5 PCR AMPLIFICATION

5.1 TECHNICAL NOTES

5.1.1 Taq DNA Polymerase, purified from *Thermus aquaticus*, attaches nucleotides in a 5'--->3' direction to extend the DNA chain.

5.1.2 **STRs differ from VNTRs and Dinucleotide repeats in that the repeat unit length for these markers range from 3 to 7 bases.**

5.1.3 **The PowerPlex® 16 BIO System contains 16 sets of primers and allows for the co-amplification and three-color detection of 16 loci (15 STR loci and Amelogenin), which include the Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818 loci.**

5.1.4 A random sample will be run with each set of convicted offender and arrestee sample amplifications to serve as a verification that the samples are successfully being entered into Combined DNA Index System (CODIS) and the search algorithm is working properly.

5.1.5 If a sample must be re-amplified, providing that the random sample has amplified, the random sample does not need to be re-amplified. However, the Control DNA (GM9947A Cell Line – positive amplification control) and negative amplification control must be amplified with the re-amplified sample.

5.1.6 If a batch of samples will be amplified using the BioMek® 2000 Automation Workstation as few as one amplification negative control and amplification positive (GM9947A) may be amplified per BioMek® 2000 Automation Workstation run. However, in order to report the typing results for the evidence samples associated with these controls, the controls must work appropriately in accordance with the procedures outlined in Chapter 9, Interpretation of The PowerPlex® 16 BIO System PCR Amplification Results.

5.2 EQUIPMENT

5.2.1 Thermal Cycler – 9600 or 9700

5.2.2 Pipettes - 2 µL, 10 µL, 20 µL, 100 µL and/or 200 µL

5.2.3 8- Channel Pipette – Range 0.5 µL to 10 µL

5.2.4 Biological Safety Hood

5.2.5 Microcentrifuge tube racks

5.3 MATERIALS

5.3.1 Sterile ART pipette tips - 2 µL, 10 µL, 20 µL, 100 µL and/or 200 µL

5.3.2 1.5 mL microcentfuge tubes

5.3.3 Kimwipes

5.3.4 Sterile MicroAmp® tubes (0.2 mL tubes)

5.3.5 Gloves

5.3.6 96 well amplification plates and strip caps

5.4 REAGENTS

5.4.1 Powerplex® 16 BIO System Primer Pairs

5.4.2 Sterile Type I Water

5.4.3 Gold ST*R 10X Buffer

5.4.4 Control DNA (GM9947A Cell Line)

5.4.5 AmpliTaq™ Gold DNA Polymerase

5.5 POWERPLEX® 16 BIO SYSTEM AMPLIFICATION PROCEDURE FOR THE 9600 AND 9700 THERMAL CYCLER

NOTE: A dedicated area, such as a biological hood or a separate room, should be used for preparing PCR amplification reactions. All equipment and supplies used to prepare amplification reactions should be kept in this dedicated "clean" area at all times. Do not use these items to handle amplified DNA or other potential sources of contaminating DNA. Trace amounts of amplified DNA, if carried over into other samples before amplification, can lead to results that are not interpretable. DO NOT bring amplified DNA, or equipment and supplies used to handle amplified DNA, into the designated "clean" area.

Wear clean disposable laboratory gloves while preparing samples for PCR amplification. Change gloves frequently or whenever there is a chance they have been contaminated with DNA.

5.5.1 Amplification Parameters for the 9600 and 9700 Thermal Cyclers:

5.5.1.1 Turn on the 9600 thermal cycler. Program as follows using the step cycle file, change the default setting to:

Set a hold file for 11 minutes at 95^0C.

Link this hold file to a separate hold file to denature at 96°C for 1 minute.

Link this preheat program to a separate program as follows:

Ramp 0 seconds to denature at 94°C for 30 seconds
Ramp 68 seconds to anneal at 60°C for 30 seconds
Ramp 50 seconds to extend at 70°C for 45 seconds

Program for 10 cycles

Link this program to a separate program as follows:

Ramp 0 seconds to denature at 90°C for 30 seconds
Ramp 60 seconds to anneal at 60°C for 30 seconds
Ramp 50 seconds to extend at 70°C for 45 seconds

Program for 21 cycles

Link to a 60°C hold file for 30 minutes
Link to a 4°C hold cycle. This program may be saved as a "User File" for later use.

5.5.1.2 Turn on the 9700 thermal cycler. Program as follows using the step cycle file, change the default setting to:

Set a hold file for 11 minutes at 95^0C.

Link this hold file to a separate hold file to denature at 96°C for 1 minute.

Link this preheat program to a separate program as follows:

Ramp 100% to denature at 94°C for 30 seconds
Ramp 29% to anneal at 60°C for 30 seconds
Ramp 23% to extend at 70°C for 45 seconds

Program for 10 cycles

Link this program to a separate program as follows:

Ramp 100% to denature at 90°C for 30 seconds
Ramp 29% to anneal at 60°C for 30 seconds
Ramp 23% to extend at 70°C for 45 seconds

Program for 21 cycles

Link to a 60°C hold file for 30 minutes
Link to a 4°C hold cycle. This program may be saved as a "User File" for later use.

NOTE: The PowerPlex® 16 BIO Allelic Ladder, Fluorescent Internal Lane Standard 600 BIO, Matrix 16 BIO and Bromophenol Blue Loading Buffer, and Tracking Dye supplied in the PowerPlex® 16 BIO System kit do not require amplification and should not be stored in the sample preparation area.

5.5.2 Transfer the PowerPlex® 16 BIO System amplification reagents to the designated "clean" area and allow the Gold ST*R 10X Buffer, GM9947A Cell Line and the PowerPlex® 16 BIO 10X Primer Pairs to thaw completely at room temperature.

5.5.3 Place the required number of labeled sterile MicroAmp® reaction tubes in a rack.

5.5.4 Calculate the required volume of each PCR amplification component to prepare a master mix by multiplying the volume by the number of samples. This should include reagent blanks, plate blanks (associated only with samples analyzed on the BioMek® 2000 Automation Workstation), a random sample (associated only with the analysis of offender and arrestee samples), and positive and negative amplification controls and two to three additional reaction volumes (depending on the number of samples being amplified) to compensate for any pipetting variation. The PCR Master Mix should be prepared in a clean 1.5 mL microcentrifuge tube.

Half Reaction Master Mix:

1.25 µL Gold ST*R 10X Buffer
1.25 µL PowerPlex® 16 BIO 10X Primer Pairs
4.60 µL Sterile Type I Water
0.40 µL AmpliTaq™ Gold Enzyme (2 Units)

5.5.5 Add 7.5 µL of PCR Master Mix to each labeled sterile MicroAmp® tube or well of the 96 well plate using sterile ART tips. Cap each tube and ensure that the solution is at the bottom of the tube by gently tapping each tube on the counter top.

NOTE: Each PowerPlex® 16 BIO System amplification is performed in a final volume of 12.5 µL. Exactly 5 µL has been allocated for the sample DNA. However, when a sample contains a low level of DNA (e.g., 0.05 ng/µL or less DNA) estimated based upon the AluQuant® Human Quantitation System, 9.6 µL of sample DNA may be used and the water removed from the Master Mix cocktail.

No more than one tube should be opened at a time when making the following additions. Use a new sterile ART tip for each addition.

5.5.6 Prepare the sample DNA and controls as follows:

For convicted offender or arrestee samples extracted using the QIAamp® manual or robotic extraction procedure, proceed to step 5.5.6.1.1.

5.5.6.1 Sample DNA (Evidence Samples): Open the MicroAmp® tube and carefully add 5 µL of DNA (0.50 to 1.0 ng/5 µL) to the appropriate labeled sample tube. Discard the pipette tip and recap the tube tightly before proceeding to the next sample. **DO NOT** vortex, mix or spin. If the DNA concentration is greater than 0.2 ng/µL, dilute a portion of it with sterile Type I Water so that no more than 1.0 ng of DNA will be added to the PCR reaction in a volume of 5 µL. If the sample DNA concentration is less than 0.10 ng/µL add 5 µL of the sample DNA to the PCR reaction mix. However, if the sample contains low level DNA (e.g., 0.05 ng/µL or less), 9.6 µL of sample DNA may be used during the amplification step.

NOTE: If a **STRONG** signal was observed using the AluQuant™ Human Quantitation System, but **NO** amplified DNA was observed on the product gel a higher concentration (i.e., 1.5 ng) of the isolated DNA may be used to enhance the possibility of obtaining amplified product from a highly degraded sample.

5.5.6.1.1 **Dried blood stains/buccal cells (Offender and Arrestee Samples ONLY):** Add 2.0 µL (blood - robotically), 1.5 µL (blood – manually), 1.0 µL (buccal -robotically), or 1.0 µL (buccal – manually) of extracted DNA to the appropriate labeled sample tube. Bring the total volume up to 5 µL by adding sterile Type 1 Water to the tube. NOTE: weaker/difficult samples may require a higher volume than is specified to obtain a complete DNA profile.

Known Standards and Random Sample (Convicted Offender and Arrestee Samples): Open the MicroAmp® tube and carefully add 5 µL of DNA for half reaction (0.50 to 1.0 ng/5 µL) to the appropriate labeled sample tube. Discard the pipette tip and recap the tube tightly before proceeding to the next sample. **DO NOT** vortex, mix or spin. If the DNA concentration is greater than 0.2 ng/µL, dilute a portion of it with sterile Type I Water so that no more than 1.0 ng of DNA will be added to the PCR reaction in a volume of 5 µL. If the sample DNA concentration is less than 0.10 ng/µL add 5µL of the sample DNA to the PCR reaction mix. However, if the sample contains low level DNA (e.g., 0.05 ng/µL or less), 9.6 µL of sample DNA may be used during the amplification step.

5.5.6.2 Positive Amplification Control: Gently mix by hand the Control DNA (GM9947A Cell Line) followed by a light tapping of the tube on the counter top to remove any liquid from the cap. Open the MicroAmp® tube and carefully add 5 µL of DNA (0.75 to 1.0 ng/5 µL) of the Control DNA to the appropriate labeled sample tube.

NOTE: When preparing the stock Control DNA (GM9947A Cell Line), dilute the sample to a concentration of 0.15 to 0.20 ng/µL with sterile Type I Water.

5.5.6.3 Negative Amplification Control: Open the MicroAmp® tube and carefully add 5 µL of sterile Type I Water to the appropriate labeled sample tube. Note: if a volume of 9.6 µL of sample DNA will be used for any samples during the amplification step, 9.6 µL of the Negative Amplification Control will be used.

5.5.6.4 Reagent Blank(s) or Plate Blank(s): Open the MicroAmp® tube and carefully add 5 µL of the reagent blank(s)/plate blank(s) to the appropriate labeled sample tube. Note: if a volume of 9.6 µL of sample DNA will be used for any samples during the amplification step, 9.6 µL of the reagent blank/plate blank will be used.

5.5.7 Place the MicroAmp® tubes into the thermal cycler. Push the tubes down completely into the block. Place the thermal cycler lid over the samples and tighten.

5.5.8 When the thermal cycler prompts the user for the sample volume, choose 13 µL.

5.5.9 Start the amplification file and verify the cycling parameters by monitoring the first cycle.

NOTE: The PCR amplification program will run for approximately 3.5 hours.

5.5.10 After the amplification process is complete remove the tubes from the thermal cycler and proceed with all samples to Section 6, Product Gel For Determining Amplified Product. **EXCEPTION: It is not necessary to evaluate convicted offender or arrestee samples that have been isolated using the QIAamp® robotic extraction procedure on a product gel. Therefore, proceed with all samples to Section 7, Vertical Gel Electrophoresis.**

5.5.11 If the sample will not be loaded into the product gel within a short time period, the samples should be stored in the dark at 2° to 8° C for a few hours or at -20° C for extended periods.

NOTE: Store amplified DNA samples separate from PCR pre-amplification reagents and extracted DNA samples.

♦**END**