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Multiplex minisequencing strategies for phenotyping M02 haplogroup-derived Y-SNPs in African populations

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Abstract. A reliable technique for biallelic phenotyping of M02-derived Y-SNPs has been carried out by means of multiplex minisequencing after laser detection of electrophoresed fragments. A tetraplex including PN1, M155, M156 and M191 provides information about the actual status of up to nine SNPs having M02 background. An application of this technique in a population study of four sub-Saharan African populations has been accomplished. © 2004 Elsevier B.V. All rights reserved.

Keywords: Single nucleotide polymorphism; SNP; Y-chromosome; Minisequencing; African populations

1. Introduction

Single nucleotide polymorphisms (SNPs) have been estimated to occur in 1 out of every 1000 nucleotides in humans, and therefore embody the most abundant source of genetic variation so far available [1]. More specifically, Y-SNPs, due to their model of inheritance, offer substantial possibilities in anthropological genetics and forensic studies. Within the YAP insertion, M02-derived lineages represent specific markers in African populations profiling [2]. Biallelic identification by polymerase mediated single-base primer extension by minisequencing, in combination with specific fluorescent targets followed by laser-automated detection, introduces new and relevant opportunities in the population screening of biallelic markers. This work deals with the development of the experimental conditions for the simultaneous screening of four Y-SNPs (PN1, M155,

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Table 1
Minisequencing primers of the Y-SNPs under study

SNP	Amplicon (bp)	Sequencing primers	Haplogroup
PN1	179	5′-CAAggg TCTT gAgA gggA gAgC-3′	E3*(xE3a)(C/T)
M155	226	5′-(C)6-gAATggA gAgg AATC CTCA CCTA TC-3′	E3a5(A/G)
M156	116	5′-CGCCGATACTTg CCTC CACg ACTT TCCT-3′	E3a6(A/G)
M191	370	5′-CCTTTTAAATTTAC ATTT TTTT CTTT ACAACTTg ACTA-3′	E3a7(T/G)

M156, M191, all them having the M02 background), by means of minisequencing strategies.

2. Materials and methods

Blood samples were obtained from donors after verbal consent. Only autochthonous individuals going back at least two generations from their respective ethnic groups were considered. The populations studied were: Fon (Benin), Bamileke (Cameroon), Wairak (Tanzania) and Tutsi (Rwanda). DNA was extracted using the standard phenol–chloroform method [3], and stored at − 20 °C before use for no longer than 1 year.

PCR multiplex coamplification was carried out at 55 °C in a GeneAmp 9700 (Applied Biosystems). Minisequencing was carried out at an annealing temperature of 60 °C, following the ABI Prism SnaPShot Multiplex kit conditions, using the sequencing primers described in Table 1. An ABI PRISM 3100 Genetic Analyzer was used for capillary electrophoresis, followed by laser detection of fluorescently labelled fragments.

3. Results and discussion

Identification of Y-SNPs phenotypes were made according to molecular size (ranging from 22 bases in PN1, to 39 in M191) (Table 1), and the fluorochrome incorporated into

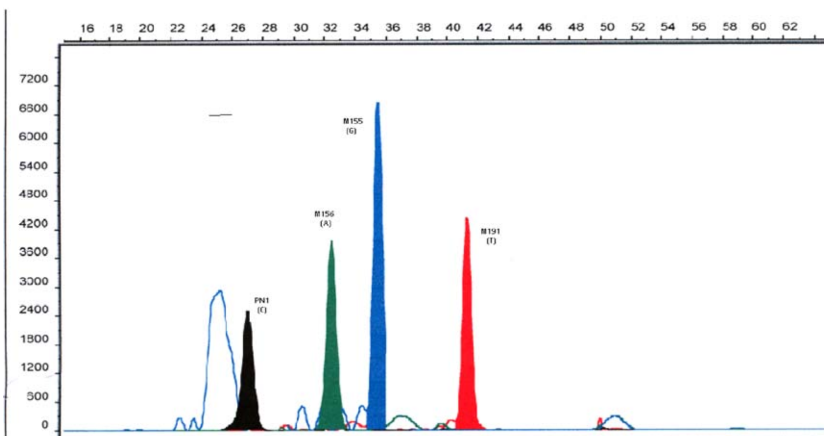

Fig. 1. Minisequencing profiles of Y-SNPs after capillary electrophoresis followed by fluorescent laser detection.

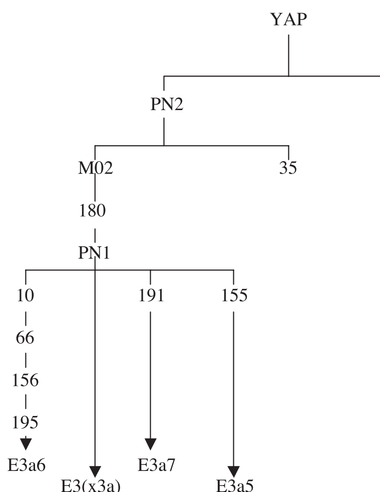

Fig. 2. Y-SNPs tree derived from M02 mutation.

the respective nucleotides. This is significant in the recognition of PN1 transitions (C–T) that may be clearly distinguished from spurious bands that may overlap the reading zone (Fig. 1) [4]. Drawn from YAP insertion, M02 gives rise to lineages that specifically characterise sub-Saharan populations, in which the incidence of PN1 mutation reaches from 0.4 up to 1.0, in contrast to Caucasoid populations in which it is virtually absent. Its presence in northern African populations points to genetic flow as the most important cause.

This multiplex provides information about the actual status up to nine single SNPs with the M02 background (Fig. 2). M02-derived haplogroups give rise to lineages that are well represented in African sub-Saharan populations. However, substantial population differences may be seen, not only in the incidence of the M02 mutation but also in the frequencies of the single derived haplogroups, which are in line with the degree of diversity reported for African populations.

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