VALIDATION OF THE AMPFLSTR® IDENTIFILER™ PCR AMPLIFICATION KIT USING THE ABI PRISM® 310 GENETIC ANALYZER.

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Fluorescent STR-based assays for human identification that originally required multiple amplifications and replicate analyses to obtain genotypes from the thirteen core STR loci have been streamlined by the development of multiplex single amplification systems. The AmpFLSTR® Identifiler™ PCR Amplification Kit (ABI) is one such megaplex system simultaneously amplifying 15 STR loci plus the Amelogenin gender determination locus utilizing 5-dye fluorescent DNA technology. The Identifiler™ system is a hybrid multiplex encompassing each of the previously validated Profiler Plus™ and COfiler™ loci plus two additional tetranucleotide loci (D2S1338 and D19S433). Prior to implementation into forensic casework and in accordance with SWGDAM guidelines, a series of validation experiments were designed to evaluate the ability of the Identifiler™ system to successfully amplify forensic evidentiary samples.

To evaluate the sensitivity of the Identifiler™ system, the 9947A positive amplification control DNA plus a second additional high molecular weight DNA sample were serially diluted such that the total DNA amount amplified ranged from 1ng to 0.0625ng. A complete profile was obtained from each sample in each of the amplification systems (Profiler Plus™, COfiler™, Identifiler™) when the amount of DNA amplified was equal to or greater than 0.125ng using a minimum peak detection threshold of 60 rfu's. Overall, the Identifiler™ system sensitivity was less than that of the Profiler Plus and Cofiler™ counterparts. The reduced sensitivity was most notable in the loci corresponding to the red (PET™) dye.

The ability of this system to successfully detect the minor component in a mixture was also investigated. DNA from two individuals (X, XY) was combined such that the ratio of major to minor components was 1:1, 1:2, 1:5 and 1:10. A minimum of 1ng of input DNA was required to obtain a full profile from the minor male component at the lowest mixture ratio of 1:10.

To evaluate the ability of the Identifiler™ multiplex to amplify degraded DNA, a high molecular weight DNA sample was subjected to DNase I exposure for multiple time points and amplified. The resulting profiles, showing increasing degrees of degradation, followed the expected motif with the higher molecular weight loci failing to amplify at a more rapid rate. Within each locus, as expected, the larger allele of a heterozygote pair exhibited reduced amplification at a rate that was equal to or greater than the smaller allele as the extent of degradation progressed.

Also considered in this validation was the efficiency with which the Identifiler™ multiplex was able to amplify inhibited DNA. Two known DNA samples were amplified with varying concentrations of hematin to inhibit AmpliTaq® Gold DNA polymerase during the PCR reaction. The larger loci were the first to display reduced amplification success in response to inhibition challenge.

Data will be presented that details these and other validation studies considered prior to the implementation of the AmpFLSTR® Identifiler™ PCR multiplex kit in our laboratory.