SEQUENCING OF 68 INSERTION/DELETION MARKERS: MOTIF AND MICROHAPLOTYPES

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Short tandem repeats (STRs) are the commonly used loci for forensic DNA typing as it applies to direct comparison human identity (HID) testing, kinship, and missing persons. These markers are valuable due to their highly polymorphic nature, relatively short amplicon size, and ability to be multiplexed. However, STRs are often still too large for DNA typing of highly degraded DNA. Small bi-allelic markers, such as insertion/deletion (INDEL) polymorphisms, overcome this limitation during analysis of compromised samples as their allelic states range in size from 2-6 basepairs, enabling small amplicons that can be reliably separated using capillary electrophoresis. In addition, their heterozygote balance may be increased due to less preferential amplification of the smaller allele, an artifact more commonly encountered with STRs. Multiplexing large panels of INDELs enables generation of discrimination power similar to those of STRs. The Nextera™ Rapid Capture Custom Enrichment Kit (Illumina, Inc., San Diego, CA) and the Illumina MiSeq™ were used to sequence 68 well-characterized HID INDELs in four major United States populations. In addition, a novel application of the STR Allele Identification Tool: Razor (STRait Razor) was used to analyze the sequence data for INDEL polymorphisms, adjacent flanking single nucleotide polymorphisms (SNPs), and other flanking-region polymorphisms. Forty-two HID INDELs were found to be part of a microhaplotype. These observations increased the allele spread and heterozygosity and decreased the single-locus random match probability of 22 INDELs making them as informative as some low-performing STR loci. These findings suggest that microhaplotypes containing HID INDELs can be powerful markers for human identity testing.