1 Sample Amplification Using ABI Identifilier™

1.1 Thaw the AmpFlSTR PCR Reaction Mix, Primer Set, and Control DNA 9947A. Vortex each tube thoroughly before using and keep cold.

1.2 Prepare the Identifiler™ Master Mix Preparation Worksheet. Determine the number of samples to be amplified. Be sure to include 9947A, the negative amplification control, and MJB.

1.3 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 Identifiler™ Master Mix Preparation Worksheet. The formulation on the amplification worksheet has already been adjusted for a sleight overfill to allow for volume lost in pipetting.

1.4 Add the final volume of each reagent to make a PCR Master Mix in a sterile 1.5 ml microcentrifuge tube and gently mix (do not vortex). Add *AmpliTaq* Gold polymerase last.

1.5 Label the appropriate number of PCR reaction tubes and place them in an amplification tray for the 9700 thermocycler.

1.6 Add the sufficient amount of sterile TE to each tube.

1.7 Add 15 µl of the PCR Master Mix to each sample tube.

1.8 Pipette samples into tubes for total DNA + TE = 10.0.

1.8.1 Pipette DNA from each sample (0.5 to 1 ng) into each respective tube to bring to the appropriate volume of 10 µl.

1.8.2 For the positive amplification control (9947A), add 10 µl of 9947A.

1.8.3 For the negative amplification control, add 10 µl of sterile TE.

1.9 Cap tubes and place rack containing tubes into the thermocycler.

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. ****

1.10 Turn on the thermocycler. Select the appropriate cycle on the 9700 thermocycler:

For Identifiler™ reactions using *AmpliTaq* Gold, use :

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 analyst takes samples out of thermocycler).

Revision History		
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