

DNA Analyst Training Laboratory Training Manual

Protocol 4.01

PCR: Amplification and Electrophoresis of STRs



This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the Illinois State Police. It has been included for training and example purposes only.

PRESIDENT'S
DNA
INITIATIVE



INTRODUCTION

Short tandem repeat (STR) genetic markers are polymorphic DNA loci that contain a repeated nucleotide sequence. The STR repeat unit can be from two to seven nucleotides in length. The number of times a unit is repeated at an STR locus differs from individual to individual, resulting in alleles of different lengths. This polymorphism makes them useful for human identification purposes.

STR loci can be amplified using the polymerase chain reaction (PCR) process. The AmpF/STR Profiler Plus PCR Amplification Kit co-amplifies the tetranucleotide repeat regions of the following nine STR loci: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820. A segment of the X-Y homologous gene amelogenin is also amplified. The AmpF/STR COfiler PCR Amplification Kit co-amplifies the tetranucleotide repeat regions of the following six STR loci: D3S1358, D16S539, TH01, TPOX, CSF1PO and D7S820. A non-coding region of the X-Y homologous gene amelogenin is also amplified.

The alleles within each locus as well as the loci themselves are separated by size using capillary electrophoresis. The use of multicolor dye-labeled primers allows loci that have alleles with overlapping size ranges to be distinguished from one another during the course of the capillary electrophoresis run.

SAFETY CONSIDERATIONS

Standard Laboratory Practices.

Warning: Potential Biohazard.

Warning: Hazardous Reagents:

Formamide: An irritant and suspected teratogen. Causes irritation to the eyes, skin and mucous membrane. Do not inhale or ingest.

Electrical Shock Hazard:

The ABI Prism 310 capillary electrophoresis unit contains a high voltage power supply. Handle with caution. Under no circumstances should any safety system be bypassed. Arcing may result from incomplete drying of instrument components.

Laser Hazard:

The ABI Prism 310 contains a laser. Operate only with doors closed. Service by ABI personnel only.

PREPARATIONS

AmpFISTR Profiler Plus and COfiler PCR Amplification Kits (Critical Reagents)

Upon receipt, the allelic ladder vial from the kit must be placed in the Post-PCR room.

(Refer to [pdi_lab_pro_2.01](#), Quality Assurance).

Deionized Formamide

Aliquot into convenient volumes and freeze with protection against defrosting. If the aliquot is not frozen, discard immediately. Formamide may be stored for a maximum of 6 months after it has been aliquoted.

GeneScan 500-ROX Internal Lane Standard

Note: Because ROX contains amplified DNA, store in the Post-PCR room.

Mix in the ratio of 1 part ROX to 24 parts formamide.

Briefly vortex and spin.

Solution must be made fresh before each use.

1X Genetic Analyzer Buffer with EDTA

Genetic Analyzer Buffer w/ EDTA (10X) 2 ml

ddi water (or equivalent) 18 ml

Mix thoroughly.

Solution must be made fresh before each use.

60% Ethanol

Ethanol 600 ml

ddi water (or equivalent) 400 ml

Amplification Master Mix

PCR Reaction Mix 21 μ l

Primer Set 11 μ l

Taq (gold) Polymerase .0 μ l

Prepare a volume sufficient for the number of samples.

Briefly vortex and spin.

Solution must be made fresh before each use.

INSTRUMENTATION

ABI Prism 310 Genetic Analyzer.

Clean the syringe and pump block and supply fresh buffer and polymer approximately every 3 - 4 days. Replace the capillary after approximately 100-150 injections. All maintenance to the 310 Genetic Analyzer must be recorded in a log book. See the Applied Biosystems Manual for maintenance instructions.

ABI 480 and 9700 Thermal Cyclers.

(Refer to [pdi_lab_pro 2.01](#), Quality Assurance).

Refer to the ABI thermal cycler operation manual for operating instructions and instructions on the Temperature Uniformity and Temperature Verification Tests.

Computer Software

- ABI Prism 310 Genetic Analyzer Firmware, version 1.0.2 or higher.
- ABI Prism 310 Collection Software, version 1.0.2 or higher.
- ABI Prism 310 Module GS STR POP4 (1 ml)F.
- GeneScan Analysis Software, version 2.1 or higher.
- Genotyper Analysis Software, version 2.0 or higher.

MINIMUM STANDARDS AND CONTROLS

Profiler Plus and COfiler Controls:

Positive Amplification Control: 20 µl of Control DNA 9947A (supplied in kit)

Purpose: To ensure that amplification has occurred successfully. The following types must be obtained:

D3S1358	14, 15
vWA	17, 18
FGA	23, 24
Amelogenin	X, X
D8S1179	13, 13
D21S11	30, 30
D18S51	15, 19
D5S818	11, 11
D13S317	11, 11
D7S820	10, 11
D16S539	11, 12
TH01	8, 9.3
TPOX	8, 8
CSF1PO	10, 12

Negative Amplification Control: 20 µl ddi water (or equivalent)

Purpose: To ensure that contamination is not present in the reagents used in the amplification.

Extraction Controls (Manipulation Blank):

Purpose: To ensure that contamination is not present in the extraction reagents or introduced during manipulation of the sample.

Every manipulation blank must be amplified in either Profiler Plus or COfiler. If the set of samples is amplified in one system only, then the manipulation blank must be amplified in that system.

The volume of the manipulation blank to be amplified will be the larger of the following values: the volume of the sample that is amplified or twenty percent of the total volume of the manipulation blank.

If any sample is injected for 10 seconds, the corresponding manipulation blank must also be injected for 10 seconds.

If any sample is analyzed at 50 RFUs, the corresponding manipulation blank must also be analyzed at 50 RFUs.

If a DNA profile is detected in a manipulation blank, the case must be brought to the attention of the Statewide Technical Leader.

PROCEDURE FOR AMPLIFICATION

1. All suspect standards must be typed in both Profiler Plus and COfiler for entry into the suspect database.
2. All samples must be amplified and typed in Profiler Plus when possible. Probative samples and samples to be entered into CODIS must also be amplified and typed in COfiler. When an F2 fraction of a differentially extracted sample is considered probative, both the F1 and F2 fraction of that sample must be amplified and typed in COfiler.
3. Determine an appropriate quantity of sample DNA to dilute or concentrate to 20 µl with autoclaved ddi water (or equivalent). This quantity is dependent upon the quality of the DNA and the sensitivity of the 310 instrument.
4. Add 20 µl of sample DNA/water to 30 µl of the Amplification Master Mix in an appropriately labeled reaction tube. Use mineral oil when amplifying with the 480 Thermal Cycler.

The 9700 block uses 0.2 ml microamp tubes which are too small to label with case specific identifiers. Create a coded identification key on the amplification worksheet and code each tube accordingly. Samples should be stored in a closed container labeled for clear identification of the specific amplification set enclosed.

5. Prepare the positive and negative amplification controls and the manipulation blanks in the same manner as case samples.
6. Amplify in a thermal cycler using the following parameters:
 - 1 cycle at 95°C for 11 minutes
 - 28 cycles (each: 1 minute at 94°C, 1 minute at 59°C and 1 minute at 72°C)
 - 1 cycle at 60°C for 45 minutes
 - Soak at 10°C
7. After amplification, the tubes can be stored in an amplified DNA dedicated refrigerator for up to 2 weeks. Samples to be stored longer should be frozen in an amplified DNA dedicated freezer.

PREPARATION OF AMPLIFIED DNA SAMPLES FOR 310 ANALYSIS

1. Mix 1.5 μ l of each amplified sample (including the amplification positive and negative controls and the manipulation blanks) and 25 μ l of formamide containing the ROX-500 internal lane standard in appropriately labeled tubes. Close with septa, vortex lightly and spin briefly.
2. Prepare samples of allelic ladders in the same manner as above.
3. Denature samples for 3-5 minutes at 95°C.
4. Snap cool denatured samples for 5-10 minutes in an ice block or equivalent.

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