Diffusion-based Microfluidic PCR for "One-pot" Analysis of Cells

Sai Ma, Despina Nelie Loufakis, Zhenning Cao, Yiwen Chang, Luke E. K. Achenie, Chang Lu

Supporting Information

Email: changlu@vt.edu

Materials and Methods

Microfluidic chip fabrication

Two-layer PDMS chip was fabricated by multilayer soft lithography [1] (Fig. 1a). Photomasks were designed by Freehand MX (Macromedia, San Francisco, CA) and printed on 4000 dpi films. The fluidic master (photoresist on a silicon wafer) was fabricated in SU-8 2025 (Microchem, Newton, MA) and AZ 9260 (Clariant, Charlotte, NC) with the thickness being 60 μm and 13 μm, respectively. The master was heated to 130℃ to form rounded cross-sectional profile for the features in AZ 9260. The control layer master was fabricated in SU-2025 with 24μm thickness. The control layer PDMS was made by spinning PDMS (RTV615A: RTV615B=20:1, R. S. Hughes, Sunnyvale, CA) at 500 rpm for 10s and then at 1700 rpm 30s, which resulted a thickness of 67 μm. The fluidic layer PDMS had the composition of RTV615A: RTV615B =5:1 and a thickness of ~0.4 cm. Both layers of PDMS were cured at 80℃ for 15 min. The two layers were then aligned, brought into contact and baked for 1h at 80℃. The two-layer PDMS structure was then peeled off from the master and access holes were punched. Finally the PDMS structure was bonded to clean cover glass (~150 μm thick, VWR, Radnor, PA) after plasma oxidation of the surfaces (Harrick Plasma, Ithaca, NY) and baked at 80℃ overnight to strengthen the device.

Device setup and operation

The solutions were delivered into the device by a syringe pump. The actuation of the two-layer valves and hydration line was controlled via solenoid valves (ASCO Scientific, Florham Park, NJ) using a DAQ card (NI SCB-68) and LabVIEW (National Instruments,

Austin, TX) programs [2,3]. The control layer and the hydration line were filled with water before experiments.

Human genomic DNA purified from a lymphoblastoid cell line (GM 12878) using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) were used in on-chip PCR testing. During the PCR, the loading chambers were filled with PCR mix of a designed composition (1x Fast Plus EvaGreen qPCR Master Mix from Biotium, 0.8% PEG 8000, 0.08% Tween-20, 0.2U/μl *Taq* polymerase, 1.2mM GAPDH primers for GM 12878 cells). The microvalves were open for 10 to 30 min to replace the original solution in the reaction chamber with desired reagent concentrations. The microfluidic device was then placed on a flat-plate thermal cycler (Techne, Bibby Scientific, Burlington, NJ) for on-chip PCR. A metal frame was used to clamp down on the microfluidic chip to ensure good contact with the flat plate. Paraffin oil (Sigma-Aldrich, St. Louis, MO) was applied in between the glass surface of the chip and the flat plate to improve heat transfer. Thermocycling conditions were 95℃ for 3min followed by 30 cycles or 45 cycles of (95℃ for 30 s, 60℃ for 30 s and 72℃ for 30 s).

When cell-lysis was included as an on-chip step, GM 12878 cells (suspended in a PBS buffer at a concentration of 10^6 to 10^7 cells/ml) flowed into the device to leave a known number of cells in the reaction chamber. A Triton X lysis buffer (1% Triton X-100, 50mM Tris-HCl, 150mM NaCl, pH 7.5) was flowed into the loading chambers. Opening the microvalves for 10 min permitted complete cell lysis. The cell lysis step was then followed by diffusion-based PCR described above.

Quantitative PCR assay on the on-chip PCR product

QPCR assays were performed in 25µl aliquots in a Real-Time PCR Detection System (CFX Connect, BIO-RAD, Hercules, CA). The product from on-chip PCR was eluted by 10 µl H_2O and then serial- diluted to the working range of the qPCR. Thermocycling conditions were 95℃ for 3min followed by 30 cycles of (95℃ for 30 s, 60℃ for 30 s and 72℃ for 30 s). The following primers were used to detect GAPDH gene in GM 12878: GAPDH forward: 5'- CCCCACACACATGCACTTACC -3', GAPDH reverse: 5'- CCTACTCCCAGGGCTTTGATT -3'.

Mathematical modelling

COMSOL Multiphysics 4.3 was used to model the diffusion process in the microfluidic device (i.e molecular exchange between the loading chambers and the reaction chamber). The physics model, namely transport of diluted species was used. A 3D time-dependent study was built to analyse the concentration variations of each species within 1h inside the microscale structure. No flux at all the boundaries was applied. The average concentration of each species in the reaction chamber was evaluated. We modelled the diffusion processes at 25 ℃ using the diffusivity values included in the main text. To model the entry of species (Triton-X 100, dNTP, *Taq* polymerase, primers, and small ions) from the loading chamber into the reaction chamber, the starting concentration in the reaction chamber was 0 and then we examined the increase in the species concentration in the reaction chamber over time in terms of its percentage of the original concentration in the loading chamber. To model the exit of the species (DNA, proteins, and Triton-X 100) out of the reaction chamber, the starting concentration in the loading chamber was 0 and then we examined the decrease in the species concentration in the reaction chamber over time in terms of its percentage of the original concentration in the reaction chamber.

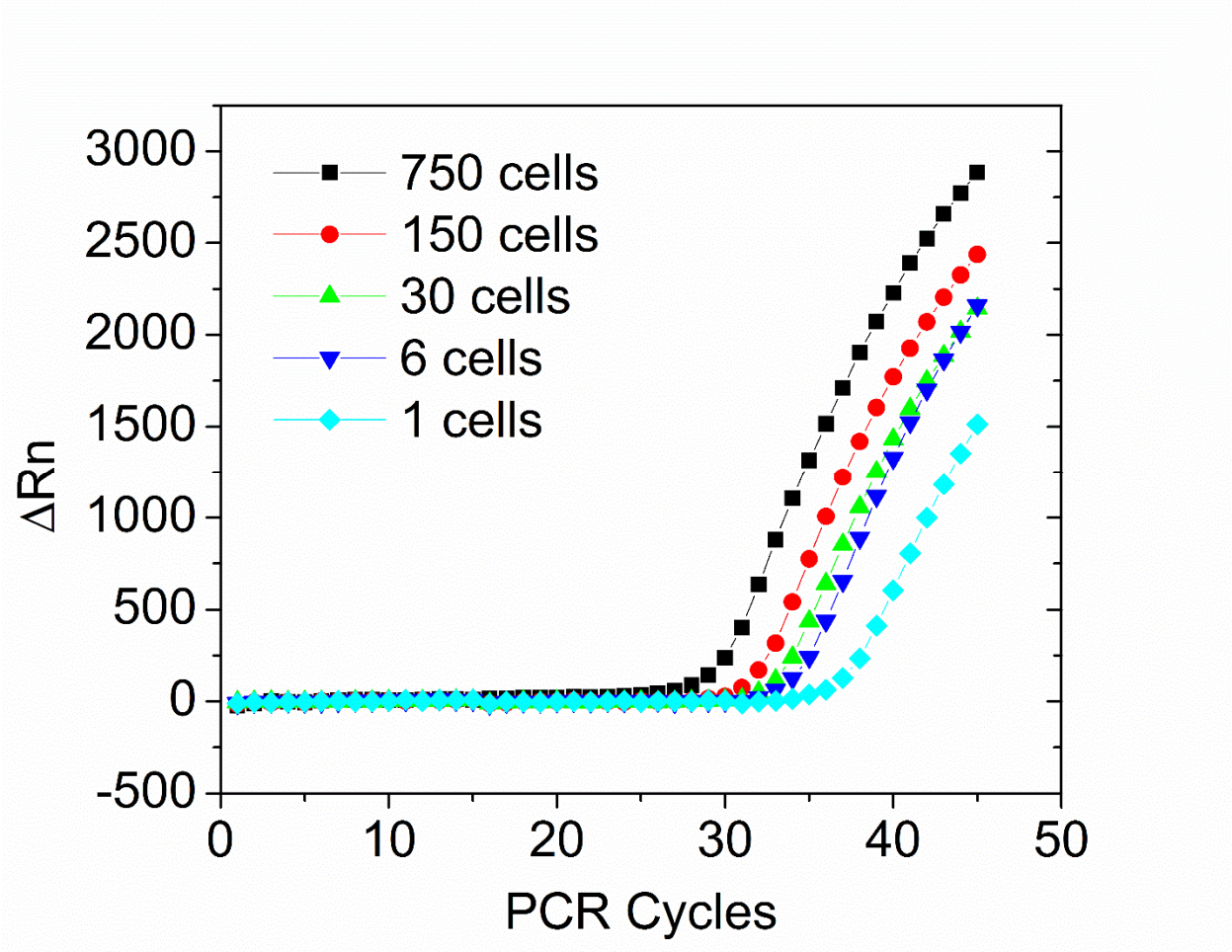

Fig. S1 Real-time PCR amplification of purified DNA from various numbers of GM12878 cells (purified by QIAamp DNA Blood Mini Kit). There is noticeable difference in the signal when the number of the cells varies.

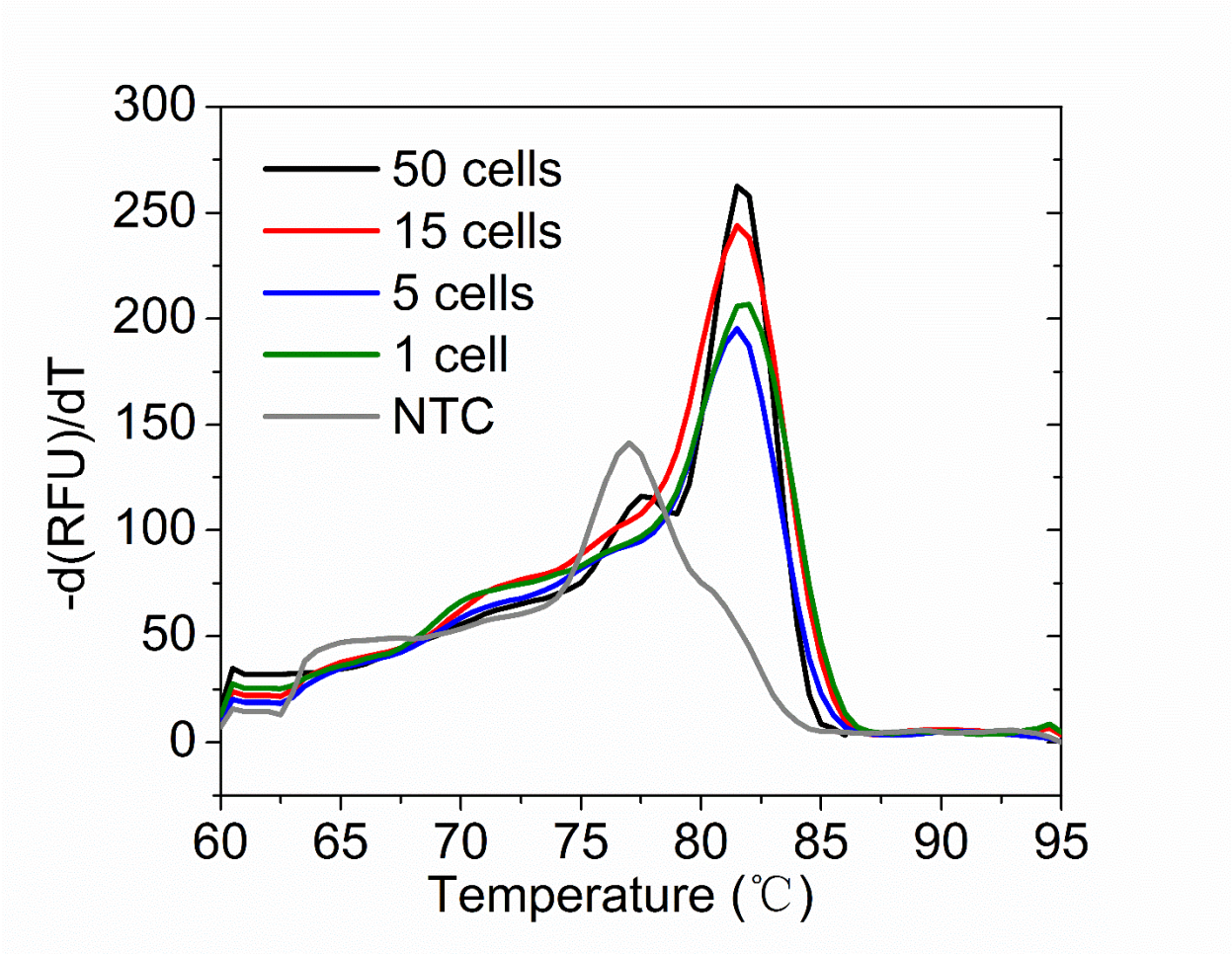

Fig. S2 The melting curves of the on-chip samples in Fig. 3C. Amplification products were eluted from the chip and measured by real time PCR. NTC is the no template control (negative control).

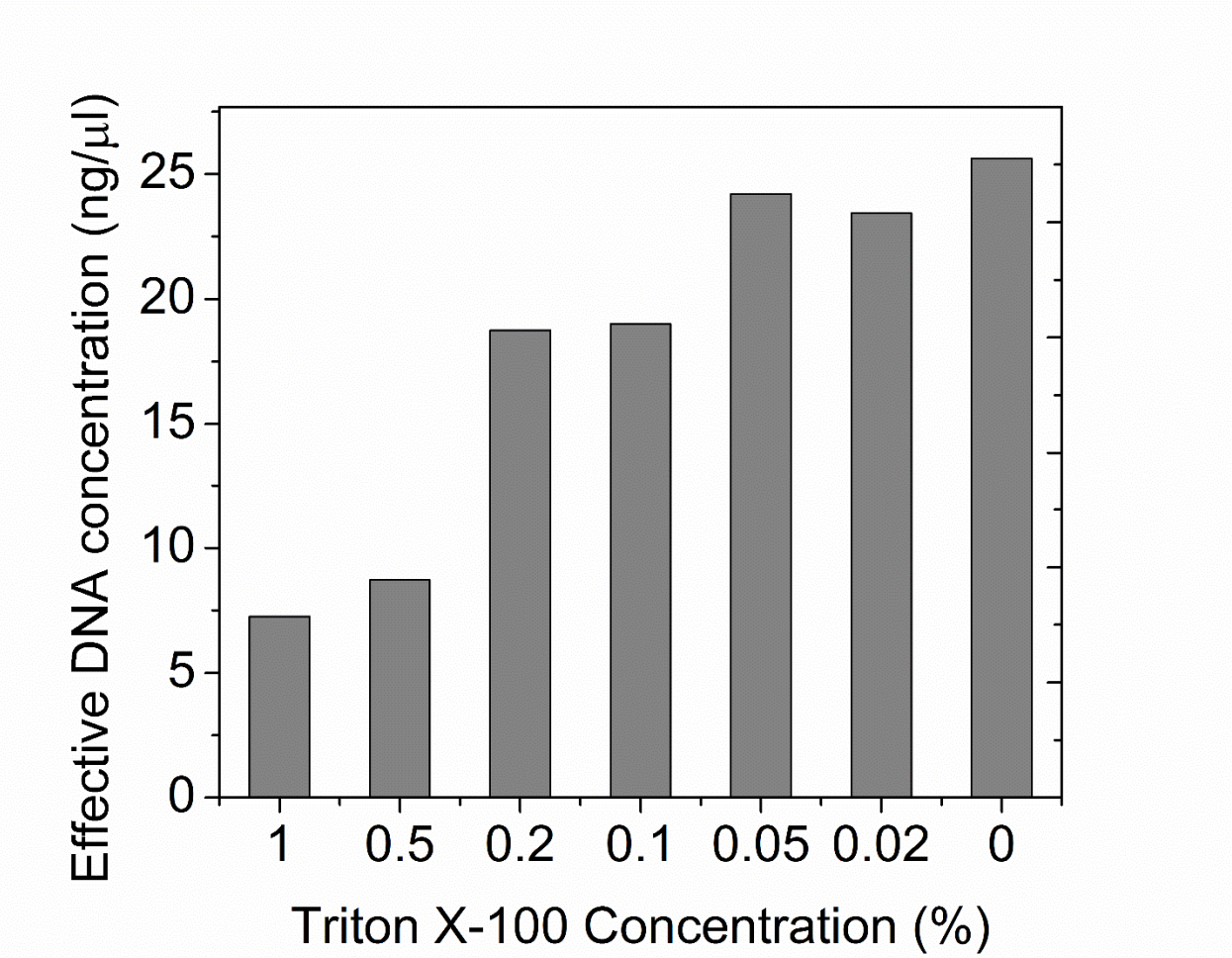

Fig. S3 The inhibition of off-chip PCR by Triton X-100. 25 ng/µl DNA template with various Triton X-100 concentrations in each test was measured by real time PCR. The measured amount of DNA (i.e. effective DNA concentration) is lower than the actual amount (25 ng/µl) due to inhibition of Triton X-100.

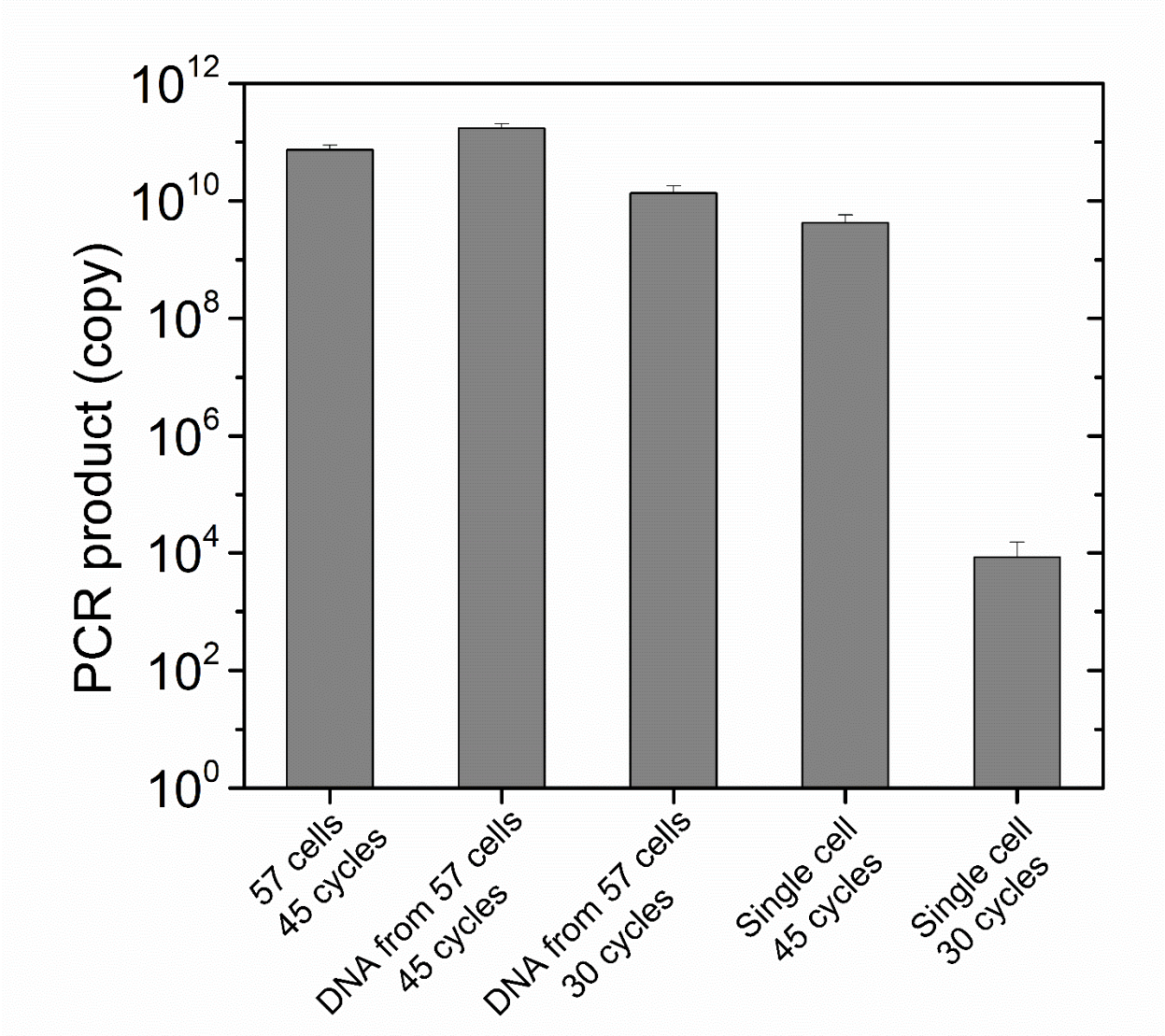

Fig. S4 The comparison among on-chip PCR tests (with 3nl reaction chamber) of various conditions. Some of these tests were conducted with combined lysis and PCR process (Loading times for Triton-X and PCR mix were 10 and 30 min, respectively), starting from GM12878 cells (e.g. "57 cells"). The others were done with purified DNA from a certain number of GM12878 cells (e.g. "DNA from 57 cells"), with the purification conducted using QIAamp DNA Blood Mini Kit. Samples were amplified for either 30 or 45 cycles.

References

(1) Unger, M. A.; Chou, H.-P.; Thorsen, T.; Scherer, A.; Quake, S. R. *Science* **2000**, *288*, 113.

(2) Geng, T.; Bao, N.; Litt, M. D.; Glaros, T. G.; Li, L.; Lu, C. *Lab Chip* **2011**, *11*, 2842.

(3) Geng, T.; Bao, N.; Sriranganathanw, N.; Li, L.; Lu, C. *Anal. Chem.* **2012**, *84*, 9632.