

# Quantitative and qualitative profiling of mitochondrial DNA length heteroplasmy

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## Introduction

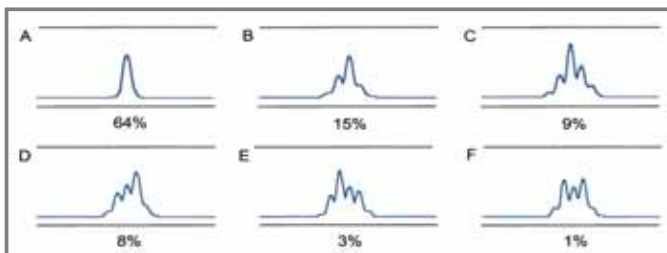
Quantitative and qualitative analysis of mitochondrial DNA length heteroplasmy for the first hypervariable segment (HV1) and the second hypervariable segment (HV2) regions were performed using size-based separation of fluorescently labelled PCR products by capillary electrophoresis. In this report, the relative proportions of length heteroplasmies in individuals were determined, and each length variant in the heteroplasmic mtDNA mixture was identified. The study demonstrated that 36% and 69% of Koreans show length heteroplasmy in the HV1 and HV2 regions, respectively. Electropherograms revealed that length heteroplasmy in the HV1 region resulted in over 5 length variants in an individual. The peak patterns of length heteroplasmy in the HV1 region were classified into 5 major types. In the HV2 region, length heteroplasmy resulted in 3 to 6 length variants in an individual, and showed 8 variant peak patterns. The increased knowledge concerning mtDNA length heteroplasmy is believed to not only offer a useful means of determining genetic identity due to increased mitochondrial DNA haplotype diversity by allowing mtDNAs to be classified into several peak patterns, but also represent a promising tool for the diagnosis of several common diseases which are etiologically or prognostically associated with mtDNA polymorphisms.

## Materials and Methods

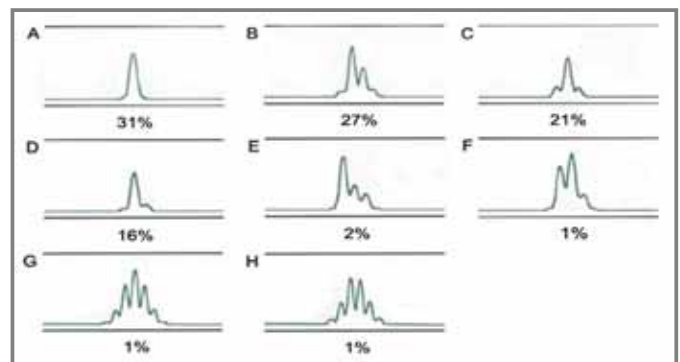
One hundred unrelated Korean DNAs were extracted from buccal swabs using QIAamp DNA Mini Kit (Qiagen). Amplification of two hypervariable regions of mitochondrial DNA (HV1 and HV2) was performed in a PCR mixture of total volume 10.0 $\mu$ l containing 0.1ng of DNA template and two sets of fluorescent primers. Thermal cycling was conducted under the conditions of 25 cycles of amplification with 56°C annealing temperature. The resultant PCR products were separated by capillary electrophoresis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

## Results

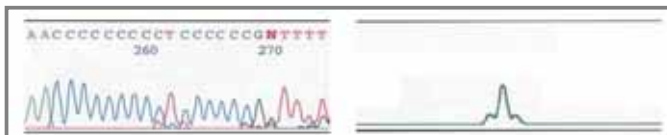
In the electropherograms, homoplasmic mtDNAs showed a single peak representing only one type of mtDNA, and heteroplasmic mtDNAs produced multiple peaks of different sizes and heights.



The mtDNA peak patterns of the HV1 region in GeneScan electropherograms. According to the analysis, 36% of samples showed length heteroplasmy in the HV1 region.



The mtDNA peak patterns of the HV2 region in GeneScan electropherograms. According to the analysis, 69% of samples showed length heteroplasmy in the HV2 region.



Sequencing and GeneScan electropherograms of HV2 length heteroplasmy. The HV2 heteroplasmic peak patterns in GeneScan analysis were very similar to multiple T peaks shown in the middle of homopolymeric C-stretch in sequencing electropherograms.

## Conclusions

We established a new strategy for profiling length heteroplasmies, which enables both the identification of all length variants in a mixture and the confirmation of the existence of a length heteroplasmy. The increased knowledge concerning mtDNA obtained in this study is believed to offer a useful means of determining genetic identity due to increased mitochondrial DNA haplotype diversity, by allowing mtDNAs to be classified into several types of peak patterns. Also, the developed method will present a promising tool for the diagnosis of several common diseases which are etiologically or prognostically associated with mtDNA polymorphisms.