B92 Y-SNPs Typing in a Japanese Population Using Allele Specific Hybridization Method and Luminex® 100™ System

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The goal of this presentation is to evaluate the forensic usefulness of commercially available Y-SNP detection kit with Luminex® 100™ flow cytometer.

This presentation will impact the forensic community and/or humanity by demonstrating Y –SNP typing is considered to be a useful tool for the prediction of population of origin from forensic materials.

Single nucleotide polymorphisms on Y chromosome (Y-SNPs) are regarded as valuable male specific genetic markers. As the haplogroups which Y-SNP markers define are highly non-randomly distributed among population, Y-SNP analysis is considered to be a useful tool for human migration and evolutionary study. As analysis of Y-SNPs markers is effective for prediction of racial and/or geographic origin of evidential sample, Y-SNPs typing could be applied for forensic purpose.

Forty-two (42) Y-SNP markers were analyzed with allele specific hybridization method using commercially available Y-SNP detection kit, Signet Y-SNP Identification System (Marligen Biosciences, Inc., Ijamsville MD). Forty two Y-SNP loci and amelogenin locus are amplified in five dif- ferent multiplex reactions and are detected using x MAP suspension array technology on Luminex 100 flow cytometer. Five (5) Y-STR markers were also examined using PowerPlex Y (Promega) and the results were com- pared to those of Y-SNP markers.

Peripheral blood samples were collected from 100 unrelated Japanese males. The DNA samples were extracted from the blood samples using QIAamp DNA Blood Mini Kit according to the manufacturer's protocol (QIAGEN). The variation 42 Y-SNP markers and amelogenin locus were detected using Signet Y-SNP Identification System kit in according to man- ufacture's protocol. ?Y-SNP types were generated using a computer software DNAsis Call (Mirai Bio). Allele calls of the biallelic marker were made based on comparison of signal intensity which was detected by Luminex 100 flow cytometer.

In order to examine the minimal quantity of template DNA for allele call, 0.1ng, 1ng and 5ng of male 9948 DNA were amplified and florescent signal intensity which was detected from each Y-SNP locus was compared. Allele calls were successfully made with 1 ng DNA for Y-SNP markers in Multiplex 1, 2, 4 and 5. As 1 ng of DNA template the signal intensity was too small and insufficient for allele calls of Y-SNP markers in Multiplex 3, above 5ng of DNA template was required in order to obtain sufficient signal intensity for complete allele calls.

Because PCR product of allele specific primer is relatively small in size, an allele call of Y-SNP type is considered to be successful from slightly decomposed forensic samples containing degraded DNA. Y-SNP typing was examined using degraded DNA extracted from aged blood stain samples which have been stored 17 to 26 years at room temperature and the haplogroup could be successfully determined from 26 year old blood stain sample.

Forty two Y-SNP markers were examined using 100 Japanese DNA samples with Signet Y-SNP Identification System. The derived alleles were observed in all Japanese samples for M42, M94 and M168 loci. Variation was observed for only 5 SNP loci, M175 , M89 , M130 , M174 and SRY+465. Only 4 different haplogroups were observed, and the hap- logroup frequencies in the Japanese sample were O*=16%, O2b=35%, C=13% and D=36%.

Y-STRs were typed from the Japanese sample using PowerPlexY, allele distribution of DYS438, DYS437, DYS392, DYS393 and DYS390 loci were characteristic relation to haplogroups defined by Y-SNP markers.

As Case work samples, tissue of victims of bombing case were ana- lyzed using Y-SNP markers in order to evaluate the presumptive capacity of ethnical of victims. Seventeen male victims including one Japanese victim were analyzed. Eleven victims were classified into haplogroup R (R, R1a and R1b) and 3 victims were classified into haplogroup O1. Three victims were unique haplogroup, C,O2b and O*. It was suggested that the Japanese male was included in these three victims.

In conclusion, allele specific hybridization method for Y-SNPs detection using Signet kit and Luminex® 100™ system was an easy and rapid system to detect the variation on Y-SNP markers and haplogroup. However, above 10 ng of template DNA is required for detection of hap- logroup, this system was considered to be not suitable for analysis of trace evidence. It might be useful for analysis of degraded forensic sample such as tissue of victim of mass disaster.

Y-SNPs, Luminex, Japanese

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