Increasing the Discriminatory Potential of Mitochondrial DNA Sequencing by Analyzing Control Region Data Outside of the Hypervariable Regions

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Mitochondrial DNA (mtDNA) sequencing is employed in forensic casework involving biological evidence that does not contain a sufficient quantity and/or quality of DNA for nuclear DNA testing. In addition, mtDNA analysis is utilized when appropriate reference specimens for nuclear DNA comparisons cannot be obtained.

The discriminatory potential of mtDNA sequencing is based upon the hypervariability of DNA located in portions of the control region and limited by the fact that the mtDNA genome is a single locus. Therefore, the ability to increase the discriminatory potential of mtDNA sequencing is dependent upon identifying the variability in other regions within the mtDNA genome. This presentation will address the variability of two additional regions within the control region. Each region is approximately 250 base pairs in length. The first region (VR1) is located between the hypervariable regions. The second region (VR2) is located adjacent to and immediately following hypervariable region two.

Approximately 100 database samples were sequenced using primers to target the entire control region. The hypervariable region sequence information was generated to demonstrate consistency with previously generated sequence data. The variable region sequence information was generated to assess the degree of variability present in these portions of the mtDNA control region. The variability observed in the variable regions was considerably less than that observed in the hypervariable regions. Even so, the variable regions of these samples exhibited sufficient variability to indicate that the additional sequence information may be useful for further discriminating among individuals having the same hypervariable region sequence. Therefore, hypervariable region database samples having common haplotypes were sequenced using primers to target the variable regions. Collectively, seven out of thirty-four United States Caucasian samples were further discriminated using variable region sequence information.

Although the variable region sequence information exhibited sufficient variability to allow for the further discrimination of some individuals having the same hypervariable region sequence, the variable region data does not exhibit adequate variability to warrant analyzing the full control region for each item of evidence. Hence, overlapping primer sets encompassing the variable regions were not utilized. Instead, primers were used to target small portions (~75 base pairs) of the variable regions, which contain polymorphic base positions (e.g., 16519, 489). Another position of interest is a simple, dinucleotide (CA) repeat between positions 514 and 523. In addition to the standard number of repeats (Anderson, *et al.* 1981. Nature 290:457-465), a repeat insertion (positions 523.1 and 523.2) and a repeat deletion (positions 522 and 523) were observed.

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