

DNA SEQUENCING BY LIGATION ON SURFACE-BOUND BEADS IN A MICROCHANNEL ENVIRONMENT

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ABSTRACT

Advancements in DNA sequencing can potentially enable genome-wide studies of associations between mutations and traits in large population pools. In this work we have developed and implemented a microchannel chip to reduce biochemical reagent volumes for sequencing by ligation on surface-bound beads by 12 \times . Further, bead binding selectivity in 8.5 μm deep channels is enhanced with C₄F₈ surface passivation. This chip integrates with a thermally controlled vacuum chuck and an automated instrument, the Polonator, to perform cyclic biochemical reactions and imaging with reagent volume and cost reduction over preceding technologies.

KEYWORDS: DNA sequencing, ligation, microchannels, surface passivation

INTRODUCTION

DNA sequencing advancements in cost and throughput are undergoing intensive development to enable widespread discovery of genomics information. Some groups have focused on conventional dideoxy sequencing, making improvements that include performing separations in microchannels [1], while others have shifted to using cyclic array sequencing technologies [2]. Our group has previously published a cyclic array technique, sequencing by ligation, using DNA templates tethered to immobilized, 1 μm diameter, ferromagnetic beads [3]. In this work we report on the extension of this technique to a microchannel chip for the open-source Polonator instrument, with resulting 12 \times reduction in reagent usage—a dominant cost.

DESIGN AND MANUFACTURE

The microchannel chip contains an array of 16-32 addressable channels etched in silicon bonded to borofloat glass. The channels are addressable through individual ports to permit multiplexing of bead arrays. Subsequently sealing these ports with polyimide tape allows a single inlet and outlet to deliver common reagents for sequencing. Beads are bound in monolayers on the channel's glass surface by selectively passivating and silanizing the silicon and glass, respectively. Passivation is achieved through C₄F₈ fluoropolymer deposition prior to anodic bonding, and silanization is performed with aminopropyltriethoxysilane that enables bead binding through NHS-ester crosslinking. When mounted on a vacuum chuck with peltier thermal control, the Polonator's epi-fluorescence microscope and reagent handling system allows sequencing from an area containing 2-4 $\times 10^9$ DNA-loaded beads.

RESULTS AND DISCUSSION

The channel layout and fabrication process are shown respectively in Figures 1a and b. Each channel is 2 mm wide with 160 mm² active, silanized area that is

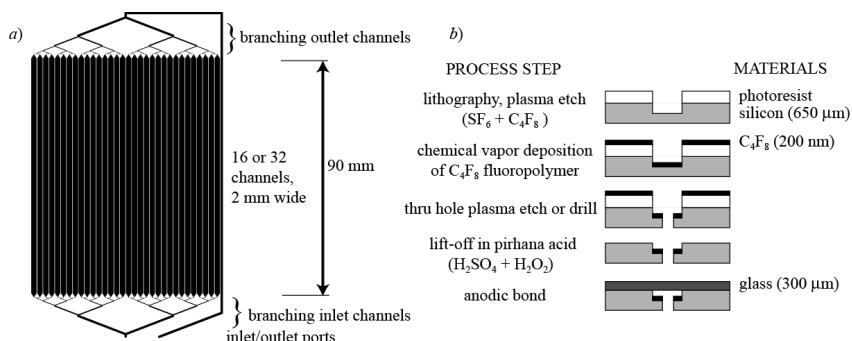


Figure 1. a) Mask design with 16-32 channels and b) fabrication process for microchannel chip, as patterned onto 150 mm diameter silicon wafers.

typically arrayed with 60×10^6 beads. Thru-hole channel ports, either plasma-etched or drilled, are located at either end and the center of each channel for bead loading.

Figure 2 shows a portion of a channel surface as fabricated using this process and design. The silicon channel is $8.5 \mu\text{m}$ deep with a 200 nm C_4F_8 passivation treatment that inhibits subsequent silanization. This passivation layer has been measured to be 5% more autofluorescent than bare silicon at 550 nm (for Cy3 fluorescent dye), which is tolerable; this surface modification mitigates bead binding by more than $18 \times$ relative to bare SiO_2 and $2 \times$ relative to bare silicon as indicated in Table 1.

The microchannel chip and its thermally controlled vacuum chuck are shown in Figure 3. Kinematic registration features on the chuck accurately and repeatably located the chip to $25 \mu\text{m}$ laterally. Thermal mapping of the chuck was performed at biochemistry temperatures to assess accuracy and uniformity as shown in Figure 3c. At the temperature extremes ($5 \text{ }^\circ\text{C}$ and $56 \text{ }^\circ\text{C}$), the total surface temperature range is 4.9 and $3.7 \text{ }^\circ\text{C}$ respectively, which is acceptable for selective DNA hybridization and refrigeration after sequencing.

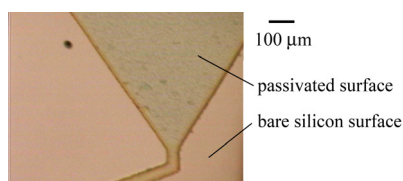


Figure 2. Photograph of channel with C_4F_8 surface passivation. The 200 nm thick passivation layer enables selective silanization for bead binding in a monolayer.

Table 1. Bead binding selectivity on microchannel walls. Gravity predisposes the beads to bind to the SiO_2 wall. Selectivity is enhanced with C_4F_8 passivation.

	Polymer (C_4F_8)	Silicon	Glass (SiO_2)
Beads bound material wall	33	66	594
Beads bound to SiO_2 wall	19,700	17,600	17,800
% spurious binding	0.17%	0.38%	3.34%

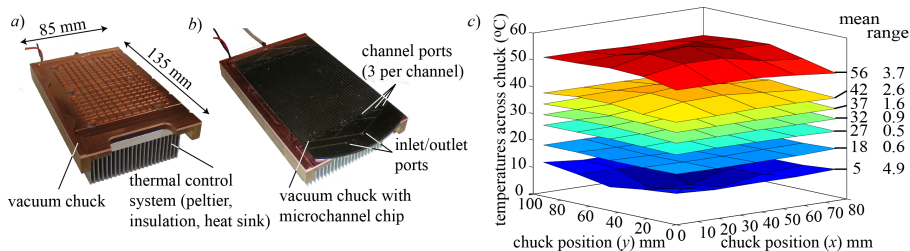


Figure 3. a) Vacuum chuck b) with microchannel chip. The assembly constrains the chip accurately and repeatably for fluorescence microscopy and c) controls its temperature between 5-56 °C sufficiently uniformly.

DNA sequencing results on surface-bound beads in the microchannel environment using sub- μ L reagent volumes are shown in Figure 4. This false-colored image is a composite of separate fluorescent images corresponding to each nucleotide base. Bead confinement in the 50 μ m wide channel is evident.

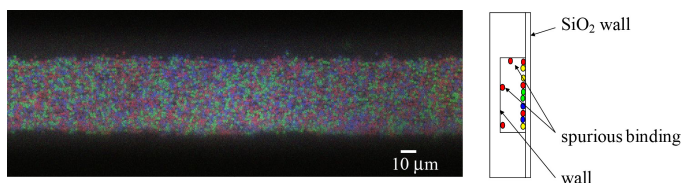


Figure 4. DNA sequencing by ligation demonstrated in a 50 μ m wide channel. The surface bound beads have unique tethered DNA templates that can be queried using biochemical protocols previously published [3].

CONCLUSIONS

This flexible, addressable array of surface-modified microchannels offers substantial reagent reduction for bead-based cyclic array sequencing, bringing us one step closer to realizing the vision for a \$1,000 sequenced human genome.

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