DNA Pyrosequencing Using Microfluidic Chips

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Abstract:

Pyrosequencing is a new method of DNA sequencing that shows promise in reducing costs by several orders of magnitude while increasing the throughput. In this project, we applied the pyrosequencing technique to a microfluidic device. The enzymes required for sequencing flowed through the channel while DNA was attached to superparamagnetic beads inside the channel. A novel bead trap was designed and tested in order to prevent the loss of DNA during the sequencing process. An oligonucleotide was used for preliminary sequencing experiments. Three base pairs were successfully sequenced. The results show potential for a revolutionary DNA sequencing technique.

Introduction:

Genome sequencing has transformed the biological sciences. For the progress to continue, cheaper and faster sequencing techniques must be developed [1]. Current sequencing techniques employ dideoxy sequencing, commonly referred to as Sanger sequencing. This process was developed over 25 years ago and has gone through major improvements over the years but has some inherent limitations to its effectiveness [2]. Using the Sanger method, the Human Genome Project took 13 years and 13 billion dollars to complete [3]. The enormous costs and time of DNA sequencing hinders a lot of genomic research.

Pyrosequencing is one possible technique that has potential to replace Sanger sequencing [2]. As shown in Figure 1, pyrosequencing works by detecting the pyrophosphate (PPi) released when a nucleotide is correctly incorporated into DNA by polymerase. ATP sulfurylase converts the released PPi into ATP. An enzyme from fireflies, called luciferase, converts the ATP into light, which can be detected by a camera. Therefore, by exposing the DNA to only one type of nucleotide at a time, one can detect whether or not the nucleotide corresponds to the next nucleotide in the sequence.

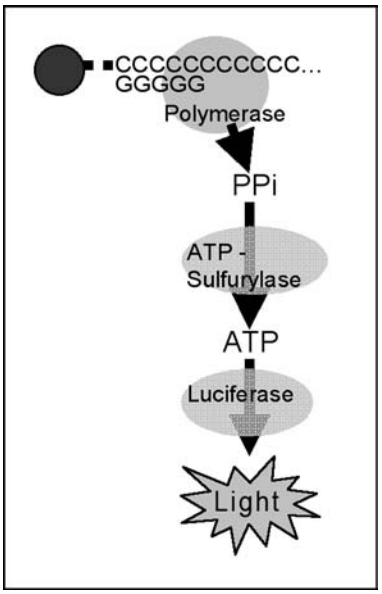

Figure 1: Pyrosequencing chain reaction.

Procedure:

In this project we created a microfluidic prototype for pyrosequencing, shown in Figure 2. The superparamagnetic beads with DNA attached were held in a bead trap with a magnet while the enzymes for pyrosequencing flowed over the beads. The microfluidic chip design consisted of a main channel with a recess to trap the beads. We at first used one 500 μm long and 14 μm deep recess as a bead trap, but testing showed that a series of 20 μm long bead traps would be more effective. The channels and bead traps were patterned on a silicon wafer using standard

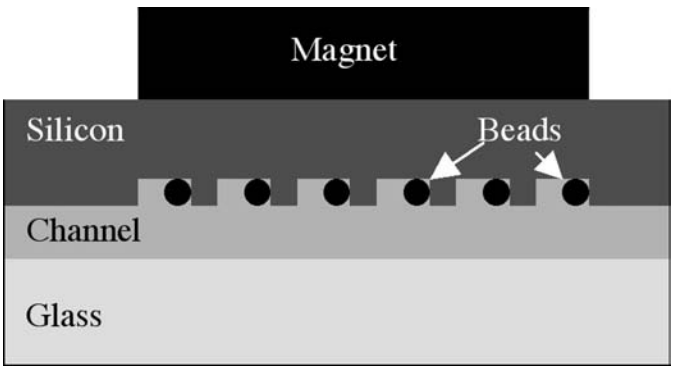

Figure 2: Diagram of bead trap design.

photolithographic techniques and etched using plasma etching. The channels were 54 μm deep and 100 μm wide. The inlet and outlet ports were created with a drill press and a 1.1 mm diamond tip drill bit. A glass wafer was anodically bonded to the silicon wafer to enclose the fluidic channel.

Four cylindrical neodymium rare earth magnets with a residual flux density between 13,400 to 13,700 Gauss [4] were stacked on the opposite side of the silicon wafer and used to pull the superparamagnetic beads into the bead traps. Superparamagnetic beads with a diameter of 2.8 μm were flowed into the channels and examined on an inverse microscope to determine the successfulness of the bead trap. Once it was shown that the bead trap design successfully retained the beads, the beads were washed out to prepare for preliminary sequencing experiments.

The glass side of the chip was placed on a CCD camera to monitor light and the magnets were returned. A single stranded oligonucleotide with a primer was biotinylated to the superparamagnetic beads and flowed into the channel. A mixture of enzymes (polymerase, ATP sulfurylase, and luciferase) along with the nucleotide corresponding with the next nucleotide in the oligonucleotide sequence were flowed into the fluidic channel. The enzymatic fluid was flowed until the light signal degraded, at which point, the next nucleotide was flowed into the channel.

Results and Discussion:

The bead traps successfully held the beads while fluid flowed through the channel. If a bubble entered the fluidic channel, it tended to remove some beads from a bead trap, but there was a high probability that the beads would be caught in another bead trap farther down the channel. This greatly increased the effectiveness of the bead trap design, because if long sequencing reads are to be successful, almost no beads can be lost. This bead trap design can be applied to many other fluidic devices and applications. It would be ideal for an electrowetting transport chip because of its ability to withstand the surface tension of bubbles.

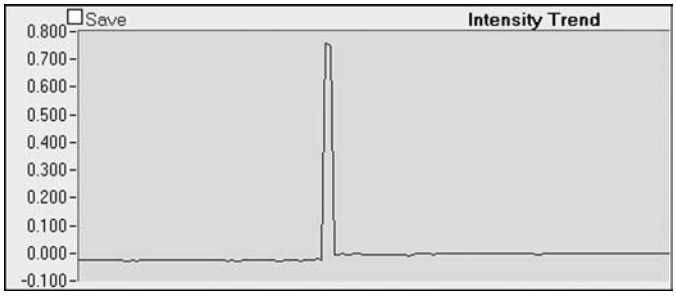

Figure 3: The peak in the light intensity corresponds to the incorporation of a nucleotide.

In our preliminary sequencing experiments, we successfully sequenced three nucleotides of an oligonucleotide. The camera detected distinct peaks in light intensity, which faded with time and then peaked with the addition of the next nucleotide. Figure 3 shows an ideal peak in light intensity we received.

One major problem was the time it took to sequence a nucleotide. The large dead volume in our inlet connectors and our extremely low flow rate of only 1 μL per minute caused a large lag in time from when the next nucleotide was introduced to when a light signal was detected. Additionally, the time it took for the light signal to degrade was unacceptably long. The pyrosequencing reaction relies on diffusion for mixing of enzymes. Either better mixing techniques must be found, or the number of beads must be reduced.

Even though only three nucleotides were sequenced, the results prove that the chip design works. Much work needs to be done to optimize the chip design and sequencing process, but this technique may revolutionize DNA sequencing.

References:

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