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Research article

Multiplex PCR electrospray-ionization mass spectrometry (ESI-MS): Application to forensic mitochondrial DNA examinations

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Abstract

Mitochondrial DNA (mtDNA) examinations play an important role in criminal investigations, identification of victims of mass disasters, and association of unidentified remains with family members. Typically, HV1 and HV2 are amplified via polymerase chain reaction (PCR) followed by fluorescent sequencing. While this method produces the highest level of resolution, it is labor intensive and unable to distinguish components of a mixture. Previously, an electrospray-ionization mass spectrometry (ESI-MS) method was described to determine the base composition profile of enzymatically digested PCR amplified fragments derived from the HV1 and HV2 regions. Advantages of ESI-MS compared to sequencing include speed of analysis, automation, and increased sensitivity, while retaining a high degree of resolution. Here, we report the next generation of this method in which a base composition profile is determined from 24 overlapping PCR reactions. Because ESI-MS provides the relative abundance of each component present, this method allows for the quantitative typing of mixtures. This ESI-MS method does not rely on a priori knowledge of variable sites, allowing the capture of private mutations and individual-specific variation. Due to the multiplex design, automation, speed of analysis, and ability to interrogate mixtures, this method provides a powerful and rapid tool for forensic mtDNA examinations.

Keywords: Mitochondrial DNA; Mass spectrometry; Base composition

Mitochondrial DNA (mtDNA) typing now can be achieved using electrospray-ionization mass spectrometry (ESI-MS). This method calculates the base composition profile for a DNA fragment by measuring the molecular masses of the light and heavy strands of a PCR product. Differences in base composition are correlated to genetic differences among individuals. The ESI-MS method described by Hall et al. [1] is robust, reliable, and automatable, and achieves approximately 85% of the resolving power of sequencing. Thus, base composition analysis is one of the most informative mtDNA SNP-based analytical methods developed to date.

Because base composition analysis does not provide discreet base location, some substitutions can balance one another and mask genetic information. For example, within a 100 base fragment two individuals (1 and 2) differ only at two positions—np 24 and np 83. Sequencing would reveal that these two individuals have different mtDNA types. However, if at position 24, individual 1 had an A (which resulted from a G to A transition) and at position 83 individual 1 had a G (which resulted from an A to G transition), individuals 1 and 2 would have the same base composition and could not be differentiated. With longer fragments there is a greater chance for two balancing substitutions to occur, reducing the resolving power per comparison. In the original procedure described by Hall et al. [1], amplicons were restriction digested to reduce the fragment sizes to less than 140 nucleotides in length, a requirement for base composition analysis. The next generation of this ESI-MS method eliminates the restriction digestion and increases the number of amplicons to 24 overlapping PCR fragments, generated in 8 triplex reactions, covering nucleotide

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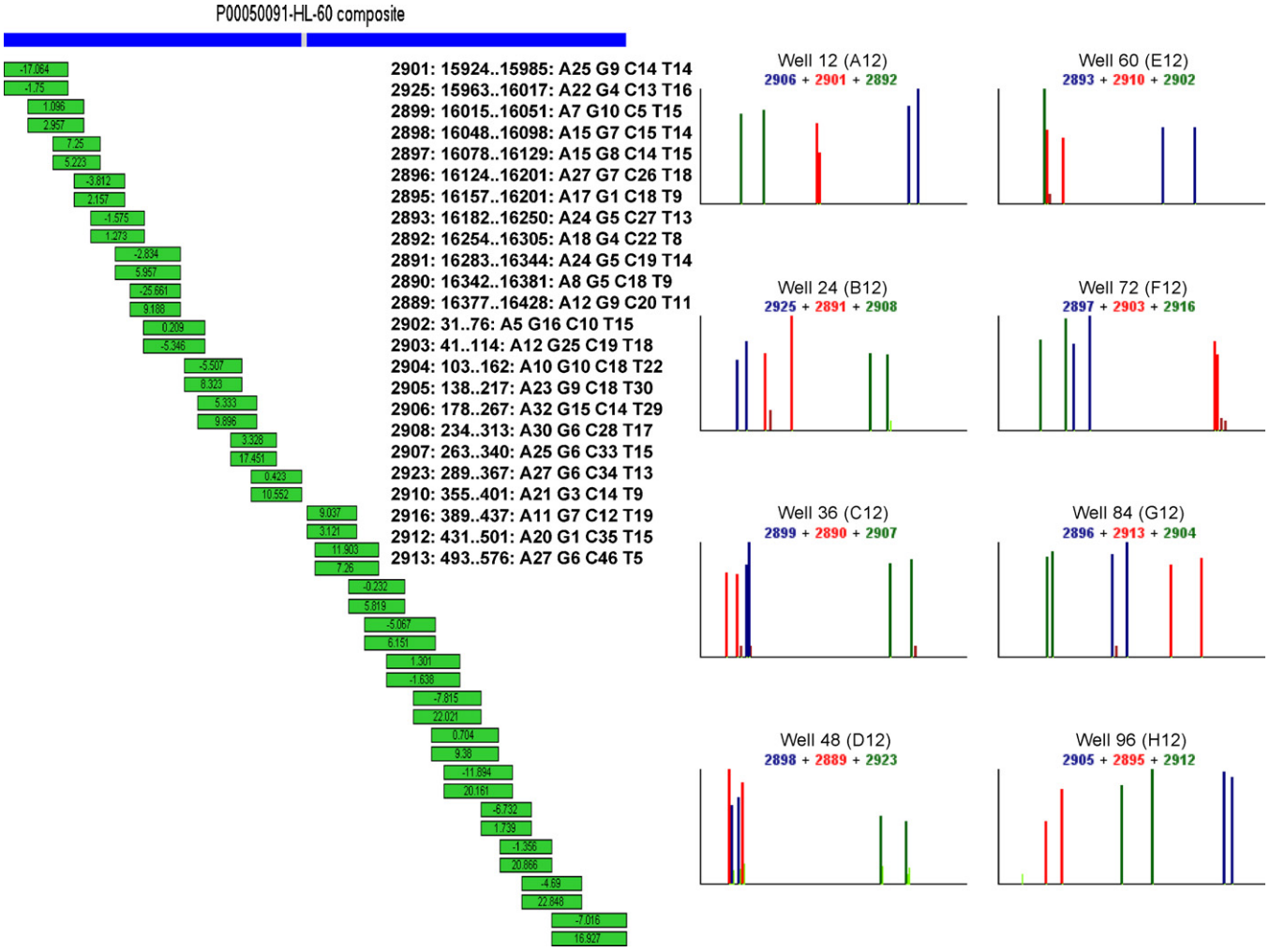

Fig. 1. The image on the left is the sample coverage map. Each green bar represents one strand of a double-stranded product. Numbers within each box are the mass measurement error for the product, expressed in parts per million (ppm). The text in the center lists the calculated base composition with corresponding nucleotide positions for each double-stranded fragment. The images on the right are spectral schematic views for each of the triplex PCR reactions. The spectral schematic view can be selected to focus on each primer pair's raw and deconvolved spectrum. Each primer pair is color coded with the corresponding key for the triplex indicated at the top of the panel.

positions 15,924–16,428 and 31–576. Amplified fragments range in size from 85 to 140 nucleotides and all triplex reactions amplify under identical reaction conditions. The greater number of small-sized fragments increases coverage and reduces the chance of two balancing substitutions within the same amplicon; this ESI-MS method achieves approximately 94% of the resolving power compared with sequencing. Amplification of small-sized amplicons has the added benefit of increased sensitivity and better success for analyzing degraded DNA.

Fig. 1 represents part of the data output from a base composition analysis. The coverage map is recreated to display all 24 amplicons (PCR products as bars) and their position in the mtDNA genome. The mass measurement error for the product is expressed in parts per million (ppm). Calculated base compositions for each double-stranded product are listed with corresponding nucleotide positions. The images on the right are spectral schematic views for each of the triplex PCR reactions. Each reaction is color coded with the corresponding primer pair indicated at the top of the panel.

This ESI-MS method consistently yields reliable results for mtDNA derived from buccal swabs, blood stains, telogen hairs, bones, and teeth. Additionally, heteroplasmic samples (both point/sequence and length) and mixtures of different known mtDNA types can be quantified and deconvolved, provided there are no balancing substitutions with in an amplicon. Base composition analysis by ESI-MS is a rapid, robust, reproducible, precise, and sensitive method capable of capturing individual-specific variation and resolving mixtures of mtDNA types.

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Conflict of interest

None.

Reference

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