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A MICROFABRICATED DEVICE FOR SEPARATING ~200 KILO-BASE-PAIR DNA MOLECULES IN ~15 SECONDS

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Abstract

We describe a thumbnail-sized "DNA prism" device that sorts large DNA fragments (~200 kb) in 15 seconds [1]. The device consists of an array of micron-scale posts as the sieving matrix, and integrated microfluidic channels that spatially shape the electric fields over the matrix. The device can be operated in conventional pulsed field electrophoresis mode, or in a novel asymmetric pulsed field mode [1], which allows for continuous-flow operation. This overcomes the limitation that microfluidic devices can only analyze small amount of sample in small plugs.

Keywords: Microchip, DNA separation, pulsed field, microfluidic, fractionation

1. Introduction

The analysis and fractionation of DNA molecules is a central step in virtually every genome project. The standard method for fractionating DNA fragments larger than ~40 kilo-base pairs (kb), pulsed-field gel electrophoresis, is extremely time-consuming, with running times of typically more than 10 hours [2]. The goal of our work is to create a microfluidic chip to reduce the separation time for 100 kb to 200 kb DNA molecules to ~15 sec [1].

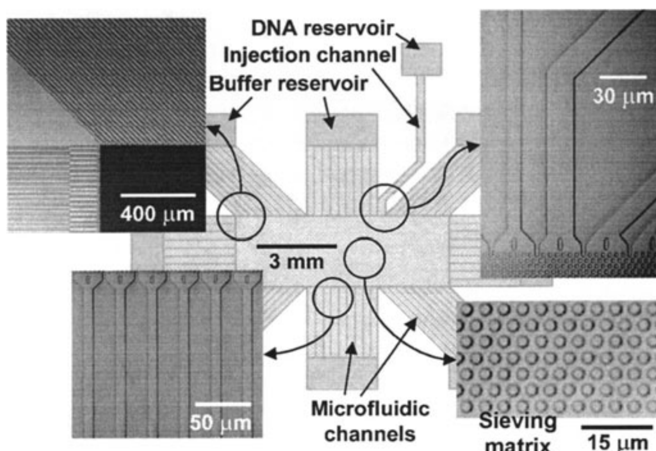

Figure 1 Structure of the device illustrating the microfabricated sieving matrix integrated with the microfluidic channels [1,3]. The post array is 3 mm x 9 mm, and the posts are 2 μm in diameter, 2 μm apart, and 2 μm tall. A single channel connecting to the DNA reservoir injects DNA through a 28 μm opening.

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2. Device Design

The device was fabricated by conventional photolithographic patterning and reactive-ion etching techniques on a fused silica wafer. It is a fully integrated analytical machine consisting of an hexagonally-packed array of micron-scale posts, sample injection and extraction channels, and structures for shaping uniform electric fields (Fig. 1) [1]. Key features are the use of microfluidic channels as electric current sources for defining boundary conditions, as it allows electric fields to be tunable in direction, yet still uniform over the entire array, with minimum number of exterior electrodes [3]. In principle, one can use two pairs of electrodes to generate electric fields in arbitrary directions, one pair for each field component (vertical or horizontal). However, such electrodes shunt electric fields generated by one another, and seriously degrade device performance. When the fluidic channels are properly inserted between the array and the electrodes, they provide electrical resistance, and eliminate shunting problems.

The device can be operated in two modes: conventional pulsed field mode [3] and the DNA prism mode [1]. In the first mode, DNA samples are injected from a channal into the post array as a ~30 μm band using vertical fields, and then separated horizontally using pulsed fields (Fig. 2) [3]. In the DNA prism mode, DNA molecules are injected, separate, and collected in microfluidic channels for further downstream analysis, using pulsed fields of different strengths or durations (Fig. 2) [1].

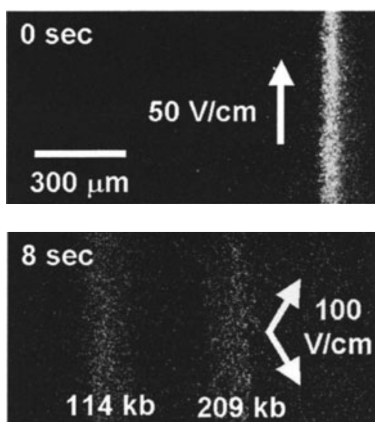

Figure 2 Conventional pulsed field separation, with loading by 50 V/cm field (0 sec), followed by separation after 8 sec with 100 V/cm square pulses 120° apart (167 ms pulse duration) [3]. Small DNA fragments (114 kb) move faster than large ones (209 kb). DNA is viewed by fluorescent microscopy.

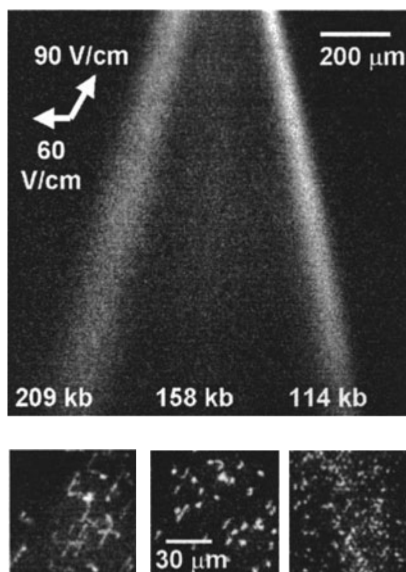

Figure 3 DNA prism separation. Top: steady-state fluorescence micrograph ~400 μm below the injection point. Bottom: close-up of individual molecules in the bands 2 sec after the fields were turned off.

3. DNA Prism

When alternating electric pulses of different strengths or durations are applied at different directions to a hexagonally-packed array of micron-scale posts, DNA molecules migrate at different directions according to their molecular weights (Fig. 3) [1]. 114, 158, and 209 kb molecules at a concentration of ~5 μg/ml each were separated using 125 ms square pulses of ~90 V/cm and ~60 V/cm. (Fig. 3 top). The straight bands implies that the electric fields are uniform. The molecular weights of each band were identified from images gathered at high magnification under elongation conditions (Fig. 3 bottom).

The time to drive the DNA fragments down 1.5 mm through the array is ~15 sec. In the novel DNA prism mode, the device separates molecules continuously at a rate of ~300 pg/hr, or ~20 nL/hr, estimated from the pulsing conditions and the DNA concentration used. By comparison, when operated in the conventional pulsed field mode, the device can only load a small volume (~200 pL) as the sample plug, an intrinsic problem of shallow-channel microfluidic devices.

4. Conclusions

The device reported here presents three major advances over previous microfabricated devices designed to separate large DNA molecules [1]. First, a new microfluidic design has been used to generate and maintain uniform electric fields tunable in direction and very precise sample injection. Second, continuous-flow mode of operation has been demonstrated, which removes the limitation of the amount of sample the device can analyze. And third, the speed: 114, 158 and 209 kb DNA molecules were separated in ~15 seconds.

Acknowledgments

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