A Lab-on-a-Chip Module for Bead Separation in DNA-Based Concept Learning

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Abstract. Affinity separation with magnetic beads is an important and widely used technique for DNA computing. We have designed and implemented an experimental lab-on-a-chip module for affinity-bead separation for DNA-based concept learning. Magnetic beads with DNA-probe sequences immobilized on their surface were used to select target strands, and these beads are restrained in the channel by a permanent magnet on top of the module. The separation process consists of two steps, i.e. hybridization and denaturation. We confirmed the separation process by a mixed solution that contains FITC modified strands, and measured the yield by UV spectrophotometer. The experimental results demonstrate a successful separation of the mixed DNA.

1 Introduction

Although one of the major attractions of DNA computing is the massive parallelism, the DNA computing operations involve a number of manual steps which require a large amount of time for bio-chemical reactions. This is one of the reasons that the application of DNA computing has been limited to the small-scale problems. Lab-on-a-chip technology provides a solution to this restriction. The miniaturization technology allows for integration and automation of experimental steps. It also reduces the amount of material necessary for the reaction and processing of the DNA. In addition, we are able to diminish the factors of errors inherent in DNA computing. Some examples for this technology can be found in [4,5,9,11,12].

Among many bio-lab techniques used in DNA computing, the affinity bead separation is an essential method for selecting the specific DNA strands. It served as a query method in a DNA-based database by associative search [2]. It was also used to check whether a path contains a specific city in HPP or TSP problems [1]. Moreover, Boolean value verification in the SAT problem [3] and attribute-value checking for hypothesis space refinement in the DNA-based concept learning [8] are accomplished by this technique.

However, in real experiment, the yield of this affinity separation is very low [6], and the information is lost or reduced while the operation is repeated. Thus, an amplification process such as PCR is indispensable to recover the information, which

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is the amount of the DNA. Furthermore, the manual experiments of the affinity separation are certain to involve some errors. Braich et al. used a separation method with a gel-filled tube module to improve the efficiency in the SAT problem [2], and other related works have been done in relation with the micro-reactor [5,9,11,12].

In the previous work [8], we have suggested version space learning with DNA molecules as a concept learning method. This learning method can be implemented by repeated application of the affinity bead separation and this is described in the next section in detail. But, in practical implementation, this method needs much effort to repeat the same procedure, which may also suffer from low yield.

In this paper, we present the design of an affinity separation module, which performs the operation in a single straight channel. The magnetic beads with DNA probe sequences immobilized on their surface were used to select target strands, and the beads are restrained in the channel by a permanent magnet on top of the module. The separation process consists of hybridization and denaturation as in the manual experiment. We have implemented a prototype of this module and some experimental results are presented to verify its working process. This module can be used as well in other applications of the DNA computing which uses affinity separation.

This paper is organized as follows. In Section 2, the workflow of the bead separation for the DNA based concept learning is presented. Section 3 describes the detailed structure of the lab-on-a-chip (LOC) module for bead separation and fabrication process. At the same time some experimental results for this module are presented in Section 4. Finally, the conclusion and future work are given in Section 5.

2 Concept Learning on LOC

A detailed description on the original version space learning in silico can be found in [10], and the DNA based method is described in [8]. In this section, we explain how a network of the bead separation modules can organize the DNA based concept learning.

Given a training example *e*, learning proceeds by selecting all hypotheses, which are consistent with *e*. This process can be achieved through the affinity separation, which examines each attribute value, as is described in Fig. 1. Let T_n be the current version space[1], T_{n+1} the one after a training example *e*, and T^e a set of hypotheses that classify *e* as positive. In this paper, we assume three attributes (A, B, C) each of which has only two values. Attribute values are denoted by a lowercase letter with subscripts 1 and 2, and the "don't care symbol" is denoted by subscript 0. Each rectangle in Fig. 1 means an affinity separation module that divides the solution in the manner of whether each hypothesis has at least one of the attribute values, which are denoted in the rectangle. Positive selection means the selection of the DNA strands that hybridize with the bead, and negative selection means the selection of the ones that does not. In principle, the learning process for a single example can be accomplished with this process in Fig. 1.

[1] In this paper, the concept 'version space' is not the one that is maintained by special and general boundaries as in [2], but simply a set of hypotheses that are consistent with training examples.

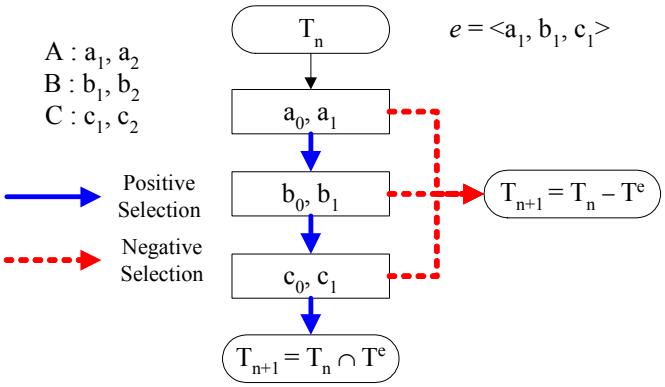

Fig. 1. Training process of DNA based version space learning for an example $<a_1, b_1, c_1>$

However, due to the possibility of false negatives in the selection process, the approach displayed above may be unreliable in the negative training examples. Therefore, this model is modified as follows for reliability, which uses only the positive selection as in Fig. 2.

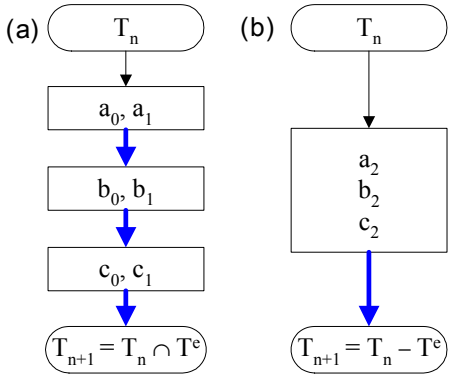

Fig. 2. Modified version of the learning process, which uses only positive selection for an example $<a_1, b_1, c_1>$. (a) is for a positive example, and (b) is for a negative example

Each learning step for a single training example can be implemented by the processes above, and therefore, the full learning process of the DNA based version space learning can be accomplished by networking the above training modules for the training examples.

3 LOC Module for Bead Separation

3.1 Layout of the Module

Basically, the module consists of a single straight channel, which is 2 mm in width, 100 μm in depth, and 44 mm in length. It should be miniaturized. This module uses

streptavidin coated magnetic beads to immobilize the probe sequences of which 5' ends are biotinylated. A permanent magnet is used to immobilize the magnetic beads. It is on top of the module and we can control the bead to flow or to make immobilized by the magnet.

To perform affinity separation, we need two steps, i.e. hybridization and denaturation as in the manual affinity separation. In the hybridization step, we first injected the mixed solution through the channel. This gave a rise to hybridize both target and probe DNA strands on the magnetic beads. After then, 0.1 M NaOH solution through the channel and the denaturized DNA strands pass through the channel.

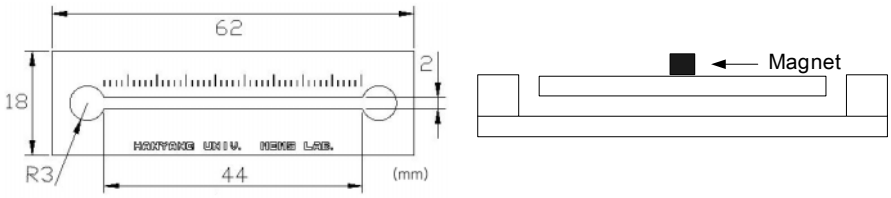

Fig. 3. Layout of the bead separation module. Top view (left) and side view (right)

3.2 Fabrication

The negative photo resistant SU-8 (Micro Chem, USA) was spun coated on the substrate 100 Si wafer (100 μm thick). And the air bubble of the coated SU-8 was removed by soft baking on a hot plate (65°C for 10 min, 95°C for 30 min). After installing and hardening the mask on the wafer, photolithography was performed on the MA-6 aligner. Finally this was cleaned with isopropyl alcohol after development by SU-8 developer. PDMS (polydimethylsiloxane, Corning, Sylgard 184) and curing agent were mixed (ratio of 10:1) and cured in oven (65°C, 4 h). PDMS replica was peeled off the mold, treated in O_2 plasma and bonded with a slide glass (Corning, #7740 Pyrex glass).

Two holes of 2 mm in diameter were drilled in the center of each reservoir port for inlet and outlet of solution. A magnet (CPG mini separator) seated on top of the device over channels was used to localize the beads in the detection area. It was ø 6 mm × 5 mm with strength 12,200 Gauss 1,220 mTesla at the pole.

The summary of the above process is shown in Fig. 4.

4 Experimental Results

In this section, we describe some experimental results for the above module. Firstly, the magnetic beads with the probe sequences were prepared, which were then installed into the module. We confirmed the separation by FITC modified strands, as well as measuring the yield by UV spectrophotometer and then compared these results with those of the manual process.

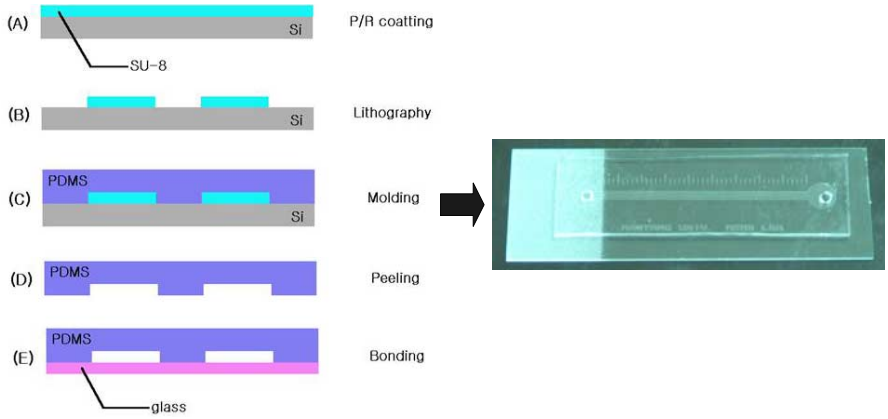

Fig. 4. Fabrication process and final module

4.1 Apparatus

Reagents were loaded into the fluid lines (silicon tube ø 1 mm) with 250 µl, ø 2.30 mm Hamilton syringe and then pushed through the device syringe pump (KD scientific, AUXETAT). The device was mounted on an inverted fluorescence microscope for detection (NIKON, DIAPHOT 300). The microscope consisted of a halogen lamp for top illumination and a 100 W xenon lamp for sample illumination from the bottom. A charge coupled device (CCD) camera (IK-642F, Toshiba) was used to monitor the transport of magnetic beads and to measure fluorescence intensity. An interference filter cube was used to detect fluorescence signals. The bandwidth of the excitation filter was 459~498 nm and 512~559 nm for the emission filter. The images of the beads and fluorescence were acquired and analyzed with imaging software (Image tool 3, UTHSCSA).

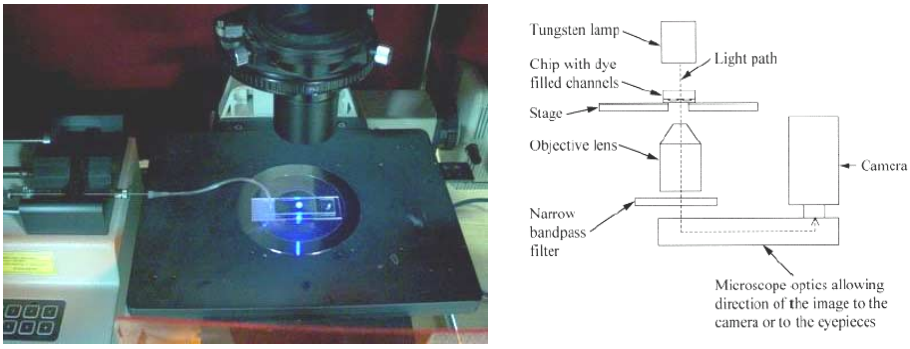

Fig. 5. Apparatus

4.2 Oligonucleotides

We used the sequence generator NACST/Seq [7] to design the DNA sequences for the experiment. When designing the oligonucleotide sequences, we considered the self-

homology and the H-measure to prevent cross hybridization. We designed three sequences of which 20 bp of 3' ends were selected as target subsequences and the probe sequences were determined from these subsequences. Table 1 shows the six sequences. All oligonucleotides were purchased from GenoTech (Daejeon, Korea) and obtained 5' FITC modified target strands for experiments to confirm the separation in the module in addition to the original three target strands. As well the probe sequences (No. 4~6 in Table 1) were 5' biotinylated for immobilization on the magnetic beads. As a result, we synthesized nine oligonucleotides for the experiment.

Table 1. Sequences of oligonucleotides used in the experiment

No.	Sequence	Tm (°C)	GC%
1	5'-AAACGGTCTTGTCGG **GGATAGGGAATGCTCGTGTT**-3'	70.1	51.4
2	5'-GTCGAGACAAGACCT **TGGCATATGTCTAAATAGAT**-3'	67.1	40.0
3	5'-GCGAATCTGATCGCT **CCAGCGAGGGCCGGTTCCAC**-3'	77.7	65.7
4	5'- AACACGAGCATTCCCTATCC -3'	57.3	50
5	5'- ATCTATTTAGACATATGCCA -3'	49.1	30
6	5'- GTGGAACCGGCCCTCGCTGG -3'	67.6	75

4.3 Probe Bead Preparation

Conjugation of biotinylated single-stranded DNA to M-280 beads (Dynal M-280 streptavidin coated, ø 2.8 μm) was performed by using the following protocol. At first, 300 μl of stock beads (6~7 × 10^8 bead/Ml) were washed three times in 200 μl of Binding and Washing (B/W) buffer which consisted of 10 mM Tris, 1 mM EDTA, and 1 M NaCl, pH 7.5 and then diluted in 90 μl of B/W buffer. And 10 μl, 100 μM biotinylated DNA solution was added to the beads solution and then incubated at room temperature for 15 min. After conjugation, the beads were washed with B/W buffer and diluted in 100 μl of TE buffer, which was made up of 10 mM Tris, 1 mM EDTA, pH 8.0.

4.4 Separation

In this experiment, oligonucleotide No. 2 in Table 1 was the target strand, the mixed solution, which contained oligonucleotides No. 1~3 were used in separation.

Before the experiments, the module and the other devices were washed with ultra sonicator (Branson) for 5 min and 0.1 M NaOH. Then the inlet port of the module was connected to the syringe pump. TE buffer was mechanically pumped into the silicon tube (ø 1 mm) and flushed the device to remove out air bubbles. The dead volume in the tube and the connectors was 10 μl in case of 250 μl Hamilton syringe.

Detection of Separation with FITC Modified Oligonucleotides

The solution that contained probe beads (30 μl of No. 5 probe attached bead + 70 μl of TE buffer) was injected into the channels by using syringe pump at the velocity of 20 μl/min. The mixed solution which contained each 10 μl of No. 1~3 that are FITC modified and 170 μl TE buffer, was injected into the channel by 5 μl/min for 40 min.

After hybridization, unbounded oligonucleotides were washed with 100 µl TE buffer by 10 µl/min, 2 times, and the intensity of fluorescence signal was detected by CCD camera. For elution of the bound target strands, 200 µl of 0.1 M NaOH was injected into the channel (10 µl/min).

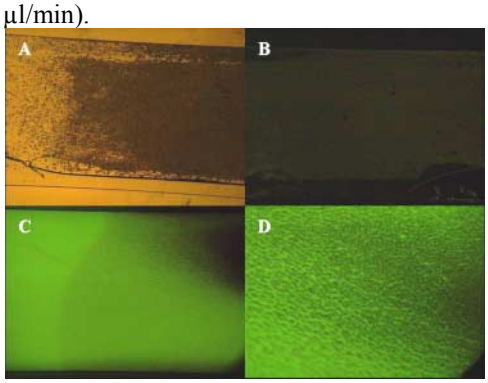

Fig. 6. Normal and fluorescent images of the beads in the module. Beads are localized in a channel by a magnet (a) Injection of M-280 bead in channel, 40X under halogen lamp, (b) 40X fluorescence image before hybridization with FITC conjugated probe, (c) 40X fluorescence image after an injection of the mixed solution which contains FITC modified target strands, (d) 100X fluorescence image, black dots are M-280 beads, green background is target solution

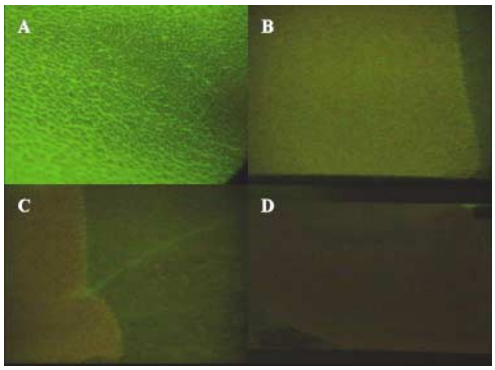

Fig. 7. Fluorescence images in washing and elution process (a) Before washing, (b) After washing, (c) Elution step: green narrow flow indicates the target oligonucleotide, (d) After completing the elution step

Figs. 6 and 7 show the separation process in the module. We can observe that the probe beads capture the target strands in hybridization step and that the strands are eluted in denaturation step.

Detection with UV Spectrophotometer

In the previous subsection, the solution, which contains the probe beads and the mixed solution were injected into the channel step by step, except that the mixed solution consisted of unmodified oligonucleotides. The waste solution of the

hybridization step containing unbounded strands was collected and then optical density of this solution was measured at 260 nm by UV spectrophotometer (UV-1601, SHIMADZU). The waste solution in the elution process was collected and optical density was measured to compare intensity profile before and after the separation.

In addition to this, we performed a manual separation process, using the same protocol without a separation module to compare the efficiency.

Table 2. Optical density of the solution at each step

Concentration	*Module*	*Manual*
Initial mixed solution	184.7 μg/ml	184.7 μg/ml
Waste in hybridization	164.8 μg/ml	146.1 μg/ml
Waste in denaturation	27.8 μg/ml	17.88 μg/ml

Table 2 is the result of the separation and the amount of the DNA strands in each step. As shown in the table, the yield is better than that of the manual process although the module misses out the much of the target strands.

5 Conclusion

In this work, we have shown that the DNA based concept learning can be performed by iterative affinity separations and also its concrete workflow has been presented. The learning process purely consists of the affinity separations, which examine each attribute value of the hypotheses in the current version space. Therefore, the network of the affinity separation modules can implement this learning scheme. For this purpose, we designed and implemented a prototype of a single LOC module for the affinity bead separation. The separation process was verified by several experiments, and the results show the separation process is performed more correctly and efficiently than manual work.

The yield of the affinity separation process can be improved further. The design or the strategy of the separation module needs to be improved to increase the efficiency of the probe beads. Undoubtedly, there may be other methods of immobilizing the probe DNA or the magnetic bead. Nevertheless, it is important that the probe sequences should be able to have more chances to hybridize with demanded target strands, and after all the proportion of the false negatives must be decreased as a result. The affinity separation used in [3] may be a good example. On the other hand, we may use the negative selection strategy to overcome the very low yield of the positive selection or the error of the positive selection in combination with a probability theory. However, we need a quantitative analysis of this process to support it. More sophisticated research about this affinity separation must be performed.

And more miniaturization, integration and automation are required for the real application. For example, Choi et al. have developed a technology to separate the magnetic beads in a micro-channel by electromagnet [4]. We may as well be able to control the beads in the module in combination with this method.

Acknowledgement

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