Analysis of Mitochondrial DNA Mixtures with Restriction Enzymes

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As repeatedly demonstrated, amplification and sequencing of mitochondrial (mt)DNA can be successfully performed even in cases where typing with genomic DNA is no longer possible. A practical consideration of great importance is the much higher copy number of mtDNA.

On the other hand direct sequencing of mtDNA has certain disadvantages. If several mtDNA molecules from a given individual are amplified in the same reaction any potential DNA mixture will remain undetectable as background noise.

In this context we present the use of restriction enzymes (e.g. Alw44I, DdeI) for the detection of DNA mixtures. DNA mixtures of two individuals (one individual with and one individual without the enzyme specific recognition site) were amplified in the same PCR reaction. After the digestion of the PCR products with several restriction enzymes, up to a ratio of 1+50 (5ng +100pg and 1ng +20pg DNA) the two individuals could be clearly detected.

In conclusion, the use of restriction enzymes is a reliable and fast method for the detection of DNA mixtures.