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Multiplex PCR development of Y-chromosomal biallelic polymorphisms for forensic applications

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Abstract. The aim of this study is to set-up multiplex PCR of NRY single nucleotide polymorphisms (SNPs) suitable for forensic purposes. A first multiplex has been developed with SNP loci defining the European haplogroups (M35, M89, M172, M170, M9, M173, M45). PCR was performed with primers designed to produce amplicons in a range between 96 and 136 bp starting from 1 ng of DNA template. PCR product was minisequenced with tailed primers of different length and run in an automated five-colour capillary electrophoresis sequencer. © 2003 Published by Elsevier B.V.

Keywords: Y-SNPs; Multiplex PCR; Minisequencing; Geographical affiliation

1. Introduction

Such binary polymorphisms, also known as single nucleotide polymorphisms (SNPs), are of special interest for forensic purposes, because the amplification of short fragments including the single-base mutation which characterizes their polymorphism potentially allows a positive result. Underhill et al. [1] recently detected more than 200 biallelic variations screening a sample of 21 populations, with many of the markers showing regional specificity. The combination of these binary polymorphisms yielded a phylogenetic tree based on the principle of maximum parsimony, defining 18 haplogroups, indicated by capital letters from A to R (Fig. 1) [2].

The aim of this study was therefore to set up a multiplex PCR of Y-SNPs suitable for forensic purposes, employing materials, methods and technologies normally available in forensic laboratories.

2. Materials and methods

DNA was extracted from whole blood of 68 Europeans and 29 non-European subjects using the QIAamp (Qiagen, Chatsworth, CA) procedure [3]. The M35, M89, M172,

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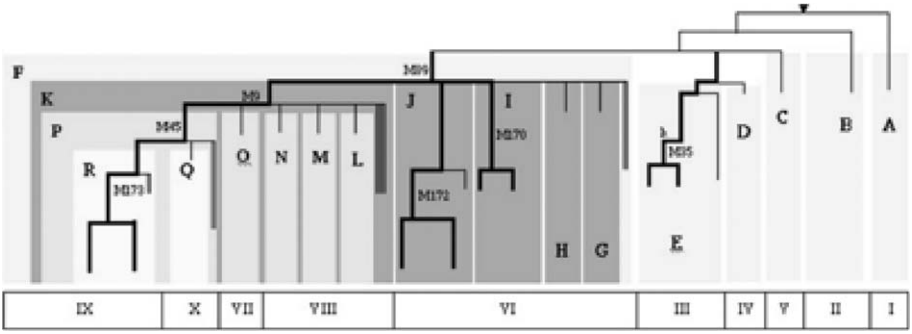

Fig. 1. Phylogeographic tree (YCC 2002, modified), showing loci investigated in this study: M35, M89, M172, M170, M9, M173 and M45.

M170, M9, M173 and M45 loci [1], characterizing European haplogroups, were chosen for this multiplex. One nanogram of DNA was submitted to amplification and 4 μl of the purified product was submitted to a dideoxy single-base extension of unlabelled oligonucleotide primers using the SnaPshot multiplex kit (AB) in the conditions recommended by the manufacturer. Moreover, multiplex was tested on low molecular weight DNA after fragmenting in a sonicator device (Labsonic, from B. Braun). Sonicated DNA was amplified with the AmpFlSTR Identifiler PCR Amplification kit (AB) and was analyzed on an ABI 310 (AB).

3. Results

For SNP genotyping, we used minisequencing, a single-base extension of an unlabeled oligonucleotide primer. Hot-start PCR was chosen for our multiplex, in place of Touchdown PCR, because it worked better and all the loci in the multiplex, starting from only 1 ng of DNA in our experimental conditions, could be amplified.

The first multiplex was designed to contain mutations capable of placing the Y chromosome of the subject under investigation within the known European haplogroups. Haplogroups F*(xK), R1 and E, that, although most highly represented in Europe, are not exclusive to Europeans. Of the 68 Europeans tested, 64 were correctly assigned to one of the three European haplogroups when typed with the multiplex we made.

Experiments to explore the suitability of the Y-SNP multiplex with low molecular weight DNA were performed by amplifying DNA fragments sized less than approximately 150 bp, produced by sonication. Positive amplifications were obtained from 1 ng of DNA for all seven SNPs included in our multiplex, whereas only pherograms with high noise background and questionable peaks below the rfu threshold of 50 were obtained (Fig. 2).

4. Discussion

Although the small number of people examined here does not allow conclusive results to be shown, the present work demonstrates the definite possibility of affiliating one subject to the geographical area of provenance by studying the biallelic markers of the Y-chromosome located in the lowest branches of phylogenetic tree.

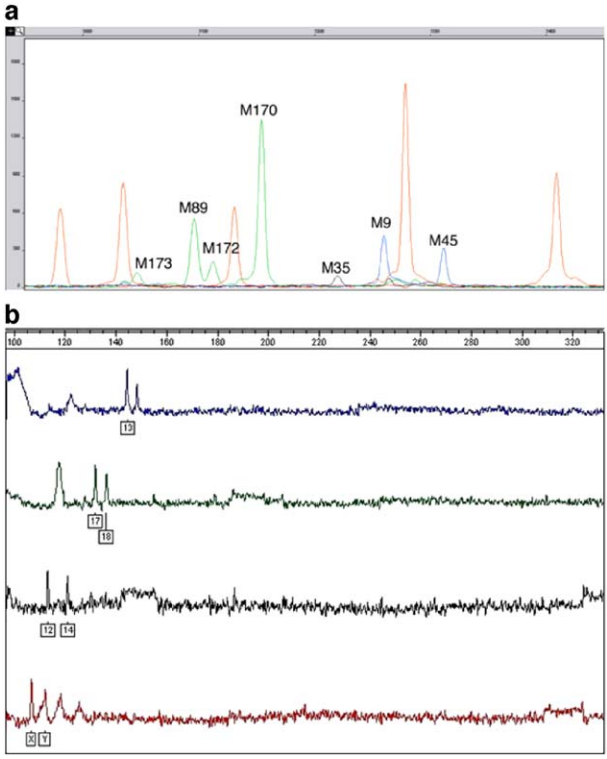

Fig. 2. Y-SNP-multiplex and microsatellites amplification from low molecular weight DNA fragmented by sonication. (a) Pherogram of Y-SNP multiplex obtained by amplification of 1 ng DNA sonicated for 200 min. All seven extension products are present in the pherogram. Typing results: K*(xP). (b) Microsatellites typing of 1 ng of fragmented DNA by sonication for 200 min from the same sample.

In conclusion, the main achievement of this work is the development of an effective, robust multiplex PCR of seven Y-chromosome SNPs starting from 1 ng of DNA template, exploring polymorphisms on fragments between 96 and 136 nucleotides in size, yielding positive results also with degraded DNA. SNPs, included in the multiplex, are hierarchically organized, capable of assigning the geographical area of the subject and guiding the choice of further polymorphisms to be analyzed for evolutionary or forensic purposes. This hierarchical strategy is an important feature of forensic work when only small amounts of DNA are available.

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