

**Supporting information for**  
**Nanoliter multiplex PCR arrays on a SlipChip**

Feng Shen, Wenbin Du, Elena K. Davydova, Mikhail A. Karymov, Janmajay Pandey,  
and Rustem F. Ismagilov

Department of Chemistry and Institute for Biophysical Dynamics  
The University of Chicago, 929 East 57<sup>th</sup> Street, Chicago, IL, 60637

**Experimental section:**

**Chemicals and Materials**

All solvents and salts purchased from commercial sources were used as received unless otherwise stated. All primers were ordered from Integrated DNA Technologies (Coralville, IA). Primer sequences are listed in Table S1. Bovine serum albumin (BSA) was purchased from Roche Diagnostics (Indianapolis, IN). SsoFast EvaGreen Supermix (2X) was purchased from Bio-Rad Laboratories (Hercules, CA). Mineral oil (DNase, RNase, Protease free), Agar, 100 bp PCR DNA ladder, and DEPC-treated and nuclease-free water were obtained from Fisher Scientific (Hanover Park, IL). Dichlorodimethylsilane was purchased from Sigma-Aldrich (St. Louis, MO). Genomic DNA (*Staphylococcus aureus* [MSSA], ATCC number 6538D-5) and organisms (*Candida albicans*, ATCC 10231; *Staphylococcus aureus* [MSSA], ATCC 25923; Methicillin-resistant *Staphylococcus aureus* [MRSA], ATCC 43300; *Escherichia coli*, ATCC 39391; and *Pseudomonas aeruginosa*, ATCC 27853) were purchased from American Type Culture Collection (Manassas, VA). YM Broth and LB Broth were purchased from Becton, Dickinson and Company (Sparks, MD). Soda–lime glass plates coated with chromium and photoresist were purchased from Telic Company (Valencia, CA). Spectrum food colors (green, red, and blue food dye) were purchased from August Thomsen Corp (Glen Cove, NY). Barrier pipette tips and PCR tubes were purchased

from Molecular BioProducts (San Diego, CA). Mastercycler and *in situ* adapter were purchased from Eppendorf (Hamburg, Germany). Teflon tubing (I.D. 370  $\mu\text{m}$ ) was obtained from Weico Wire & Cable (Edgewood, NY), and teflon tubing (O.D. 250  $\mu\text{m}$ , I.D. 200  $\mu\text{m}$ ) was purchased from Zeus (Orangeburg, SC). Photomasks were purchased from CAD/Art Services, Inc. (Bandon, OR). Red quantum dots (QDs), Qdot 655 ITK, and kit for pBad His B plasmid were purchased from Invitrogen (Carlsbad, CA). Green QDs were obtained from Ocean Nanotech (Springdale, AR). MinElute PCR Purification Kit was obtained from Qiagen (Valencia, CA).

### **Fabrication of SlipChip for PCR**

The procedure for fabrication of SlipChip from glass was based on previous work.<sup>1</sup> The soda-lime glass slide with chromium and photoresist coating was aligned with a photomask containing the design for the wells and the ducts, and the AZ 1500 photoresist was exposed using standard exposure protocols. The glass plate was immersed in 0.1 mol/L NaOH immediately after exposure to remove the areas of the photoresist exposed to UV light. The underlying chromium layer that was exposed was removed by applying a chromium etchant (a solution of 0.6:0.365 mol/L  $\text{HClO}_4$  /  $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$ ). The glass plate was then rinsed with Millipore water and dried with nitrogen gas. The back of the glass plate was taped with PVC sealing tape, and then the glass plate was immersed in a glass etching solution (1:0.5:0.75 mol/L  $\text{HF}/\text{NH}_4\text{F}/\text{HNO}_3$ ) to etch the exposed glass surface where chromium coating was removed in the previous step. The etching speed was controlled by the etching temperature. The top plate for both the 40-well design and the 384-well design contained the square wells (Figure 1C) that were etched to be 70  $\mu\text{m}$  deep. The bottom slide for both the 40-well design and the 384-well design contained the

circular wells (Figure 1D) that were etched to be 30  $\mu\text{m}$  deep. A through hole was drilled in the top plate as an inlet for the solution for both 40-well and 384-well designs. Four through holes were drilled in the top plate as outlet for solution for 40-well design. A dead-end filling method was used in 384-well design.<sup>2</sup> The final volume of a single compartment (a pair of overlapping square and circular wells) for the 40-well design was around 26 nL and for the 384-well design was around 7 nL.

The glass plate with etched wells was thoroughly rinsed with Millipore water and ethanol and they dried with nitrogen gas. The glass plate was oxidized in a plasma cleaner for 100 seconds and then immediately transferred into a desiccator. 50  $\mu\text{L}$  of dichlorodimethylsilane was injected into the desiccator and a vacuum was then applied to perform gas phase silanization for an hour. The silanized glass plate was cleaned with chloroform, acetone, and ethanol, and then dried with nitrogen gas. The silanized glass plate was used for PCR experiment within one day. The patterned glass plate could be re-used after it was cleaned with piranha solution (3:1 sulfuric acid:hydrogen peroxide) and silanized again as described above.

### **Deposition of primers on SlipChip**

For the 40-well SlipChip design (shown in Figure 1, 3, and 4), the concentration of each primer in solutions used for loading was 0.05  $\mu\text{M}$ . The primer solution was flowed in Teflon tubing (200  $\mu\text{m}$  ID) connected to a 50  $\mu\text{L}$  Hamilton glass syringe. A volume of 0.1  $\mu\text{L}$  of primer solution, controlled by a Harvard syringe pump, was deposited into each of the circular wells. For the 384-well SlipChip design, (shown in Figure 5), a volume of 0.02  $\mu\text{L}$  of 0.1  $\mu\text{M}$  primer solution was deposited into the circular wells on the bottom

plate. In both cases, the primer solution was allowed to dry at room temperature, and the preloaded SlipChip was used within one day. All primer sequences are listed in Table S1.

### **Culturing bacteria and fungi for PCR experiments**

MSSA, MRSA *Escherichia coli*, and *Pseudomonas aeruginosa* were cultured in LB broth for 6-8 hours to an exponential phase. *Candida albicans* was cultured in YM broth for 8 hours. The cells were collected and washed with 1X PBS buffer. The number of cells was counted under a microscope and the concentration was normalized to be approximately  $1 \times 10^7$  cfu/mL. The final concentration of pathogens was  $1 \times 10^6$  cfu/mL after mixing with the PCR master mixture.

### **Assembling the SlipChip**

The SlipChip was assembled under mineral oil, which was filtered and de-gased before experiments. The bottom plate was first immersed into the oil in a Petri dish, with the patterned side facing up. The top plate was then laid on top of the bottom plate with the patterned side facing down. The two plates were aligned as shown in Figure 1E and stabilized using binder clips.

### **Observation of thermal expansion inside the SlipChip.**

Thermal expansion was studied using a fluorescence stereomicroscope, MZ FLIII (Leica, Germany), equipped with a GFP filter set and 11.2 Color Mosaic camera (Diagnostic Instruments Inc., MI). This stereomicroscope allowed simultaneous observation of red and green quantum dots, both excited with a blue light. The gap between the two plates of the SlipChip was filled with mineral oil stained with green fluorescent quantum dots (QDs). To stain the oil, the original 1% QDs solution in toluene was filtered through 0.22 micron microcentrifuge Amicon filters (Millipore, MA) and

sonicated in ultrasonic bath (Fisher Scientific, NJ) for 10 min. A 10% solution of QDs in mineral oil was thoroughly vortexed and kept for at least 10 min under vacuum before filling the device.

Stained mineral oil was deposited between the slides of the SlipChip; excess oil was removed by rinsing the assembled device sequentially with chloroform, acetone, and ethanol. The SlipChip wells were filled by injecting an aqueous solution of red QDs through the fluidic path created by the wells and ducts. Red QDs (Qdots 655 ITK) were diluted 1:10 in 10 mM Tris-HCl buffer, pH 8.0, containing 1 mM EDTA and 50 mM NaCl. The SlipChip was placed under the stereoscope on the Mastercycler and multiple heating cycles were performed to observe aqueous thermal expansion.

### **PCR amplification**

For reactions in the 40-well SlipChip (Figure 3, 4), the reaction master mixture consisted of 10  $\mu$ L of 2X SsoFast EvaGreen SuperMix, 2  $\mu$ L of 10 mg/mL BSA solution, 6  $\mu$ L of RNase free water, and 2  $\mu$ L of 1 ng/ $\mu$ L MSSA gDNA (replaced with 2  $\mu$ L of nuclease-free water in control experiments). The final concentration of gDNA template was 100 pg/ $\mu$ L. A portion of the *nuc* gene (~270bp) was amplified from *S aureus* genomic DNA (Primer1: GCG ATT GAT GGT GAT ACG GTT; Primer 2: AGC CAA GCC TTG ACG AA CTA AAG C).<sup>3</sup>

For reactions in the 384-well SlipChip, a 331-bp long portion of dsDNA amplified from His B plasmid (pBad template) was used as a template for a PCR control reaction (Primer 1: GCG TCA CAC TTT GCT ATG CC; Primer 2: GCT TCT GCG TTC TGA TTT AAT CTG). The pBad template was purified using a MinElute PCR Purification Kit (Qiagen). The reaction master mixture for the 384-well SlipChip consisted of 10  $\mu$ L of

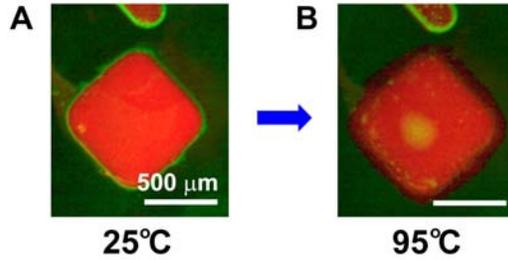
2X SsoFast EvaGreen SuperMix, 2  $\mu\text{L}$  of 10 mg/mL BSA solution, 1  $\mu\text{L}$  of 100 pg/ $\mu\text{L}$  pBad template, 2  $\mu\text{L}$  of cell suspension, and 5  $\mu\text{L}$  of nuclease-free water.

The PCR master mixture was injected into the SlipChip by pipetting. The square wells on the top plate were slipped to overlay the circular wells on the bottom plate. The SlipChip was then placed on an *in situ* adaptor in the Mastercycler (Eppendorf) for thermal cycling.

An initial step of 15 min at 94 °C was used to lyse the cells and activate the enzyme for reaction. Next, a total 35 cycles of amplification were performed as follows: a DNA denaturation step of 1 min at 94 °C, a primer annealing step of 30 sec at 55 °C, and a DNA extension step of 30 sec at 72 °C. After the final cycle, the DNA extension step was performed for 5 min at 72 °C. Then the SlipChip was kept in the cycler at 4 °C before imaging.

### **Image acquisition and analysis**

Bright field images in Figure 1 were acquired by using Leica stereoscope. All fluorescence images were acquired by using a Leica DMI 6000 B epi-fluorescence microscope with a 5X / 0.15 NA objective and L5 filter at room temperature. The intensity level of fluorescence images was adjusted to be the same values for all images. All fluorescence images were corrected by a background image obtained with a standard fluorescent slide. For Figure 5, fluorescence images were stitched together using MetaMorph software (Molecular Devices, Sunnyvale, CA).



**Figure S1.** Example of the thermal expansion event observed during PCR thermal cycling. Mineral oil was stained with green quantum dots (QDs), aqueous phase was stained with red QDs, as described in Materials and Methods section. A) Aqueous PCR solution completely filled the square wells at 25 °C. B) Significant leakage of the aqueous solution was observed at 95 °C.

**Table S1.** Position, name and sequence of deposited primer pairs in the 384-well SlipChip (positions correspond to those in Figure 5). Primer pairs used in the 40-well SlipChip (Figures 3 and 4) are marked in bold.

Position	Pathogen and Target gene	Primer sequence
A1, B1, C1, D1	pBad control	GCGTCA CACTTT GCT ATG CC GCT TCT GCGTTC TGA TTT AAT CTG
A2, B2, C2, D2	Blank for leakage control test	
A3	<i>E coli nlp</i>	ATA ATC CTC GTC ATT TGC AG <sup>4</sup> GACTTC GGGTGA TTG ATA AG
A4	<i>S pyogene fah</i>	TTA AAT ACG CTA AAG CCC TCT <sup>4</sup> AGG GTG CTT AAT TTG ACA AG
A5	<i>S pyogene OppA</i>	CCC AGT TCA ATT AGA TTA CCC <sup>4</sup> TTG ACT TAG CCT TTG CTT TC
A6	<i>S pneumoniae cinASP</i>	GGCTGT AGG AGA CAATGA AG <sup>4</sup> CTT TGT TGA CAG ACGTAG AGT G
A7	<i>S pneumoniae plySP</i>	ATT TCG AGT GTT GCT TAT GG <sup>4</sup> GTA AAGTGA GCC GTC AAATC
B3	<i>E faecium bglB</i>	TCT TCA TTT GTT GAA TAT GCT G <sup>4</sup> TGG AAT CGA ACC TGT TTATC

Position	Pathogen and Target gene	Primer sequence
B4	<i>E faecalis ace</i>	TAG TTG GAA TGA CCG AGA AC <sup>4</sup> AGT GTA ACG GAC GAT AAA GG
B5	<i>P aeruginos vic</i>	TTC CCT CGC AGA GAA AAC ATC <sup>5</sup> CCT GGT TGA TCA GGT CGA TCT
B6	<i>S agalactia cpsY</i>	CGA CGA TAA TTC CTT AAT TGC <sup>4</sup> TCA GGA CTG TTT ATT TTT ATG ATT
B7	<i>Pseu general 16S</i>	GAC GGG TGA GTA ATG CCT A <sup>5</sup> CAC TGG TGT TCC TTC CTA TA
C3	<i>S aureus nuc</i>	<b>GCGATTGATGGTGATAACGGTT<sup>3</sup></b> <b>AGCCAAGCCTTGACGAACTAAAGC</b>
C4	<i>S epid agrC</i>	GAT GAT ATT AAT CTA TTT CCG TTT G <sup>4</sup> TCA GGA CTG TTT ATT TTT ATG ATT
C5	<i>S mutans dltA</i>	AGATAT GAT TGC AAC AAT TGA A <sup>4</sup> CGC ATG ATT GAT TTG ATA AG
C6	<i>P mirabil aad</i>	CGCTAT TAA CCT TGC TGA AC <sup>4</sup> CCT TTC TCA CTC ACC ACATC
C7	<b>MRSA mecA</b>	<b>CAAGATATGAAGTGGTAAATGGT<sup>6</sup></b> <b>TTTACGACTTGTTCATACCATC</b>
D3	<i>C tropicalis ctr</i>	CAA TCC TAC CGC CAG AGG TTA T <sup>7</sup> TGG CCA CTA GCA AAA TAA GCG T
D4	<i>C glabrata cgl</i>	TTA TCA CAC GAC TCG ACA CT <sup>7</sup> CCC ACA TAC TGA TAT GGC CTA CAA
D5	<i>C albicans calb</i>	TTT ATC AAC TTG TCA CAC CAG A <sup>7</sup> ATC CCG CCT TAC CAC TAC CG
D6	<i>K pneumonia cim</i>	AAT TTA ACC TGG TTT GAT AAG AA <sup>4</sup> CAA AAT ATG AAC TAT CAG AAA GAT TG
D7	<i>K pneumonia acoA</i>	TAA CGG CAA AGA CGC TAA <sup>4</sup> TGA CCA GGG CTT CTA CTT C

## References:

- (1) Du, W. B.; Li, L.; Nichols, K. P.; Ismagilov, R. F., *Lab Chip* **2009**, *9*, 2286-2292.
- (2) Li, L.; Karymov, M.; Nichols, K. P.; Ismagilov, R. F., *Langmuir* **2010**, *submitted*.
- (3) Brakstad, O. G.; Aasbakk, K.; Maeland, J. A., *J. Clin. Microbiol.* **1992**, *30*, 1654-1660.
- (4) Palka-Santini, M.; Cleven, B. E.; Eichinger, L.; Kronke, M.; Krut, O., *BMC Microbiol.* **2009**, *9*, 1.
- (5) Qin, X.; Emerson, J.; Stapp, J.; Stapp, L.; Abe, P.; Burns, J. L., *J. Clin. Microbiol.* **2003**, *41*, 4312-4317.
- (6) Shrestha, N. K.; Tuohy, M. J.; Hall, G. S.; Isada, C. M.; Procop, G. W., *J. Clin. Microbiol.* **2002**, *40*, 2659-2661.
- (7) Luo, G. Z.; Mitchell, T. G., *J. Clin. Microbiol.* **2002**, *40*, 2860-2865.