DNA: Amplification

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 *AmpFℓSTR Identifiler® Plus* PCR Amplification Kit is a multiplex assay that amplifies 15 autosomal STR loci and amelogenin, a gender identification locus.

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.
- Wash 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:

- *AmpFℓSTR Identifiler® Plus* PCR Amplification Kit containing:
  - Master Mix
  - Primer Set
  - Control DNA 9947A
- *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
- 0.2 mL thin-walled PCR reaction tubes

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 **Identifiler® Plus**: DNA 9947A
  - For **Yfiler®**: DNA 007
- negative control: sterile water or TE Buffer

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DNA Manager

QAS 9.5.2

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.
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. – The maximum volume for each sample 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 that contains 1.0 ng DNA. • Positive control: The volume that contains 0.5 ng to 1.0 ng DNA. • Negative control: 10 µL of sterile water or TE buffer The worksheet will calculate a volume of TE buffer or sterile water necessary for a final volume of 10 µL.

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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 master mix as follows: STANDARD amplification • **Identifiler® Plus** – (number of samples + 15%) x 10.0 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 amplification.

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Amplification procedure

Use the following procedure to amplify the samples.

Step	Action
1	Label the reaction tubes.
2	Using a fresh pipette tip for each tube, add the volumes determined in *DNA Manager, Step 2* into separate reaction tubes.
3	Vortex and centrifuge the reagents, including BSA if using a modified Yfiler® amplification.
4	Place the appropriate volumes of reagents listed in *DNA Manager, Step 3* into a single sterile microcentrifuge tube to make a reagent mixture.
5	With one tube open at a time, add the volume of reagent mixture listed below to each reaction tube. • 15 µL for a STANDARD amplification • 17.25 µL for a MODIFIED amplification
6	Amplify the samples in the thermal cycler using the pre-set program for the kit used.
7	Following amplification, transfer the tubes to the refrigerator or the freezer. Refer to *DNA: Sample Storage* for additional information. Amplified samples will be discarded after the case is released.