# DNA Database Procedure for PCR Amplification with Identifiler™

**1.0 Purpose**- To specify the steps for performing PCR amplification using Identifiler™.

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

**3.0 Definitions** – N/A

**4.0 Equipment, Materials, and Reagents**

- Centrifuge
- 9700 Thermal cycler
- Calibrated Pipettes
- ART Pipette 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
- Biosafety amplification cabinet
- 10 % Bleach
- 70 % Isopropyl Alcohol
- Bleached and clean amp trays
- Vortex

**5.0 Procedure**

**5.1** Obtain the AmpFℓSTR PCR Reaction Mix, Primer Set, and Control DNA 9947A. Vortex each tube 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.

**5.2** 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 DNA Database Forensic Scientist may amplify a lower or higher amount based on training and experience.

**5.3** 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.

**5.4** 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 DNA Database 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.

**5.5** 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.

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

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

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

**5.9** 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.

**5.10** Place amplification tray containing tubes onto the thermal cycler.

**5.11** 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 database amplifications shall have a 25 µL volume set on the thermal cycler, except for amplifications set up using the Qiagen BioRobot®in which the reaction volume is set for a 12.5 ul reaction. If the thermal cycler requires reaction volumes to be entered as whole numbers, set the reaction volume to 13 ul. 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 DNA Database Forensic Scientist 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 DNA Database Forensic Scientist 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 DNA Database Forensic Scientist shall re-amplify the DNA extracts. If there is not enough DNA extract to re-amplify, the DNA Database Forensic Scientist shall consult with the DNA Technical Leader and thereafter proceed as directed.

**7.0 Safety** - N/A

**8.0 References**

DNA Database Section Procedure for DNA Database Training

DNA Database Section Procedure for Calibration and Equipment Maintenance

DNA Database Section Procedure for Qiagen 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 |
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