

Procedure for PCR Amplification with Identifiler™

1.0 Purpose- This procedure specifies the steps for performing PCR amplification using ABI Identifiler™.

2.0 Scope- This procedure applies to analysts in the Forensic Biology Section who perform PCR amplification using ABI Identifiler™.

3.0 Definitions – N/A

4.0 Equipment, Materials, and Reagents

- Centrifuge
- 9700 Thermal cycler
- Calibrated Pipets
- ART Pipet Tips (or equivalent, various sizes)
- Sterile 1.5 µL microcentrifuge tubes
- PCR reaction tubes (including individual tubes, or strip tubes with caps)
- ABI AmpFℓSTR Identifiler™ Reagents
- AmpliTaq Gold polymerase (Taq)
- Extracted DNA
- TE
- BSA (optional)
- Biosafety amplification cabinet
- Bleach
- bleached and clean amp trays
- Vortex

5.0 Procedure

5.1 For casework, a maximum of four (4) cases can be amplified at the same time.

5.2 Thaw the AmpFℓSTR PCR Reaction Mix, Primer Set, and Control DNA 9947A. Vortex each tube thoroughly before using and keep cold. New kits are kept in the freezer; once the kit has been thawed it may be stored at 4 °C. Taq is always stored at -15 °C to -25 °C, except when in use for preparation of the master mix.

5.3 Prepare the Amplification Worksheet. Determine the number of samples to be amplified. Include 9947A, the negative amplification control, and MJB if applicable. For database samples amplified manually, 0.5 µL of DNA is the standard amplification volume; however, the Forensic Scientist/Database Analyst may amplify a lower or higher amount based on training and experience.

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). See the Amplification Worksheet for volumes for each component. Additionally, up to 1 µL total of Taq and 0.5 µL to 1 µL of BSA may be added per sample to samples and corresponding negative controls where inhibitors may be present. If only added to certain samples and associated controls, then it must be noted which samples received extra.

5.5 Label the appropriate number of PCR reaction tubes 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 containing sample shall be labeled using a sticker-type label. If the sample is bar-coded, the label shall consist of the entire barcode number (NC may be removed) or distinguishable portion of the batch number and sample present in the tube (e.g., Ladder, 9947A, etc.). If the sample is not bar-coded, the label shall consist of the last two digits of the year and the specimen identification number. 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 as described above.

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. (Add Taq last.) Spin down 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 ABI 7000 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, using the arrows on the thermal cycler and move them until "Identifiler" is highlighted. All amplifications except for database amplifications set up using the Qiagen BioRobot® shall have a 25 µL volume set on the thermal cycler; database amplifications set up using the Qiagen BioRobot® shall have a 13 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 1 minute

59 °C for 1 minute

72 °C for 1 minute

For 28 cycles, then:

60 °C for 60 minutes, then:

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

6.0 Limitations- Amplified products have an expiration date of one month after they are generated; however, if it is necessary for the Forensic Scientist/DNA Database Analyst to use amplified product longer than one month, the approval of the DNA Technical Leader shall be obtained for both the use of the amplified product and the

resulting data. Additionally, if Identifiler™ kit lot numbers change during the one month period (due to expiration or supply exhaustion), the Forensic Scientist/DNA Database Analyst shall re-amplify the DNA extracts. If there is not enough DNA extract to reamplify, the Forensic Scientist/DNA Database Analyst 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 DNA Database Training

Forensic Biology Section Procedure for Performance Check and Equipment Maintenance

Forensic Biology Section Procedure for Qiagent BioRobot® Universal

9.0 Records

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

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document
05/30/2013	2	5.5 – clarified labeling requirements