DEVELOPMENT OF mtDNA MULTIPLEX SNP PANELS

Dixie Hybki, M.S.[1], Shelley Crispin, M.S.[1], Suzanne Shaffer, M.S.[1], John V. Planz, Ph.D.[1], Arthur Eisenberg, Ph.D.[1], Kerri Dugan Ph.D.[2]
[1]DNA Identity Laboratory, Department of Pathology and Anatomy, University of North Texas Health Science at Fort Worth, Texas.
[2]FBI Laboratory, Counterterrorism and Forensic Science Research Unit, Quantico, Virginia

The standard method for mitochondrial DNA (mtDNA) sequencing is laborious, involves significant analysis time, and often requires multiple amplifications of limited genetic material. Methods that can improve mtDNA typing are needed. A single nucleotide polymorphism (SNP) assay is one approach that has the capability of assaying degraded DNA and the potential for reducing sample consumption.

We have developed four mtDNA multiplexes to analyze the hypervariable (HV) regions of the mtDNA genome, two for HV1 and two for HV2, using Applied Biosystem's SNaPshot™ multiplex kit. SNaPshot is a single-base extension methodology utilizing specific primers designed to anneal immediately adjacent to SNP sites. A single fluorescently-labeled ddNTP, complementary to the SNP site, is incorporated into the primer, and the primers are then separated by size via capillary electrophoresis.

Our multiplex panels interrogate a total of 39 mtDNA polymorphic sites that demonstrate higher frequencies of substitution in the control region of the mitochondrial genome. For HV1, we have designed a 13-plex and an 11-plex and for HV2, we developed an 11-plex and a 10-plex SNP panel. We designed our panels such that 3 extension primers specific for each region (HV1 or HV2) are the same in each multiplex panel, providing a double check system. Haplotypes can be obtained with as little as 50pg starting material for PCR amplification. Due to the sensitivity of this SNP assay, only small amounts of amplicon are required for analysis, leaving plenty for downstream sequencing. Additionally, we have optimized a HV1/HV2 multiplex PCR amplification and are currently testing this single tube amplification product with our SNP array to further streamline the assay.