Multiplex mutagenically separated PCR assays for simple and rapid screening of East Asian mtDNA haplogroups on forensic samples

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Introduction

Nucleotide polymorphisms in human mitochondria DNA (mtDNA) have been one of the main issues in population genetics, clinical medicine, and forensic science. Especially, determination of human mtDNA haplogroup has become a useful tool to study human evolutionary history and to infer the matrilineal bio-geographic ancestry. In forensic field, the screening of mtDNA haplogroups by genotyping of mtDNA single nucleotide polymorphisms (SNPs) can help guarantee the quality of mtDNA sequence data as well as can reduce the need to sequence samples that do not match. Here we describe a multiplex MS PCR system for hierarchical determination of the mtDNA haplogroups that are frequent in East Asians. This method is a simple, rapid, and reliable detection system using multiplex allele-specific PCR primers and four types of fluorescence.

Materials and Methods

DNA Samples

Buccal or blood samples from 120 unrelated Koreans whose mtDNA haplogroups were previously determined from direct sequencing and single base primer extension were analyzed. Genomic DNA from buccal swabs and blood samples was extracted using a QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction.

Design of MS PCR for East Asian mtDNA haplogroup determination

A multiplex mutagenically separated (MS) PCR system was developed for simultaneous rapid detection of 14 coding region SNPs and one deletion motif representing common mtDNA haplogroups of East Asia; mtDNA haplogroups M, G, D, D4, D5, M7, M8, M9, M10, N, A, N9, R, F, and B. Additional multiplex MS PCR system was also developed for the determination of four coding region SNPs to further define D4 subhaplogroups D4a, D4b, D4e, and D4j, as the D4 haplotypes occur most frequently (> 25% in Koreans).

PCR amplification and genotyping

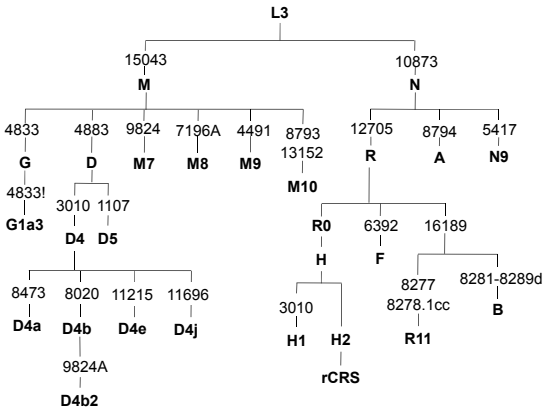
Multiplex MS PCR mixture : 10 µL reaction containing 100 pg of DNA, 1.5~2.5 U AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA, USA), 1.0 µL Gold ST°R 10X buffer (Promega, Madison, WI, USA) and appropriate concentration of primers.

Thermal cycling conditions : 95°C for 11 min; 27~28 cycles of 94°C for 20 sec, 60°C for 1min, and 72°C for 30 sec; and a final extension at 60°C for 45 min.

Detection system : ABI PRISM 310 Genetic Analyzer (Applied Biosystems), GeneScan 3.7 software (Applied Biosystems), GeneMapper ID 3.2 software (Applied Biosystems).

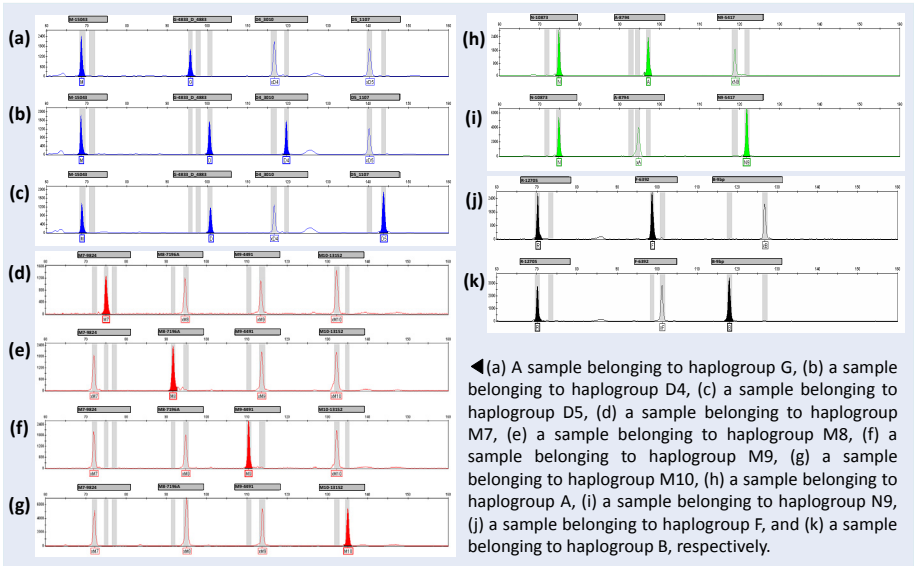
Results

Fig. 1. Selection of East Asian mtDNA haplogroups and corresponding SNPs



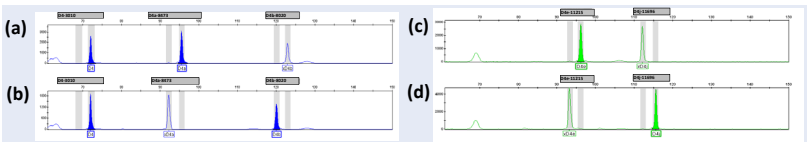
▲The mtDNA haplogroups in the East Asian mtDNA phylogenetic tree that can be defined using the multiplex MS PCR system.

Fig. 2. Electropherograms of multiplex MS PCR system for 11 common East Asian mtDNA haplogroups



◀(a) A sample belonging to haplogroup G, (b) a sample belonging to haplogroup D4, (c) a sample belonging to haplogroup D5, (d) a sample belonging to haplogroup M7, (e) a sample belonging to haplogroup M8, (f) a sample belonging to haplogroup M9, (g) a sample belonging to haplogroup M10, (h) a sample belonging to haplogroup A, (i) a sample belonging to haplogroup N9, (j) a sample belonging to haplogroup F, and (k) a sample belonging to haplogroup B, respectively.

Fig. 3. Electropherograms of multiplex MS PCR system for four common mtDNA D4 subhaplogroups



◀ (a) A sample belonging to haplogroup D4a, (b) a sample belonging to haplogroup D4b, (c) a sample belonging to haplogroup D4e, and (d) a sample belonging to haplogroup D4j, respectively.

Discussion

- **The use of different length allele-specific primers and four types of fluorescence made it possible to easily identify PCR products by fragment analysis on an automatic DNA analyzer like general forensic STR typing method and to simultaneously analyze a great number of SNPs in a single tube, which are the advantages of the developed multiplex MS PCR system.**
- **The PCR amplicons in the electropherogram of the multiplex MS PCR system were arranged in hierarchical order. Therefore, the developed method enabled researchers to type mtDNA haplogroups along the branches of phylogenetic tree through the sequential interpretation of each fluorescent signal shown in the electropherograms, which makes the haplogroup designation straightforward and less confusing.**
- **The multiplex MS PCR system could easily be applied to mtDNA haplogroup typing to help guarantee mtDNA data quality as well as to screening samples that do not match in forensic casework. In addition, the SNPs selected to discriminate major haplogroups in East Asians will enable the method to be applicable for classifying many samples, determining haplogroup of rare sequence and reconfirming the maternal lineage of the known data.**

For more information or detailed protocols, please refer to the paper *Leg Med (Tokyo)*. 2012; In press.
(http://forensic.yonsei.ac.kr/)