic Band Detection Parameters.

5.17.4 Select "Mono" on the image setting dialog box to view the black and white image and edit the bands which have been automatically placed by either double clicking on the lane or clicking on the band edit icon while the lane is selected.

5.17.5 The band edit tool can be used to add or delete existing bands by placing it over the desired location and selecting the band for deletion and pressing the small delete key on the keyboard.

5.17.6 Bands can be placed by clicking on the desired location, then clicking and dragging down to move the lower volume line then clicking again and dragging up to move the upper volume line. Failure to accurately move these volume lines will adversely affect the way which the optical density (OD) of the band is calculated.

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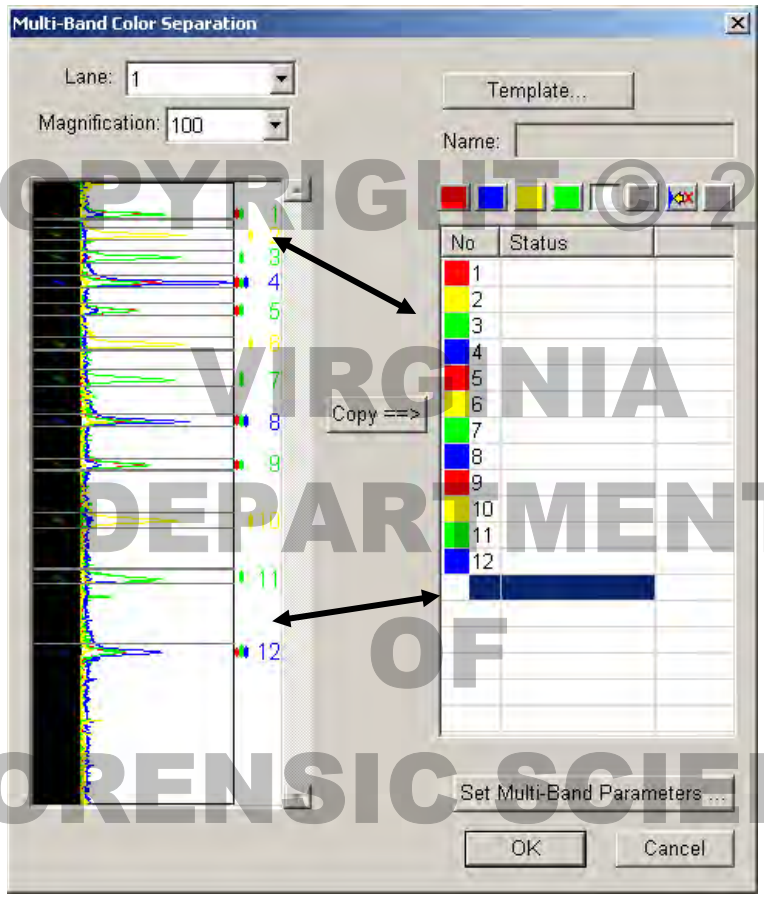
NOTE: If using the Matrix sample, ensure that all the bands which are visualized on the mono image have bands placed on their location.

5.17.7 Click on the "Multi Band Color Separation" icon and the following window appears:

NOTE: Colors represented on the gel may not be in the same order as they appear in the "Multi-Band Color Separation" preview window.



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5.17.8 All bands present in the lane will appear both as bands and peaks in the Band/Lane Trace window. Ensure that the correct twelve bands are banded then click on "Copy". Click "OK" when it asks "Do you want to copy the lanes…?" The bands will be represented as colored boxes in the Color Change box. These boxes can be changed to any of the colors represented and should reflect the known color of the band. To change the color of the box, highlight the incorrect color box and then click on the correct color from the color bar above. The Matrix should contain 12 bands total and will appear in the order; Red, Yellow, Green, Blue, Red, Yellow, Green, Blue, etc.

5.17.9 Once the bands have been set, click on "OK", the color separation values will be reflected in the main color separation window.

5.17.10 Further adjustments can be made by changing the "Target Image Overlap %" values as described in the manual measured color separation method. See 4.18.5 for starting adjustment values.

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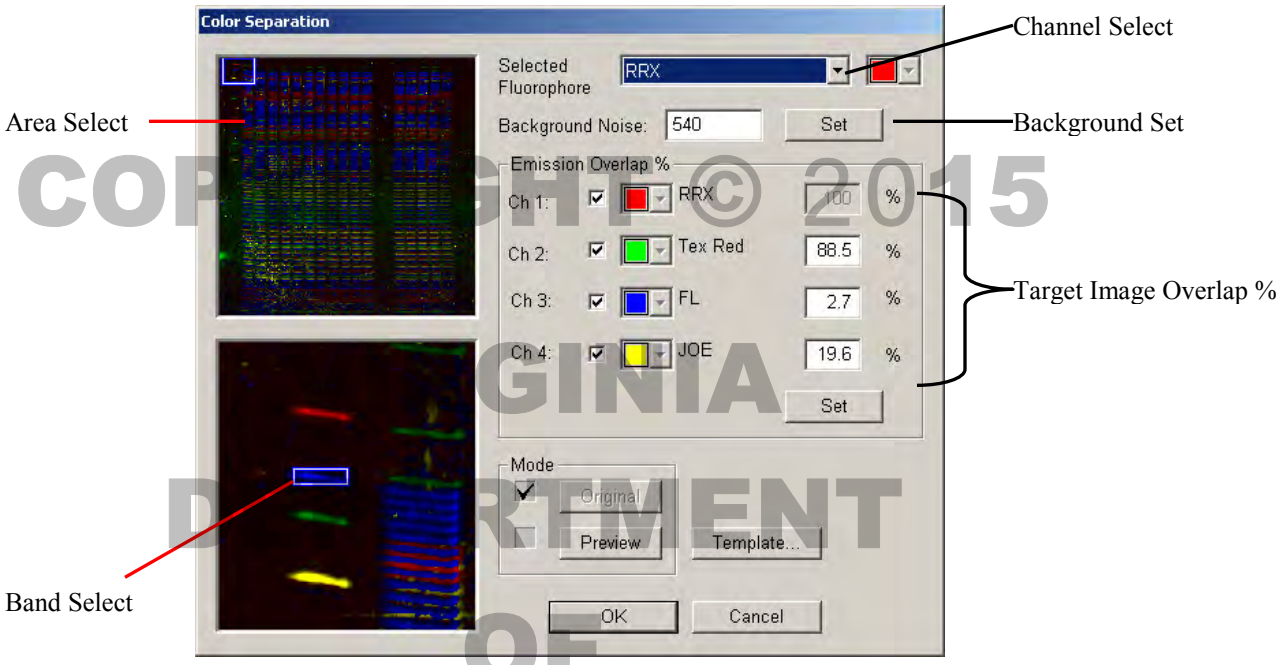

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5.18 The Manual Measured color separation is performed by selecting bands that represent each channel then measuring the amount of bleed-through. Ensure that the 'Over' mode is selected. Then to perform the Manual Measured color separation, select "Color Separation" from the "Image" menu or click on the "Color Separation" icon.

5.18.1 The "Area Select" view window is used to identify the portion of the gel to select bands. The blue box in the "Area Select" view window is used to reduce or enlarge the "Band Select" view window. Use this box to control the size of the area zoomed by moving the borders of the blue box with the mouse, the area can also be moved by clicking and dragging the center of the box to a different area of the gel.

5.18.2 Position the blue box in the "Band Select" view window over an area of background where no bands are observed. Then click on the "Background Set" button. This sets the background value for the channel displayed in the "Channel Select" drop down box. Select each of the other channels and click on the "Background Set" until all channels have had the background set.

5.18.3 Make the necessary adjustments with the "Area Select" view window and the "Band Select" cursor to select a red band. Ensure that channel 1 is selected in the "Channel Select" box and click on the Target Image Overlap % Set button. Repeat this for each colored band/channel combination, ensuring that the band selected is the same color as the "Channel Select" box color.

NOTE: The channels listed in the "Emission Overlap %" boxes represent the amount of signal that the selected fluorophore overlaps (bleeds-through) to the listed channels. Increasing the percentage number for one of these channels will increase the amount that the selected fluorophore is color separated from that particular channel. A percentage value of "0" means that there is no measured overlap of the selected fluorophore into that channel.

5.18.4 Follow these general guidelines for PowerPlex® 16 BIO gels:

Selected Fluorophore	Change to Emission Overlap %	
	FMBIO® II	FMBIO® III Plus
RRX	Increase JOE by 30-40% points	Increase JOE by 10-20% points
RRX	Increase Tex Red by 30-40% points	Increase Tex Red by 10-20% points
JOE	Increase RRX by 30-40% points	Increase RRX by 10-20% points
JOE	Decrease Tex Red to 0% points	Decrease Tex Red to 0% points
JOE	Decrease FL to 0-4% points	Decrease FL to 0-4% points
FL	Decrease Tex Red to 0% points	Decrease Tex Red to 0% points

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5.18.5 Click "OK" to perform the color separation. Ensure that there is no bleed-through seen from other channels in each channel of the gel and that over-separation has not occurred. If changes need to be made, uncheck the "Show color-separated images" box in the "Image Setting" dialog box and click on the "Color Separation" icon or select it from the Image menu.

5.18.6 Make adjustments to the Emission Overlap % values for each channel (increase or decrease) based on the effectiveness of the measured values on the color separation. Once the adjustments are made, click on "OK" to perform color separation.

NOTE: Once color separation parameters have been established, these values can be saved as a template and used in future color separations by clicking "Template…" Select the preset parameter from the list and click on "Apply".

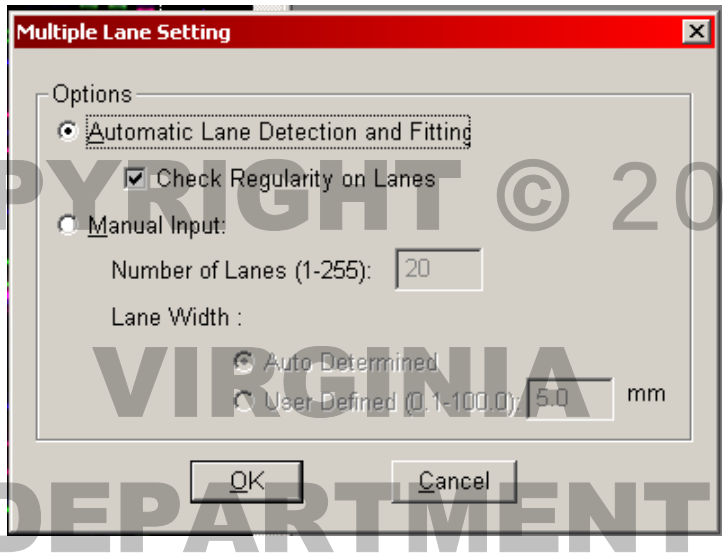
5.19 After color separation, it may be necessary to fine-tune the gray-scale for each channel. Once the desired gray levels have been selected, record the gray level values in the format as depicted in section 9 of this chapter.

5.20 Select "Save Project" from the "file" menu and save the project. Do this often so as to prevent loss of data in the event of a computer crash.

5.21 Next the lane and bands need to be placed on each analyst's project file.

5.21.1 Set the upper and lower migration lines to define the migration area of the gel. These migration lines will be seen at the top and bottom of the scanned image. Position the upper migration line just above the 475 bp ILS fragment, and the lower limit line just below the 100 bp ILS fragment. Adjust for angle deviation as required. To do this, hover the cursor over the migration line and a small four-sided arrow appears. Click on the line and drag up or down to the desired position. Notice the enlarged boxes or handles at each end of the migration limit lines. Hover the cursor over one of these boxes and notice that an enlarged four-sided arrow appears. As long as the enlarged arrow is showing, changes to the angle of the migration limit line with respect to the migration direction of the axis of the gel can be made with any of the four boxes or handles. Use this function only for gels that are misaligned.

5.21.2 Select the "Multiple Lane Selection" tool from the "Tool" menu in the "1D Gel" menu, or click on the "Multiple Lane Selection" tool icon. Position the "Multiple Lane" icon above the upper migration limit and at the upper left-hand corner of the first lane (do not include the matrix lane). Click and drag the cursor to the bottom right-hand corner of the last lane to finish the selection and release the mouse button. A dialog box will appear. You can choose Automatic Lane Detection or enter the number of lanes manually. If you enter the number of lanes manually, you may also wish to specify the lane width. Then click "OK".



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5.21.3 All lanes will be marked with lane lines as well as lane numbers at the top of each lane. Use the "Lane Style" icon to toggle the lane lines "off"; leaving only the lane numbers visible. The separated images with the desired headings must be printed to reflect the allele calls that are being made.

5.22 Once lane creation is complete, create the 2ND sizing file. Select "Save Project As…" from the "file" menu and save project to the appropriate destination file adding "2ND" to the file name.

NOTE: Failure to add "2ND" to the file name could result in future overwriting problems and possible loss of sizing data.

5.23 Select "Close" from the "File" menu to close the "2ND" file. Next, re-open the saved project by selecting "Open Project…" from the "File" menu.

5.24 Printing the separated images at this time allows the lane numbers to be automatically inserted onto the images. Click on the "Display Select" drop down menu and select "Mono" in the Image Setting box.

5.25 The check-marked channel will appear in black and white. Make any changes to the text at this time.

5.26 To print the separated images, under the "File" menu, select "Print Setup…" and ensure that the settings are the same as in the following image.

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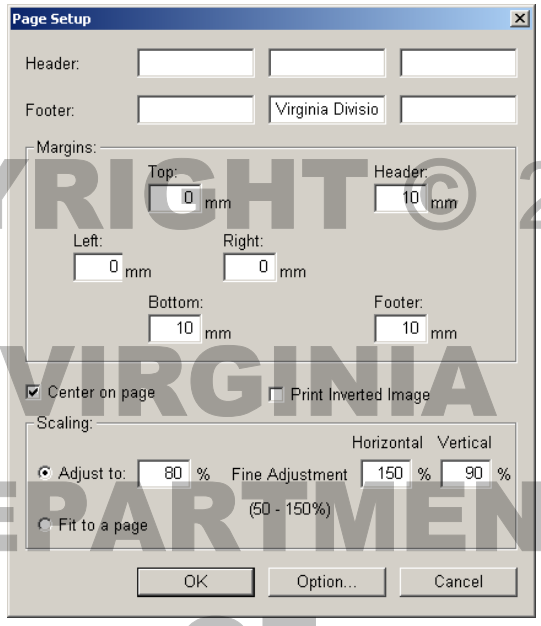

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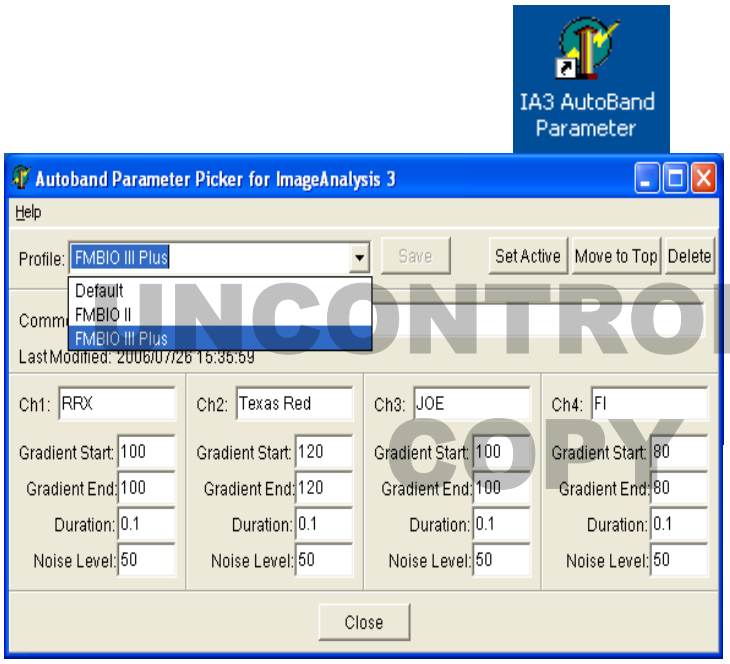
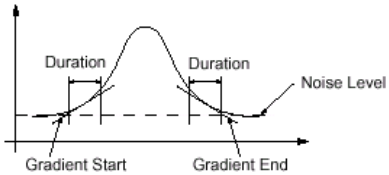
5.27 Use "Print Preview" under the "File" menu to ensure the printout is appropriately sized. Print each image by selecting it with a check mark in the "Image Setting" box and selecting "Print" from the "File" menu.

NOTE: Band recognition in the FMBIO® system depends on values derived from calculation of the linear differential coefficient of the spectrum curve. Whether a band will be recognized as standing out from the background depends on four parameters: gradient start, gradient end, duration, and noise level.

5.28 The auto band parameters are modified through an input program separate from Image Analysis. Open the IA3 AutoBand Parameter program by selecting if from the desktop.





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5.28.1 **GRADIENT START**: The gradient start determines the signal intensity in the "leading edge" of the band. The parameter is the vertical component of the leading edge, which together with the duration parameter defines the slope of the function.

5.28.2 **GRADIENT END:** The gradient end determines the intensity in the "trailing edge" of the band. The parameter is the vertical component of the function, which together with the duration parameter defines the slope of the function.

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5.28.3 **DURATION:** The duration is used in combination with the noise level to determine how much distance can exist between bands which are detected.

5.28.4 **NOISE Level:** The noise level is used in combination with the duration to determine when to finally stop detecting a single band and look for another one. That condition is set when a signal drops below noise level or the "gradient end" or the signal value at the starting position.

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Parameter	Suggested Starting Value	
	FMBIO® II	FMBIO® III Plus
Gradient Start	1-100	100-120
Gradient End	1-100	100-120
Duration	0.1	0.1
Noise	50	50

NOTE: The preset auto-band parameter values for the system can be saved as a template by clicking in the text/drop down box "Profile:" which contains the default settings. Select the preset parameter from the list and click on "Set Active". Alternatively, new templates can be saved by renaming the text/drop down box and c licking on "Save". Then click on "Set Active" to set the auto band parameters for the gel being analyzed. Click on "Close" when finished modifying the auto-band parameters.

5.28.5 Return to the Image Analysis Preferences window. Now click on the "Calculation" tab and ensure that the OD calculation type is Average Pixel Intensity.

5.28.6 Now click on the "Band Information" tab and ensure that only "OD" and "bp" are selected in the "Variables in spreadsheet" section. This is important only with gels greater than 25 lanes.

NOTE: Preset "Band information" values can be used. Click the "template" button and either create a new template or retrieve a previously saved template. To use the template, highlight the desired file and click "apply".

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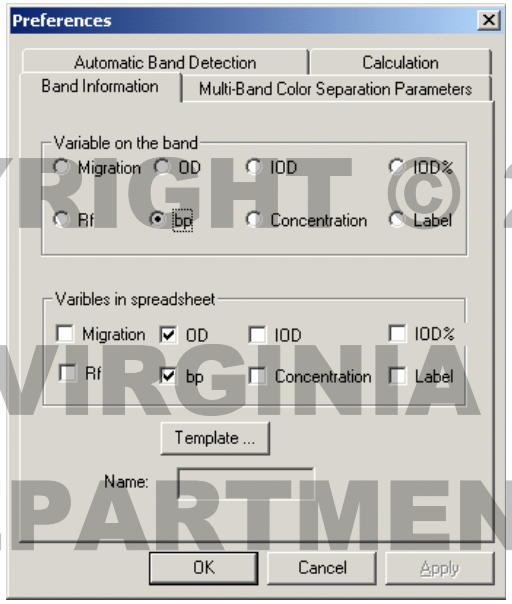

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5.28.7 Click "OK" when the parameters have been set.

5.28.8 Activate all the lane lines by pressing the **"Ctrl" and "A"** key simultaneously or use the "Edit" menu and then "Select All". All lane lines must be activated in order for the Image Analysis software to recognize the bands in the combined image.

5.28.9 Now click on the "Automatic Band Detection" icon and allow the software to automatically find and mark the bands.

5.28.10 Once the bands have been marked, click the "Lane Style" icon to toggle on the preferred lane view style.

5.28.11 Select "Save Project" from the "file" menu and save the project. Do this often so as to prevent loss of data in the event of a computer crash.

5.28.12 To determine if all bands have been selected for a particular channel, select the desired channel in the "Image Setting" dialog box. Check each channels band placement.

5.28.13 The bands can be edited by either selecting the lane by clicking on the lane, then clicking on the "Band Edit" icon, or alternatively select the lane by double clicking on it. Then click on the desired band to delete the band or click onto an area of the lane to add a band as described in 5.18.5.

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NOTE: There is no automated computer assistance with manual band placement. Proper placement of the band is critical. It may be more beneficial to delete the bands on this lane and change the auto-band parameters then redo the auto-band placement manually.

5.28.14 The volume calculation bands (yellow bands) should be displayed above and below each band. If these bands are not located in the proximity of the allelic band, delete all the bands by pressing the **"Ctrl"** and **"A"** keys together, then the "Delete Band" icon. This deletes the bands in all channels. Once the bands have been cleared, adjust the gradient start and end as described in 5.29. Activate the lane line again or all the lanes by pressing the **"Ctrl"** and **"A"** key then press the "Automatic Band Detection" icon.

5.28.15 Using the cursor tool, edit multiple bands by drawing a box around the band(s) to be deleted. Be cautious to not select a lane in this process. Press the Delete key and click "OK" to delete

the selected bands. This function can be used to delete incorrectly recognized bands, such as spots or marks on the image.

5.28.16 An advanced method for band editing involves the use of the Overlay Trace. To use this, click on the "New Overlay Trace" icon. All lanes of the gel are displayed in columns. The bands also appear for each channel.

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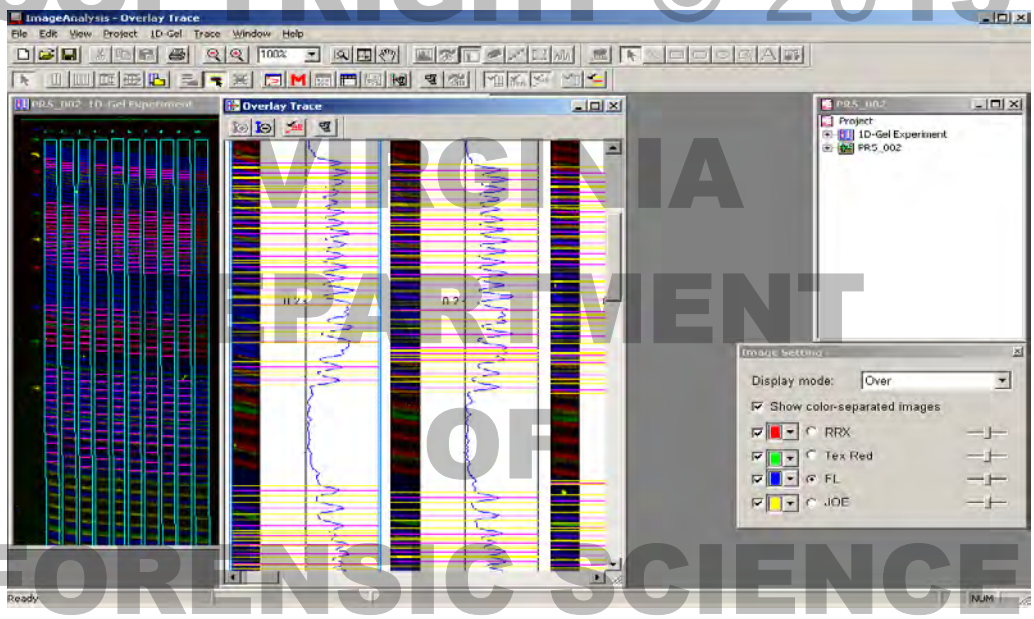

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5.28.17 The channel display and band display can be changed by clicking on the "Preferences" icon in the Lane Trace window.

5.28.18 The size of the lane trace may be extended or reduced by clicking on the (+) and (-) icons located in the overlay trace window. The size of the entire lane trace can be altered by making adjustment to the main zoom in/zoom out tools.

NOTE: Do not change the colors in the image setting box. The only change that can be made is to display mono instead of overlay display to preview the grayscale of the gel lanes being evaluated.

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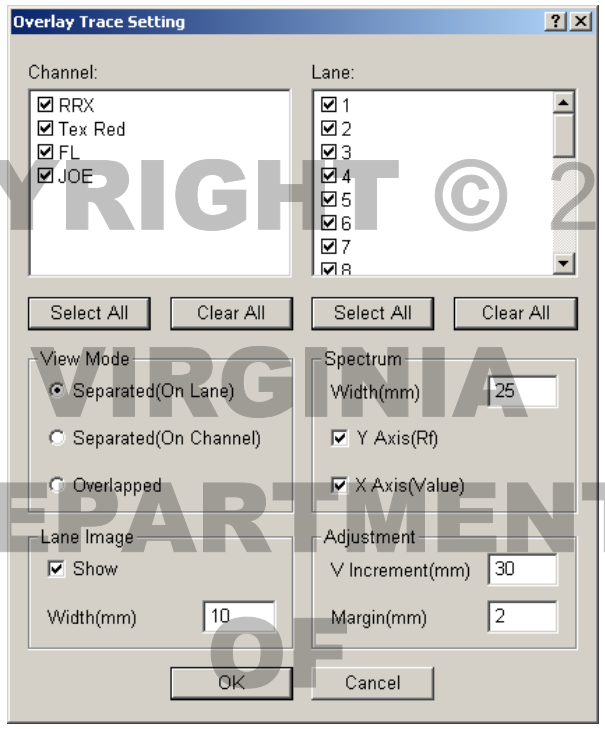

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5.28.19 Each channel, when selected separately is viewed in the Lane Trace window. The bands corresponding to the lane trace are selected from the black dots on the "Image Setting" dialog box.

5.28.20 The bands are edited by highlighting the lane, or double clicking the lane. Using the band edit cursor, click on the band to move. The yellow volume lines as well as the magenta band line can each be moved to be aligned to the optimal point of the bands peak intensity.

NOTE: It is best to make adjustments to a single channel at a time, exiting from the lane trace window and re-opening the lane trace window before selecting each different channel.

5.28.21 Select "Save Project" from the "file" menu and save project numerous times during this process.

5.28.22 Once all the bands are in their desired locations, the Internal Lane Standard needs to be set.

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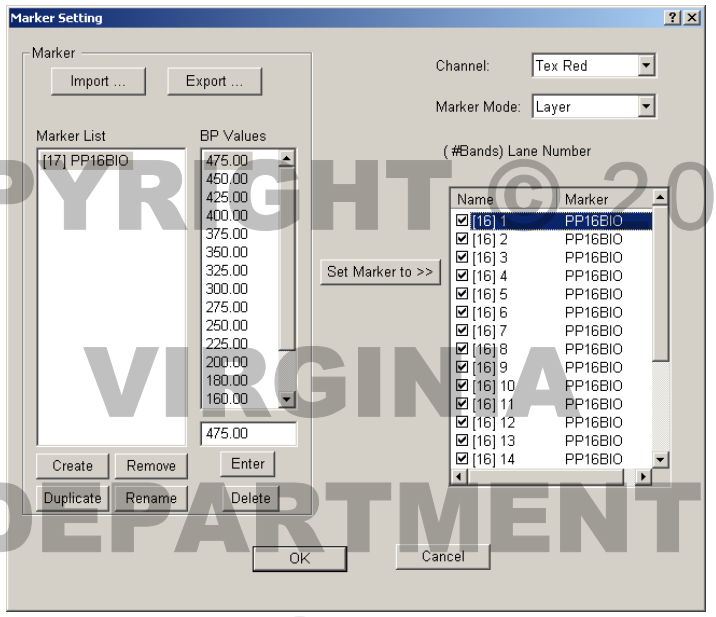

5.28.23 Click on the "Set Marker" icon. The "Marker Setting" window appears. Select the channel containing the internal lane standard (Tex Red) in the channel drop down box. Ensure that the "Marker Mode" drop down box reads "Layer". Highlight the appropriate marker from the Marker List (PP16BIO for PowerPlex® 16 BIO) and then click on the lane to apply the marker. Multiple lanes may be checked by checking the first lane, then holding down the **Shift key**, scroll to the bottom of the lane list and click the last lane. Click on the "Set Marker to>>" button, the highlighted lanes will use the marker as the internal lane standard. When finished, click "OK".

5.28.24 Click the "Volume Calculation" icon or select "Volume Calculation" located under the "1D-Gel" menu. The marked lanes will be automatically compared to the internal lane standard and the base pair size will be assigned to each band.

5.28.25 To display the quantitative values for the base pairs (bp) derived from the migration data for each separate gel image select "Open Spreadsheet" from the "1D-Gel" menu or click on the "Open Spreadsheet" icon.

5.28.26 To prepare the data for analysis with the STaRCall™ software, each channel of the spreadsheet must be saved in a separate file. Select the tab at the bottom of the sheet for each channel to be analyzed and select "Export…" from the "Spreadsheet" menu. Save each file as separate filenames.

IMPORTANT: Name each gel image spreadsheet separately (Example: 203-11-426RRX or 203-11-426JOE).

5.28.27 Once the spreadsheets for each of the images of the combined image file have been assigned a name and saved, the spreadsheet data can be opened in the Excel software program for allele designation. Close the image and save the newly created file. Then quit Image Analysis by selecting "Exit" under the File menu. Proceed to Section 6, DETERMINATION OF ALLELE DESIGNATIONS USING THE MICROSOFT EXCEL SOFTWARE PROGRAM.

6 Determination of Allele Designations Using the Microsoft Excel Software Program

6.1 Open the EXCEL program using the desktop icon.

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6.2 If the STR OD/IOD CUTOFF TABLE does not contain the following values for the appropriate STR system, change the cutoff values to those specified in the appropriate table.

6.3 This can be checked by selecting "STR Lookup Table…" from the "Star Call" menu.

6.4 Select the appropriate table and click "edit".

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6.5 Make the changes in the correct location and select "Save" from the "File" menu.

PowerPlex® 16 BIO

	Cutoff (%)			Cutoff (%)			Cutoff (%)	
Locus	**From**	**To**	**Locus**	**From**	**To**	**Locus**	**From**	**To**
FGA	0	13	Penta E	0	2	Penta D	0	2
TPOX	0	11	D18S51	0	13	CSF1PO	0	13
D8S1179	0	12	D21S11	0	15	D16S539	0	13
vWA	0	16	TH01	0	5	D7S820	0	12
			D3S1358	0	16	D13S317	0	11
						D5S818	0	13

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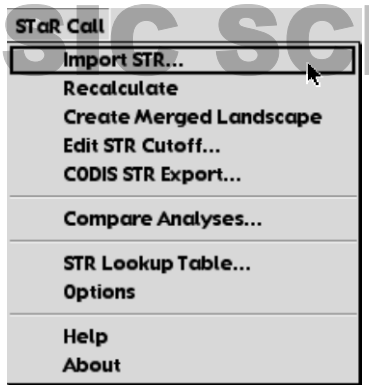
6.6 Under the "STaRCall" menu choose "Import STR…"



6.7 Select the desired files by clicking on "Browse". Find each of the related data files and select "Open". (Example: 022900ASR-RRX and 022900ASR-JOE). Also, select the appropriate look-up tables, which will be used to evaluate the data in these files. Ensure that the "STR with OD values" button is selected and then click on "OK".

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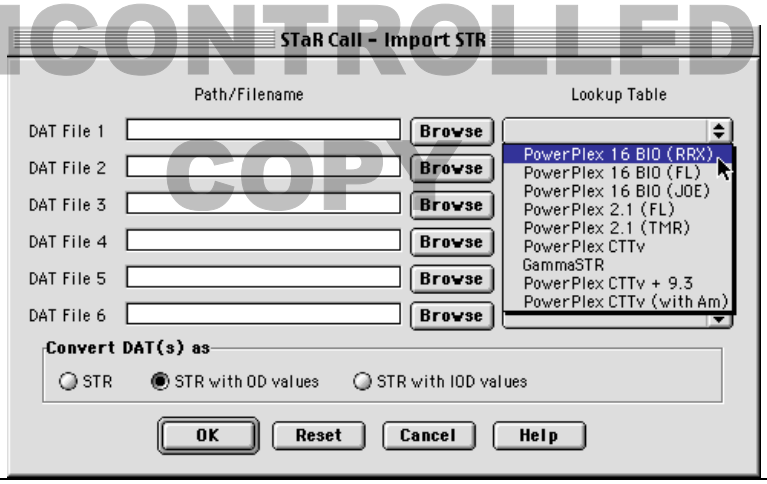
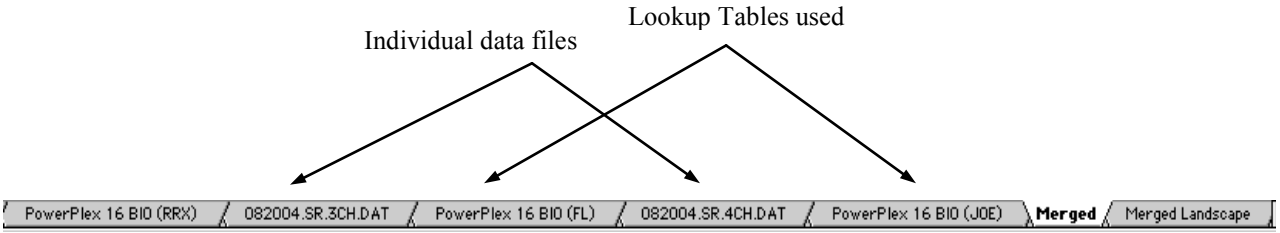


6.8 The allelic ladder lanes of the first data file should be highlighted, as in the following diagram. These lanes will be used as allelic ladders in all subsequent data files selected in the Import STR window. Select "OK". If the allelic ladder lanes are not highlighted click the "Auto Select" button, then select "OK".

6.9 The STaRCall™ program creates a workbook, which details the evaluation of the data files. It contains an Analysis Summary sheet, a Merged sheet, a Landscape sheet, as well as the converted individual data files and the associated lookup tables. This workbook must be saved.



6.10 Under the "File" menu, select "Save". Save the workbook as a "Microsoft Excel Workbook" in the appropriate folder either on the file hard drive. For example, 022900-SR includes all the data needed in this analysis.

6.11 Proceed to step 6.12 for instruction on how to label the individual lanes (this step is conducted by the examiner/analyst). IF AN INDEPENDENT ANALYST IS CONDUCTING THE STaRCall™ ANALYSIS, PROCEED TO 6.13.

6.12 Specimen information must be entered for each sample. This may be accomplished by manual entry or through the use of "CODIS STR Export…".

6.12.1 A screen will appear labeled "STaRCall - CODIS STR Header Information".

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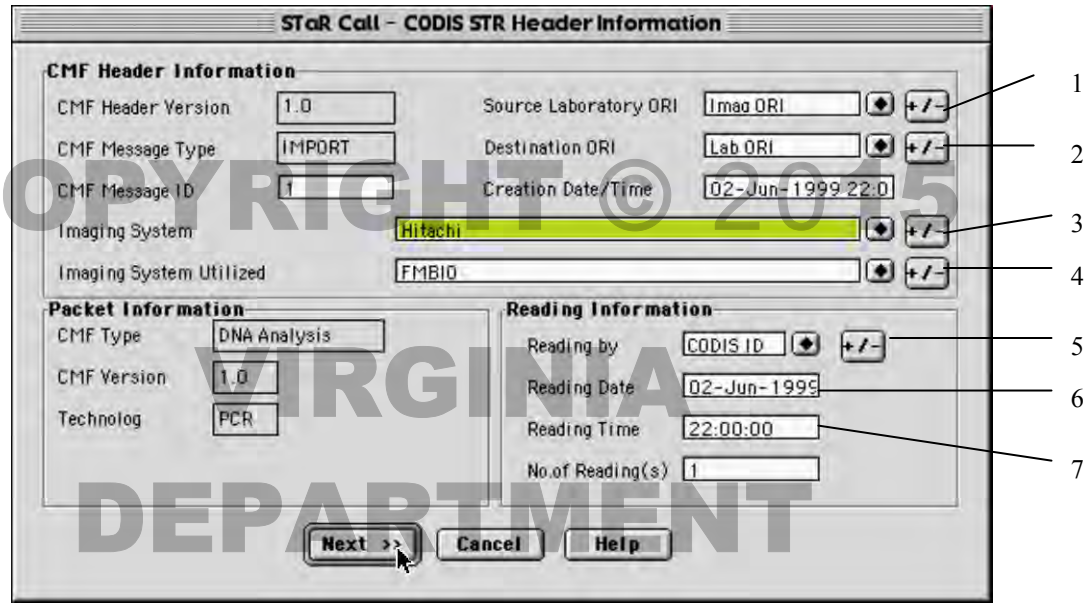

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Number	Feature Name	Description
1	Source Laboratory ORI	Laboratory ORI –see CODIS Administrator
2	Destination ORI	Laboratory ORI – See CODIS Administrator
3	Imaging System	Manufacturer of system (Hitachi)
4	System Utilized	Type of scanning instrument (FMBIO®)
5	Reading by	Analyst's CODIS user identification
6	Reading Date	Date of gel scan/ analysis
7	Reading Time	Time gel was scanned
**	+ / - Button	Creates or deletes items from the pull down menu

6.12.2 Click on the "Next" button and the "STaRCall-CODIS STR Specimen Information" screen will appear.

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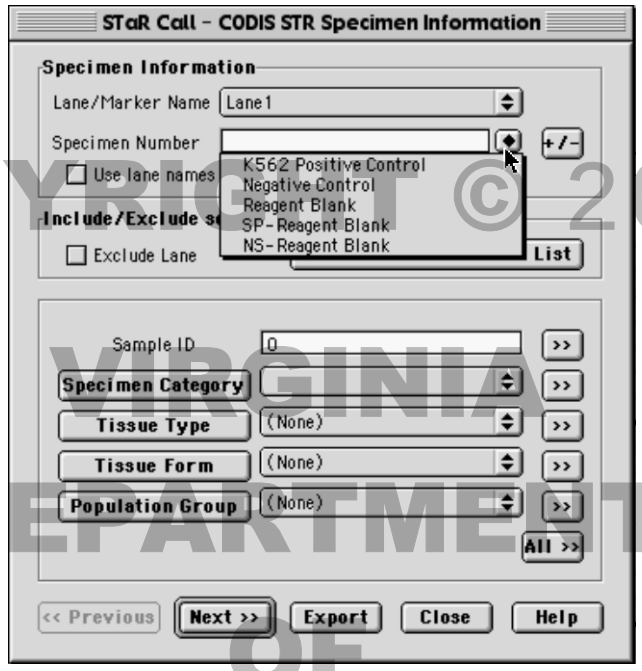

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6.12.3 Enter the sample identifier (Example: W98-12345V, 98-12345, or Victim/Suspect, etc. into the "Specimen Number" field. For commonly used lane identifiers, such as controls, the pull down menu may be used.

NOTE: If importing the specimen into CODIS, the specimen identification should be the same nomenclature discussed in the CODIS OPERATING POLICIES AND PROCEDURES MANUAL). If the specimen will be imported into a table for use in an Appendix associated with the Certificate of Analysis containing the typing results, the specimen must have enough distinguishing information to identify it in the chart.

6.12.4 Using the pull down menus next to the Specimen Category, highlight the appropriate category. If the sample identifier is a control sample, a blank lane, or Random Sample, use “OTHER” as the specimen category and skip to 6.12.6

6.12.5 Using the pull down menus next to the Tissue Type, Tissue Form, and Population Group, highlight the appropriate category.

6.12.6 After all categories have been identified, click "Next". The “STaRCall - CODIS STR Specimen Information” screen will move to the next sample lane. Repeat steps 6.12.3 through 6.12.5 until all sample lanes have been provided with specimen identification.

NOTE: If the specimen has already been analyzed by another system and the specimen information already exists in CODIS, the specimen information must be identical.

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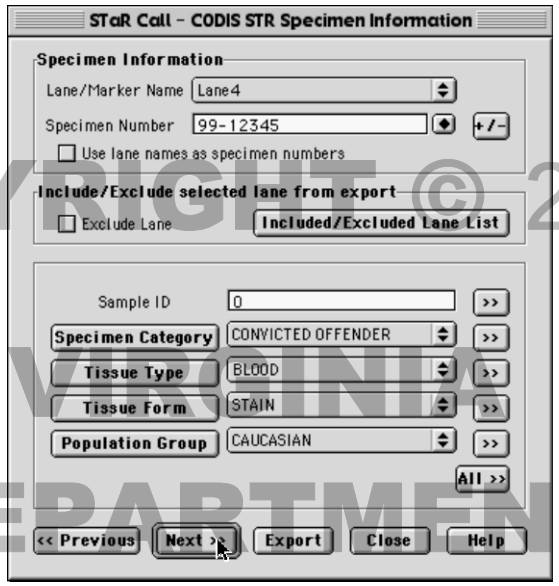

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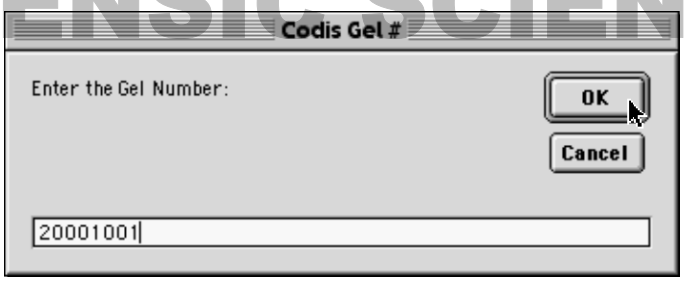
6.12.7 Once all sample lanes have been assigned specimen identification, close the "STaRCall – CODIS STR Specimen Information" screen by clicking on "Close".

6.13 If a gel number needs to be assigned to the specimens being exported to CODIS, under the "STR Setup" menu, select "Gel Number". When prompted, enter the gel number and click "OK".



6.14 If a microvariant is detected during analysis, an allele may be called as "Out of Range" by the STaRCall™ software. To see if this allele is a microvariant, select the base pair value for it in the individual data worksheets. Then single click on the M icon located on the toolbar. This will evaluate the base pair to see if it is a microvariant, assign cut off values for adjacent microvariants and report those values in the appropriate look up table for each microvariant reported.

NOTE: The M icon will not work when allelic ladder bands are greater then 4 bps apart (i.e., Penta D and E)

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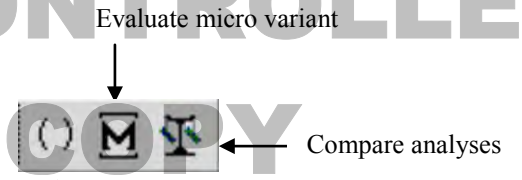
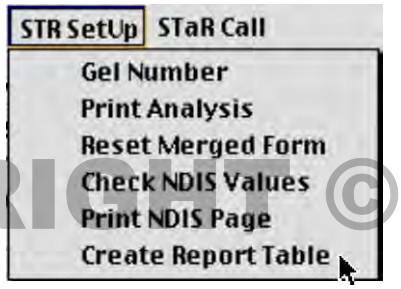


6.15 To place or remove parentheses on the alleles, select the "Merged Landscape" worksheet, highlight the allele and single click on the ( ) icon. Alternatively, alleles may be parenthesized on the Merged worksheet, and then selecting 'create merged landscape' from the "STaRCall" menu will update the changes on the "Merged Landscape" worksheet.

6.16 After the analyst has checked all data for accuracy, the entire analysis can be printed. Under the "STR Setup" menu, select "Print Analysis".



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6.17 When prompted enter the analyst's initials, the gel number/case number associated with the data, and the date analysis was conducted-, then click "OK".

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6.18 When the Custom Print Box asks, "Do you want to continue?" Click "Yes" if all information entered is correct.

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NOTE: Two separate landscape sheets may be printed for PowerPlex® 16 BIO data. Choose "Yes" when prompted. Choosing "No" will print the single landscape page.

6.19 If reprinting is required, (i.e. changes are made due to microvariants, stutter, questions marks, etc), repeat steps 6.16-6.18 and select "No" to format the workbook without printing. If reprinting of individual worksheets of the analysis is required, select the desired worksheet from the tab bar at the bottom of the workbook. Then under the file menu, select "Print…"

OF

6.20 Once the analysis has been completed (including microvariant calls) and all pages printed, select "Check NDIS Values" from the "STR SetUp" menu. All data values will be corrected for uploading to NDIS. Also, an "Archived Merged Landscape" sheet will be created which has all of the actual/original allele values.

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6.21 The NDIS corrected landscape can be printed by selecting "Print NDIS Landscape" from the "STR SetUp" menu.

6.22 If the analyst will be exporting data to CODIS, all controls, blank lanes, and failed sample lanes, need to be excluded. Under the "STaRCall" menu, select "CODIS STR Export…"

6.22.1 A screen will appear labeled "STaRCall - CODIS STR Header Information". Click on the "Next" button and the "STaRCall-CODIS STR Specimen Information" screen will appear.

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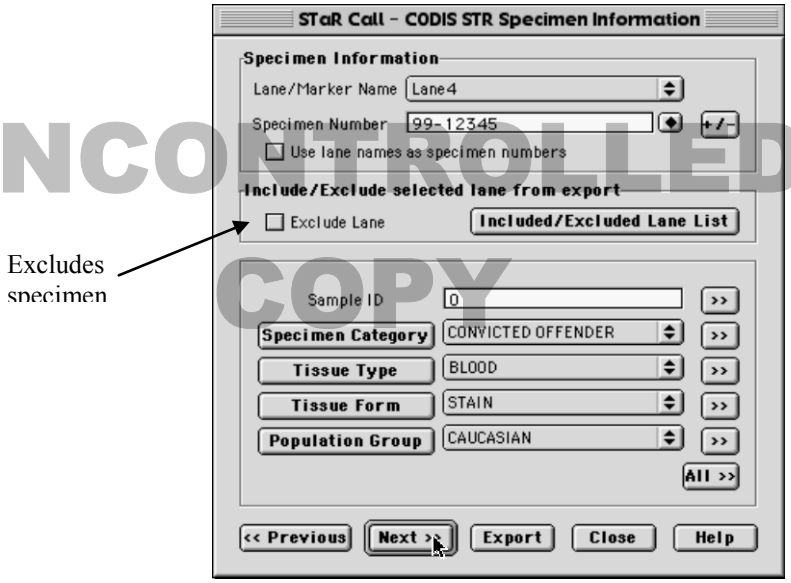

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6.22.2 If sample identifier is a control sample blank lane, or failed sample, click on the "Exclude" box to prevent this lane from being exported into CODIS.

6.22.3 Click "Next" and repeat 6.22.2 for each lane of the gel that is to be excluded. Alternatively click on “Included/Excluded Lane List” and highlight the lanes to be excluded.

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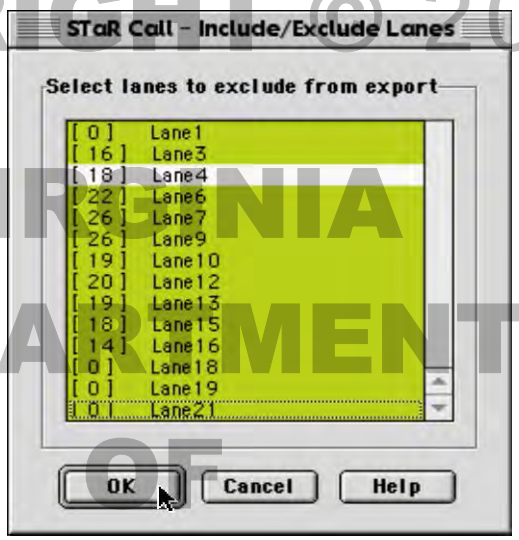

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6.22.4 When finished, click "Close" to close the “STaRCall - CODIS STR Specimen Information” screen.

6.23 If no further analysis will be performed, under the "File" menu, select "Save", then close the merged file.

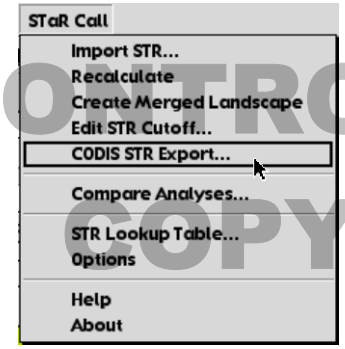
6.24 Under the "File" menu, select "Exit", to quit using Microsoft Excel.

7 Exporting STaRCalll™ Data into CODIS

7.1 Open the EXCEL program using the desktop icon.

7.2 Under the "File" menu, select "Open". Choose the file name that you wish to work with and click Open.

7.3 Under the "STaRCall" menu, select "CODIS STR Export…”



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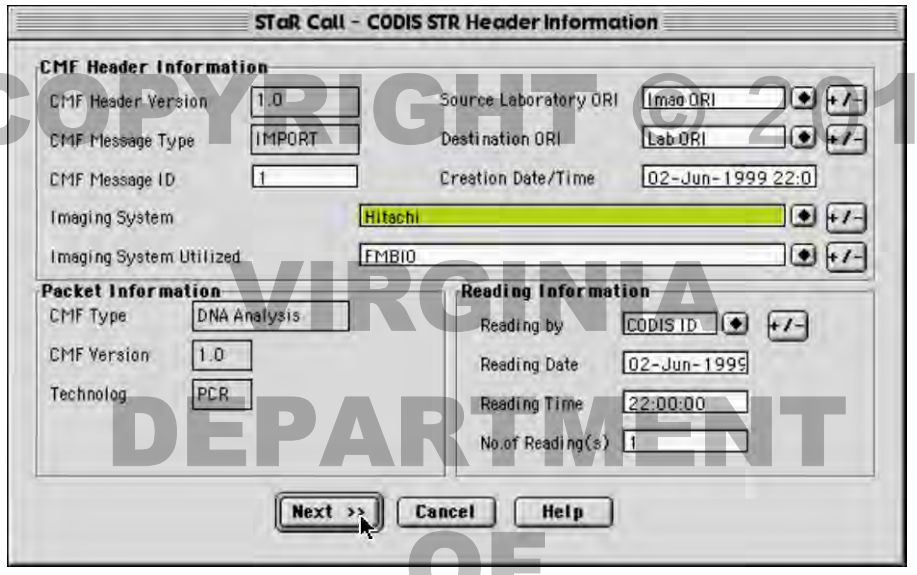
7.4 A screen will appear labeled "STaRCall - CODIS STR Header Information".

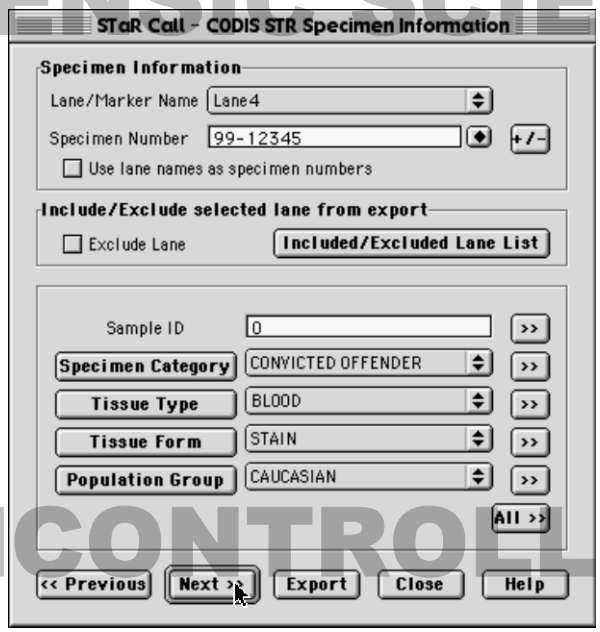
7.5 Ensure that the "Source Laboratory ORI” and “Destination ORI", have the appropriate workstation/location and laboratory identifications, and that the "Reading By" has the analyst’s CODIS

user identification. Change the "Read Date" to reflect the date the data is being exported to CODIS. Use the pull down menus to make any appropriate change.



7.6 Click on the "Next" button and the "STaRCall-CODIS STR Specimen Information" screen will appear.

7.7 Ensure that all the proper specimens have been excluded. (See 6.22 for excluding sample lanes)



7.8 To create the export file containing the STaRCall™ data for transfer to CODIS, click on "Export".

7.9 In the "Save As" field, enter the name of the file (Example: Filename-IMP) which will be exported to CODIS. Make sure the file is saved into the appropriate file folder on the file server or transferable media.

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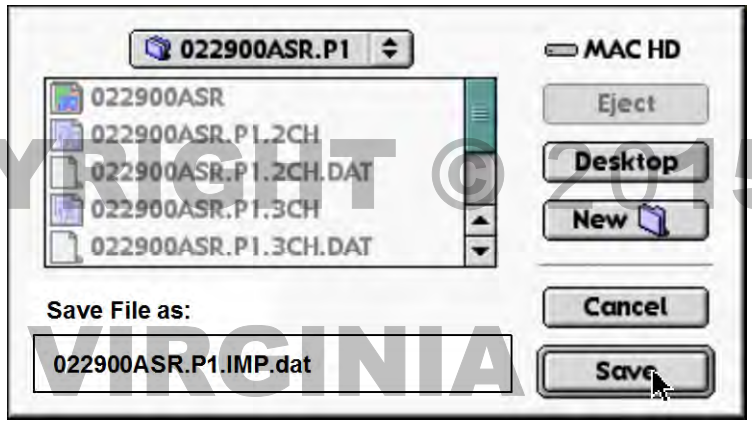

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7.10 If no further export files will be created, under the "File" menu, select "Save" to include added file information. Then under the "File" menu, select "Close" to close the workbook file. Repeat 6.2-6.10 to create additional export files.

7.11 Under the "File" menu, select "Exit", to quit using Microsoft Excel.

8 Creating Appendix of DNA Typing Results for Certificate of Analysis

8.1 Once the landscape spreadsheet file has been saved as outlined in step 6.23, delete from the landscape spreadsheet the DNA profiles that do not pertain to the case file of interest.

IMPORTANT: Ensure that all changes or modifications to the landscape sheet have been electronically documented so that the allele calls on the landscape sheet in the case file are identical to the Appendix of DNA Typing Results that is being created in step 8.4.

8.2 Select "Save As" from the file menu and save this modified workbook using the case number in the filename to distinguish it from the original gel's data.

8.3 Ensure that the Specimen identification is complete enough to properly describe the evidence. Do not exceed 24 characters, including spaces. See section 10 for example of Abbreviations Appendix.

8.4 In the "STR SetUp" menu choose "Create Report Table".

NOTE: Once the report table has been created it cannot be edited and both the Appendix of DNA Typing Results and the Appendix of Abbreviations are automatically printed together.

8.5 Continue this process for all of the cases on the landscape spreadsheet.

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9 Documentation of Gray Levels Used During Image Analysis

NON-SEPARATED IMAGE GRAY LEVEL

Channel:	RRX	Channel:	Texas Red
Mapping Type:	Equal Range	Mapping Type:	Equal Range
Background		Background	
High Signal		High Signal	

Channel:	JOE	Channel:	FL
Mapping Type:	Equal Range	Mapping Type:	Equal Range
Background		Background	
High Signal		High Signal	

SEPARATED IMAGE GRAY LEVEL

Channel:	RRX	Channel:	Texas Red
Mapping Type:	Equal Range	Mapping Type:	Equal Range
Background		Background	
High Signal		High Signal	

Channel:	JOE	Channel:	FL
Mapping Type:	Equal Range	Mapping Type:	Equal Range
Background		Background	
High Signal		High Signal	

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10 Abbreviations for Appendix of DNA Typing Results for Certificate of Analysis

ANO	Anorectal Sample
BLD	Blood Stain

ELIM	Elimation
ENV	Envelope
FNC	Finger Nail Clippings
FNS	Finger Nail Scrapings
K	Known
L	Left
LLA	Lips/Lip Area Sample
MAT	Material
NSP	Non-Sperm Fraction
OR	Oral Rinse Sample
PA	Pubic Area Sample
PB	Perianal/Buttocks Sample
PH	Pubic Hair
Q	Questioned
R	Right
SC	Stain Card
SMPL	Sample
SP	Sperm Fraction
STN	Stain
STN'D	Stained
SUS	Suspect
SWB	Swab
TEG	Thighs/External Genitalia Sample
UPS	Underpants
VC	Vaginal/Cervical sample
VIC	Victim

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Appendix H – STR Population Frequencies

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
CSF1P0, TPOX, TH01 AND vWA ALLELE FREQUENCY TABLE

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DATE: JUNE 1, 1998 **N = 194**

ALLELE CSF1P0	OBSERVATION	FREQUENCY		ALLELE TH01	OBSERVATION	FREQUENCY
15	0*	0.01289		11	0*	0.01289
14	2*	0.01289		10	4*	0.01289
13	24	0.06186		9.3	34	0.08762
12	107	0.27577		9	50	0.12886
11	90	0.23196		8	96	0.24742
10	92	0.23711		7	154	0.39690
9	14	0.03608		6	47	0.12113
8	29	0.07474		5	3*	0.01289
7	30	0.07732				
6	0*	0.01289				
n = 388				n = 388		
ALLELE TPOX	**OBSERVATION**	**FREQUENCY**		**ALLELE VWA**	**OBSERVATION**	**FREQUENCY**
13	1*	0.01289		21	1*	0.01289
12	8	0.02062		20	11	0.02835
11	88	0.22680		19	28	0.07216
10	32	0.08247		18	44	0.11340
9	86	0.22165		17	61	0.15722
8	138	0.35567		16	110	0.28351
7	6	0.01546		15	96	0.24742
6	29	0.07474		14	29	0.07474
				13	6	0.01546
				12	0*	0.01289
				11	2*	0.01289
n = 388				n = 388		

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Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 388, or 0.01289. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
D16S539, D7S820, D13S317, AND D5S818 ALLELE FREQUENCY TABLE

DATE: JUNE 1, 1998 **N = 194**

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ALLELE D16S539	OBSERVATION	FREQUENCY		ALLELE D13S317	OBSERVATION	FREQUENCY
15	1*	0.01289		15	1*	0.01289
14	12	0.03093		14	24	0.06190
13	56	0.14433		13	54	0.13918
12	73	0.18814		12	158	0.40722
11	113	0.29124		11	127	0.32732
10	37	0.09536		10	7	0.01804
9	81	0.20876		9	10	0.02577
8	15	0.03866		8	7	0.01804
5	0*	0.01289		7	0*	0.01289
n = 388				n = 388		
ALLELE D7S820	OBSERVATION	FREQUENCY		ALLELE D5S818	OBSERVATION	FREQUENCY
14	1*	0.01289		15	0*	0.01289
13	7	0.01804		14	10	0.02577
12	41	0.10567		13	89	0.22938
11	90	0.23196		12	141	0.36340
10	132	0.34021		11	99	0.25515
9	41	0.10567		10	26	0.06701
8	73	0.18814		9	8	0.02062
7	3*	0.01289		8	15	0.03866
6	0*	0.01289		7	0*	0.01289
n = 388				n = 388		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 388, or 0.01289. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

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VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
D18S51 and D21S11

DATE: MAY 19, 2000 N = 192

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ALLELE D18S51	OBSERVATION	FREQUENCY		ALLELE D18S51 CONT.	OBSERVATION	FREQUENCY
17	60	0.15625		27	0*	0.01302
16	68	0.17708		26	0*	0.01302
15	66	0.17188		25	0*	0.01302
14	25	0.06510		24	0*	0.01302
13.2	1*	0.01302		23	1*	0.01302
13	27	0.07031		22	4*	0.01302
12	28	0.07292		21	6	0.01563
11	0*	0.01302		20	18	0.04688
10.2	3*	0.01302		19	28	0.07292
10	3*	0.01302		18	46	0.11979
9	0*	0.01302				
8	0*	0.01302		n = 384		
ALLELE D21S11	OBSERVATION	FREQUENCY		ALLELE D21S11 CONT.	OBSERVATION	FREQUENCY
31.2	19	0.04948		38	0*	0.01302
31	26	0.06771		37	0*	0.01302
30.2	7	0.01823		36	3*	0.01302
30	74	0.19271		35.2	0*	0.01302
29.2	0*	0.01302		35	11	0.02865
29	79	0.20573		34.2	0*	0.01302
28	97	0.25260		34	4*	0.01302
27	16	0.04167		33.2	11	0.02865
26	1*	0.01302		33	3*	0.01302
25.2	0*	0.01302		32.2	30	0.07813
25	0*	0.01302		32.1	0*	0.01302
24.2	0*	0.01302		32	3*	0.01302
24	0*	0.01302		n = 384		

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Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 384, or 0.01302 Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
Penta E and D3S1358

DATE: MAY 19, 2000 N = 168 (Penta E) and 192 (D3S1358)

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ALLELE Penta E	OBSERVATION	FREQUENCY		ALLELE Penta E cont.	OBSERVATION	FREQUENCY
15	18	0.05357		25	0*	0.01488
14	20	0.05952		24	0*	0.01488
13	46	0.13691		23	0*	0.01488
12	33	0.09821		22	0*	0.01488
11	26	0.07738		21	1*	0.01488
10	16	0.04762		20.3	0*	0.01488
9	12	0.03571		20	3*	0.01488
8	64	0.19048		19	0*	0.01488
7	37	0.11012		18	4*	0.01488
6	0*	0.01488		17	12	0.03571
5	34	0.10119		16	10	0.02976
				n = 336		
ALLELE D3S1358	OBSERVATION	FREQUENCY		ALLELE D3S1358 cont.	OBSERVATION	FREQUENCY
16.2	1*	0.01302		21	0*	0.01302
16	103	0.26823		20	0*	0.01302
15	124	0.32292		19	1*	0.01302
14	45	0.11719		18	21	0.05469
13	4*	0.01302		17	81	0.21094
12	3*	0.01302				
11	1*	0.01302		n = 384		

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Note: * = Alleles for Penta E with fewer than 5 observations are defaulted to a frequency of 5 per 336, or 0.01488. Alleles for D3S1358 with fewer than 5 observations are defaulted to a frequency of 5 per 384, or 0.01302. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
FGA AND D8S1179

DATE: MAY 19, 2000 N = 192

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ALLELE FGA	OBSERVATION	FREQUENCY		ALLELE FGA, CONT.	OBSERVATION	FREQUENCY
25	33	0.08594		46.2	1*	0.01302
24.2	0*	0.01302		45.2	0*	0.01302
24	54	0.14063		44.2	2*	0.01302
23.2	0*	0.01302		43.2	0*	0.01302
23	66	0.17188		42.2	1*	0.01302
22.2	0*	0.01302		31.2	0*	0.01302
22	77	0.20052		31	1*	0.01302
21.2	1*	0.01302		30.2	1*	0.01302
21	39	0.10156		30	0*	0.01302
20.2	1*	0.01302		29	2*	0.01302
20	30	0.07813		28	7	0.01823
19.2	1*	0.01302		27	11	0.02865
19	25	0.06510		26.1	0*	0.01302
18.2	9	0.02344		26	18	0.04688
18	4*	0.01302		25.2	0*	0.01302
17	0*	0.01302				
				n = 384		
ALLELE D8S1179	OBSERVATION	FREQUENCY		ALLELE D8S1179	OBSERVATION	FREQUENCY
12	42	0.10938		18	0*	0.01302
11	19	0.04948		17	7	0.01823
10	11	0.02865		16	23	0.05990
9	2*	0.01302		15	71	0.18490
8	2*	0.01302		14	114	0.29688
7	0*	0.01302		13	93	0.24219
				n = 384		

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Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 384, or 0.01302. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
Penta D

DATE: JANUARY 7, 2002 N = 100

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ALLELE Penta D	OBSERVATION	FREQUENCY		ALLELE Penta D cont.	OBSERVATION	FREQUENCY
10	22	0.11000		17	0*	0.02500
9	34	0.17000		16	0*	0.02500
8	23	0.11500		15	2*	0.02500
7	9	0.04500		14	4*	0.02500
5	8	0.04000		13	15	0.07500
3.2	3*	0.02500		12	20	0.10000
2.2	27	0.13500		11	33	0.16500
				n = 200		

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Note: * = Alleles for Penta D with fewer than 5 observations are defaulted to a frequency of 5 per 200, or 0.0250. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

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VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
CSF1P0, TPOX, THO1 AND vWA ALLELE FREQUENCY TABLE

DATE: JUNE 1, 1998 N = 174

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ALLELE CSF1P0	OBSERVATION	FREQUENCY		ALLELE THO1	OBSERVATION	FREQUENCY
15	0*	0.01437		11	0*	0.01437
14	3*	0.01437		10	4*	0.01437
13	27	0.07759		9.3	108	0.31034
12	125	0.35919		9	50	0.14368
11	97	0.27874		8	35	0.10057
10	83	0.23851		7	66	0.18966
9	11	0.03161		6	83	0.23851
8	1*	0.01437		5	2*	0.01437
7	1*	0.01437				
6	0*	0.01437				
n = 348				n = 348		
ALLELE TPOX	OBSERVATION	FREQUENCY		ALLELE Vwa	OBSERVATION	FREQUENCY
13	0*	0.01437		21	0*	0.01437
12	18	0.05172		20	2*	0.01437
11	86	0.24713		19	29	0.08333
10	23	0.06609		18	81	0.23276
9	31	0.08908		17	96	0.27586
8	190	0.54598		16	73	0.20977
7	0*	0.01437		15	33	0.09483
6	0*	0.01437		14	33	0.09483
				13	1*	0.01437
				12	0*	0.01437
				11	0*	0.01437
n = 348				n = 348		

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Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 348, or 0.01437. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
D16S539, D7S820, D13S317, AND D5S818 ALLELE FREQUENCY TABLE

DATE: JUNE 1, 1998 N = 174

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ALLELE D16S539	OBSERVATION	FREQUENCY		ALLELE D13S317	OBSERVATION	FREQUENCY
15	1*	0.01437		15	0*	0.01437
14	6	0.01724		14	15	0.04310
13	62	0.17816		13	40	0.11494
12	112	0.32184		12	100	0.28736
11	89	0.25575		11	102	0.29310
10	24	0.06897		10	20	0.05747
9	48	0.13793		9	23	0.06609
8	6	0.01724		8	48	0.13793
5	0*	0.01437		7	0*	0.01437
n = 348				n = 348		
ALLELE D7S820	OBSERVATION	FREQUENCY		ALLELE D5S818	OBSERVATION	FREQUENCY
14	3*	0.01437		15	0*	0.01437
13	8	0.02299		14	8	0.02299
12	54	0.15517		13	55	0.15805
11	66	0.18966		12	121	0.34770
10	108	0.31034		11	128	0.36782
9	47	0.13506		10	20	0.05747
8	52	0.14943		9	15	0.04310
7	10	0.02874		8	1*	0.01437
6	0*	0.01437		7	0*	0.01437
n = 348				n = 348		

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Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 348, or 0.01437. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
D18S51 and D21S11

DATE: MAY 19, 2000 N = 173

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ALLELE D18S51	OBSERVATION	FREQUENCY		ALLELE D18S51 CONT.	OBSERVATION	FREQUENCY
17	51	0.14740		27	0*	0.01445
16	47	0.13584		26	0*	0.01445
15	55	0.15896		25	0*	0.01445
14	55	0.15896		24	0*	0.01445
13.2	0*	0.01445		23	0*	0.01445
13	49	0.14162		22	2*	0.01445
12	43	0.12428		21	1*	0.01445
11	6	0.01734		20	5	0.01445
10.2	0*	0.01445		19	6	0.01734
10	1*	0.01445		18	25	0.07225
9	0*	0.01445				
8	0*	0.01445		n = 346		
ALLELE D21S11	OBSERVATION	FREQUENCY		ALLELE D21S11 CONT.	OBSERVATION	FREQUENCY
31.2	27	0.07804		38	0*	0.01445
31	24	0.06936		37	0*	0.01445
30.2	13	0.03757		36	0*	0.01445
30	91	0.26301		35.2	1*	0.01445
29.2	0*	0.01445		35	0*	0.01445
29	73	0.21098		34.2	3*	0.01445
28	40	0.11561		34	0*	0.01445
27	7	0.02023		33.2	13	0.03757
26	0*	0.01445		33	0*	0.01445
25.2	1*	0.01445		32.2	43	0.12428
25	1*	0.01445		32.1	1*	0.01445
24.2	0*	0.01445		32	8	0.02312
24	0*	0.01445		n = 346		

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Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 346, or 0.01445. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
Penta E and D3S1358

DATE: MAY 19, 2000 N =120 (Penta E) and 173 (D3S1358)

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ALLELE Penta E	OBSERVATION	FREQUENCY		ALLELE Penta E cont.	OBSERVATION	FREQUENCY
15	20	0.08333		25	0*	0.02083
14	11	0.04583		24	0*	0.02083
13	18	0.07500		23	0*	0.02083
12	41	0.17083		22	0*	0.02083
11	30	0.12500		21	0*	0.02083
10	19	0.07917		20.3	0*	0.02083
9	2*	0.02083		20	3*	0.02083
8	2*	0.02083		19	5	0.02083
7	50	0.20833		18	3*	0.02083
6	0*	0.02083		17	11	0.04583
5	16	0.06667		16	9	0.03750
				n = 240		
ALLELE D3S1358	OBSERVATION	FREQUENCY		ALLELE D3S1358 cont.	OBSERVATION	FREQUENCY
16.2	0*	0.01445		21	0*	0.01445
16	89	0.25723		20	2*	0.01445
15	82	0.23699		19	7	0.02023
14	48	0.13873		18	51	0.14734
13	0*	0.01445		17	66	0.19075
12	0*	0.01445				
11	1*	0.01445		n = 346		

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Note: * = Alleles for Penta E with fewer than 5 observations are defaulted to a frequency of 5 per 240, or 0.02083. Alleles for D3S1358 with fewer than 5 observations are defaulted to a frequency of 5 per 346, or 0.01445. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
FGA AND D8S1179

DATE: MAY 19, 2000 N = 173

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ALLELE FGA	OBSERVATION	FREQUENCY		ALLELE FGA, CONT.	OBSERVATION	FREQUENCY
25	31	0.08960		46.2	0*	0.01445
24.2	0*	0.01445		45.2	0*	0.01445
24	43	0.12428		44.2	0*	0.01445
23.2	0*	0.01445		43.2	0*	0.01445
23	45	0.13006		42.2	0*	0.01445
22.2	4*	0.01445		31.2	0*	0.01445
22	56	0.16185		31	0*	0.01445
21.2	0*	0.01445		30.2	0*	0.01445
21	66	0.19075		30	0*	0.01445
20.2	0*	0.01445		29	0*	0.01445
20	58	0.16763		28	0*	0.01445
19.2	1*	0.01445		27	4*	0.01445
19	20	0.05780		26.1	0*	0.01445
18.2	0*	0.01445		26	14	0.04046
18	3*	0.01445		25.2	0*	0.01445
17	1*	0.01445				
				n = 346		
ALLELE D8S1179	OBSERVATION	FREQUENCY		ALLELE D8S1179	OBSERVATION	FREQUENCY
12	49	0.14162		18	0*	0.01445
11	32	0.09249		17	3*	0.01445
10	37	0.10694		16	12	0.03468
9	2*	0.01445		15	31	0.08960
8	5	0.01445		14	68	0.19653
7	0*	0.01445		13	107	0.30925
				n = 346		

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Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 346, or 0.01445 Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
Penta D

DATE: JANUARY 7, 2002 N = 101

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ALLELE Penta D	OBSERVATION	FREQUENCY		ALLELE Penta D cont.	OBSERVATION	FREQUENCY
10	27	0.13366		17	0*	0.02475
9	46	0.22772		16	0*	0.02475
8	2*	0.02475		15	2*	0.02475
7	1*	0.02475		14	11	0.05446
5	0*	0.02475		13	43	0.21287
3.2	0*	0.02475		12	41	0.20297
2.2	0*	0.02475		11	29	0.14356
				n = 202		

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

Note: * = Alleles for Penta D with fewer than 5 observations are defaulted to a frequency of 5 per 202, or 0.02475. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

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VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
HISPANIC POPULATION DATA
CSF1P0, TPOX, THO1 AND vWA ALLELE FREQUENCY TABLE

DATE: JUNE 1, 1998 N = 181

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ALLELE CSF1P0	OBSERVATION	FREQUENCY		ALLELE THO1	OBSERVATION	FREQUENCY
15	1*	0.01381		11	0*	0.01381
14	3*	0.01381		10	2*	0.01381
13	20	0.05525		9.3	73	0.20166
12	120	0.33149		9	49	0.13536
11	109	0.30111		8	34	0.09392
10	90	0.24862		7	100	0.27624
9	12	0.03315		6	104	0.28729
8	3*	0.01381		5	0*	0.01381
7	4*	0.01381				
6	0*	0.01381				
n = 362				n = 362		
ALLELE TPOX	**OBSERVATION**	**FREQUENCY**		**ALLELE VWA**	**OBSERVATION**	**FREQUENCY**
13	2*	0.01381		21	0*	0.01381
12	31	0.08564		20	5	0.01381
11	100	0.27624		19	24	0.06630
10	19	0.05249		18	52	0.14365
9	31	0.08564		17	89	0.24590
8	169	0.46685		16	106	0.29282
7	3*	0.01381		15	53	0.14641
6	7	0.01934		14	31	0.08564
				13	1*	0.01381
				12	0*	0.01381
				11	1*	0.01381
n = 362				n = 362		

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Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 362, or 0.01381. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

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HISPANIC POPULATION DATA
D16S539, D7S820, D13S317, AND D5S818 ALLELE FREQUENCY TABLE

DATE: JUNE 1, 1998 N = 181

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ALLELE D16S539	OBSERVATION	FREQUENCY		ALLELE D13S317	OBSERVATION	FREQUENCY
15	0*	0.01381		15	0*	0.01381
14	9	0.02486		14	27	0.07459
13	58	0.16022		13	56	0.15470
12	97	0.26796		12	80	0.22099
11	73	0.20166		11	79	0.21823
10	55	0.15193		10	20	0.05525
9	62	0.17127		9	61	0.16851
8	7	0.01934		8	39	0.10773
5	1*	0.01381		7	0*	0.01381
n = 362				n = 362		

ALLELE D7S820	OBSERVATION	FREQUENCY		ALLELE D5S818	OBSERVATION	FREQUENCY
14	4*	0.01381		15	0*	0.01381
13	6	0.01657		14	3*	0.01381
12	72	0.19890		13	58	0.16022
11	94	0.25967		12	104	0.28729
10	94	0.25967		11	126	0.34807
9	34	0.09392		10	21	0.05801
8	55	0.15193		9	27	0.07459
7	3*	0.01381		8	5	0.01381
6	0*	0.01381		7	18	0.04972
n = 362				n = 362		

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Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 362, or 0.01381. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

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HISPANIC POPULATION DATA
D18S51 and D21S11

DATE: MAY 19, 2000 N = 183

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ALLELE D18S51	OBSERVATION	FREQUENCY		ALLELE D18S51 CONT.	OBSERVATION	FREQUENCY
17	55	0.15027		27	0*	0.01366
16	43	0.11749		26	0*	0.01366
15	51	0.13934		25	0*	0.01366
14	60	0.16393		24	0*	0.01366
13.2	0*	0.01366		23	2*	0.01366
13	51	0.13934		22	1*	0.01366
12	37	0.10109		21	1*	0.01366
11	4*	0.01366		20	10	0.02732
10.2	1*	0.01366		19	23	0.06284
10	1*	0.01366		18	26	0.07104
9	0*	0.01366				
8	0*	0.01366		n = 366		
ALLELE D21S11	OBSERVATIONS	FREQUENCY		ALLELE D21S11 CONT.	OBSERVATION	FREQUENCY
31.2	42	0.11475		38	0*	0.01366
31	30	0.08197		37	0*	0.01366
30.2	4*	0.01366		36	1*	0.01366
30	88	0.24044		35.2	0*	0.01366
29.2	1*	0.01366		35	3*	0.01366
29	78	0.21312		34.2	2*	0.01366
28	42	0.11475		34	1*	0.01366
27	7	0.01913		33.2	16	0.04372
26	0*	0.01366		33	0*	0.01366
25.2	0*	0.01366		32.2	46	0.12568
25	0*	0.01366		32.1	0*	0.01366
24.2	0*	0.01366		32	5	0.01366
24	0*	0.01366		n = 366		

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Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 366, or 0.01366 Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
HISPANIC POPULATION DATA
Penta E and D3S1358

DATE: MAY 19, 2000 N = 181 (Penta E) and 183(D3S1358)

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ALLELE Penta E	OBSERVATION	FREQUENCY		ALLELE Penta E cont.	OBSERVATION	FREQUENCY
15	32	0.08840		25	1*	0.01381
14	28	0.07735		24	0*	0.01381
13	29	0.08011		23	1*	0.01381
12	58	0.16022		22	4*	0.01381
11	21	0.05801		21	8	0.02210
10	27	0.07459		20.3	0*	0.01381
9	6	0.01658		20	4*	0.01381
8	21	0.05801		19	12	0.03315
7	34	0.09392		18	14	0.03867
6	0*	0.01381		17	16	0.04420
5	19	0.05249		16	27	0.07459
				n = 362		
ALLELE D3S1358	OBSERVATION	FREQUENCY		ALLELE D3S1358 cont.	OBSERVATION	FREQUENCY
16	93	0.25410		21	1*	0.01366
15	143	0.39071		20	0*	0.01366
14	26	0.07104		19	3*	0.01366
13	1*	0.01366		18	30	0.08197
12	0*	0.01366		17	69	0.18853
11	0*	0.01366		16.2	0*	0.01366
				n = 366		

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Note: * = Alleles for Penta E with fewer than 5 observations are defaulted to a frequency of 5 per 362, or 0.01381. Alleles for D3S1358 with fewer than 5 observations are defaulted to a frequency of 5 per 366, or 0.01366. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

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HISPANIC POPULATION DATA
FGA AND D8S1179

DATE: MAY 19, 2000 N = 183

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ALLELE FGA	OBSERVATION	FREQUENCY	ALLELE FGA, CONT.	OBSERVATION	FREQUENCY
25	47	0.12842	46.2	0*	0.01366
24.2	0*	0.01366	45.2	1*	0.01366
24	58	0.15847	44.2	0*	0.01366
23.2	0*	0.01366	43.2	0*	0.01366
23	46	0.12568	42.2	0*	0.01366
22.2	1*	0.01366	31.2	0*	0.01366
22	36	0.09836	31	0*	0.01366
21.2	1*	0.01366	30.2	0*	0.01366
21	65	0.17760	30	1*	0.01366
20.2	0*	0.01366	29	1*	0.01366
20	40	0.10929	28	2*	0.01366
19.2	0*	0.01366	27	9	0.02459
19	31	0.08470	26.1	1*	0.01366
18.2	0*	0.01366	26	25	0.06831
18	1*	0.01366	25.2	0*	0.01366
17	0*	0.01366			
			n = 366		

ALLELE D8S1179	OBSERVATION	FREQUENCY	ALLELE D8S1179	OBSERVATION	FREQUENCY
12	40	0.10929	18	0*	0.01366
11	17	0.04645	17	0*	0.01366
10	30	0.08197	16	17	0.04645
9	1*	0.01366	15	44	0.12022
8	3*	0.01366	14	96	0.26230
7	0*	0.01366	13	118	0.32240
			n = 366		

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Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 366, or 0.01366. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

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HISPANIC POPULATION DATA
Penta D

DATE: NOVEMBER 13, 2001 N = 157

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ALLELE Penta D	OBSERVATION	FREQUENCY		ALLELE Penta D cont.	OBSERVATION	FREQUENCY
10	49	0.15556		17	1*	0.01587
9	66	0.20952		16	0*	0.01587
8	9	0.02857		15	1*	0.01587
7	3*	0.01587		14	16	0.05079
5	3*	0.01587		13	56	0.17778
3.2	0	0.01587		12	59	0.18730
2.2	9	0.02857		11	43	0.13651
				n = 315**		

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Note: * = Alleles for Penta D with fewer than 5 observations are defaulted to a frequency of 5 per 315, or 0.01587. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

** One of the samples analyzed for the creation of the Hispanic population database contained a 3 banded pattern at Penta D. Therefore, the number of alleles observed in the 157 individuals was 315.

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Appendix I – Likelihood Ratio Formulas

The following formulas address the question what is the likelihood that the suspect left the DNA that is foreign to the victim and/or the likelihood that the suspect is a co-contributor of the genetic material identified on the item of evidence. These formulas may also be used to answer the converse question what is the likelihood that the victim left the DNA foreign to the suspect. However, the appropriate alleles must be plugged into the formulas to obtain this information.

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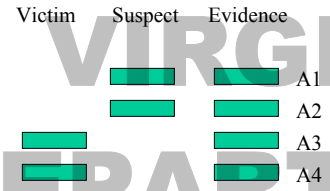
1.



$LR = 1/ 2P_1P_2$

C1 column 0 unknown - leave field blank
C2 column 1 unknown - enter alleles A1 & A2

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2.

$LR = 1/ 2P_1P_2$

C1 column 0 unknown - leave field blank
C2 column 1 unknown - enter alleles A1 & A2

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3.

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$LR = 1/P_1^2 + 2P_1P_2 + 2P_1P_3$

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C1 column 0 unknown - leave field blank
C2 column 1 unknown - enter allele A1

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4.

$LR = 1/P_1^2 + 2P_1P_2 + P_2^2$

C1 column 0 unknown - leave field blank
C2 column 1 unknown - leave field blank

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5.

VIRGINIA

$LR = 1/P_1^2 + 2P_1P_2 + P_2^2$

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C1 column 0 unknown - leave field blank
C2 column 1 unknown - leave field blank

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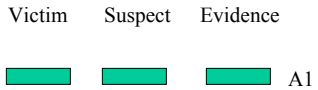
6.



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$LR = 1/P_1^2$

C1 column 0 unknown - leave field blank
C2 column 1 unknown - leave field blank

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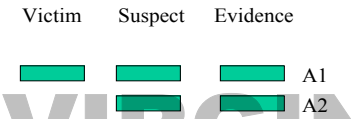
7.



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$LR = 1/2P_1P_2 + P_2^2$

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C1 column 0 unknown - leave field blank
C2 column 1 unknown - enter allele A2

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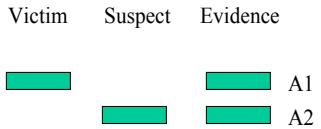
8.



$LR = 1/2P_1P_2 + P_2^2$

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C1 column 0 unknown - leave field blank
C2 column 1 unknown - enter allele A2

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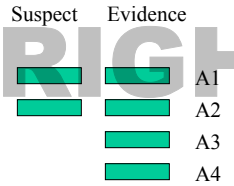
9.



$$LR = 2P_3P_4/24P_1P_2P_3P_4$$
$$= 1/12P_1P_2$$

C1 column 1 unknown - enter alleles A3 & A4
C2 column 2 unknown - enter alleles A1, A2, A3, & A4

10.

$$LR = 2P_1 + 2P_2 + P_3/ 12P_1P_2(P_1 + P_2 + P_3)$$

C1 column 1 unknown - enter alleles A3
C2 column 2 unknown - enter alleles A1, A2, & A3

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The formulas provided above were obtained from Ian W. Evett and Bruce S Weir's 1998 book, Interpreting DNA Evidence. These are general formula that cover most of the cases that are handled on a day-to-day basis by the Virginia Division of Forensic Science. However, depending on the scenario of the case other likelihood ratio formulas not listed above may need to be used.

Appendix J – Procedure for Drying Down and Resolubilizing Extracted DNA

1 Equipment

1.1 DNA concentrator/vaporator

1.2 Microcentrifuge

1.3 Pipettes - 100 µL and 1000 µL

2 Materials

2.1 Plastic ziploc bags

2.2 Microcentrifuge tubes, 1.5 mL

2.3 Kimwipes

3 Reagents

3.1 Sterile Type 1 Water

4 Procedure

4.1 For permanent storage and preservation of the extracted DNA, all evidence and known standard samples will be dried down.

NOTE: Once the DNA analysis has been completed the reagent blanks should be discarded.

4.1.1 Open the cap of the microcentrifuge tube and place either the open evidence sample tubes or the reference sample tubes, **BUT NOT BOTH TOGETHER AT THE SAME TIME**, into a DNA concentrator/evaporator until the sample has been dried down. To prevent contamination the open evidence sample tubes should be placed into the DNA concentrator/evaporator at a different time or a different DNA concentrator/evaporator than the open reference sample tubes.

4.1.2 Once the DNA sample has been dried down close the microcentrifuge cap and store in a plastic ziploc bag or similar storage medium.

4.2 To resolubilize a dried down extracted DNA sample the following procedure will be used.

4.2.1 Resolubilizing Extracted DNA: To resolubilize the dried down extracted DNA sample, add a volume of sterile Type 1 Water to the microcentrifuge tube that is consistent with the sample volume prior to drying down the extracted DNA. Place the tube in a 56 C incubator or heat block for a minimum of 2 hours, but no more than 3 hours.

4.2.2 A reagent blank consisting of sterile Type 1 Water consistent in volume with the amount added to the sample(s) should be prepared along with the reconstituted sample(s).

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Appendix K - 6% PAGE PLUS™ - Optional Procedure

1 While working in a fume hood prepare a 6% PAGE Plus™ gel matrix solution as follows:

1.1 Add 18 g of urea to a 50 mL conical tube.

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CAUTION: Urea dust particles should not be inhaled.

1.2 Add 24 mL of Type 1 Water to the container.

1.3 Add 7.5 mL of 40% PAGE Plus™ gel solution to the container.

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1.4 Add 5 mL of 10X TBE to the container.

1.5 Swirl the 50 mL conical tube to mix the solution thoroughly, then heat the PAGE Plus™ gel solution briefly in a 50°C water bath to dissolve the urea particles.

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1.6 In a well ventilated area, add 300 µL of 10% ammonium persulfate and 30 µL of TEMED to the PAGE Plus™ gel solution.

NOTE: 10% ammonium persulfate should be prepared the day of use.

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1.7 Using a 50 mL disposable pipette and an auto pipette, pipette the gel mix between the glass plates. Pipette the gel mixture slowly and maintain a continuous flow. Avoid creating bubbles. Carefully tap the plates to dislodge any air bubbles.

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1.8 Insert the 30 or 38 well comb at the top of the gel. Be careful not to form bubbles around the comb teeth.

1.9 Allow the gel to polymerize in a horizontal position for at least 1 hour prior to setting up for electrophoresis.

NOTE: The polymerized gel can be stored in the dark at room temperature by wrapping the gel in plastic wrap. Do not store the gel in the refrigerator once it has polymerized. To prevent the gel from drying, add a small amount of 1X TBE buffer or water to several paper towels and then lay the paper towel on the comb, and both sides and the bottom of the glass plates before wrapping with plastic wrap.

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Appendix L – Code of Virginia - Paternity

§ 20-49.1. How parent and child relationship established.

The parent and child relationship between a child and a woman may be established prima facie by proof of her having given birth to the child, or as otherwise provided in this chapter.

The parent and child relationship between a child and a man may be established by:

Scientifically reliable genetic tests, including blood tests, which affirm at least a ninety-eight percent probability of paternity. Such genetic test results shall have the same legal effect as a judgment entered pursuant to § 20-49.8.

In the absence of such acknowledgment or if the probability of paternity is less than ninety-eight percent, such relationship may be established as otherwise provided in this chapter.

§ 63.1-250.1:2. Administrative establishment of paternity.

Paternity may be established by a written statement of the father and mother made under oath acknowledging paternity or scientifically reliable genetic tests, including blood tests, which affirm at least a ninety-eight percent probability of paternity.

A genetic test result affirming at least a ninety-eight percent probability of paternity shall have the same legal effect as a judgment entered pursuant to § 20-49.8.

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Appendix M – Storage of Electronic Data

Saving Digital Images

Typing gel images (scanned and color separated) generated using the FMBIO II or FMBIO III Fluorescent Image Analysis System and FMBIO Analysis Software which are saved digitally on a CD-R will be stored according to the following procedures.

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- Once the case has been completed and the Certificate of Analysis issued, the scanned and separated typing gel images (if the typing gel image has been sized) will be transferred by the case examiner to a specified folder on the CODIS server that has been established by the Laboratory's CODIS Administrator. The supervisor in each laboratory will be responsible for establishing a deadline each month for the data to be transferred by the casework examiner to the specified folder. The folder will be labeled with the month and year to correlate with the month in which the files were transferred.

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- The file name containing the typing gel images for a particular case will be assigned based upon either the typing gel number (i.e., the typing gel contains several cases) or the case number.

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- The date of the Certificate of Analysis will dictate the month when the typing gel images for a particular case will be saved regardless of when the gel was poured or run. For example, if a typing gel was run and analyzed in January 2003 and another typing gel was run and analyzed in March 2003 and the Certificate of Analysis is dated March 26, 2003, then all typing gels used in the analysis of this case will be burned to a CD containing images from cases completed in March.2003.

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- The outside jacket of the CD will be labeled with the Month and Year, as well as will be labeled with the number of the CD (i.e., 1 of 2 and 2 of 2) to indicate the number of CDs that were created during the particular month. In addition, on the backside of the outside jacket the cases that are stored on the CD will be listed. If the file name is based upon the typing gel number the case numbers will be listed on the backside of the jacket followed by the typing gel number (i.e., C03-01234/012303BH).

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- If the file name is based upon the typing gel number it will be the responsibility of the casework examiner to provide the supervisor or his/her designee with a list of the case numbers that are stored under a particular typing gel number.

- Before the 16th of each month the supervisor or his/her designee will burn all of the files in a specified folder that were transferred during the previous month to a CD or CDs. At this point the folder will be deleted from the CODIS server.

- The CDs 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 needs to be added to the CD after the 16th of the month, the examiner will make arrangements with his/her supervisor to have the CD retrieved from storage and have the file burned to the appropriate CD.

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- If typing gel images must be retrieved and a copy made, the examiner will pull the CD containing the images based upon the date of the Certificate of Analysis. The file containing the typing gel images will be copied to the desktop of one of the Macintosh/PC based sizing computers. Subsequently the file will be transferred to a new CD.

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Appendix N – Reuse of a Polyacrylamide Electrophoresis Gel

1 Equipment

1.1 SA-43 vertical electrophoresis tank

1.2 Power Supply

2 Materials

2.1 Kimwipes

2.2 Disposable 60 cc syringe

2.3 Disposable, large bore transfer pipettes

2.4 Plastic wrap

2.5 Paper Towels

3 Reagents

3.1 Ethanol, 95%

3.2 1X TBE buffer

4 Procedure

4.1 After the gel has been scanned in the FMBIO, place it into the SA-43 vertical electrophoresis tank.

4.2 Secure the gel in place and add 1X TBE buffer to the upper and lower tank reservoirs.

4.3 To run the previously loaded samples out of the top of the gel, connect the leads from the power supply so that the red (+) electrode is attached to the top black electrode and the black (-) electrode is attached to the red lower electrode of the electrophoresis tank.

4.4 Turn the power supply on and begin to electrophoresis at 60 Watts for a minimum of 2½ hours. It is recommended that the typing gel be run in the reverse direction for approximately ½ hour longer than the typing gel was run in the forward direction. This will ensure that all of the DNA has run out of the top of the gel prior to loading new samples.

NOTE: If the DNA samples are not run out of the top of the gel on the same day as they were loaded, wrap the typing gel in plastic wrap with a moistened paper towel wrapped around the top, bottom, and sides of the gel and then store at room temperature in the dark until the reverse electrophoresis is conducted.

4.5 Once the samples have run out of the top of the gel into the top tank buffer reservoir, drain the upper tank buffer reservoir. Remove the gel from the SA-43 electrophoresis tank and discard the tank buffer in the lower buffer reservoir.

4.6 Rinse the upper tank reservoir with water and wipe with a Kimwipe to remove any remaining buffer.

NOTE: If the gel will not be reused the same day, wrap it in plastic wrap with a moistened paper towel wrapped around the top, bottom, and sides of the gel and then store at room temperature in the dark.

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4.7 Place the gel into the SA 43 vertical gel electrophoresis tank, large plate 'facing out', and turn the clamps to secure the gel in place. Add 1X buffer to the upper reservoir of the gel tank. Flush the wells with the tank buffer using a syringe or a large bore transfer pipet.

4.8 Fill the lower reservoir of the gel tank with 1X TBE buffer. Remove any air bubbles that are trapped between the bottom of the gel and the buffer in the lower reservoir. Then proceed with steps 7.5.10 through 7.5.20 for PowerPlex® 16 BIO System typing gels, steps 7.6.10 through 7.6.19 for PowerPlex® 1.1 System typing gels, and steps 7.7.10 through 7.7.19 for PowerPlex® 2.1System typing gels.

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