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Analysis of 29 Y-chromosome SNPs in a single multiplex useful to predict the geographic origin of male lineages☆

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Abstract. The European Consortium "High throughput analysis of single nucleotide polymorphisms for the forensic identification of persons—SNP*for*ID" has performed a selection of candidate Y-chromosome SNPs (single nucleotide polymorphisms) for making inferences on the geographic origin of an unknown sample. A "Major Y chromosome haplogroup typing kit" has been developed, which allows the multiplex amplification of 29 SNPs in a single reaction followed by a single base extension (SBE) reaction (minisequencing) and separation of the resulting extension products by capillary electrophoresis.
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Keywords: Y-chromosome; SNP; Multiplex; Geographic origin

1. Introduction

Markers located on the Y-chromosome have specific properties useful for many different areas. Their genetic diversity can be used to provide information on male-specific patterns of migration in the past and on the origin and diversity of specific populations [1]. In addition, in the forensic field, they have shown to be useful for screening samples from sexual assault cases as well as to provide evidence on the male lineage in deficiency paternity cases [2]. Based on this, we have selected a group of candidate Y-SNPs to provide clues on the possible

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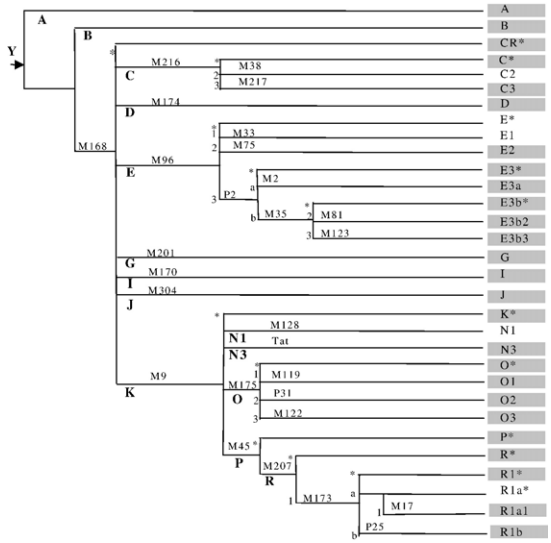

Fig. 1. Phylogenetic tree of binary Y-chromosomal haplogroups.

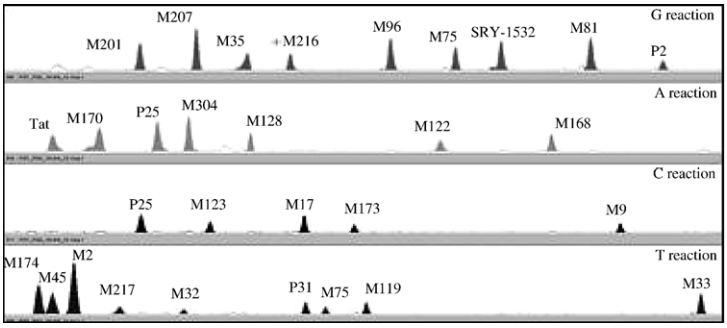

Fig. 2. Electropherogram with 29 Y-chromosome SNP profile from a male donor belonging to the Hg R1b.

Table 1
Haplogroup frequencies in each population

Haplogroup	A	B	CR* (xCE,G,IK)	C* (xC2,3)	C3	D	E2	E3* (xE3ab)	E3a	E3b* (xE3b2,3)	E3b2	E3b3
Galicia			1					1		6	6	1
Colombia									5	4	2	2
Mozambique	1	23					9		93		1	
Argentina												1
Thais			3	3	3							
China					3	1						
Japan			1	1	3	12						
Germany								**3**	3	10		2
Denmark										3		1
Greenland					**1**					1		
Somalia		1	3						1	78	3	
Turkey										1		3

geographic origin of a sample. We have developed multiplex strategies, allowing the analysis of as many SNPs as possible in a single reaction. Finally, we have validated the new multiplex in a sample of more than one thousand unrelated males distributed all around the world.

2. Material and methods

A total of 1126 unrelated males from Denmark (150), Greenland (90), Turkey (51), Thailand (84), China (55), Japan (40), Germany (150), Galicia (130), Mozambique (130), Somalia (105), Argentina (81 racially mixed individuals), and Colombia (60 ethnically interbred individuals) were typed.

In order to identify the major population haplogroups present all around the world, a total of 29 SNPs were selected [3]. Fig. 1 represents the haplogroup tree.

SNP genotyping was performed in a single multiplex PCR reaction followed by a single SBE reaction using the SNaPshot™ multiplex kit (Applied Biosystems). Primer sequences, PCR conditions and minisequencing conditions are available from the authors upon request. Detection of SBE products was performed by capillary electrophoresis using an AB 310 or AB 3100 genetic analyzer (Applied Biosystems).

3. Results and discussion

A stable multiplex PCR was developed with 28 amplicons ranging in size from 79 to 188 bp (M122 and M123 are located on the same amplicon). The single base extension products in the final multiplex design ranged from 18 to 95 nucleotides in sizes, which include the target-specific sequences of the SBE primers and non-complementary tails with different sizes to separate the SBE products in the subsequent electrophoresis. In Fig. 2, a representative electropherogram of the 29-plex is shown.

The major Y-chromosome haplogroup typing kit seems to have utility to make inferences about the possible geographic origin of any sample of interest (Table 1). With this first panel of SNPs, it can be possible to discriminate only between the major human groups present all around the world. It is evident, however, that a Y haplotype-based prediction will always be limited to the elucidation of the patrilineage and thus could be quite misleading regarding the phenotype of a given individual. It cannot be excluded that a person exhibiting a European phenotype may carry a sub-Saharan or Asian Y-chromosome which has been passed on over many generations.

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Haplogroup	G	I	J	K* (xN1,3,OP)	N3	O* (xO1,2,3)	O1	O2	O3	P* (xR)	R* (xR1)	R1* (xR1ab)	R1a	R1b
Galicia	2	17	19	2								1	1	73
Colombia	1	2	4	1						3	3		1	32
Mozambique														3
Argentina	6	5	8	1	1					28				31
Thais			1	4		2	9	21	36				2	
China				3			9	9	30					
Japan				1			1	14	7					
Germany	3	29	10	1	2					3			20	64
Denmark	1	56	4							2		3	26	54
Greenland		20	2							48			5	13
Somalia	1		2	15									1	
Turkey	5	4	17	2						1	1		7	10