1 Thaw the STR 10X Buffer, Amelogenin Primers, PowerPlex™ Primer Pair Mix, PowerPlex™ D16 Add-In primers (if appropriate) and BSA if not already thawed. Vortex each tube thoroughly before using and keep cold.

2 Prepare the PowerPlex™ Master Mix Preparation Worksheet for 1.1 or 2.1. Determine the number of samples to be amplified. Be sure to include K562, the negative amplification control, and MJB. Add four (4) additional reactions to this number to compensate for pipetting variations.

3 Label the appropriate number of PCR reaction tubes and place them in the amplification tray.

4 Add the sufficient amount of sterile dH_20 to each tube.

5 Pipette samples into tubes.

5.1 PowerPlex 1.1

5.1.1 Pipette DNA from each sample (0.5 to 1 ng) into each respective tube to bring to the appropriate volume of 13.5 µl.

5.1.2 For the positive amplification control (K562), add 0.1 to 0.25 µl of K562 DNA to an appropriate amount of sterile dH_20 to equal 13.5 µl.

5.1.3 For the negative amplification control, add 13.5 µl of sterile dH_20.

5.2 PowerPlex 2.1

5.2.1 Pipette DNA from each sample (0.5 to 1 ng) into each respective tube to bring to the appropriate volume of 18.5 µl.

5.2.2 For the positive amplification control (K562), add 0.1 to 0.25 µl of K562 DNA to appropriate amount of sterile dH_20 to equal 18.5 µl.

5.3 For the negative amplification control, add 18.5 µl of sterile dH_20.

****NOTE - The volumes above are based on a 25 µl amplification volume. Analysts may elect to double the amplification volume if they feel that inhibitors may be present. Members of the DNA database may use 12.5 µl reactions in which 2.75 µl of master mix is added to 9.75 µl of water/template. For the positive amplification control (K562), add 0.15 µl of K562. ****

6 Calculate the required amount of each component of the PCR master mix. Multiply the volume (µl) per sample by the total number of reactions to obtain the final volume (µl) per the Powerplex Master Mix Preparation Worksheet.

7 Add the final volume of each reagent into a sterile tube and gently mix (do not vortex). Add *Taq* Gold polymerase last.

8 Add 11.5 µl of the PCR Master Mix to each sample tube for Powerplex 1.1 reactions, and 6.5 µl to each for Powerplex 2.1 reactions. Cap tubes and place rack containing tubes into the thermocycler. Members of the DNA Database use 2.75 µl for both.

9 Turn on the thermocycler. Select the appropriate cycle on the:

For Powerplex 1.1 and 2.1 reactions using *Taq* Gold, use :

95°C for 11 minutes
96°C for 2 minutes, then:

94°C for 60 seconds
Ramp to 60°C, hold for 60 seconds
Ramp to 70°C, hold for 90 seconds
For 10 cycles, then:

90°C for 60 seconds
Ramp to 60°C, hold for 60 seconds
Ramp to 70°C, hold for 90 seconds
For 20 cycles, then:

60°C for 30 minutes, then:

4°C for infinite hold (to refrigerate until analyst takes samples out of thermocycler).

NCSBI Forensic Biology Section	DNA SOP	Effective Date: July 19, 2004
Title: **PowerPlex™ 1.1 and 2.1 Amplification**		Revision 04

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 "DNA Amplification" Procedure into separate document. Changed name to "PowerPlex™ 1.1 and 2.1 Amplification". No technical changes in SOP.

APPROVAL SIGNATURES	Date
Author/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	