PCR AMPLIFICATION USING INVESTIGATOR® 24PLEX QS DNA PROFILING KIT

Background

This latest kit is a 24-locus multiplex from Qiagen in Hilden, Germany. It allows for the amplification of the 22 autosomal loci in the expanded CODIS core loci (CSF1PO, FGA, TH01, TPOX, vWA, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, and SE33), Amelogenin and DYS391 for gender determination, and two internal control loci (Q and S) that can help distinguish sample degradation from sample inhibition.

Summary of Procedure

A master mix of Investigator® 24plex QS Master Mix and Primer Set is prepared and added to each amplification tube. The DNA samples are amplified using any thermal cycler with specific cycling parameters. The total reaction volume is 25 uL with the option to add up to 15uL extracted DNA sample targeting 500-1000pg total human DNA.

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

Investigator® 24plex QS DNA Profiling Kit:
- Investigator® 24plex QS Master Mix
- Investigator® 24plex QS DNA Primer Set
- DNA Control 9948

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 or Veriti 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 **Investigator® 24plex QS DNA Profiling Kit 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 (9948): Ensures that the amplification and typing processes are working as expected when amplifying liquid extraction samples. This control is included in the Investigator® 24plex QS DNA Profiling 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 Investigator® 24plex QS Master Mix, Investigator® 24plex QS Primer Set and kit positive amplification control (9948). Spin the tubes in a microcentrifuge to remove any liquid from the caps.

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 (9948).

    - Total number of samples + ___ × 7.5uL Investigator® 24plex QS Master Mix
    - Total number of samples + ___ × 2.5uL Investigator® 24plex QS 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

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 0.5 - 1.0 ng of DNA in a total volume of 15 uL and add to the corresponding sample tube. The target amount of DNA may vary according to sample quality; recommended target range is 0. 5 - 1.0 ng.

Extraction Positive Control (Optional): 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.

Kit Positive Amplification Control (9948): Vortex the control DNA and spin briefly in a microcentrifuge before use to remove any liquid from the cap. Add 5 uL of the control DNA (to target 0.5ng DNA) and 10 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: Add 15 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, place tray thermal cycler, 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 –"24plex" is a shortcut on the main screen.
    - Enter reaction volume
    - 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 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 Qiagen using their website: www.qiagen.com.

Reference

Qiagen. Investigator® 24Plex QS Handbook. July, 2016.