ANALYSIS OF SINGLE-SOURCE SHORT TANDEM REPEAT (STR) AND SINGLE NUCLEOTIDE POLYMORPHISM (SNP) LOCI USING A CUSTOM HALOPLEX TARGET ENRICHMENT SYSTEM PANEL

Frank Wendt[1], Xiangpei Zeng[1], Jennifer Churchill[1], Jonathan King[1], Bruce Budowle[1,2]

[1]University of North Texas Health Science Center, 3500 Camp Bowie Blvd. Fort Worth, TX 76107, USA
[2]Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia

The forensic science community relies heavily on the use of short tandem repeats (STRs) and, more recently, single nucleotide polymorphisms (SNPs) to individualize biological evidence samples. Limitations of capillary electrophoresis (CE), the current mainstay in STR typing, include amplicons, that are labeled with the same fluorescent molecule but from different loci, must be of different size to unequivocally assign alleles to the correct locus; there is a limit of the number of STR markers that can be multiplexed (currently 24-27 loci); and SNPs assays designed for this instrument are cumbersome and labor intensive. Massively parallel sequencing (MPS) offers a potential solution to these limitations because it relies on sequence information rather than size and has a substantially higher throughput. MPS sample preparation entails enrichment of desired targets from other genomic DNA followed by preparation of sample libraries by addition of adapters, sequencing priming sites, and sample barcodes. Since nanogram and subnanogram quantities of input DNA can be typed by MPS, PCR enrichment has become the method of choice for studies involving forensic applications. However, capture enrichment, with the HaloPlex Target Enrichment System (Agilent Technologies), was used in this study because minimal PCR amplification is required. Therefore, primer binding site mismatch issues would not impact multiplex design and amplification success. In addition, PCR generated errors would be reduced and thus minimize potential artifacts. Lastly, alternate approaches can provide comparison data to support or refute reliability of MPS systems when full validation studies are undertaken. The efficacy of this HaloPlex method was assessed by designing a panel of 275 human identity SNPs and 89 STRs that include the markers in the Identifiler®, GlobalFiler®, Yfiler®, PowerPlex® 16HS, PowerPlex® Fusion, and PowerPlex® Y23 kits. The metrics analyzed were typing success of each locus, depth of sequence coverage, heterozygote allele coverage ratio, and concordance of the system. The data indicate that the HaloPlex Target Enrichment System performs similarly to conventional PCR-CE methods with the advantage of analyzing substantially more markers in one sequencing run. The performance of the HaloPlex method was compared with other MPS-based sample preparation systems that utilize primer-based target enrichment.