B185 Multiplexed SNP Detection System for Mitochondrial DNA

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After attending this presentation, attendees will learn about a novel SNP genotyping method and its use in the detection of mitochondrial SNPs.

This presentation will impact the forensic community and/or humanity by presenting optimization of a multiplexed SNP detection system and its application to mitochondrial DNA.

Single nucleotide polymorphisms (SNPs) are the most prevalent form of genetic variations in the human genome. Because of their low mutation rate, SNPs have the potential to be used as genetic markers in human identity applications such as paternity testing, genealogy studies, and human population genetics. In addition, the ability to be genotyped using very short DNA fragments, SNP genotyping may become a very important tool to analyze highly degraded and aged samples commonly seen in the forensic field. For the analysis of aged sample (e.g., bones) or samples that contain a small amount of biological material (e.g., hair), SNP markers on mitochondrial DNA are preferred over SNP markers on nuclear DNA because of multiple copies of mitochondrial DNA in a cell. The major goal of present study was to develop a sensitive, easy to use, rapid, and robust SNP detection method for forensic applications. A detection system for identification of mutations and SNPs was optimized. The method involves a multiplexed PCR amplification of as many as 48 regions in the mitochondrial genome, followed by detection of mutations or SNPs in these amplicons using an oligonucleotide ligation assay (OLA). Optimization of the multiplex PCR was achieved using primer titration and minimizing primer-dimer formation. The ligated products were hybridized to coded sequences with mobility modifiers and detected by capillary electrophoresis. Direct detection by CE following ligation is also possible. To define the accuracy of the system total mitochondrial genomes were sequenced from 20 individuals using M13 tailed primers for PCR amplification designed to cover the entire genome.

A SNP detection multiplex assay comprised of multiple SNPs outside the hypervariable region was developed for mitochondrial SNP detection. Performance of the assay was evaluated by comparison with sequence approach. Complete concordance for more than 200 allele calls made with the PCR/OLA method and sequencing method was observed. The method was further evaluated for forensic application by using forensic type samples, such as vaginal swab, saliva, hair, urine, and blood stains. The results indicate that the SNP system is accurate, sensitive, fast (< 7 hour time for analysis), easy to perform, medium through-put, and robust.

Genotyping, Mitochondrial DNA, SNP

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