1. Remove the clamps from the polymerized acrylamide gel and clean the plates with dH_2O and kimwipes.

2. Shave any excess acrylamide from the plates using a razor blade.

3. Place foam blocks on the top of the short plate (press sticky side against the longer plate) then place the gel in the SA32 tanks with the longer plate outside and the shorter plate towards the tank. Be sure that the foam spacers are flush with the shorter plate before tightening the top clamps and that the drain for the top tank is closed. (This is the knob on the top right side of the tank.)

4. Place ~300 ml of .5X Tris Borate buffer in the top and bottom chambers of the tank. Be sure the seal is tight and there is no buffer leaking between the tank and the shorter glass plate.

5. Remove the comb and pre-run the gel for 10-20 minutes using the same conditions as shown in step 11 below to achieve a gel surface temperature of approximately 50°C. Remove the comb and rinse the wells with tank buffer.

6. Sample Preparation

   6.1 Each sample must be bracketed by Allelic Ladders provided by the manufacturer of the kit. Amelogenin ladder does not have to be included in each ladder lane. However, Amelogenin ladder must bracket all forensic samples. Should an analyst run out of Amelogenin Ladder or inadvertently forget to include the amelogenin ladder on an analytical gel, cells could still be made from the K562 (female) and MJB (male) positive controls (this is not a standard practice).

   6.2 Two-Color Detection (not using Fluorescent Ladder, CXR)

      6.2.1 Prepare an electrophoresis worksheet.
      6.2.2 In a biosafety cabinet, label sterile PCR amplification tubes and place in a thermocycler tray
      6.2.3 Prepare the samples by adding 2.5 ul loading dye to 2.5 ul amplified sample to each respective tube.
      6.2.4 Prepare the ladder samples by adding 2.5ul prepared loading cocktail, approximately 0.2-0.5 ul Amelogenin ladder and approximately 0.5-1.5 ul PowerPlex™ allelic ladder to each respective tube.

      Note: Ladder amounts may vary more depending on the quality/strength of the ladder. A tube of Ladder that has been

through many freeze/thaw cycles may be "weaker" than a fresh tube.

6.3 Two-Color Detection (using Fluorescent Ladder, CXR)

6.3.1 Prepare electrophoresis worksheet. In a biosafety cabinet, label sterile PCR amplification tubes and place in a thermocycler tray.

6.3.2 Prepare a loading cocktail by combining and mixing the Fluorescent Ladder (CXR) and load dye as follows:

[(1ul Fluorescent Ladder) x (# lanes)] + [3ul Load Dye) x (# lanes)]

6.3.3 Prepare the samples by adding 2.5ul of the prepared loading cocktail and 2.5 ul amplified sample to each respective tube.

6.3.4 Prepare the ladder samples by adding 2.5ul prepared loading cocktail, 0.3 ul Amelogenin ladder and 0.6 ul PowerPlex™ allelic ladder to each respective tube.

Note: Ladder amounts may vary depending on the quality/strength of the ladder. A tube of Ladder that has been through many freeze/thaw cycles may be "weaker" than a fresh tube.

7. Denature the samples by heating to 96°C for 2 minutes in the thermocycler. Immediately remove and chill on an ice block.

8. While denaturing, flush the wells with tank buffer using a 10 ml syringe with a needle to remove the excess urea.

9. Immediately after completion of denaturation, place the samples on an ice block. Load approximately 2 - 5 ul of each sample/dye mixture into the respective wells using a fresh flat tip pipette for each sample. Sample amount loaded can vary depending on sample strength

10. Load 3 ul of Gel Tracking Dye in the gel. This dye is used as a visual indicator of migration.

11. At the completion of loading, attach the tank to the power supply and run at the following conditions:

3,500 V	300 mA	45 - 60 W - 1 gel
3,500 V	300 mA	60 - 120 W - 2 gels

Run the gel for approximately 80 to 100 minutes or until the second dye front at least passes the bottom gel fastener.

Revision History		
Effective Date	Revision Number	Reason
Unknown	00	Original Document for DNA Tech Procedures
January 4, 2002	01	Addition of Speed-Vac Procedure (Change in DNA Technical Procedures)
November 26, 2002	02	Procedure Updates; Collation of Procedures and use of WP Outline; Change in page numbering (Change in DNA Technical Procedures)
August 7, 2003	03	Addition of Table of Contents; no substantive changes in any procedure. (Change in DNA Technical Procedures)
July 19, 2004	04	Made "Gel Electrophoresis" Procedure a separate document. No technical change in SOP.

Approval Signatures	Date
Author/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	