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Supplementary information

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Living circuit boards built by printing bacterial transistors

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Living circuit boards built by printing bacterial transistors

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Table of Contents

Supplementary Note 1: Detailed Protocol to Print Bacterial Circuit Boards

Equipment

- Echo 550 Liquid Handler (Beckman Coulter Life Sciences, USA)
- 30°C shaking incubator (ELMI DTS-4 Digital Thermo shaking incubator)
- 30°C static incubator (Fisher Scientific 750F Isotemp Oven)
- Imaging system (Bio-Rad ChemiDoc MP)
- Refrigerated Centrifuge (Eppendorf 5910R)

Materials

- Echo Qualified 384-Well Polypropylene Microplate (Beckman Coulter Life Sciences, USA)
- Nunc OmniTray plates (ThermoFisher 267060), Single-Well, 4-well, and 8-well plates
- Nunc MicroWell 96-well plate (Thermo Scientific 249662)
- LB-Miller broth (BD #244620). Autoclave to sterilize (121°C for 40 min).
- Bacteriological Agar (BD #214530)
- Kanamycin (GoldBio #25389-94-0) stock solution: 50 mg/mL in deionized water (store in freezer). Final working concentration in plates: 50 µg/mL.
- Disposable sterile loop (Fisher Scientific, Cat. No. 22-363-600)

Step 1 – Design the print layout

The print layout is designed using the electronic circuit layout. The circuits in this paper are not very large, therefore, both electronic and print layouts were designed by hand and are not unique. Software packages (Synopsys, Cadence, etc.) can perform electronic circuit layout for larger circuit boards.

1. Obtain the electronic layout using established software, the following or a similar approach. Write the desired logic function as a Boolean expression decomposed into sum-of-products. Implement each product by one or multiple transistors and connect their output to the appropriate nodes. Draw the layout on a 2D surface with no crossing wires, placing connected nodes as close to each other as possible. Occasionally, a 2D layout may be possible by simplifying the sum-of-products using common factors, this decreases the number of transistors required.
2. Obtain colony arrangement. Position Colony locations as similarly as possible to the electronic layout with 5 mm as the characteristic distance for connected colonies. Ensure there is at least >10 mm distance between non-connected colonies. Multiple layouts may be possible with the conditions given, the layouts are not unique.
3. Open Adobe Illustrator and set the document to RGB color mode.
4. Create an artboard that represents the destination agar plate (size = 151 x 211 pixels). Each pixel corresponds to one possible spot on the agar.
5. Use eight defined colors to specify each of the eight strains placed in odd column wells.
    - #FF0000 -> column 1, *P. agglomerans* S
    - #AA8000 -> column 3, *P. agglomerans* G
    - #FFFF00 -> column 5, *P. agglomerans* Ntype
    - #00FF00 -> column 7, *P. agglomerans* Ptype
    - #00FFFF -> column 9, *P. agglomerans* R_{DI}
    - #0000FF -> column 11, *P. agglomerans* R_{IS}
    - #FF00FF -> column 13, *P. agglomerans* R_{IG}
    - #000000 -> column 15, *P. agglomerans* Null

2. Create a print layout by placing a pixel for each printed colony, of the strain's corresponding color, in the desired location.
6. Export the design as a .png file with the naming convention: "1_A_platename.png" where 1 specifies the number of the destination plate (Nunc OmniTray plate) and A specifies the row of the source plate (Echo-qualified 384-well plate) used.
7. Run the provided Python script (gen_picklist.py, https://github.com/VoigtLab/Living_circuit_boards), converting .png into a layout .csv file. For smaller designs the .csv file with the following columns can be created manually with one row for each colony printed. Column titles: Source Plate, Source Well, Destination Plate, Destination Well, Transfer Volume.
8. Open Echo Cherry Picker software, in the tools menu under labware definitions, create and save a new Plate Type named "omnitray151p". Input the size specification of a single-well Nunc OmniTray plate and 151 x 211 wells.

Step 2 – Grow strains

Day 1

1. Remove glycerol stocks from the –80°C freezer.
2. For each strain, using a sterile loop, scrape a small quantity of frozen bacteria and streak it onto an LB agar plate containing 50 µg/mL Kanamycin.
3. Place plates in a 30°C static incubator for 16 to 24 hours until colonies are visible.

Day 2

4. For each strain, pick a single colony from the Day 1 plate using a sterile loop.
5. Inoculate into 200 µL LB medium with 50 µg/mL Kanamycin in a sterile Nunc MicroWell 96-well plate.
6. Place plate in an ELMI DTS-4 Digital Thermo shaking incubator at 30°C and 1000 rpm for 16 hrs.
7. Pour 60 mL of sterilized LB-Miller broth media with 1.5% agar with 50 µg/mL Kan in a single-well Nunc OmniTray plate. For 4-well plates, pour 15 mL in each well and for 8-well plates, pour 7.5 mL in each well in a hood. Leave plates to solidify for 30 min, then cover with a lid, remove from the hood and keep at room temperature overnight.

Day 3

8. Dilute cultures by taking 1 µL of each culture and adding into 99 µL of fresh LB-Miller broth (1:100 dilution) in a sterile Nunc MicroWell 96-well plate.
9. Transfer 50 µL of the diluted cultures using a multi-channel pipette into the assigned wells (A1, A3, A5, A7, A9, A11, A13, A15) of the Echo Qualified 384-Well Plate.
10. Ensure there are no bubbles and that no droplets reside on the sides of the well, use a sterile pipette tip to burst bubbles or move droplets into the liquid if needed. Ensure the liquid has a symmetric and concave meniscus in all wells, gently tap the plate on all four sides. If needed, centrifuge plates at 250xg for 30 sec for liquid to form symmetric meniscus.
11. Open the Echo Cherry Picker software. In the Protocol window, select Echo Qualified 384-Well Polypropylene Microplate as the source plate and omnitray151p as the destination plate. In the Pick List window, load the layout.csv file generated in Step 1. Press run and in the Run Status window, press Start.
12. Place the Echo Qualified 384-Well Plate containing the diluted cultures in the Echo when asked for the Source Plate.
13. Place the Nunc OmniTray plate into the Echo when asked for the Destination Plate. It is important to ensure there are no visible liquid droplets on the agar surface in this step to prevent spreading of the printed colonies. If droplets are visible, the plate can be stored in a sterile environment to fully dry.
14. The Echo will transfer droplets (2.5 nL or the volume specified in the .csv file) from the source wells to the positions on the agar plate.

For overlayed fluorescent images proceed to Step 14. For videos, proceed to Supplementary Note 2.

15. Place a lid on the printed destination plate and place it upside down in a static incubator at 30 ^{o}C for 5 days. Place an empty Nunc OmniTray plate at the bottom and top of a stack of destination plates to ensure uniform heat distribution across all the plates in the incubator.
16. Every 24 hr after printing, remove the plates from the static incubator and image using the Bio-Rad ChemiDoc MP Imaging system. To image, open Image Lab 4.0 software and create a multi-channel protocol with 3 channels: (a) with Epi-white illumination and a standard filter, (b) with Epi-blue (460 – 490 nm excitation) illumination and 530/28 nm filter, and (c) with Epi-green (520 – 545 nm excitation) illumination and a 695/55 nm filter. Save images as .scn files and Export images using 'for analysis' option to obtain single channel images for quantification.
17. Overlayed images were created from the three individual images with false-coloring for each plate. Images shown are after 72 hr of growth unless otherwise specified.

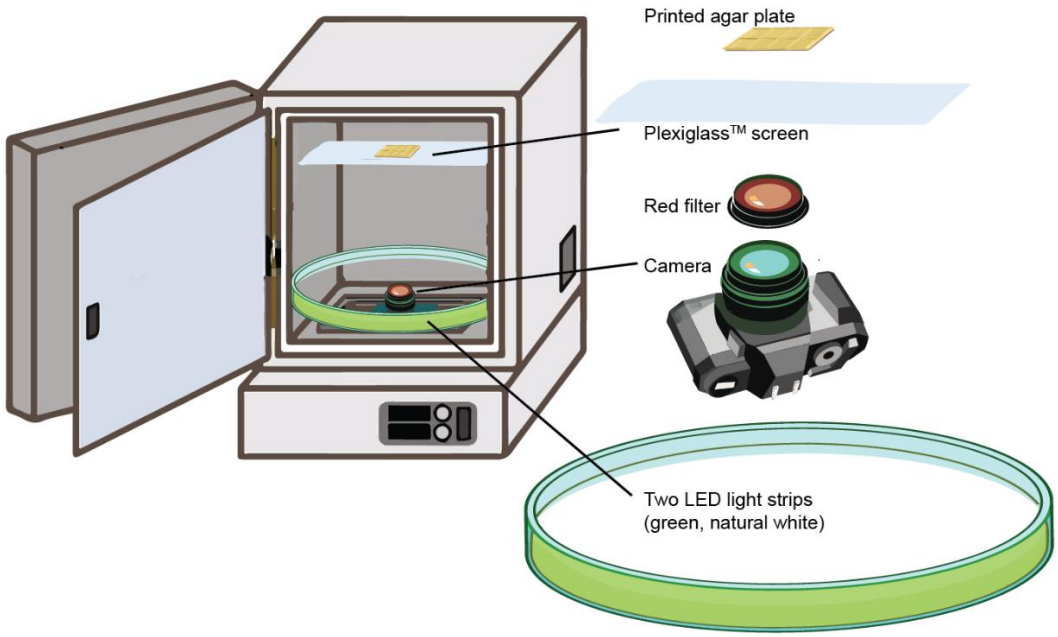
Supplementary Note 2: Imaging Setup

Time-lapse imaging of bacterial colonies was performed using a custom-built incubator and camera system. Plates were imaged *in situ* under controlled illumination to capture both colony morphology and fluorescence over time.

Illumination inside the incubator (Fisher Scientific 750F Isotemp Oven) was provided using an 80 CRI LED strip with 2835 SMD LEDs (Epistar Corp, Hsinchu, Taiwan) for natural white light and a green LED strip with 3528 SMD LEDs, peak wavelength λ = 515–535 nm (Epistar Corp, Hsinchu, Taiwan), for fluorescent excitation of reporter proteins. The LED strips were affixed in a circular layout to the interior of the incubator surrounding the camera to ensure uniform light distribution and minimize shadowing artifacts across the plate surface.

A ZWO ASI178MM telescope camera (ZWO, Suzhou, China) equipped with a red-light emission filter (λ = 630 nm) was mounted in the center of the incubator, beneath the agar plates, to capture images of plates placed inverted on a clear Plexiglas support (Röhm GmbH, Darmstadt, Germany). This arrangement ensured consistent focal distance and minimized distortion across the plate. The optical path is schematically represented showing LED placement, camera position, and plate orientation.

Both the LED strips and camera were connected to an Arduino microcontroller, programmed to take four images at each 30 min interval (time_series_imager.py, https://github.com/VoigtLab/Living_circuit_boards). One brightfield image was taken after activating the white light LED strip, after which the LED strip was turned off. Three images under green LED illumination were then taken with varying exposure times to capture fluorescent intensity without saturation. This multi-exposure approach enabled clear images of both dim and bright colonies while minimizing photobleaching. Captured images were timestamped and stored in a structured directory. Image analysis pipelines were applied post-acquisition to extract colony metrics and fluorescence intensity profiles. The temporal resolution allowed continuous monitoring of colony growth and reporter protein expression over the experimental timeframe and the generation of videos (Supplementary Videos 1-4).



Supplementary Note 3: Mathematical models and simulation details

We modeled multicellular communication using reaction–diffusion equations. Note that these simulations were not performed as part of the design of the colony patterns; rather, they were used to analyze the empirical data. Colonies of engineered bacteria produce, sense, and degrade signaling molecules OC12, OC6, OHC14, and DAPG and perform computational operations dictated by their internal genetic circuits. Intracellular transcription, translation, and repression events are combined into a single effective ordinary differential equation (ODE) for enzyme expression (mRNA dynamics were implicit). Extracellular signaling molecule (AHLs/DAPG) were produced at rate g and were proportional to enzyme concentration. Intracellular regulation in each strain was modeled by Hill functions (from Equations 1-3). The hill function was assumed to be at pseudo-steady state with respect to gene expression, signaling molecule production, and diffusion.

There are five response functions for the five *P. agglomerans* strains. For N-type,

$$f_{\mathrm{N}}(C_{\mathrm{OC12}}, C_{\mathrm{OC6}}) = f_{min,N} + \left(f_{max,\mathrm{N}} - f_{min,\mathrm{N}}\right)\left(\frac{C_{\mathrm{OC12}}}{C_{\mathrm{OC12}}+K_{\mathrm{OC12}}}\right)^{n_{\mathrm{OC12}}}\left(\frac{C_{\mathrm{OC6}}}{C_{\mathrm{OC6}}+K_{\mathrm{OC6}}}\right)^{n_{\mathrm{OC6}}} , \quad \text{(S.1)}$$

where C_i is the local concentration of the small molecule i, f_{N} is the promoter activity of cI434 (au), for which $f_{min,\mathrm{N}}$ and $f_{max,\mathrm{N}}$ were the minimum and maximum activity. The responses to the small molecules have half-activation thresholds K_i and cooperativities n_i. For the P-type strain, it follows

$$f_{\mathrm{P}}(C_{\mathrm{OC12}}, C_{\mathrm{OC6}}) = f_{min,\mathrm{P}} + \left(f_{max,\mathrm{P}} - f_{min,\mathrm{P}}\right)\left(\frac{C_{\mathrm{OC12}}}{C_{\mathrm{OC12}}+K_{\mathrm{OC12}}}\right)^{n_{\mathrm{OC12}}}\left(\frac{K_{\mathrm{OC6}}}{C_{\mathrm{OC6}}+K_{\mathrm{OC6}}}\right)^{n_{\mathrm{OC6}}} . \quad \text{(S.2)}$$

Then, for the RDI, RIS, and RIG relay strains:

$$f_{\mathrm{DI}}(C_{\mathrm{OHC14}}) = f_{min,\mathrm{DI}} + \left(f_{max,\mathrm{DI}} - f_{min,\mathrm{DI}}\right)\left(\frac{C_{\mathrm{OHC14}}}{C_{\mathrm{OHC14}}+K_{\mathrm{OHC14}}}\right)^{n_{\mathrm{OHC14}}} , \quad \text{(S.3)}$$

$$f_{\mathrm{IS}}(C_{\mathrm{DAPG}}) = f_{min,\mathrm{IS}} + \left(f_{max,\mathrm{IS}} - f_{min,\mathrm{IS}}\right)\left(\frac{C_{\mathrm{DAPG}}}{C_{\mathrm{DAPG}}+K_{\mathrm{DAPG}}}\right)^{n_{\mathrm{DAPG}}} , \text{ and} \quad \text{(S.4)}$$

$$f_{\mathrm{IG}}(C_{\mathrm{DAPG}}) = f_{min,\mathrm{IG}} + \left(f_{max,\mathrm{IG}} - f_{min,\mathrm{IG}}\right)\left(\frac{[\mathrm{DAPG}]}{C_{\mathrm{DAPG}}+K_{\mathrm{DAPG}}}\right)^{n_{\mathrm{DAPG}}} . \quad \text{(S.5)}$$

The parameters f_{max}, f_{min}, K, n were obtained empirically from best fits to flow cytometry data (Table 1).

The genes encoding enzymes E were controlled by promoters that respond to the signaling molecules (Equations S.1 to S.5). $\hat{E}$ was defined as the ratio of enzyme concentration E to the steady-state enzyme concentration at maximal expression E_{max}. We assume the decay of enzyme concentration is governed by cellular dilution. Then,

$$\hat{E} = \frac{E}{E_{max}} \quad \text{and} \quad \text{(S.6)}$$

$$\frac{d\hat{E}}{dt} = \left(\frac{f}{f_{max}} - \hat{E}\right)\frac{\ln(2)}{t_d} , \quad \text{(S.7)}$$

where f and f_{max} are from Equations S.1 to S.5, and t_d is the doubling time which determines the enzyme dilution rate. $\hat{E}$ has a value between 0 and 1.

Each signaling molecule i was represented by a concentration field $C_i(x, y, t)$ in 2-dimensional space

on the plate surface *x,y* and time *t*. Its evolution is modeled as

$$\frac{\partial C_i(x,y,t)}{\partial t} = \frac{\alpha_i N\left(1-\frac{N}{N_{max}}\right)\hat{E}_i}{L\,dx\,dy} + D\nabla^2 C_i - \delta_i C_i(x,y,t) \quad , \qquad \text{(S.8)}$$

where α_i is the signaling molecule production rate per cell at maximal enzyme expression ($\hat{E}$ = 1), *N* is the number of cells in the colony and is multiplied by the ratio of cells actively growing (1-N/N_{max}), $\hat{E}$ is the ratio of enzyme concentration to maximum enzyme concentration, *L* = 5.5 mm is the thickness of the agar layer, δ_i is the signaling molecule's degradation rate, and *D* is the diffusion coefficient of the signaling molecules on the plate. *D* was assumed to be the same for all the signaling molecules. α_i, δ_i, and D were estimated from literature sources. For the simulations, these equations were discretized with the plate surface defined as a lattice with widths *dx* = *dy*. "No flux" boundary conditions were enforced at the plate edges and bottom:

$$\left.\frac{\partial C_i(x,y,t)}{\partial x}\right|_{x=0\text{mm},\,86\text{mm}} = 0 \qquad \text{and} \qquad \text{(S.9)}$$

$$\left.\frac{\partial C_i(x,y,t)}{\partial y}\right|_{y=0\text{mm},\,128\text{mm}} = 0 \quad , \qquad \text{(S.10)}$$

where 86 mm and 128 mm are the measured width and length of the plate.

The number of cells in a colony on agar with limited nutrients was described with the logistic growth function,

$$\frac{dN}{dt} = \mu N\left(1-\frac{N}{N_{max}}\right) \quad , \qquad \text{(S.11)}$$

where μ is the intrinsic growth rate and N_{max} is the carrying capacity (*i.e.*, the maximum number of cells reached with the current experimental conditions and nutrient availability).

To obtain the intrinsic growth rate μ and carrying capacity N_{max}, the cell number can be described using the analytical solution to Equation S.11 with N_0 as the initial number of cells at *t* = 0:

$$N(t) = \frac{N_{max}}{1+\left(\frac{N_{max}-N_0}{N_0}\right)e^{-\mu t}} \quad . \qquad \text{(S.12)}$$

This equation assumes that signaling molecule concentrations do not significantly impact the colony growth rate.

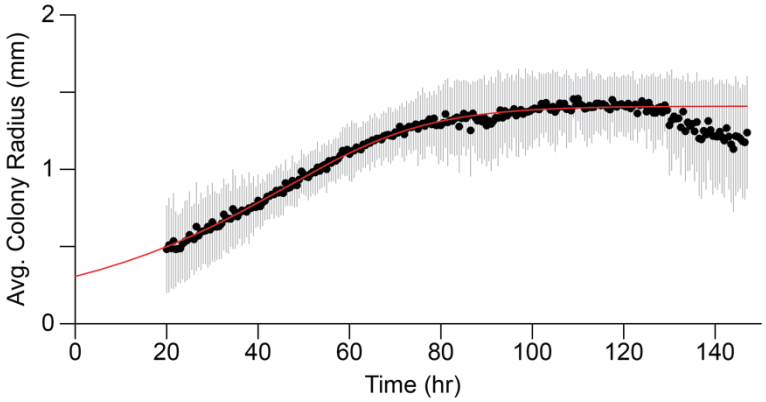
N can also be described as a function of colony radius:

$$N(t) = \frac{4}{6}\pi R(t)^3 \rho \qquad \text{(S.13)}$$

where ρ = 1 cell/μm^3 for a densely-packed colony on a solid surface. This equation assumes that the colony is a densely packed hemisphere. Substituting Equation S.14 into Equation S.13 yields

$$R(t) = \frac{R_{max}}{\sqrt[3]{1+\left(\frac{R_{max}^3}{R_0^3}-1\right)e^{-\mu t}}} \quad . \qquad \text{(S.14)}$$

Time-series images were obtained for the signal propagation circuit corresponding to Supplementary Figure 4b and average colony size was measured at each time point. We fit Equation S.14 to average colony radius data over time to obtain R_0 = 0.3 mm, R_{max}= 1.4 mm, and μ = 0.075 hr^{-1}. The doubling time t_d = 9.3 hr was calculated from the intrinsic growth rate μ. N_{max}= 5.7 × 10^9 was calculated from Equation S.13 using R_{max}. For simulations, we set N_0 = 2.5 × 10^6 at t = 0 corresponding to a 2.5 nL colony, assuming the time for the droplet to become a densely-packed colony is negligible.



Empirical measurement of μ. The average colony sizes measured from time-series imaging of signal propagation circuit (Supplementary Figure 4b) are shown as black dots. The best fit to Equation S.14 to the data is shown as a red line (R^2 = 0.93). The parameters R_{max} = 1400 μm and growth rate μ = 0.075 h^{-1} were obtained from the fit. The data represent all colonies on one plate imaged over six days as described in the Methods, with each black dot indicating the average colony radius across all colonies. The grey bars are standard deviations of these data.

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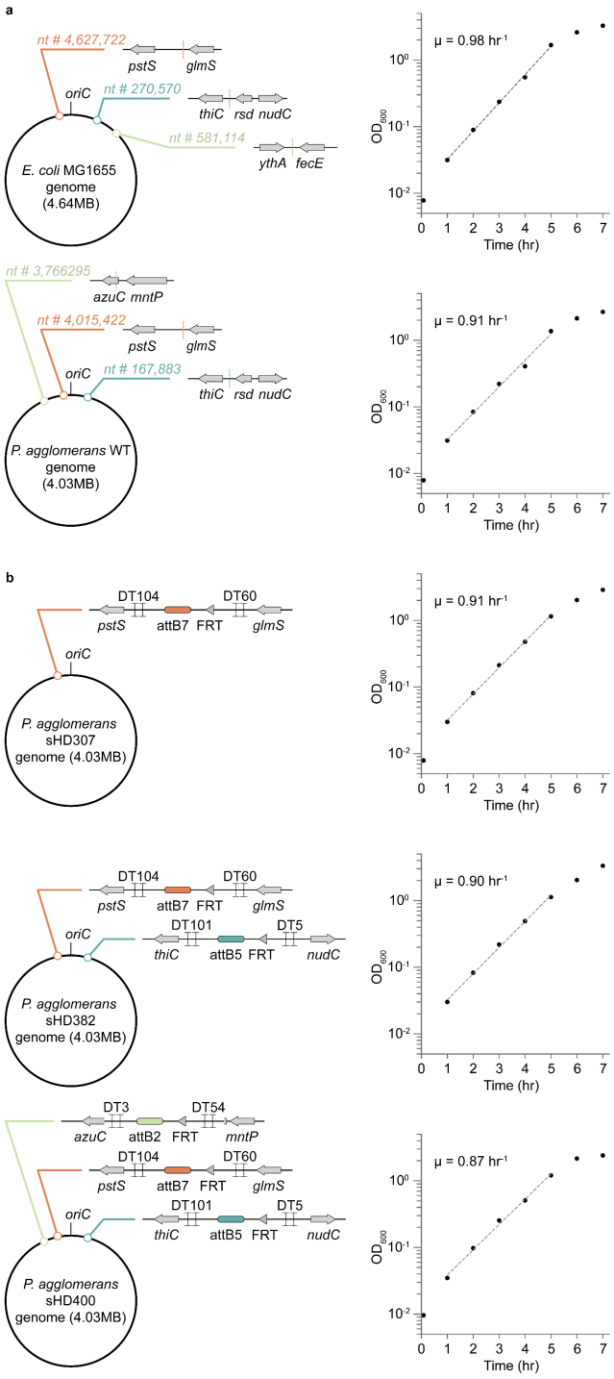
Parameters used for modeling computation performed by interacting colonies

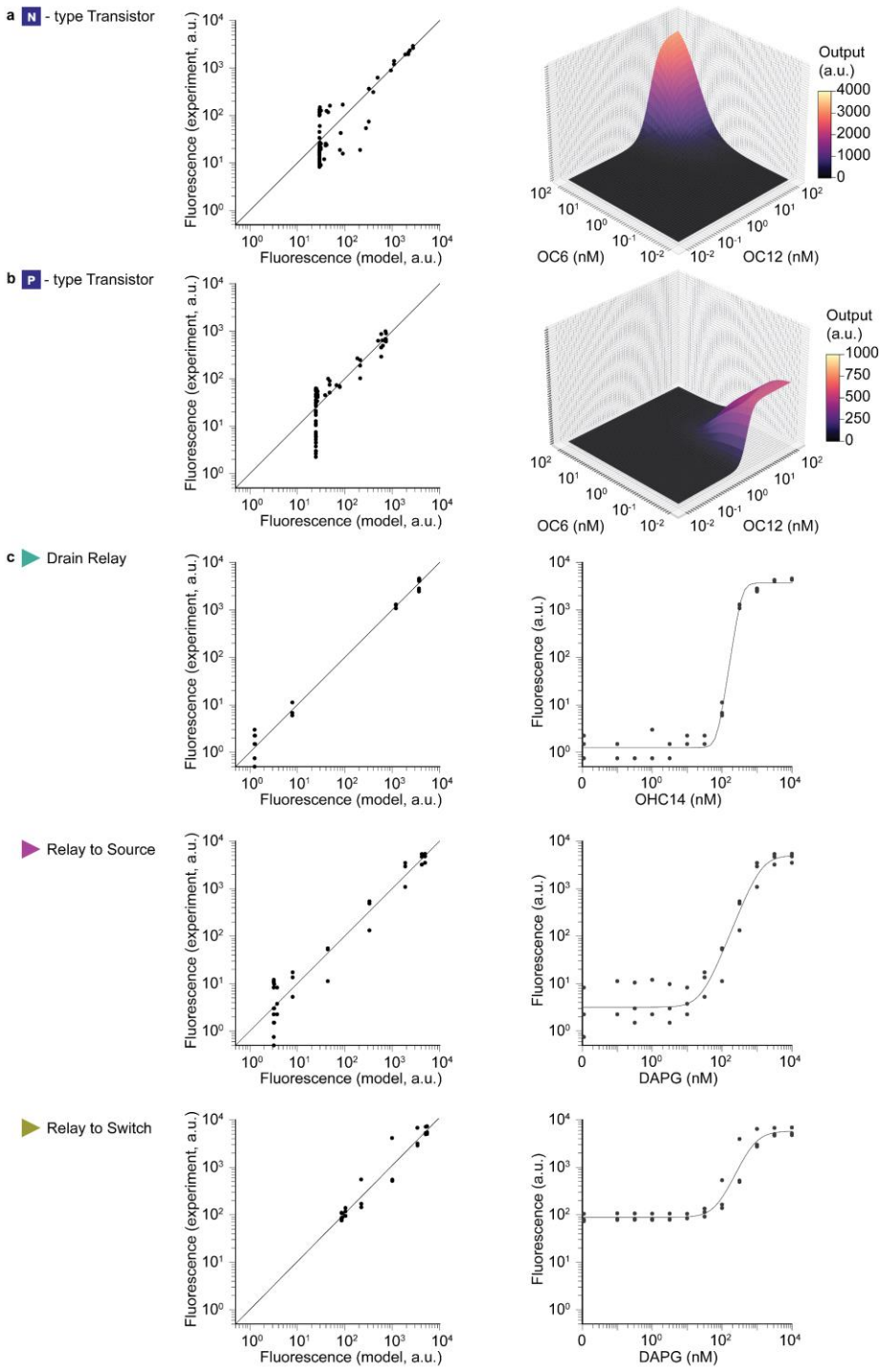
Parameter	Description	Value	Refs
t_d	Doubling time	9.3 hr	This work
μ	Colony growth rate	0.075 hr^{-1}	This work
α_{OC12}	OC12 maximal production rate[1]	10^{-9} nmol/hr per cell	1
α_{OC6}	OC6 maximal production rate[1]	10^{-9} nmol/hr per cell	1
α_{OHC14}	OHC14 maximal production rate[1]	10^{-9} nmol/hr per cell	1
α_{DAPG}	DAPG maximal production rate[2]	3 × 10^{-9} nmol/hr per cell	2
N_0	Colony cell count at start of simulation	2.5 × 10^6	This work
N_{max}	Colony cell count at end of simulation	5.7 × 10^9	This work
L	Agar film depth	5.5 mm	This work
D	Signaling molecule diffusion coefficient[1]	0.5 mm^2/hr	1
δ_{OC12}	OC12 degradation rate[3,4]	0.12 hr^{-1}	3,4
δ_{OC6}	OC6 degradation rate[3]	0.45 hr^{-1}	3
δ_{OHC14}	OHC14 degradation rate[3]	0.07 hr^{-1}	3
δ_{DAPG}	DAPG degradation rate[5]	0 hr^{-1}	5

Simulations

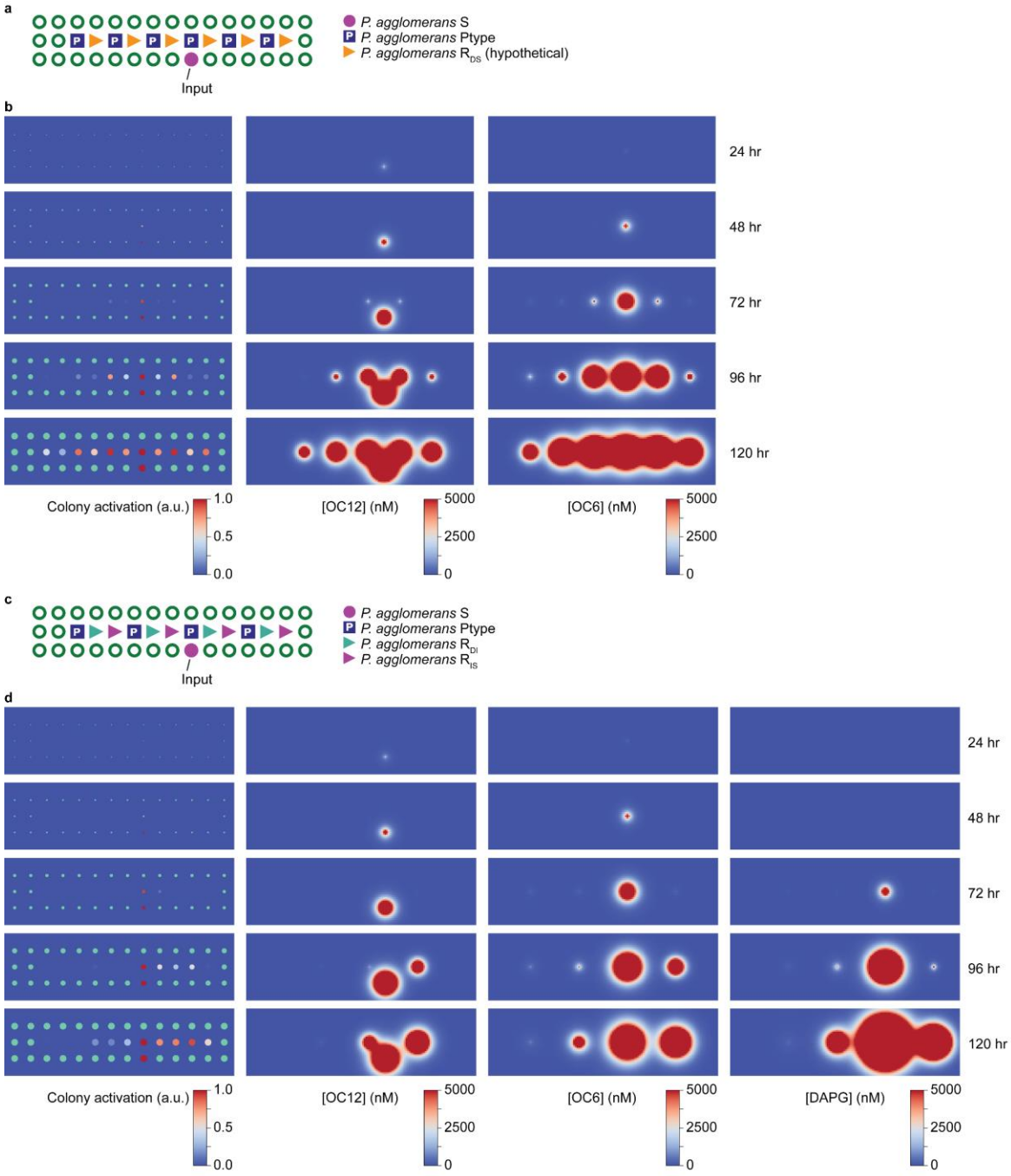
Equations S.1 through S.11 were integrated using a forward Euler scheme with timestep Δt = 0.01 hr for T = 120 hr. Diffusion was discretized using a 2D five-point Laplacian operator with lattice dimension dx = dy = 0.6 mm. All variables were updated synchronously at each iteration. Colonies were represented as fixed coordinates on a 2-D lattice of size (211,151). Extracellular signaling molecules diffuse between lattice sites according to a finite-difference Laplacian, with reflecting (no-flux) boundary conditions for the agar plate. (simulator.ipynb, github.com/VoigtLab/Living_circuit_boards). Simulation results for the signal propagation circuit are shown in Supplementary Figure 15. Simulation results for the transistors, demultiplexor, and half adder are shown in Supplementary Videos 4, 5, and 6, respectively.



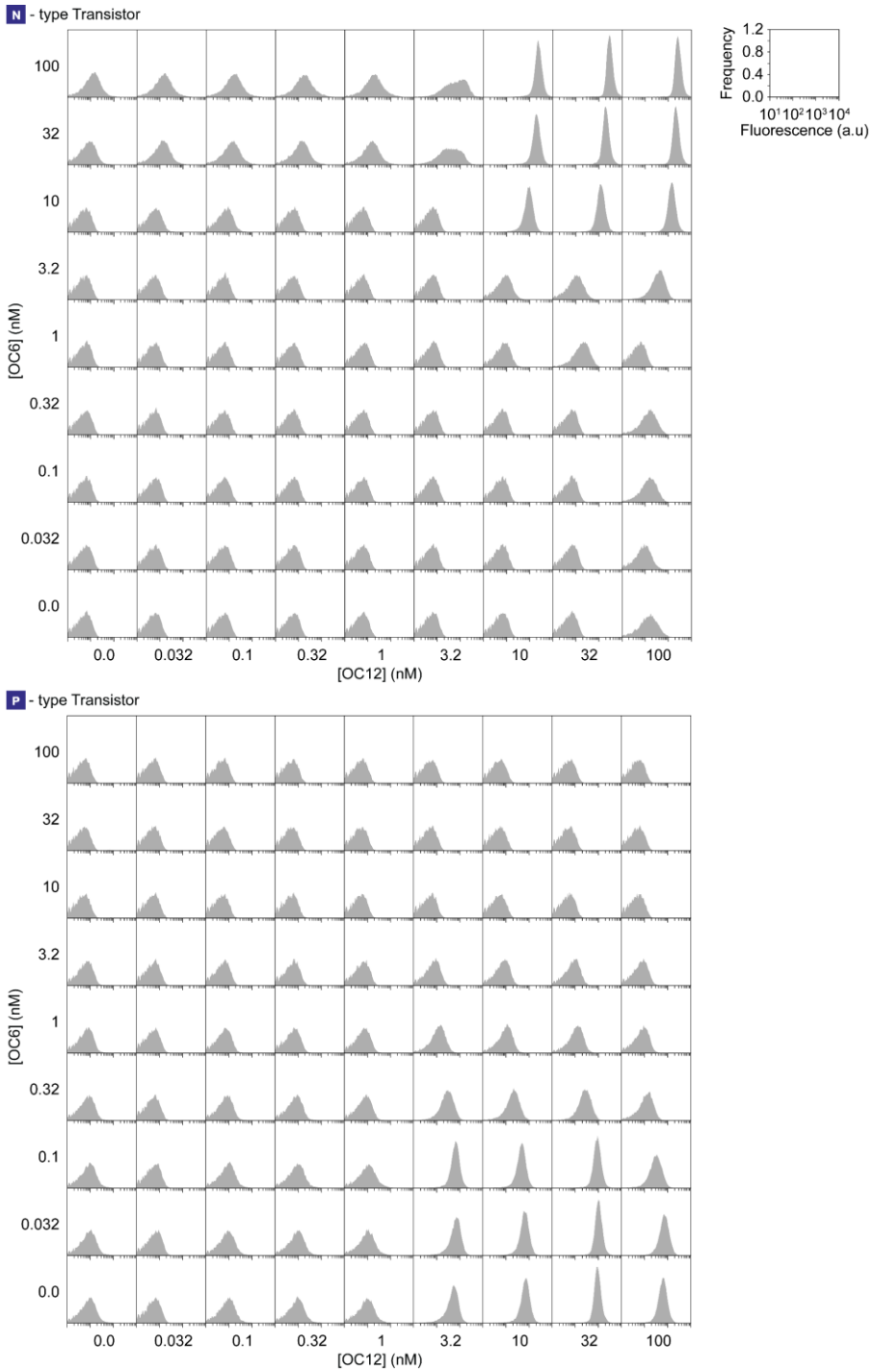
Supplementary Figure 1: **Integration of landing pads and growth impact. (a)** Left: Schematics of the genome sites used to carry landing pads. Dashed lines show the location relative to neighboring annotated features. The numbers above are the nucleotide positions. Right: OD_{600} measurements of the wild-type strain grown in LB medium at 30 °C in a 96-well plate (Methods). The dashed lines indicate the exponential growth model during exponential phase growth from t =1 to 5 hr (Methods, Eq. 4). The growth rate μ resulting from these fits are shown on the graphs. R^2 = 0.81 for *E. coli* MG1655 (top) and 0.90 for *P. agglomerans* (bottom). **(b)** Left: Schematics of the genomic landing pads. Dashed lines show integrated constructs relative to neighboring annotated features. The attB sites mark where the payloads are inserted. Strain genotypes are described in Supplementary Table 1, genetic parts for the landing pads in Supplementary Table 2, and payloads in Supplementary Table 3. Right: OD_{600} measurements of engineered strains grown in LB medium at 30 °C in a 96-well plate (Methods). The same growth model was used as in part a: R^2 = 0.97 for *P. agglomerans* sHD307, 0.99 for sHD382, and 0.98 for sHD400.



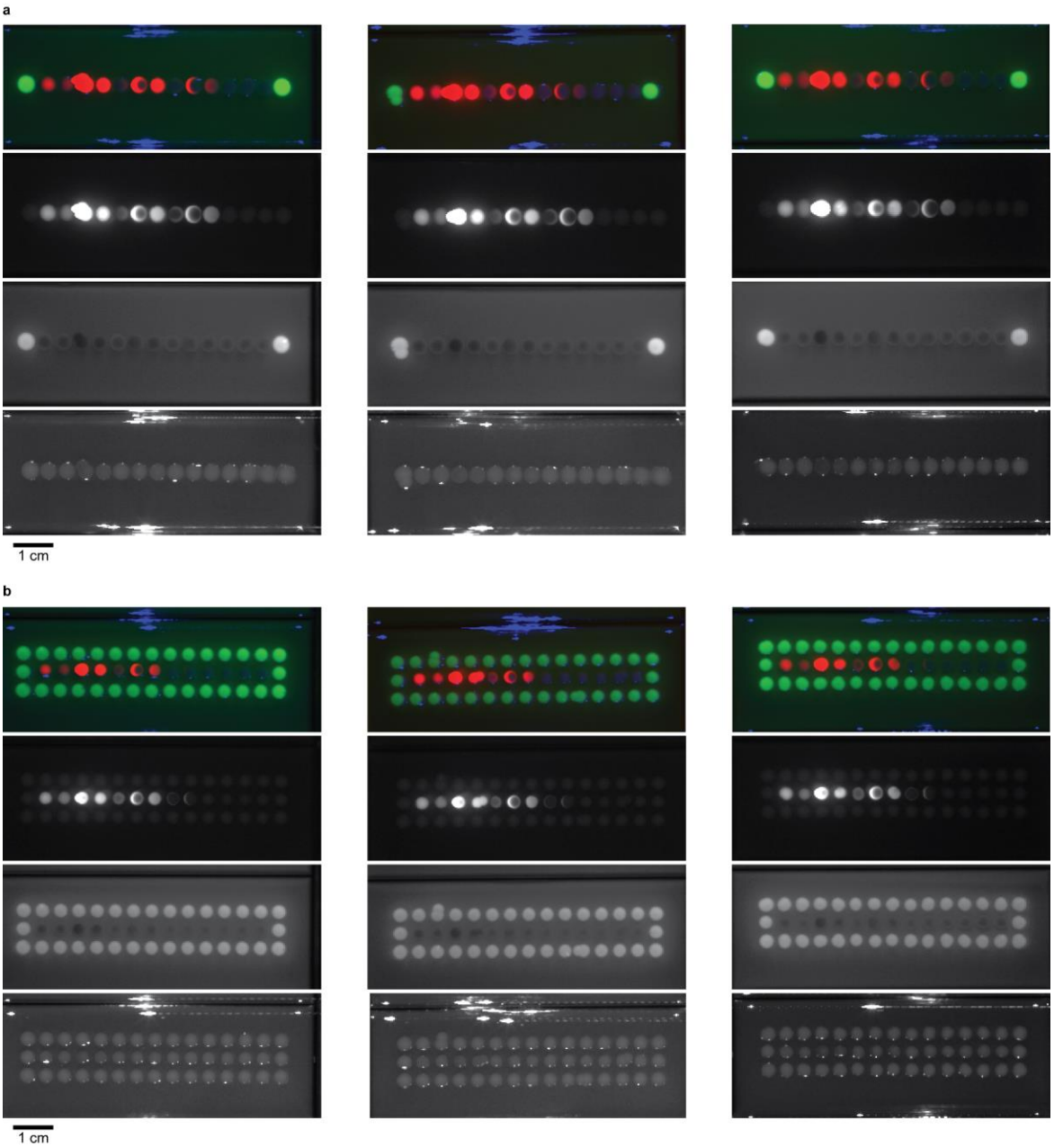
Supplementary Figure 2: **Fits to the models of the sensor and circuit response functions.** The fit parameters are provided in Table 1. **(a)** *P. agglomerans* Ntype. Empirical values obtained with flow cytometry compared to a fit to Equation 1. The line is $x = y$ ($R^2 = 0.98$). **(b)** *P. agglomerans* Ptype. Empirical values obtained with flow cytometry compared to a fit to Equation 2. The line is $x = y$ ($R^2 = 0.90$). **(c)** Relay strains. Empirical values obtained with flow cytometry compared to a fit to Equation 3 with $R^2 = 0.97$ for the drain relay (*P. agglomerans* R_{DI}), $R^2 = 0.91$ for the relay-to-source (*P. agglomerans* R_{IS}), and $R^2 = 0.85$ for the relay-to-switch (*P. agglomerans* R_{DI}).



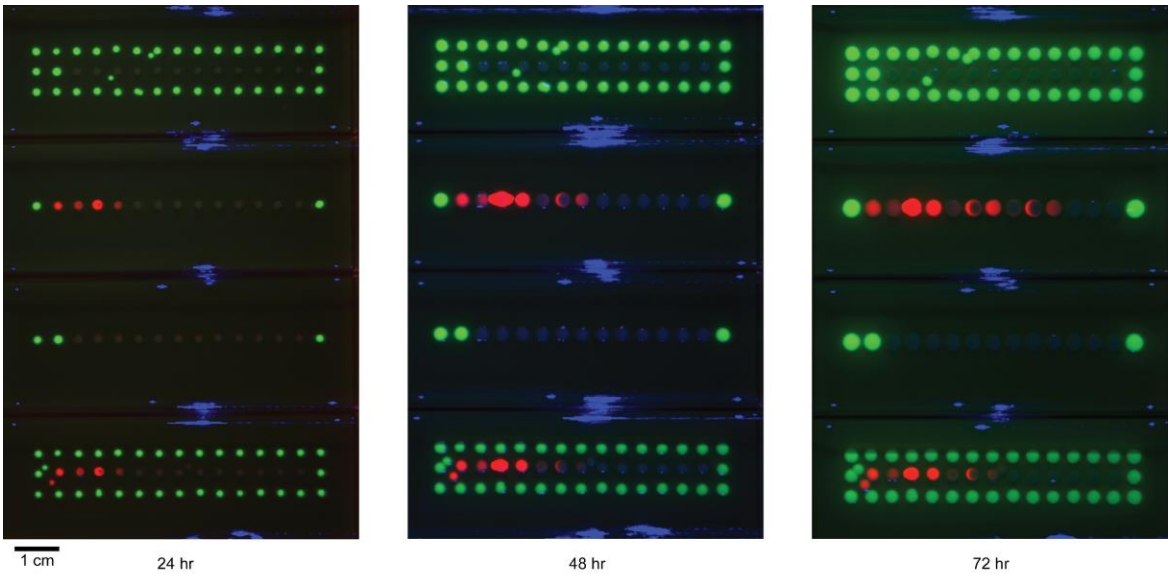
Supplementary Figure 3: **Simulated signal propagation circuit using a single relay.** **(a)** The layout of the circuits with a single relay is shown. *P. agglomerans* S was printed as input to a P-type transistor in the center of a repeating pattern of *P. agglomerans* Ptype and R_{DS}. **(b)** Simulated output images of the signal propagation after 24, 48, 72, 96, and 120 hr from top to bottom, respectively. Colony activation and signaling molecule concentrations across the plate are shown. **(c)** The layout of the circuits with alternating relays with an input to an internal P-type transistor is shown. P. agglomerans S was printed as input. **(d)** Simulated output images of the signal propagation after 24, 48, 72, 96, and 120 hr from top to bottom, respectively. Colony activation and signaling molecule concentrations across the plate are shown.



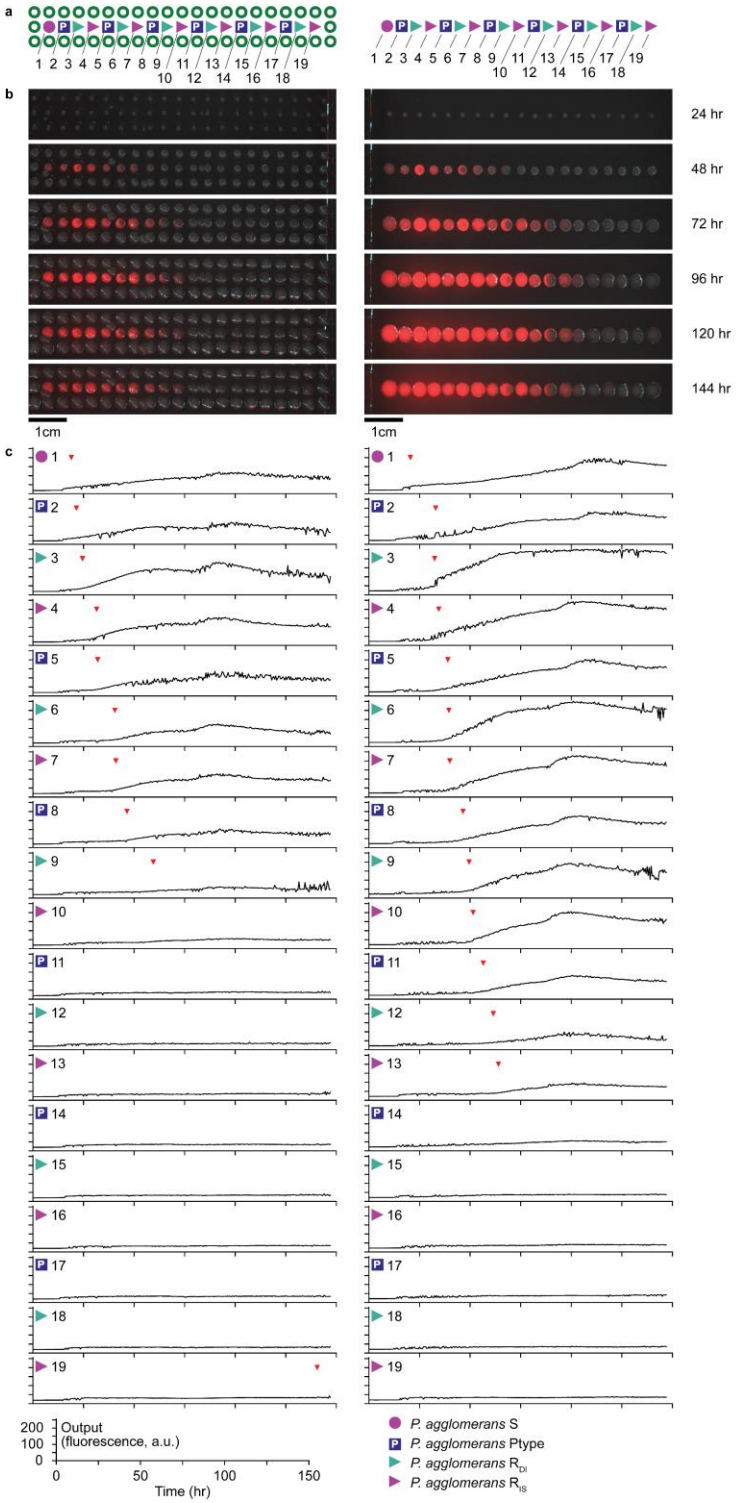
<u>Supplementary Figure 4:</u> **Cytometry distributions for N-type and P-type Strains.** These representative plots correspond to Figure 1a and 1b.



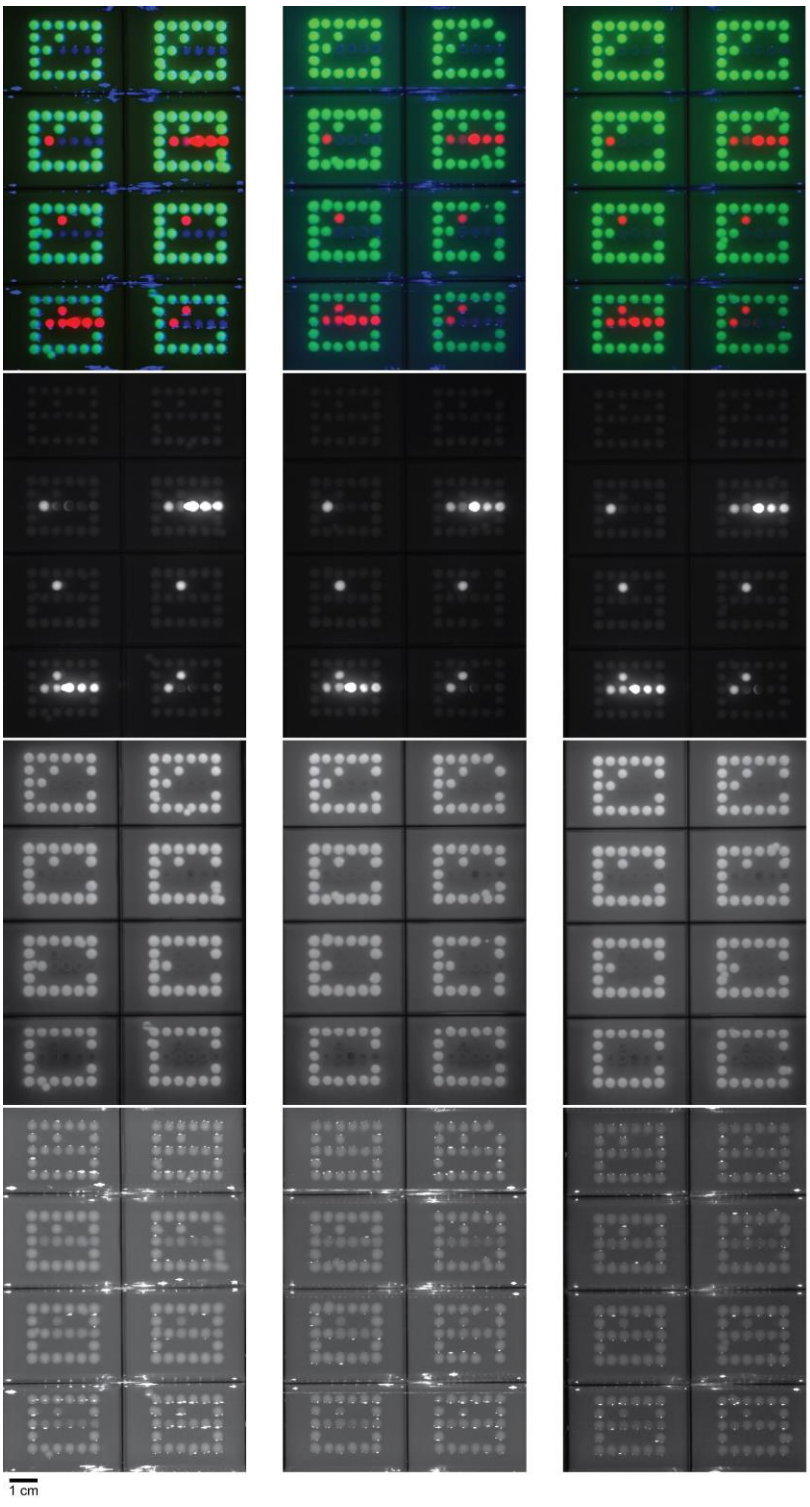
Supplementary Figure 5: **Replicates of signal propagation circuit plates.** **(a)** Signal propagation circuit without borders. (These images correspond to replicates of Figure 1). Images of three replicates printed on different days. **(b)** Signal propagation circuit with a printed border of P. agglomerans Null colonies. Images in a and b were taken after 72 hr (Methods). From top to bottom: overlayed false-colored, red fluorescence, green fluorescence, and white light images. Green colonies are *P. agglomerans* Null and transistor/relay colonies are red if they are in the ON state (mCherry).



Supplementary Figure 6: **Signal propagation circuit control plates.** These plates show relays do not self-activate in the absence of an input strain. Signal propagation circuit plate imaged for three days (top to bottom): with a border without source input, without border with source input, without border without source input, and with border with source input. The presence of a source input activates relays and signal is propagated, however, no signal is observed in any relay colony when source input is absent.

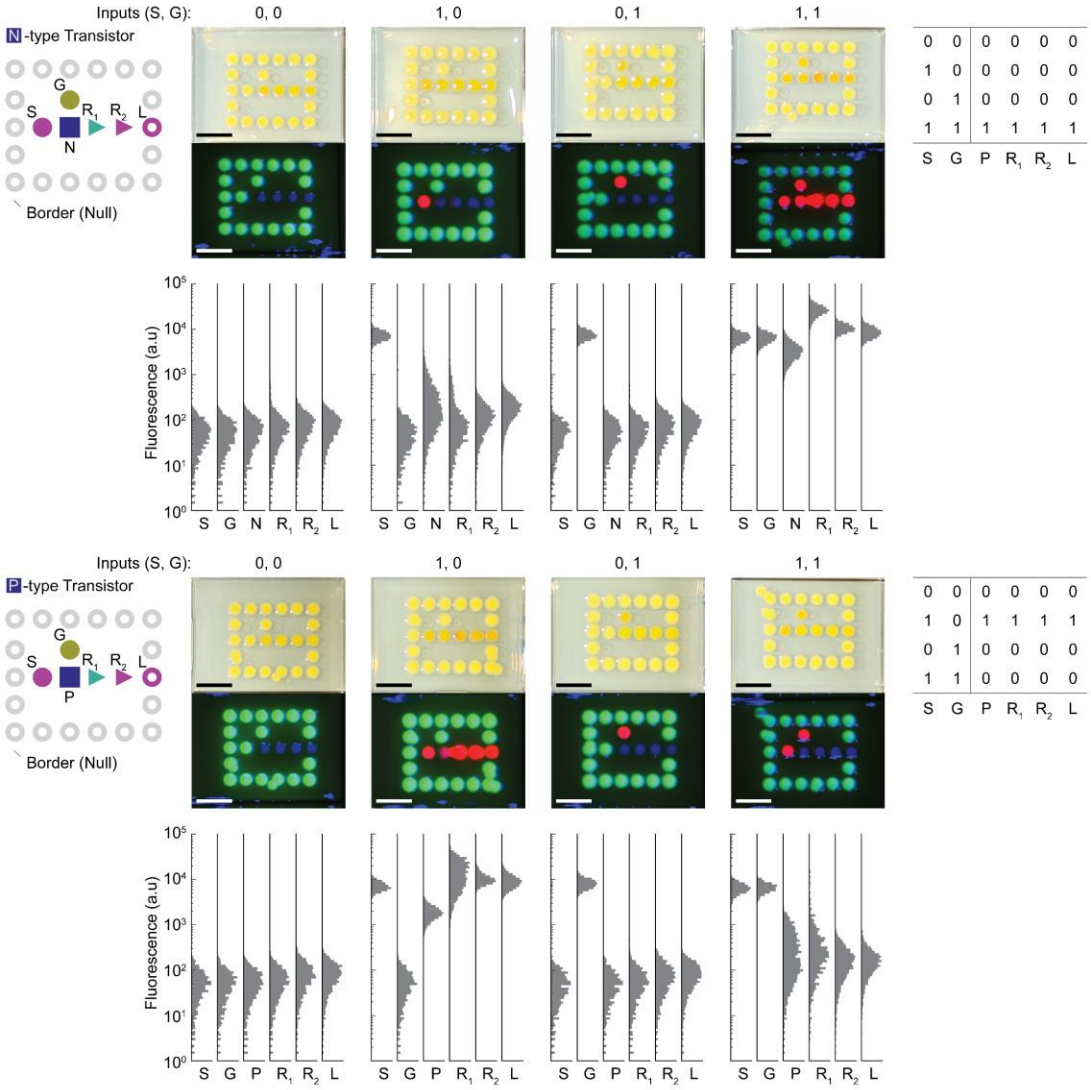


Supplementary Figure 7: **Time-course of the signal propagation circuit. (a)** The layout of the circuit with (left) and without (right) a border of *P. agglomerans* Null strains. *P. agglomerans* S was printed in position 1 followed by a repeating pattern of *P. agglomerans* Ptype, R_{DI}, and R_{IS}, respectively. **(b)** Time course imaging of the signal propagation (Methods). These images correspond to Supplementary Videos 1 and 2. **(c)** Response times. The average colony pixel intensity of the red fluorescence images is shown. Upside-down triangles represent the response time defined as the first time-point where pixel intensity reaches 15% above baseline. The propagation delay τ reported in the main text is the average difference in response times of two adjacent colonies.



S

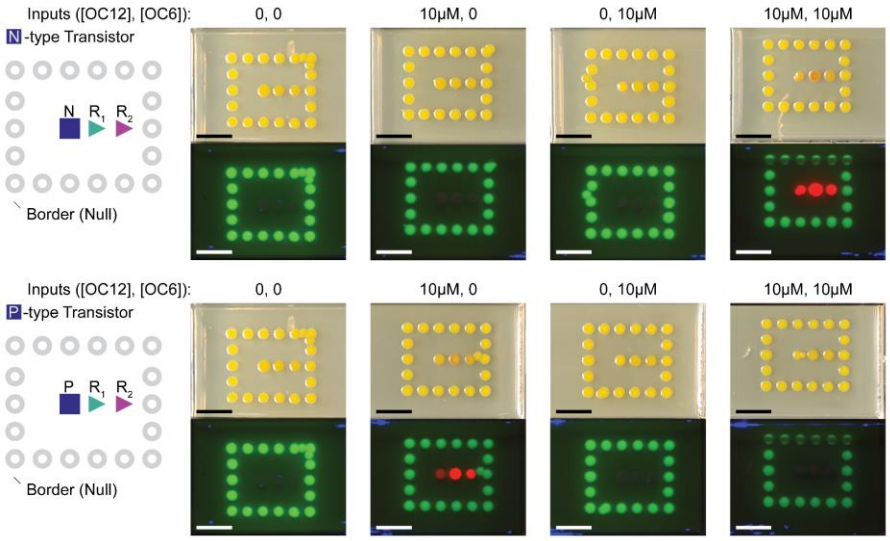
Supplementary Figure 8: **Transistor plate replicates.** These images correspond to Figure 2. Images of three replicates printed on different days. Images were taken after 72 hr (Methods). From top to bottom: overlayed false-colored, red fluorescence, green fluorescence, and white light images. Green colonies are *P. agglomerans* Null and transistor/relay colonies are red if they are in the ON state (mCherry).



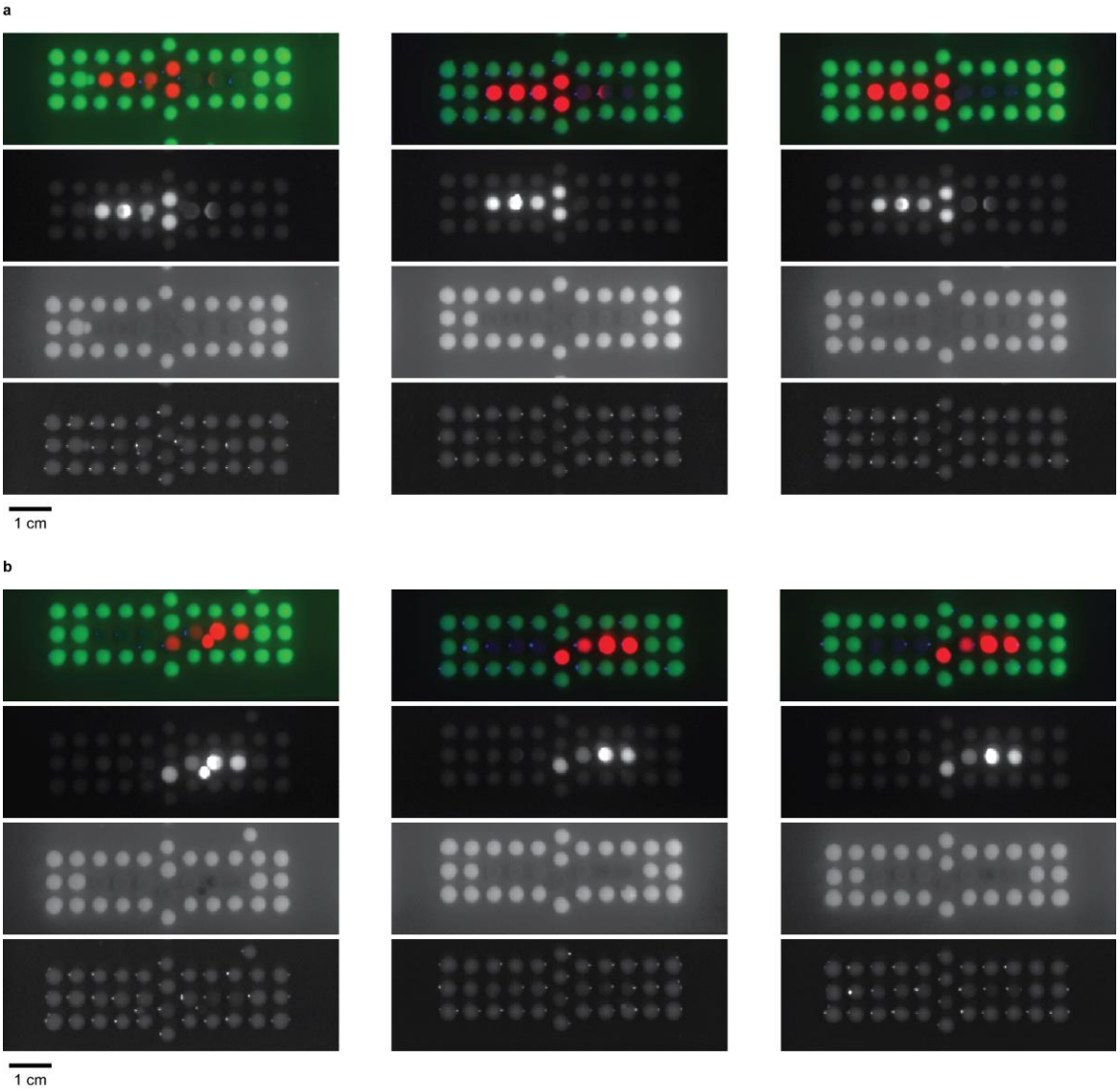
S	G	P	R_1	R_2	L
0	0	0	0	0	0
1	0	0	0	0	0
0	1	0	0	0	0
1	1	1	1	1	1

S	G	P	R_1	R_2	L
0	0	0	0	0	0
1	0	1	1	1	1
0	1	0	0	0	0
1	1	0	0	0	0

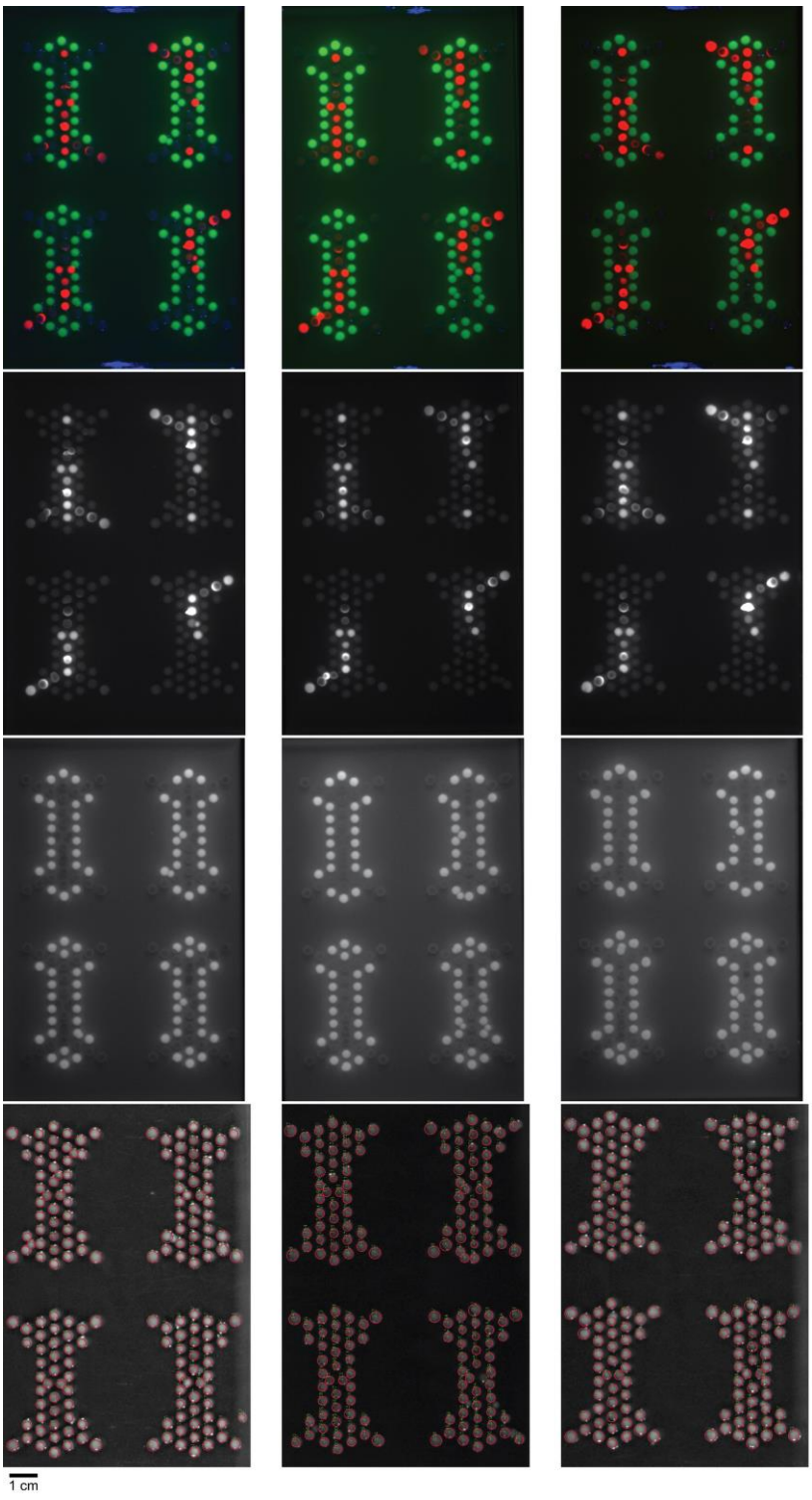
<u>Supplementary Figure 9:</u> **Transistor flow cytometry distributions** These images and data correspond to Figure 2. **(a)** Left: colony layouts. These examples show the *P. agglomerans* S/G strains as the ON inputs. For different combinations of inputs, *P. agglomerans* Null was used for the OFF state. Center: On-plate responses. The bright light and fluorescent images of the colonies are shown. Green colonies were *P. agglomerans* Null and transistor/relay colonies are red if they were in the ON state (mCherry). Scale bars are 1 cm. Colonies were scraped and analyzed by flow cytometry, distributions are shown below images. Right: The truth tables of the circuits.



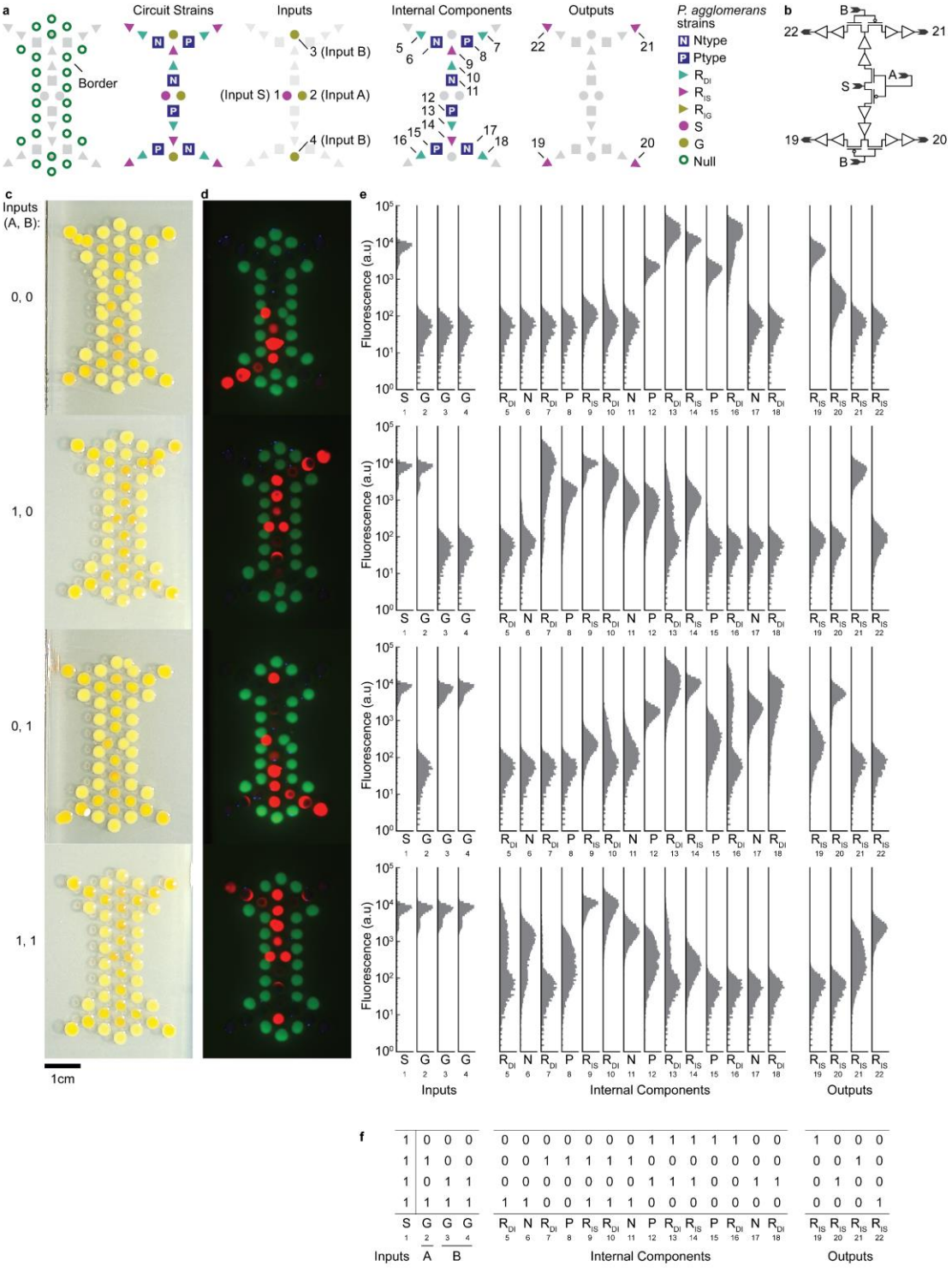
Supplementary Figure 10: **Transistor response to inducers mixed in agar.** Other experiments in this paper use cells in colonies to produce the input molecules to the circuit; here, they are added to the agar to show that the system can respond to an "environmental" signal. Four different combinations of OC12 and OC6 were mixed into the agar prior to printing the colonies on the surface. Center: On-plate responses. The bright light and fluorescent images of the colonies are shown. Green colonies were *P. agglomerans* Null and transistor/relay colonies are red if they were in the ON state (mCherry).



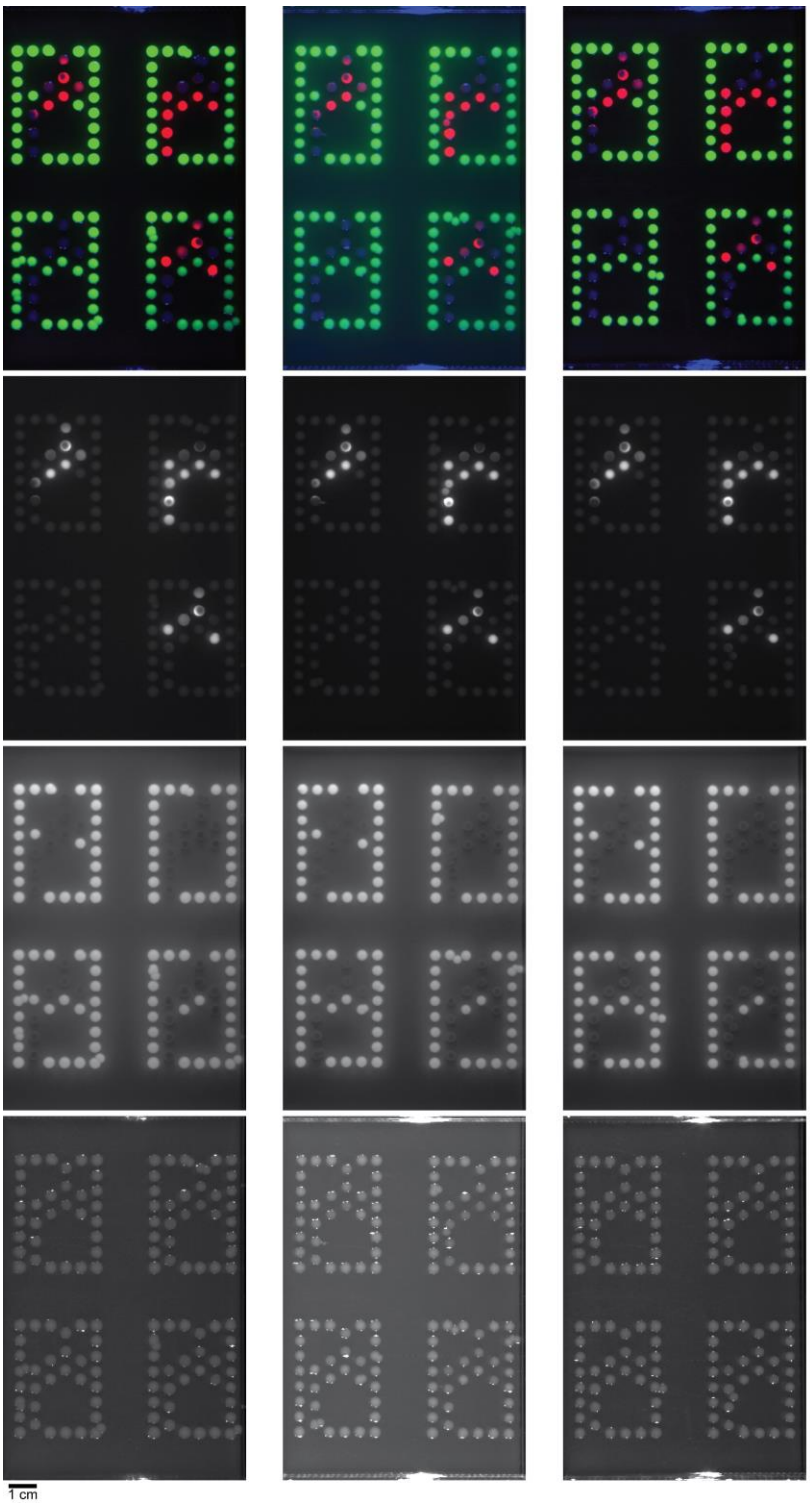
Supplementary Figure 11: **Replicates of bi-directional switch plates.** These images correspond to Figure 2. Images of three replicates printed on different days. Images were taken after 72 hr (Methods). From top to bottom: overlayed false-colored, red fluorescence, green fluorescence, and white light images. Green colonies are *P. agglomerans* Null and transistor/relay colonies are red if they are in the ON state (mCherry).



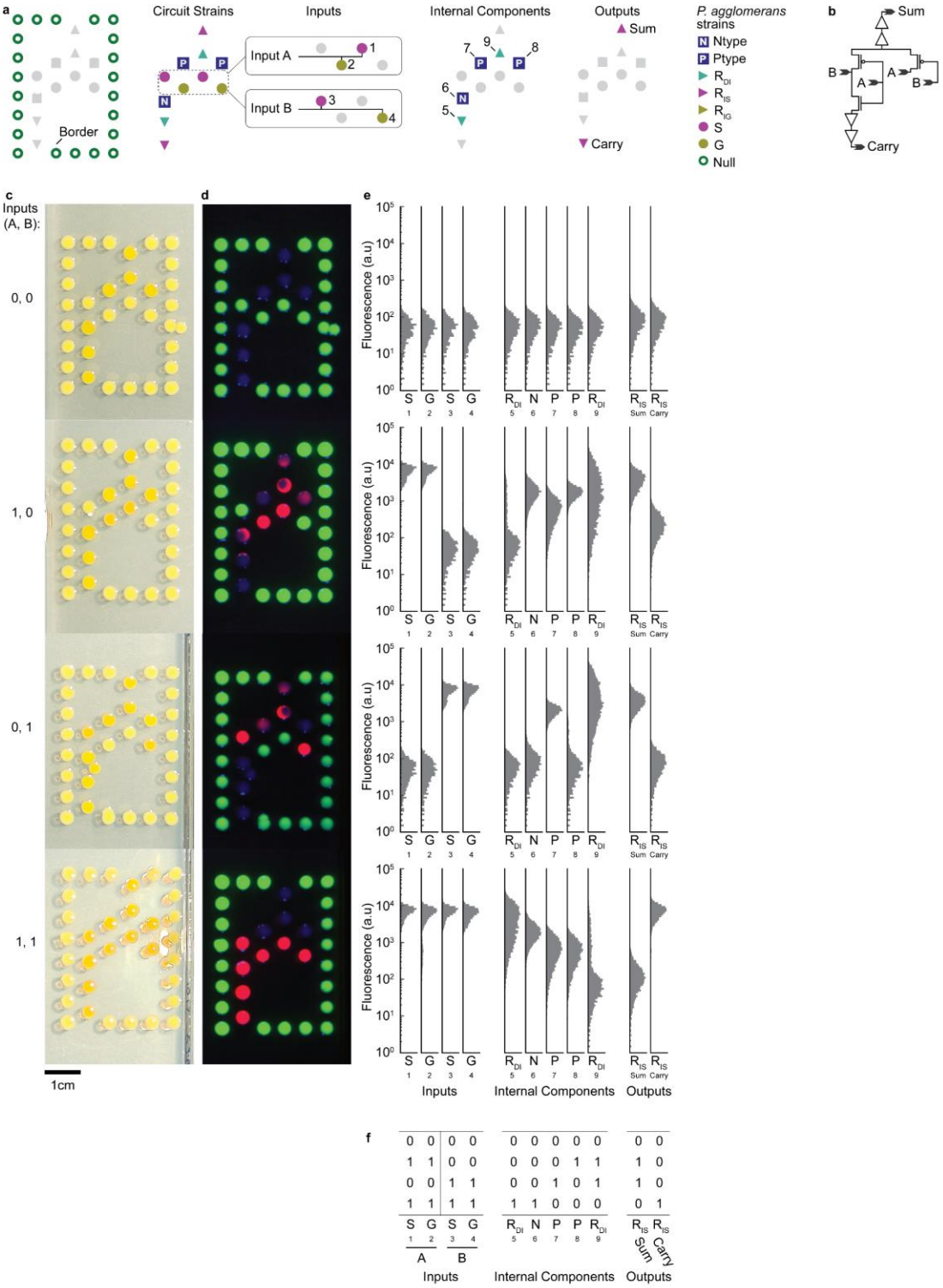
Supplementary Figure 12: **Replicates of 1-to-4 de-multiplexor plates.** These images correspond to Figure 3. Images of three replicates printed on different days. Images were taken after 72 hr (Methods). From top to bottom: overlayed false-colored, red fluorescence, green fluorescence, and white light images. Green colonies are *P. agglomerans* Null and transistor/relay colonies are red if they are in the ON state (mCherry). White light images are shown with automatically detected colonies circled in red.



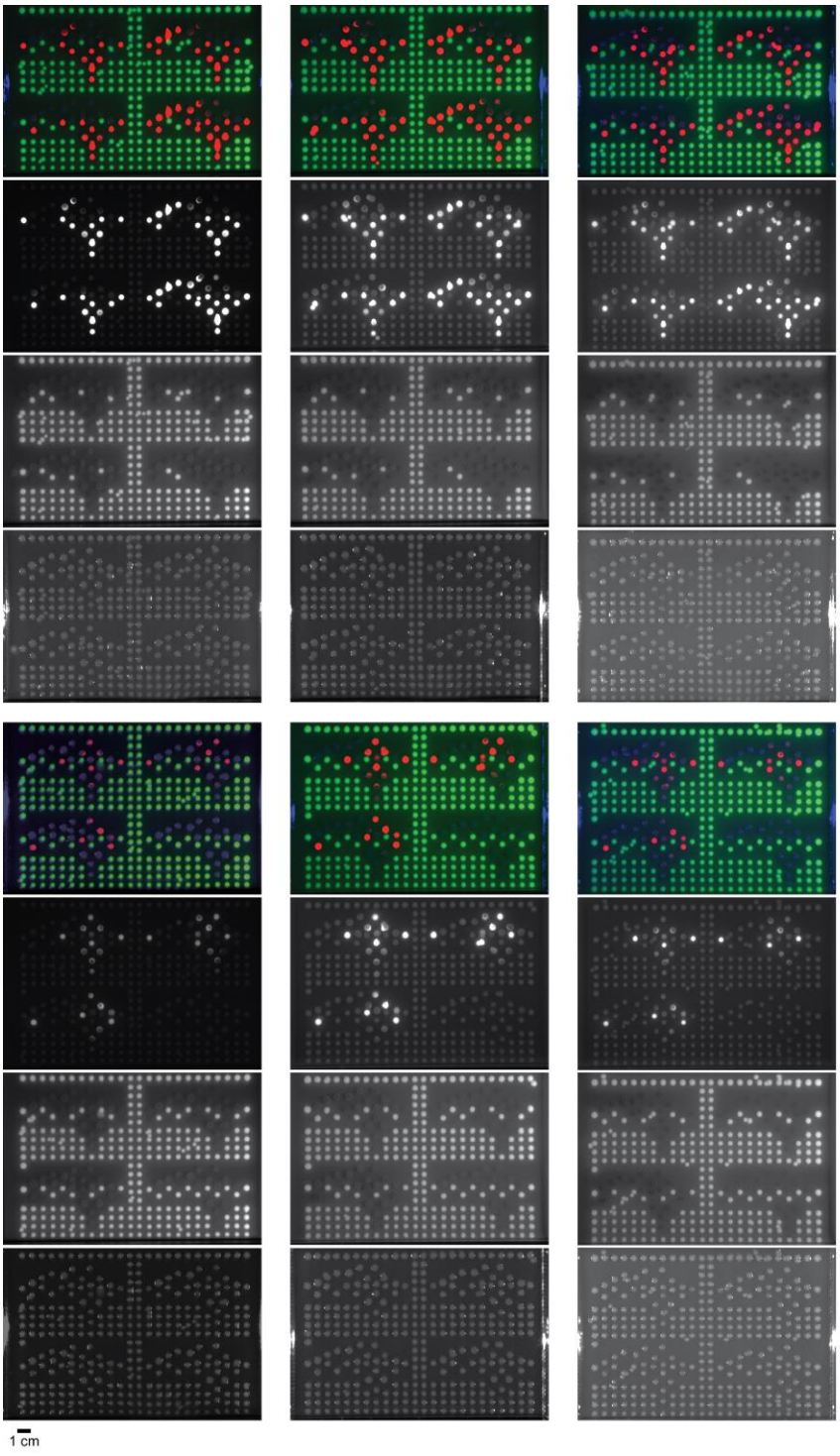
Supplementary Figure 13: **1-to-4 de-multiplexor flow cytometry distributions.** These images and data correspond to Figure 3. **(a)** Colony layouts. These examples show the *P. agglomerans* S strain as the ON input S and *P. agglomerans* G strains as the ON/ON inputs A/B. For different combinations of inputs, *P. agglomerans* Null was used for the OFF state. **(b)** The circuit diagram for the de-multiplexor is shown. Inputs S/A/B and outputs 19/20/21/22 are shown as black tags. **(c,d)** On-plate responses. The bright light (c) and fluorescent images (d) of the colonies are shown. Green colonies were *P. agglomerans* Null and transistor/relay colonies are red if they were in the ON state (mCherry). Scale bars are 1 cm. **(e)** Colonies were scraped and analyzed by flow cytometry, distributions are shown **(f)** The truth tables of the circuits.



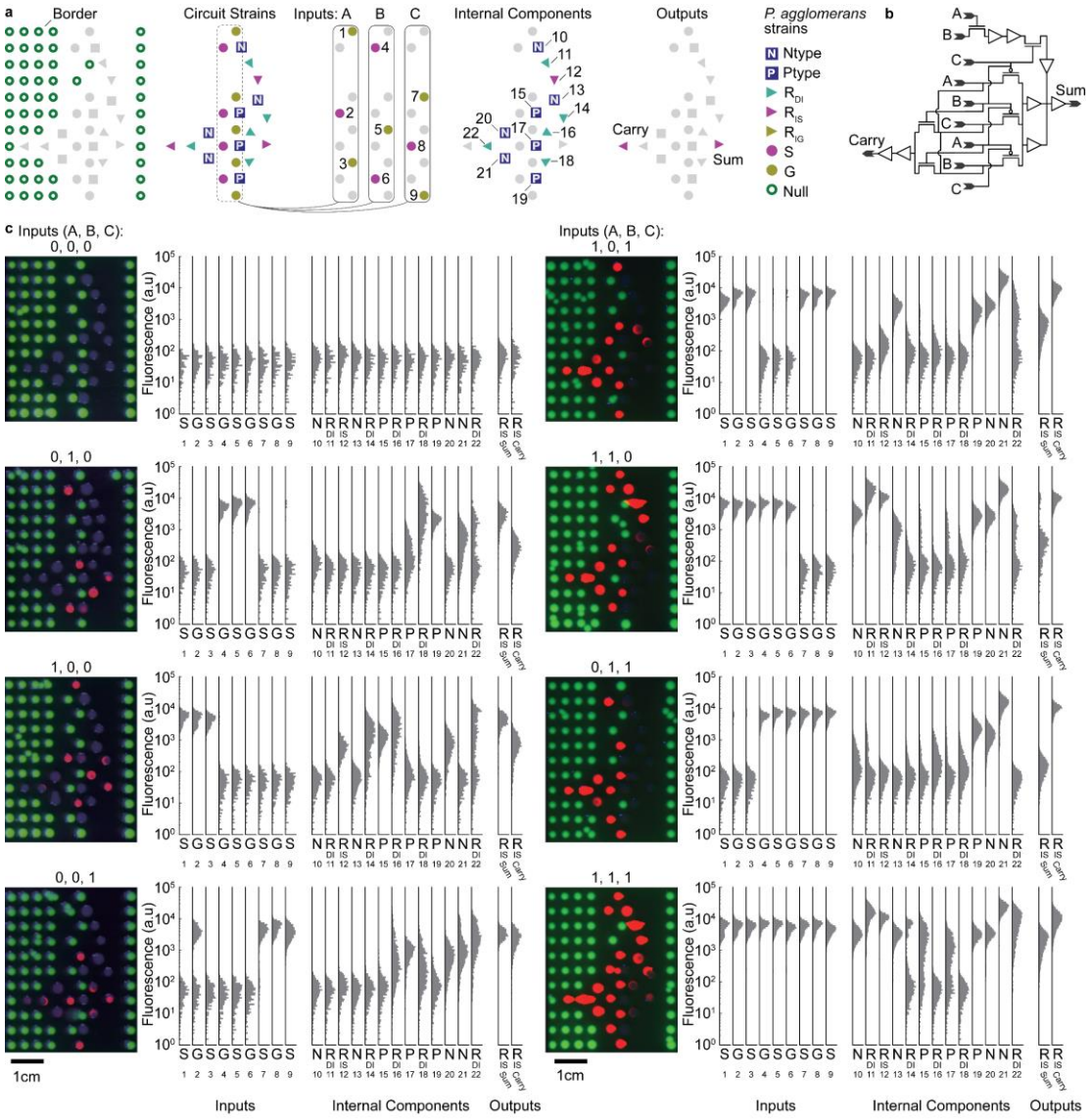
Supplementary Figure 14: **Replicates of half adder plates.** These images correspond to Figure 4. Images of three replicates printed on different days. Images were taken after 72 hr (Methods). From top to bottom: overlayed false-colored, red fluorescence, green fluorescence, and white light images. Green colonies are *P. agglomerans* Null and transistor/relay colonies are red if they are in the ON state (mCherry).

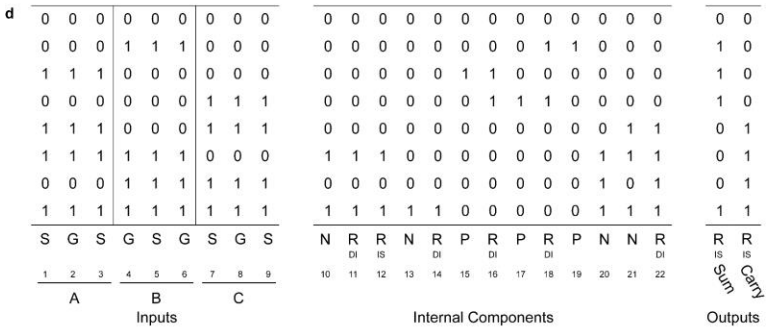


Supplementary Figure 15: **Half-adder flow cytometry distributions.** These images and data correspond to Figure 4. **(a)** Colony layouts. These examples show the *P. agglomerans* S/G strains as the ON/ON inputs A/B. For different combinations of inputs, *P. agglomerans* Null was used for the OFF state. **(b)** The circuit diagram for the half-adder is shown. Inputs A/B and outputs Sum/Carry are shown as black tags. **(c,d)** On-plate responses. The bright light (c) and fluorescent images (d) of the colonies are shown. Green colonies were *P. agglomerans* Null and transistor/relay colonies are red if they were in the ON state (mCherry). Scale bars are 1 cm. **(e)** Colonies were scraped and analyzed by flow cytometry. **(f)** The truth tables of the circuits.

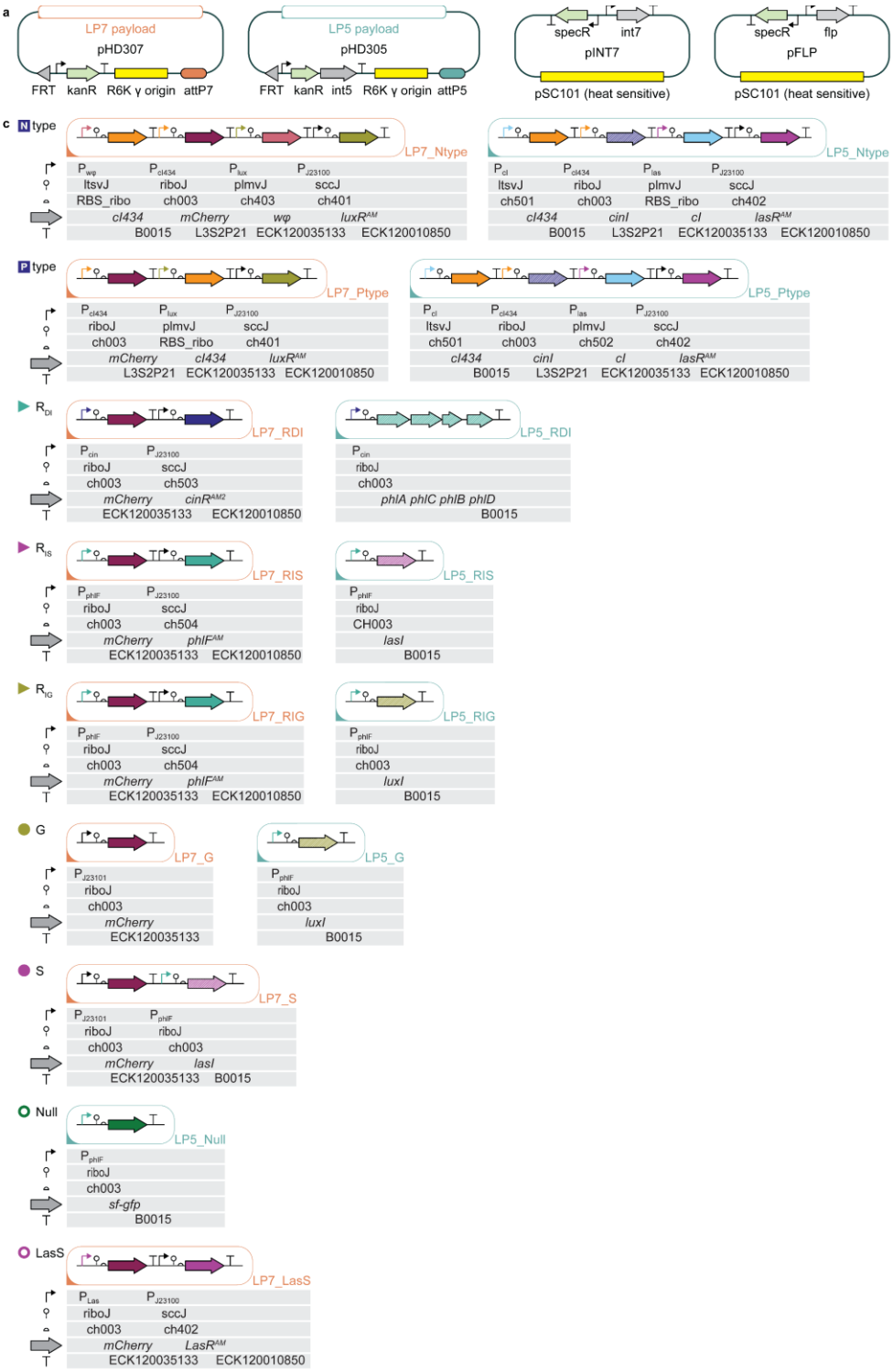


Supplementary Figure 16: **Replicates of full adder plates.** These images correspond to Figure 5. Images of three replicates printed on different days. Images were taken after 72 hr (Methods). From top to bottom: overlayed false-colored, red fluorescence, green fluorescence, and white light images. Green colonies are *P. agglomerans* Null and transistor/relay colonies are red if they are in the ON state (mCherry).





Supplementary Figure 17: **Full adder flow cytometry distributions.** These images and data correspond to Figure 5. **(a)** Colony layouts. These examples show the *P. agglomerans* S/G strains as the ON/ON/ON inputs A/B/C. For different combinations of inputs, *P. agglomerans* Null was used for the OFF state. **(b)** The circuit diagram for the full adder is shown. Inputs A/B/C and outputs Sum/Carry are shown as black tags. **(c) (left)** On-plate responses. The fluorescent images of the colonies are shown. Green colonies were *P. agglomerans* Null and transistor/relay colonies are red if they were in the ON state (mCherry). Scale bars are 1 cm. **(right)** Colonies were scraped and analyzed by flow cytometry. **(d)** The truth tables of the circuits.



<u>Supplementary Figure 18:</u> **Plasmids and genetic constructs used for strain engineering.** **(a)** Cloning plasmids used to move DNA payloads into and out of landing pads, including regulatory elements and genes. **(b)** Schematic of genetic inserts integrated into two chromosomal landing pads, LP7 (left) and LP5 (right), in *P. agglomerans*. Constructs show regulatory elements and genes for cellular transistors (N-type and P-type), relays (drain relay, gate relay, and source relay), and input strains. For each strain x, a pHD307 plasmid containing LP7_x as payload and a pHD305 plasmid containing LP5_x as payload were built. Full sequences of the inserts are provided in Supplementary Table 3 and all genetic parts are in Supplementary Table 2.

Supplementary Table 1: Strain genotypes		
Strain name	**Genotype**	**Notes**
P. agglomerans sHD307	glmS-pstS::{DT60 FRT attB7 DT104}	
P. agglomerans sHD382	glmS-pstS::{DT60 FRT attB7 DT104} rsd-thiC::{DT5 FRT attB5 DT101}	
P. agglomerans sHD400	glmS-pstS::{DT60 FRT attB7 DT104} rsd-thiC::{DT5 FRT attB5 DT101} azuC::{DT54 FRT attB2 DT3}	
P. agglomerans S	glmS-pstS::{DT60 FRT pJ23101-mCherry RSF-ori pAmp-KanR FRT pWΦ-LasI DT104}	
P. agglomerans G	glmS-pstS::{DT60 FRT pJ23101-mCherry DT104} rsd-thiC::{DT5 FRT DT54 RSF-ori DT60 pAmp-KanR FRT pPhlF-luxI DT101}	
P. agglomerans Ptype	inv(azuC-glmS) mntP-pstS::{DT54 FRT RSF-ori pAmp-KanR FRT pWΦ-cI434 pCI434-mCherry pLux-WΦ pJ23100-LuxR^{AM} DT104} rsd-thiC::{DT5 FRT pCI-cI434 pCI434-cinI pLas-cI pJ23100-LasR^{AM} DT101} azuC::{DT60 FRT attB2 DT3}	a
P. agglomerans Ntype	inv(azuC-glmS) mntP-pstS::{DT54 FRT RSF-ori pAmp-KanR FRT pCI434-mCherry pLux-cI434 pJ23100-LuxR^{AM} DT104} rsd-thiC::{DT5 FRT pCI-cI434 pCI434-cinI pLas-cI pJ23100-LasR^{AM} DT101} azuC::{DT60 FRT attB2 DT3}	a
P. agglomerans R_{DI}	inv(azuC-glmS) mntP-pstS::{DT54 RSF-ori pAmp-KanR FRT pCin-mCherry pJ23100-CinR^{AM} DT104} rsd-thiC::{DT5 pCin-phlACBD DT101} azuC::{DT60 FRT attB2 DT3}	a
P. agglomerans R_{IS}	glmS-pstS::{DT60 FRT RSF-ori int7 pAmp-KanR FRT pPhlF-mCherry pJ23100-PhlF^{AM} DT104} rsd-thiC::{DT5 FRT pPhlF-LasI DT101} azuC::{DT54 FRT attB2 DT3}	b
P. agglomerans R_{IG}	inv(azuC-glmS) mntP-pstS::{DT54 pPhlF-mCherry pJ23100-phlF^{AM} DT104} rsd-thiC::{DT5 FRT DT54 RSF-ori DT60 int5 pAmp-KanR FRT pPhlF-luxI DT101} azuC::{DT60 FRT attB2 DT3}	a
P. agglomerans Null	glmS-pstS::{DT60 FRT attB7 DT104} rsd-thiC::{DT5 FRT DT54 RSF-ori DT60 pAmp-KanR FRT pPhlF-sfGFP DT101} azuC::{DT54 FRT attB2 DT3}	
P. agglomerans LasS	glmS-pstS::{DT60 FRT RSF-ori pAmp-KanR FRT pLas-mCherry pJ23100-LasR^{AM} DT104} rsd-thiC::{DT5 FRT attB5 DT101}	

a. *Sequencing revealed inversion of the genomic sequence between azuC and glmS after strain construction*
b. *In this strain, pHD307 was modified to include int7 and used for integration*

Supplementary Table 2: Sequences of genetic parts		
Name	**Sequence**	**Reference**
Promoters		
P_{J23100}	TTGACGGCTAGCTCAGTCCTAGGTACAGTGCTAGC	iGEM J23100
P_{J23101}	GATAAGTCCCTAACTTTTACAGCTAGCTCAGTCCTAGGTATTATGCTAGC	6
P_{J23105}	GGCGCGCCTTTACGGCTAGCTCAGTCCTAGGTACTATGCTAGCAAGGT	6
P_{ampR}	TTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGA	*P. aeruginosa* genome
P_{cI}	TAACACCGTGCGTGTTGACTATTTTACCTCTGGCGGTGATAAT	7
P_{cI434}	TTGACAAACAAGATACATTGTATGAAAATACAAGAAAGTTTGT	7
$P_{w\phi}$	CATTTGACAGTAACCTATTGGTGACTTATATTCCCGTCAAAAGGTTGTGTATTGGTGACCTT	*Escherichia phage* vB_EcoM-12474III
P_{lux*H}	GCTACGGAACTCTTGTGCGTACCTGTAGGATCTTACAAGTTTACGCAAGAAAATGGTTTGTTATTTTCGAATAAA	8, 9
P_{las}	AACTAGCAAATGAGATAGATTTCGGTGAACCCGGACCCTTGCTAGGCTCGAAGAA	9
P_{cin}	GACATGCTCTGATCCCCCTCATCTGGGGGGGCCTATCTGAGGGAATTTCCGATCCGGCTCGCCTGAACCATTCTGCTTTCCACGAACTTGAAAACGCT	iGEM R0078
P_{phlF}	CGACGTACGGTGGAATCTGATTCGTTACCAATTGACATGATACGAAACGTACCGTATCGTTAAGGT	10
Ribosome Binding Sites		
BB0032	TCACACAGGAAAG	IGEM B0032
RBS_ch401	AGGTTCCAAAGACAGGTAATAAGGAGGTAGC	This work
RBS_ch402	ATATAATAATAACACTAAGGAGGT	This work
RBS_ch403	GAGCGATACTTATGCTAC	This work
RBS_ch003	TGGTTCCAAAGCCAGATACTAAGGAGGTCCC	This work
RBS_ch501	GAGCGATACTTAGGCTACCAG	This work
RBS_ch502	CCTCTACAAATAATTTTGTTTAAGGATAA	This work
RBS_ch503	ATATAATAATAACAATTAGGACGT	This work
RBS_ch504	ATATAATAATAACAATTAGGAGGTAGTT	This work
RBS_ribo	CTCTACAAATAATTTTGTTTAA	11
Ribozymes		
riboJ	AGCTGTCACCGGATGTGCTTTCCGGTCTGATGAGTCCGTGAGGACGAAACAGCCTCTACAAATAATTTTGTTTAA	11
sccJ	AGATGCTGTAGTGGGATGTGTGTCTCACCTGAAGAGTACAAAAGTCCGAAACGGTATCCTCTACAAATAATTTTGTTTAA	11
plmvJ	AGGAAGAGTCTGTGCTAAGCACACTGACGAGTCTCTGAGATGAGACGAAACTCTTCCCTCTACAAATAATTTTGTTTAA	11
ltsvJ	AGTACGTCTGAGCGTGATACCCGCTCACTGAAGATGGCCCGGTAGGGCCGAAACGTACCTCTACAAATAATTTTGTTTAA	11
Genes		
lasR^{AM}	ATGGCCTTGGTTGACGGTTTTCTCGAGCTGGAACGCTCAAGTGGAAAATTGGAGTTGAGCGCCATCCTGCAGAAGATGGCGAGCGACCTCGGATTCT CGAAGATCCTGTTCGGCCTGTTGCCTAAGGACAGCCAGGACTACGAGAACGCCTTCATCGTCAGCAACTACCCGGCCGCCTGGCGCGAGCATTACGA CCGAACTGGCTACGCGCGGGTCGACCCGACGGTCAGTCACTGTACCCAGAGCGTACTGCCGATTTTCTGGGAACCGCCCATCTACCAGACGCGAAAG CAGCACGAGTTCTTCGAGGAAGCCTTGGCCGCTGGCCTGGTGTATGGACTGACCATGCCGCTGCATGGTGCTCGCGGCGAACTCGGCGCGCTGAGCC TCAGCGTGGAAGCGGAAAACCGGGCCGAGGCCAACCGTTTCATGGAGTCGGTCCTGCCGACCCTGTGGATGCTCAAGGACTACGCACTGCAGAGCGG TGTCGGAATGGCCTTCGAACATCCGATCAGCAAACCGGTGGTTCTGACCAGCCGAGAGAAGGAAGTGTTACAGTGGTGCGCCATCGGCAAGACCAGC TGGGAGATATCGGTTATCTGCAACTGCTCGGAAGCCAATGTGAACTTCCATATGGGAAATATTCGGCGGAAGTTCGGTGTGACCTCCCGCCGCGTAG CGGCCATTATGGCCGTTAATTTGGGTCTTATTACTCTC	9
lasI	ATCGTTCAGATCGGTCGTCGTGAAGAGTTCGACAAAAAACTGCTGGGTGAAATGCACAAACTGCGTGCTCAGGTTTTCAAAGAACGTAAAGGTTGGG ACGTTTCCGTTATCGACGAAATGGAAATCGACGGTTACGACGCTCTGTCCCCGTACTACATGCTGATCCAGGAAGATACCCCGGAAGCTCAGGTTTT CGGTTGCTGGCGTATCTTCGACACCACCGGTCCGTACATGCTGAAAAACACCTTCCCGGAACTGCTGCACGGTAAAGAAGCTCCGTGCTCCCCGCAC ATCTGGGAACTGTCCCGTTTCGCTATCAACTCCGGTCAGAAAGGTTCCCTGGGTTTCTCCGACTGCACCCTGGAAGCTATGCGTGCTCTGGCTCGTT ACTCCTTGCAGAACGACATCCAGACCCTGGTTACCGTTACCACCGTTGGTGTTGAAAAAATGATGATCCGTGCTGGTCTGGACGTTTCCCGTTTCGG TCCGCACCTGAAAATCGGTATCGAACGTGCTGTTGCTCTGCGTATCGAACTGAACGCTAAAACCCAGATCGCTCTGTACGGTGGTGTTCTGGTTGAA CAGCGTCTGGCTGTTTCC	12
luxR^{AM}	ATGAAAAACATAAATGCCGACGACACATACAGAATAATTAATAAAATTAAAGCTTTTAGAAGCAATAATGATATTAATCAATGCTTATCTGATATGA CTAAAATGGTACATTGTGAATATTATTTACTCGCGATCATTTATCCTCATTCTATGGTTAAATCTGATATTTCAATCCTAGATAATTACCCTAAAAA ATGGAGGCAATATTATGATGACGCTAATTTAATAAAATATGATCCTATAGTAGATTATTCTAACTCCAATCATTCACCAATTAATTGGAATATATTT GAAAACAATGCTGTAAATAAAAAATCTCCAAATGTAATTAAAGAAGCGAAAACATCAGGTCTTATCACTGGGTTTAGTTTCCCTATTCATATGGCTA ACAATGGCTTCGGAATGCTTAGTTTTGCATATTCAGAAAAAGACAACTATATAGATAGTTTATTTTTACATGCGTGTATGAACATACCATTAATTGT TCCTTCTCTAGTTGATAATTATCGAAAAATAAATATAGCAAATAATAAATCAAACAACGATTTAACCAAAAGAGAAAAAGAATGTTTAGCGTGGGCA TGCGAAGGAAAAAGCTCTTGGGATATTTCAAAAATATTAGGATGCAGTGAGCGTACTGTCACTTTCCATTTAACCAATGCGCAAATGAAACTCAATA CAACAAACCGCTGCCAAAGTATTTCTAAAGCAATTTTAACAGGAGCAATTGATTGCCCATACTTTAAAAAT	9
luxI	ATGACTATAATGATAAAAAAATCGGATTTTTTGGCAATTCCATCGGAGGAGTATAAAGGTATTCTAAGCCTTCGTTATCAAGTGTTTAAGCAAAGAC TTGAGTGGGACTTAGTTGTAGAAAATAACCTTGAATCAGATGAGTATGATAACTCAAATGCAGAATATATTTATGCTTGTGATGATACTGAAAATGT AAGTGGATGCTGGCGTTTATTACCTACAACAGGTGATTATATGCTGAAAAGTGTTTTTCCTGAATTGCTTGGTCAACAGAGTGCTCCCAAAGATCCT AATATAGTCGAATTAAGTCGTTTTGCTGTAGGTAAAAATAGCTCAAAGATAAATAACTCTGCTAGTGAAATTACAATGAAACTATTTGAAGCTATAT ATAAACACGCTGTTAGTCAAGGTATTACAGAATATGTAACAGTAACATCAACAGCAATAGAGCGATTTTTAAAGCGTATTAAAGTTCCTTGTCATCG TATTGGAGACAAAGAAATTCATGTATTAGGTGATACTAAATCGGTTGTATTGTCTATGCCTATTAATGAACAGTTTAAAAAAGCAGTCTTAAATT	9
cinR^{AM2}	ATGATTGAGAATACCTATAGCGAAAAGTTCGAGTCCGCGTTCGAACAGATCAAAGCGGCGGCCAACGTGGATGCCGCCATCCGTATTCTCCAGGCGG AATATAACCTCGATTTCGTCACCTACCATCTCGCCCAGACAATCGCGAGCAAGATCGATTCGCCCTTCGTGCGCACCACCTATCCGGATGCCTGGGT TTCCCGTTACCTCCTCAACTGCTATGTGAAGGTCGATCCGATCATCAAGCAGGGCTTCGAACGCCAGCTGCCCTTCGACTGGAGCGAGGTCGAACCG ACGCCGGAGGCCTATGCCATGCTGGTCGACGCCCAGAAACACGGCATCGATGACAATGGCTACTCCATCCCCGTCGCCGACAAGGCGCAGCGCCGCG CCCTGCTGTCGCTGAATGCCCATATACCGGCCGACGAATGGACCGAGCTCGTGCGCCGCTACCGCAACGAGTGGATCGAGATCGCCCATCTGATCCA CCGCAAGGCCGTATATGAGCTGCATGGCGAAAACGATCCAGTGCCGGCATTGTCGCCGCGCGAGATCGAGTGTCTGCACTGGACCGCCCTCGGCAAG	9

	GATTACAAGGATATTTCGGTCATCCTGGGCATATCAGAGCATACCACACGCGATTACCTGAAAACCGCCCGCTTCAGGCTCGGCTGCACCACGATCT CGGCCGCCGCGTCGCGGGCTGTTCAATTGTGCATCATCAATCCCTATAGGATCCGCATGACGCGACGTAATTGG	
cinI	TTCGTTATCATCCAGGCTCACGAATACCAGAAATACGCTGCTGTTCTGGACCAGATGTTCCGTCTGCGTAAAAAAGTTTTCGCTGACACCCTGTGCT GGGACGTTCCGGTTATCGGTCCGTACGAACGTGACTCCTACGACTCCCTGGCTCCGGCTTACCTGGTTTGGTGCAACGACTCCCGTACCCGTCTGTA CGGTGGTATGCGTCTGATGCCGACCACCGGTCCGACCCTGCTGTACGACGTTTTCCGTGAAACCTTCCCGGACGCTGCTGACCTGATCGCTCCGGGT ATCTGGGAAGGTACCCGTATGTGCATCGACGAAGAAGCTATCGCTAAAGACTTCCCGGAAATCGACGCTGGTCGTGCTTTCTCCATGATGCTGCTGG CTCTGTGCGAATGCGCTCTGGACCACGGTATCCACACCATGATCTCCAACTACGAACCGTACCTGAAACGTGTTTACAAACGTGCTGGTGCTGAAGT TGAAGAACTGGGTCGTGCTGACGGTTACGGTAAATACCCGGTTTGCTGCGGTGCTTTCGAAGTTTCCGACCGTGTTCTGCGTAAAATGCGTGCTGCT CTGGGTCTGACCCTGCCGCTGTACGTTCGTCACGTTCCGGCTCGTTCCGTTGTTACCCAGTTCCTGGAAATGGCTGCT	13
phlF	GCACGTACCCCGAGCCGTAGCAGCATTGGTAGCCTGCGTAGTCCGCATACCCATAAAGCAATTCTGACCAGCACCATTGAAATCCTGAAAGAATGTG GTTATAGCGGTCTGAGCATTGAAAGCGTGGCACGTCGCGCCGGTGCAGGCAAACCGACCATTTATCGTTGGTGGACCAACAAAGCAGCACTGATTGC CGAAGTGTATGAAAATGAAATCGAACAGGTACGTAAATTTCCGGATTTGGGTAGCTTTAAAGCCGATCTGGATTTTCTGCTGCATAATCTGTGGAAA GTTTGGCGTGAAACCATTTGTGGTGAAGCATTTCGTTGTGTTATTGCAGAAGCACAGTTGGACCCTGTAACCCTGACCCAACTGAAAGATCAGTTTA TGGAACGTCGTCGTGAGATACCGAAAAAACTGGTTGAAGATGCCATTAGCAATGGTGAACTGCCGAAAGATATCAATCGTGAACTGCTGCTGGATAT GATTTTTGGTTTTTGTTGGTATCGCCTGCTGACCGAACAGTTGACCGTTGAACAGGATATTGAAGAATTTACCTTCCTGCTGATTAATGGTGTTTGT CCGGGTACACAGTGT	14
phlA	AACGTGAAAAAGATAGGTATTGTCAGCTATGGCGCGGGTATTCCGGTATGCCGCCTGAAAGTCCAGGAAGTGATCAACGTCTGGAAAAACACCGACC TCAAGCTGGTGGAGGAAAACCTCGGCGTCACGGAAAGAGCCGTGCTGCAACCGGATGAAGATGTCATCACCCTCGGGGTGCTGGCGGCGCAACGGGC CCTGGATAAAGTCCCCGGTCATCAGATCGAAGCCCTGTACCTGGGCACCTGCACCAATCCCTATGACTCACGGGCCTCGGCTTCGATCATCCTGGAA ATGCTCGGCAGCGGCTACGACGCCTATTGCGCCGACGTGCAGTTCGCCGGCAAGTCCGGAACTTCTGCCCTGCAGATCTGCCAGGCGCTGGTGGCTT CCGGCATGACGGGCAGCGCCCTGGCCATCGGCGCCGACACCATCAACCGCAACACCGCGCCGGGCGACCTGACCGAATCCTATGCCGGGGCCGGAGC CGCTGCGCTGCTGATCGGCAGCCAGGATGTCATCGCCGAATTCGACGCCAGCTTCTCCTGCGCCGCCGATGTCGCCGACAACATCCGCCCCCAGGGC GATCGCTACATTCGTTCGGGCATGGGCCTGGGCTCGGACAAGAACAGCATCGGCCTGGAAGATCAGACCCGCCGCGCCGCCGAAGGCCTGATGGCCA AGCTGCACACCAGCCCCGCCGACTATGACTATGTGGTGTTCCAGCAAAACCTGGTGTCCACGCCCTACTCCCTGGCCAAGCACCTGGGCTTCAACCC CAAGCAGGTGGAACCGGGCATCTATGCCGGCAACGTTGGCGATGCCGGATCGGCCAGCCCGCTGCTCGGCCTGATCAACGTACTGGACCAGGCACGC CCGGGGCAGAAAATTCTTTTGGTGTCCTACGGTTTCGGCGCCGGCAGCGACGCCATCGCCCTGACCGTCACCGACGCCATCGAGCAGTACCAGAAGC ACAACAAGCCTCTGCGCGAACTGCTGGAATCCAAGATCTACGTCGACTACGGCACGTCCATCAAGTACGAGTTCAAGTACCTGCGGGCTGACTACGC CCTGACCGCCTATCTC	*P. fluorescens* genome
phlC	ATGTGCGCACGTCGTGTTGCAATCGTTTCGGCCGCCTATACGCCGAAACCCGGAAGTTCACGAGTTCGGCAGACGTTCAAGGAAATGATCGTCGAGT CCGCCTACAAGGCACTCAAGGACGCCAAGATGCACCCCCGGGAAATCCAGGCCGTGGCCTATGGTTATCACGGTGAAGGCATTTCCGAATATGGCGG CCTGGGCCCGACCATTTCCGACGCCCTGGGCATCAGCCCGGCGCCGACCTTCATGAGCACCGCCAACTGCACCAGCAGCTCGGTGTCGTTCCAGATG GGCCACCAGATGGTGGCGTCCGGGGAGTACGACATCGTCCTATGCGGCGGCTTCGAGAAGATGACCGATCACTTCAACTACGCCGAATACATCGGCT CCAGCACTGAATGTGAATACGATTATTTCCTCGGCATTTCCCACACCGATGCCTTCGCCCTGGCCACCGCCGAGTACTTCCAGAAGTTCGGCTATGC CGGTCGCGAGGCCGACGTACTGGCCACCTTCGGCCGGCAGATGCGCATCTACGCACAGAACACCCCCACCGCCACCCGTTATGGCCAGCCGATTCCA TCCCTGGAAGTGTTGAAGAACAGCGAAGCCTGCGGCTCGATGCTGGCCTGGGGCGAAGCCAGTGGCTGCGCGATCCTGGTGGCCGAACACCTGGCCC ACAAGTACACCGATAAACCGGTGTTCGTACGCGGTTGCGCCTATACCGGGGTTTCCCACTATTTCGGTACGCGCTTTCAACAACCGACCCTGCATCA TCCGGGCCTGCCCAAGGATGTGGGCATGGCGGTTTCGGCCAACTCCATCGCCTGTGCGGAGATCGCCTACAAGAAAGCCGGGATCACCGCCAAGGAC ATCGACGTGGCCCAGGTCTACGACCTGCTCGGCGCCGGGCTGATCCAGATGGAGTCCATGGGCATCTGTGGCAAGGGCCAGGCCGGCGACTTCGTGC TCGAAGGCGGCATCGCCCTGGATGGCCAGCTGCCGCTCAATACCGACGGCGGCAACATCGGCCGCGGCCATGCCTCCGGCTGCGACGGCATCCTGCA CATCACCGAGCTGTTCCGGCAGCTGAGGGGTGAATCCGACAACCAGGTCAAGGGCGCGCGCATCGGCGTGTCGCAGAACCTTGGCGGTTACGCGGCG CACAACTCAGTGATTGTCCTCTCGAACGAC	*P. fluorescens* genome
phlB	ATGTCCATGTACCCAGAACAGATCCACAGAATGACCACCGCCAGCATGCTTCGCGAATGGCGCGAGCATGGCGGCAAGTACCGCCTCGAAGGCAGCC AGTGCGAAGAATGCAACGAAATCTTCTTCCCCCGGCGCACCGTCTGCGGCGCTTGCAACTCCCTGAGTGTGAAGCCGTATCGCTGCGCGCGCAGTGG CAAGATCGAGGTCATGGCCCCGGCCGAGAACCCGATCCTGGCCGCCATGGGCTATGGCGAAACCGTGCCGCGCATCATGGCCATGGTGCGCCTGGAC GACGGCCTGGTGATCGCTTCGGAAATCGTCGATGTGTGCGATCAGCAACAGCTGAAAGTCGGTGCGCCGGTGCGCATGGTGATCCGCAAGCATGTGC GCGAAAGCAACCTGGCCTGGCAATACGCTTATAAGTTCGTACTCGATATA	*P. fluorescens* genome
phlD	ATGTCTACACTTTGCCTTCCACACGTCATGTTTCCGCAACACAAGATCACCCAGCAACAGATGGTCGATCACCTGGAAAACCTGCACGCCGACCATC CACGCATGGCCCTGGCCAAGCGCATGATCGCCAACACCGAAGTCAACGAGCGCCACCTGGTGTTGCCGATCGACGAACTGGCAGTGCACACCGGTTT CACCCACCGCAGCATCGTCTACGAGCGTGAAGCCCGGCAGATGTCGTCGGCCGCGGCGCCGCCAGGCCATCGAGAATGCCGGGCTGCAGATCAGCGAC ATTCGCATGGTGATCGTCACTTCCTGCACCGGCTTCATGATGCCGTCGCTGACCGCGCACCTGATCAACGACCTGGCCCTGCCAACCTCCACCGTGC AGTTGCCGATCGCCCAGCTGGGCTGCGTGGCCGGTGCCGCGGCCATCAACCGCGCCAACGACTTCGCCCGGCTCGATGCCCGCAACCACGTACTGAT CGTGTCCCTGGAATTCTCCTCGCTGTGCTACCAGCCGGACGACACCAAGCTGCACGCCTTCATCTCCGCGGCGCTGTTCGGCGATGCGGTATCCGCC TGCGTGCTGCGCGCCGATGACCAGGCCGGCGGCTTCAAGATCAAGAAAACCGAGTCGTACTTCCTGCCCAAGAGCGAGCACTACATCAAGTACGACG TGAAGGACACCGGCTTTCACTTCACCCTCGACAAGGCGGTGATGAACTCCATCAAGGACGTGGCACCGGTCATGGAGCGGCTCAACTACGAGAGCTT CGAACAGAACTGTGCGCACAACGACTTCTTCATCTTCCACACCGGTGGTCGCAAGATCCTCGACGAGCTGGTGATGCACCTGGACCTGGCATCCAAC CGGGTTTCGCAATCGCGCAGCAGCCTGTCGGAAGCCGGCAACATTGCCAGCGTGGTGGTGTTCGACGTACTCAAGCGGCAGTTCGATTCCAACCTCA ATCGCGGCGACATCGGCCTGCTGGCAGCCTTCGGCCCGGGGTTCACCGCGGAAATGGCGGTGGGCGAGTGGACCGCC	*P. fluorescens* genome
cI	AGCACAAAAAAGAAACCATTAACACAAGAGCAGCTTGAGGACGCACGTCGCCTTAAAGCAATTTATGAAAAAAAGAAAAATGAACTTGGCTTATCCC AGGAATCTGTCGCAGACAAGATGGGGATGGGGCAGTCAGGCGTTGGTGCTTTATTTAATGGCATCAATGCATTAAATGCTTATAACGCCGCATTGCT TGCAAAAATTCTCAAAGTTAGCGTTGAAGAATTTAGCCCTTCAATCGCCAGAGAAATCTACGAGATGTATGAAGCGGTTAGTATGCAGCCGTCACTT AGAAGTGAGTATGAGTACCCTGTTTTTTCTCATGTTCAGGCAGGGATGTTCTCACCTGAGCTTAGAACCTTTACCAAAGGTGATGCGGAGAGATGGG TAAGCACAACCAAAAAAGCCAGTGATTCTGCATTCTGGCTTGAGGTTGAAGGTAATTCCATGACCGCACCAACAGGCTCCAAGCCAAGCTTTCCTGA CGGAATGTTAATTCTCGTTGACCCTGAGCAGGCTGTTGAGCCAGGTGATTTCTGCATAGCCAGACTTGGGGGTGATGAGTTTACCTTCAAGAAACTG ATCAGGGATAGCGGTCAGGTGTTTTTACAACCACTAAACCCACAGTACCCAATGATCCCATGCAATGAGAGTTGTTCCGTTGTGGGGAAAGTTATCG CTAGTCAGTGGCCTGAAGAGACGTTTGGCTGA	7
cI434	ATGAGTATTTCGAGCCGTGTGAAAAGCAAACGCATTCAGCTTGGACTTAATCAAGCCGAACTGGCCCAAAAAGTCGGCACCACTCAGCAGAGCATCG AGCAACTTGAAAACGGCAAAACAAAACGCCCCCGTTTTCTTCCGGAGTTAGCGAGCGCCTTGGGTGTATCAGTCGACTGGCTGCTTAACGGCACATC GGACTCAAATGTGCGTTTTGTAGGTCATGTAGAGCCTAAAGGTAAGTATCCGTTAATTAGCATGGTACGCGCTGGAAGTTGGTGCGAAGCGTGTGAG CCCTATGATATTAAAGACATCGACGAATGGTATGATAGCGATGTGAACCTTCTTGGAAACGGCTTCTGGCTTAAAGTCGAGGGGGACTCAATGACGT CGCCCGTTGGCCAGTCAATCCCTGAGGGTCACATGGTACTTGTAGATACGGGTCGTGAACCAGTCAATGGCTCTTTGGTTGTAGCCAAGCTGACAGA TGCAAACGAAGCTACCTTTAAGAAACTGGTCATCGACGGGGGGCAGAAGTATTTAAAAGGGTTAAATCCAAGCTGGCCTATGACCCCCATCAATGGC AACTGTAAAATCATCGGAGTTGTGGTGGAAGCCCGCGTTAAATTCGTC	7
wφ	CAGACATTCGAAAAACTGAAAGCGATTAGGAAAGCAGAAGGCTTAACACAGGCGAAATTCAGCGAAATTAGCGGGATAGCTCTAGGAACAGTCAAAA ATTACGAAAGTGGGCATAAAGACCCTGGTCTGAGCATCGTTATGCGAGTCACAAATACGCCTTTATTTAAAAAATATACGCTCTGGTTAATGACTGG TGATACGTCACCACAAGCTGGTCAGATCGCGCCGGCTCTCGCACACATTGGGCAAAAACCAACAGAATCAGACCACTCCGAAAAACAGACTGGTTA	Escherichia phage vB_EcoM-12474III
int5	ATGCCTGGTATGACCACCGAAACCGGTCCGGATCCTGCAGGTCTGATTGACCTGTTTTGTCGTAAAAGCAAAGCAGTTAAAAGCCGTGCAAATGGTG CAGGTCAGCGTCGTAAACAAGAAATTAGCATTGCAGCACAAGAAACCCTGGGTCGTAAAGTTGCAGCACTGCTGGGTATGCAGGTTCGTCATGTTTG GAAAGAAGTTGGTAGCGCAAGCCGTTTTCGTAAAGGTAAAGCACGTGATGATCAGAGCAAAGCACTGAAAGCCCTGGAAAGCGGTGAAGTTGGTGCA CTGTGGTGTTATCGTCTGGATCGTTGGGATCGTGGTGGTGCCGGTGCAATTCTGAAAATTATCGAACCGGAAGATGGTATGCCTCGTCGTCTGCTGT TTGGTTGGGATGAAGATACCGGTCGTCCGGTTCTGGATAGCACCAATAAACGTGATCGCGGTGAACTGATTCGTCGTGCAGAAGAAGCACGCGAAGA AGCAGAAAAACTGAGCGAACGTGTTCGTGATACCAAAGCACATCAGCGTGAAAATGGTGAATGGGTTAATGCCCGTGCACCGTATGGTCTGCGTGTT GTTCTGGTTACCGTTAGTGATGAAGAGGGTGATGAATATGATGAACGTAAACTGGCAGCAGATGATGAAGATGCGGGTGGTCCTGATGGTCTGACCA AAGCAGAAGCAGCCCGTCTGGTTTTTACCCTGCCGGTTACCGATCGTCTGAGCTATGCAGGCACCGCACATGCAATGAATACCCGTGAAATTCCGAG CCCGACCGGTGGTCCGTGGATTGCAGTTACCGTGCGTGATATGATTCAGAATCCGGCATATGCGGGTTGGCAGACCACAGGTCGTCAGGATGGTAAA CAGCGTCGTCTGACCTTTTATAACGGTGAAGGTAAACGTGTTAGCGTTATGCATGGTCCTCCGCTGGTGACCGATGAAGAACAAGAAGCCGCAAAAG	6

	CAGCCGTTAAAGGTGAAGATGGTGTTGGTGTTCCGCTGGATGGTAGCGATCATGATACCCGTCGCAAACATCTGCTGAGCGGTCGTATGCGTTGTCC GGGTTGTGGTGGTAGCTGTAGCTATAGCGGTAATGGTTATCGTTGTTGGCGTAGCAGTGTGAAAGGTGGTTGTCCGGCACCGACCTATGTTGCACGT AAAAGCGTTGAAGAATATGTTGCATTTCGTTGGGCAGCAAAATTAGCAGCAAGCGAACCGGATGATCCGTTTGTTATTGCAGTTGCAGATCGCTGGG CAGCACTGACCCATCCGCAGGCAAGCGAAGATGAAAAGTATGCAAAAGCCGCAGTTCGTGAAGCCGAAAAAAATCTGGGTCGCCTGCTGCGTGATCG TCAGAATGGTGTTTATGATGGTCCGGCAGAACAGTTTTTTGCCCCTGCATATCAAGAAGCACTGAGCACCCTGCAGGCAGCCAAAGATGCAGTTAGC GAAAGCAGCGCAAGCGCAGCAGTTGATGTTAGCTGGATTGTTGATAGCAGCGATTATGAAGAACTGTGGCTGCGTGCAACCCCGACCATGCGTAATG CAATTATTGATACCTGCATCGATGAAATTTGGGTTGCAAAAGGCCAGCGTGGTCGTCCGTTTGATGGTGATGAACGCGTTAAAATCAAATGGGCAGC CCGTACC	
Int7	ATGAAAGTGGCCATTTATGTTCGTGTTAGCACCGATGAACAGGCCAAAGAAGGTTTTAGCATTCCGGCACAGCGTGAACGTCTGCGTGCATTTTGTG CAAGCCAGGGTTGGGAAATTGTGCAAGAATATATTGAAGAAGGTTGGAGCGCAAAAGATCTGGATCGTCCGCAGATGCAGCGTCTGCTGAAAGATAT CAAAAAAGGCAACATTGATATTGTGCTGGTGTATCGTCTGGATCGCCTGACCCGTAGCGTTCTGGATCTGTATCTGCTGCTGCAGACCTTTGAAAAA TACAATGTGGCATTTCGTAGCGCCACCGAAGTTTATGATACCAGCACCGCAATGGGTCGTCTGTTTATTACCCTGGTTGCAGCACTGGCACAGTGGG AACGTGAAAATCTGGCAGAACGTGTTAAATTTGGTATCGAGCAGATGATCGATGAAGGTAAAAAACCGGGTGGTCATAGCCCGTATGGTTACAAATT TGATAAAGACTTCAATTGCACCATTATTGAGGAAGAAGCAGACGTTGTTCGTATGATCTATCGCATGTATTGTGATGGTTATGGCTATCGTAGCATT GCAGATCGTCTGAATGAACTGATGGTTAAACCGCGTATTGCCAAAGAATGGAATCATAATAGCGTGCGTGATATCCTGACCAACGATATCTATATTG GCACCTATCGTTGGGGTGATAAAGTTGTTCCGAATAATCATCCGCCTATTATTAGCGAAACCCTGTTCAAAAAAGCCCAGAAAGAAAAAGAAAAACG TGGCGTTGATCGTAAACGCGTTGGTAAATTTCTGTTTACCGGTCTGCTGCAGTGTGGTAATTGTGGTGGCCATAAAATGCAGGGCCATTTTGATAAA CGTGAGCAGAAAACCTATTACCGTTGTACCAAATGTCACCGCATTACCAACGAAAAAAACATTCTGGAACCGCTGCTGGATGAAATTCAGCTGCTGA TTACCAGCAAAGAATACTTTATGAGCAAATTCAGCGACCGCTATGATCAGCAAGAGGTTGTTGATGTTAGCGCACTGACAAAAGAACTGGAAAAAAT CAAACGCCAGAAAGAGAAATGGTACGATCTGTATATGGATGATCGTAACCCGATTCCGAAAGAAGAACTGTTTGCCAAAATTAACGAACTGAACAAA AAAGAAGAAGAAATCTATAGCAAGCTGAGCGAAGTGGAAGAAGATAAAGAACCGGTTGAAGAGAAATATAACCGCCTGAGCAAAATGATCGATTTTA AACAGCAGTTTGAGCAGGCCAACGACTTTACCAAAAAAGAGCTGCTGTTCAGCATCTTCGAAAAGATTGTGATTTATCGCGAGAAAGGCAAGCTGAA AAAAATCACCCTGGATTACACCCTGAAATAA	6
specR	ATGGCTTGTTATGACTGTTTTTTTGGGGTACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGTCGATGTTTGATGTTATGGA GCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCTAAAACAAAGTTAAACATCATGAGGGAAGCGGTGATCGCCGAAGTATCGACTCAACTATCAGA GGTAGTTGGCGTCATCGAGCGCCATCTCGAACCGACGTTGCTGGCCGTACATTTGTACGGCTCCGCAGTGGATGGCGGCCTGAAGCCACACAGTGAT ATTGATTTGCTGGTTACGGTGACCGTAAGGCTTGATGAAACAACGCGGCGAGCTTTGATCAACGACCTTTTGGAAACTTCGGCTTCCCCTGGAGAGA GCGAGATTCTCCGCGCTGTAGAAGTCACCATTGTTGTGCACGACGACATCATTCCGTGGCGTTATCCAGCTAAGCGCGAACTGCAATTTGGAGAATG GCAGCGCAATGACATTCTTGCAGGTATCTTCGAGCCAGCCACGATCGACATTGATCTGGCTATCTTGCTGACAAAAGCAAGAGAACATAGCGTTGCC TTGGTAGGTCCAGCGGCGGAGGAACTCTTTGATCCGGTTCCTGAACAGGATCTATTTGAGGCGCTAAATGAAACCTTAACGCTATGGAACTCGCCGC CCGACTGGGCTGGCGATGAGCGAAATGTAGTGCTTACGTTGTCCCGCATTTGGTACAGCGCAGTAACCGGCAAAATCGCGCCGAAGGATGTCGCTGC CGACTGGGCAATGGAGCGCCTGCCGGCCCAGTATCAGCCCGTCATACTTGAAGCTAGACAGGCTTATCTTGGACAAGAAGAAGATCGCTTGGCCTCG CGCGCAGATCAGTTGGAAGAATTTGTCCACTACGTGAAAGGCGAGATCACCAAGGTAGTCGGCAAA	6
kanR	ATGAGCCATATTCAACGGGAAACGTCTTGCTCTAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATG TCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGT TACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTC ACCACTGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCC GGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGA TGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTCAGTCGTC ACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACC AGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAA TAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTC	6
mCherry	GTGAGCAAGGGCGAAGAAGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGA TCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCT GTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGG GAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCTTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCG GCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAAACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAA GGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCC GGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCA CCGGCGGCATGGACGAGCTGTACAAG	6
sf-gfp	AGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGGTG AAGGTGATGCTACAAACGGAAAACTCACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCGTGGCCAACACTTGTCACTACTCTGACCTA TGGTGTTCAATGCTTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATA TCTTTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAGGGTATTGATTTTA AAGAAGATGGAAACATTCTTGGACACAAACTCGAGTACAACTTTAACTCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGC TAACTTCAAAATTCGCCACAACGTTGAAGATGGTTCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTA CCAGACAACCATTACCTGTCGACACAATCTGTCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCTGCTG GGATTACACATGGCATGGATGAGCTCTACAAA	15
Terminators		
L3S2P21	CTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC	16
DT5	TCCGGCAATTAAAAAAGCGGCTAACCACGCCGCTTTTTTTACGTCTGCACTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGGGCCTT TTTTCGTTTTGGTCC	6
DT101	TCTTTAAAAAGAAACCTCCGCATTGCGGAGGTTTCGCCTTTTGATACTCTGTCTGAAGTAATTCTTGCCGCAGTGAAAAATGGCGCCCATCGGCG CCATTTTTTTATGCTTCCATTAGAAAGCAAAAAGCCTGCTAGAAAGCAGGCTTTTTTGAATTTGGCTCCTCTGAC	6
DT104	GCAGACAAAAAAAATGGCGCACAATGTGCGCCATTTTTCACTTCACAGGTACTATTGTTTTGAATTGAAAAGGGCGCTTCGGCGCCCTTTTTGCA TTTGTTGACGGCATATATTTGTATATCGAAGCGCCCTGATGGGCGCTTTTTTTATTTAATCGATAACCAGA	6
DT54	GGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGGCTCGGTACCAAATTCCAGAAAAGACACCCGAAAGGGTG TTTTTTCGTTTTGGTCC	6
DT60	ACATTTAATAAAAAAAGGGCGGTCGCAAGATCGCCCTTTTTTACGTATGACACAGTGAAAAATGGCGCCCATCGGCGCCATTTTTTTATG	6
ECK120010850	AGTTAACCAAAAAGGGGGGATTTTATCTCCCCTTTAATTTTTCCT	16
ECK120035133	ACTGATTTTTAAGGCGACTGATGAGTCGCCTTTTTTTTGTCT	16
lambda t0	GACTCCTGTTGATAGATCCAGTAATGACCTCAGAACTCCATCTGGATTTGTTCAGAACGCTCGGTTGCCGCCGGGCGTTTTTTATTGGTGAGAAT	iGEM K3257021
B0015	CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACT GGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATA	iGEM B0015
Primers		
LP2_322F	CGAAGACTTGGAGTGAGACCGGCTGAGAAATGGTCAGAAAACGCTTTGC	
LP2_322R	GAGACCTTATATTCCCCAGAACATCAGGCAGTATCCTGATCCCACAATTAAGCG	
LP2_323F	GACAGGGCGGGGTTTTTTTTGACGTGTTCCGCGCTTACGTTG	
LP2_323R	TTCCCGTTAACAGCGCAGAACTCGTTTAAAAATACGTTTCATGGAACACCTG	
LP2_324F	GGTGTTCCATGAAACGTATTTTTAAACGAGTTCTGCGCTGTTAACGGGAAAC	

LP2_324R	GAACGTCTCAACGTAAGCGCGGAACACGTCAAAAAAAACCCCGCCCTGTC
LP5_305F	CGAAGACTTGGAGTGAGACCGGCTGTTTCCGTGGGGTAAATCACCTTG
LP5_305R	GAGACCTTATATTCCCCAGAACATCAGGCAGCAATACCTCACTGCGGATTCG
LP5_306F	GGGGCATTTTTCTTCCTGTTATGTAGCCCTTTTATGCTGAACAGGTTCTG
LP5_306R	TCCGCCCACACCCGCTTACGCAGGGCCGGGATAATCTCAACCCCTTTGCC
LP5_307F	GGCAAAGGGGTTGAGATTATCCCGGCCCTGCGTAAGCGGGTGTG
LP5_307R	CAGAACCTGTTCAGCATAAAAGGGCTACATAACAGGAAGAAAAATGCCCCG
LP7_296F	TGATTTTTGCGGTATAAGAATATATACTGCCTCTACCAAAGCGTTCACCACCC
LP7_296R	AGACCTTATATTCCCCAGAACATCAGGCTGGTGAAGACGAACGAGGTGCC

Supplementary Table 3: Plasmids and genomic insertions

Name	Sequence[a]
Plasmids	
pHD305 (without payload)	GAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAATAGGAACTTCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACCATGCCTATTTGTTTATTTTTCTAA ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGCCATATTCAACGGGAAACGTCTTGCTCTAGGCCGCG ATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGT TTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCT GATGATGCATGGTTACTCACCACTGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCG GTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGACCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACG AGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGAC GAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACG GCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCACACAGGAAAGTACTAGATGCCTGGTATGACCACC GAAACCGGTCCGGATCCTGCAGGTCTGATTGACCTGTTTTGTCGTAAAAGCAAAGCAGTTAAAAGCCGTGCAAATGGTGCAGGTCAGCGTCGTAAACAAGAAATTAGCATTGCAGC ACAAGAAACCCTGGGTCGTAAAGTTGCAGCACTGCTGGGTATGCAGGTTCGTCATGTTTGGAAAGAAGTTGGTAGCGCAAGCCGTTTTCGTAAAGGTAAAGCACGTGATGATCAGA GCAAAGCACTGAAAGCCCTGGAAAGCGGTGAAGTTGGTGCACTGTGGTGTTATCGTCTGCGATCGTTGGGATCGTGGTGGTGCCGGTGCAATTCTCGAAAATTATCGAACCGGAAGAT GGTATGCCTCGTCGTCTGCTGTTTGGTTGGGATGAAGATACCGGTCGTCCGGTTCTGGATAGCACCAATAAACGTGATCGCGGTGAACTGATTCGTCGTGCAGAAGAAGCACGCGA AGAAGCAGAAAAACTGAGCGAACGTGTTCGTGATACCAAAGCACATCAGCGTGAAAATGGTGAATGGGTTAATGCCCGTGCACCGTATGGTCTGCGTGTTGTTCTGGTTACCGTTA GTGATGAAGAGGGTGATGAATATGATGAACGTAAACTGGCAGCAGATGATGAAGATGCGGGTGGTCCTGATGGTCTGACCAAAGCAGAAGCAGCCCGTCTGGTTTTTACCCTGCCG GTTACCGATCGTCTGAGCTATGCAGGCACCGCACATGCAATGAATACCCGTGAAATTCCGAGCCCGACCGGTGGTCCGTGGATTGCAGTTACCGTGCGTGATATGATTCAGAATCC GGCATATGCGGGTTGGCAGACCACAGGTCGTCAGGATGGTAAACAGCGTCGTCTGACCTTTTATAACGGTGAAGGTAAACGTGTTAGCGTTATGCATGGTCCTCCGCTGGTGACCG ATGAAGAACAAGAAGCCGCAAAAGCAGCCGTTAAAGGTGAAGATGGTGTTGGTGTTCCGCTGGATGGTAGCGATCATGATACCCGTCGCAAACATCTGCTGAGCGGTCGTATGCGT TGTCCGGGTTGTGGTGGTAGCTGTAGCTATAGCGGTAATGGTTATCGTTGTTGGCGTAGCAGTGTGAAAGGTGGTTGTCCGGCACCGACCTATGTTGCACGTAAAAGCGTTGAAGA ATATGTTGCATTTCGTTGGGCAGCAAAATTAGCAGCAAGCGAACCGGATGATCCGTTTGTTATTGCAGTTGCAGATCGCTGGGCAGCACTGACCCATCCGCAGGCAAGCGAAGATG AAAAGTATGCAAAAGCCGCAGTTCGTGAAGCCGAAAAAAATCTGGGTCGCCTGCTGCGTGATCGTCAGAATGGTGTTTATGATGGTCCGGCAGAACAGTTTTTTGCCCCTGCATAT CAAGAAGCACTGAGCACCCTGCAGGCAGCCAAAGATGCAGTTAGCGAAAGCAGCGCAAGCGCAGCAGTTGATGTTAGCTGGATTGTTGATAGCAGCGATTATGAAGAACTGTGGCT GCGTGCAACCCGACCATGCGTAATGCAATTATTGATACCTGCATCGATGAAATTTGGGTTGCAAAAGGCCAGGCGTGGTCGTCCGTTTGATGGTGATGAACGCGTTAAAATCAAAT GGGCAGCCCGTACCTAACATTACAGTTTATCGATGATAAGCTGTCAATCCGATTCGCCAGATGATAAGGAACCATAAAAAAATGGCGCCGATGGGCGCCATTTTTCACTGGTTGAT TGCTTTGGTTGAGACGAGGTGTCATACGTAAAAAAGGGCGATCTTGCGACCGCCCTTTTTTTATTAAATGTTGGATTTGTTCAGAACGCTCGGTTGCCGCCGGGCGTTTTTTATTG GTGAGAATCCAAGCACAAGAAGATCCGGCCACGATGCGTCCGGCGTAGAGGATCTGAAGATCAGCAGTTCAACCTGTTGATAGTACGTACTAAGCTCTCATGTTTCACGTACTAAG CTCTCATGTTTAACGTACTAAGCTCTCATGTTTAACGAACTAAACCCTCATGGCTAACGTACTAAGCTCTCATGGCTAACGTACTAAGCTCTCATGTTTCACGTACTAAGCTCTCA TGTTTGAACAATAAAATTAATATAAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAAAGAATATATAAGGCTTTTAAAGCTTTTAAGGTTTAACGGTTG TGGACAACAAGCCAGGGATGTAACGCACTGAGAAGCCCTTAGAGCCTCTCAAAGCAATTTTGAGTGACACAGGAACACTTAACGGCTGACATGGGAATTAGCCATGGGCCCGTGGC AATCATTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCGTGTGGTAACCGTATTACCGCCTGGACCAAAACGAAAAACAC CCTTTCGGGTGTCTTTTCTGGAATTTGGTACCGAGCCTTTGGTCGAAAAAAAAAGCCCGCACTGTCAGGTGCGGGCTTTTTCTGTGTTTCCCAGATCAGGGTGCGCAAGTTGTCA ACGCTCCCAGGAGAGTTATCGACTTGCGTATTAGGG
pHD307 (without payload)	GAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAATAGGAACTTCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACCATGCCTATTTGTTTATTTTTCTAA ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGCCATATTCAACGGGAAACGTCTTGCTCTAGGCCGCG ATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGT TTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCT GATGATGCATGGTTACTCACCACTGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCG GTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGACCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACG AGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGAC GAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACG GCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAAAGCTACTAGTCGCTTGGACTCCTGTTGATAGATCCAG TAATGACCTCAGAACTCCATCTGGATTTGTTCAGAACGCTCGGTTGCCGCCGGGCGTTTTTTATTGGTGAGAATCCAAGCACAAGAAGATCCGGCCACGATGCGTCCGGCGTAGAG GATCTGAAGATCAGCAGTTCAACCTGTTGATAGTACGTACTAAGCTCTCATGTTTCACGTACTAAGCTCTCATGTTTAACGTACTAAGCTCTCATGTTTAACGAACTAAACCCTCA TGGCTAACGTACTAAGCTCTCATGGCTAACGTACTAAGCTCTCATGTTTCACGTACTAAGCTCTCATGTTTAACGAACTAAACCCTCATGGCTAACGTACTAAGCTCTCATGTTTA GTTTTAAGTTTTATAAGAAAAAAAAGAATATATAAGGCTTTTAAAGCTTTTAAGGTTTAACGGTTGTGGACAACAAGCCAGGGATGTAACGCACTGAGAAGCCCTTAGAGCCTCTC AAAGCAATTTTGAGTGACACAGGAACACTTAACGGCTGACATGGGAATTAGCCATGGGCCCGTGCGAATCATTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTC TTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTAAGCTGAACCTGCGTAAAAGTTAAGGTTAGGCATGTAAACTTAACTCTCACACAGGTTTATAACAC
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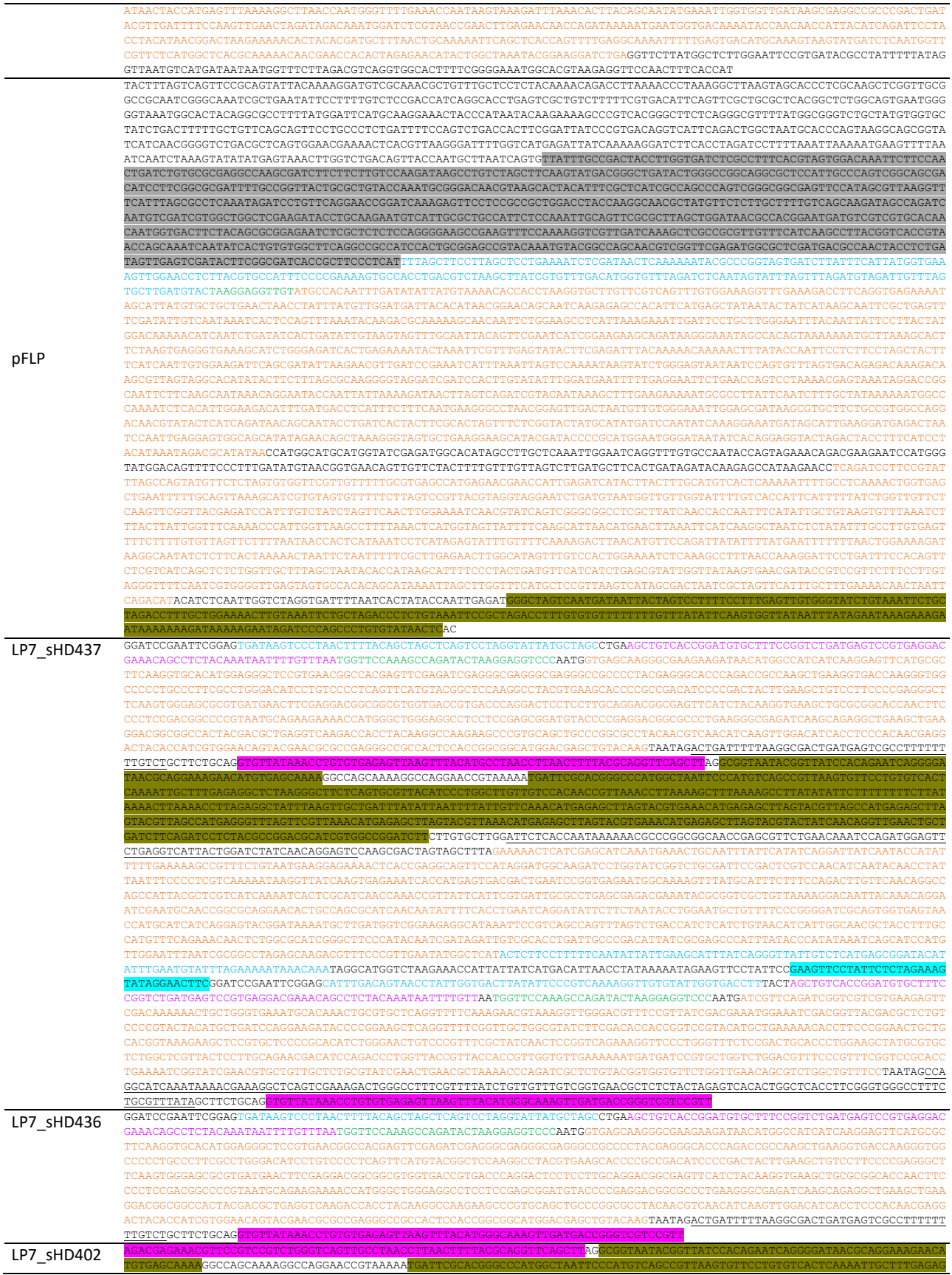
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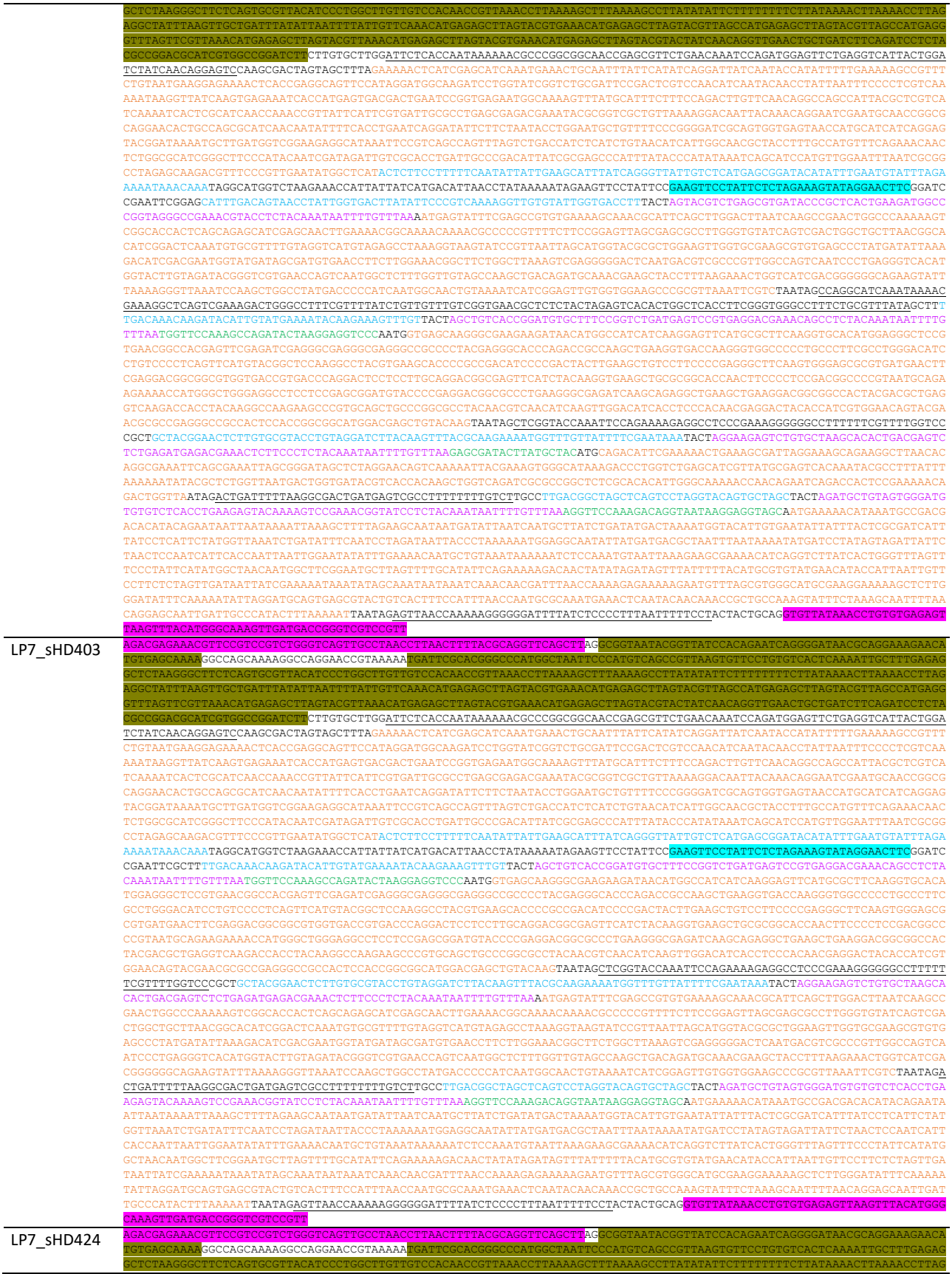
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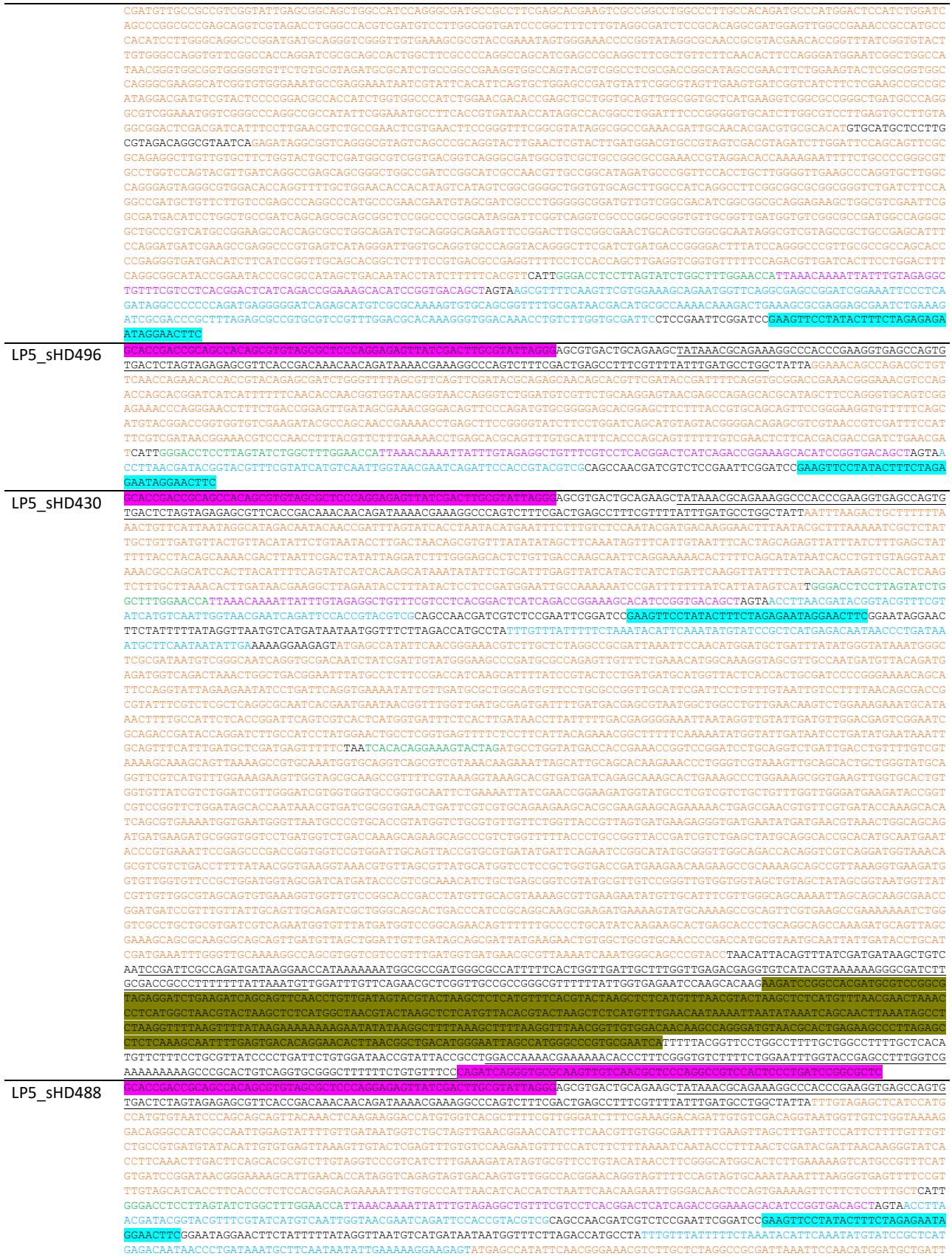
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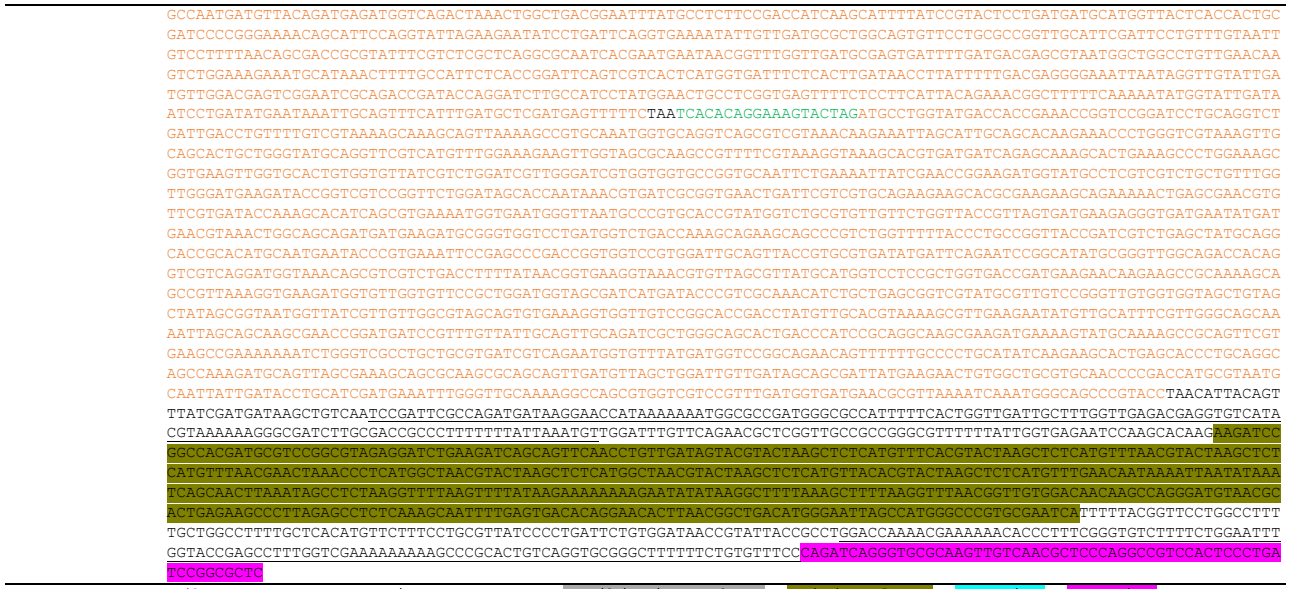

a. Promoters; Ribozymes; CDS; Terminatorrs; RBS; antibiotic markers; origin of rep; FRT site; att site

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