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EVALUATION OF THE APPLIED BIOSYSTEMS AMPFLSTR® MINIFILER™ PCR AMPLIFICATION KIT

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The Armed Forces DNA Identification Laboratory participated in a study to evaluate the performance of the Applied Biosystems AmpFℓSTR® MiniFiler™ PCR Amplification kit. The AmpFℓSTR® MiniFiler™ kit contains a five dye chemistry, which amplifies 8 autosomal STR loci (D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, FGA) and the sex determining marker Amelogenin. The loci span a range of 71 to 283 base pairs. We will present the results of this study and discuss how this kit compared to the Applied Biosystems AmpFℓSTR® Identifiler® PCR amplification kit and the Promega PowerPlex® 16 amplification kit. The study consisted of four components: reproducibility, sensitivity, mixtures, and challenged non-probative casework samples. Reproducibility was tested by amplifying 0.5ng/5µl of target DNA from four samples along with a negative and positive control (DNA 007). The sensitivity of the kit was assessed using a 2-fold serial dilution of the control DNA from 1ng to 31.25pg. The third component was a mixture study that targeted 1ng of input DNA for the following mixture ratios: 0:1, 15:1, 10:1, 3:1, 1:1, 1:0. The fourth component involved the testing of 18 challenged samples from previously processed AFDIL casework. The samples were comprised of extracts from touched objects (LCN), degraded bone and tissue specimens, and 60 year old aged skeletal remains which previously generated mtDNA profiles. All experiments were run in triplicate except the non-probative samples. Additionally, AFDIL compared the MiniFiler™ non-probative results to the AmpFℓSTR® Identifiler® and PowerPlex® 16 results obtained for the same samples. Results demonstrated that the AmpFℓSTR® MiniFiler™ kit is highly reproducible and sensitive to 62pg, which is approximately four times more sensitive than traditional STR kits. Peak imbalance was detected at D21S11, CSF1PO, and D2S1338 in high quality DNA amplicons that was not present with degraded or challenged samples. The MiniFiler™ kit detected mixtures at a 15:1 ratio and more importantly detected the presence of a fourth person in one of the mixed touched samples that was not identified using traditional STR kits. During the non-probative portion, the MiniFiler™ kit out preformed the Identifiler® and Powerplex® 16 kits on challenged and degraded samples including the ability to generate full profiles from 60 year aged bone samples. In conclusion, the MiniFiler™ kit offers four times the sensitivity of traditional STR kits when processing challenged or degraded samples. This translates into the ability to detect minor contributors in mixed samples (e.g. gang rapes or touched objects), or obtain full profiles from samples that did not generate results from traditional STR kits (i.e. charred remains or environmental challenged remains). The single greatest advantage of this kit is the ability to obtain results without having to go to a low copy number approach, thus alleviating interpretation issues due to elevated stutter, allelic drop in and drop out as well as the need for multiple amplifications. The increased sensitivity is due to a combination of smaller locus sizes and optimization toward challenged samples as seen by the absence of peak imbalance.