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Typing mtDNA SNPs of forensic and population interest with snapshot

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Abstract. In last years, interest in Single Nucleotide Polymorphism (SNP) has increased due to their numerous applications: in medical genetics, in human and evolutionary genetics but also in forensic field. Polymorphisms placed in the mitochondrial genome are particularly important in a forensic context in part due to the fact that it is in high copy number per cell. Consequently, mtDNA is especially useful to the study of degraded samples with little or no nuclear DNA as being old skeletal remains and hair shafts. On the other hand, mtDNA SNPs have demonstrate its usefulness for human population studies. The development of new methodologies for high-throughput SNPs analysis is one of the most promising areas in genetics. Here, we describe a rapid and robust assay to simultaneously genotype 17 SNPs of mitochondrial DNA by minisequencing reaction using SNaPshot (Applied Biosystems). The methodology here described shows high accuracy and sensitivity, and avoid the use of alternative time-consuming classical strategies (i.e. RFLP typing) and the requirement of high quantities of DNA template.

Keywords: SNaPshot; SNPs; mtDNA; Hypervariable region; Human identification

1. Introduction

The development of new methodologies for high-throughput SNPs analysis is one of the most challenging areas in genetic research because their numerous applications. Most of these high-throughput technologies as DNA microarrays or MALDI-TOF are extremely expensive and therefore cannot be used by a great majority of standard labs. Furthermore, many of them are still in phase of development. Classical methods are time-consuming, costly and require high amounts of DNA. Here, we describe a rapid and robust assay to simultaneously genotyping 17 mitochondrial DNA coding region SNPs by minisequencing reaction using SNaPshot (Applied Biosystems), a methodology based on the dideoxy

Abbreviations: HVS-I, the first hypervariable region; HVS-II, the second hypervariable region; rCRS, revised Cambridge Reference Sequence.

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(ddNTP) single base extension of an unlabeled oligonucleotide at the 3′ -end of the base immediately adjacent to the SNP. Each ddNTP is labeled with different fluorescent dyes. We have incorporated a set of SNPs in a multiplex that allow allocate common mitochondrial European types into the mtDNA phylogeny, including haplogroup H, the most frequent lineage in Europe (40–50%) and the worst phylogeneticaly characterized in the first and second hypervariable segments as well.

2. Materials and methods

A total of 266 individuals from Galicia (nothwest Iberia) were analyzed. First hypervariable segment (HVS-I) was sequenced from position 16024 to 16400 following conditions described by Salas et al. [1]. A preliminary classification of the sequences into haplogroups was done following phylogenetic criteria according to Richards et al. [2].

Two reactions of PCR were performed: Multiplex 1 includes SNPs defining common European haplogroups (4216, 4529, 4580, 7028, 10,398, 10,400, 10,873, 12,308, 12,705 and 14,766). Multiplex 2 includes SNPs defining subhaplogroups inside haplogroup H (3010, 3915, 3992, 4336, 4769, 4793 and 6776). After post-PCR purification with ExoSapIT (Amersham Biosciences), minisequencing reaction of 17 SNPs was carried out simultaneously. After post extension treatment with Sap (Amersham Biosciences), products were electrophoresed on an ABI PRISM 3100® Genetic Analyzer.

In some cases, two SNPs are placed very closely to each other within the mtDNA molecule and this allows its co-amplification in the same amplicon. The size of the PCR products ranged from 80 to 224 bp, which facilitate the analysis of degraded samples or with low quantities of DNA. The extended SNaPshot primers for different SNPs differ between them by size and color and they can be detected by capillary electrophoresis. The length of the extension primers were modified by the addition of non-homologous tails at the 5′ -end.

3. Results and discussion

Fig. 1 shows the electropherogram for the 17-coding region mtDNA SNPs minisequenced in a single reaction.

The advantages of the used of this set of SNPs in a forensic and anthropological context clearly manifest when looking at the most common European lineage (H) that represents 45% of the Galician samples. We can subtype this haplogroup in 6 different sublineages [3] (frequencies in Fig. 2). H7 (4793G) did not found. With the sub-classification of rCRS

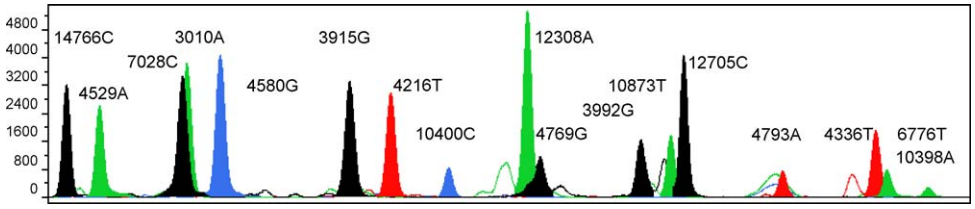

Fig. 1. Electropherogram for the minisequenced 17-coding region mtDNA SNPs.

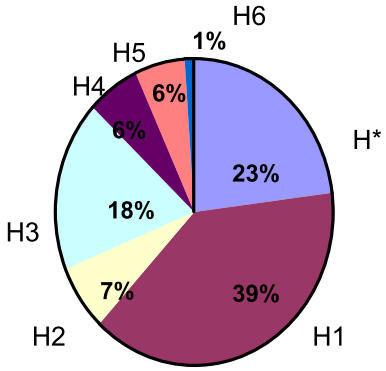

Fig. 2. Frequencies of sublineages of haplogroup H in Galician sample.

in HVS-I, the haplotype diversity increase from 0 to 0.737 in this set of samples. Other HVS-I haplotypes also gain in discrimanation power when using this coding region SNPs. In those cases where HVS-I is phylogenetically informative [2], we found a complete correlation with the coding region mtDNA SNP status.

The method for typing of 17 mitochondrial DNA coding region SNPs of forensic and population interest with SNaPshot shows to be rapid, robust and accurate and avoids both the use of alternative time-consuming classical strategies (i.e. RFLP typing) and the requirement of high quantities of DNA template. The electrophoresis performed on an ABI PRISM 3100® Genetic Analyzer providing and automatic, standardized and sensitive mode of detection and process of the results.

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