VALIDATION OF THE AMPF*l*STR™ PROFILER PLUS™ AND COFILER™ PCR AMPLIFICATION KITS USING THE ABI™ PRISM® 310 GENETIC ANALYZER

E.J. Kriss MFS, T.D. Ferguson, D. E. Snipes M.S., R. W. Cotton Ph.D.
Cellmark Diagnostics

Use of the ABI™ Prism® 310 Genetic Analyzer in the DNA analysis of biological crime scene evidence has been a valuable addition to the forensic DNA laboratory. Since the introduction of fluorescent STRs, post-amplification analysis methods were predominately slab-gel based. A more versatile and semi-automated technology known as capillary electrophoresis (CE) has further advanced the analysis of fluorescently-tagged PCR products.

While this innovative separation technology utilizes the basic principles of conventional electrophoresis, fragment separation on the 310 occurs in a polymer contained within a thin capillary. Dye-labeled PCR products are excited by a laser producing fluorescent emissions which are then detected by a CCD camera and displayed as raw data for subsequent analysis.

Prior to implementation into forensic casework, a series of validation experiments were performed which were designed to meet TWGDAM guidelines and demonstrate reproducibility and proficiency in the use of the ABI™ Prism® 310 Genetic Analyzer. A summary of the validation studies performed at Cellmark Diagnostics will be presented.

Experiments were performed to determine optimal operating conditions for forensic casework on the 310. These experiments included differences in sample loading volume, capillary longevity, different types of formamide, and variations in electrokinetic injection time.

DNA from a wide range of biological samples including whole blood, bloodstains, saliva stains, hair, tissue, and mixed stains were amplified for Profiler Plus™and COfiler™ and typed successfully on the 310. In addition, results obtained from DNA extracted by the organic, FTA® and Chelex® methods were compared.

Sensitivity studies were done by amplifying a dilution series of high molecular weight DNA's. Although detectable results were observed as low as 0.15ng of input DNA, the optimal range for amplification was determined to be 0.5ng to 2.5ng. Variations in amplification conditions including comparisons of different reaction volumes, units of *Taq* DNA Polymerase, and variations in cycling parameters (i.e. extension time) were also evaluated.

The usefulness of this system in identifying mixtures was investigated. Using a mixture of DNA from two individuals with the component ratios varied from 1:1 to 1:20, the minor component could be detected at a 1:5-1:10 ration when amplifying at least 1.25ng of sample DNA. The ability to detect the minor component was gradually reduced as the amount of DNA amplified was reduced.

As a basis for statistical comparison, base pair size differences were calculated in order to demonstrate reproducibility within a single run and between multiple runs. These data demonstrate that high levels of reproducibility exist both within and between runs. Stutter percent and heterozygote peak height ratio calculations were performed on a set of 100 Profiler Plus™ and COfiler™ amplified samples. The average stutter percent for each of the thirteen loci was calculated and applied to the Genotyper® macro for utilization in forensic casework. Additionally, stutter percent and heterozygote peak height ratios were calculated for samples extracted by the organic, FTA® and Chelex® methods. These values were compared to determine whether variations exist in results obtained between extraction methods.

Our data showed that the capillary-based ABI™ Prism® 310 Genetic Analyzer is a reliable and sensitive instrument for the analysis of crime scene evidence. Validation and optimization of experimental protocols is required in order to perform a comprehensive assessment of instrument performance.