B100 Base Composition Analysis of Human Mitochondrial DNA by Electrospray Ionization Mass Spectrometry: Applications for Forensic Examinations

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The goal of this presentation is to demonstrate the power and utility of base composition analysis of human mitochondrial DNA (mtDNA) by electrospray ionization mass spectrometry (ESI-MS).

Base composition analysis by ESI-MS is highly reproducible, precise, and sensitive. Moreover, the method provides the ability to detect and quantify heteroplasmy and mixtures of different mtDNA types. This presentation will impact the forensic science community by demonstrating how base composition analysis by ESI-MS is a rapid, robust method capable of capturing individual specific variation and resolving mixtures of mtDNA types.

Mitochondrial DNA analysis plays an important role in criminal investigations, identification of victims of mass disasters, and association of unidentified remains with family members. The conventional method of typing human mitochondrial DNA relies upon amplification of hypervariable regions 1 and 2 (HV1 and HV2) of the control region by the polymerase chain reaction (PCR) followed by cycle sequencing. Fluorescently labeled sequencing fragments are then separated by capillary electrophoresis. The resulting sequence is described relative to the published revised Cambridge Reference Sequence (rCRS). Sequencing of human mtDNA is often considered the gold standard for detecting variation due to the single base resolution achieved. However, sequencing is a time consuming and laborious process that is not quantitative. Moreover, when confronted with a mixture of different mtDNA types, sequencing results are often difficult to interpret. Additionally, sequencing requires substantial post-PCR processing of samples.

Electrospray ionization mass spectrometry (ESI-MS) offers an attractive alternative to traditional sequencing because it is a rapid, sensitive, automatable method for the quantitative analysis of human mtDNA that affords exquisite mass resolution. With this process, PCR generated fragments are purified and ionized. The molecular masses of the ionized amplicons are used to determine the base compositions of DNA products which are directly correlated to sequence variation. The molecular mass measurement obtained for each product strand is used to derive a list of possible base compositions. By exploiting the base complimentary nature of DNA, the masses of both the forward and the reverse strands are used to constrain the list of potential base compositions to one configuration. The reliability of the base composition determination is due to the fact that it is derived from an intrinsic property of the PCR product, the molecular mass, which is independent of environmental conditions. A feature of ESI-MS analysis is that two samples with different molecular masses will have different sequences and each ionized component is detected independently. Each signal in a mixture can be deconvolved and the intensities can be used to measure the relative amounts of mtDNA types. Thus, quantitation and resolution of mixed mtDNA samples is possible.

To investigate the utility of this method, known mtDNA types were analyzed by ESI-MS base composition analysis. Known mtDNA types were selected to include types with insertions, deletions, point (or sequence) heteroplasmy, and length heteroplasmy. Also, biological samples were selected to mimic those typically encountered in forensic mtDNA casework. Samples types included blood, hair, saliva, and bone. Matching buccal swabs and hair samples from the same donor were processed to explore tissue-specific variation. Samples were extracted and amplified using a multiplex PCR consisting of 24 overlapping reactions. The sensitivity of the method was assessed by processing diluted samples of known mtDNA types. Mixed mtDNA samples were generated by mixing two known mtDNA types at predetermined ratios prior to multiplex PCR amplification.

Base composition analysis by ESI-MS is highly reproducible, precise, and sensitive. Moreover, this method provides the ability to detect and quantify heteroplasmy and mixtures of different mtDNA types. Base composition analysis by ESI-MS is a rapid, robust method capable of capturing individual specific variation and resolving mixtures of mtDNA types.

Mitochondrial DNA, Mass Spectrometry, Base Composition

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