A57 High-Throughput Human Mitochondrial DNA Database Development Using Automated Electrospray-Ionization Mass Spectrometry

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The goal of this presentation is to demonstrate the use of a fully-automated electrospray-ionization mass spectrometry (ESI-MS) system in the high-throughput development of a human mitochondrial DNA profile database.

This presentation will impact the forensic science community by revealing how, over the course of five years, the Ibis platform was used to develop a database of one hundred thousand human mitochondrial DNA profiles at a single site, allowing an unprecedented ability to interrogate the prevalence and extent of genetic variation in the non-coding region, including length and point heteroplasmy.

Forensic mitochondrial DNA (mtDNA) analysis is performed when DNA quantity/quality is insufficient for nuclear DNA analysis, or maternal-lineage DNA is required. Analysis involves sequencing mtDNA segments, a lengthy and labor intensive technique. Ibis Biosciences has developed a high-throughput mass spectrometry-based mitochondrial DNA profiling assay suitable for automated analysis of mtDNA control region segments that retains approximately 94% of the individually-discriminating information of sequencing the same regions. The assay amplifies HVI 15924-16428 and HVII 31-576 and provides more discriminating information than sequencing the minimum HVI and HVII regions of 16024-16356 and 73-34, respectively. Twenty four (24) overlapping PCR primer pairs are used to amplify 1051 bases of mtDNA hypervariable regions HVI and HVII in eight triplexed PCR reactions. Pre-fabricated 96-well kit plates are configured such that one sample occupies one column of an assay plate and 12 samples may be run on a single plate. All reaction components are supplied in frozen kit plates such that only DNA extract (5ul per well) needs to be added prior to thermal cycling. After thermal cycling, assay plates are placed directly onto an Ibis T5000™ or PLEX-ID™ instrument and all analytical steps through mass spectrometry data processing are automated. Amplified PCR products are desalted using an anion-exchange matrix coupled to paramagnetic beads and analyzed by ESI-MS in a time-of-flight (TOF) mass spectrometer. Forward and reverse strands of each PCR product are weighed with sufficient accuracy to calculate the base composition (A, G, C, and T count) of each amplicon in multiplexed PCR reactions. Base composition indexed by rCRS coordinates of amplified regions define 24-module mtDNA profiles that can be compared directly to each other or used to search a lookup or population database derived from mass spectrometry, sequence data, or both. Likewise, sequence data can be used to search a base composition-based database by converting the sequence(s) to base compositions corresponding to the primer pairs employed in the assay. Base composition profile comparisons to a population database are amenable to the same counting and confidence interval (upper bound frequency) estimates used for sequence comparisons.

The Ibis platform was used over the course of five years at a single laboratory to generate a searchable database of 117,278 human mitochondrial profiles. Profiles were developed as a list of PCR product base compositions referenced by revised Cambridge Reference (rCRS) coordinates. Profiles may be directly compared to each other, compared to a database of base compositions, or compared to a population database of sequence profile by converting sequence profiles to base compositions prior to comparisons. Frequency statistics for profile matches within a population database may be calculated in a manner identical to sequence database comparisons. The assay is not hindered by the presence of sequence length heteroplasmy, is capable of resolving mixtures, and can be used to quantify the relative contributions of both length and point heteroplasmic variants. Statistics will be presented detailing the development of an mtDNA profiling system, sample processing rates over time using two Ibis instruments in parallel, rates of profile completion, heteroplasmic variants, and precision and reliability of assay controls.

mtDNA, Mass Spectrometry, Base Composition Analysis

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