

DNA Analyst Training Laboratory Training Manual

Protocol 6.01

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



PREPARATION OF AMPLIFIED DNA SAMPLES FOR 310 ANALYSIS

1. Mix 1.5 µl of each amplified sample (including the amplification positive and negative controls and the manipulation blanks) and 25 µl 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.

310 DATA COLLECTION

1. Create a Sample Sheet and Injection List for the run.
2. Verify the following analysis parameters in the Injection List:
 - a) Module: GS STR POP4 1ml F
 - b) Injection time: 5 seconds or 10 seconds at the analyst's discretion. Injection time can be reduced to 1 to 4 seconds if a sample is found to be overloaded at 5 seconds.
 - c) Run temperature: 60°C.
 - d) Run time: instrument dependent. The run time must be sufficient so that ROX is present between 75 and 400 basepairs for all Profiler Plus runs, and between 75 to 350 basepairs for all COfiler runs.

GENESCAN ANALYSIS

1. Analyze data using the current matrix.
2. Create a size standard file. A new size standard file must be used for each run.
3. Use the appropriate Analysis Parameters. Start analysis after the primer peaks, with the smoothing option at light. Routine analysis is performed at 150 RFU threshold, with a minimum peak half width of 3. Analysis must contain values from 75 to 400 basepairs. All sizing is performed using the Local Southern method.

GENOTYPER ANALYSIS

1. Analyze the data from the GeneScan project using appropriate analysis parameters (Profiler Plus or Cofiler).
2. The electropherograms must display the internal lane standard peaks 75-400 bp for Profiler Plus loci and 75-350 bp for COfiler loci.
3. Do not unlabeled any peak. Labels for sample peaks must include base pairs, peak height and allele call.

ELECTRONIC DATA

1. Insure that more than one copy of electronic data exists in different locations. This can be accomplished with an archive CD, a backup CD, or backup on another computer.
2. Electronic data for each run will be retained and archived on a CD. When multiple cases are analyzed together, a run folder can be created for each individual case.
3. The electronic data should contain a copy of the GeneScan project containing the sample files, the Genotyper files, the Sample Sheet and the Injection List.

[Return to Laboratory Training Manual User Guide](#)