MULTIPLEX SNP ANALYSIS MICRODEVICE USING ALLELE-SPECIFIC POLYMERASE CHAIN REACTION-MICROARRAY

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ABSTRACT

In this study, we developed an allele-specific polymerase chain reaction-microarray (AS PCR-microarray) chip to perform the allele-specific gene amplification and microarray-based detection on a single wafer to identify Korean indigenous beef cattle, called Hanwoo. For allele-specific discrimination and amplification, Hanwoo-specific ten homozygous single nucleotide polymorphism (SNP) sites were chosen and analyzed by AS PCR on a chip, and the resultant amplicons were verified by the downstream microarray assay. We could identify Hanwoo with 99.3% reliability by detecting the fluorescence signals of the microarray less than three among eleven spots.

KEYWORDS

Integrated microdevice, allele-specific polymerase chain reaction, microarray, SNP typing

INTRODUCTION

SNPs which are major variants on genome have been useful genetic markers for investigating inter-individual differences in drug response and common diseases [1], and genetic variations in animals as well as human [2]. Among SNP typing methods, AS PCR provides high allele-specificity during the PCR reaction [3]. Regarding the detection system of the AS PCR amplicons, the microarray format has been widely employed for SNP analysis due to its ability of multiplex and high-throughput screening. Thus the integrated AS PCR-microarray microdevice would be ideal for SNP typing with advantages of high-speed analysis, high-throughput capability, low reagent consumption, and portability.

Here, we fabricated an AS PCR-microarray chip to identify the DNA fingerprint of Hanwoo with allele-specific DNA amplification. Hanwoo-specific ten homozygous SNP sites were selected with a house-keeping gene as a control (MDH2). The integrated AS PCR-microarray chip mainly consisted of two parts: the integrated AS PCR chip and a disposable microarray chip. The integrated AS PCR chip had a micropump, a PCR reactor, and a micromixer channel on a 4-inch borofloat glass wafer. The disposable microarray chip could be attached to the end of the micromixer unit of the AS PCR chip, and replaced with new one after use. As a result, ten SNP sites were successfully analyzed with high speed and accuracy on a portable instrumentation within 100 min.

THEORY

AS PCR method provides high allele-specificity during the PCR reaction [3]. Selective amplification is achieved by designing a primer such that the primer will match/mismatch one of the alleles at the 3'-end of the primer. By adding multiplex primer sets in one PCR reaction, we can obtain multiple PCR amplicons which represent each SNP site. AS primers only binding to heterozygous SNP sites which Hanwoo does not have. As a result, Hanwoo should show relatively low frequency of AS product on selected SNPs, while imported beef cattle (IBC) has high one in the microarray analysis.

EXPERIMENT

The fabrication process of a microdevice was followed by the previous literature [4]. The integrated AS PCR-microarray chip was composed of the AS PCR unit and a disposable microarray chip (Figure 1). The AS PCR chip has a micropump, microvalves, a PCR reactor, and a micromixer channel on a 4-inch borofloat glass wafer. For temperature-sensing during the AS PCR, four-point resistance temperature detector (RTD) was patterned close to the PCR chamber. A film heater and a mini-cooling fan were used for heating and cooling during thermal cycling. A poly(dimethylsiloxane) (PDMS) membrane was sandwiched between a channel wafer and a glasss manifold to function as microvalves and a micropump. The manifold glass has the patterned pneumatic channels for vacuum/pressure access, and linked with a solenoid valve for fluidic control. In the case of microarray chip, it was located at the end of a micromixer channel and attached to the assembled microdevice from the bottom. A thin and sticky PDMS membrane was plasma-treated and used as an adhesive layer between the AS PCR chip and the microarray chip.

Firstly, the extracted DNA was mixed with a PCR cocktail, which was injected into the PCR chamber. After closing the microvalves, thermal cycling reaction was carried out. After AS PCR, the hybridization buffer (6.9M urea in saline sodium citrate buffer) was loaded on the inlet well with the valve open, and injected into the PCR chamber by continuous micropumping operation. The resultant amplicons and hybridization buffer was mixed together in the micromixer channel and placed on the microarray chip. After incubation and washing process, the fluorescence of each spot was scanned using a miniaturized fluorescence scanner.

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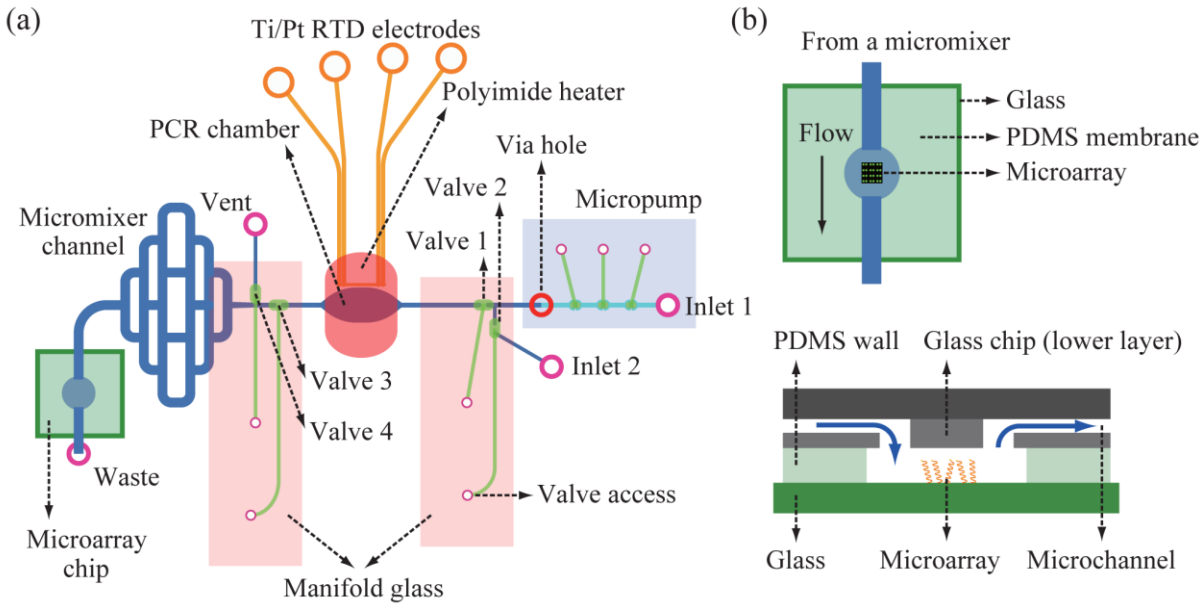

Figure 1. A schematic illustration of the AS PCR-microarray chip (a) and the microarray (b).

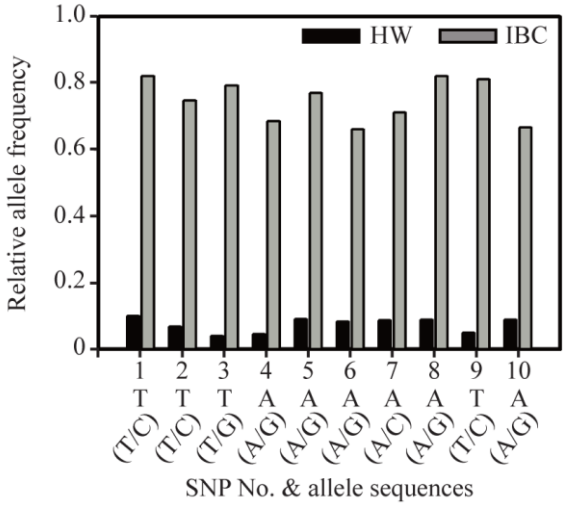

Figure 2. Relative allele frequency of beef cattle for each SNP site. Allele sequences are shown under each bar (HW: Hanwoo; IBC: imported beef cattle).

RESULTS AND DISCUSSION

For evaluation of selected SNPs, the genotype on each SNP allele were confirmed using a MassARRAY system with 606 heads of Hanwoo and 384 heads of IBC samples which were guaranteed by National Institute of Animal Science of Korea (Figure 2). Since the target SNP was adjusted for non-Hanwoo type, the imported beef cattle always showed high frequency on each marker (from 0.661 for SNP6 to 0.859 for SNP8), while Hanwoo had relatively low values (from 0.041 for SNP3 to 0.101 for SNP1). If the criteria number of the resultant amplicons is determined as three or under among eleven products, we can identify Hanwoo with 99.3% fidelity.

The AS PCR on a conventional thermal block was also carried out with the genomic DNA of beef cattle samples, and the resultant amplicons were analyzed using both the microarray assay and the capillary electrophoresis (Figure 3). Figure 3(a) shows the representative microarray and electropherogram of Hanwoo samples having SNP1 and 8 in addition to the MDH2 control. The microarray result was matched with that of the electropherogram data, meaning that the sample included T, C, G, G, G, G, C, A, C, and G from SNP1 to 10. Based on the criteria number of three, it was proven to be Hanwoo. Figure 3(b) shows the IBC SNP typing. The microarray had all fluorescent spots on each probes, which was exactly matched with the electropherogram result. Thus, it could be deduced that the nucleotide on the SNP site was T, T, T, A, A, A, A, A, T, and A from SNP 1 to 10. The produced ten amplicons among the multiplex AS PCR confirmed that the sample is from an IBC.

Finally, we performed the integrated AS PCR amplification and microarray assay on a portable genetic analyzer which consists of the integrated AS PCR-microarray chip, a miniaturized chip operation device, and a portable fluorescence scanner. Figure 4(a) represents the Hanwoo sample, displaying only an MDH2 positive control (less than three). Meanwhile, the IBC sample (Figure 4(b)) produced SNP3, 4, 6, 8, 9, and 10 with a positive control (more than three). These results imply that the multiplex AS PCR and microarray assay on a chip could be

successfully completed to be applied for identifying the animal species.

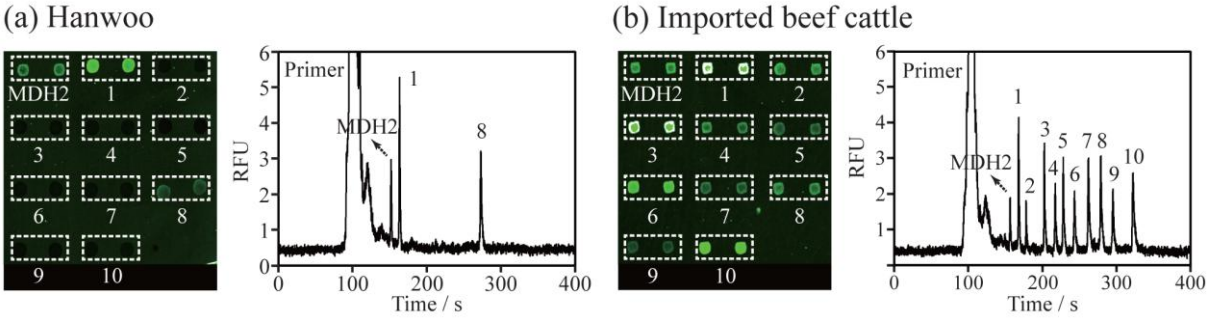

Figure 3. Microarray and electropherogram analysis using AS PCR products produced by a conventional thermal cycler. (a) Hanwoo; (b) imported beef cattle

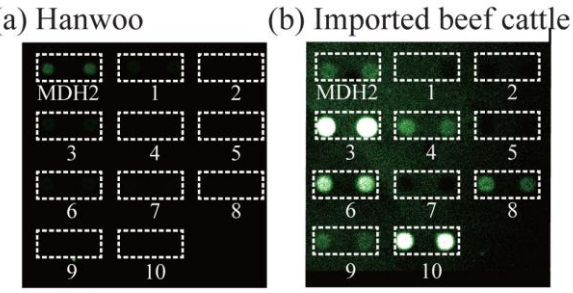

Figure 4. Microarray analysis on a portable integrated AS PCR-microarray system. (a) Hanwoo; (b) imported beef cattle

CONCLUSION

We developed the microfluidic-based AS PCR-microarray chip for multiplex SNP typing with high speed and accuracy on a portable instrumentation. Ten SNP sites were targeted with an MDH2 control, and the resulting three peaks or less informs us of the Hanwoo identification with high fidelity. This advanced genetic analysis microsystem can be utilized for a variety of biomedical research fields including point-of-care DNA diagnostics, food safety testing, and pathogen detection.

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