

# Measuring by fragment analysis the proportion of length variants in samples carrying length heteroplasmy at the homopolymeric C-stretch in mitochondrial HVII region

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**Abstract.** We describe a method to estimate the proportion of length variants in samples carrying mtDNA homopolymeric C-stretch (303 to 315) originated by a T310C transition. Sequencing analysis of these samples produces blurred electropherograms and the exact number of C-residues remains ambiguous. This has important consequences in forensic casework, since one of the aims in the interpretation of mtDNA profiles is the determination of the existence of a match or a mismatch between two or more sequences under study. HV-2B region (positions 172 to 408) was amplified with two sets of labelled primers. Electrophoresis of fluorescently labelled amplicons was performed on an ABI310 or ABI377. Results were reproducible in repeated PCR experiments, using the two sets of primers and using either capillary or slab-gel electrophoresis. © 2003 Elsevier B.V. All rights reserved.

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## 1. Introduction

MtDNA control region contains three C-homopolymeric tracts frequently associated with length heteroplasmy. This length variability should not be taken into account when comparing mtDNA profiles, whilst point heteroplasmy is regarded as strength of the evidence [1]. However, in particular cases, it would be desirable to characterize these length variants in order to gain an additional support for the identification by mtDNA analysis.

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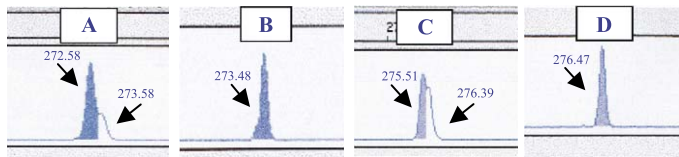


Fig. 1. Sizes of L amplicons (263G 315.1C haplotype) on ABI 310 (A and B) and ABI 377 (C and D) with final extension step (B and D) and without final extension step (A and C).

## 2. Materials and methods

### 2.1. Samples

Blood from a female and her daughter (individuals 1 and 2, respectively); two hairs from the daughter; and saliva samples from three maternally related donors (a female and two sons; individuals 3, 4 and 5, respectively). All samples exhibited a partial haplotype 195T 263G 310C. A sample with haplotype 263G 315.1C was used as reference.

### 2.2. PCR

Two PCRs, using primer pairs 6' FAM-L 172/H 408 and L 172/ NED-H 408, were carried out to generate fluorescent labelled L and H amplicons. After routine protocol, an additional extension step (72 °C for 45 min) was performed to promote the addition of an extranucleotide by Taq polymerase at the 3' end of the PCR product.

### 2.3. Electrophoresis

ABI377 and ABI310 equipments. ROX-500 was used as size standard. The data were analysed using GeneScan software.

## 3. Results

As expected, PCR fragments with the same length showed differences in size due to different electrophoretic mobility of attached fluorochromes and different nucleotide com-

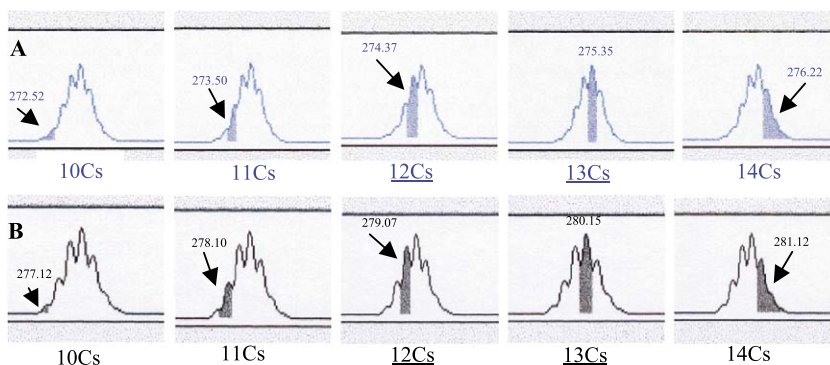


Fig. 2. ABI 377 electrophoretic profiles from individual 1: (A) L strand, and (B) H strand. A low level of 15Cs variant can be observed. Predominant length variants are underlined. Similar patterns were obtained in ABI 310 platform and also from individual 2.

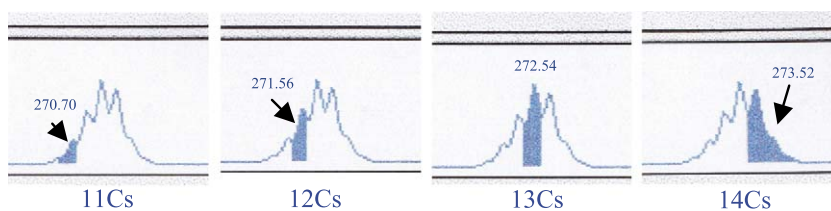


Fig. 3. ABI 310 electrophoretic profile from hair sample of individual 2. Probably 10 and 15Cs variants (first and last shoulder peaks) are present. Predominant length variants are underlined.

position of L and H strands. The differences found between the two platforms may be attributed to the influence of physical electrophoresis conditions [2] (Fig. 1). Sizes of the reference PCR products (14 residues in the C-stretch) were approximately 273.5 bp (L strand) and 278 bp (H strand) on ABI 310 and 276.5 bp (L strand) and 281 bp (H strand) on ABI 377.

Samples with T–C transition rendered electropherograms with multiple peaks differing in size by a single nucleotide (heteroplasmic pattern). The relative proportions of the various length variants were estimated by peak heights. The predominant variants from individual 2 contained 12 and 13C residues in blood sample (Fig. 2) whereas in hair sample contained 13 and 14 (Fig. 3). This is in accordance with intra-individual heteroplasmic variability previously described [3]. A slight difference in the proportion of length variants could be observed between individuals within a single maternal lineage (data not shown).

#### 4. Discussion

The measurement of length variant proportions by fragment analysis showed a great reproducibility since the same pattern was obtained with both strands and on 310 or 377 sequencers. Repeated PCR experiments from the same sample provided similar heteroplasmic patterns, giving further support to the fact that the heteroplasmic state is present in the sample and does not arise during the *in vitro* replication process. In the forensic practise, the estimation of proportion of length variants could increase the usefulness of this region in order to support or refuse an identification (e.g. when the evidence and known samples come from the same tissue).

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