B113 Developments in SNP Analysis via Quadrupole MS for Forensic Applications

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The goals of this presentation are to present an approach to DNA SNP genotyping for forensics that is based on specific and mass adjustable molecular tags with APCI quadrupole MS detection.

As the field of forensic DNA analysis advances and genetic complexity is further defined, there will be a need for instrumentation and methods that can both accurately genotype novel genetic systems in individuals as well as provide this information in a timely and cost-effective manner. The determination of a single-nucleotide polymorphism (SNP) or a set of SNPs located within the mitochondrial or nuclear DNA can prove useful in a variety of forensic applications including mass disaster situations where substantive degraded DNA is present, cases involving paternal or maternal family lines for missing persons, or mixed samples from multiple male donors in the case of rape or abuse. Many SNPs that may prove beneficial for these forensic applications are becoming evident. In addition, there are several different contending approaches for assessing SNPs. Some of the current approaches include DNA microarrays both suspension/solution based and solid support based, fluorescence tagging, mass spectrometry, pyrosequencing, and direct sequencing. One potential high-throughput approach identifies SNPs and the allelic state by labeling with small molecular weight tags, i.e., Masscodes. This allele specific discrimination assay will be explored and presented, primarily in regard to forensic applications.

The current casework mtDNA assay analyzes approximately 610bp of mtDNA by sequencing the HVI and HVII regions of the control region. However, currently between 4 and 11% of mtDNA analyses in Caucasians yield identical DNA sequences. Analysis of regions of the mitochondrial genome outside of the HVI and HVII could improve resolution. A mtSNP analysis system could analyze SNPs from the remainder of the mitochondrial control region, as well as polymorphic sites outside of the control region. A proof-of-concept study of the Masscode assay utilizing known mitochondrial DNA (mtDNA) sequenced samples and Y-chromosome SNP (Y-SNP) determinations have been initiated. A mtSNP or a Y-SNP analysis may provide discrimination information not currently available and can also provide a method for rapidly excluding samples.

The Masscode assay employs cleavable mass spectrometry tags (CMSTs) that are conjugated at the 5' end with a SNP specific oligodeoxynucleotide. The CMST includes a photolabile linker, a mass spectrometry sensitivity enhancer, and a variable mass unit, all connected through a scaffold constructed around a central lysine. Each tagged oligonucleotide has a different cleavable mass unit that can be uniquely associated with the specific DNA sequence being interrogated. Identification of the polymorphic state is determined by photolytic cleavage of the tag from the amplicon, followed by detection with a standard single quadrupole mass spectrometer using positive-mode atmospheric pressure chemical ionization (APCI). The assay provides a high level of sensitivity (femtomole range), can be designed for multiplex analyses, can be completely automated, and can be scaled from highthroughput to medium-to-low throughput which may be more applicable to forensic analyses.

Three groups of mtDNA-sequenced samples were selected for this pilot study: 25 Caucasian, 25 African American, and 25 Hispanic samples. A few samples were purposely mixed to test the assay in another dimension. Ten mtSNPs were probed: 73A, 16126C, 16069T, 16270T, 16298C, 16223T, 16189C, 16311C, 204C, and 195C. In addition, foren-
sically important Y-SNPs were investigated.

Genotyping, SNP Analysis, Mass Spectrometry

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