Supporting Information For

Synthetic Biology in a Chip: A Digital Microfluidic Platform for Cell Transformation, Culture and Expression

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METHODS

Reagents and materials. Unless otherwise specified, general-use reagents were purchased from Sigma Aldrich. Fabrication reagents and supplies included SU-8-5, SU-8-2025, SU-8-2075, S-1811 and SU-8 Developer from Microchem (Newton, MA), MF-321 positive photoresist developer from Rohm and Haas (Marlborough, MA), CR-4 chromium etchant was from OM Group (Cleveland, OH), and AZ-300T photoresist stripper from AZ Electronic Materials (Somerville, NJ).

Device fabrication. The digital microfluidic heat shock devices were fabricated using standard photolithography as described previously with moderate adjustments.[1] The device consisted of a glass bottom, gold electrodes, dielectric layer, channel layer and an ITO cover. All photolithography was performed by exposing a photopolymer through a photomask (Fineline Imaging Inc., Colorado Springs, CO) by an Oriel® 500 W Hg exposure system (Newport Corporation, Irvine, CA). Gold electrodes were initially micropatterned on a glass slide by exposing, developing and etching a gold and S1811 coated glass slide (Telic Company, Valencia, CA). Following cleaning with acetone, IPA and O_2 plasma (Harrick Plasma, Ithaca, NY): devices were coated with a 5 μm layer of SU-8 5. After selective removal of the SU-8 5 over the side contacts and another cleaning step, 150 μm channels composed of SU-8 2075 were fabricated over the dielectric layer. Following development, devices were rinsed with IPA and DI water, dried with N_2 gas and hard-baked for 15 min. at 200 °C. Prior to assembly, the microfluidic device and ITO coated glass slide (Delta Technologies, Stillwater, MN) were coated with 0.2 Fm filtered Aquapel (TCP Global, San Diego, CA) for 15 min. A kimwipe was then used to

remove the Aquapel and the device was rinsed with DI water, dried with N_2 gas and kept at 21 °C for 1h.

Cell Preparation. *Chemically competent E. coli.* A common strain of *Escherichia coli (E. coli)* for laboratory cloning (DH5α) was acquired from the JBEI public registry, JBEI-4930 (https://public-registry.jbei.org/folders/205)[2] and made chemically competent using a standard $CaCl_2$ procedure.[3] Reagents and materials were kept chilled at 4 °C during all aspects of cell preparation. Briefly, a bacterial cell colony in Luria-Bertani (LB) broth was cultured in a 37 °C incubator at 200 rpm until early log phase was achieved ($OD_{600} = 0.3$) measured with a spectrophotometer. At this p0oint, the cell suspension was placed on ice for 15 min then centrifuged for 10 min at 3300 g. The medium was then replaced with 0.1 M $CaCl_2$ in which the cells suspended on ice for 30 min. The suspension was again centrifuged for 10 min at 3300 g and the supernatant exchanged for a 0.1 M $CaCl_2$ solution containing 15% glycerol. The cell suspension at a final concentration of 1 x 10^{10} cells/mL was then aliquoted into micro-centrifuge tubes and stored at -80 °C for future use.

Chemically competent yeast. A commercially available kit (Frozen-EZ Yeast Transformation IITM) from Zymo Research Corp. (Irvine, CA) was used to prepare competent *Saccharomyces cerevisiae* (JBEI-4714) cells obtained from the JBEI registry. Following the manufactures protocol, cells were grown at 30 °C at 200 rpm until mid-log phase was reached ($OD_{600} = 0.9$). The cell suspension was then centrifuged at 500 g for 4 min and media replaced with EZ 1 solution (≤1% Tris and ≤1% EDTA in water). Suspended cells were again centrifuged at 500 g for 4 min and the supernatant replaced with EZ 2 solution (1 M sorbitol and 7% DMSO in water). Competent cells at a density

of 2 x 10^7 cells/mL are then aliquoted into micro-centrifuge tubes and kept frozen at -70 ^{o}C until needed.

Protoplast preparation. Aspergillus niger protoplasts were prepared using a previous protocol with minor modifications.[4] Briefly, 10^6 *A. niger* (ATCC® 11414™) conidia in 1 mL H_2O were added to 100 mL YEPD broth and cultured in a 30 ^{o}C incubator while shaking at 150 rpm for 12 hrs. A Miracloth was used to filter the mycelia, which were then washed with stile H_2O. Mycelia were digested in 40 mL Vinoflow solution (30 mg/mL Vino Taste Pro, 0.6 M Ammonium Sulphate, 50 mM Maleic acid, pH 5.5) at 30 ^{o}C, shaking at 70 rpm for 4 hrs. At this point, the culture was filtered through a Miracloth and the filtrate centrifuged at 800 g for 10 min. The supernatant was then removed and the pellet washed twice with 25 mL of ST solution (1 M sorbitol, 50 mM Tris, pH 8.0) then resuspended in 10 mL STC solution (1 M sorbitol, 50 mM Tris, pH 8.0, 50 mM $CaCl_2$). This suspension was centrifuged at 800 g for 10 min and then supernatant replaced with fresh STC solution until a protoplast concentration of 1.2×10^7 spores/mL was achieved. 40% PEG 4000 in STC solution is then gently mixed with this suspension (20% v/v), which is then mixed with DMSO (7% v/v). Protoplasts at a final concentration of 9 x 10^6 cells/mL are then aliquoted into micro-centrifuge tubes and frozen at -80 ^{o}C.

Plasmid Preparation. Plasmid DNA was extracted from *E. coli* cells expressing blue fluorescence protein (pBAD-mTag BFP2)[5] acquired from Addgene (Cambridge, MA), green fluorescence protein (pRSET-EmGFP) from Life Technologies (Grand Island, NY) or red fluorescence protein (pDsRed_T3-S4T)[6] and kanamycin resistant green fluorescence protein (pBbB2k-GFP) obtained from the JBEI public registry

(https://registry.jbei.org/folders/205) using QIA miniprep from Qiagen (Valencia, CA). pL211 containing a TEF promotor and ADH1, URA- and mCherry (pL211-mCherry) for transformation into *S. cerevisiae* was provided by the JBEI public registry (https://public-registry.jbei.org/folders/205). GFP genes were cloned into an *A. Niger / E. coli* shuttle vector (peGFP-glaA) and are available at the JBEI public registry (https://public-registry.jbei.org/folders/205).

pProm1_BCD1-GFP DNA fragments used for Golden Gate assembly were prepared by PCR using primers and plasmids described elsewhere.[7] Briefly, 50 μL PCR reactions consisted of 2.5 μL (2.5 μM) of each forward and reverse primer, 1 μL template, 1 μL dNTPs (10 mM), 0.5 μL high fidelity iProof phusion polymerase (BioRad; Hercules, CA), 10 μL 5Å~ high fidelity phusion buffer, and 32.5 μL deionized water. Four 50 μL PCR reactions (200 μL total) were performed for each fragment amplified. The following PCR thermocycling conditions were used: denaturation at 98 °C for 30 s, 38 cycles of denaturation at 98 °C for 20 s, annealing at 68 °C for 30 s, and elongation at 72 °C for 15 s each kb, and a final extension at 72 °C for 10 min. Following PCR amplification the samples were DpnI-digested and gel purified. At this point a modified Golden Gate DNA assembly method was performed. 1 μL BsaI-digested BCD1-GFP fragment was mixed with 1 μL BsaI-digested pProm1 promoter fragment, 1 μL BsaI-digested vector backbone, 1 μL T4 ligase enzyme (Thermo Scientific), 1 μL of T4 ligase buffer and 5 μL deionized water for 30 min at room temperature.

Supplementary Table 1. JBEI public registry

Strain name	Strain ID	Plasmid name	Plasmid ID	About
JBEI-4930	JPUB_006854			DH5a *E.coli*
JBEI-11346	JPUB_006511	pRSET-EmGFP	JPUB_006512	pRSET-EmGFP
Keasling-1512	JPUB_006504	pDsRed_T3-S4T	JPUB_006505	DsRed variant
JBEI-11345	JPUB_006509	pBAD-mTag BFP2	JPUB_006510	pBAD-mTag BFP2
JBEI-7336	JPUB_004978	pProm1_BCD1-GFP	JPUB_001384	pProm1_BCD1-GFP
JBEI-7893	JPUB_006507	pBbB2k-GFP	JPUB_006508	pBbB2k-GFP
JBEI-4714	JPUB_006506			CEN.PK2-1D *S.cerevisiae*
JBEI-11341	JPUB_006515	pL211-mCherry	JPUB_006516	mCherry expressing S.cerevisiae
ATCC11414	JPUB_006514			*A. niger*
JBEI-11353	JPUB_006851	peGFP-glaA	JPUB_006513	eGFP expressing *A. niger*

Physical samples and additional information of strains and plasmids are available on the JBEI public registry (https://public-registry.jbei.org/folders/205).

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