7 VERTICAL GEL ELECTROPHORESIS

7.1 TECHNICAL NOTES

7.1.1 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.

7.1.2 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.

7.1.3 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 with the samples that are associated with this control. 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.

7.1.4 The amplification negative control(s), the reagent blanks, 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.

7.1.5 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.

7.2 EQUIPMENT

7.2.1 SA-43 vertical electrophoresis tank

7.2.2 Power supply

7.2.3 Electrophoresis glass plate set, 19 cm X 43 cm

7.2.4 Delrin spacer sets, 0.4 mm thick

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

7.2.6 Binder clips, 1 inch

7.2.7 Autopipet

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

7.2.9 8 –Channel Pipette – Range 0.5 µL to 10 µL

7.2.10 Microcentrifuge

7.2.11 Ice Bucket/Pack

7.2.12 Heat Block, 95^0C

7.2.13 Fume Hood

7.2.14 Analytical Balance

7.2.15 Hot Water Bath, 50^0C

7.3 MATERIALS

7.3.1 Flat gel loading capillary pipette tips, 10 µL

7.3.2 Kimwipes

7.3.3 Disposable 60 cc syringe and needle

7.3.4 Disposable, large bore transfer pipettes

7.3.5 Disposable plastic pipettes, 50 mL

7.3.6 Microcentrifuge tubes, 0.5 mL and 1.5 mL

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

7.3.8 Plastic wrap

7.3.9 Conical tubes, 50 mL

7.3.10 Paper towels

7.3.11 Nitrile Gloves

7.4 REAGENTS

7.4.1 Ethanol, 95%

7.4.2 Type I Water

7.4.3 10X and 1X TBE buffer

7.4.4 10% Ammonium Persulfate

7.4.5 TEMED

7.4.6 Urea

7.4.7 6.0% Gene PAGE Plus™ Gel Mix

7.4.8 40% PAGE Plus™ Gel Mix

7.4.9 Bind Silane

7.4.11 Acetic Acid, 0.5% in 95% Ethanol

7.4.12 Bromophenol Blue Loading Buffer

7.4.13 STR 2X Loading Buffer (Tracking Dye)

7.4.14 PowerPlex® 16 BIO System allelic ladder

7.4.15 Fluorescent Internal Lane Standard 600 BIO

7.4.16 Matrix 16 BIO

7.4.17 Gold ST*R 1X Buffer

7.5 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.

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.

7.5.1 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.

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

7.5.3 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.

7.5.4 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.

7.5.5 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.

7.5.6 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.**

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

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

7.5.7.2 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.

7.5.7.3 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.

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

7.5.7.9 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 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.

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™

7.5.8 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.

7.5.9 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.

7.5.10 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.

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

7.5.12 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. **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.

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.

7.5.13 Casework Samples:

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.

Convicted Offender or Arrestee Samples:

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.

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

7.5.14.1 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.

7.5.14.2 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: All casework samples (i.e., evidence samples, known standards, ~~random samples~~, and positive amplification controls) must be adjacent to an allelic ladder and an internal lane standard should be used to determine the allele designation. It is not necessary for the reagent blanks, negative amplification control or plate blanks to be loaded next to an allelic ladder.

When loading the samples into the typing gel ensure that the evidence samples and known standards are not loaded next to each other. The known standards and evidence samples must be separated from each other by an allelic ladder, reagent blank, negative amplification control or plate blank.

7.5.15 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 2 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.

7.5.16 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.

7.5.17 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.

7.5.18 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.

7.5.19 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.

7.5.20 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.

7.5.21 Proceed to section 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 A Polyacrylamide Electrophoresis Gels, for the procedure.**

♦END