Procedure for PCR Amplification for Casework

1.0 Purpose - This procedure specifies the steps for performing PCR amplification with AmpFℓSTR® Identifiler® Plus.

2.0 Scope - This procedure applies to casework analysts and trainees in the Forensic Biology Section who perform amplification with AmpFℓSTR® Identifiler® Plus.

3.0 Definitions - N/A

4.0 Equipment, Materials and Reagents

- Centrifuge
- GeneAmp® PCR System 9700 Thermal Cycler
- Calibrated Pipets
- ART Pipet Tips (or equivalent, various sizes)
- Sterile 1.5 mL microcentrifuge tubes
- PCR reaction tubes (including individual tubes or strip tubes with caps)
- AmpFℓSTR® Identifiler® Plus Reagents
- Extracted DNA
- STR TE
- Biosafety amplification cabinet
- Bleach
- Bleached and clean amp trays
- Vortex

5.0 Procedure

5.1 No more than 30 total items (including controls) per analyst may be processed simultaneously.

5.2 Thaw the AmpFℓSTR® Identifiler® Plus Master Mix, Primer Set and Control DNA 9947A. Vortex each tube thoroughly before using and keep cold. New kits shall be kept in the freezer; once a kit has been thawed, it may be stored at 4 °C.

5.3 Prepare the Amplification Worksheet. Determine the number of samples to be amplified. Include 9947A and the negative amplification control. The Forensic Scientist may amplify a lower or higher amount based on training and experience if inhibition is noted.

5.4 Calculate the required amount of each component for the PCR Master Mix. Multiply the volume (µL) per sample by the total number of reactions to obtain the final volume (µL). To account for pipetting variation, add 2 for every set of 16 samples. The volumes for the PCR Master Mix are 10 µL per sample for the Identifiler® Plus Master Mix and 5 µL per sample for the Primer Set.

5.5 Label the appropriate number of PCR reaction tubes (individual or strip) and place them in an amplification tray for the 9700 thermal cycler. If strip tubes are used, the first and last tube in each strip that contain sample shall be labeled using a sticker-type label. The label shall contain the distinguishable

portion of the case number (e.g., R11-12345) and the item number or sample contained within (e.g., 9947A, Ladder). The intermediate tubes shall be labeled to permit the Forensic Scientist to determine the sample contained in each tube. This determination can be made in conjunction with the plate map (3130XL set-up sheet). If individual tubes are used, each tube shall be labeled using a sticker-type label with the distinguishable portion of the case number (e.g., R11-12345) and the item number or sample contained within (e.g., 9947A, Ladder).

5.6 Add the final volume of each reagent to make a PCR Master Mix in a sterile 1.5 mL microcentrifuge tube and mix. Spin down the tube to remove any liquid from the lid.

5.7 Add 15 µL of the PCR Master Mix to each sample tube.

5.8 Add the amount of sterile TE to each tube as calculated per the Amplification Worksheet.

5.9 Pipette DNA samples and controls into each tube as calculated per the Amplification Worksheet.

5.9.1 Make dilutions for DNA samples as required by the DUO Dilution Calculation Worksheet. If dilutions are made, use the same TE for the negative amplification control as used for the dilutions.

5.10 Cap tubes and spin amplification tray containing tubes to ensure all sample is seated at the bottom of the tubes and no bubbles are present.

5.11 Place amplification tray containing tubes onto the thermal cycler.

5.12 Turn on the thermal cycler. To select the appropriate cycle on the 9700 thermal cycler, press F1, use the arrows on the thermal cycler and move them until "if plus 28cyc" is highlighted. All amplifications shall have a 25 µL volume set on the thermal cycler. Start the thermal cycler run. The program is pre-recorded as follows:

95 °C for 11 minutes, then:

94 °C for 20 seconds
59 °C for 3 minutes

For 28 cycles, then:

60 °C for 10 minutes, then;

4 °C for infinite hold (to refrigerate until the Forensic Scientist takes the samples out of the thermal cycler).

6.0 Limitations - Amplified products cannot be used after one month of being generated without the approval of the DNA Technical Leader. Additionally, if AmpFℓSTR® Identifiler® Plus kit lot numbers change during the one month period (due to expiration or supply exhaustion), the Forensic Scientist shall re-amplify the

DNA extracts. If there is not enough DNA extract to re-amplify, the Forensic Scientist shall discuss how to proceed with the DNA Technical Leader.

7.0 Safety - N/A

8.0 References

Forensic Biology Section Procedure for DNA Casework Training

Forensic Biology Section Procedure for Calibration and Equipment Maintenance

Forensic Biology Section Procedure for DNA Reagent Preparation and Quality Control

9.0 Records

- Amplification set-up worksheet (to be used for QC and training)

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
12/31/2012	1	Original Document
05/30/2013	2	5.5 – clarified labeling requirement
07/31/2013	3	5.1 – changed 4 case batch limit to sample limit of 30 samples at a time