HAPLOGROUP DETERMINATION OF MITOCHONDRIAL DNA IN JAPANESE USING SINGLE NUCLEOTIDE POLYMORPHISMS ANALYSIS

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Mitochondrial DNA (mtDNA) polymorphism has some characteristics which are very appropriate for individual or species identification in the field of forensic science, that is, its maternal inheritance, high copy number per cell, and deficiency of recombination. In this study, we investigated single nucleotide polymorphisms (SNPs) of mtDNA using FAST TaqMan® method for the purpose of the rapid and efficient analysis.

Genomic DNA was extracted from 404 blood or buccal swab samples of Japanese and 31 mtDNA SNPs (mtSNPs) were selected from MITOMAP (http://www.mitomap.org/) to determine mtDNA haplogroups. The details of these markers are as follows; three mtSNPs for the haplogroup L1/L2 (African), M and N, 22 mtSNPs for the haplogroup D*, D4, D4a, D4b, D4e, D5, G, M7*, M7a, M7b, M8, M9, M10, M11, M12, A, N9*, N9a, N9b, Y, F and B (Asian), 6 mtSNPs for the haplogroup I/X/W, K/U, V, H, J and T (European). Typings of SNPs were performed by the TaqMan® SNP Genotyping Assays using the 7500 FAST and StepOnePlus Real-Time PCR System (AppliedBiosystems) and analysis software equipped each machine. After an initial 20 sec denaturation at 95°C, 40 amplification cycles were carried out, each consisting of 3 sec at 95°C and 30 sec at 62°C using 2~5ng template DNA.

Of the 404 subjects, 19 haplogroup were found, in which 266 and 138 samples were classified to the major clades, haplogroup M and N, respectively. In addition, we designed a spreadsheet package using Microsoft® EXCEL to analyze 31 mtSNPs at the same time.