PCR AMPLIFICATION USING PROMEGA® POWERPLEX® FUSION 6C KIT

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, DYS576, DYS570, Penta D, Penta E, and Amelogenin loci in a single amplification reaction.

The PowerPlex® Fusion 6C kit combines the 13 original CODIS loci with 7 from the expanded European Standard Set of Loci (ESSL), the highly discriminating SE33 locus, three Y-STR loci, and the sex determining marker, Amelogenin. The kit delivers a 27-locus multiplex with the highest discrimination power of any Promega® Amplification Kit, along with high sensitivity and tolerance to inhibitors. The concentration of 8 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 ~60 minutes. The Fusion 6C kit uses the same improved process for synthesis and purification of the amplification primers developed for other next-generation Promega® STR chemistries. The improved amplification primers deliver clean electrophoretic backgrounds that assist interpretation.

Summary of Procedure

A master mix of PowerPlex® Fusion 6C Master Mix and PowerPlex® Fusion 6C Primer Set is prepared and added to each amplification tube. The DNA samples are added and amplified using the Veriti™ 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

PowerPlex® Fusion 6C Amplification Kit:
PowerPlex® Fusion 6C 5X Master Mix
PowerPlex® Fusion 6C 5X Primer Set
2800M Control DNA 10ng/µl
10% bleach (or other decontaminate solution)
Molecular Biology Grade Water (MBG Water)
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
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 **Fusion 6C 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 (2800M): Ensures that the amplification and typing processes are working as expected when amplifying liquid extraction samples. This control is included in the Fusion 6C 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 PowerPlex® Fusion 6C 5X Master Mix, PowerPlex® Fusion 6C 5X Primer Set and kit positive amplification control (2800M). 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 (2800M).

    - Total number of samples + ___ × 5µL PowerPlex® Fusion 6C 5X Master Mix
    - Total number of samples + ___ × 5µL PowerPlex® Fusion 6C 5X 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 MBG Water 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 1.25 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 0.25 - 3.0 ng.

If the following targets cannot be achieved for a full reaction volume (25 uL) PowerPlex® Fusion 6C amplification, it is appropriate to cease testing (unless otherwise specified by the client):

- Total Small Autosomal DNA: **≥0.0500ng**
- Total Human Male DNA: **≥0.0200ng**

These are considered the "stop at quant" values from Quantifiler® Trio for PowerPlex® Fusion 6C.

If the maximum obtainable ***small autosomal amp target is ≥ 0.050ng***, but the maximum obtainable ***Human Male amp target is < 0.0200ng***, 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.

Unless otherwise specified by the client, MUFSC will utilize the stop at quant threshold values for 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 should be amplified.
- If the sperm fraction is stopped at quant and there is an insufficient amount of male DNA in the epithelial fraction, then both fractions may stop at quant.
- Sperm fractions with a sufficient amount of small autosomal DNA that could stop at quant based on the male DNA results should only be amplified if there is a sufficient quantity of male DNA in the epithelial fraction. In this case, *both* fractions should be amplified for quality control purposes (to verify that the fractions originated from the same source).
- *Any* fraction/sample with a maximum small autosomal amp target less than 0.0500ng may stop at quant, regardless of the male DNA results.

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 (2800M): Vortex the control DNA and spin briefly in a microcentrifuge before use to remove any liquid from the cap. Dilute the Positive Control to 0.1 ng/µL in 50 µL of MBG Water by taking 0.5 µL from the 2800M control DNA tube and adding it to 49.5 µl of MBG Water. Add 5 µL of the diluted aliquot and 10 µL of MBG Water to each amplification 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 µL of MBG Water 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
    - 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 Promega® between 7:00 a.m. and 6:00 p.m. CST at 1-800-356-9526. Or contact Promega® by email at techserv@promega.com

Reference

PowerPlex® Fusion 6C System for Use on the Applied Biosystems® Genetic Analysers Technical Manual-Promega®