Supplementary Information

A Versatile Microfluidic Device for Automating Synthetic Biology

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Table S1. Strains and plasmids used in this study*

Plasmids and strains used for Golden Gate and Gibson assembly		
Type	**Name**	**JBEI Part ID**
Plasmid	pProm1_BCD1-GFP	JPUB_001384
Plasmid	pProm1_BCD2-GFP	JPUB_001383
Plasmid	pProm1_BCD20-GFP	JPUB_001382
Plasmid	pProm1_BCD21-GFP	JPUB_001381
Plasmid	pProm2_BCD1-GFP	JPUB_001380
Plasmid	pProm2_BCD2-GFP	JPUB_004950
Plasmid	pProm2_BCD20-GFP	JPUB_001378
Plasmid	pProm2_BCD21-GFP	JPUB_004951
Plasmid	pProm9_BCD1-GFP	JPUB_001376
Plasmid	pProm9_BCD2-GFP	JPUB_004952
Plasmid	pProm9_BCD20-GFP	JPUB_004953
Plasmid	pProm9_BCD21-GFP	JPUB_004954
Plasmid	pProm11_BCD1-GFP	JPUB_001372
Plasmid	pProm11_BCD2-GFP	JPUB_004955
Plasmid	pProm11_BCD20-GFP	JPUB_004956
Plasmid	pProm11_BCD21-GFP	JPUB_004957
Strain containing pProm1_BCD1-GFP	JBEI-7336	JPUB_004978
Strain containing pProm1_BCD2-GFP	JBEI-7337	JPUB_004979
Strain containing pProm1_BCD20-GFP	JBEI-7338	JPUB_004980
Strain containing pProm1_BCD21-GFP	JBEI-7339	JPUB_004981
Strain containing pProm2_BCD1-GFP	JBEI-7340	JPUB_004982
Strain containing pProm2_BCD2-GFP	JBEI-7341	JPUB_004983
Strain containing pProm2_BCD20-GFP	JBEI-7342	JPUB_004984
Strain containing pProm2_BCD21-GFP	JBEI-7343	JPUB_004985
Strain containing pProm9_BCD1-GFP	JBEI-7344	JPUB_004986
Strain containing pProm9_BCD2-GFP	JBEI-7345	JPUB_004987
Strain containing pProm9_BCD20-GFP	JBEI-7346	JPUB_004988
Strain containing pProm9_BCD21-GFP	JBEI-7347	JPUB_004989
Strain containing pProm11_BCD1-GFP	JBEI-7348	JPUB_004990

Strain containing pProm11_BCD2-GFP	JBEI-7349	JPUB_004991
Strain containing pProm11_BCD20-GFP	JBEI-7350	JPUB_004992
Strain containing pProm11_BCD21-GFP	JBEI-7351	JPUB_004993
Plasmids and strains used for yeast assembly		
Plasmid	pProm1_BCD1_yeast	JPUB_004995
Plasmid	pProm1_BCD2_yeast	JPUB_004997
Plasmid	pProm1_BCD20_yeast	JPUB_004999
Plasmid	pProm1_BCD21_yeast	JPUB_005001
Plasmid	pProm2_BCD1_yeast	JPUB_005003
Plasmid	pProm2_BCD2_yeast	JPUB_005005
Plasmid	pProm2_BCD20_yeast	JPUB_005007
Plasmid	pProm2_BCD21_yeast	JPUB_005009
Plasmid	pProm9_BCD1_yeast	JPUB_005011
Plasmid	pProm9_BCD2_yeast	JPUB_005013
Plasmid	pProm9_BCD20_yeast	JPUB_005015
Plasmid	pProm9_BCD21_yeast	JPUB_005017
Plasmid	pProm11_BCD1_yeast	JPUB_005019
Plasmid	pProm11_BCD2_yeast	JPUB_005023
Plasmid	pProm11_BCD20_yeast	JPUB_005025
Plasmid	pProm11_BCD21_yeast	JPUB_005021
Strain containing pProm1_BCD1-yeast	JBEI-10697	JPUB_004994
Strain containing pProm1_BCD2-yeast	JBEI-10698	JPUB_004996
Strain containing pProm1_BCD20-yeast	JBEI-10695	JPUB_004998
Strain containing pProm1_BCD21-yeast	JBEI-10696	JPUB_005000
Strain containing pProm2_BCD1-yeast	JBEI-10702	JPUB_005002
Strain containing pProm2_BCD2-yeast	JBEI-10708	JPUB_005004
Strain containing pProm2_BCD20-yeast	JBEI-10709	JPUB_005006
Strain containing pProm2_BCD21-yeast	JBEI-10710	JPUB_005008
Strain containing pProm9_BCD1-yeast	JBEI-10707	JPUB_005010
Strain containing pProm9_BCD2-yeast	JBEI-10706	JPUB_005012
Strain containing pProm9_BCD20-yeast	JBEI-10705	JPUB_005014

Strain containing pProm9_BCD21-yeast	JBEI-10704	JPUB_005016
Strain containing pProm11_BCD1-yeast	JBEI-10703	JPUB_005018
Strain containing pProm11_BCD2-yeast	JBEI-10700	JPUB_005020
Strain containing pProm11_BCD20-yeast	JBEI-10699	JPUB_005024
Strain containing pProm11_BCD21-yeast	JBEI-10701	JPUB_005020

*To obtain strains and plasmids, go to the JBEI's public registry https://public-registry.jbei.org/login

Table S2. DNA oligos used for PCR

Name	Length (bp)	T_m (°C)	3' T_m (°C)	Sequence
Golden Gate assembly primers				
MS_02148_(Backbone_p4001)_forward	38	67.37	60.04	CACACCAGGTCTCAATAAGGATCGGTTGTCGAGTAAGG
MS_02149_(Backbone_p4001)_reverse	46	65.44	60.49	CACACCAGGTCTCATCTTACTTACTTAAGATCTTTTGAATTCG ACG
MS_02150_(P1)_forward	43	65.93	60.15	CACACCAGGTCTCAAAGAGTATACGTATATCGGCTAATAACGT
MS_02151_(P1)_reverse	43	67.06	59.43	CACACCAGGTCTCACCTCCACACATTATACCTATAGGTTAGAC
MS_02152_(BCD1-GFP)_forward	39	69.29	62.26	CACACCAGGTCTCAGAGGGCCCAAGTTCACTTAAAAAGG
MS_02153_(BCD1-GFP)_reverse	38	66.74	60.87	CACACCAGGTCTCATTATTTGTAGAGCTCATCCATGCC
MS_02154_(P2)_forward	50	66.25	62.84	CACACCAGGTCTCAAAGAGTATACGTATATCGGCTAATAACGT ATTAAGG
MS_02161_(P11)_reverse	36	74.47	60.11	CACACCAGGTCTCACCTCCACAAACACTAAGAGCCG
MS_02149_(Backbone_p4001)_reverse	46	65.44	60.49	CACACCAGGTCTCATCTTACTTACTTAAGATCTTTTGAATTCG ACG
MS_02150_(P1)_forward	43	65.93	60.15	CACACCAGGTCTCAAAGAGTATACGTATATCGGCTAATAACGT
Gibson assembly primers				
DVA00220_(vector_BB)_forward	45	70.08	69.144	TGAGCTCTACAAATAAGGATCGGTTGTCGAGTAAGGATCTCC AGG
DVA00221_(vector_BB)_reverse	53	66.31	65.198	AGCCGATATACGTATACTCTTACTTACTTAAGATCTTTTGAATT CGACGTCGG
DVA00222_(Prom1)_forward	57	65.28	64.538	TCAAAAGATCTTAAGTAAGTAAGAGTATACGTATATCGGCTAA TAACGTATTAAGGC
DVA00223_(Prom1)_reverse	55	64.07	63.612	TTTTAAGTGAACTTGGGCCCTCCACACATTATACCTATAGGTT AGACTTTAAGTC
DVA00224_(BCD1)_forward	29	68.75	62.259	TGTGGAGGGCCCAAGTTCACTTAAAAAGG
DVA00225_(BCD1)_reverse	37	70.78	60.869	CGACAACCGATCCTTATTTGTAGAGCTCATCCATGCC
DVA00226_(Prom2)_reverse	49	71.99	63.73	TTTTAAGTGAACTTGGGCCCTCCACACATTATAGGTACAAAAA GATGCG
DVA00227_(Prom9)_reverse	53	71.69	69.104	TTTTAAGTGAACTTGGGCCCTCCACACATTATACGAGCCGAT GATTAAGAGGC
DVA00228_(Prom11)_reverse	41	75.83	62.461	TTTTAAGTGAACTTGGGCCCTCCACAAACACTAAGAGCCGG
Yeast assembly primers				
DVA00321_(vector_Bb)_forward	48	70.17	69.744	GATGAGCTCTACAAATAAGTGAGCGCGCGTAATACGACTCAC TATAGG
DVA00322_(vector_Bb)_reverse	37	74.82	69.399	GCCGATATACGTATACTCATGGCGAATGGCGCGACGC
DVA00323_(Prom1)_forward	53	65.43	64.538	CGTCGCGCCATTCGCCATGAGTATACGTATATCGGCTAATAA CGTATTAAGGC
DVA00324_(Prom1)_reverse	65	64.07	63.612	TTGATCTCCTTTTTAAGTGAACTTGGGCCCTCCACACATTATA CCTATAGGTTAGACTTTAAGTC
DVA00224_(BCD1)_forward	29	68.75	62.259	TGTGGAGGGCCCAAGTTCACTTAAAAAGG
DVA00325_(BCD1)_reverse	42	75.02	60.869	CGTATTACGCGCGCTCACTTATTTGTAGAGCTCATCCATGCC
DVA00326_(Prom2)_reverse	59	71.99	63.73	TTGATCTCCTTTTTAAGTGAACTTGGGCCCTCCACACATTATA GGTACAAAAAGATGCG
DVA00327_(Prom9)_reverse	63	71.69	69.104	TTGATCTCCTTTTTAAGTGAACTTGGGCCCTCCACACATTATA CGAGCCGATGATTAAGAGGC
DVA00328_(Prom11)_reverse	51	75.83	62.461	TTGATCTCCTTTTTAAGTGAACTTGGGCCCTCCACAAACACTA AGAGCCGG

Table S3. DNA oligos used for sequencing

Name	Length (bp)	T_m (°C)	3' T_m (°C)	Sequence
QB3284_fwd	24	56.5	56.5	CGATCCTCATCCTGTCTCTTGATC
QB3810_rev	19	57.9	57.9	CGAGCGTAGCGAGTCAGTG
yeast_fwd	20	57.1	57.1	AGAACGTGGACTCCAACGTC
yeast_rev	20	57.2	57.2	CGGCCGCTCTAGAACTAGTG

Protocol to prepare electrocompetent yeast cells

Before starting, prepare the following:

**Make YPD/0.02M HEPES solution and 1.0M dithiothreitol solution.
**Ensure all solutions are chilled or 4°C before use unless stated otherwise.
**Set incubator temperature for 30°C.

1. Streak yeast cells on an YPD-agar plate and incubate at 30°C overnight.
2. Pick one colony and place it in 5 mL YPD media at 30°C. Grow overnight.
3. Obtain an aliquot from the overnight culture and place it in fresh YPD to a final volume of 50 mL. Ensure starting O.D. is 0.25 (600 nm)
4. Grow the cells to O.D. 0.7-0.8. (~4-5 h)
5. Add another 50 mL of YPD media and grow it for an addition 1 h at 30°C. Incubate longer if O.D. is not between 0.7-0.8 O.D.
6. Centrifuge the cells at 4000g for 10 min in 50 mL falcon tubes.
7. Discard supernatant. Add 10 mL of YPD/0.02M HEPES and then 250 μL of 1.0M dithiothreitol (DTT) into each falcon tube.
8. Incubate for 15 min with shaking at 85 rpm 30°C.
9. Add room temperature water to a final volume of 50 mL.
10. Centrifuge cells for 10 min at 4000g.
11. Discard supernatant. Add 50 mL of water. Spin at 4000g for 10 min.
12. Discard supernatant. Add 25 mL of water. Spin at 4000g for 10 min.
13. Discard supernatant. Add 10 mL of 1.0M sorbitol. Spin at 4000g for 10 min.
14. Discard supernatant. Add 5 mL of 1.0M sorbitol. Spin at 4000g for 10 min.
15. Add 2mL of 1.0M sorbitol and aliquot 40 μL into 1.5mL tubes.
16. Place in -80°C freezer immediately until needed for transformation.

Statistical analysis for cross-contamination study

If x is the probability of obtaining an incorrect assembled plasmid then the probabilities for correctly and incorrectly assembling the plasmids are as follows:

$P_{wrong} = x$
$P_{right} = 1 - x$

For our experiment, we picked 30 clones and all of them were showing the correct plasmid.

Hence, we would like to determine what is the worst-case scenario for failure rate such that we would have had at least a 95% chance of observing at least one incorrect clone.

Given the failure rate x, the probability of observing at least one out of N colonies is mathematically,

$1-(1-x)^N \geq 0.95$ (This is showing 100% minus the chance that ALL of them are correct)

Rearranging and solving for 'x'…

$(1-x)^N \geq 0.05$

$(1-x)^{30} \geq 0.05$ (Since we picked 30 clones, N=30)

$1-x \geq (0.05)^{1/30}$

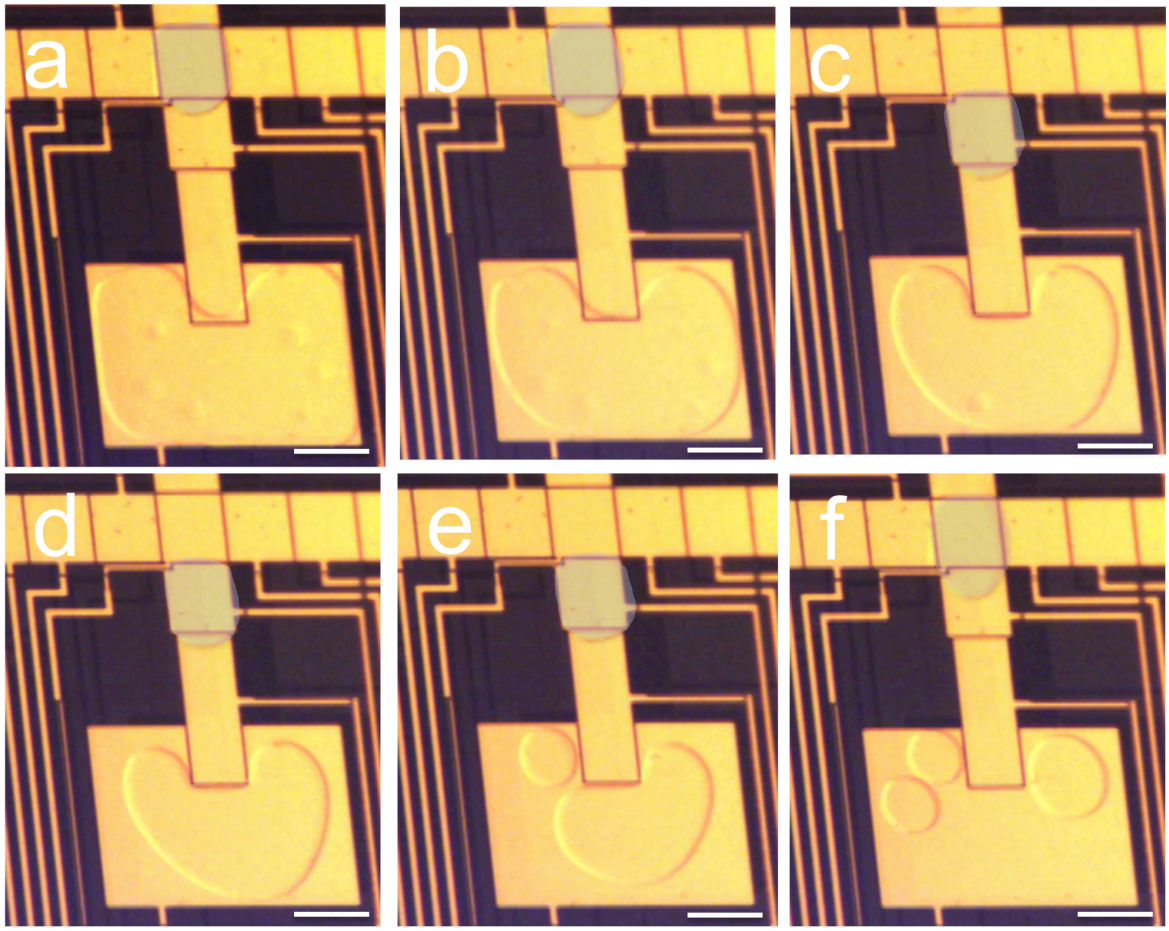
$-x \geq (0.05)^{1/30}-1$

$x \leq 1- (0.05)^{1/30}$

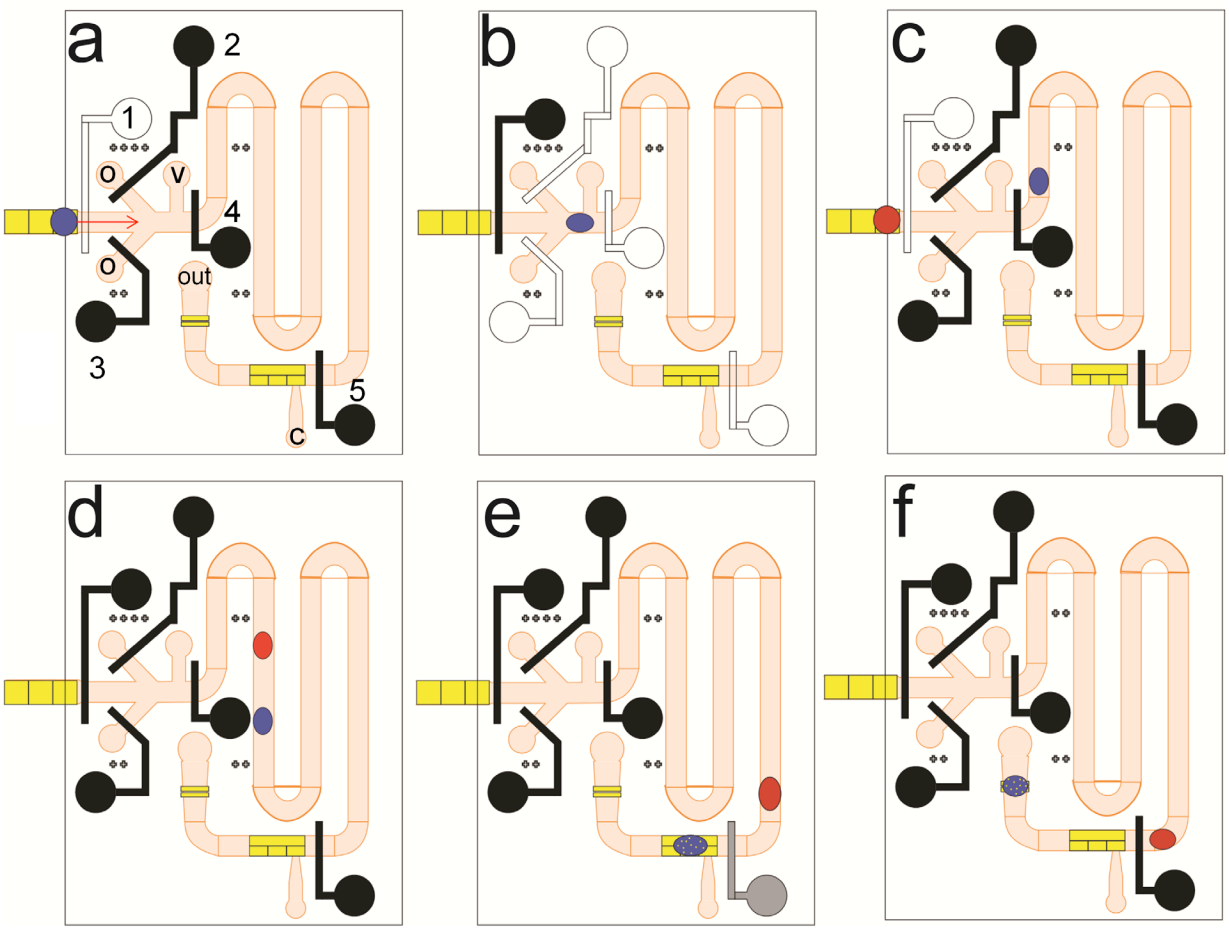
$x \leq 0.095$

$x \approx 9.5\%$ (worst-case scenario)

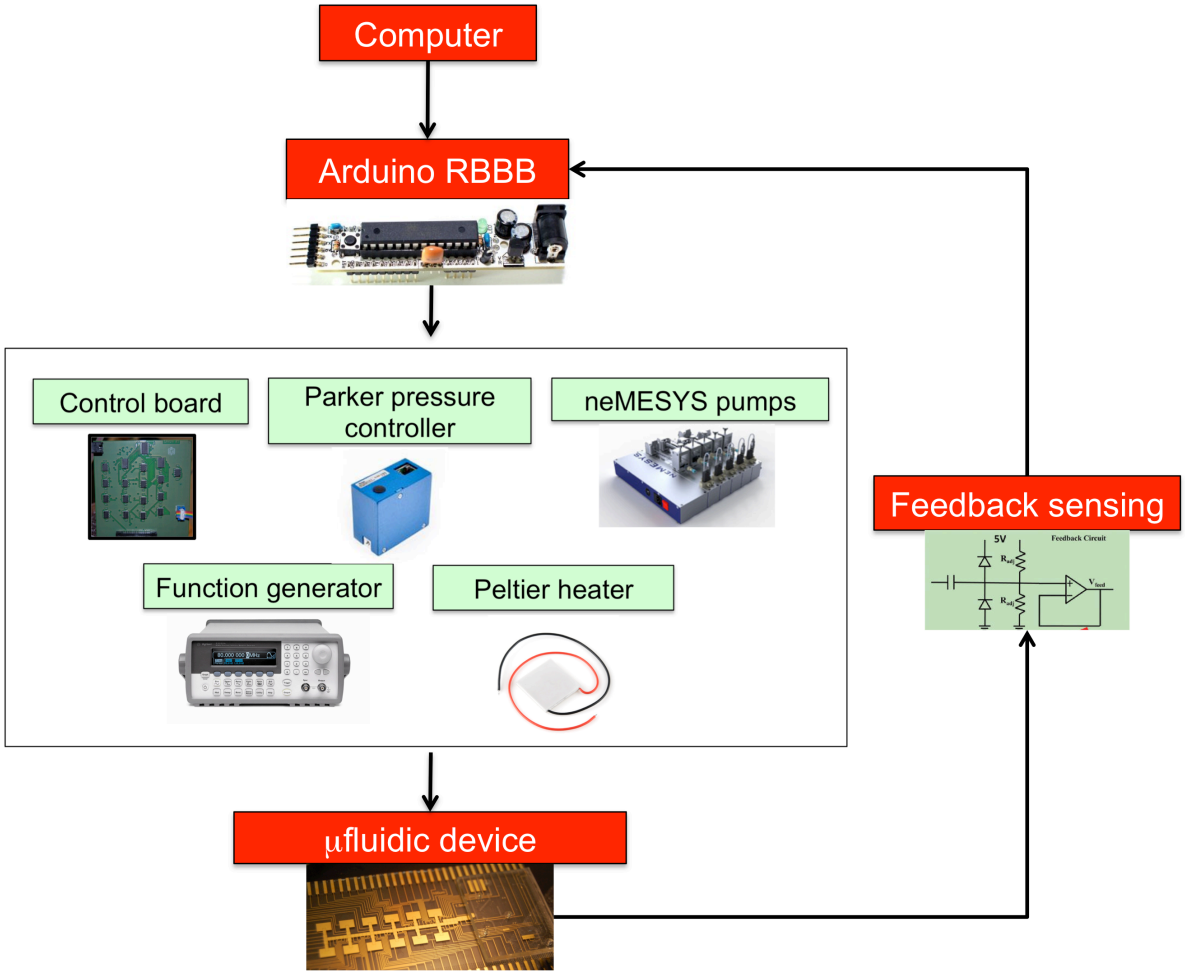
Since we did not see an incorrect colony, we are 95% confident that the error rate is less than 9.5%.



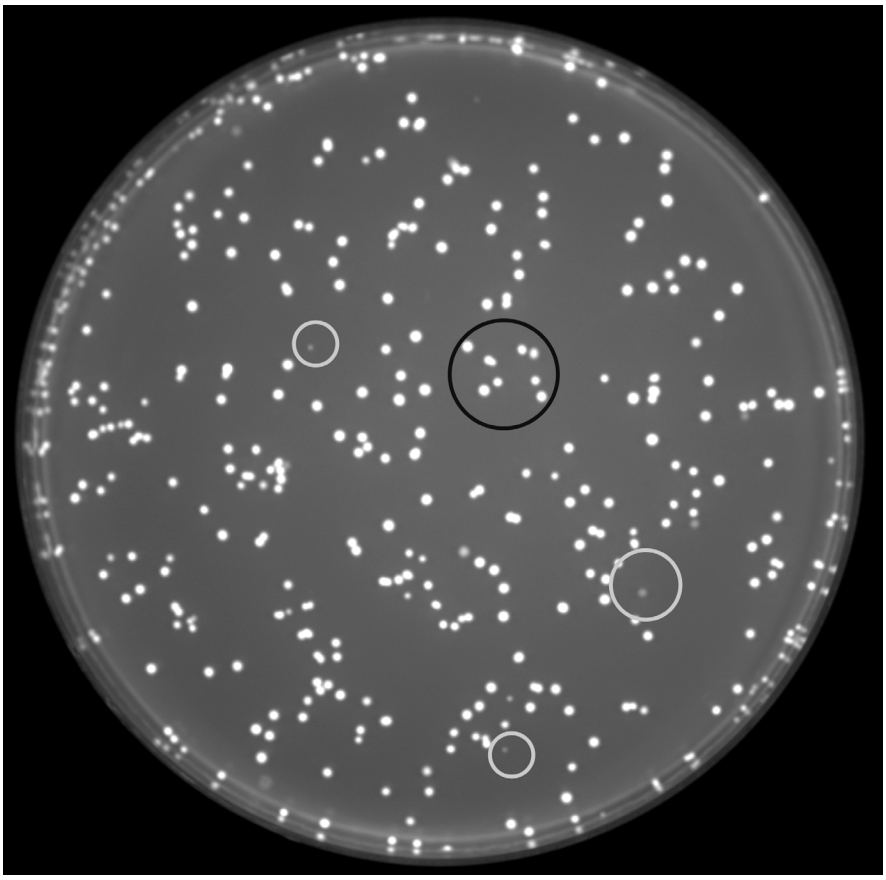
Supplementary Figure 1: *Volumetric dispensing reproducibility.* Frames from a movie (a-f) depicting six successive dispensed droplets (shown in faint blue). The area of each dispensed droplet was calculated by ImageJ (a - 1.391, b – 1.384, c – 1.371, d – 1.422, e – 1.369, f - 1.411 mm^2). These areas were used to approximate the droplet volumes (volume in μL = area of dispensed droplet * gap height of device). We obtained volumes that were reproducible (~1.5% precision deviation). Scale bar = 1 mm.



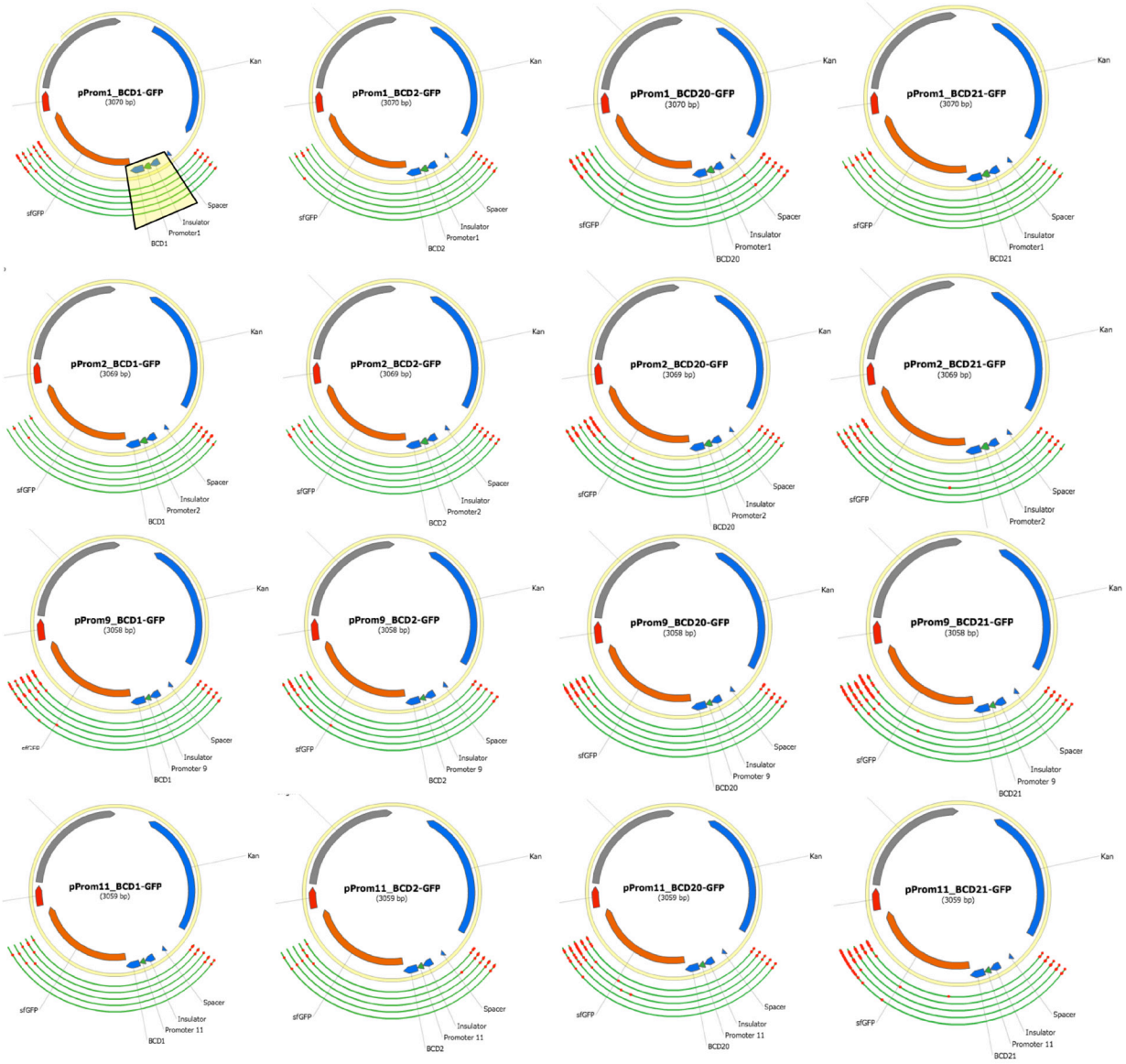
Supplementary Figure 2: *Valve operation*. Schematics (a-f) show the valve operations required for DNA assembly and electroporation. In (a), the mixed droplet containing DNA fragments (shown in blue) is ready to be suctioned into the channel by inlet V (vacuum). Valve 1 is turned on (showed in white) while all others are turned off (shown in black). Next (b), the droplet is driven to the incubation channel by the oil inlet (O). Valve 1 is now closed while valves 2-5 open. (c) This process is repeated for other droplets (show only one red droplet for clarity). (d) The droplets incubate inside the channel and all valves close. After incubation, (e) valve 5 opens for the blue droplet to actuate to the electrode and quickly turns off (shown in grey). The blue droplet is driven to the electrodes by syringe-vacuum from the outlet and is mixed with a droplet of cells from the inlet C. Valves 1-5 close to prevent red droplet coalescence with the blue droplet. (f) To electroporate the cells, the blue droplet is driven to the microelectrodes by replacing the inlet containing cells with an oil phase. With all valves closed, the oil phase will flow towards the outlet driving the electroporated droplet to a tube with media or water. This process (e-f) is repeated for other droplets.



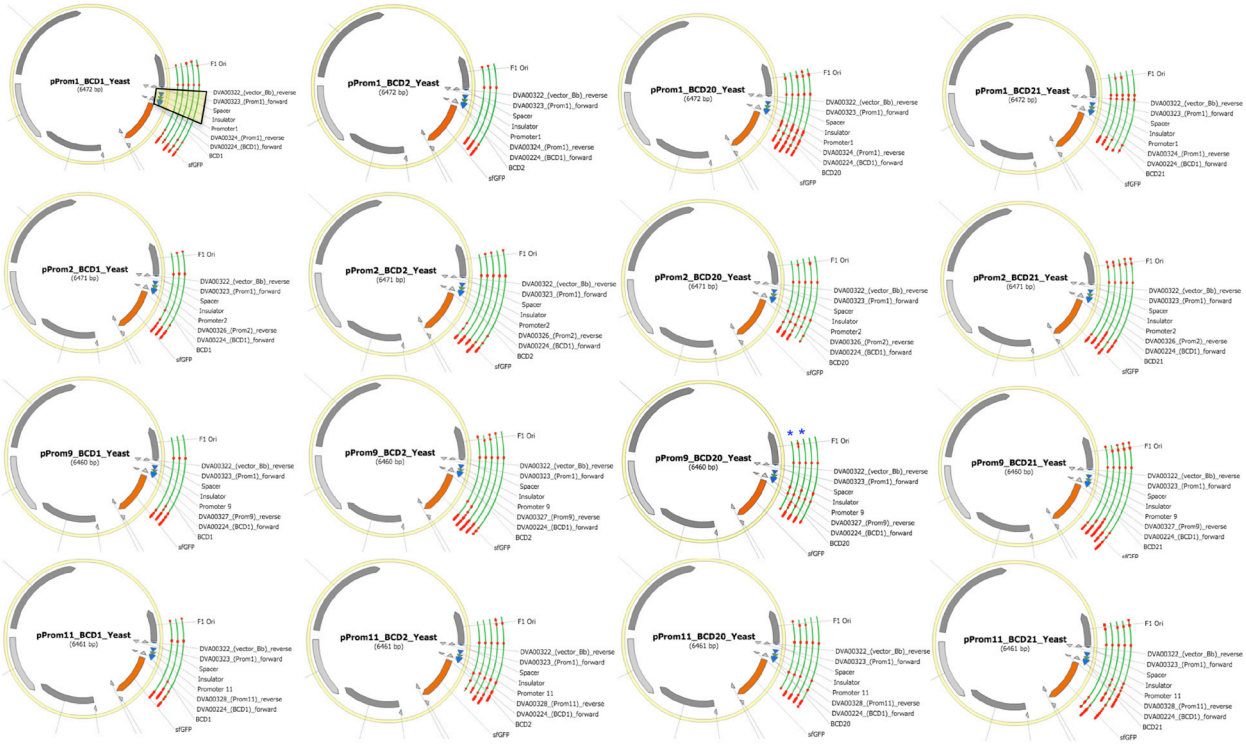
Supplementary Figure 3. *Microfluidic automation system for synthetic biology*. A computer is connected to the Arduino Really Bare Bones Board (RBBB). This microcontroller is connected to different hardware components: 1) the control board which actuates the droplets on the microfluidic device (see (*1-3*) for more details), 2) the function generator to control the applied voltage and frequency, 3) Parker pressure controllers to actuate valves, 4) neMESYS pumps to control the vacuum and the oil flow rate, and 5) a peltier heater to turn on the temperature to anneal the DNA parts for Gibson assembly. Droplet position on the microfluidic device is detected by the feedback sensing circuit and a decision is made by the Arduino to continue actuating the activated electrode (if movement was not successful) or to start the next step in the sequence (if movement is successful).



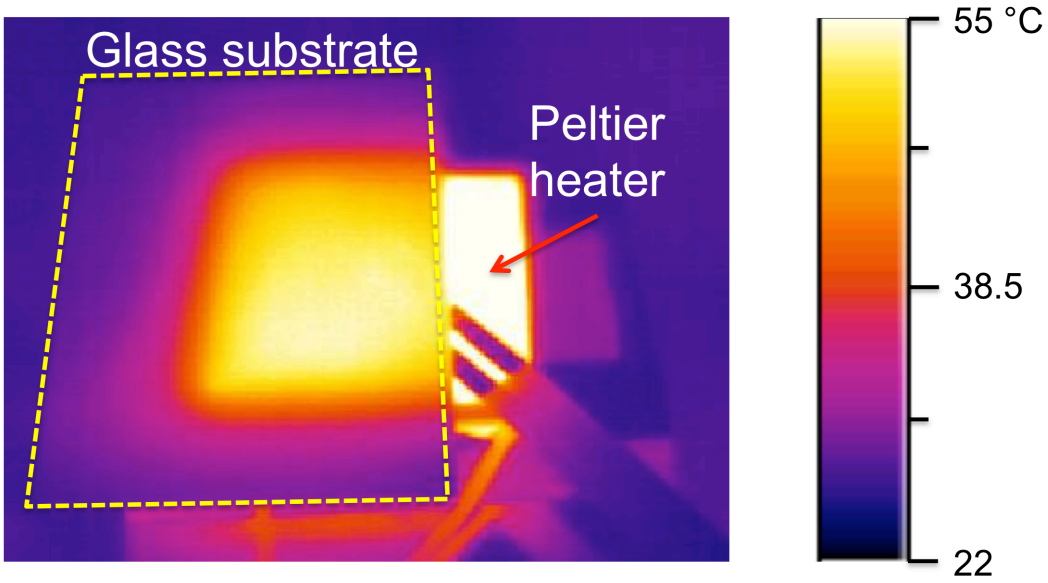
Supplementary Figure 4. *Grayscale image of the colonies after DNA assembly and electroporation.* The dark circle represents colonies that fluoresce green under blue excitation (~95% of the colonies). Light gray circles show colonies that are white which represent colonies that did not uptake the correct plasmid.



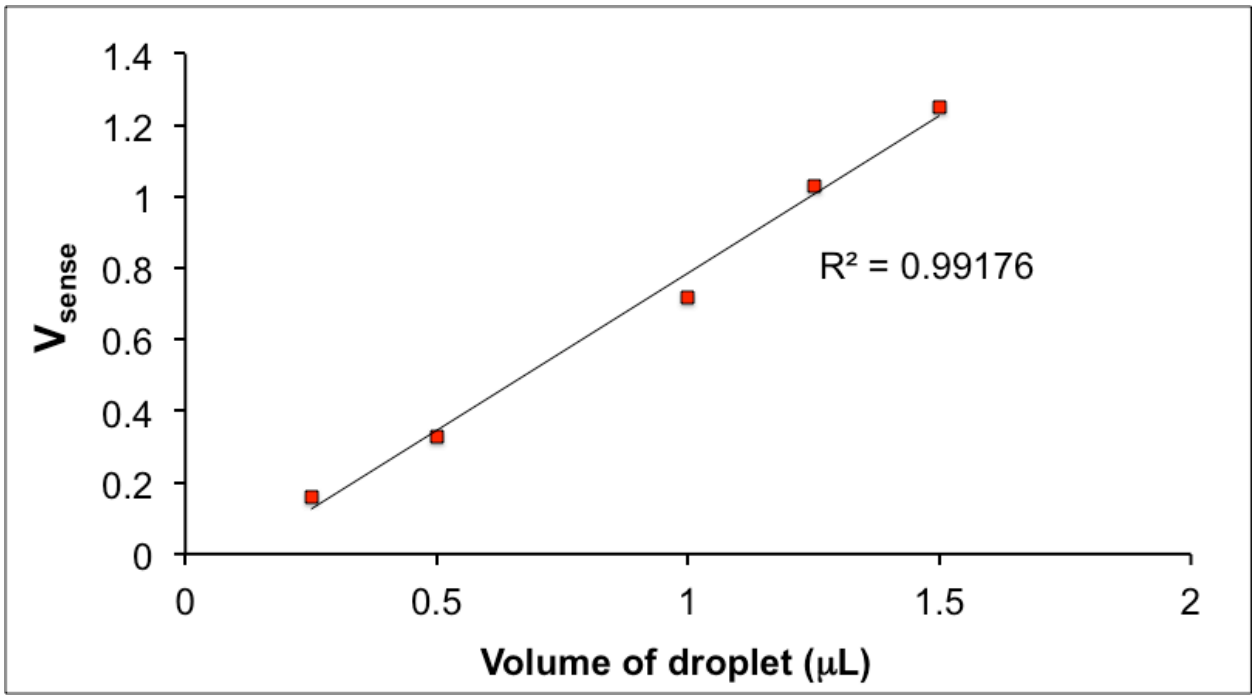
Supplementary Figure 5. *Sequencing results for Gibson assembly.* A library of 16 DNA plasmids was created using our microfluidic platform by Gibson assembly. Colonies were picked following the protocol for Golden Gate assembly. The green portions of the sequencing arcs show regions where the sequencing results matches the expected sequence while the red portions of the sequencing arcs show regions where the sequencing result does not matches the expected sequence.(*4*) The assembly region spanning the BCD, insulator, Promoter (Prom), and neighboring portions of the vector backbone showed perfect sequence matching (highlighted by a yellow box). The mutations in the *gfp* gene and spacer regions are not included in the calculation.



Supplementary Figure 6. *Sequencing results for yeast assembly.* A library of 16 DNA plasmids was created using our microfluidic platform by yeast assembly (containing TRP and AMP selection markers). Three yeast colonies were picked from CSM ΔTRP agar plates. After mini-prep and transformation in Turbo *E. coli*, five *E. coli* colonies were chosen for analysis. The green portions of the sequencing arcs show regions where the sequencing results matches the expected sequence while the red portions of the sequencing arcs show regions where the sequencing result does not matches the expected sequence.(*4*) The assembly region spanning the BCD, insulator, Promoter (Prom), and neighboring portions of the vector backbone showed high sequence matching (~ 97.2%; highlighted by a yellow box). The blue asterisk symbol beside the sequencing arc represents incorrect clones and is considered as a failed assembly.



Supplementary Figure 7. *Infrared image of a glass substrate showing the region-specific temperature zone (50 °C).* A glass substrate (the same as our devices) was placed on a peltier heater to heat our incubation region to 50 °C for Gibson assembly.



Supplementary Figure 8. *A plot showing the linearity between the output sensing voltage (V_{sense}) and droplet volume.* Water droplets of different volumes were manually pipetted onto a reservoir and V_{sense} values were recorded by applying potential to the reservoir electrode.

References

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