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MITOCHONDRIAL DNA MIXTURE RESOLUTION BY BASE COMPOSITION ANALYSIS

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The analysis of human mitochondrial DNA (mtDNA) is commonly used in forensic investigations to assist in identifying the source of evidentiary items, uniting unidentified remains with family members, and identifying victims of mass disasters. Typically, hypervariable regions 1 and 2 (HV1 and HV2) are amplified by polymerase chain reaction (PCR) and subsequently analyzed by Sanger sequencing of fluorescently labeled products. While Sanger sequencing is considered the gold standard methodology for mtDNA analysis, it is a laborious and time intensive process. Moreover, in some cases, evidentiary items may contain biological material from multiple contributors, resulting in mixtures of DNA types. Mixed samples are problematic for Sanger sequencing; this method typically does not allow for accurate quantification. Therefore, deconvolving the components of a mixed sample by Sanger sequencing is challenging and often leads to uninterpretable results. Methods capable of detecting and resolving mixtures are needed to fully maximize the informative value of evidence. Base composition analysis of mtDNA using electrospray ionization mass spectrometry (ESI-MS) allows for quantitation and resolution of the components of mtDNA mixtures. In this method, PCR amplified mtDNA fragments are ionized and separated according to the intrinsic molecular mass of the products present. By exploiting the base-complimentary nature of DNA, the masses of both the forward and the reverse strands are used to deduce the base composition of the amplified species. Differences in base composition indicate genetic differences between samples. The relative signal intensities of products produced by the same primer pairs are used to measure the relative amounts of each mtDNA type in the mixed sample. This presentation describes the application of base composition analysis using ESI-MS to the detection and resolution of mixtures of biological samples.