



Analysis of the single nucleotide polymorphisms of mitochondrial DNA by liquid bead array technology

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Abstract. Thirty single nucleotide polymorphisms (SNPs) in mitochondrial DNA were investigated in 105 unrelated Japanese by liquid bead array technology which is a detection method using microsphere and a flow cytometer. Nineteen SNPs were observed, and 50 different mt DNA types were observed, and 35 types were seen only once, and 15 types in multiple individuals. Genetic diversity was estimated as 0.943. This array system is simple and rapid, as well as suitable for high-throughput applications in a forensic investigative screening. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

DNA typing of mitochondria has been used as a useful technique in forensic caseworks. Signet™ Mitochondrial DNA Screening System (Marligen Biosciences, Ijamsville, USA) is a new array system based on liquid bead array technology. Thirty single nucleotide polymorphisms (SNPs) in human mitochondrial DNA can be analyzed rapidly by this system.

2. Materials and methods

DNA samples of 105 unrelated Japanese volunteers were extracted from buccal swabs using Chelex 100 method.

The assay consists of five steps as follows: (1) Amplify the mitochondrial DNA hypervariable regions. (2) Remove the PCR primers by digesting with Exonuclease I. (3) Label the PCR products with a fluorescently tagged oligonucleotide by primer extension. (4) Hybridize the labeled DNA with allele-specific oligonucleotide in a multiplex beads array. (5) Read the hybridized microspheres in a Luminex 100 System. Collected data was analyzed by a MasterPlex GT software (MiraiBaio, Alameda, USA). In this study, IB,

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Table 1
mt DNA types observed in 105 Japanese

IB	IC1	IC2	ID	IIA1	IIA2	IIB	IIC	IID	N
CRS	CRS	CRS	CRS	73G	CRS	CRS	CRS	263G	1
CRS	CRS	CRS	CRS	73G	CRS	CRS	195C	263G	1
CRS	CRS	CRS	16362C	73G	CRS	CRS	CRS	263G	2
CRS	CRS	CRS	16362C	73G	CRS	152C	CRS	263G	1
CRS	CRS	16311C	16362C	73G	CRS	CRS	CRS	263G	1
CRS	16304C	16311C	CRS	73G	CRS	CRS	CRS	263G	1
CRS	16304C	16311C	CRS	73G	CRS	CRS	195C	263G	1
CRS	16304C	16311C	CRS	73G	CRS	152C	CRS	263G	10
CRS	16304C	CRS	CRS	73G	CRS	CRS	CRS	263G	2
16217C	CRS	CRS	CRS	73G	CRS	CRS	CRS	263G	2
16217C	CRS	CRS	CRS	73G	CRS	CRS	195C	263G	1
16217C	CRS	CRS	CRS	73G	CRS	CRS	200G	263G	2
16217C	CRS	CRS	CRS	73G	CRS	146C	CRS	263G	1
16217C	CRS	CRS	CRS	73G	CRS	150T	CRS	263G	1
16217C	CRS	CRS	CRS	73G	CRS	150T	195C	263G	1
16217C	CRS	CRS	CRS	73G	CRS	150T/152C	CRS	263G	2
16217C	CRS	16311C	CRS	73G	CRS	CRS	CRS	263G	1
16217C	CRS	16311C	CRS	73G	CRS	150T	195C	263G	1
16217C	16298C	CRS	16362C	73G	CRS	152C	CRS	263G	1
16223T	CRS	CRS	CRS	73G	CRS	CRS	CRS	263G	4
16223T	CRS	CRS	CRS	73G	CRS	CRS	195C	263G	1
16223T	CRS	CRS	CRS	73G	CRS	146C	CRS	263G	1
16223T	CRS	CRS	CRS	73G	CRS	146C	199C	263G	1
16223T	CRS	CRS	CRS	73G	CRS	150T	CRS	263G	5
16223T	CRS	CRS	16362C	73G	CRS	CRS	CRS	263G	18
16223T	CRS	CRS	16362C	73G	93G	CRS	CRS	263G	1
16223T	CRS	CRS	16362C	CRS	CRS	CRS	CRS	263G	1
16223T	CRS	CRS	16362C	73G	CRS	CRS	CRS	CRS	1
16223T	CRS	CRS	16362C	73G	CRS	150T	CRS	263G	6
16223T	CRS	CRS	16362C	CRS	CRS	150T	CRS	263G	1
16223T	CRS	CRS	16362C	73G	CRS	152C	CRS	263G	7
16223T	CRS	CRS	16362C	73G	CRS	152C	CRS	CRS	1
16223T	CRS	CRS	16362C	73G	CRS	CRS	195C	263G	2
16223T	CRS	CRS	16362C	CRS	CRS	CRS	195C	263G	1
16223T	CRS	CRS	16362C	73G	CRS	CRS	199C	263G	1
16223T	CRS	CRS	16362C	73G	CRS	146C	CRS	263G	1
16223T	CRS	16311C	CRS	73G	CRS	146C/152C	CRS	263G	1
16223T	CRS	16311C	16362C	73G	CRS	150T	CRS	263G	1
16223T	CRS	16319A	CRS	73G	CRS	CRS	CRS	263G	4
16223T	CRS	16319A	CRS	73G	CRS	146C	195C	263G	2
16223T	CRS	16319A	CRS	CRS	CRS	146C	195C	263G	1
16223T	CRS	16319A	CRS	73G	CRS	152C	CRS	263G	1
16223T	CRS	16319A	16362C	73G	CRS	CRS	CRS	263G	2
16223T	CRS	16319A	16362C	73G	CRS	152C	200G	263G	1
16223T	16298C	CRS	CRS	73G	CRS	146C	195C	263G	1
16223T	16298C	16311C	CRS	73G	CRS	150T	199C	263G	1
16223T	16298C	16319A	CRS	73G	CRS	152C	CRS	263G	1
16223T	16304C	CRS	CRS	73G	CRS	150T	CRS	263G	1
16223T/16224C ^a	CRS	CRS	16362C	73G	CRS	146C	195C	263G	1
16224C	16294T	16309G	CRS	73G	CRS	152C	200G	263G	1

^a No difference of relative intensity was observed between 16223T and 16224C.

IC1, IC2, and ID in HV1 regions, and IIA1, IIA2, IIB, IIC, and IID in HV2 regions were analyzed. IA in HV1 region was not analyzed.

3. Results and discussion

Nineteen SNPs were observed, and the frequencies of observed variations were approximately similar to previous report [1,2]. Fifty different mtDNA types were observed of which 35 types were seen only once. Fifteen types were seen in multiple individuals (Table 1). Genetic diversity [3] was estimated as 0.952. Seo et al. [2] and Imaizumi et al. [4] reported that the genetic diversity of Japanese mt DNA was estimated as 0.999 and 0.996, respectively. As compared to them, this study is not high polymorphic, because this system is designed to rapidly screening, not to detailed analyses. In forensic case work, this system excludes irrelevant mtDNA samples, and then, further sequencing analysis would be carried out about unexcluded samples. This array system is simple and rapid, as well as suitable for high-throughput applications in a forensic investigative screening.

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