A48 High Throughput SNP Typing Based on Invader® Assay on Integrated Fluidic Circuits

Hiroaki Nakahara, PhD, Koji Fujii, PhD, Tetsushi Kitayama, MS, and Kazumasa Sekiguchi, PhD, N'tl Research Institute of Police Sci, 6-3-1, Kashiwanoha, Kashiwa, JAPAN*

After attending this presentation, attendees will understand the effectiveness of a high-throughput multiplex SNP (single nucleotide polymorphism) typing system utilizing integrated fluidic circuits (IFCs), which are fluidic lines designed on a detection disc forming 2,304 reaction chambers.

This presentation will impact the forensic science community by providing an approach for multiplex invader assay SNP typing in thousands of reactions. Dozens of SNP loci in dozens of DNA samples can be quickly detected at low cost.

SNP typing has advantages in forensic identification, because SNPs are abundant in genomic DNA and are easily detected using an automated high-throughput system. The primary benefit of SNP typing is more successful typing of degraded samples compared with other methods, including short tandem repeat (STR) typing, because of their small target size. SNPs, however, are bi-allelic and large numbers of loci must be analyzed to increase the power of discrimination. To obtain a comparable discriminatory power to that of the commonly used fifteen loci STR typing kit, more than 40 SNP loci must be typed. In addition, the analysis of this many loci should be simple, cheap, and rapid. Common SNP typing procedures, such as the TaqMan assay, the cycling probe assay, the single primer extension assay, or the Invader assay, cost a few dollars for each locus. Thus, typing 40 loci would cost more than a hundred dollars.

In this presentation, high throughput and low cost multiplex SNP typing using the Invader assay in IFCs on a BioMark HD System produced by the Fluidigm Corporation are introduced. In this system, reactions proceed in a few nanoliters of solution enclosed in small chambers, rather than the commonly used microliter-scale reaction tubes. The flow of reaction mixtures and DNA sample solutions are automatically controlled and filled in separated chambers by air pressure, without interdiffusion. The volume of each reaction mixture is very small and thus the total cost of analysis is low. In the device, the temperature of the reactions is controlled and the fluorescence of each chamber is detected and recorded in real time. Using this system, we attempted to analyze 32 SNPs that had been validated in our previous study.[1]

DNA samples were collected from 48 individuals who had given their informed consent to our experiments. Their SNPs were analyzed in our previous study and confirmed by direct sequencing. The Invader assay for the amplified products of the 32 SNP loci by multiplex PCR were carried using Universal General Purpose Reagent (Third Wave Japan Co.) injected into the chambers on the detection disc. The intensities of two types of fluorescence, FAM and Yakima Yellow, in each chamber were detected every 20 seconds during the reaction, which used isothermal heating at 63°C for 30 minutes. The genotypes of each locus were determined from the increasing fluorescence curve of the chart in which both fluorescence intensities were plotted, as reported previously.[2] Triplicate typing for each sample was carried out to verify the results. Using the IFCs system, high-throughput and low cost multiplex SNP typing could be achieved. The reaction and detection time for 48 samples in the device was only approximately 40 minutes, not including one hour of PCR amplification and the 30 minutes taken to inject the samples into the chip. The cost for each SNP locus was less than 25 cents. Furthermore, this system can also perform digital PCR as another application of IFCs. Digital PCR is a quantitative analysis method commonly used in gene expression studies. It is expected that the application of IFCs will be effective for resolving the problem of DNA mixtures in forensic casework analyses, when used in the quantitative detection of different alleles.

References:

1. Nakahara H., Hosono N., Kitayama T., Sekiguchi K., Kubo M., Takahashi A., Nakamura Y., Yamano Y. and Kai K. (2009) Automated SNPs typing system based on the Invader assay. Leg. Med. 11(Supplement 1): S111-S114
2. Nakahara H., Sekiguchi K., Hosono N., Kubo M., Takahashi A., Nakamura Y. and Kasai K. (2009) Criterion values for multiplex SNP genotyping by the Invader assay. Forensic Sci. Int.: Genetics 4: 130-136

SNP, Invader Assay, Fluidic Circuits

 ** Presenting Author*