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Fast Screening of Single-Nucleotide Polymorphisms Using Chip-Based Temperature Gradient Capillary Electrophoresis

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Abstract: Recently, the analysis of single-nucleotide polymorphisms has attracted much attention. Although many techniques have been reported, new methods with high resolving power, low-cost and fast speed are still in demand. We present a fast SNP detection scheme using chip-based temperature gradient capillary electrophoresis to separate the homoduplex and heteroduplex PCR products which contain one or two SNP sites. The total time of a single run was only 8 minutes.

Key words: Single-nucleotide polymorphisms, temperature gradient, capillary electrophoresis chip.

1. Introduction

As the most common type of human genetic variation, single-nucleotide polymorphisms (SNPs) have attracted much attention. It is estimated that there is almost 1 SNP/1000bp [1]. SNPs are important for understanding the relationship between genetic variants and diseases. It can also be used for identification purposes, such as forensics. Low-cost, reliable, fast speed and high-throughput methods for analyzing SNPs become increasingly more important [2].

Direct sequencing of a gene is the ultimate way of identifying the variants. However, this approach is not commonly used because of its high cost and long duration. For this reason, many other methods have been developed, such as DNA chip [3], mass spectroscopy [4]. The techniques based on

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conformational differences of DNA are very important in this field. They include Single-strand Conformational Polymorphism (SSCP) [5], Consistent Denaturant Gel Electrophoresis (CDGE) [6], Denaturant Gradient Gel Electrophoresis (DGGE) [7], Temperature Gradient Gel Electrophoresis (TGGE) [8], Denaturant High-performance Liquid Chromatography (DHPLC) [9], etc. In order to improve their speed and separation efficiency, these methods have been adapted to capillary electrophoresis, including Consistent Denaturant Capillary Electrophoresis (CDCE) [10, 11], Denaturant Gradient Capillary Electrophoresis (DGCE) [12], and Temperature Gradient Capillary Electrophoresis (TGCE) [13].

Chip-based capillary electrophoresis is a powerful separation technique and has become an attractive alternative to slab-gel electrophoresis and capillary electrophoresis in many fields [14]. Several groups have used this approach to detect SNPs, yet found that the separation efficiency and convenience in practice are still inadequate [11]. In the current study, we developed a method for conducting the SNPs detection by using a normal crossed-channel electrophoresis chip which is featured with temperature gradient programmed by a computer.

2. Experimental Section

2.1 Chemical Reagent

The sieving matrix used was a 2.5% hydroxyethylcellulose (HEC, 200-300 cps, 2% in water, at 20 °C) (Tokyo Kasei, Tokyo, Japan) solution prepared in 1×TBE buffer (89mM Tris-boric acid, 2mM EDTA, pH=8.0) and was vacuumed to remove bubbles. The samples were labeled in the PCR procedure with Cy5-dCTP, which was purchased from Amersham Pharmacia (Piscataway, NJ).

2.2 Capillary Electrophoresis Chip

The crossed-channel electrophoresis chip is made of poly-(dimethylsiloxane) (PDMS). The cross-section dimension of the channel is 50×20 μm and the length of the effective separation channel is 50 mm (Figure 8.1).

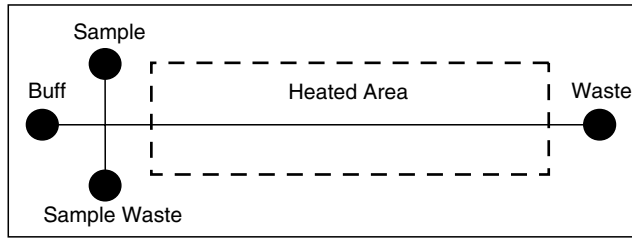

FIGURE 8.1. Scheme of the crossed-channel electrophoresis chip.

2.3 Experimental Setup

A laboratory-assembled chip-based CE system with laser-induced fluorescence (LIF) detection and heaters was used in our work. The design of the CE system was similar to that reported in previous papers [14]. Briefly, a 632 nm He-Ne laser was used as the excitation source. The fluorescence was collected by a PMT with a 670 nm band-pass filer and the frequency of sampling was 10 Hz.

To achieve the temperature gradient along the separation channel, three heaters (DN515, ThermOptics, Carson City, NV) were placed against the backside of the chip. The temperature of the heaters was controlled with a digital potentiometer tuned by a computer. All the parameters for generating the temperature gradient can be easily programmed via the computer. The precision for temperature gradient control reaches 0.1 ˚C.

2.4 DNA Samples

Two Cy5-labeled PCR products with the same length of 101bp containing one or two SNP sites were analyzed. The characteristics of the samples are listed in Table 8.1.

Before the analysis, heteroduplex PCR products were generated by heating the wild type and mutant PCR products at a ratio of 1:1 in the same test tube at 94 ˚C for 5 min and then by decreasing the temperature to 56 ˚C for 1 h to facilitate the reannealing of the DNA amplicons.

3. Result and Discussion

3.1 Results

The results in Figure 8.2 and 8.3 show that a particular homoduplex DNA fraction was base-line resolved from its corresponding heteroduplex composites through the crossed-channel chip in only 8 minutes by applying a temperature gradient along the separation channel. The samples were also run separately through the same chip with the same temperature gradient. As a result only a single band was detected. The samples containing two SNP sites were tested under the temperature gradient of 62-67 ˚C for 240 s with a precision

TABLE 8.1. Characteristics of the DNA Samples

No.	Length (bp)	SNP type	SNP position	Sample source
1	101	C to T, G to A	50, 60	HLA_A1101 HLA_A2501
2	101	C to T	53	HLA_B2703 HLA_B2705

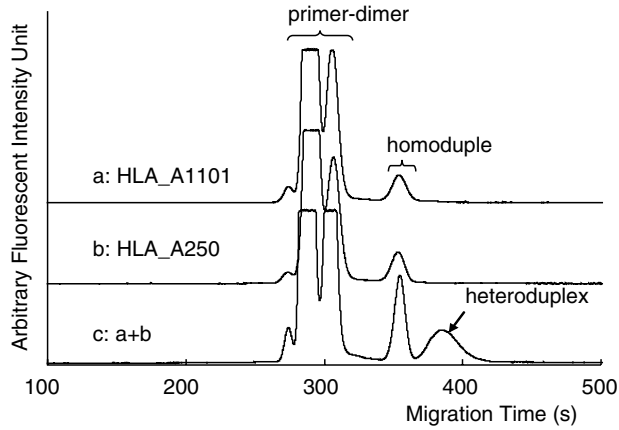

FIGURE 8.2. The electropherograms of DNA samples containing two SNP sites.

of 0.1 ˚C per step and the separation electric field of 150 V/cm. The temperature gradient for the samples containing one SNP site was 62.5-67.5 ˚C and the other conditions were the same as above. In our experiment, the sieving power and the heat durability of the separation media were satisfactory.

3.2 Different Temperature Gradient

We also analyzed the samples with two SNP sites under different temperature gradients. As shown in Figure 8.4, with only one degree C difference in the

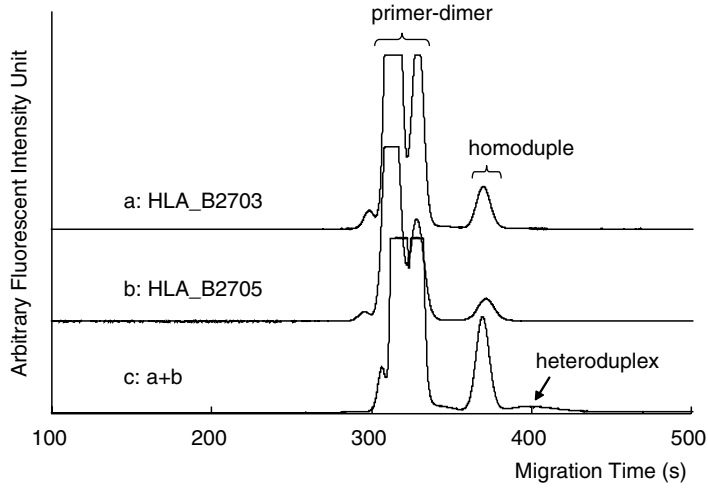

FIGURE 8.3. The electropherograms of DNA samples containing one SNP site.

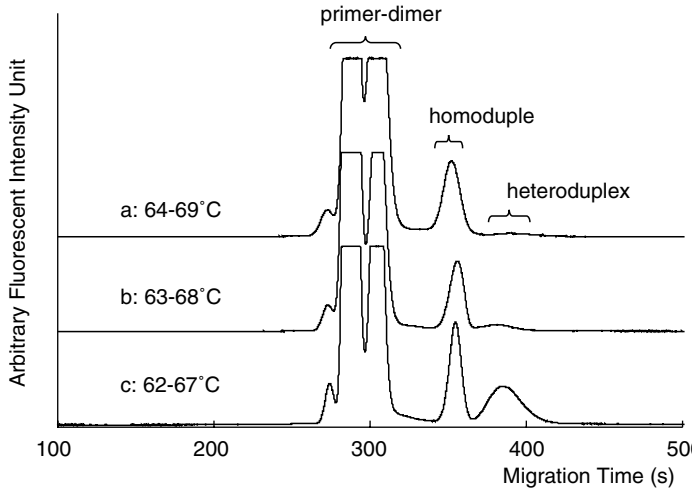

FIGURE 8.4. The electropherograms of DNA samples under different temperature gradients.

temperature gradient, the patterns of electropherograms changed dramatically. So in practice, we should choose the most suitable temperature gradient for each sample to obtain the best results.

3.3 Separation Efficiency

It is obvious that, in our experiment, only partial resolution was achieved, and two peaks, homoduplexes and heteroduplexes, were obtained. But this is good enough for us to differentiate homoduplexes from heteroduplexes. So we conclude that this method is very suitable to rapidly recognize the presence of a broad range of SNPs prior to further characterization.

4. Conclusion

We have demonstrated a reliable chip-based temperature gradient capillary electrophoresis system for fast SNPs detection. Using this method, DNA samples can be analyzed for their SNPs in a single run. It can thus simplify the analysis process and shorten the total time needed for analysis.

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