A50 Internal Validation of the AmpFℓSTR® Yfiler™ Amplification Kit on a Life Technologies™ 3130 Genetic Analyzer

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After attending this presentation, attendees will understand the process of an internal validation of the AmpFℓSTR® Yfiler™ PCR Amplification Kit on a Life Technologies™ 3130 Genetic Analyzer and the efficiency of the amplification kit to reliably amplify and produce complete and accurate Y-Short Tandem Repeat (Y-STR) profiles. The following properties are taken into account: threshold, sensitivity, contamination, precision, concordance, reproducibility, male-female mixtures, male-male mixtures, and stutter.

This presentation will impact the forensic science community by increasing throughput of casework and reducing backlog, which is one of the main problems many laboratories face. By utilizing the Y-STR selectivity to amplify STRs, the AmpFℓSTR® Yfiler™ PCR Amplification Kit can be utilized in mixture samples to help reduce interpretation time. Before implementing the AmpFℓSTR® Yfiler™ PCR Amplification Kit, an internal validation is necessary to ensure that performance in the laboratory is concordant with the performance demonstrated by the manufacturer.

The successful validation of the AmpFℓSTR® Yfiler™ PCR Amplification Kit may reduce the time spent on mixture interpretation and can increase the throughput of the Prince George's County Police Department DNA Laboratory. Y chromosomal short-tandem repeat (Y-STR) amplification is of interest at Prince George's County Police Department DNA Laboratory due to the overwhelming number of cases with complex mixtures that the analysts encounter annually. Y-STR amplification targets the male component, the Y chromosome, and can be utilized in cases where the male component is of interest. The AmpFℓSTR® Yfiler™ PCR Amplification Kit is a multiplex assay that amplifies 17 loci located on the Y chromosome: DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385a/b, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438, and DYS448. The resulting profile is a human male haplotype. In autosomal amplification, loci from several different chromosomes are amplified in one reaction, and in the case of mixture samples can be difficult to interpret. Using a male specific kit, such as the YFiler™ kit, male profiles can be isolated and aid in interpretation, especially in conjunction with a genotype produced from autosomal analysis. The internal validation of the Yfiler™ kit followed the SWGDAM validation guidelines along with a comparison of analytical threshold calculations. The analytical threshold calculations were completed using two different sample type runs using a 5second capillary electrophoresis injection. The samples included capillary electrophoresis run negative samples and YFiler™ amplified samples (including samples, positive controls, and reagent blanks). For the run negative analytical threshold calculations, the average and standard deviation of the baseline height (disregarding internal size standard pull up or known artifacts) and separated by dye channel. The analytical threshold using YFiler™ amplified samples was calculated by evaluating average height and standard deviation of the baseline between allelic peaks (disregarding artifacts such as pull up and stutter). Through both sample type calculations, the optimal analytical threshold was found to be 150 relative fluorescent units (RFU) for a 5-second injection time. A 10-second injection time also utilized a 150 RFU analytical threshold, while a 200 RFU analytical threshold was utilized for a 15second injection time. Full profiles were produced for a 5-second injection time from a DNA input concentration of 2.0ng/µL to 0.250ng/µL, with full profiles produced from 0.125ng/µL in six of 10 samples, and an average of 11 loci for 0.0625ng/µL. For a 10 second injection time, a DNA input concentration from 1.0ng/µL to 0.250ng/µL produced full profiles, with full profiles produced from 0.125ng/µL in all but one of 10 samples, and an average of 14 loci for 0.0625ng/µL. Full profiles were produced for a 15-second injection time from 0.50ng/µL to 0.125ng/µL, with full profiles produced from 0.0625ng/µL in two of ten samples. In the male-male mixtures, a full profile from the minor male component was identified up to a 1:5 ratio and a 1:1:1 male mixture produced full profiles for all male contributors. In the female-male mixtures, a full Y-STR profile was obtained at ratios up to 1:1. Inhibition was observed at ratios above 1:1, with an average of six loci called in the 1:100 male to female ratio. A full male profile was produced from a 1:1:1 male to female to female mixture. The studies illustrate that the Yfiler™ kit successfully amplifies evidence samples from adjudicated cases, is male specific, and precise.

Y-STRs, Internal Validation, Y-Chromosome

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