FORENSIC mtDNA ANALYSIS AND MIXTURE SEPARATION BY DENATURING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Mitochondrial DNA analysis using Denaturing high-performance liquid chromatography (DHPLC) is a novel approach to the detection of sequence variability in the mitochondrial D-loop. The technique relies on cross-hybridization of reference and questioned samples followed by high-resolution chromatographic separation of the resulting homo- and heteroduplices under partially denaturing conditions. The approach uses PCR primer sets that have been validated in forensic science for amplification of the HVI and HVII regions. The resulting chromatographic profile serves as a reproducible means of mitochondrial DNA sequence polymorphisms, heteroplasmy and multi-component mixtures. Pair-wise comparisons of hypervariable regions of human mitochondrial DNA from 96 unrelated human volunteers has demonstrated the ability to accurately identify sequence differences for each of four mtDNA amplicons in approximately seven minutes per sample. Thus, DHPLC analyses yield reproducible chromatographic profiles for maternally related individuals that are distinct from non-maternally related individuals possessing mitotypes that differ by at least one basepair.

The laborious nature, high cost and complexity of separating mtDNA mixtures has also been an obstacle to the broader use of mtDNA in forensics. Denaturing HPLC is being developed as a method for the efficient separation of DNA mixtures in preparation for DNA sequencing. The technology enables sequence-specific separation of mtDNA mixtures by high-resolution chromatography of cross-hybridized DNA. DHPLC can be used to isolate the individual components of natural (heteroplasmic) and situational (multi-contributor) mixtures. This is achieved without secondary amplification or excessive manipulation prior to DNA sequencing and will reduce the time and money required to obtain conclusive mitochondrial DNA results. DHPLC technology has the potential to benefit criminal and mass disaster investigations requiring timely analysis of mitochondrial DNA.