Quantitative measurement of mtDNA heteroplasmy by electrospray ionization time of flight mass spectrometry (ESI-TOF-MS)

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Mitochondrial DNA mixtures may be the result of heteroplasmy (the presence of multiple mtDNA genomes within an individual) or due to multiple contributors. Heteroplasmy can exist as multiple nucleotides at a particular location or as variable lengths of DNA. Whether the mixture is natural or situational, the accuracy of analysis and interpretation of a mixed mtDNA sample is a function of the technology employed. Previous studies have estimated the prevalence of heteroplasmy within the general population, described tissue specific variation within an individual (hair versus blood or saliva), and estimated that a 20% minor component is required for detection by sequencing. However, there is a paucity of true quantitative measures of intra-individual variation. While Sanger sequencing is routinely used for mtDNA analysis, the method is inadequate with mixed samples due to its unreliable quantitative performance and limited detection capabilities. We have previously described a multiplex PCR electrospray ionization time of flight mass spectrometry (ESI-TOF-MS) assay that produces a base composition profile for a given mtDNA sequence and retains approximately 94% of the resolving power compared to direct sequencing. While analysis of length heteroplasmy by sequencing often results in limited data due to degraded electropherogram quality, ESI-TOF-MS detects each DNA fragment independently, providing a sensitive quantitative measure of each species present. The examination of multiple hair segments from known heteroplasmic individuals demonstrates that ESI-TOF-MS is a reliable and reproducible measure of heteroplasmic species.