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INTERNAL VALIDATION OF THE APPLIED BIOSYSTEMS AMPFLSTR® YFILER™ PCR AMPLIFICATION KIT

Cindy A. Carroll, Ph.D., Michelle R. Madrid, M.S., Flynn E. Lamas, M.S. and Steve R. Renteria, B.S.
Los Angeles County Sheriff's Department, 2020 W Beverly Blvd., Los Angeles, CA 90057

An internal validation of the AmpFISTR® Yfiler™ Kit was performed for case work using ABI's 310 Genetic Analyzer and GeneMapper ID v3.2 software. The kit was used following ABI's protocol for reagent setup and thermocycling conditions. The optimal peak detection threshold was determined to be 75 Relative Fluorescent Units (RFU). Using a 75 RFU peak detection threshold, a full profile was detected at DNA amounts as low as 100pg. The optimal target input was determined to be 500pg of total male DNA, with a range of 350pg to 750pg. Two different male to female DNA mixture experiments were performed at 1:500, 1:1000, 1:2000, 1:4000, 1:8000 and 1:10,000. In one experiment, the amount of female DNA remained constant at 500ng and the amount of male DNA was varied from 1000pg to 50pg. A full male profile was obtained up through 1:4000 and partial profiles were detected at 1:8000 and 1:10,000. In the other experiment, the amount of male DNA remained constant at 500pg and the amount of female DNA was varied from 250ng to 5000ng. A full male profile was detectable up through a 10,000 fold excess of female DNA, however, a decrease in some of the allele peak heights was observed at the higher ratios. The cross reactivity with female DNA was evaluated using 500ng and 1000ng of female DNA without the presence of male DNA, and no cross reactivity was detected. In addition, male to female mixtures at ratios of 1:20, 1:100 and 1:400 were analyzed with Identifiler and Yfiler. With Identifiler, the Y peak at Amelogenin was not detected in any of the samples. Only some of the minor alleles of the male donor were detected at 1:20 and 1:100 with no indication of a mixture at 1:400. In contrast, a full male profile was obtained with Yfiler for all three mixture samples. In mixtures consisting of two male sources, all alleles from both contributors were observed in ratios out to 1:10. Ratios greater than 1:10 and out to 1:50 resulted in partial profiles of the minor contributor. Stutter percentages at each locus were calculated and similar results as ABI were obtained when comparing the maximum observed stutter values. The N-2 stutter at DYS19 and N+3 stutter at DYS392 were routinely observed. Additional stutter peaks were also observed at DYS456, DYS389II, DYS458, DYS19, DYS393, DYS439, and DYS635 that were not reported by ABI. In general, more stutter artifacts were present in Yfiler than what has been observed in the laboratory with Identifiler using the same 75 RFU detection threshold. The precision of allele sizing (basepairs) and peak heights (RFUs) were evaluated using the Yfiler ladder. Three times the highest standard deviation value for the allele sizing was 0.27 basepairs. The coefficient of variation of the peak heights ranged from 4.32% to 7.62%. Lastly, multiple previously characterized samples were typed with Yfiler and all yielded concordant results.