

SUPPLEMENTARY INFORMATION

A Microfluidic Device for Multiplex Single-Nucleotide Polymorphism Genotyping

Jing Zhu,^a Chunmei Qiu,^b Mirkó Palla,^{a,b} ThaiHuu Nguyen,^a James J. Russo,^b

Jingyue Ju^b and Qiao Lin^{a,*}

^aDepartment of Mechanical Engineering, Columbia University, New York, NY 10027, United States

^bDepartment of Chemical Engineering, Columbia University, New York, NY 10027, United States

*** To whom correspondence should be addressed. Email: qlin@columbia.edu**

1. Fabrication

The temperature control chip was fabricated using standard microfabrication techniques. Briefly, gold (150 nm) and chrome (5 nm) thin films were thermally evaporated onto the glass substrate, and patterned by photolithography and wet etching, resulting in resistive temperature sensors and resistive heaters (Fig. S1A). Then, 1 μm of silicon dioxide was deposited using plasma-enhanced chemical vapor deposition (PECVD) to passivate the sensors and heaters (Fig. S1B). The contact regions for electrical connections were opened by etching the oxide layer away with hydrofluoric acid.

The polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning Inc., Midland, MI) sheet bearing microfluidic features was fabricated using soft lithography techniques. A layer of AZ-4620 positive photoresist (20 μm , Clariant Corp., Somerville, NJ) was spin-coated on a silicon wafer (Silicon Quest International, Inc., San Jose, CA), exposed to ultraviolet light through a photomask, developed, and baked to form the weirs as well as the flow channels which have a semi-circular cross section that can be sealed completely (Fig. S1C). Then, layers of SU-8 photoresist (MicroChem Corp., Newton, MA) were patterned to finalize the mold with microfluidic features. Next, a PDMS prepolymer solution (base and curing agent mixed in a 10:1 ratio) was cast onto the mold and cured on a hotplate at 72 °C for 1 h (Fig. S1D). The resulting PDMS sheet was peeled off from the mold, and fluidic inlets and outlets were established via punching holes (Fig. S1E).

In parallel, a layer of SU-8 photoresist was patterned on another silicon wafer to establish oil-filled channels for microvalve actuation (Fig. S1F). Next, a thin PDMS membrane was spin-coated onto the silicon wafer (Fig. S1G) to which the PDMS sheet with the microchannels was then bonded after treating the bonding interfaces with oxygen plasma for 20 seconds (Fig. S1H). Afterward, the PDMS sheet together with the thin PDMS membrane was peeled off (Fig. S1I), punched to form inlets and then

bonded irreversibly onto the temperature-controlled chip after another oxygen plasma treatment (Fig. S1J).

Finally, the surfaces of the SBE microchamber were coated with a thin layer of Parylene C via chemical vapor deposition (Fig. S1K), and streptavidin beads and C₁₈ bonded silica microspheres were packed in the SPP chamber and desalting channel (Fig. S1L).

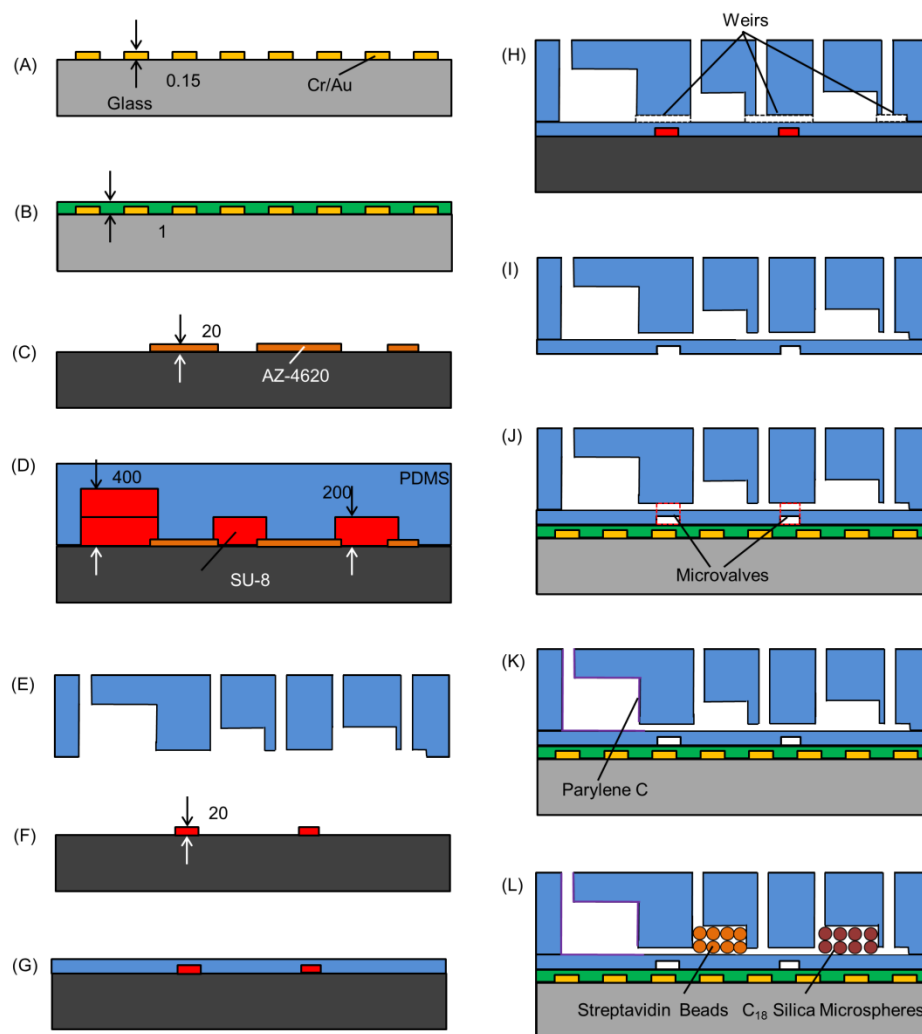


Figure S1. (A) Deposition and patterning of gold sensors and heaters. (B) Passivation of gold sensors and heaters. (C) Fabrication of fluidic channel mold for on-chip valves using positive photoresist AZ-4620. (D) Finalization of mold for microfluidic chambers and channels using SU-8. (E) Demolding of PDMS microfluidic channels. (F) Fabrication of SU-8 mold for oil-filled channels. (G) PDMS spin-

coating. (H) Bonding of PDMS microfluidic channel to PDMS-coated mold for oil-filled channels. (I) Peeling off of PDMS sheet containing fluidic channels and oil-filled valve actuation channels. (J) Bonding of PDMS sheet to temperature control chip. (K) Deposition of Parylene C. (L) Packing of streptavidin beads and C₁₈ bonded silica microspheres.

2. Mass and structure of chemically cleavable biotinylated dideoxynucleotide²⁴

Table S1 Mass of ddNTPs-N₃-biotins (Da)

ddATP-N ₃ -biotin	ddGTP-N ₃ -biotin	ddCTP-N ₃ -biotin	ddUTP-N ₃ -biotin
1101.89	1231.04	1078.85	1192.99

Chemical structures of the four biotinylated dideoxynucleotides are shown below the table:

- ddATP-N₃-biotin**: Structure showing the adenine base, dideoxyribose sugar, and triazole linker.
- ddGTP-N₃-biotin**: Structure showing the guanine base, dideoxyribose sugar, and triazole linker.
- ddCTP-N₃-biotin**: Structure showing the cytosine base, dideoxyribose sugar, and triazole linker.
- ddUTP-N₃-biotin**: Structure showing the uracil base, dideoxyribose sugar, and triazole linker.

Figure S2. Chemical structure of chemically cleavable biotinylated dideoxynucleotide set, ddNTPs-N₃-biotins (ddATP-N₃-biotin, ddGTP-N₃-biotin, ddCTP-N₃-biotin and ddUTP-N₃-biotin)

3. Experimental Procedure

The C₁₈ silica microspheres suspended in methanol were first packed in the desalting channel and dried overnight at 60 °C. Then, the fluidic channels were filled with DI water, and the streptavidin beads

were packed in the SPP chamber (Fig. S3A, Fig. S4A). Pressure-driven microvalves (V1 - V4) were closed by default with 200 kPa pressure.

During an experiment, the C₁₈ silica beads were rinsed with 50% ACN (Fig. S3B, Fig. S4B, 20 μ L/min for 1 min) followed by 0.1M TEAA (Fig. S3C, Figure S4C, 20 μ L/min for 2 min) for wetting and equilibration. Meanwhile, by opening valve V3, B&W buffer was introduced to rinse the streptavidin beads (Fig. S3D, Fig. S4D, 10 μ L/min for 5 min) to provide the optimal binding condition for biotin and streptavidin.

Then, by opening valve V1, a 10 μ L reagent composed of 2 μ L target template, 75 pmoles of ddATP-N₃-biotin, 50 pmoles of ddUTP-N₃-biotin, 50 pmoles of ddCTP-N₃-biotin, 75 pmoles of ddGTP-N₃-biotin, 40 pmoles of each locus specific primer, 1 $\times$ Reaction Buffer and 8 unit Thermo Sequenase™ was introduced into the SBE chamber (Fig. S3E, Fig. S4E) and went through 30 thermal cycles of 94 °C for 15 s, 40 °C for 60 s and 69 °C for 30 s (Fig. S3F).

Next, by opening valves V2 and V3, the SBE product was transferred into the SPP chamber and extracted under continuous flow conditions by the streptavidin beads at a flow rate of 5 μ L/min for 2 minutes (Fig. S3G, Fig. S4F), followed by rinsing with 50 μ L of B&W buffer at a flow rate of 10 μ L/min (Fig. S3H, Fig. S4G). Subsequently, by opening valve V3, 10 μ L of 100 mM TCEP (pH 9.0) was introduced into the SPP chamber (Fig. S3I, Fig. S4H), and incubated at 65 °C for 10 minutes to cleave the linker and release the primer extension product from the beads (Fig. S3J).

Finally, by opening valve V4, the cleaved SBE products were transferred to the desalting channel at a flow rate of 1 μ L/min for 5 min (Fig. S3K, Fig. S4I), followed by rinsing with 100 μ L of 0.1 M TEAA (Fig. S3L, Fig. S4J) and 50 μ L of DI water (Fig. S3M, Fig. S4K) at a flow rate of 20 μ L/min to eliminate any impurities and salts. The desalted product was then eluted with 2 μ L of 50% ACN (Fig.

S3N, Fig. S4L) and characterized with a Voyager DETM MALDI-TOF mass spectrometer (Applied Biosystems[®], Life Technologies, Grand Island, NY).

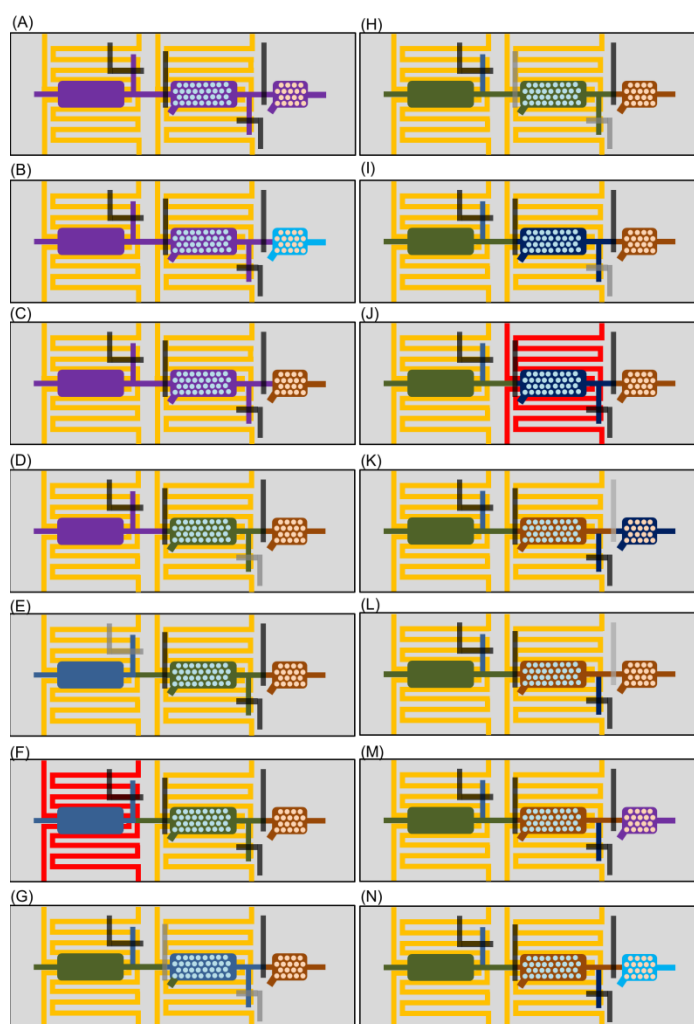


Figure S3. (A) Filling of the microfluidic channels with DI water. (B) Rinsing of C₁₈ bonded beads with 50% ACN. (C) Rinsing of C₁₈ bonded beads with 0.1M TEAA. (D) Rinsing of streptavidin beads with B&W buffer. (E) Introduction of SBE reaction solution. (F) Thermal cycling. (G) Extraction of SBE product by streptavidin beads in SPP chamber. (H) Rinsing of streptavidin beads with B&W buffer to wash off the unextended primer and other impurities. (I) Introduction of TCEP. (J) Incubation at 65 °C to release the SBE product. (K) Transfer of cleaved SBE fragments to desalting channel. (L) Rinsing of C₁₈ bonded beads with 0.1M TEAA. (M) Rinsing of C₁₈ bonded beads with DI water. (N) Elution of desalted SBE fragments with 50% ACN. Colored ink is used to represent the actual reagents. Purple: DI water; Dark Orange: TEAA; Light Blue: ACN; Green: B&W buffer; Blue: SBE sample; Dark Blue: TCEP.

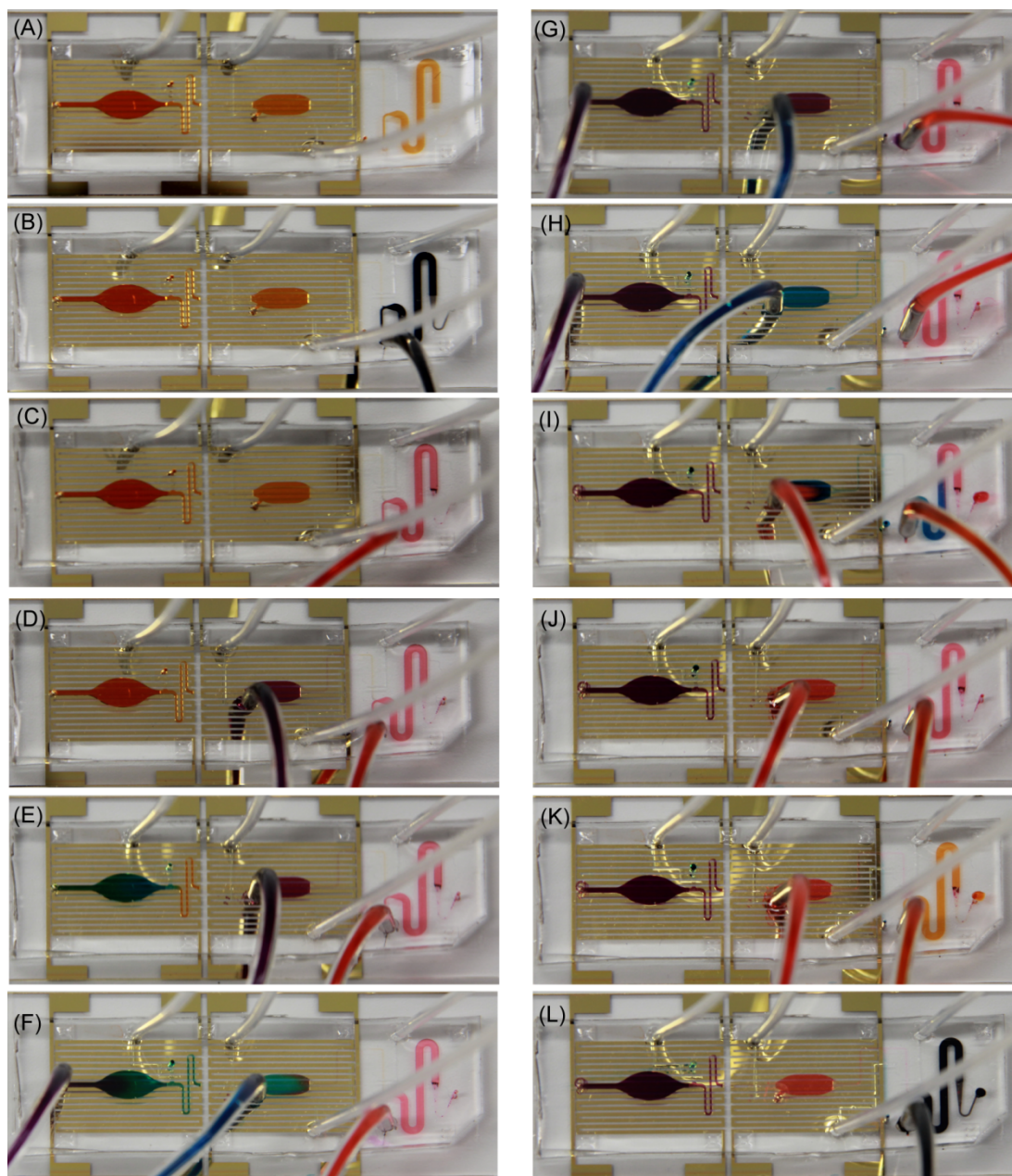


Figure S4. (A) Filling of the microfluidic channels with DI water. (B) Rinsing of C_{18} bonded beads with 50% ACN. (C) Rinsing of C_{18} bonded beads with 0.1M TEAA. (D) Rinsing of streptavidin beads with B&W buffer. (E) Introduction of SBE reaction solution and thermal cycling. (F) Extraction of SBE product by streptavidin beads in SPP chamber. (G) Rinsing of streptavidin beads with B&W buffer to wash off the unextended primer and other impurities. (H) Introduction of TCEP and incubation at 65 °C to release the SBE product. (I) Transfer of cleaved SBE fragments to desalting channel. (J) Rinsing of C_{18} bonded beads with 0.1M TEAA. (K) Rinsing of C_{18} bonded beads with DI water. (L) Elution of desalted SBE fragments with 50% ACN. Colored ink is used to represent the actual reagents. Orange: DI water; Pink: TEAA; Black: ACN; Purple: B&W buffer; Green: SBE sample; Blue: TCEP.

4. Characterization of Temperature Control

The temperature-resistance relationships of the resistive temperature sensors for both the SBE and SPP chambers were calibrated following fabrication to provide accurate temperature control. The resistance of the thin film gold resistor is linearly dependent on temperature, as given by $R=R_0[1+\alpha(T-T_0)]$, where R is the sensor resistance at temperature T , R_0 is the sensor resistance at reference temperature T_0 , and α is the sensor's temperature coefficient of resistance (TCR). Measurements of SBE sensor resistance at varying temperatures displayed highly linear temperature dependence and the TCR was calculated to be $2.74 \times 10^{-3} \text{ }^{\circ}\text{C}^{-1}$. The temperature-resistance relationship of the SPP sensor also exhibited linear behavior with a TCR value of $2.76 \times 10^{-3} \text{ }^{\circ}\text{C}^{-1}$.

We then characterized the temperature control of the buffer-filled SBE chamber to simulate thermal cycling in the SBE process. The SBE chamber temperature was controlled by an integrated heater and a fan beneath the chip via a closed-feedback loop. In accordance with the experimental fits (Fig. S5A, 10 cycles are shown), the control algorithm was able to ramp rapidly to the specified temperature with approximate closed-loop time constants of 2.13 s from 40 °C to 69 °C, 2.25 s from 69 °C to 94 °C and 10.51 s from 94 °C to 40 °C, respectively. During the thermal cycling for the SBE chamber, the highest temperature reached in the SPP chamber was 33.27 °C (Fig. S5A), which would not affect the streptavidin affinity [1]. Fig. S5B shows a temperature profile in the buffer-filled SPP chamber during heating, using the closed-loop temperature control. The channel temperature increased from room temperature (25 °C) to 65 °C rapidly in about 13.7 s (with an insignificant overshoot of $\sim 0.25 \text{ }^{\circ}\text{C}$) where it remained for approximately 10 min, which was sufficient for the chemical cleavage reaction to release the captured SBE products.

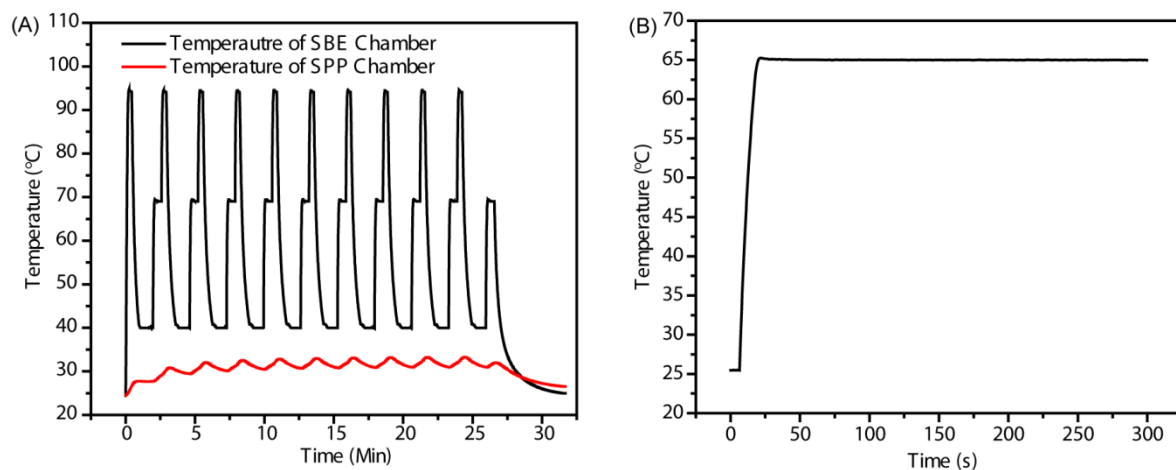


Figure S5. Time-resolved tracking of (A) the temperatures inside the buffer-filled SBE chamber and SPP chamber during thermal cycling, and (B) the temperature inside the buffer-filled SPP chamber during the chemical cleavage procedure.

[1] M. Gonzalez, L. A. Bagatolli, I. Echabe, J. L. Arrondo, C. E. Argarana, C. R. Cantor and G. D. Fidelio, *J Biol Chem*, 1997, **272**, 11288-11294.