

SIZING PRECISION AND REPRODUCIBILITY STUDIES OF AMPF/STR KITS WITH THE ABI PRISM® 3700 DNA ANALYZER

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The ABI Prism® 3700 DNA Analyzer is a fully automated high-throughput capillary electrophoresis system that can separate, detect, and analyze the fluorescently labeled DNA contents of up to 96 capillaries in one run of 2 hr. 40 min. duration. POP-6 polymer (for fragment analysis) is pumped automatically from a large polymer bottle into a 96-capillary array that makes it possible for the instrument to run seven 96-well plates per day for 3 days without addition of polymer or other reagents. Immediately at the end of each run, the analyzed data can be viewed in 96 sample files by ABI Prism® GeneScan® software, Genotyper® software, or GeneMapper™ software.

To evaluate the performance of the 3700 DNA Analyzer for human identification applications, 3 Ampf/STR kits for multiplex STR analysis were used to analyze sizing accuracy and precision, peak resolution, and run to run reproductability. The 3 kits were: Profiler Plus™, COfiler™, and SGM Plus.

In the first study, two 3700 DNA Analyzers were tested, each with two 96-capillary arrays, using DNA samples from 6 different individuals at 2 different DNA concentrations (1.5 ng and 3 ng). After PCR amplification with Profiler Plus™ kit, the DNA sample from each individual was loaded in all 96 wells of a microplate and injected into a 96-capillary array. GeneScan® 500 ROX Size Standard was used in this study. This procedure was repeated 4 times for each DNA sample (at 2 concentrations), every time with a new 96-capillary array. To analyze the data, the average fragment size (for 96 capillaries of each run) and standard deviation (SD) of each amplified allele of each DNA sample, as well as the minimum and maximum deviations from each mean allele size were calculated using the statistical features of Genotyper® software.

The results showed that sizing accuracy was comparable to those obtained with other platforms for all 50 alleles amplified from the DNA samples of the 6 tested individuals (16 to 18 amplified alleles per individual). With regard to sizing precision, of about 77,000 allelic peaks obtained from 4 arrays, 29 peaks, that is < 0.04%, showed deviation from mean allele size > +/- 0.50 nucleotide (nt). It should be noted that 17 out of these 29 peaks occurred when 1.5 ng DNA was used as a template. In terms of the number of injections carried out, 7 injections out of about 4,500 injected DNA samples, that is < 0.2%, showed deviation from mean allele size > +/- 0.50 nt and SD > 0.15 nt. These 7 unsuccessful injections could be easily identified and flagged out by first checking the size accuracy of different peaks of the DNA size standard.

In the second study, SGM Plus Allelic Ladder (labeled with fluorescent dyes in 3 colors, and containing 154 peaks) was loaded in all 96 wells of a microplate and injected into a 96-capillary array, once with GeneScan® 500 ROX Size Standard, and once with GeneScan 400HD ROX Size Standard. The results of analyzing about 15,000 allelic peaks (for each different DNA size standard) showed that when GeneScan® 400HD ROX Size Standard was used, the mean allele size deviation for every peak and every injection was < +/- 0.50 nt, and the SD of every injection was < 0.15 nt.

The 3700 DNA Analyzer showed excellent one bp peak resolution, very good color balance within and between different multiplex STR loci, and due to the fully automated operation, ease of use, and high-throughput features is an ideal platform for DNA databanking applications.

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