CHEMICAL-MEDIATED MELTING CURVE ANALYSIS FOR GENOTYPING OF SINGLE NUCLEOTIDE POLYMORPHISMS

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ABSTRACT

This report describes a new microfluidic solid-phase chemical-mediated melting curve analysis method for Single Nucleotide Polymorphism (SNP) genotyping. The method is based on allele-specific denaturation of DNA duplexes upon exposure to dynamic chemical gradient. DNA duplexes conjugated on beads are captured in a microfluidic gradient generator device. The device is designed with dams, keeping the beads trapped perpendicular to the gradient generating channel. Upon exposure to a gradient of formamide or urea, rapid denaturing profile is monitored in real time or in a single end-point analysis.

KEYWORDS: Single Nucleotide Polymorphism, Chemical Gradient, Formamide, Urea

INTRODUCTION

A fundamental principle involved in most DNA analysis chemistries in practice today is DNA hybridization. Various enzymatic and processing steps typically accompany the hybridization reaction. DNA hybridization may also be tracked in real time (dynamically) as reaction stringencies are altered (e.g., by raising the reaction temperature). We presented earlier melting curve analysis method based on monolayers of beads immobilized on chips with integrated heaters and sensors for detecting differences in melting points between matched and mismatched duplex configurations at a single base [1, 2]. However, the system increases the complexity, and is limited to laboratories with sophisticated microfabrication facilities. Here, we report a simple isothermal “melting curve analysis” at room temperature - simply by exposing DNA duplexes to a chemical gradient generated and precisely controlled by a microfluidic device. Formamide is well-known DNA denaturing agent and has previously been used in solution based DNA analysis assay [3] and is frequently used to lower the DNA melting temperature in PCR reactions. Here, we use formamide for solid-phase SNP detection to monitor dynamical denaturation process. Furthermore, we introduce the use of urea as a non-toxic and cheap alternative for isothermal solid-phase SNP analysis.

EXPERIMENTAL

The microfluidic gradient generating device was fabricated by casting PDMS according to standard soft lithographic techniques. The device is designed with dams, keeping the beads trapped perpendicular to the gradient generating channel (Figure 1). 10 μm beads were conjugated to single stranded DNA followed by hybridization...
to allele-specific probe labelled with Cy3. The beads were captured and then exposed to the chemical gradient (see Figure 1). An inverted fluorescence microscope was used for real time monitoring of the denaturation process.

RESULTS AND DISCUSSION

We designed a simple, valve-less, gradient generator device to trap and expose DNA duplexes to chemical (Figure 1). By precise controlling the liquid and beads inside the device, we were able to generate stable gradients and control of the initiation of the gradient without sophisticated valves. Figure 2 shows results of gradient generated by mixing water (inlet 1) and fluoresceine dye solution (inlet 2). The gradient is constant before and after passing the perpendicular cross-channel, indicating that the gradient is not affected by the presence of the channel where the beads are trapped.

![Figure 1. (A) Image of microfluidic gradient generator. Dam filters effectively trapped the beads perpendicular to the gradient flow.](image1)

![Figure 2. (A) Gradient generator device with one cross-channel (area ii) perpendicular to the gradient. (B) Gradient of fluoresceine dye mixed with pure water before, at and after the cross-channel.](image2)

Device with two parallel cross-channels perpendicular flow was designed and evaluated for pair-wise comparison to score the presence of a SNP position in a single run. Figure 3 shows beads conjugated with cy-3-labeled DNA trapped in the device before exposure to formamide and after exposure. The denaturants destabilizes the DNA duplex by competing with Watson-Crick pairing. A rapid fall in fluorescence indicates the denaturing concentration or “melting temperature” of the probe-target duplex. As can be seen in Figure 3, the mismatched DNA duplex is clearly denatured at a lower formamide concentration compared to the matched DNA duplex, enabling unambiguous SNP calling. Figure 4 shows the resulting fluorescence changes using formamide and urea as denaturants. In the formamide case (Figure 4A), both the matched and the mismatched alleles are denatured at a formamide concentration lower than 50%, while it requires higher than 8M urea concentration to denature the matched target DNA-probe duplexes. Formamide as denaturant is rapid, takes less than 2 minutes exposure time, while the urea is takes 5 to 10 minutes to complete the denaturation. In addition, formamide had better optical properties enabling effective real-time monitoring. On the other hand urea is non-toxic and readily available in any laboratory.

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Figure 3. Two parallel cross-flow channel device filled with beads conjugated with DNA before (top) and after exposure to formamide gradient. The mismatched DNA duplex has lower denaturation concentration.

Figure 4. Chemical-mediated melting curve analysis using (A) formamide and (B) urea as denaturants.

CONCLUSIONS
In conclusion, we report a simple and rapid chemical-mediated solid-phase method for SNP analysis. We demonstrate arraying capabilities for potential high-throughput genotyping and the use of non-toxic urea as a denaturant.

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