CHARACTERIZATION OF A 41-PLEX PCR AMPLIFICATION ASSAY FOR MALE-SPECIFIC DATABASING APPLICATIONS

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DNA databases are indispensable tools in forensics to help solve crimes by matching autosomal STR profiles obtained from crime scene samples with known crime offenders. In more recent years the forensic community has been debating the inclusion of Y-STR markers to existing databases to help determine or exclude relationships, identify missing persons, infer ancestry and interpret mixture. We developed a 41-plex that simultaneously amplifies the 27 Y-STR markers included in the Applied Biosystems Yfiler™ Plus PCR Amplification Kit plus 11 new Y-STRs (DYS549, DYS645, DYS557, DYS593, DYS522, DYS444, DYS596, DYS643, DYS447 and DYS527a/b) and 3 Y-indels, which together can provide extremely high discriminating power. This multiplex is designed to process single-source reference samples using direct PCR amplification from blood samples on paper substrates without the need for sample purification. This 41-plex was built in a 6-dye multiplex format with PCR products ranging from 68-570 base pairs and it is compatible with detections on the 3130xl, 3500xL and 3730xl instrument platforms. Particularly, the Y-indels can be used to quickly exclude male lineages, as the mutation rates of the Y-indels are significantly lower as compared to STR's (i.e., ~10^{-9} vs 10^{-3} per locus per generation). This study shows the feasibility of amplifying 41-markers simultaneously and describes the optimization of the multiplex assay to deliver high first pass success rate and assay performance when amplifying blood samples on paper substrates. In addition, the haplotype diversity and discriminatory capacity calculations with the expanded multiplex will be presented.