NEXT-GENERATION SEQUENCING OF STR AND SNP LOCI FOR HUMAN IDENTIFICATION AND MIXTURE ANALYSIS

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High throughput DNA sequencing technologies enable the analysis of far more of the human genome than is currently possible via capillary electrophoresis. This allows the analysis of thousands of single nucleotide polymorphisms (SNPs) and new forensic capabilities to estimate biogeographic ancestry, extended kinship, phenotype and complex mixture analysis while maintaining backwards compatibility with the sizing of combined DNA index system (CODIS) loci. Highly multiplexed PCR, combined with sample barcoding, could provide the throughput and speed to make current sequencing technologies an attractive alternative to traditional capillary electrophoresis (CE) sizing. There are several technical hurdles that need to be addressed to ensure comparable performance to CE methods and maintain backwards compatibility with current STR databases, including balanced multiplex PCR reactions, adequate read length, barcoding of pooled samples, and allele calling based on sequence instead of CE sizing. In addition, because commercial DNA analysis programs do not align STR alleles accurately, a custom program was developed to identify STR alleles. Two people who appear to share the same allele have been distinguished by sequencing, and individuals who appear to be homozygous at a locus have been shown to be heterozygous. Of 36 people sequenced so far in this study, more than half have one or more novel STR alleles.

Complex DNA mixtures were tested using panels of SNPs with low minor allele frequencies (MAF) and were sequenced on an Ion Torrent PGM or Proton. These results demonstrate that the SNP profile of an individual can be resolved in an equimolar mixture of DNA from 8 unrelated people with a RMNE probability of 4×10^{-9} (as calculated in Voskoboinik, et al., 2011, Forensic Sci. Int. Genetics, 5:428-435). Results from additional mixture analyses will be presented.

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