Supporting information for:

Microfluidic single cell mRNA isolation and analysis

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Table of Contents

Supporting methods

Master mold fabrication

All photomasks, which define device features, were designed with AutoCAD software (AutoDesk, Sausalito, CA) and printed at a resolution of 20,000 dots per inch on transparency films. In all optical lithography processes, 3'' silicon wafers were utilized as substrates; and mold exposures were under UV light on an MJB mask aligner (7 mW/cm^2) for the indicated time.

Twenty four μm high features present on control molds were fabricated with a single lithographic step. SU8-2025 (Microchem) was spun on a wafer (3,000 rpm, 45 s.), baked before exposure to evaporate excess solvent (2 min./5 min. at 65°C /95°C), exposed under a negative mask for 75 s., baked after exposure (2 min./5 min. at 65°C /95°C) to facilitate additional resist polymerization, and developed in NanoSU8 developer (Microchem). Once features were developed, the mold was baked again for 40 s. at 95 °C to remove remaining solvent.

A two step lithographic process, was utilized for 4plex flow mold fabrication. The first step was undertaken was to define column construction flow channels (10 μm high). For these channels, SU8-2010 (Microchem) was spun onto a wafer (3,000 rpm, 45 s.), baked before exposure to evaporate excess solvent (1 min./3 min. at 65°C /95°C), exposed under a negative mask for 45 s., baked after exposure (1 min./3 min. at 65°C /95°C) to facilitate additional resist polymerization, and developed in NanoSU8 developer (Microchem). Upon visualization of developed features, the mold is subjected to a 90 min. hard baked at 150 °C. When the mold has cooled to room temperature, the

2nd step defining the remaining 40 μm high channels is carried out. First, to promote resist adhesion, the mold was exposed to HMDS vapor for 2 min. AZ-50 (Clariant) was then spun onto the mold (1,600 rpm, 60 s.). The mold was subsequntly soft baked (2 min./5 min. at 65°C /115°C), aligned to the column construction flow channels, exposed under a positive mask for 4 min., and developed in 25% 2401 developer (Microchem), diluted with 18 mΩ dI H_2O. Once features were developed, the mold was annealed/hard baked for 3 hr. 200°C for the aforementioned reasons stated above. Additionally, if the AZ-50 resist does not undergo a chemical change (color turns from red to black), the features will crack when subjected to subsequent soft lithography baking steps.

Device Fabrication

All devices are fabricated by multilayer soft lithography (MSL) with the silicone elastomer polydimethylsiloxane (PDMS, General Electric). Each device employs push-up valve geometry and is a three layer elastomeric structure bonded to a 3'' X 1'' RCA cleaned coverslip. Negative master molds were first exposed to chlortrimethylsilane (TMCS, Aldrich) vapor for 2 min. to promote elastomer release from molds after baking steps. Thirty g of liquid PDMS (5 parts A:1 part B) was poured onto the flow master, degassed under vacuum and baked for 45 min. at 80°C. Liquid PDMS (20 parts A:1 part B) was spun onto the control master mold (2,000 rpm for 60 s.) and allowed to settle for 30 min. in order to obtain a uniform elastomer membrane on top of the control features. The mold was then baked for 30 min. at 80°C. Upon completion of the baking steps, the partially cured flow layer was peeled from its mold and 650 μm diameter flow channel access holes punched (Technical Innovations, part# CR0350255N20R4). The layer is

then aligned to the partially cured control layer, still on the control master. The two layer structure was then baked for 45 min. The third layer, a featureless elastomeric membrane, was fabricated by spinning liquid PDMS (20 parts A:1 part B) onto a clean silicon wafer (1,600 rpm for 60 s.) followed by baking for 30 min. at 80°C. Once baking was completed, the two layer structure was peeled from the control master, control channel access holes punched (Technical Innovations, part# CR0350255N20R4) and mounted onto the third partially cured PDMS layer. The three layer structure was then baked for 5 hr. at 80°C. The assembled three layer structure was then peeled from the clean silicon wafer, output holes punched (Technical Innovations, part #CR0830655N14R4), cut to size and bonded to an RCA cleaned coverslip and baked overnight at 80°C.

Figure S-1

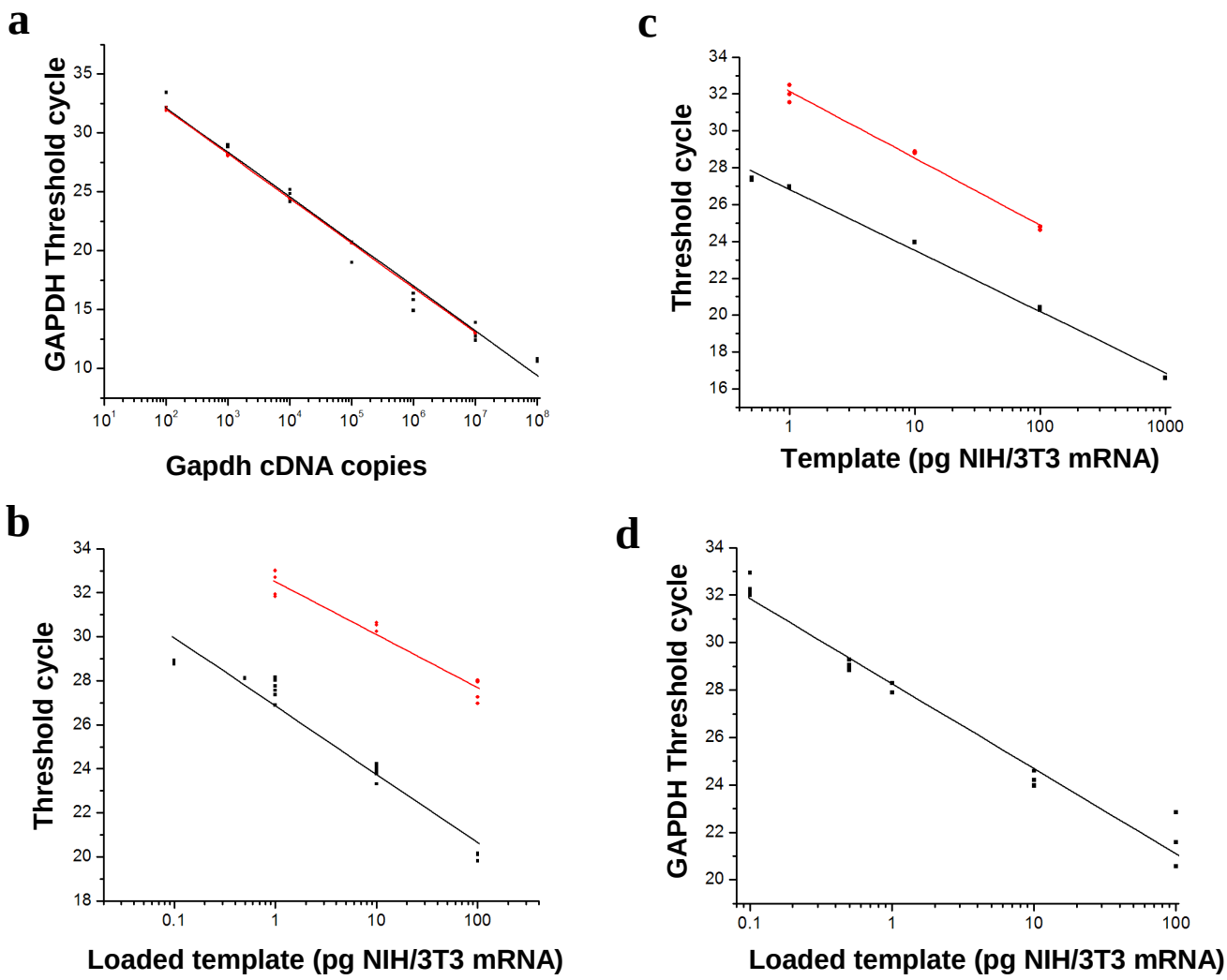
a
GAPDH Threshold cycle
Gapdh cDNA copies
c
Threshold cycle
Template (pg NIH/3T3 mRNA)
b
Threshold cycle
Loaded template (pg NIH/3T3 mRNA)
d
GAPDH Threshold cycle
Loaded template (pg NIH/3T3 mRNA)

Table S-1

Yields from various mRNA isolation procedures

Method	Total RNA yield per NIH/3T3 cell	mRNA yield per NIH/3T3 cell
Microfluidic 4plex	N/A	1pg
Dynal Bulk isolation	N/A	0.5pg
Promega PolyA Tract mRNA	N/A	0.33pg
Sigma GenElute Mammalian total	15pg	0.15pg-0.75pg*
Stratagene Absolutely RNA kit[3]	17.7pg	0.18pg-0.89pg*

*Range is 1-5% of total RNA

Supporting References

1. http://www.promega.com/pnotes/56/5338f/5338f_core.pdf

2. http://www.sigmaaldrich.com/sigma/bulletin/rtn10bul.pdf

3. http://www.stratagene.com/newsletter/pdf/13_3_p104-105.pdf

Supporting Figure Legends

Supporting Figure 1: qPCR and qRT-PCR standard curves. **a**. Standard curves utilized to extract GAPDH copy number from cell samples. Black: qPCR standard curve was generated with known GAPDH cDNA templates using the icycler platform ($Y = -3.78X + 39.67$. $r^2=0.99$). The regression line was utilized to extrapolate average GAPDH copy number in bulk preparations of NIH/3T3 mRNA by running qRT-PCR on seven mRNA samples, each extracted from 2500 NIH/3T3 cells. Red: Results from the identical experiment in (a) utilizing the Roche Lightcycler ($Y = -3.79X + 39.57$. $r^2=0.99$). **b**. RT-qPCR standard curve generated with microfluidic mRNA samples (NIH/3T3 mRNA loaded on-chip) . A 132 bp portion of GAPDH (black, $Y = -3.11X + 26.86$. $r^2=0.95$), and a 294 bp portion of HPRT (red, $Y = -2.40X + 32.51$. $r^2=0.96$) were amplified and detected. **c**. RT-qPCR standard curves generated with NIH/3T3 mRNA. A 132 bp portion of GAPDH (black: $Y = -3.31X + 26.82$. $r^2=0.99$) or a 294 bp portion of HPRT (red: $Y = -3.63X + 32.16$. $r^2=0.99$) were amplified and detected. **d.** qPCR standard curve generated with microfluidic NIH/3T3 mRNA standards subjected to mRNA isolation/1st strand cDNA synthesis. $Y = -3.59X + 28.27$. $r^2=0.98$.