



## 29 Y-chromosome SNP analysis in European populations

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**Abstract.** Single Nucleotide Polymorphisms (SNPs) located on the Y chromosome have a specific interest as forensic tools, and based on these facts, we have designed a strategy, which allows to identify the most frequent haplogroups in Europe. Among the 245 binary polymorphisms described in the Y Chromosome Consortium tree, we have selected 29 markers. The whole set has been grouped into four multiplexes, in order to determine the final haplogroup using only one or two multiplexes. In this way, we avoid typing unnecessarily SNPs to define the final haplogroup saving effort and cost, since we only type in the best case 9 SNPs and in the worst possible combination 17 SNPs. One hundred fifty samples from Galicia (Northwest Spain) have been analysed. Data obtained by using the SNaPshot multiplex kit (Applied Biosystems) will be presented. © 2003 Elsevier B.V. All rights reserved.

*Keywords:* Y-chromosome; SNP; Multiplex analysis; Europe

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### 1. Introduction

Analysis of binary polymorphisms, and especially Single Nucleotide Polymorphisms (SNPs) is becoming an extensive routine. [1]. In particular, Y-SNPs have a specific interest as forensic tools for several reasons. Firstly, the SNPs are the most frequent kind of polymorphism. Secondly, because of their simplicity, SNPs can be analysed from a very low quantity of DNA in small fragments and are amenable to analysis with high throughput technologies. Thirdly, the low mutation rate allows their use in paternity testing. Nevertheless, the presence of only two alleles in addition to the lack of recombination and the highly population-specific distribution, shown in the Y-chromosome, often needs the analysis of large numbers of SNPs.

Despite slow progress in the discovery of Y-chromosome variation, now a large number of SNPs have been described, defining a highly resolved tree of binary haplogroups [2,3] Checking the literature, an extensive search has been performed looking for the allele

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frequencies of each SNP in Europeans. As a result of this search, a set of 29 SNPs was selected in order to determine the most frequent haplogroups present in Europe.

A large number of SNP genotyping methods are now available; however, the selected method was the SNaPshot multiplex system (Applied Biosystems).

## 2. Material and methods

PCR multiplexes were performed in 25  $\mu$ l of final volume, with 1  $\times$  buffer, 300  $\mu$ M of dNTPs, 2 mM of MgCl<sub>2</sub>, 2 U of Taq Gold polymerase and 10 ng of genomic DNA, except multiplex 2 which was performed with 200  $\mu$ M, 1.5 mM of MgCl<sub>2</sub> and 0.5 U of Taq Gold. The cycling conditions were 95 °C 10 min, 32 cycles of 94 °C 30 s, 59 °C 30 s and 70 °C 30 s, and a final extension at 65 °C during 15 min. Primer concentrations range between 0.1 and 0.5 (primer sequences and concentrations are available from the authors upon request). After PCR, 1  $\mu$ l of the product was cleaned with 0.5  $\mu$ l of ExoSAP-IT (Amersham Biosciences), in order to eliminate the unincorporated primers and dNTPs.

Multiplex SNaPshot reactions were performed in 5  $\mu$ l final volume, including 2  $\mu$ l of multiplex SNaPshot kit (Applied Biosystems) and 1.5  $\mu$ l of cleaned PCR product. The cycling conditions were 96 °C 10 s, 50 °C 5 s and 60 °C 30 s, during 25 cycles. Primer sequences and concentrations are available from the authors upon request. To remove the unincorporated ddNTPs, the final product was incubated with 1 U of SAP (Amersham Biosciences). Snapshot products were run on an ABI 3100 and analysed using Genescan 3.7 software (Applied Biosystems).

## 3. Results and discussion

The 29 SNPs were divided in four multiplex PCRs, according to their location on the Y-chromosome consortium tree. Multiplex 1 allows for the detection of the major clades more frequent in Europe [2,3], multiplex 2 determines haplogroups G, I and J, multiplex 3 subdivides haplogroup E, and multiplex 4 subdivides haplogroup R1b. The strategy adopted for typing the SNPs includes on one hand the development of four multiplex PCRs, and on the other hand the development of four multiplex SNaPshot reactions (Fig. 1).

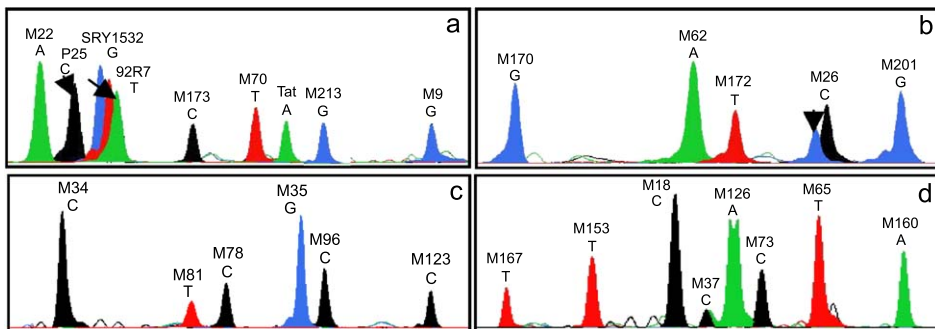


Fig. 1. SNaPshot multiplexes from different samples. (a) Multiplex 1, the P25 shows duplicated pattern; (b) multiplex 2, M26 shows an artefact blue peak; (c) multiplex 3; (d) multiplex 4.

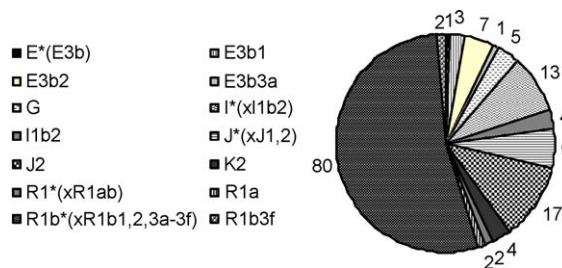


Fig. 2. Haplogroup frequencies in Galicia.

The products of the PCR multiplexes were checked in polyacrylamide gels (data not shown). In the SNaPshot reactions, the products were spatially separated tailing the 5' end of the minisequencing primers with varying lengths of poly (dC) non-homologous tails.

The reproducibility of these four multiplexes was checked in 150 samples from Galicia. For all the samples, the results agree with the Y-chromosome Consortium tree [2,3]. Only four samples were assigned to the final haplogroup using one multiplex, the remaining samples needed two multiplexes.

The 29 SNPs analysed describe a total of 32 haplogroups; however, in the 150 samples analysed only 14 of these haplogroups were detected (Fig. 2).

With this hierarchical strategy adapted for Europeans the massive typing of SNPs was avoided, and therefore the time and money involved in the study was also reduced. Once more the high degree of population homogeneity present in Europe has been confirmed, since more than half the samples belong to the same paragroup.

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