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Y-SNPs analysis in Japanese using liquid bead array technology

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Abstract. Forty-two Y chromosome single nucleotide polymorphism (Y-SNP) loci were investigated in 142 unrelated Japanese males by liquid bead array technology, which is a detection method using microspheres and a flow cytometer. Six haplogroups were found in this study. About two-thirds of Japanese were classified into 'Haplogroup O,' which is widely distributed over East Eurasia, and one-third were 'Haplogroup D,' which is different from the haplogroup distribution for other East Asian races. © 2003 Elsevier B.V. All rights reserved.

Keywords: Single nucleotide polymorphism (SNP); Y chromosome; Haplogroup; Japanese population

1. Introduction

Analysis of single nucleotide polymorphism markers on the Y chromosome (Y-SNPs) could provide useful information in some forensic cases or paternal genetic relationships. However, comparing with STR, analyses of many SNPs loci are required to get information of individual identification because SNPs are biallelic loci. There are several different methods for analyzing SNPs. One of them is a liquid bead array system, which is a rapid, cost-effective, high-throughput readout system performed on a flow cytometer, LuminexTM 100 (Luminex, Austin, USA).

2. Materials and methods

Genomic DNA samples were extracted from buccal swabs using Chelex 100 method. A Signet Y-SNP Identification System (Marligen Biosciences, USA) provides the PCR mixture, 43 SNPs primers and specific oligonucleotide complementarily bind the beads.

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Table 1
Polymorphic loci included in the Y-SNP identification system and their multiplex assignments

Multiplex	1	2	3	4	5
Markers	M42	M2	M11	M3	M18
	M45	M31	M52	M5	M37
	M60	M32	M130	M95	M87
	M89	M33	M170	M119	M153
	M94	M35	M172	M124	M157
	M168	DYS391	M174	SRY+465	P25
	M175	M75	M201	SRY9138	SRY10831
	M207	M150		Tat	
	Amelogenin	M146			
		M182			
		P3			
		P4			

Forty-three loci cannot be amplified by one tube, but the template DNA is amplified by dividing into five tubes, taking advantage of the characteristic of a marker. Analyzed markers were listed in Table 1. The flow of experiment is as follows: first, the 5-ng template DNA is amplified, then the primers are removed by Exonuclease I enzyme digestion, the PCR products are labeled and the beads hybridized in accordance with manufacturer's

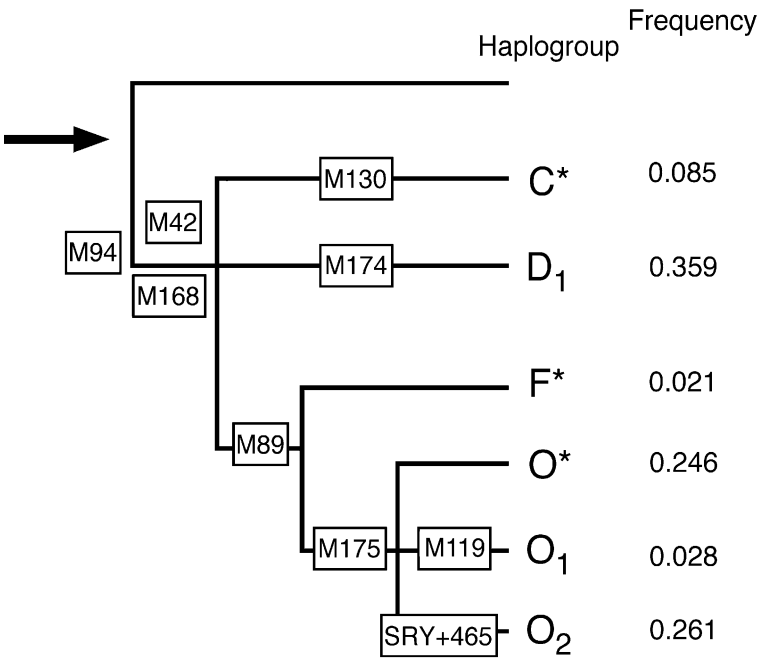

Fig. 1. The genealogical tree and frequency created using the nine markers in which changes was found in Japanese. The root of the tree is denoted with an arrow. An Asterisk means that it belongs to the clade, but not to subclade [1].

protocols. Finally, collected LuminexTM data were analyzed by MasterPlex GT software (MiraiBaio, Alameda, USA).

3. Results and discussion

This Y-SNPs kit is said to determine the ethnic groups using the tree of human Y-chromosomal binary haplogroups, which was made by the Y Chromosome Consortium [1]. Of the 42 Y-SNP markers (without amelogenin), the changes were observed in nine markers in which six haplogroups in 142 subjects analyzed can be classified (Fig. 1). All Japanese samples have changes in the M42, M94 and M168 markers. Haplogroup D and haplogroup O were classified to the major clades defined by M175 and M174 markers in addition to above markers, respectively. Further, 41 samples classified into haplogroup O fell into the subclade O1 and O2 by M119 and SRY+465 markers, respectively. This haplogroup O is widely distributed over East Eurasia, but is hardly found in Africa and Europe [2]. Otherwise, the other major haplogroup D is close to African clade according to genealogical tree [1] and different from the haplogroup distribution for other East Asian races [2]. It might be thought that D lines spread in Japan influenced by random genetic drift. The other two small clades are haplogroups C and F, defined by M130 and M89 markers, respectively.

This system was very effective in SNP genotyping; however, for individual identification, appropriate SNP loci would have to be chosen.

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