A62 Internal Validation of the PowerPlex® Y23 Amplification Kit for Use in Forensic Casework

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After attending this presentation, attendees will understand the added advantage for using a Y-Chromosome Short Tandem Repeat (Y-STR) amplification kit in forensic DNA testing and the ability of PowerPlex® Y23 to perform effectively in the Colorado Springs Metro Crime Laboratory.

This presentation will impact the forensic community by demonstrating the robustness, reliability, and reproducibility of PowerPlex® Y23 in a forensic DNA laboratory setting through comparison with other commercially available Y-STR amplification kits and full validation.

Over the past decade, forensic laboratories have come to rely on commercially available Y-STR kits as a fundamental tool in forensic DNA casework where the identification of male specific DNA is essential. In an admixed sample, foreign DNA from a male is often masked by high levels of female DNA. Autosomal STR (short tandem repeat) analysis in admixtures often results in a DNA profile with major female alleles and minor male alleles. The minor male alleles are either difficult to distinguish from stutter peaks in the mixed profile or absent due to preferential amplification of the female DNA. However, the male-specific nature of Y-STR analysis allows for targeted amplification of male DNA with no amplification of female DNA, even when present in the mixture at high levels. Additionally, Y-STR analysis is advantageous in sexual assault cases involving multiple male assailants where autosomal STR analysis is ambiguous regarding the number of contributors to the mixture. Furthermore, Y-STR analysis is valuable in cases where mixed gender DNA cannot be segregated by differential extraction, such as evidence from azoospermic or vasectomized males and blood-blood or blood-saliva mixtures.

The initial stage of this study focused on the comparison of three commercially available Y-STR kits: the eleven loci PowerPlex® Y kit (Promega Corporation), the sixteen loci Yfiler™ kit (Applied Biosystems-Life Technologies), and the twenty-three loci PowerPlex® Y23 kit (Promega Corporation). Precision, sensitivity, and mixture interpretation capabilities were compared for the purpose of identifying the optimal kit for implementation at the Colorado Springs Metro Crime Laboratory. In these comparisons, all three kits demonstrated comparable performance in precision and mixture interpretation. On the other hand, PowerPlex® Y exhibited superior sensitivity over Yfiler™ and PowerPlex® Y23, with less allelic drop out at DNA target concentrations below 0.125ng/µL. Despite an increase in allelic drop out at low concentrations, however, Yfiler™ and PowerPlex® Y23 exhibited an increase in the number of allele calls overall compared to PowerPlex® Y at the same concentrations. PowerPlex® Y23 provided significantly more allele calls for all concentrations tested and was therefore chosen for full internal validation.

During internal validation, PowerPlex® Y23 was examined for precision, sensitivity, mixture interpretation, stutter, analytical and stochastic thresholds, reproducibility, concordance, and contamination. Precision of the 3130 AB Genetic Analyzer using PowerPlex® Y23 was evaluated using an allelic ladder. The average standard deviation for each allele at all loci was calculated. All loci as well as all alleles within each locus exhibited an average standard deviation value less than 0.15, indicating compliance with the 95% confidence interval. Sensitivity data was collected for DNA target concentrations of 0.0312, 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0ng/µL with amplicon loads of 1, 2, and 3µL at injection times of two, five, and ten seconds. For standard run parameters of 1µL amplicon load and a five second injection, the target DNA concentration range capable of producing a full profile was 0.125 to 2.0ng/µL. However, the recommended target DNA concentration range is 0.5 to 1. 0ng/µL. Increasing the amplicon load resulted in little success in generating additional allele calls where allelic drop-out had occurred. However, increasing injection time generally increased the number of allele calls, but did not result in a full profile at concentrations lower than 0.125 ng/µL. Decreasing the injection time from five to two seconds successfully decreased pull-up and extraneous artifacts in overloaded samples. Previously extracted non-probative samples as well as DNA standards were used to determine reproducibility and concordance. Since the Colorado Springs Metro Crime Laboratory does not currently perform Y-STR typing, each sample was amplified using Yfiler™ and PowerPlex® Y23. All samples produced reproducible results, and all alleles shared between Yfiler™ and PowerPlex® Y23 kits exhibited 100% concordance.

Y-STR, Forensic DNA Testing, PowerPlex® Y23

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