An Automated Induction Microfluidics System (AIMS) for Synthetic Biology

Supplementary Information

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Fabrication of the 3D enclosure

The 3D printed enclosure that includes our LED and sensor with integrated pogo pins for delivering electric potential (Fig. S1) was designed using the Fusion 360 software from Autodesk (file included in the GitHub repository – shihmicrolab/AIMS/AIMS3D.zip) and produced by 3D printing using an Ultimaker 2 Extended+ (3DShop, Mississauga, ON, Canada). To print the design, the 3D drawing file was converted to a STL file. Using Cura (software incl. with the Ultimaker), the STL file was opened and a 3D model of the setup appears. In the parameters settings, the printer was selected to Ultimaker 2 Extended+, nozzle – 0.4 mm, material – PLA, and profile - high quality. In the print setup settings, the recommended button was checked, infill checked as Dense and helper parts checked as Print Build Plate Adhesion and Print Support Structure. Once completed, the converted STL file was saved on a SD card as a G-code file. Before printing, glue has been applied on the build-plate to ensure adhesion of the PLA material. The storage card is then inserted into the 3D printer and the setup is then selected for printing using the interactive monitor.

Table S1 – Strains and plasmids used in this study

Strains	**Genotype**	**Source**
E. coli DH5α	fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17	V. Martin
E. coli BL21(DE3)	F– ompT gal dcm lon hsdSB(rB- mB-) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])	V. Martin
Plasmids	**Relevant characteristics**	**Source**
pET16b	AmpR, pBR322 origin	V. Martin
pET_RFP	AmpR, mRFP	This study
pET_BGL1	AmpR, BGL1	This study
pET_BGL2	AmpR, BGL2	This study
pET_BGL3	AmpR, BGL3	This study

Table S2 – Primer Sequences

Gene	**Orientation**	**Sequence**
RFP	Forward	TGACTGACTCTAGAAATAATTTTGTTTAACTTTAAGAAGGA GATATACCATGGCTTCCTCCGAAGACGT
RFP	Reverse	GCATGCATGGATCCTTAAGCACCGGTGGAGTGAC
BGL1	Forward	TGACTGACTCTAGAAATAATTTTGTTTAACTTTAAGAAGGA GATATACCATGAAACATCTGGTTACCACACTGC
BGL1	Reverse	GCATGCATGGATCCCTACTGAACATCAATTTCGGTCTGCA
BGL2	Forward	TGACTGACTCTAGAAATAATTTTGTTTAACTTTAAGAAGGA GATATACCATGGACCCGTATGAAGATCCGC
BGL2	Reverse	GCATGCATGGATCCCTACAGGGTCAGACCATGACCG
BGL3	Forward	TGACTGACTCTAGAAATAATTTTGTTTAACTTTAAGAAGGA GATATACCATGAGCGTAGCGCGGTTT
BGL3	Reverse	GCATGCATGGATCCCTAACCTTCCACCAAAGCATTTCTTG

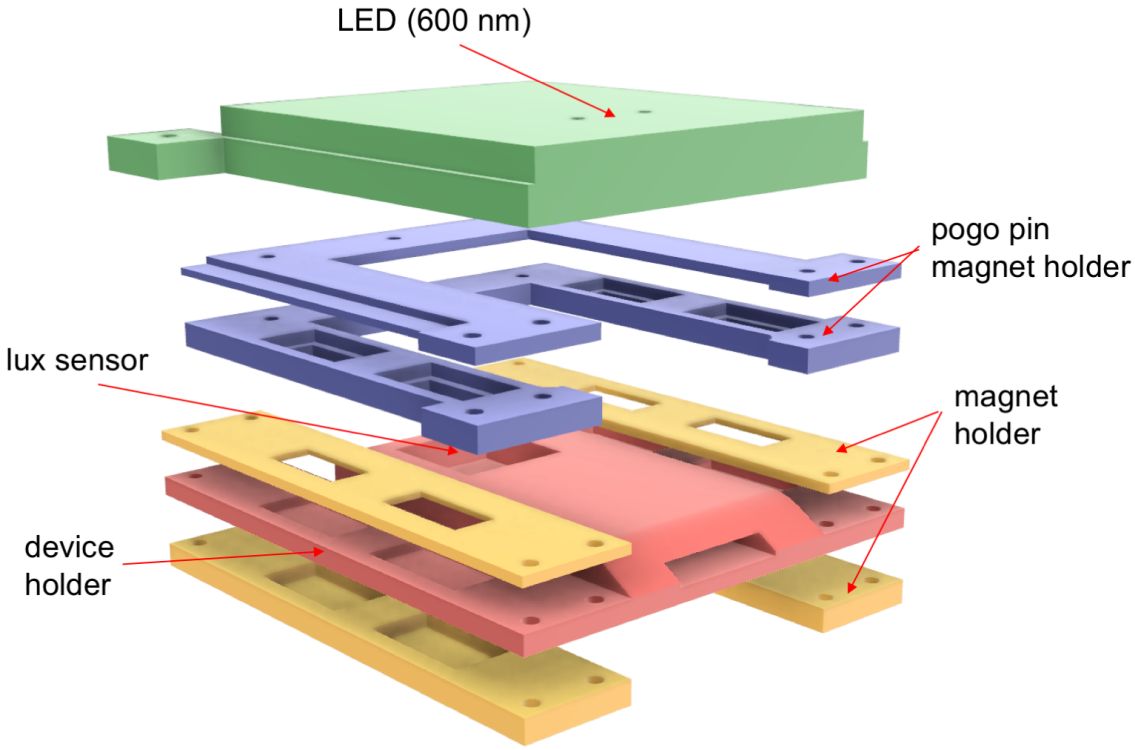

Fig. S1. – Fabrication of the 3D enclosure for the AIMS. It consists of four layers (top to bottom): Layer 1 (shown in green) to hold the LED, Layer 2 (shown in blue) is to support the pogo pin board that will apply electric potentials to the device, Layer 3 (shown in orange) is used to support the device in place and Layer 4 (shown in red) is to position the sensor directly below the device. The .stl file is included in the supplementary information which is available for download.

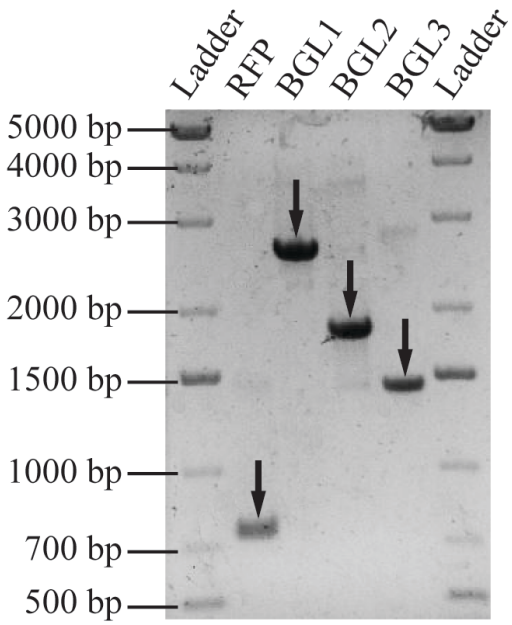

Fig. S2 – Gel electrophoresis of the PCR products derived from amplification of the pET16b vector containing the synthetic inserts *RFP, BGL1, BGL2* and *BGL3*. Arrows show the bands with the expected weight for each PCR products, which were 678 bp (RFP), 2520 bp (BGL1), 1761 bp (BGL2), and 1359 bp (BGL3).

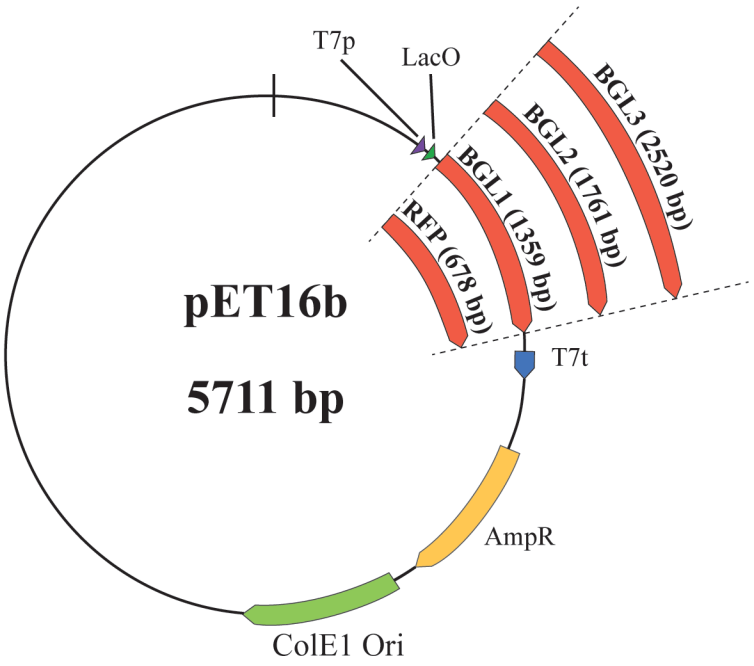

Fig. S3 – A schematic of the plasmid used in this study. BGL and RFP were inserted downstream of a T7 promoter.

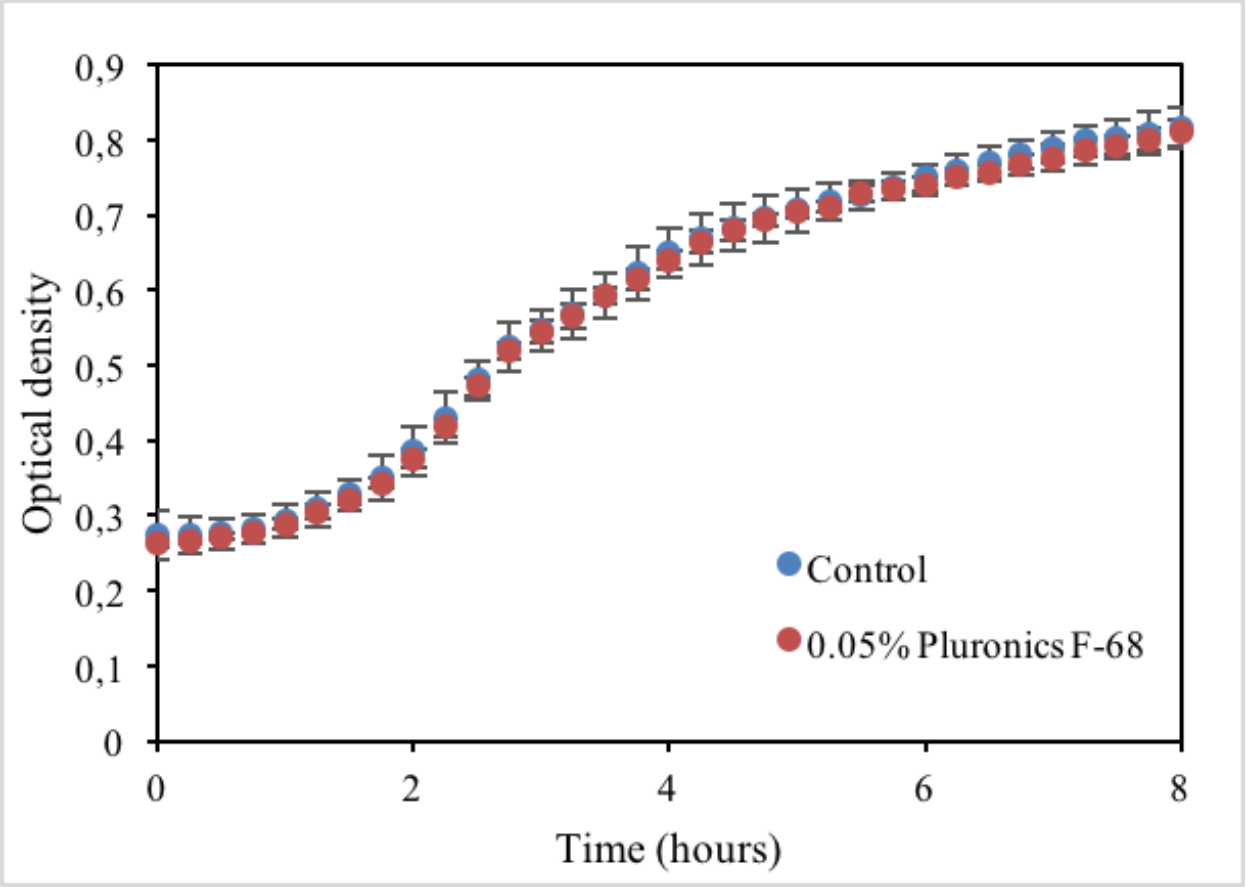

Fig. S4 – A growth curve for BL21 *E.coli* cultured under normal culturing conditions with (red) and without (blue) 0.05% Pluronics F-68

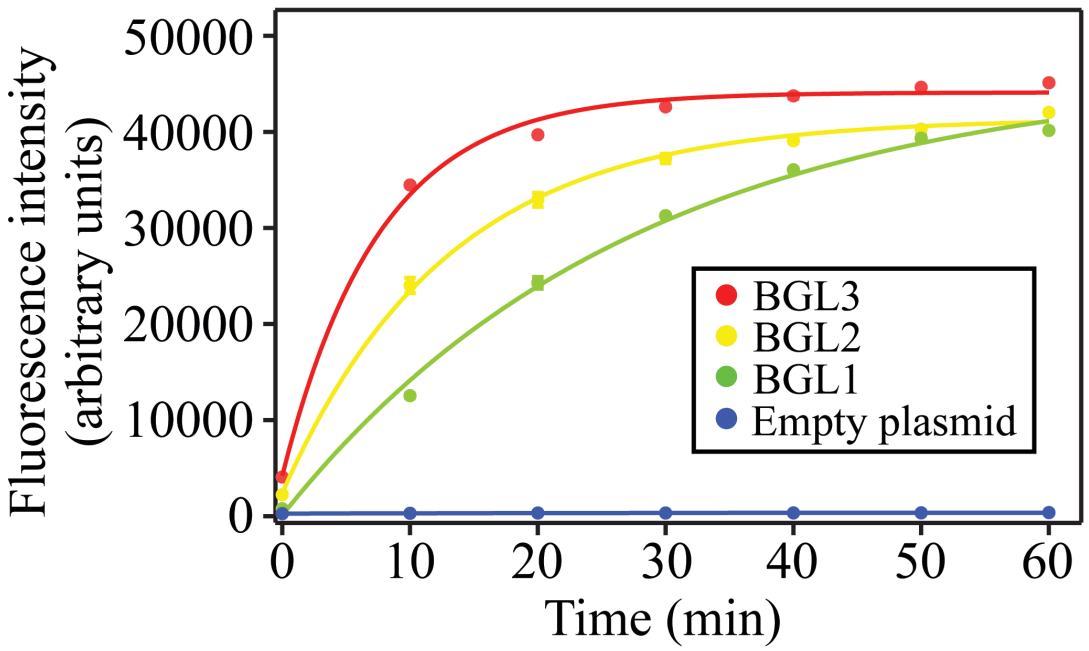

Fig. S5 – Expression optimization assay to discover highly active BGL conducted in well-plates. The activity of three different BGLs in the presence of 2 mM MUG were measured by fluorescence intensity (λ_{ex} = 369 nm and λ_{em} = 449 nm) over 60 min.

Table S3 - Electronic components required for manfacturing the control system

Symbols	Item	Description	Ref Des	Qty/ board	Symbols on the board	Manufacturer Part Number	Manufacturer	BOARDS
R	1	4.7k Ohm ±5% 0.063W, 1/16W Chip Resistor 0402 (1005 Metric) Moisture Resistant Thick Film	R1, R2, R47, R48	4	R1,R2,R47,R48	RC0402JR-074K7L	Yageo	CONTROL BOARD
R	2	39k Ohm ±1% 0.063W, 1/16W Chip Resistor 0402 (1005 Metric) Moisture Resistant Thick Film	R3, R49	2	R3, R49	RC0402FR-0739KL	Yageo	CONTROL BOARD
R	3	220 Ohm ±5% 0.063W, 1/16W Chip Resistor 0402 (1005 Metric) Moisture Resistant Thick Film	R17, R18, R19, R20, R21, R22, R23, R24, R25, R26, R27, R28, R29, R30, R31, R32, R33, R34, R35, R36, R63, R64, R65, R66, R67, R68, R69, R70, R71, R72, R73, R74, R75, R76, R77, R78, R79, R80, R81, R82	40	[R17-R36] , [R63-R82]	RC0402JR-07220RL	Yageo	CONTROL BOARD
R	4	1k Ohm ±0.5% 0.063W, 1/16W Chip Resistor 0402 (1005 Metric) Moisture Resistant Thick Film	R4, R5, R6, R7, R8, R9, R10, R11, R12, R13, R37, R38, R39, R40, R41, R42, R43, R44, R45, R46, R50, R51, R52, R53, R54, R55, R56, R57, R58, R59, R83, R84, R85, R86, R87, R88, R89, R90, R91, R92	40	[R4 - R16] , [R37 - R46] , [R50 - R62] , [R83 - R92]	RT0402DRD071KL	Yageo	CONTROL BOARD
C	5	CAP CER 0.1UF 16V X7R 0402	C1, C2, C3, C4, C5, C6, C7, C8	8	[C1 - C 8]	CC0402KRX7R7BB104	Yageo	CONTROL BOARD
M	6	I/O Expander 28 I²C 400kHz 28-SSOP	M1,MX2	2	MAX7300	MAX7300AAI+	Maxim Integrated	CONTROL BOARD
I	7	Buffer, Inverting Element 8 Bit per Element Push-Pull Output 20-SOIC	I1, I2, I3, I4, I5, I6	6	[I1 - I6]	74AC540SC	Fairchild/ON Semiconductor	CONTROL BOARD
U	8	RELAY OPTO 600V 0.04A 8SMD	U1, U2, U3, U4, U5, U6, U7, U8, U9, U10, U11, U12, U13, U14, U15, U16, U17, U18, U19, U20, U21, U22, U23, U24, U25, U26, U27, U28, U29, U30, U31, U32, U33, U34, U35, U36, U37, U38, U39, U40	40	[U1 - U40]	AQW216EHA	Panasonic Electric Works	CONTROL BOARD
AD	9	CONN HEADER 8POS .100" VERT TIN	AD0, AD0., AD1, AD1.	3	AD0 , AD0. , AD1 , AD1.	15912080	Molex, LLC	CONTROL BOARD
Header	10	STACKING HEADER ARDUINO SHIELD	HEADER	1	Header	85	Adafruit Industries LLC	CONTROL BOARD
H1	11	CONN HEADER RT/A 40POS .100 GOLD	H1	1	H1	5499913-9	TE Connectivity AMP Connectors	CONTROL BOARD
CONX	1	CONN HEADER RT/A 40POS .100 GOLD	CONX1, CONX2, CONX3	3	CONX1 , CONX2 , CONX3	5499913-9	TE Connectivity AMP Connectors	POGO PIN BOARD
N/A	2	CONN PIN SPRING-LOADED PCB GOLD	U$1	104		0906-1-15-20-75-14-11-0	Mill-Max Manufacturing Corp.	POGO PIN BOARD

The breakdown for 5 different conditions from this work:

On-device:
20 µL of starting culture on device → **LB** at \$7.5/L → \$0.00015
2 µL of 11mM **IPTG** on reservoir at \$32/g --> $5.3x10^{-6}$ g → \$0.000169
15 µL total → 6 mM **MUG** at \$400/g → $30x10^{-6}$ g → \$0.012
Device substrates = \$4.50
Total: 0.00015+0.000169+0.012+4.50 = **\$4.51**

Macro-scale:
150 mL **LB** at \$7.5/L → \$1.125
150 µL 1M **IPTG** at \$32/g → 36 mg → \$1.152
5 x 90 µL per well = 450 uL total; 4 mM **MUG** at \$400/g → 0.609 mg → \$0.24
Well-plates = \$5.50
Total: 1.125+1.152+0.24+\$5.50 = **\$8.02**

An estimated breakdown for 100 different conditions for potential scale-up:

On-device:
20 µL per reservoir * 4 cultures * 5 refills to the reservoir → 400 µL **LB** at \$7.5/L → \$0.003
15 µL to dispense 4 droplets →14.3 mM **IPTG** at \$32/g → $5.11x10^{-5}$ g → \$0.00164
100 conditions x 1.5 µL= 150 µL at 6mM **MUG** at \$400/g → $3x10^{-4}$ g → \$0.12
For the additive, we assume a similar price (and same volumes) as MUG → \$0.12
Device substrates = \$4.50
Total: 0.003+0.00164+0.12+0.12+4.50 = **\$4.74**

Macro-scale:
4 cultures x 150 mL = 600 mL **LB** at \$7.5/L → \$4.5
~6 µL per well * 100 conditions = 600 µL 1M **IPTG** at \$32/g → 144 mg → \$4.608
100 conditions x 90 µL = 9 mL 4 mM **MUG** at \$400/g → 12.186 mg → \$4.86
For the additive, we assume a similar price (and same volumes) as MUG → \$4.86
Well-plates = \$5.50
Total: 4.5+4.608+4.86+4.86+5.50 = **\$24.33**

An estimated breakdown for 1000 different conditions for potential scale-up:

On-device – we will require 10 devices:
10 devices x 400 µL each device = 4 mL **LB** at \$7.5/L → \$0.03
10 devices x 15 µL/device = 150 µL 1M **IPTG** at \$32/g → $5.11x10^{-4}$ g → \$0.0164
1000 conditions x 1.5 µL = 1.5 mL 6mM **MUG** at \$400/g --> $3x10^{-3}$ g --> \$1.2
For the additive, we assume a similar price (and volumes) as MUG → \$1.2
Device substrates = \$4.50 *10 = \$45
Total: 0.03+0.0164+1.2+1.2+0.45 = **\$47.45**

Macro-scale:
4 cultures x 150 mL = 600 mL **LB** at $7.5/L → $4.5
~6 μL per well * 1000 conditions = 6000 μL 1M **IPTG** at $32/g → 1.44 g → $46.08
1000 conditions x 90 μL per well = 90 mL 4 mM **MUG** at $400/g --> 121.86 mg --> $48.6
For the additive, we assume a similar price (and volumes) as MUG → $48.6
Well-plates = $5.50*11 = $60.5
Total: 45+46.08+48.6+48.6+60.5 = **$248.78**

A summary of each step is shown below for testing 5 conditions:

Macro-scale:

-For the preparation of the starter culture, an overnight culture of the transformed E.coli BL21(DE3) cells in LB Amp was diluted to OD 0.1 in 150 mL of fresh media (2 min; 1 pipetting step per flask).

-Frequent OD readings were taken to monitor growth and involved taking a 1mL sample of the culture and measuring OD against a blank of LB at 600nm (10 min; 1 pipetting step per reading and 1 for the blank).

-Induction was carried out by adding 150 μL of 1M IPTG to the culture flask (0.5 min; 1 pipetting step per flask)

-The induced culture was sampled at different times after induction by removing 1 mL samples from the growing flask and check OD (10 min; 5 pipetting steps per flask).

-Lysis was done by adding 1 mL of lysis solution to each sample and leaving at room temperature for 15 min (2 min of hands-on time; 1 pipetting step per sample).

-The assay was started by adding 50 μL of lysate and 130 μL of substrate solution to individual wells of a 96-well plate (10 min; 2 pipetting step per sample). It was stopped by the addition of 20 μL of stop solution (1 pipetting step per sample).

AIMS:

-Prepare device - washing with EtOH and drying (10 min).

-For the preparation of the starter culture, an overnight culture of the transformed E.coli BL21(DE3) cells in LB Amp was diluted to OD 0.1 in 1mL of fresh media with 0.05% Pluronics F-68 (1 min; 3 pipetting steps).

-Before starting the experiment, a droplet of starter culture, LB and IPTG were pipetted onto the device (1 min; 3 pipetting steps).

-All subsequent OD readings and sampling of the induced culture are automated and do not require pipetting. (5 min to setup software)

-In preparation for the assay, a droplet of lysis solution, substrate solution and stop solution were pipetted onto the device and actuated to their reservoirs (1 min; 3 pipetting steps).

-All mixing steps for the assay are automated and do not require manual pipetting steps.

For 100 conditions, we estimated for 4 different cultures that are interrogated with 5 different IPTG concentrations and 5 additive concentrations for the macro- and micro-scale. For the macroscale, cultures were started in flasks and then aliquoted into 96 well-plates. For the chip, the culture, buffers for dilutions, lysis, substrate, and stop solutions required refilling of the reservoir, hence the higher number of pipetting steps.

For 1000 conditions, we estimated for 4 different cultures that are interrogated with 5 different IPTG concentrations and 50 additive concentrations. Pipetting steps were scaled linearly from 100 conditions while hands-on time are generally 3x more while the chip has been scaled linearly.

More information is given in the next page.

5 conditions on 1 chip

Macro-scale			AIMS		
Step	*Solution*	*Pipetting required*	*Step*	*Solution*	*Pipetting required*
Culture preparation	Overnight culture	1		LB media	1
OD readings	LB blank	1	Culture preparation	Overnight culture	1
	4 OD readings	4		Pluronics	1
Induction	IPTG	1		Diluted culture	1
Sampling the induced culture	Culture aliquots	5	Setup on device	LB blank	1
Lysing each aliquot	Lysis solution	5		IPTG	1
	Transfer to 5 wells	5		Lysis buffer	1
Assay in 96-well plate	Substrate solution	5	Adding assay reagents	Substrate solution	1
	Stop solution	5		Stop solution	1
Total		**32**	**Total**		**9**

100 conditions on 1 chip

Macro-scale			AIMS		
Step	*Solution*	*Pipetting required*	*Step*	*Solution*	*Pipetting required*
Culture preparation	Overnight culture	4		LB media	4
OD readings	LB blank	1	Culture preparation	Overnight culture	4
	4 OD readings per flask	16		Pluronics	4
	Aliquot culture into 96-plate	100	Setup on device	Diluted culture	20
5 induction concentrations (for each culture)	Adding to plate	100		LB blank	1
5 additive concentrations (for each culture)	Adding to plate	100		IPTG	1
Lysing each aliquot	Lysis solution	100		Additive	1
Assay in 96-well plate	Substrate solution	100		Buffer for dilutions	40
	Stop solution	100	Adding assay reagents	Lysis buffer	20
				Substrate solution	20
				Stop solution	20
Total		**621**	**Total**		**135**

1000 conditions on 10 chips

Macro-scale			AIMS		
Step	*Solution*	*Pipetting required*	*Step*	*Solution*	*Pipetting required*
Culture preparation	Overnight culture	4	Culture preparation	LB media	4
OD readings	LB blank	1		Overnight culture	4
	4 OD readings per flask	16		Pluronics	4
	Aliquot culture into 96-plate	1000	Setup on device	Diluted culture	200
5 induction concentrations (for each culture)	Adding to plate	1000		LB blank	1
50 additive concentrations (for each culture)	Adding to plate	1000		IPTG	1
Lysing each aliquot	Lysis solution	1000		Additive	1
Assay in 96-well plate	Substrate solution	1000		Buffer for dilutions	400
	Stop solution	1000	Adding assay reagents	Lysis buffer	200
				Substrate solution	200
				Stop solution	200
Total		**6021**	**Total**		**1215**

5 conditions on 1 chip

Macro-scale		AIMS	
Step	*Time (min)*	*Step*	*Time (min)*
Starter culture dilution	2	Chip preparation	10
Taking 4X1 OD readings; plate reader setup	10	Starter culture dilution	1
Inducing 1 culture	1	Automation system setup	5
Taking 5 aliquots of the culture	10	Reagent setup	1
Cell lysis	2	Assay setup	1
Assay setup	10		
Total	**35**	**Total**	**18**

100 conditions on 1 chip

Macro-scale		AIMS	
Step	*Time (min)*	*Step*	*Time (min)*
Starter culture dilution (into 4 flasks)	10	Chip preparation	10
Taking 4X4 OD readings readings; plate reader setup	40	Starter culture dilution (incl. multiple cultures)	10
Generating 5 inducer concentrations and inducing	40	Automation system setup	10
Generating 5 additive concentrations and adding to culture	40	Reagent setup	5
Cell lysis	40	Assay setup	15
Assay setup	200		
Total	**370**	**Total**	**50**

1000 conditions on 10 chip

Macro-scale		AIMS	
Step	*Time (min)*	*Step*	*Time (min)*
Starter culture dilution (into 4 flasks)	10	10 chip preparation (can wash in parallel)	15
Taking 4X4 OD readings readings; plate reader setup	40	Starter culture dilution (1 time)	10
Generating 5 inducer concentrations and inducing	40	Automation system setup (1 time)	10
Generating 50 additive concentrations and adding to culture	120	Reagent setup (10 times)	50
Cell lysis	120	Assay setup (10 times)	150
Assay setup	600		
Total	**930**	**Total**	**235**