MICROFLUIDIC CHIP OF FAST DNA HYBRIDIZATION USING DENATURE AND MOTION OF NUCLEIC ACIDS

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ABSTRACT

We demonstrate that the efficiency of DNA hybridization could be improved by introducing elevated temperature in the hot region and higher velocities in the cold region of a microfluidic chip. Compared with the conventional methods, this hybridization microchip was shown to increase the hybridization signal 4.6-fold within 30 minutes using a 1.4 kb target DNA as the test material. The increase in fluorescence intensity was apparent when the temperature was higher than 82°C, and the fluorescence intensity reached an asymptotic value as T > 90°C. A mathematical model was proposed to relate the fluorescence intensity of DNA hybridization with the temperature of hot region and the velocity of cold region.

KEYWORDS: hybridization, temperature, velocity

INTRODUCTION

Previously, we demonstrated that the effect of extensional strain rate was larger than that of velocity on the hybridization reaction [1]. A fiber-optic DNA microarray using microsphere-immobilized oligonucleotide probes was developed, and the hybridization time reached a plateau after 30 minutes [2]. Magnetic nanoparticles were applied in electrochemical detection, and could be used to control the DNA hybridization process [3]. The simultaneous multicolor array hybridization of eight samples provided a 4-fold increase in throughput over standard two-color assays [4].

METHOD AND DESIGN

Two factors are used to affect the DNA hybridization. The first factor is increasing the velocity of the target DNA, the second factor is increasing the temperature of the target mixture in the hot (denature) region, as shown in Figure 1.

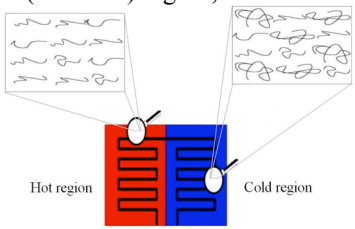

Figure 1. Mechanism of DNA stretch caused by the high temperature.

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The hybridization chip included a glass slide with probes and a micro-reactor with flow channels, as shown in Figure 2. The distances between heater route turns were placed uniformly in the micro-heater. The temperature of the cold (hybridization) region was set to be 42°C, and the temperatures of the hot (denature) region were set to be different values varying from 42°C to 94°C.

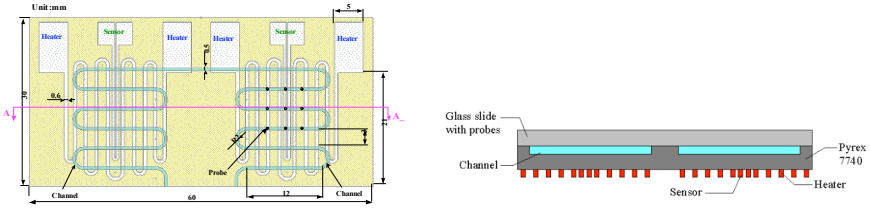

Figure 2. (a) Layout of the hybridization chip, the dots on the channel are the locations of probes, (b) Schematic drawing of the cross-section A-A' in (a). (unit: mm)

ANALYSIS AND RESULTS

In Figure 3, the differences in the fluorescence images between T = 82 and 94 °C were fairly apparent. The images between T = 62 and 82 °C could be distinguished, but the differences were not apparent. Higher temperatures in the hot region resulted in brighter fluorescence images in the cold region.

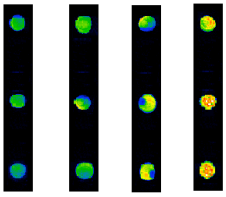

Figure 3. Images of hybridization experiments after 30-minute hybridization at u= 0.5 cm/s in the cold region and T = 42, 62, 82, 94°C in the hot region, respectively.

The fluorescence intensity distributions of the cold region as a result of different temperatures in the hot region are shown in Figure 4. The fluorescence intensity in the cold region increased as temperature increased in the hot region. The fluorescence intensity increased sharply when the temperature was higher than 80°C.

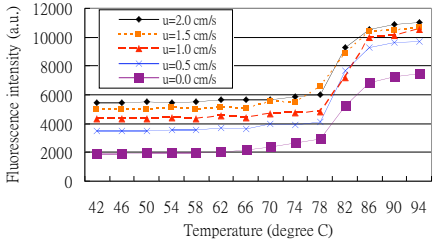

Figure 4. Comparison of fluorescence intensity after 30-minute hybridization at different hot-region temperatures and u = 0, 0.5, 1, 1.5, 2 cm/s in the cold region.

The fluorescence intensity distributions of the cold region at different velocities in the cold region are shown in Figure 5. One can see that the fluorescence intensity increased gradually when the velocities increased, and the tendency was similar as increasing temperatures except for the different values. Higher velocities in the cold region resulted in higher fluorescence intensities.

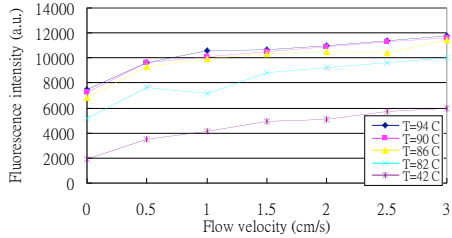

Figure 5. Comparison of fluorescence intensity after 30-minute hybridization at different velocities in the cold region and T = 82, 86, 90, 94°C in the hot region.

A mathematical model was proposed to relate the fluorescence intensity (F) with the hot-region temperature (T) and the cold region velocity (u):

$$F = 2798\, u^{1/2} + 3245 \tanh [0.132\,(T-81.8)] + 5056 \qquad (1)$$

The relationships between predicted and experimental fluorescence intensities of DNA hybridization are shown in Figure 6. Good correlation between experimental data and predicted data by Equation (1) was obtained.

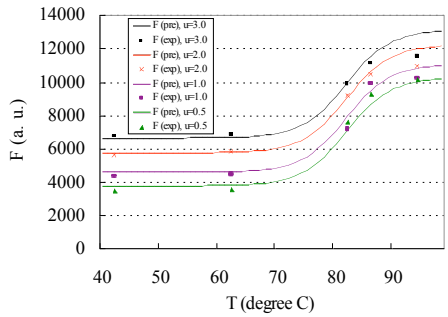

Figure 6. Comparison of predicted and experimental fluorescence intensities.

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