INTERNAL VALIDATION OF THE PowerPlex Y23 SYSTEM ON THE APPLIED BIOSYSTEMS 3500 GENETIC ANALYZER

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Many forensic laboratories currently utilize the amplification of Y-chromosome specific STRs with sexual assault kit evidence, in order to aid in the examination of male DNA that may be found in low quantity, as compared to the high level of female DNA that is often seen in evidentiary samples. In these cases, as well as in other cases such as homicides, an autosomal profile may not be obtained, but Y-STR analysis can be used to analyze a detected male contributor.

The PowerPlex Y23 System utilizes a 23-loci, 5-color Y-STR multiplex, including six loci with high genetic diversity. This diversity allows for a greater distinction in unrelated males. Our validation experiments followed all studies required by the FBI (QAS Standard 8.3.1) and SWGDAM (Validation Guidelines for DNA Analysis Methods – Guideline 4). The studies conducted included tests for sensitivity, variation of injection time, amplicon volume variation, precision and reproducibility, concordance using various known samples, and mixture analysis. These experiments were duplicated, using two different 3500 Genetic Analyzers.

A sensitivity of the system was seen using 0.062 ng DNA template, with the best results seen with 0.250 ng template DNA, and an injection time of 10 seconds. Genotypes were not affected by the amount of amplicon volume used, and reproducible results were seen when the same sample was amplified and injected multiple times. Amplification of samples with varying mixture ratios demonstrated that the system can be used to distinguish between two male components within a mixture. Samples amplified from two brothers demonstrated a variant at the locus DYS481, causing an off-ladder allele call in one sample, and a "25" allele call in the other. Sequencing of each sample later identified a G to A base change in the flanking region, thereby accounting for the off-ladder allele calls.

Throughout these experiments consistent and reproducible results were seen, demonstrating that PowerPlex Y23 is a kit that could successfully be used in casework analysis, allowing our laboratory to aid in solving sexual assault cases where semen is not identified.