PCR AMPLIFICATION USING APPLIED BIOSYSTEMS® GLOBALFILER™ KITS

Background

This protocol allows for the amplification of DNA at the D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338, DYS391, Y indel, and Amelogenin loci in a single amplification reaction.

The GlobalFiler™ kit combines the 13 original CODIS loci with 7 from the expanded European Standard Set of Loci (ESSL) and the highly discriminating SE33 locus. The kit delivers a 24-locus multiplex with the highest discrimination power of any Thermo Fisher Scientific Human Identification Kit, along with high sensitivity and tolerance to inhibitors. The concentration of 10 mini-STR loci that are entirely below 220 bp maximizes performance on degraded samples. The highly optimized buffer formulation contains an enzyme that allows completion of amplification in ~80 minutes. The GlobalFiler™ kit uses the same improved process for synthesis and purification of the amplification primers developed for other next-generation Thermo Fisher Scientific STR chemistries. The improved amplification primers deliver clean electrophoretic backgrounds that assist interpretation.

Summary of Procedure

A master mix of GlobalFiler™ Master Mix and GlobalFiler™ Primer Set is prepared and added to each amplification tube. The DNA samples are added and amplified using the GeneAmp 9700 Thermal Cycler with specific cycling parameters. The total reaction volume is 25 uL.

Sample Handling

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- ***Safety Manual***, *Universal Precautions*
- ***Quality Assurance Manual***, *General Sample Control and Forensic Sample Preservation Policy*
- ***Analytical Procedures Manual***, **Forensic Evidence Handling**

Reagents and Materials

See **Appendix B** for reagent preparation

GlobalFiler™ Amplification Kit:
- **GlobalFiler™** Master Mix
- **GlobalFiler™** Primer Set
- DNA Control 007

10% bleach (or other decontaminate solution)
DNA Suspension Buffer
Gloves
Lab Coat
Kimwipes
Rainin Repeat Pipettor, EDP-2
Single Channel Pipettors (0.5 - 10uL, 5 - 40uL, 20 - 200uL)
Multi-Channel Pipettor (0.5-10uL)
Universal Tips, sterile
0.5 – 10uL tips, sterile (ART – not required)
Microcentrifuge tubes, 1.7mL
0.2mL 8/strip amplification tubes and singles

8/strip amplification caps and singles
Optical 96 well reaction plate
96 well – base
Tube rack
Permanent marker
Microcentrifuge
CL3 Centrifuge
Vortex
9700 GeneAmp Thermal Cycler

Reagents and Materials – Storage and Handling

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the **GlobalFiler Amplification Worksheet** and an appropriate **Sample Orientation Worksheet** is required for documentation. All information must be completed.

Positive Control

Extraction Positive Control (Optional): Used to ensure that the extraction, amplification and typing procedures are working as expected.

Kit Positive Amplification Control (007): Ensures that the amplification and typing processes are working as expected when amplifying liquid extraction samples. This control is included in the GlobalFiler Kit.

Negative Control

Extraction Negative Control: Reagent negative control(s) are processed and run with each set of extractions. This negative control consists of all reagents used in the procedure but contains no DNA sample.

Amplification Negative Control: Reagent negative control processed and run with each set of amplifications. This negative control consists of all reagents used in the amplification procedure but contains no DNA sample.

Procedure

Before starting:

- Clean the work area using decontaminate solution and Kimwipes before starting.

1. Briefly vortex the GlobalFiler™ Master Mix, GlobalFiler™ Primer Set and kit positive amplification control (007). Spin the tubes in a microcentrifuge to remove any liquid from the caps.

Full Reaction Volume Procedure

2. Prepare a master mix in a 1.7mL-microcentrifuge tube based on the number of samples, including an extraction positive control if applicable, amplification negative control, extraction negative control and Kit Positive Control (007).

    - Total number of samples + ___ × 7.5uL GlobalFiler™ Master Mix
    - Total number of samples + ___ × 2.5uL GlobalFiler™ Primer Set

3. Mix thoroughly with a brief vortex and spin the tube in a microcentrifuge to remove any liquid from the cap.

4. Dispense 10uL of the master mix into each amplification tube.

5. Aliquot DNA Suspension Buffer into a 1.7mL microcentrifuge tube (about 10uL times the number of samples).

6. Prepare samples in the following manner below

Half Reaction Volume Procedure

2. Prepare a master mix in a 1.7mL-microcentrifuge tube based on the number of samples, including an extraction positive control if applicable, amplification negative control, extraction negative control and Kit Positive Control (007).

   - Total number of samples + ___ × 3.75 uL GlobalFiler™ Master Mix
   - Total number of samples + ___ × 1.25 uL GlobalFiler™ Primer Set

3. Mix thoroughly with a brief vortex and spin the tube in a microcentrifuge to remove any liquid from the cap.

4. Dispense 5 uL of the master mix into each amplification tube.

5. Aliquot DNA Suspension Buffer into a 1.7 mL microcentrifuge tube (about 10uL times the number of samples).

6. Prepare samples in the following manner below

Note: Complete the extract transfer process by capping each tube (or strip of tubes) of extract before proceeding to the next tube (or strips of tubes), if applicable.

Sample tubes: For each sample, determine the volume of sample and volume of DNA Suspension Buffer that would constitute a recommended target of 1.0 ng of DNA in a total volume of 15 uL for full reaction or target 0.5 – 2.0 ng in a total volume of 7.5 uL for half reaction then add to the corresponding sample tube. The target amount of DNA may vary according to sample quality; recommended target range is 0.25 - 2.0 ng. An across the board load for samples extracted on the QIAsymphony is 4 uL.

If the following targets cannot be achieved for a full reaction GlobalFiler™ amplification (25 uL):

- Total Small Autosomal DNA: **≥0.038ng**
- Total Human Male DNA: **≥0.0266ng**

It is appropriate to cease testing at this point. These are considered the "stop at quant" values from Quantifiler® Trio for GlobalFiler™.

If the maximum obtainable ***small autosomal amp target is ≥ 0.038ng***, but the maximum obtainable ***Human Male amp target is < 0.0266ng***, a meaningful male DNA profile (or mixture) is unlikely to be produced. If the male DNA profile is the probative profile, the Human Male stop at quant value will be utilized.

The "stop at quant" values will be utilized for a differential (sperm and epithelial fractions), when male DNA is probative, as follows:

- If a sufficient quantity of male DNA is detected in the sperm fraction, *both* fractions will be amplified.
- If the sperm fraction gets stopped at quant and there is an insufficient amount of male DNA in the epithelial fraction, then both fractions will stop at quant.

- If the sperm fraction gets stopped at quant but there is a sufficient quantity of male DNA in the epithelial fraction, only the epithelial fraction will be amplified.

Extraction Positive Control (Optional):

- For full reaction volume: Determine the volume of sample and volume of DNA Suspension Buffer that would constitute a recommended target 1.0 ng of DNA in a total volume of 15 uL and add to each positive control tube.
- For half reaction volume: Determine the volume of sample and volume of DNA Suspension Buffer that would constitute a recommended target 0.2 – 2.0 ng of DNA in a total volume of 7.5 uL and add to each positive control tube

Kit Positive Amplification Control (007): Vortex the control DNA and spin briefly in a microcentrifuge before use to remove any liquid from the cap.

- For full reaction volume: Add 10 uL of the control DNA and 5 uL of DNA Suspension Buffer to each kit positive control tube.
- For half reaction volume: Add 5 uL of the control DNA and 2.5 uL of DNA Suspension Buffer to each kit positive control tube

Extraction Negative Control: Amplify using same concentration conditions as required by the samples containing the least amount of DNA template.

Amplification Negative Control:

- For full reaction volume: Add 15 uL of DNA Suspension Buffer to each amplification negative control tube.
- For half reaction volume: Add 7.5 uL of DNA Suspension Buffer to each amplification negative control tube.

A witness must observe ALL above sample additions when manually added and initial the appropriate space on the worksheet.

7. Cover the tubes with a 96 well full plate cover or caps. Clean the work area using decontaminate solution and Kimwipes and expose to UV light for 30 minutes.

8. Wrap the tray in a Kimwipe; remove lab coat and gloves, before carrying the tray into the PCR Laboratory vestibule. Put on dedicated lab coat before entering the PCR lab.

9. Briefly vortex the tray and spin. Make sure all bubbles are removed from the bottom of the tubes after the spin.

10. Remove the tray from the base and place tray on thermal cycler. Place a compression pad over the caps to prevent melting of the plastic and close the lid.

    If using a Veriti thermal cycler:

    - Touch the screen to wake up the thermal cycler.
    - Touch the power button in the lower left corner (if not already powered on).
    - Touch the amplification program you want to use –Globalfiler is a shortcut on the main screen.
    - Enter 25uL reaction volume (regardless of full or half amp)
    - Touch run
    - At the end of the amplification run, the screen will appear for the post-run report. Save the report on the USB drive and transfer the file to the network in the appropriate amplification ID folder using the computer in the PCR lab. This report may be printed and included with the amplification worksheets.

Once amplification is complete, continue on to “Preparing the Samples for Analysis”. If not immediately running samples on the genetic analyzer, remove the retainer clip and cap the tubes or seal the 96 well plate with a foil cover. Store as described below in “Comments on Storage”.

Comments on Storage

Amplified product must be stored separately from reagents used in PCR set-up and non-amplified DNA. Store at -15 to -25°C. The post-amplification product cannot leave the PCR laboratory.

Technical Assistance

For information and assistance regarding the performance or applications, contact Applied Biosystems between 5:30 a.m. and 5:00 p.m. pacific time at 1-800-955-6288 (option 4). Or contact Applied Biosystems by email at customerservice@lifetech.com.

Reference

GlobalFiler™ PCR Amplification Kit User Guide – Applied Biosystems