

The Detection of C-stretch in Mitochondrial Control Region Using Melting Curve Analysis.

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Sequencing analysis of mitochondrial DNA (mtDNA) hypervariable region is effective tool for forensic individual identification because of its high rate of mutation and unique maternal inheritance. But the presence of C-stretch sometimes makes the complicated interpretation of the sequence which shows the mixture of the different length variants (length heteroplasmy) in its downstream. The C-stretch is caused by the nucleotide T-C replacement at #16,189 in hypervariable region 1(HV1) or by the insertion of nucleotide C after #309 in hypervariable region 2 (HV2). In this report, we describe about the prediction of C-stretch using melting curve analysis prior to sequencing analysis. The DNA was extracted from 125 individuals and mtDNA HV1 or HV2 region was amplified in the real-time PCR apparatus LightCycler (Roche diagnostics). Following amplification, melting curve analysis for the products were carried out with fluorescent labeled probes which were designed to anneal with the sequence including nucleotide #16,189 in HV1 or #309 in HV2. It is expected that the nucleotide replacement or insertion at the positions makes the melting temperature of the probe lower or higher. In order to confirm the analysis, the sequence of each product was verified in routine procedure. As a result, the melting temperatures of the samples were classified into three groups of HV1 and two groups of HV2. And the sequencing analysis of the products clarified that the difference of the temperature reflected the presence or absence of C-stretch or other nucleotide replacement predictably. It means that this melting curve analysis makes the brief typing of mtDNA possible without any other extra steps including electrophoresis. The rapid and simple method described in this report must be useful to construct the effective analysis strategies.