学 位 論 文 の 要 旨

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学 位 論 文 名　Simultaneous Determination of Seven Informative Y Chromosome SNPs to Differentiate East Asian, European, and African Populations.

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INTRODUCTION

When specimens regarded as evidence are collected from crime scenes, it is frequently desirable to use them to narrow down the ancestral and geographic origins of the individuals to whom they belong. Therefore, informative genetic markers with large differences in frequency among ethnically defined populations, such as haplogroups of mitochondrial DNA and single nucleotide polymorphisms (SNPs), have been studied. However, most markers are unsuitable for forensic purposes because they are expensive, are intended for large-scale analysis, or require large amounts of DNA. Here, attention is focused on the Y chromosomal polymorphism. Y-SNP markers are suitable for identifying stable paternal lineages and reconstructing an ancestral state from which to explore the history of human evolution and reconstruct family relationships by lineage analysis.

The first main objective of the present study was to select candidate Y-SNPs to differentiate the three major population groups (East Asian, European, and African) in a cost- and time-effective manner. The second main objective was to develop a Multiplex Single Base Extension (MSBE) assay in a single reaction to detect seven Y-SNPs that can be utilized to

analyze forensic samples. The last main objective was to assess the accuracy and reproducibility of the multiplex method as well as its potential to differentiate the three major population groups.

MATERIALS AND METHODS

DNA samples With the use of a QIAamp® DNA mini kit, genomic DNA was extracted from blood or blood-stains randomly collected from healthy subjects. The East Asian populations included 295 Japanese, 225 Korean, and 121 Chinese males and 3 Japanese females; the European population included 49 German males; and the African populations included 52 Ovambo and 38 Ghanaian males.

SNP selection Seven potentially informative SNPs (M168, M130, JST021355, M96, P126, P196, and P234) were selected to differentiate the three major population groups.

Multiplex PCR Primers for multiplex PCR were designed with the Primer Express software. To optimize the multiplex PCR conditions, the expected amplicon size (between 80 and 152 bp) was chosen. Multiplex PCR for seven Y-SNPs was carried out as follows: The PCR reaction mixture (15 μl) contained approximately 0.5-1 ng of DNA, 1X supplemented buffer, a mixture of 14 primers, 0.2 mM of each dNTP, and 0.75 units of AmpliTaq Gold® DNA polymerase. PCR was performed in a GeneAmp® 9700 thermal cycler at 95°C for 11 min, followed by 33 cycles of 94°C for 30 s, 63°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 7 min.

MSBE Reaction and SNP typing The MSBE reaction was carried out using the SNaPshot® Multiplex Kit according to the manufacturer's protocol. To prepare samples for capillary electrophoresis, purified extension products were mixed with Hi-Di™ formamide containing the internal size standard GeneScan-120 Liz™. After denaturation, samples were run on an ABI PRISM® 3130*xl* Genetic Analyzer. Data were analyzed using GeneMapper™ ID Software (Ver. 3.7.1).

RESULTS AND DISCUSSION

Y-SNP typing and population validation We were able to reliably genotype seven Y-SNPs for all male samples by the multiplex PCR and MSBE reaction. The allelic state of each marker was definitively determined from a total of 791 males from the three major population groups. As expected, samples from the three groups showed haplogroups common in the region of provenance: haplogroups C, D, and O for East Asians; IJ and R1 for Europeans; and AB and E for Africans. The haplogroup frequencies observed were similar to those reported in previous studies. We were able to differentiate the three major populations in this study, but the seven haplogroups are not always exclusive to the East Asian, European, and African populations. With this method, it is not possible to define the origin of an individual but only to infer it.

Forensic samples No PCR amplification was observed in any of the samples of the three females. These samples were amplified using the input of 20 ng of DNA as a template. In order to evaluate female DNA interference on the amplification of Y-SNP typing, experiments with male-female mixtures were also performed. Y-SNPs were typed correctly, although the female DNA concentration was more than 400 times that of the male. Environmental insults on forensic samples may result in DNA degradation at random locations. Therefore, the ability of Y-SNP typing in this study to amplify degraded DNA was investigated and compared to that of the Identifiler® kit. A total of 0.5 ng degraded DNA was amplified. While only partial DNA profiles were obtained using the Identifiler® kit, positive amplifications were obtained for the seven Y-SNP PCR multiplex developed in this study.

CONCLUSION

In conclusion, we developed a simple and rapid method for the analysis of seven informative Y-SNPs that can help infer the population of origin of a male subject within the three major population groups. This method is cost-effective and saves materials, which is an important feature of forensic work.