DNA: Amplification – Fusion 6C/Yfiler

Introduction

Short tandem repeat (STR) markers are polymorphic DNA loci that contain a repeated nucleotide sequence. The majority of the STRs that have been evaluated by the forensic community are composed of four nucleotide repeat units.

STR markers on the Y chromosome (Y-STR) are found on the non-recombining region of the Y chromosome (NRY) and produce a haploid profile when amplified from male DNA. This quality simplifies male and female mixture interpretation by removing the female contribution from an amplification profile.

The Promega PowerPlex® Fusion 6C PCR Amplification Kit is a multiplex assay that amplifies twenty-six loci and amelogenin, a gender identification locus. The Fusion 6C kit contains twenty-three autosomal loci including the original thirteen core CODIS loci (D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX and vWA), seven expanded core loci (D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433 and D22S1045), SE33, Penta D, and Penta E. Fusion 6C also amplifies three Y-STR loci (DYS391, DYS576, and DYS570).

The AmpFℓSTR Yfiler® PCR Amplification Kit is a short tandem repeat multiplex assay that amplifies 17 Y-STR loci in human male DNA.

Modified Y-STR amplification for inhibited or vacufuged samples

The presence of inhibitors in samples can interfere with the DNA amplification process. Vacufuging samples concentrates both the DNA and any inhibitors that may be present in an extract. These samples exhibit poor or no results when amplified using the laboratory's standard casework protocols for STR amplification. Improved results for these samples can be obtained by using a modified amplification protocol.

The modified amplification is performed using the Yfiler® PCR amplification kit with the addition of supplementary Bovine Serum Albumin (BSA) and additional AmpliTaq Gold DNA Polymerase.

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Procedural safeguards

The following is a list of procedural safeguards for amplification.

- Access to the amplification and typing room is restricted to authorized personnel only.
- Gloves and a dedicated lab coat must be worn at all times.
- All amplified DNA is to remain in the amplification and typing room.
- No equipment used in the amplification and typing room is to be removed from this area of the laboratory.
- Clean the tube openers, thermal cycler, and pipette barrels with ethanol and dry with a clean lab wipe.

Equipment

This procedure uses the following laboratory equipment:

- GeneAmp PCR System 9700 Thermal Cycler
- pipettes and barrier tips
- vortex
- centrifuge

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Prepared reagents and supplies

This procedure uses the following chemicals and supplies:

- PowerPlex® Fusion 6C PCR Amplification Kit containing:
  - Master Mix
  - Primer Set
  - Amplification grade water
  - Control DNA 2800M
- AmpFℓSTR Yfiler® PCR Amplification Kit containing:
  - Reaction Mix
  - Primer Set
  - AmpliTaq Gold DNA Polymerase
  - Control DNA 007 (human male DNA)
  - Control DNA 9947A (human female DNA)
- BSA solution
  - Mix together 32.0 mg of BSA and 10 mL sterile water.
  - Aliquot the solution and store at -20º C.
- Additional AmpliTaq Gold DNA Polymerase
- TE Buffer or sterile water
- sterile microcentrifuge tubes
- 96-well reaction plates or 0.2 mL thin-walled PCR reaction tubes
- 8-well strip caps
- 96-well plate covers

Blanks and controls

This procedure uses the following blanks and controls for **each** analysis:

- reagent blank
- QC stain control (included with reference samples only)
- positive control
- For **Fusion 6C**: 2800M
- For **Yfiler®**: DNA 007
- Negative control
  - For **Fusion 6C**: Promega Amplification grade water
- For **Yfiler®**: sterile water or TE Buffer

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DNA Manager

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Use the following procedure to calculate sample requirements using *DNA Manager*. Alternatively, sample names and hand-calculated values can be manually entered onto the worksheet.

Step	Action
1	Using *DNA Manager*, add the sample names to the appropriate worksheet for the kit being used. Select whether amplifying in 'Plate' or 'No Plate' format for Fusion 6C amplification.
2	For each sample, fill in the volume to be amplified. The volume to be amplified is determined as follows: • Test samples and QC stain control: Using quantitation results, calculate the volume required to obtain 0.5 to 1.0 ng of DNA. – If appropriate, the analyst may amplify an amount of DNA outside the ranges noted above. When a mixture is expected based on sample type, case scenario, or quantitation results, the analyst should consider amplifying greater than 1.0 ng of DNA. – The maximum sample volume for Fusion 6C is 15 µL. The maximum sample volume for Yfiler® is 10 µL. – CAUTION: The DNA concentration of an inhibited sample cannot be reliably determined during quantitation. • Reagent blank: The volume will be the same as the least diluted test sample. • Human female control (**During Yfiler® QC only**): The volume of DNA 9947A that contains 1.0 ng of DNA. • Positive control: The volume that contains 0.5 ng to 1.0 ng of DNA. NOTE: The positive control DNA concentration differs between Fusion 6C and Yfiler® kits. Always be aware of starting concentration. • Negative control: – For Fusion 6C: 15 µL Promega Amplification grade water – For Yfiler®: 10 µL of sterile water or TE buffer The worksheet will calculate a volume of TE buffer or water necessary for a final sample volume of 15 µL (Fusion 6C) or 10 µL (Yfiler®).

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DNA Manager
(continued)

Step	Action
3	When all of the sample names are filled in, the worksheet will calculate the volumes used to prepare the reagent mixture as follows: STANDARD amplification • **PowerPlex® Fusion 6C** – (number of samples + 15%) x 5.0 µL of Master Mix – (number of samples + 15%) x 5.0 µL of Primer Set • **Yfiler®** – (number of samples + 2) x 9.2 µL of Reaction Mix – (number of samples + 2) x 5.0 µL of Primer Set – (number of samples + 2) x 0.8 µL of DNA Polymerase MODIFIED amplification using **Yfiler®** (inhibited/vacufuged samples) • (number of samples + 2) x 9.2 µL of Reaction Mix • (number of samples + 2) x 5.0 µL of Primer Set • (number of samples + 2) x 2.4 µL of DNA Polymerase (see note below) • (number of samples + 2) x 1.4 µL of BSA solution NOTE: Any lot of DNA polymerase that has passed quality control testing is suitable for the preparation of samples for Yfiler® amplification.

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Amplification procedure

Use the following procedure to amplify the samples.

Step	Action
1	Label the reaction tubes or the 96-well reaction plate.
2	Vortex the reagents, including BSA if using a modified Yfiler® amplification.
3	Place the appropriate volumes of reagents listed in *DNA Manager, Step 3* into a single sterile microcentrifuge tube to make a reagent mixture.
4	For samples prepared in 96-well plates, designate one well for allelic ladder and at least one formamide blank for each 24-sample injection. *DNA Manager* will automatically designate these wells when 'Plate' amplification is selected for Fusion 6C amplification.
5	Into each reaction tube or individual plate well, add the appropriate volumes as follows: • Reagent mixture – 10 µL for a PowerPlex® Fusion 6C amplification – 15 µL for a STANDARD Yfiler® amplification – 17.25 µL for a MODIFIED Yfiler® amplification • For Fusion 6C - Promega Amplification grade water determined in *DNA Manager, Step 2* • For Yfiler® - Water or TE determined in *DNA Manager, Step 2* • Sample determined in *DNA Manager, Step 2* NOTE: To minimize the consumption of tips, the pipetting order would be: water or TE → reagent mixture → sample.
6	Seal reaction tubes with attached caps. Seal 96-well plates with 8-well strip caps or appropriate 96-well plate mats.
7	Amplify the samples in the thermal cycler using the pre-set program for the kit used.
8	Following amplification, proceed to Capillary Electrophoresis or transfer the tubes or 96-well plate to the refrigerator or the freezer for storage.

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Amplification procedure (continued)

Step	Action
9	For storage of 96-well amplification plates: • If amplified with 8-well strip caps, re-seal with original or new strip caps after loading the CE plate. • If amplified with 96-well plate mat, discard plate mat and use new strip caps to seal the plate after loading the CE plate.
10	Amplified samples will be discarded after the case is released. Refer to *DNA: Sample Storage* for additional information.