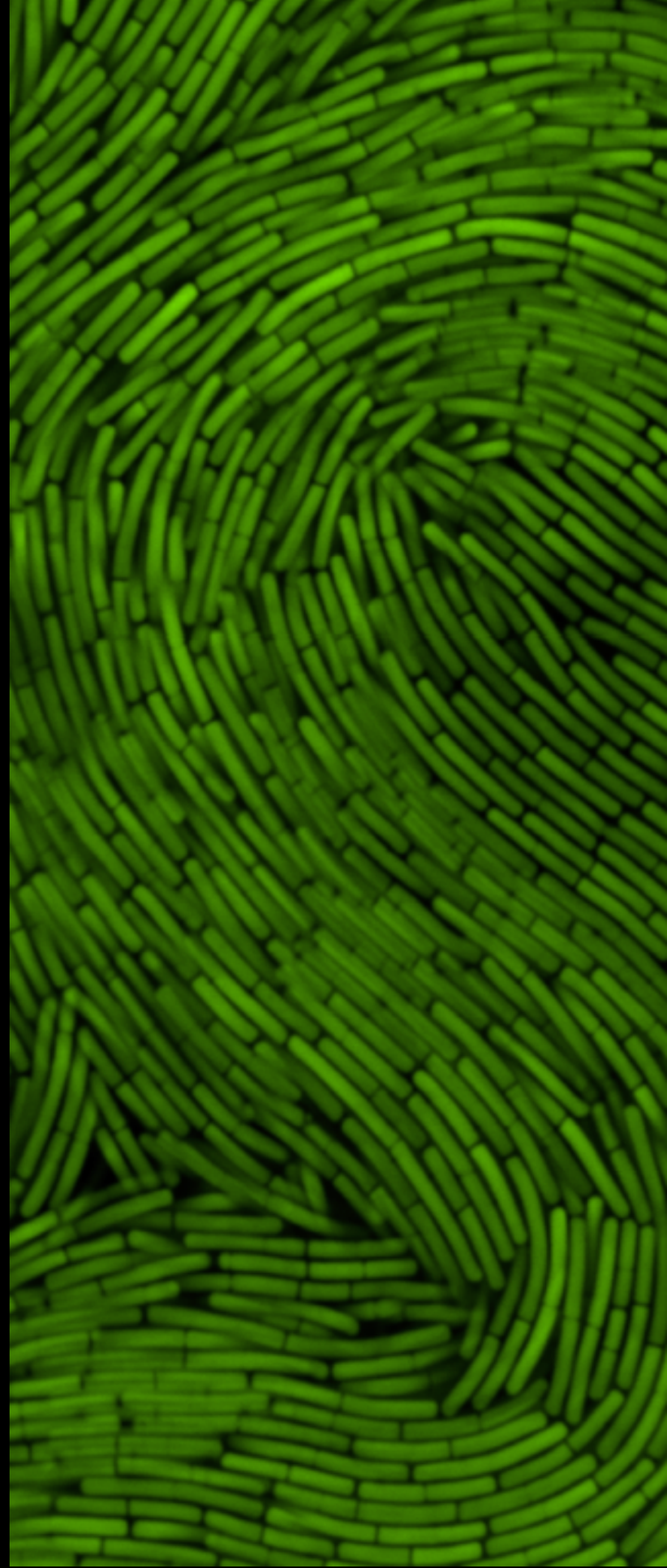
Genomics, Epigenetics & Synthetic Biology

Lecture 3:
Engineered logic and the control of gene expression.

Jim Haseloff
www.haseloff-lab.org (Education)



Applications of Synthetic Biology

Lecture 3

Cell autonomous genetic circuits with self-regulating properties

e.g. microbial engineering,

enviromental and biomedical sensors

engineering novel metabolic pathways

Lecture 4

Morphogenetic circuits with self organising properties

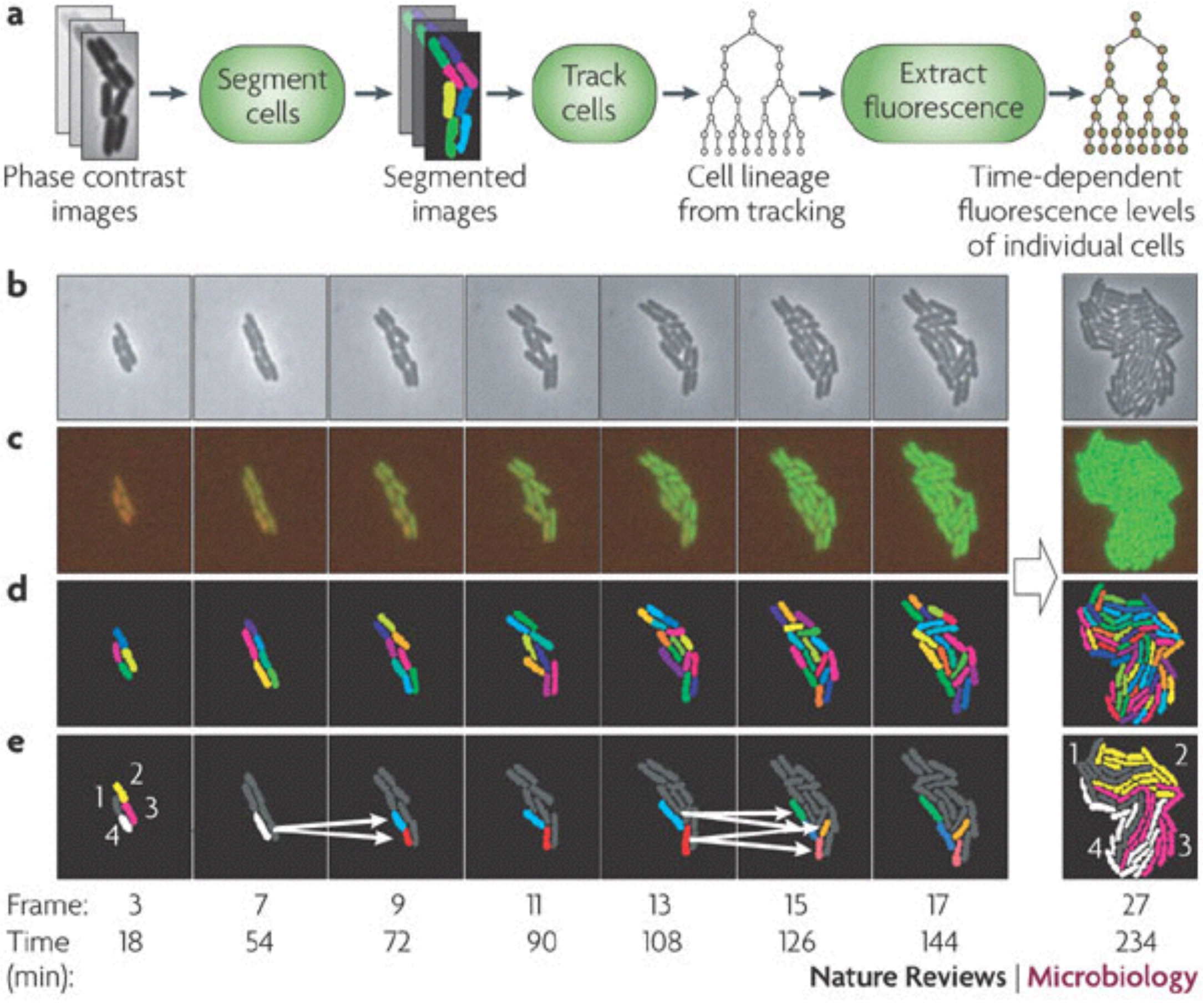
e.g. microbial biofilms or self-organising communities for

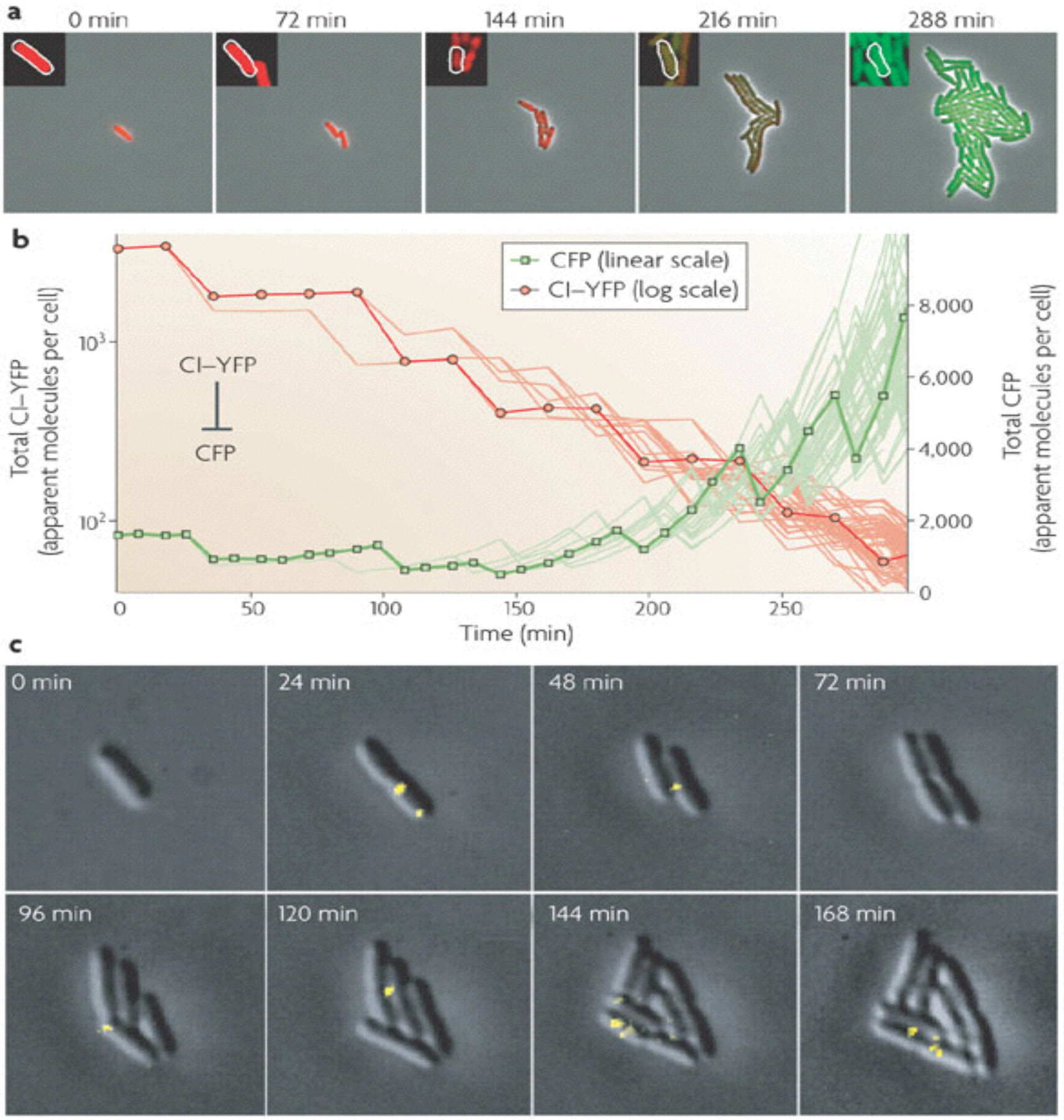
bioremediation and bio catalysis

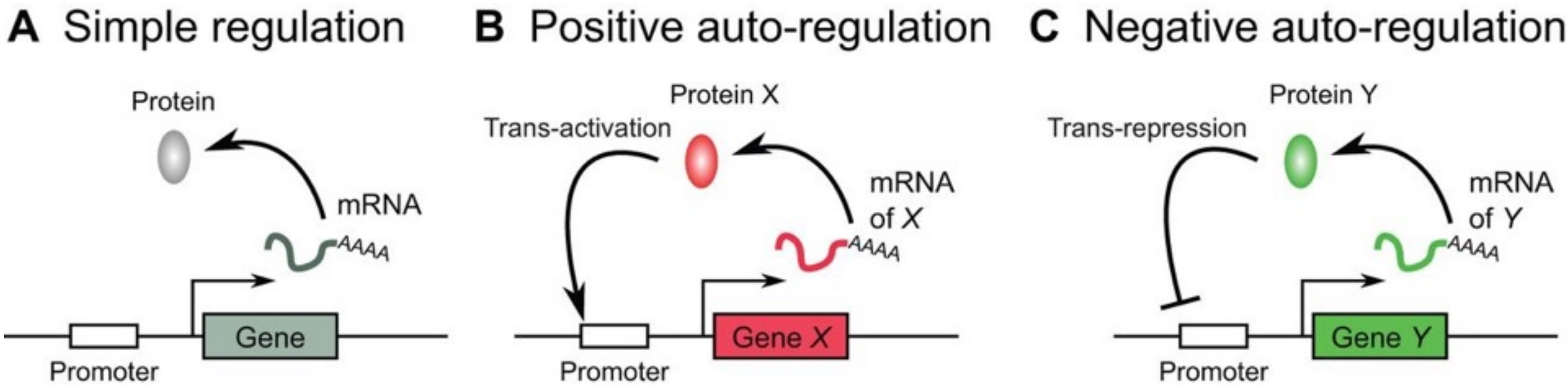
novel plant and algal feedstocks for bioproduction and bioenergy

tissue engineering

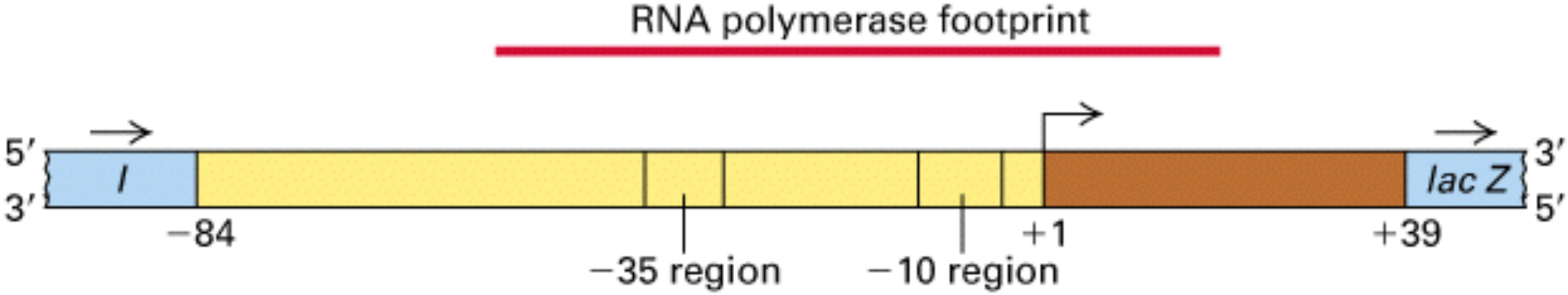
Microbes as test-beds for circuit testing


a
Phase contrast images
Segment cells
Segmented images
Track cells
Cell lineage from tracking
Extract fluorescence
Time-dependent fluorescence levels of individual cells
b
c
d
e
1
2
3
4
Frame: 3 7 9 11 13 15 17 27
Time (min): 18 54 72 90 108 126 144 234
Nature Reviews | Microbiology


a
0 min
72 min
144 min
216 min
288 min
b
CFP (linear scale)
CI–YFP (log scale)
CI–YFP
CFP
Total CI–YFP (apparent molecules per cell)
10^3
10^2
8,000
6,000
4,000
2,000
0
Total CFP (apparent molecules per cell)
0
50
100
150
200
250
Time (min)
c
0 min
24 min
48 min
72 min
96 min
120 min
144 min
168 min


A Simple regulation
Protein
mRNA
AAAA
Gene
Promoter
B Positive auto-regulation
Protein X
Trans-activation
mRNA
of X
AAAA
Gene X
Promoter
C Negative auto-regulation
Protein Y
Trans-repression
mRNA
of Y
AAAA
Gene Y
Promoter
D Cell-cell variability
Population frequency
Gene expression levels
Simple regulation
Positive feedback
Negative feedback (damping)
E No oscillation (damping)
Gene Y expression
Time
F Oscillation
Gene Y expression
Time

In prokaryotes, RNA polymerase binds to the -10 and -35 regions of the promoter relative to the start site of transcription (+1)



promoter operator

Operon	–35 region	–10 region (Pribnow box)	Initiation site (+1)
lac	ACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGG		
lacI	CCATCGAATGGCGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTC		
galP2	ATTTATTCCATGTCACACTTTTCGCATCTTTGTTATGCTATGGTTATTTCATACCAT		
araBAD	GGATCCTACCTGACGCTTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTT		
araC	GCCGTGATTATAGACACTTTTGTTACGCGTTTTTGTCATGGCTTTGGTCCCGCTTTG		
trp	AAATGAGCTGTTGACAATTAATCATCGAACTAGTTAACTAGTACGCAAGTTCACGTA		
bioA	TTCCAAAACGTGTTTTTTTGTTGTTAATTCGGTGTAGACTTGTAAACCTAAATCTTTT		
bioB	CATAATCGACTTGTAAACCAAATTGAAAAGATTTAGGTTTACAAGTCTACACCGAAT		
$tRNA^{Tyr}$	CAACGTAACACTTTACAGCGGCGCGTCATTTGATATGATGCGCCCCGCTTCCCGATA		
rrnD1	CAAAAAAATACTTGTGCAAAAAATTGGGATCCCTATAATGCGCCTCCGTTGAGACGA		
rrnE1	CAATTTTTCTATTGCGGCCTGCGGAGAACTCCCTATAATGCGCCTCCATCGACACGG		
rrnA1	AAAATAAATGCTTGACTCTGTAGCGGGAAGGCGTATTATGCACACCCCGCGCCGCTG		

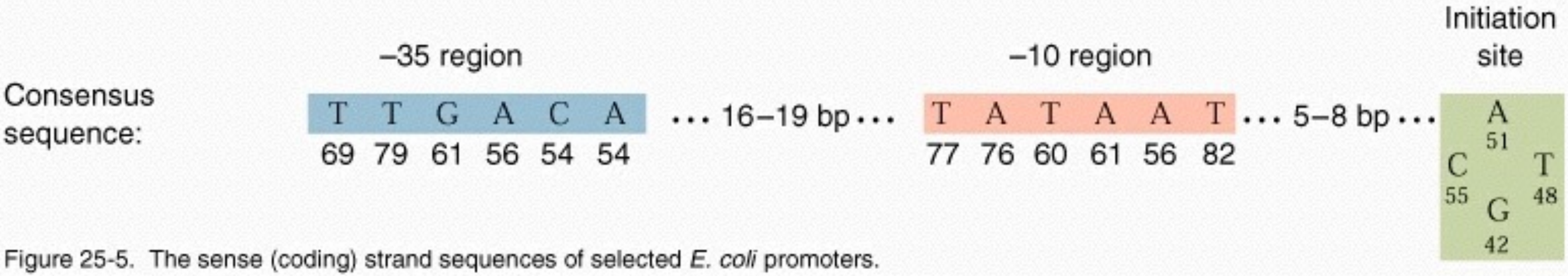
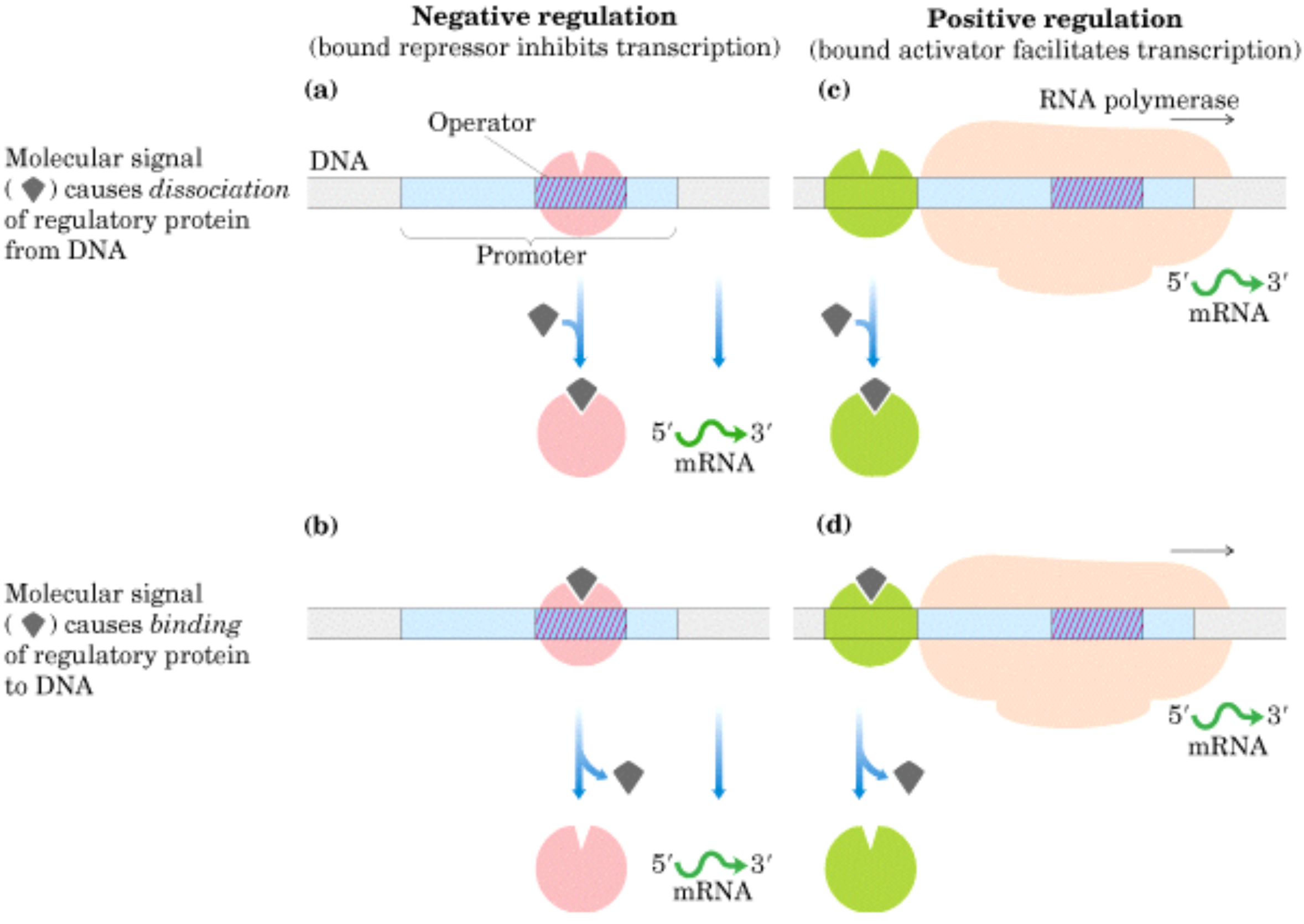
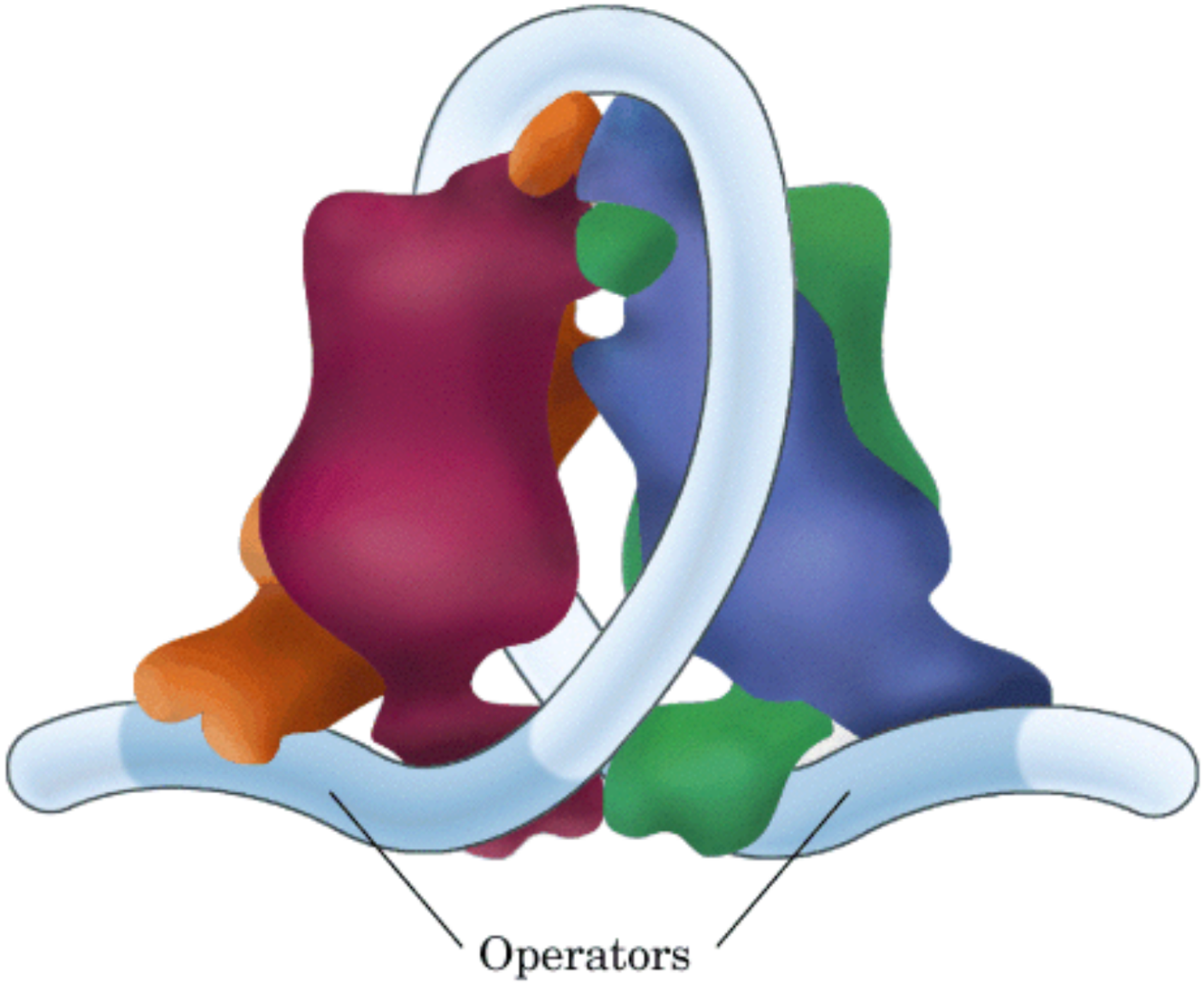


Figure 25-5. The sense (coding) strand sequences of selected *E. coli* promoters. [After Rosenberg, M. and Court, D., *Annu. Rev. Genet.* 13, 321-323 (1979). Consensus sequence from Lisser, D. and Margalit, H., *Nucleic Acids Res.* 21, 1512 (1993).]

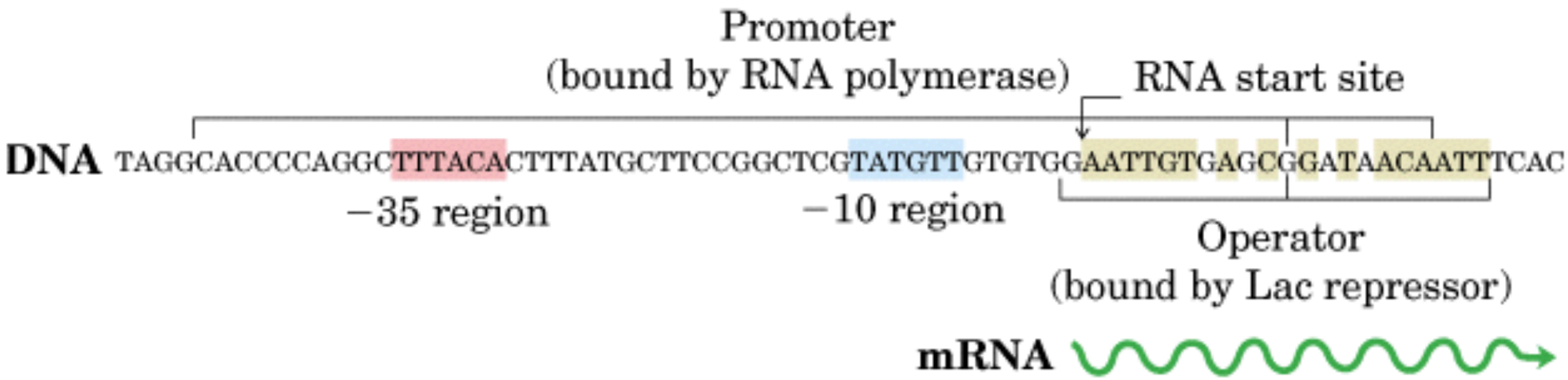
Regulation of bacterial gene expression



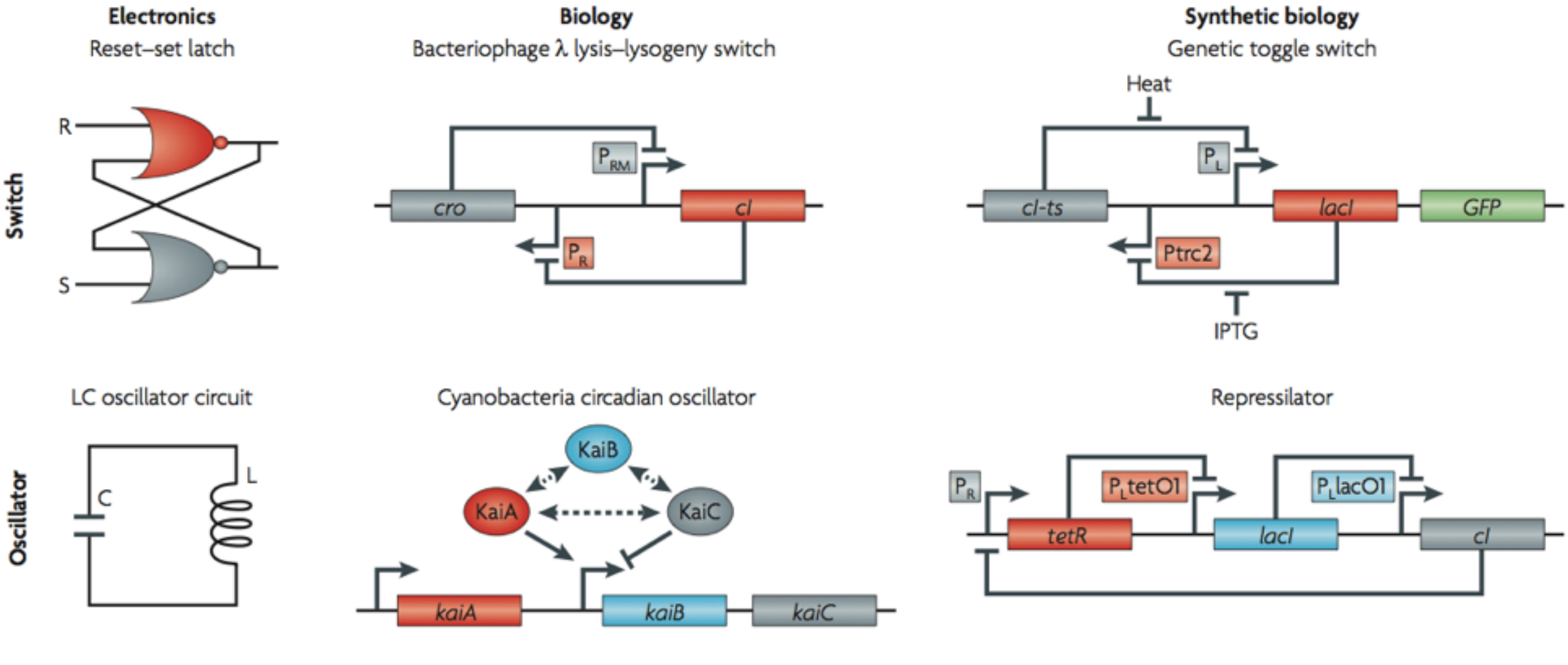
Repressor binding



(b)



Box 1 | **Early synthetic biology designs: switches and oscillators**

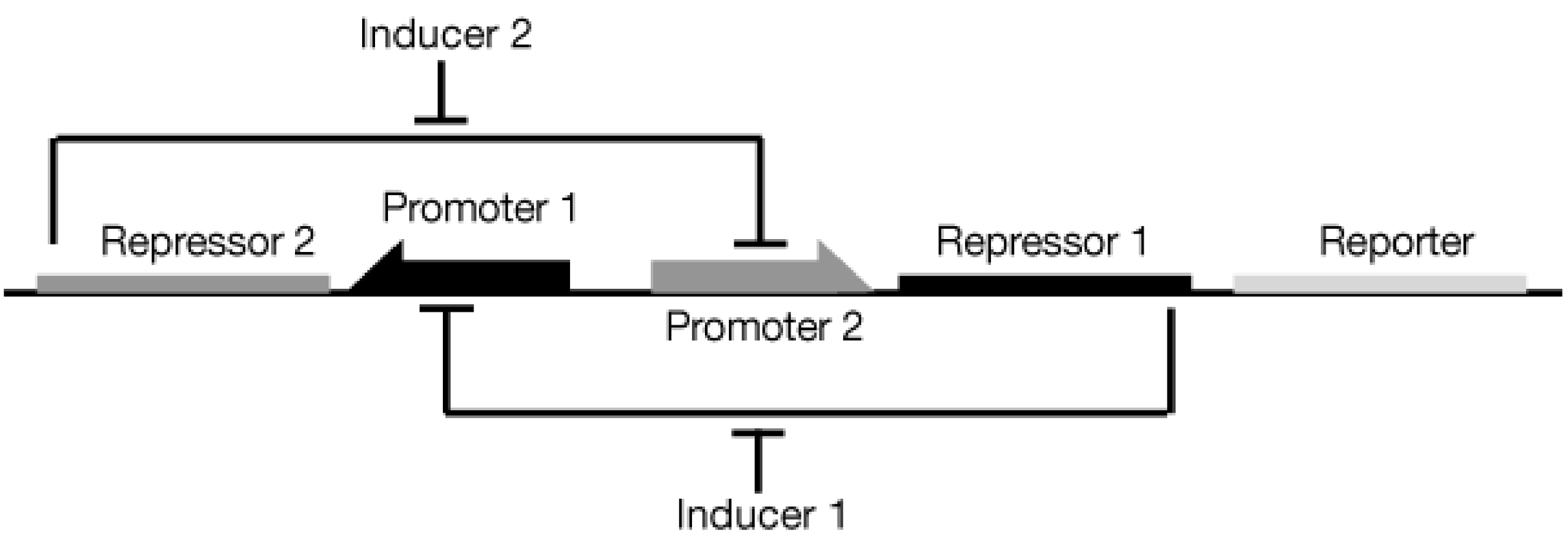


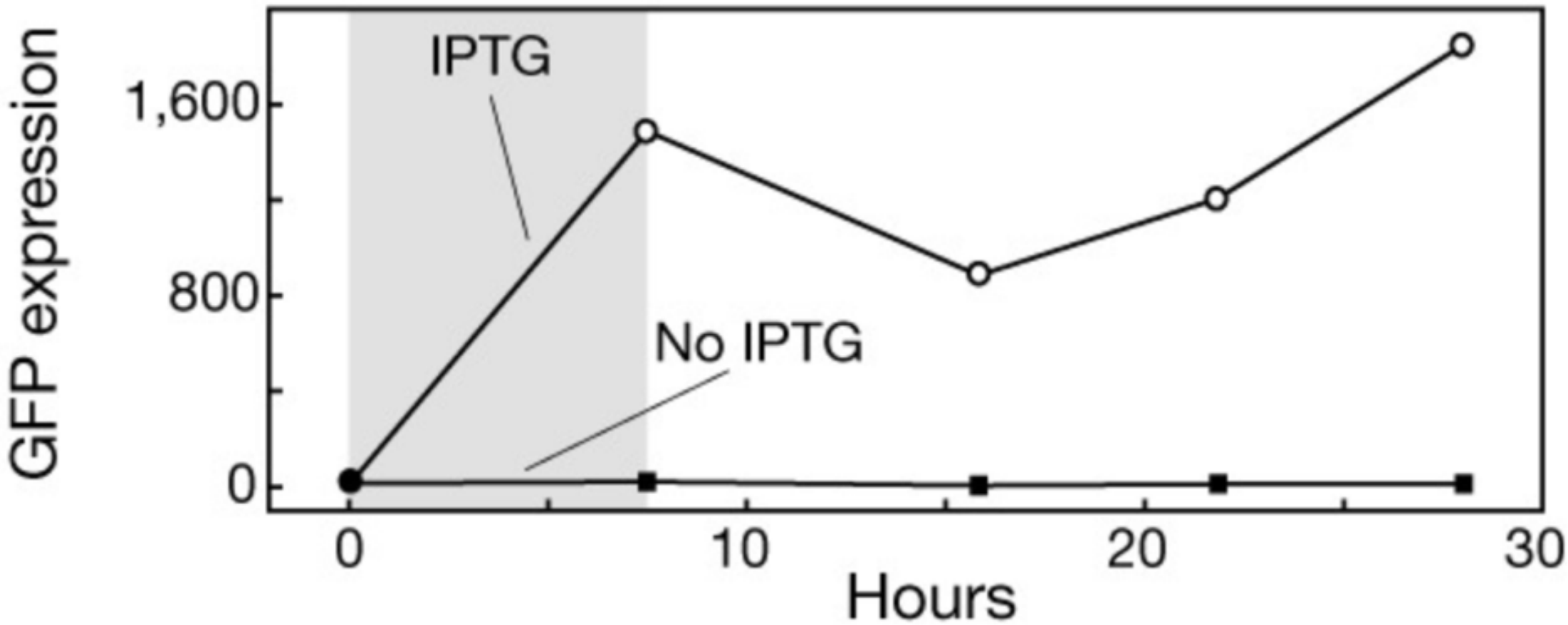
Examples: genetic switches and oscillators

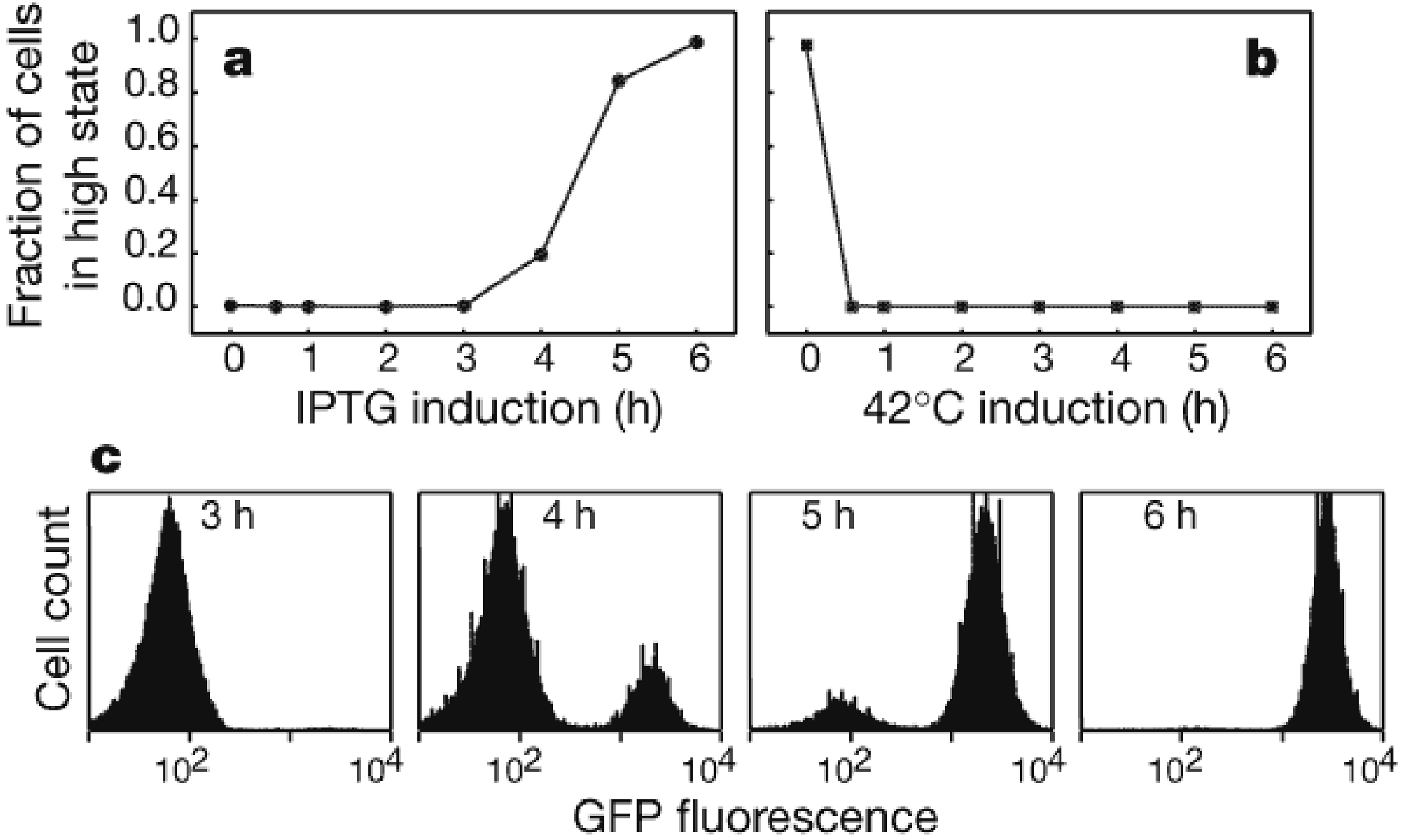
Construction of a genetic toggle switch in *Escherichia coli*

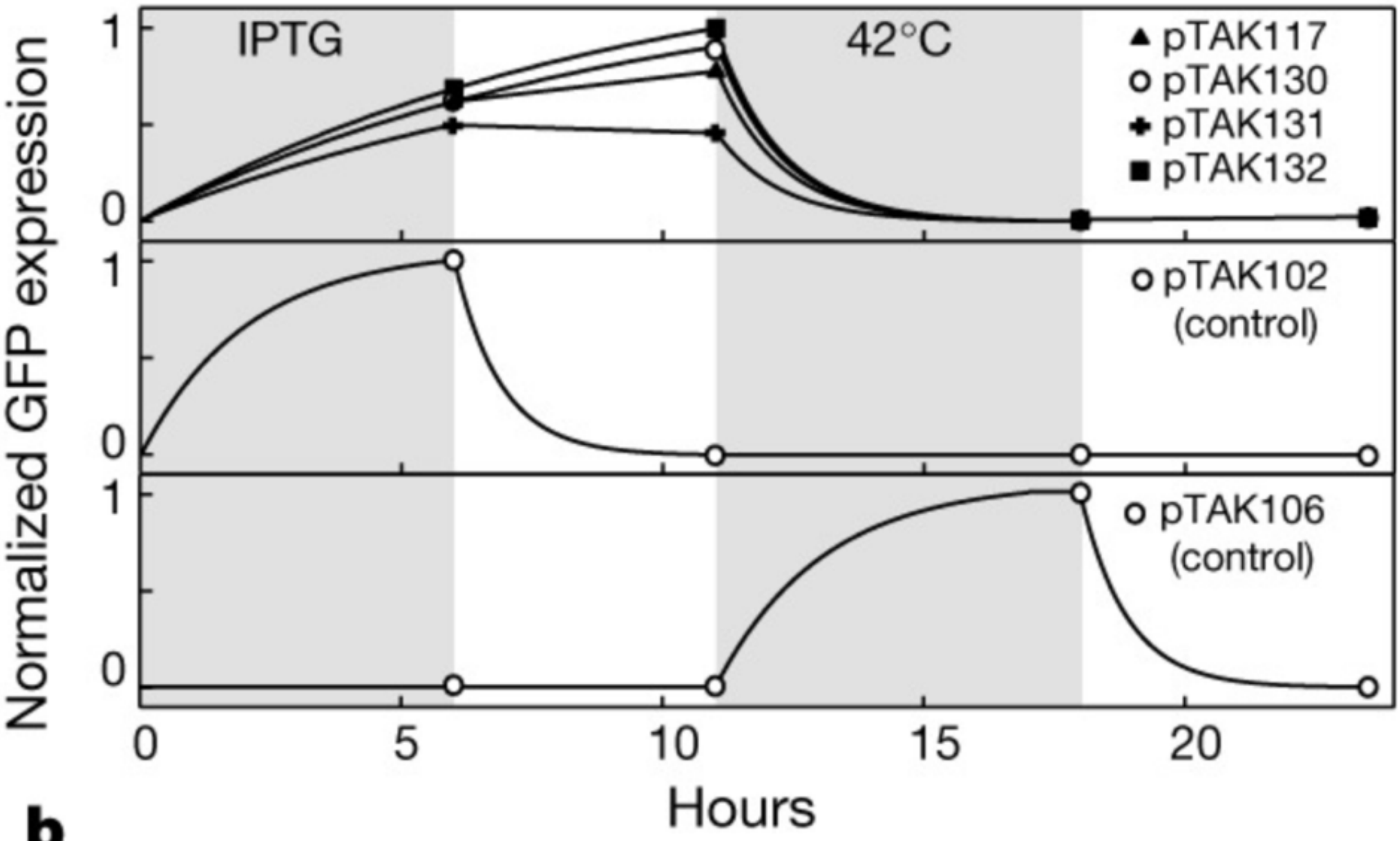
Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

** Department of Biomedical Engineering, † Center for BioDynamics and ‡ Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA*




GFP expression
1,600
800
0
IPTG
No IPTG
0
10
20
30
Hours

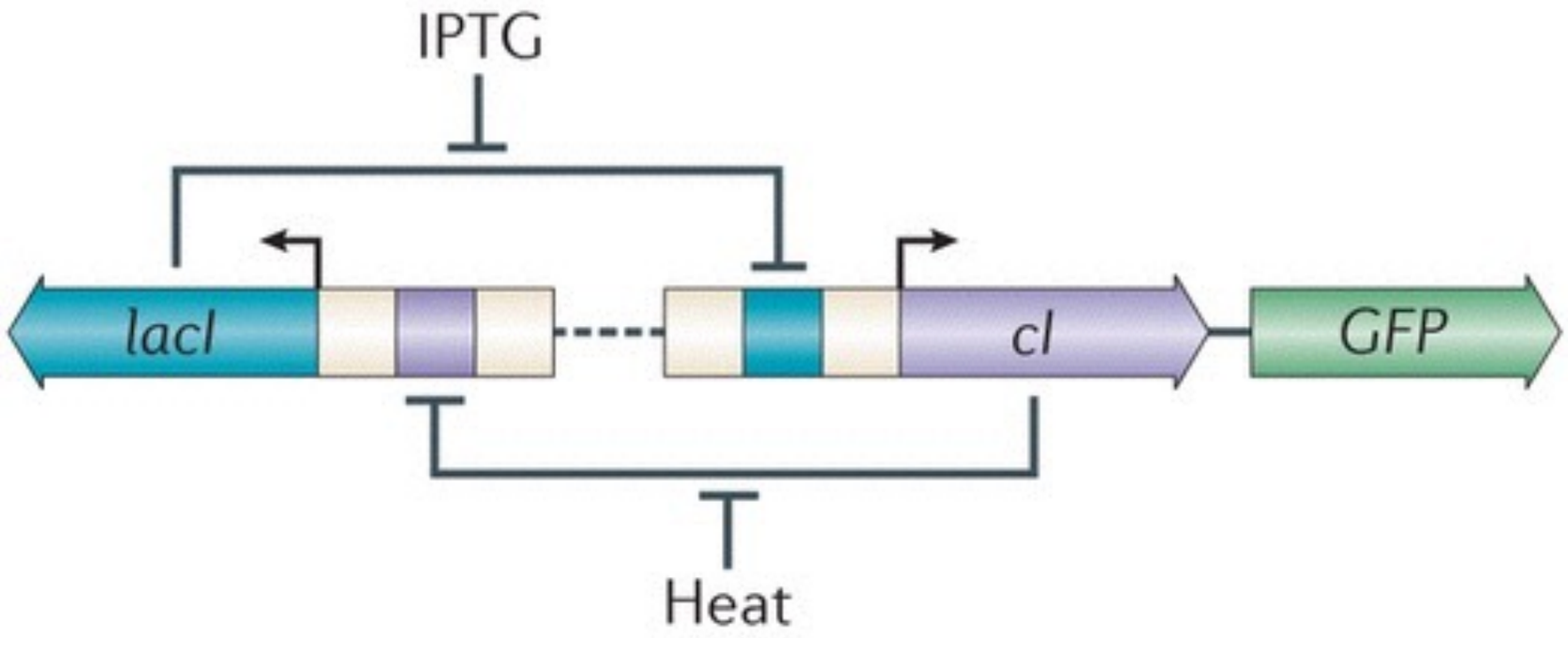

a
b
c
Fraction of cells in high state
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
IPTG induction (h)
42°C induction (h)
Cell count
3 h
4 h
5 h
6 h
10^2
10^4
GFP fluorescence


IPTG
42°C
pTAK117
pTAK130
pTAK131
pTAK132
pTAK102
(control)
pTAK106
(control)
Normalized GFP expression
1
0
1
0
1
0
0
5
10
15
20
Hours

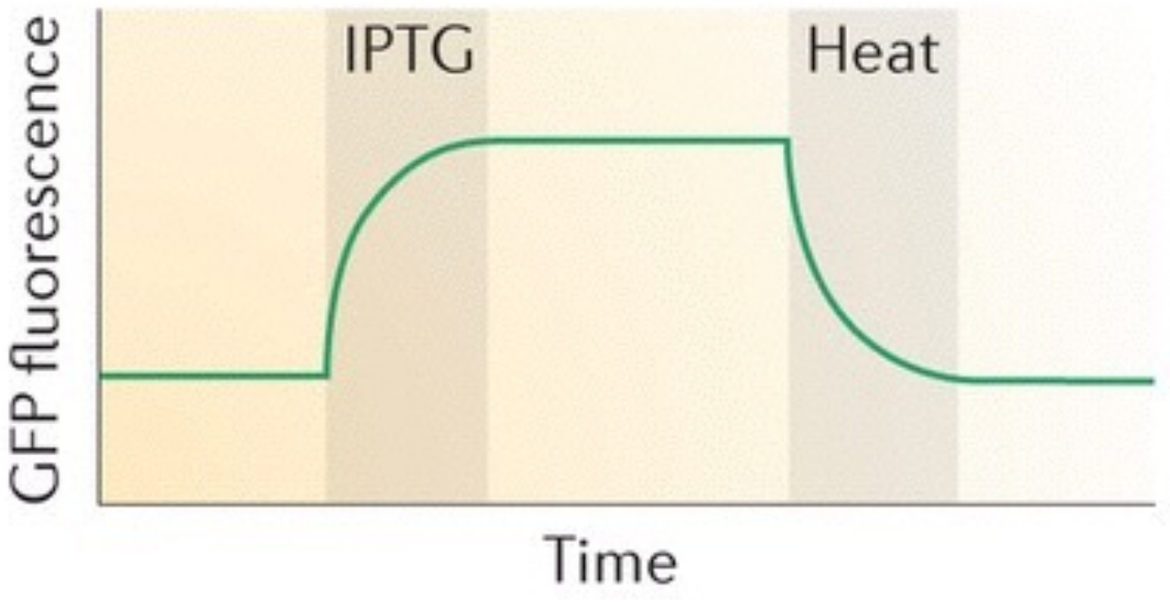
b

Toggle switch

Design



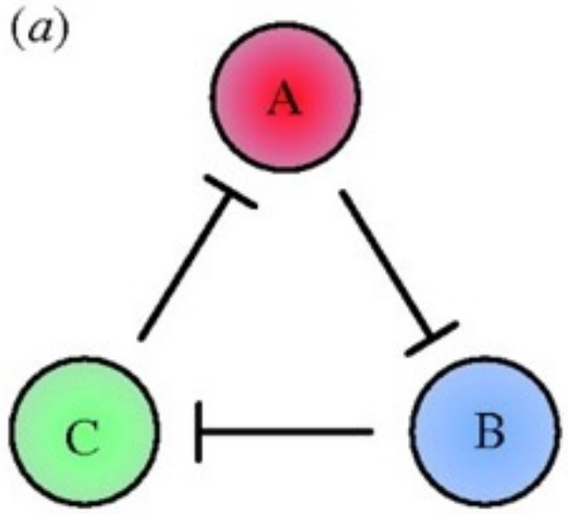
Behaviour

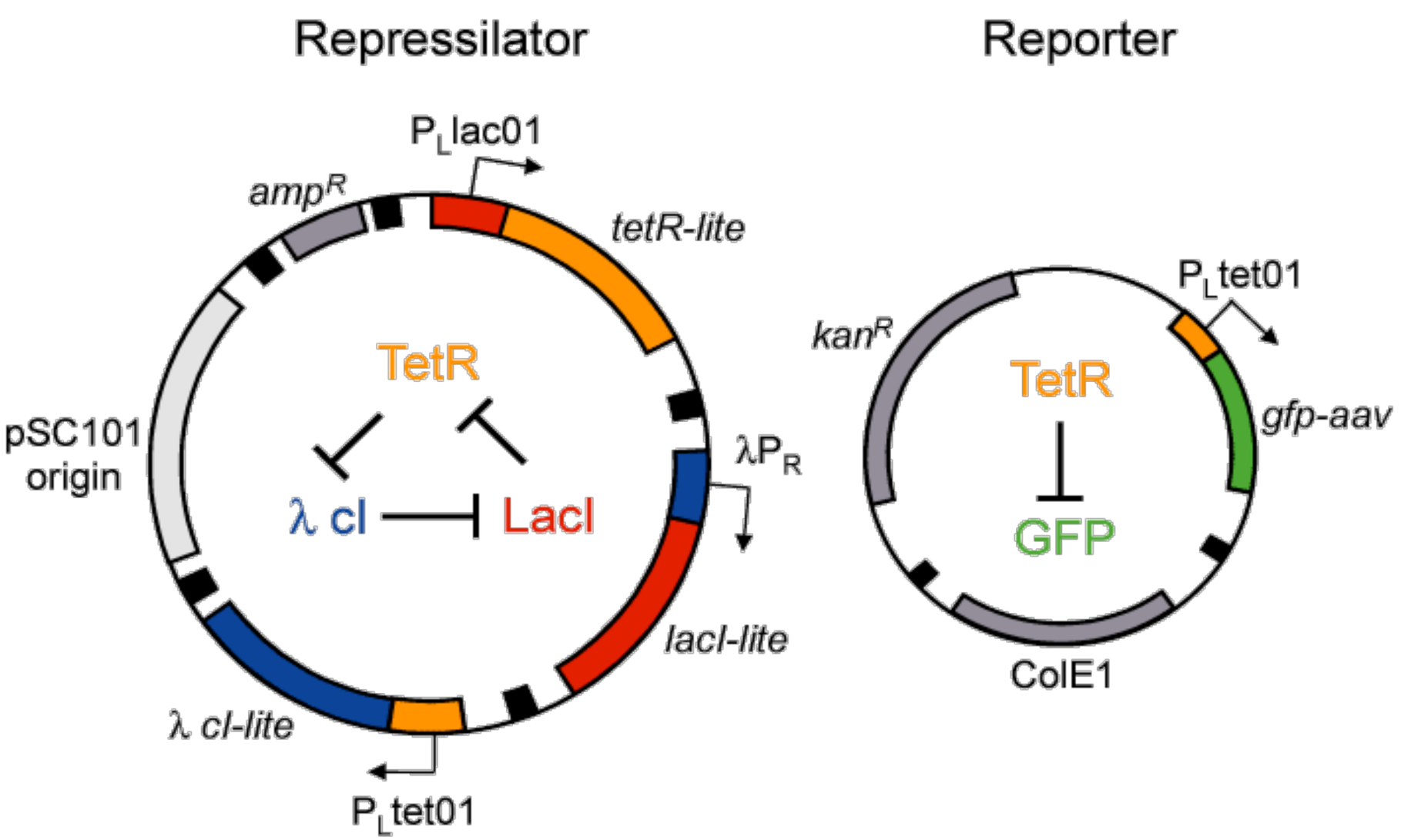


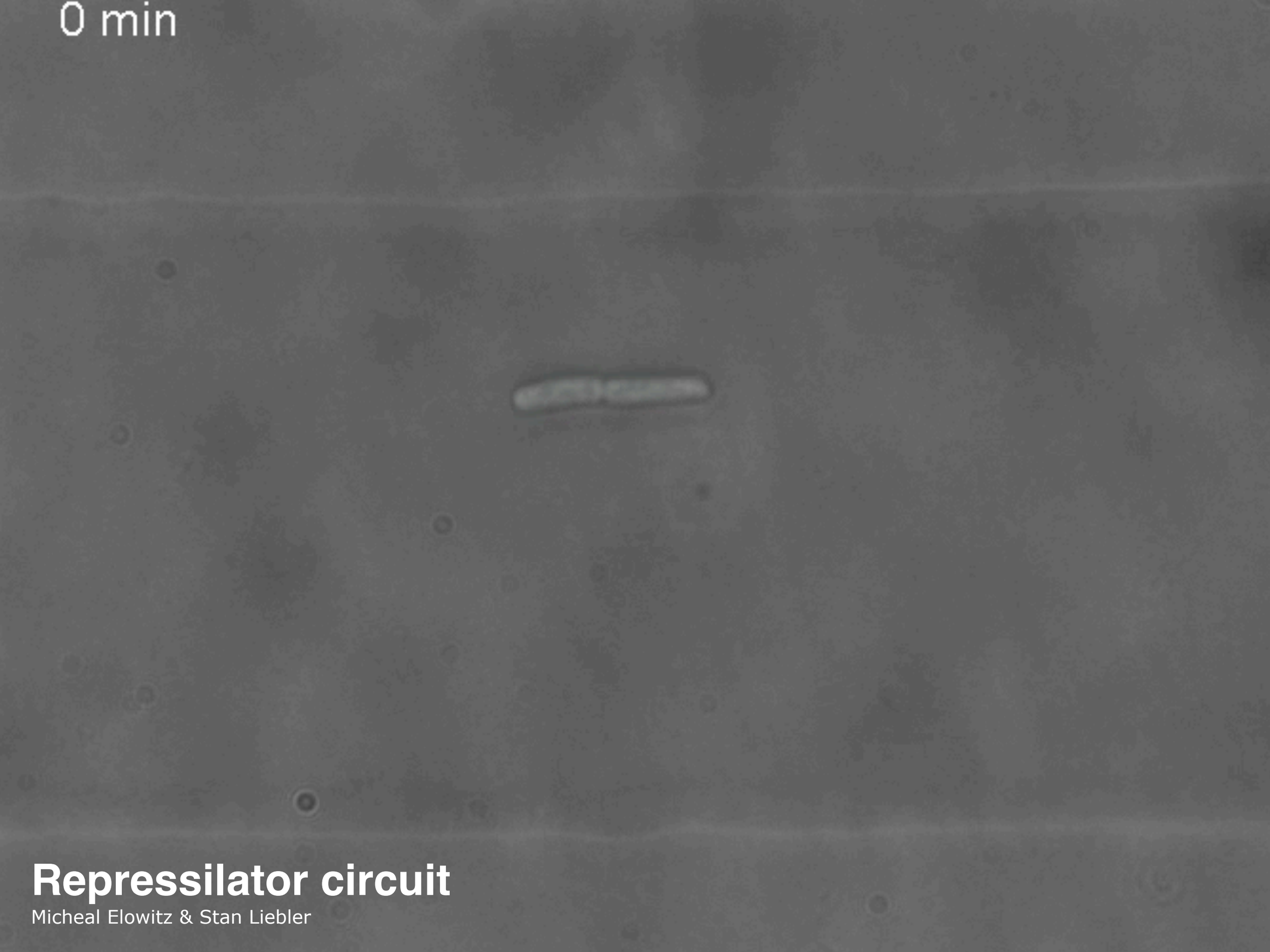
The Repressilator

- Cellular clocks oscillate with defined periods
  - Circadian clocks oscillate with 24-hour period
- Elowitz and Leibler set out to build oscillator with components not found in cellular clocks
- Used three transcription factors in mutual-repression network
  - LacI
  - TetR
  - cI from lambda phage
- Readout: GFP controlled by Tet repressor

Repressilator design



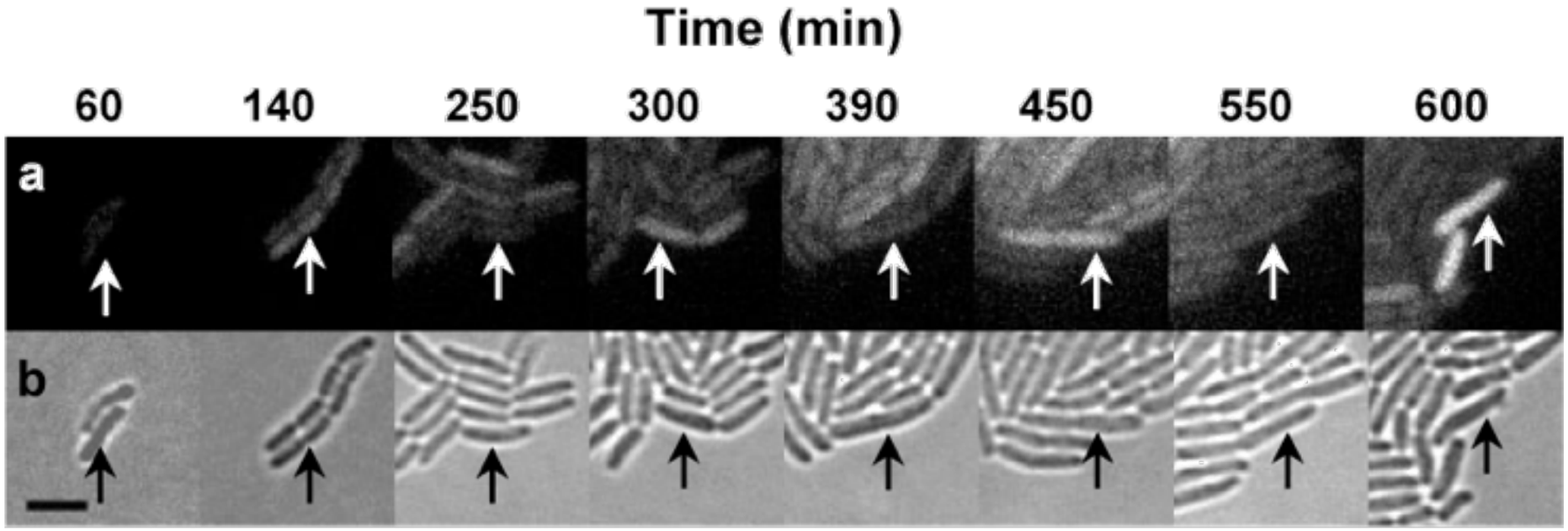


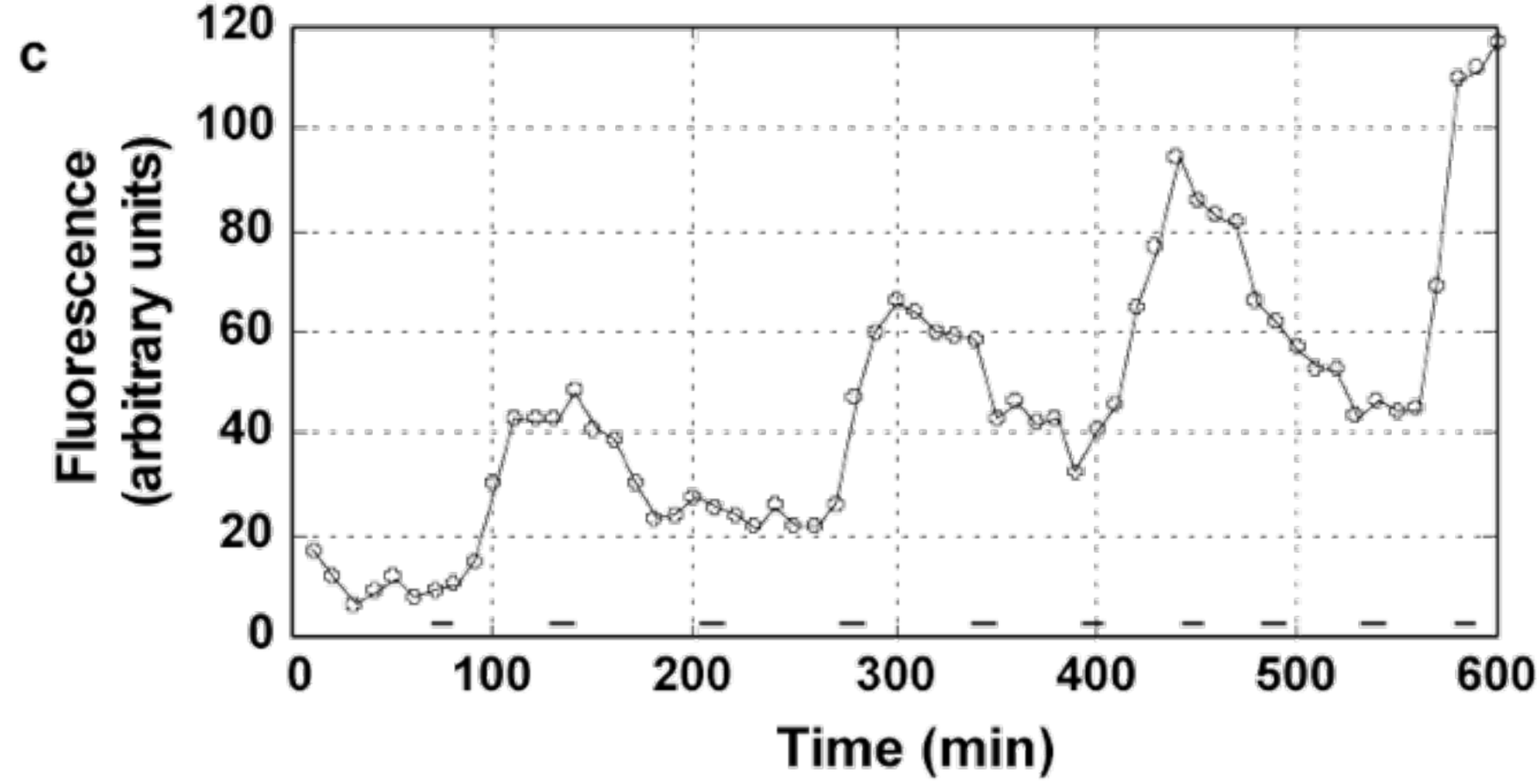


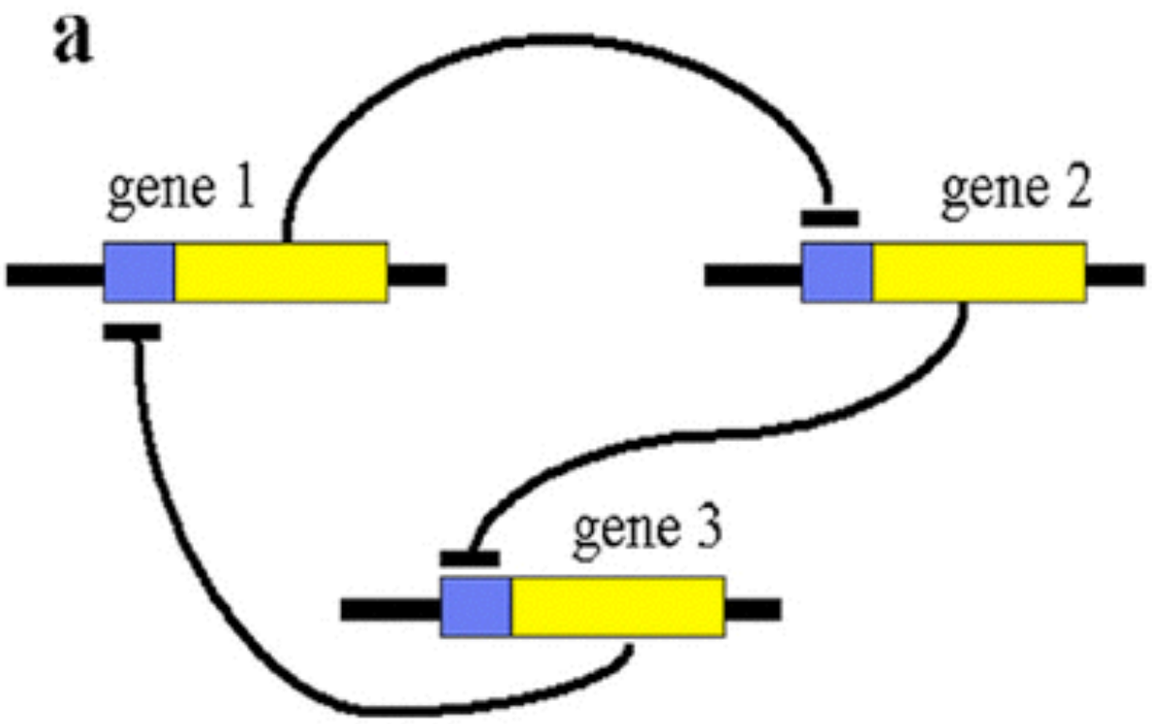
Repressilator circuit

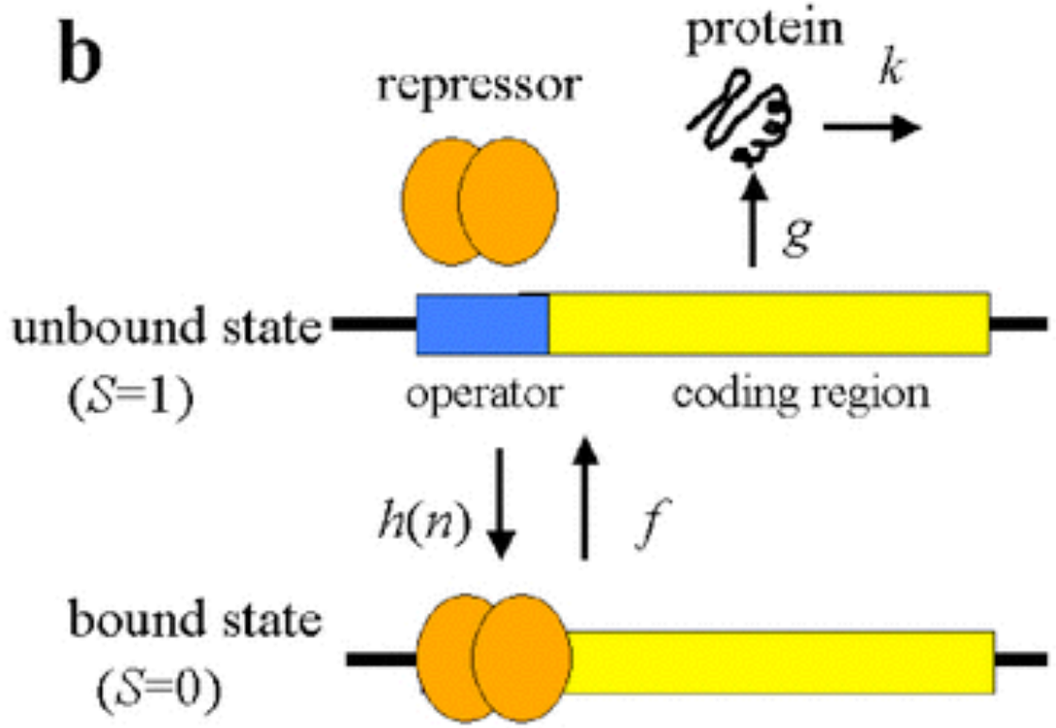
Micheal Elowitz & Stan Liebler

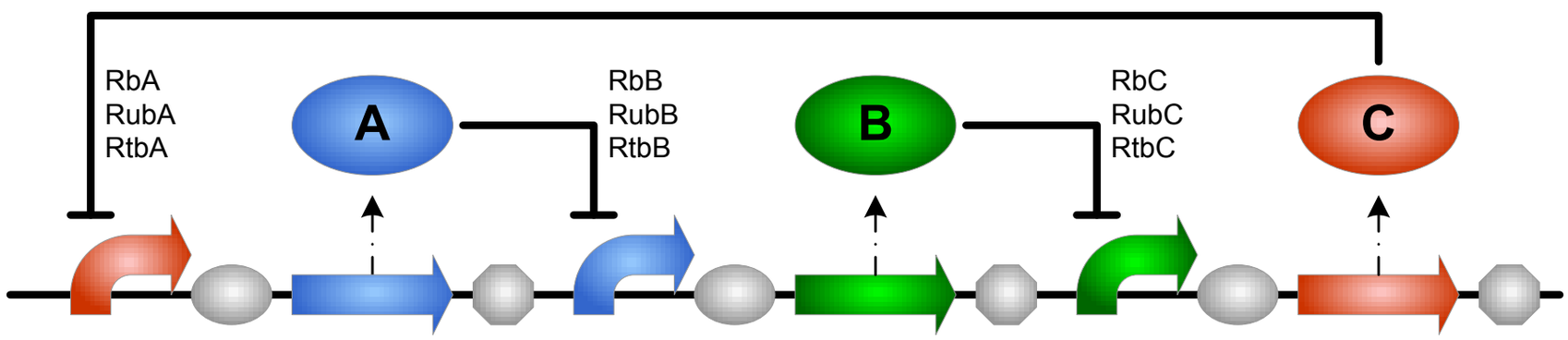
Activity of repressilator











prom<neg(C,RbA,RubA,RtbA)>; rbs; pcr<codes(A)>; ter;
prom<neg(A,RbB,RubB,RtbB)>; rbs; pcr<codes(B)>; ter;
prom<neg(B,RbC,RubC,RtbC)>; rbs; pcr<codes(C)>; ter

| 0.4 < RubB | RubB < 0.6
| 0.4 < RubC | RubC < 0.6
| 0.4 < RubA | RubA < 0.6

The Repressilator

Visual GEC - localhost

GEC Examples: Repressilator | Solve GEC | LBS Examples: | Solve LBS | v0.1-20100820-1240 | License | Update

Simulate LBS | Pause | Simulation: Stochastic

Database | GEC | LBS

Zoom | Save | Load

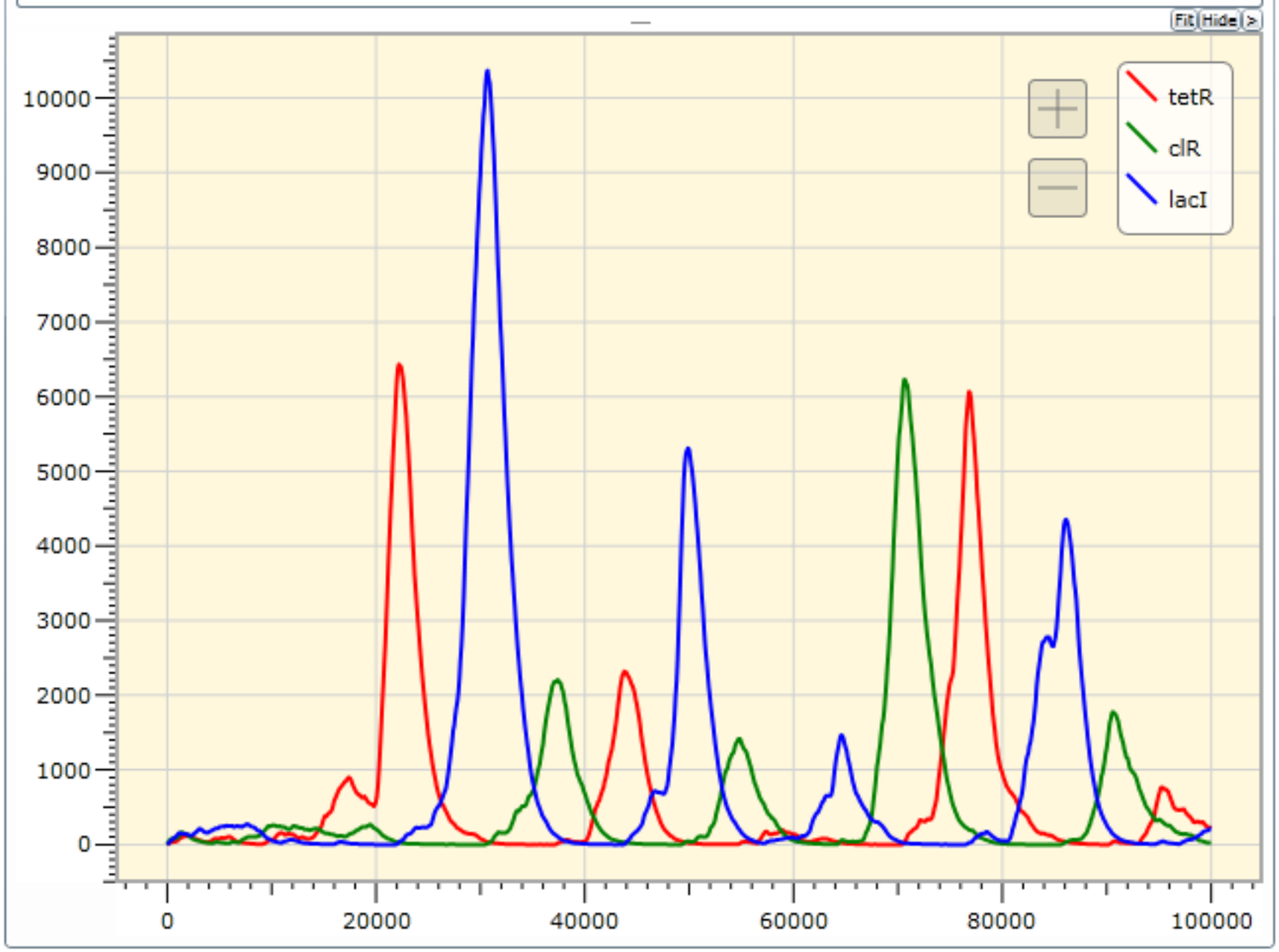
```
directive sample 100000.0 1000
directive plot tetR; clR; lacI

rate RMRNADeg = 0.001;

init g9 1 |
mrna10 ->{RMRNADeg}   |
g9 ->{0.1} g9 + mrna10  |
g9 + lacI ->{1.0} g9-lacI  |
g9-lacI ->{0.5} g9 + lacI  |
g9-lacI ->{5E-05} g9-lacI + mrna10  |
init g31 1 |
mrna32 ->{RMRNADeg}   |
g31 ->{0.09} g31 + mrna32  |
g31 + tetR ->{1.0} g31-tetR  |
g31-tetR ->{0.5} g31 + tetR  |
g31-tetR ->{5E-05} g31-tetR + mrna32  |
init g53 1 |
mrna54 ->{RMRNADeg}   |
g53 ->{0.12} g53 + mrna54  |
g53 + clR ->{1.0} g53-clR  |
g53-clR ->{0.5} g53 + clR  |
g53-clR ->{5E-05} g53-clR + mrna54  |
mrna54 ->{0.1} mrna54 + lacI  |
```

Weak Typing

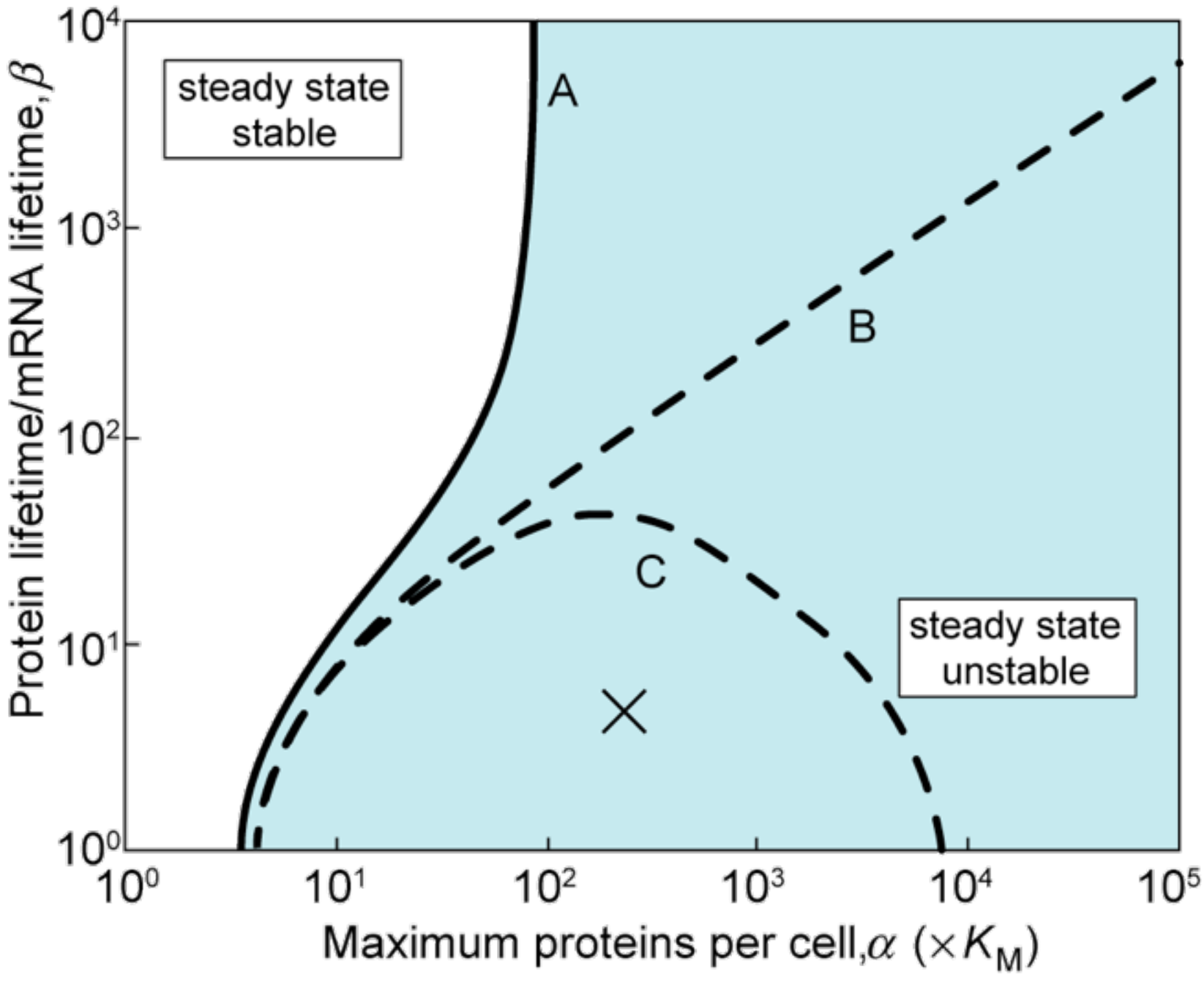
Units: Concentrations | Molecules

Reaction Graph | Reactions Text | Reactions | Kappa | SBML | MATLAB | Table | Plot

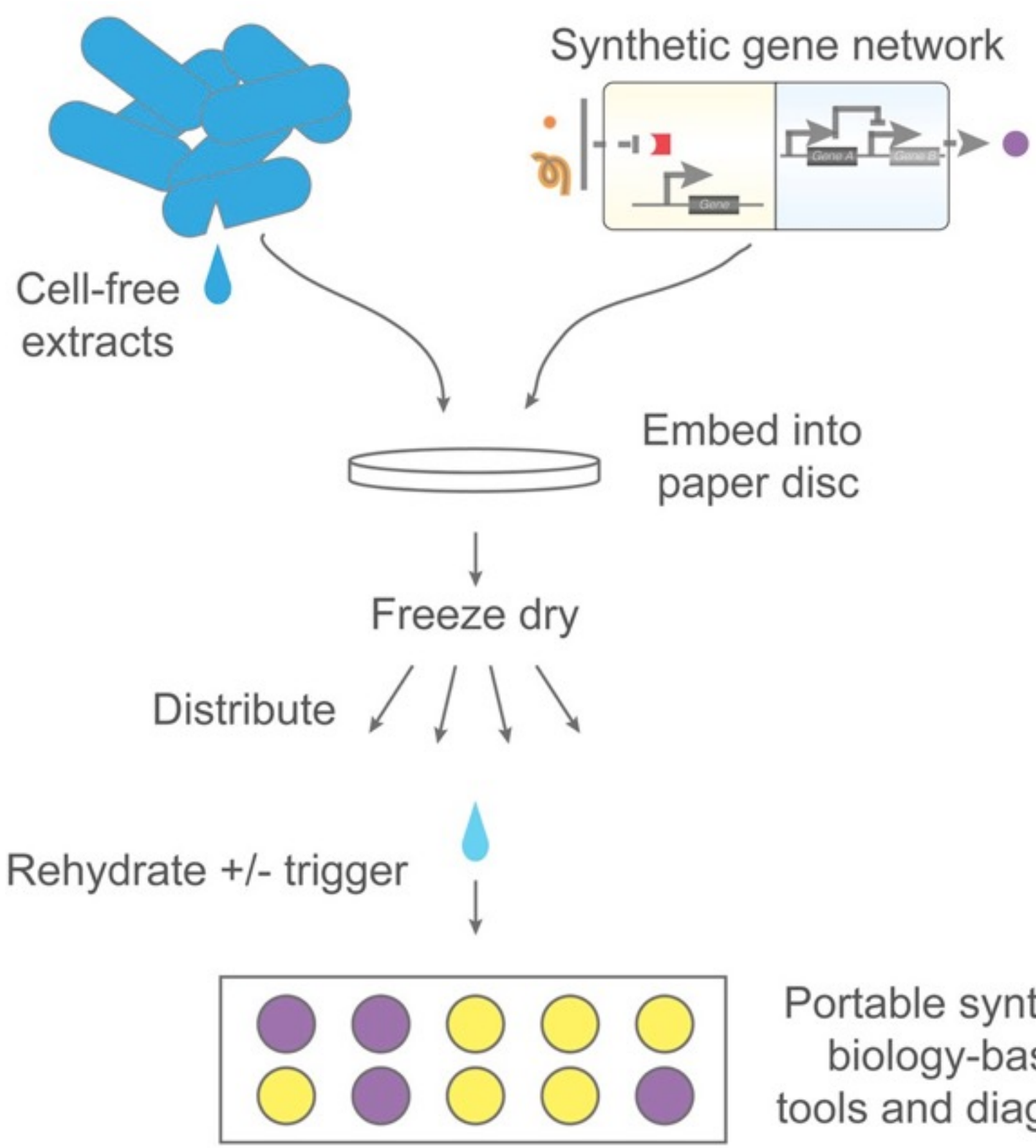
Show all | Hide all | tetR | clR | lacI



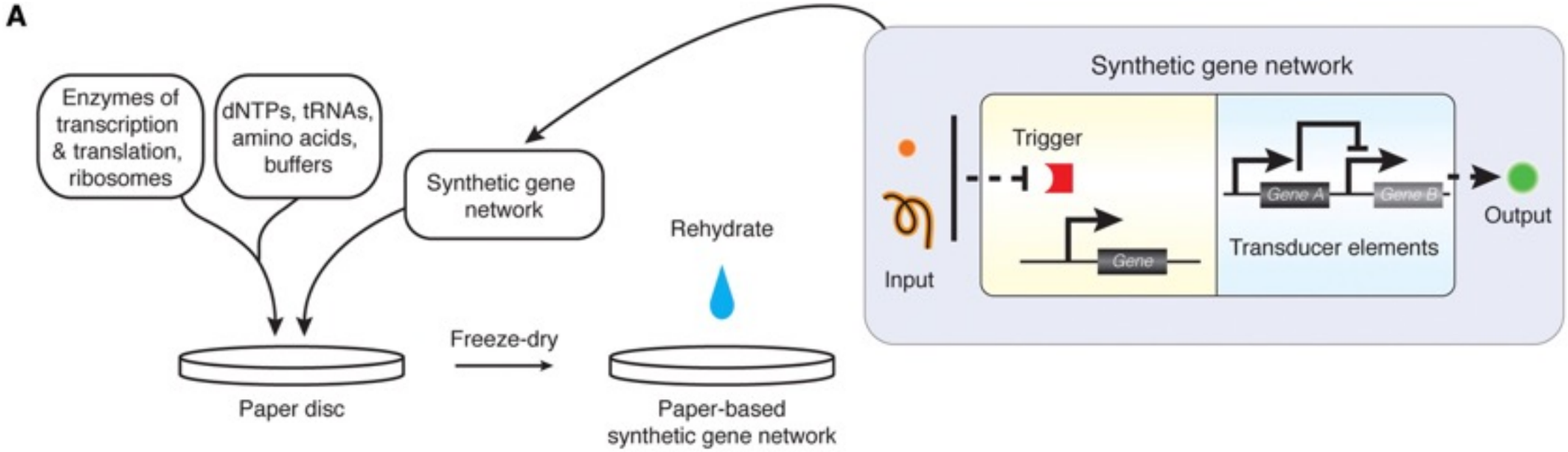
Network behavior



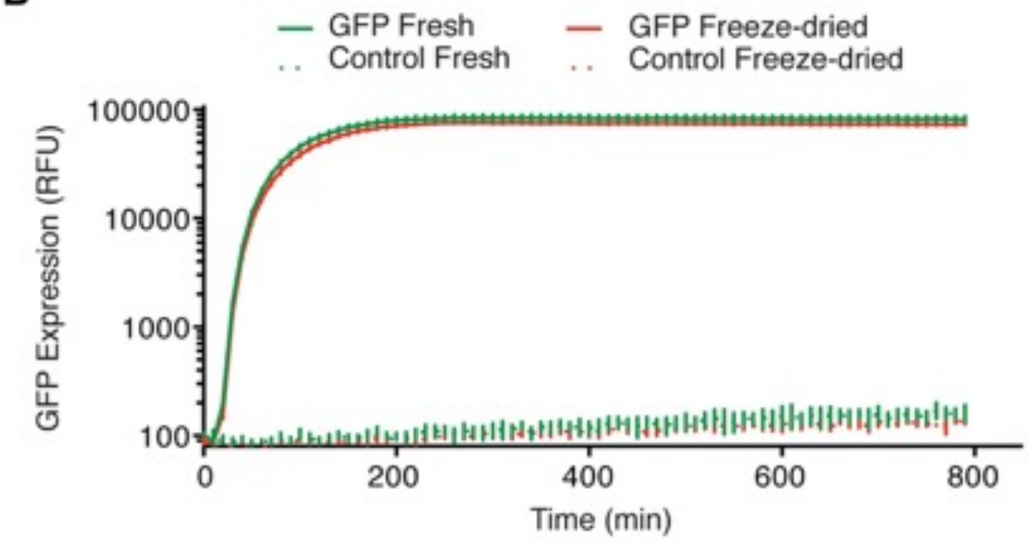
In vitro implementation of synthetic gene circuits



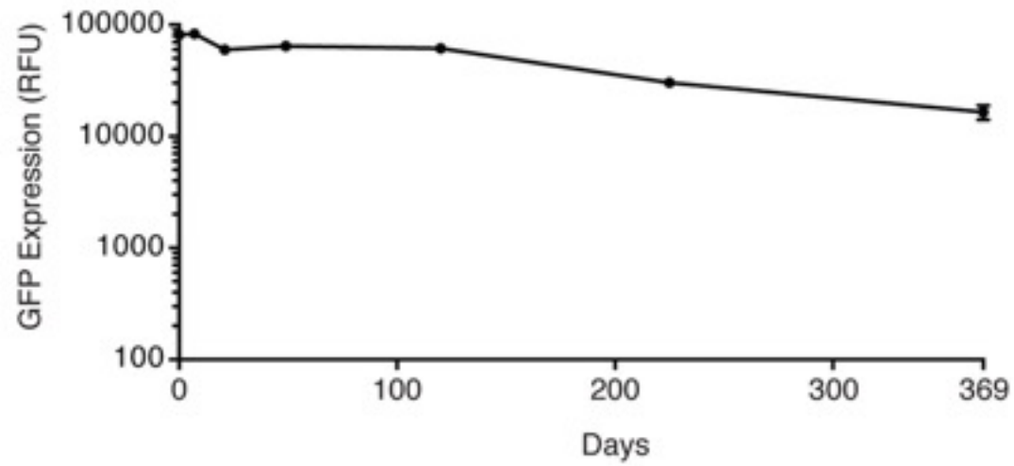
A


Enzymes of transcription & translation, ribosomes
dNTPs, tRNAs, amino acids, buffers
Synthetic gene network
Paper disc
Freeze-dry
Rehydrate
Paper-based synthetic gene network
Synthetic gene network
Input
Trigger
Gene
Gene A
Gene B
Transducer elements
Output

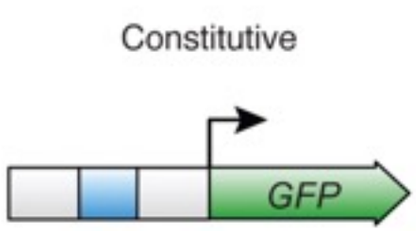
B


GFP Fresh
Control Fresh
GFP Freeze-dried
Control Freeze-dried
GFP Expression (RFU)
100000
10000
1000
100
0
200
400
600
800
Time (min)

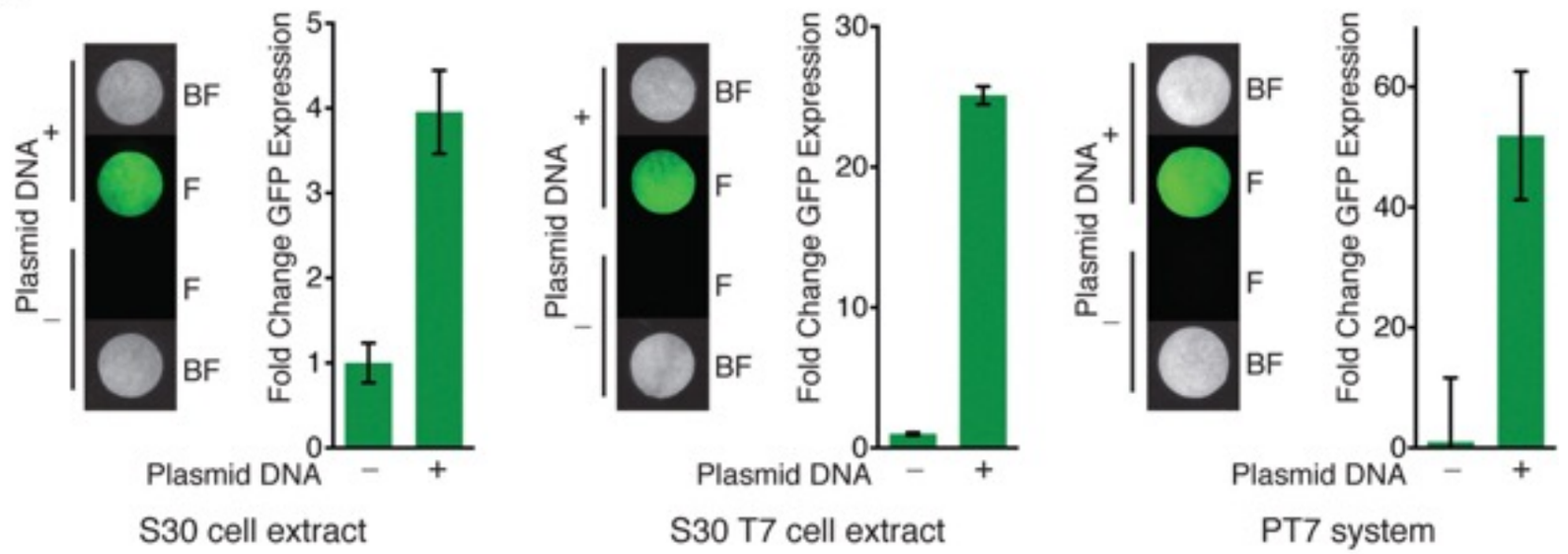
C


GFP Expression (RFU)
100000
10000
1000
100
0
100
200
300
369
Days

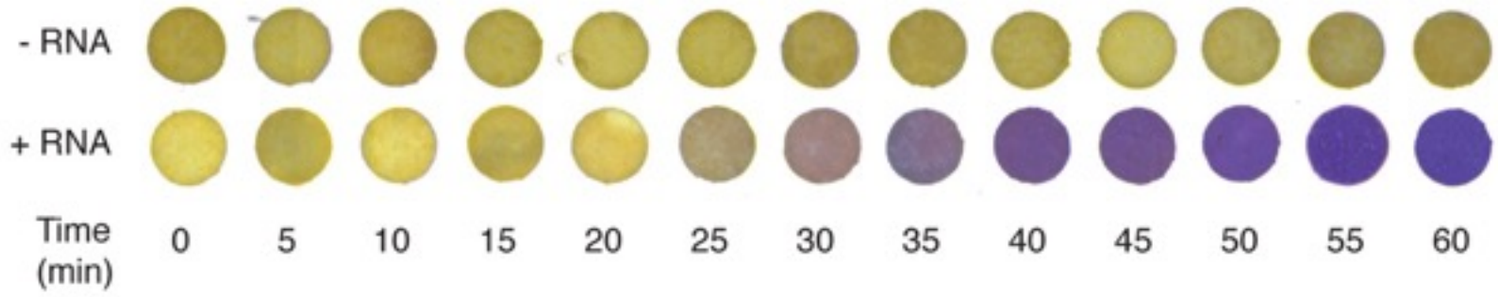
D


Constitutive
GFP

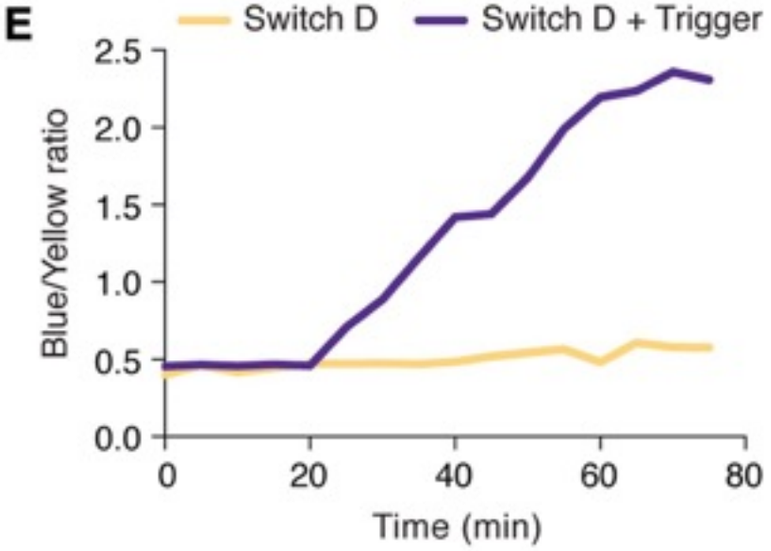
E


Plasmid DNA
+
−
BF
F
BF
Fold Change GFP Expression
Plasmid DNA
S30 cell extract
S30 T7 cell extract
PT7 system

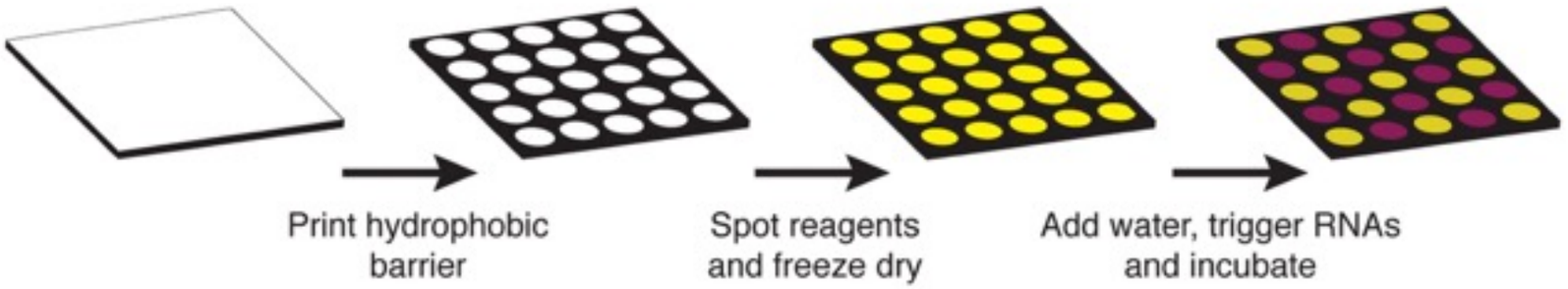
D


- RNA
+ RNA
Time (min)
0
5
10
15
20
25
30
35
40
45
50
55
60

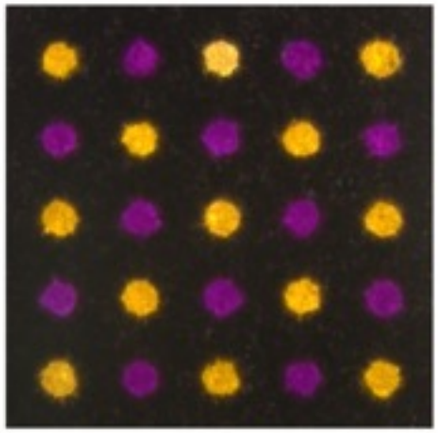
E


Switch D
Switch D + Trigger
Blue/Yellow ratio
2.5
2.0
1.5
1.0
0.5
0.0
0
20
40
60
80
Time (min)

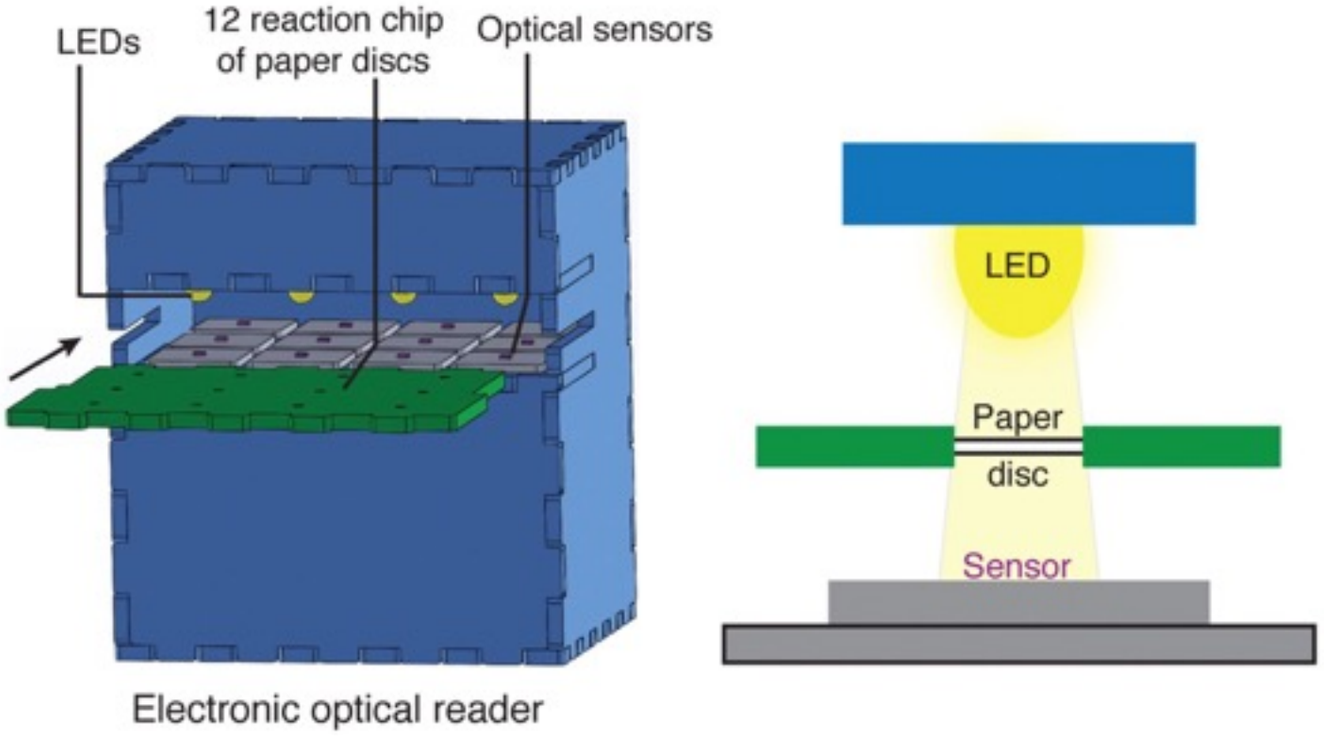
F


Print hydrophobic barrier
Spot reagents and freeze dry
Add water, trigger RNAs and incubate

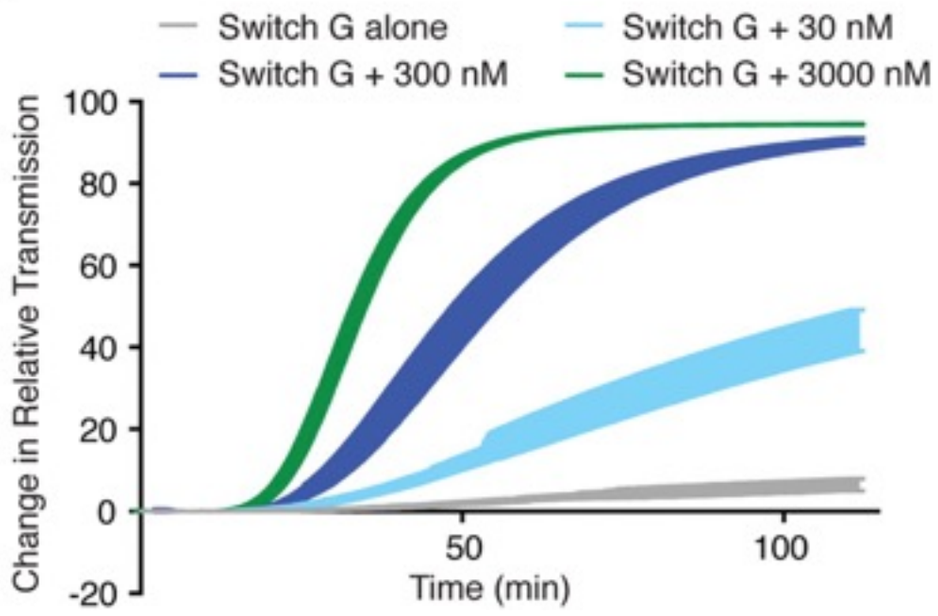
G



H


LEDs
12 reaction chip of paper discs
Optical sensors
Electronic optical reader
LED
Paper disc
Sensor

I

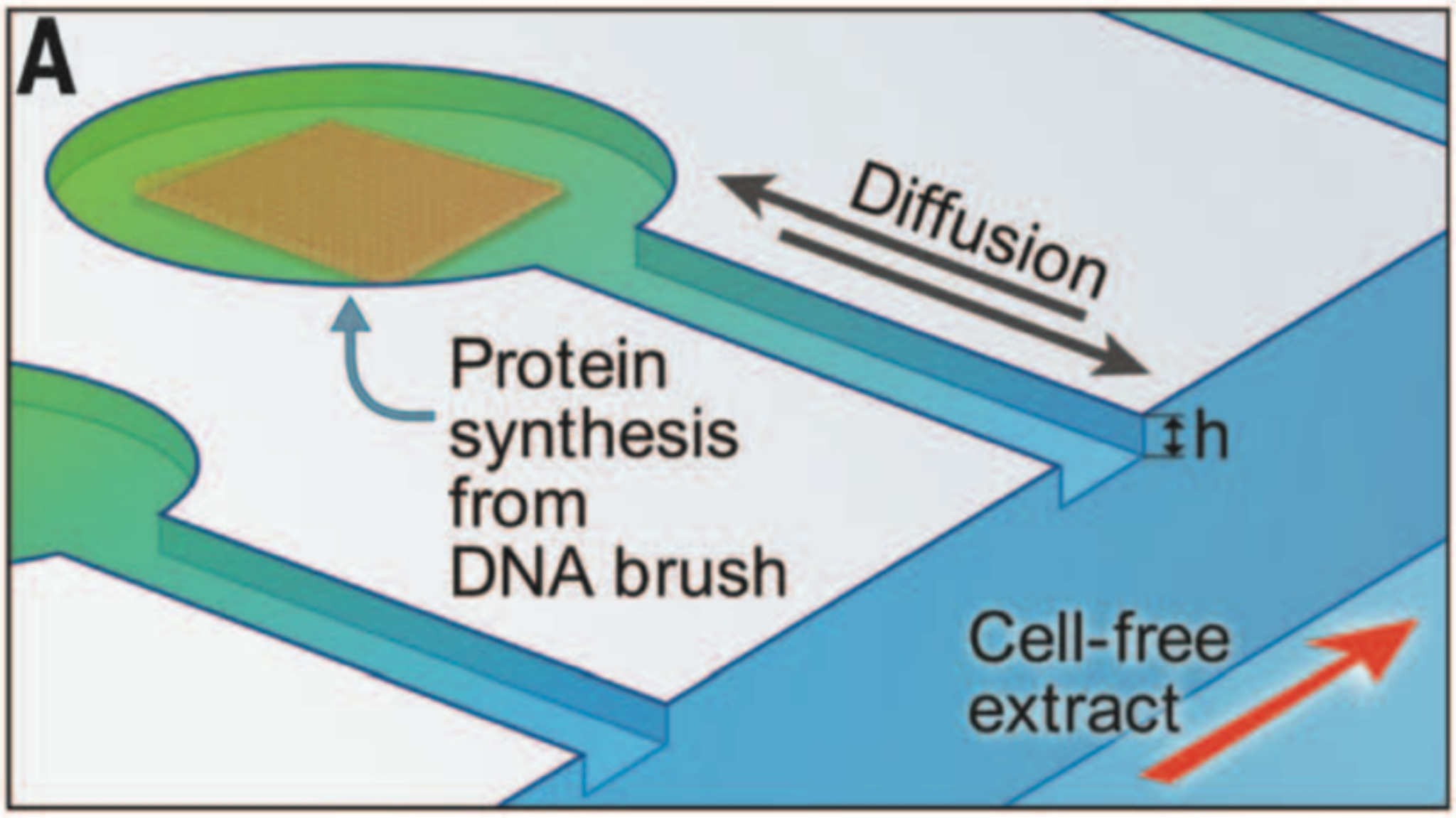

Switch G alone
Switch G + 30 nM
Switch G + 300 nM
Switch G + 3000 nM
Change in Relative Transmission
100
80
60
40
20
0
-20
50
100
Time (min)

SYNTHETIC BIOLOGY

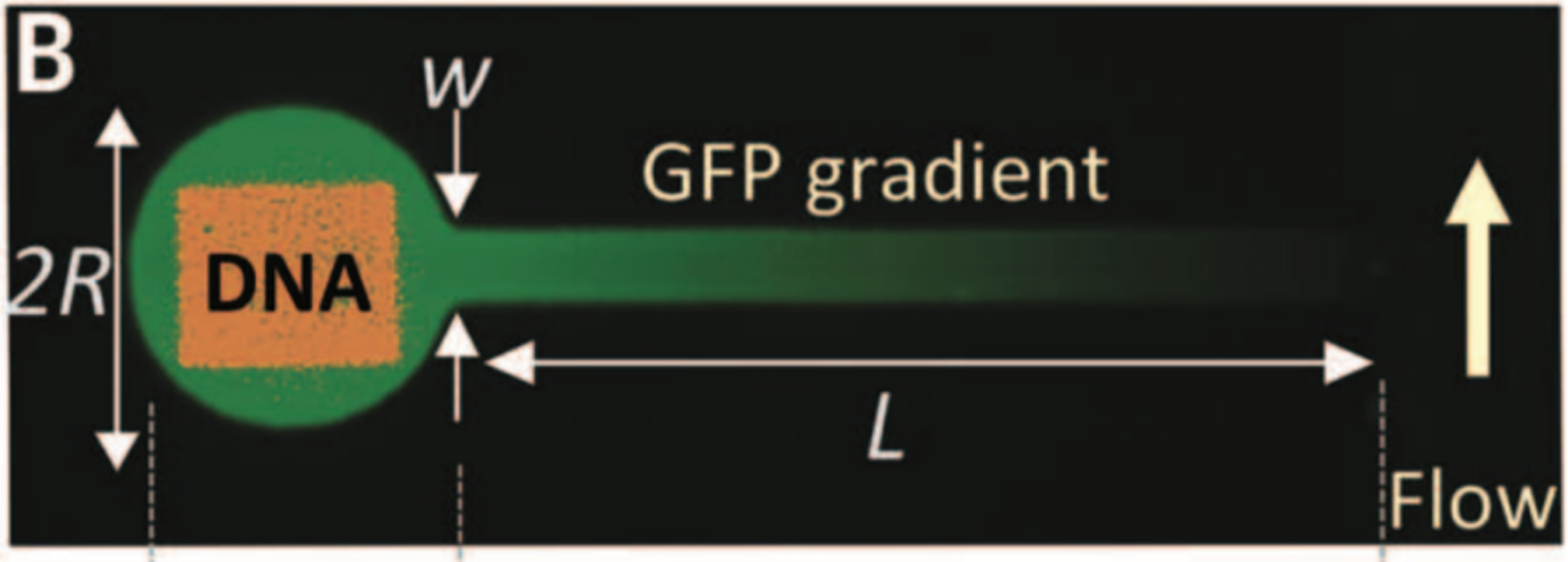
Programmable on-chip DNA compartments as artificial cells

Eyal Karzbrun,[1*] Alexandra M. Tayar,[1*] Vincent Noireaux,[2] Roy H. Bar-Ziv[1]†

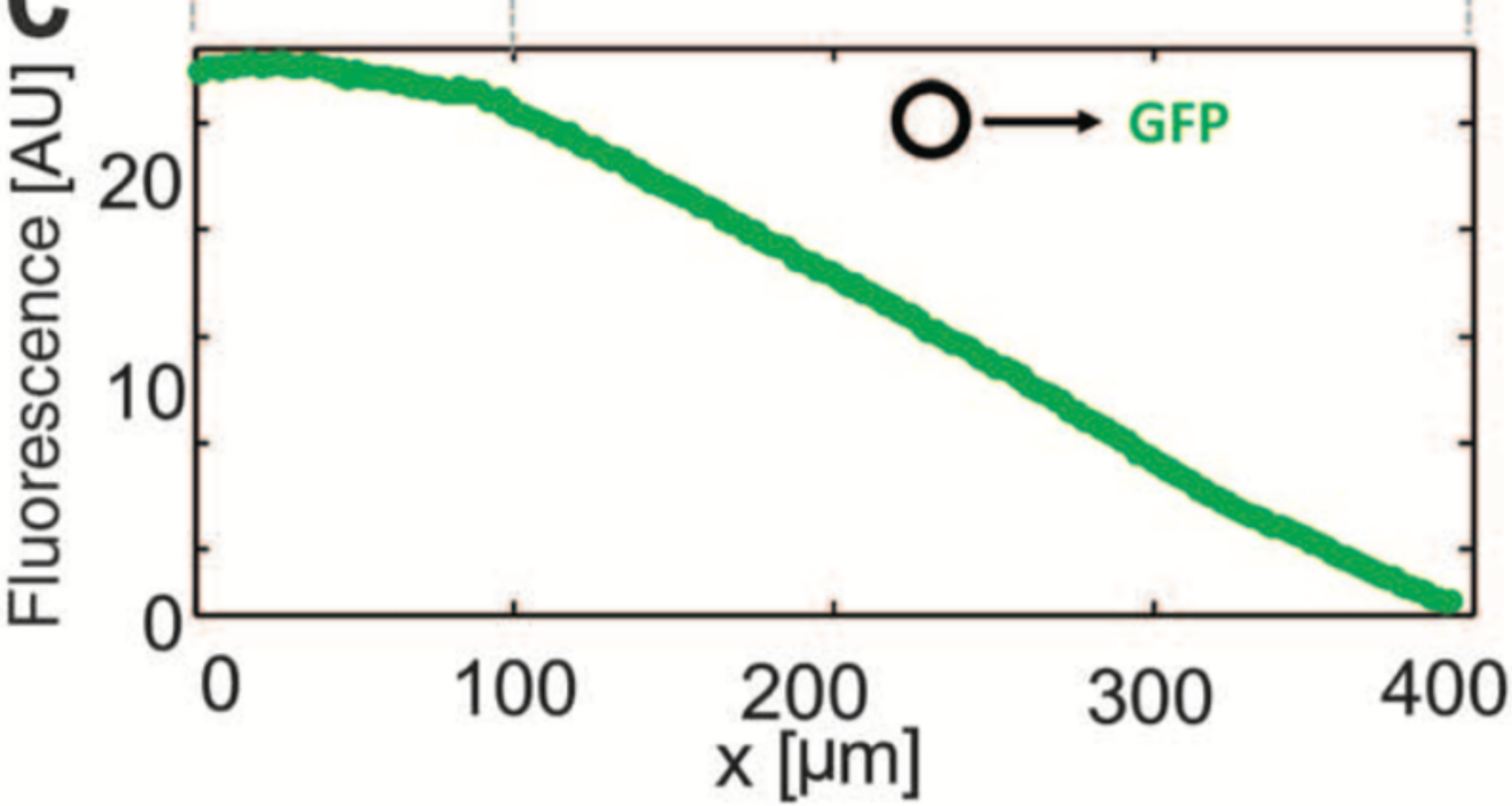
The assembly of artificial cells capable of executing synthetic DNA programs has been an important goal for basic research and biotechnology. We assembled two-dimensional DNA compartments fabricated in silicon as artificial cells capable of metabolism, programmable protein synthesis, and communication. Metabolism is maintained by continuous diffusion of nutrients and products through a thin capillary, connecting protein synthesis in the DNA compartment with the environment. We programmed protein expression cycles, autoregulated protein levels, and a signaling expression gradient, equivalent to a morphogen, in an array of interconnected compartments at the scale of an embryo. Gene expression in the DNA compartment reveals a rich, dynamic system that is controlled by geometry, offering a means for studying biological networks outside a living cell.



B

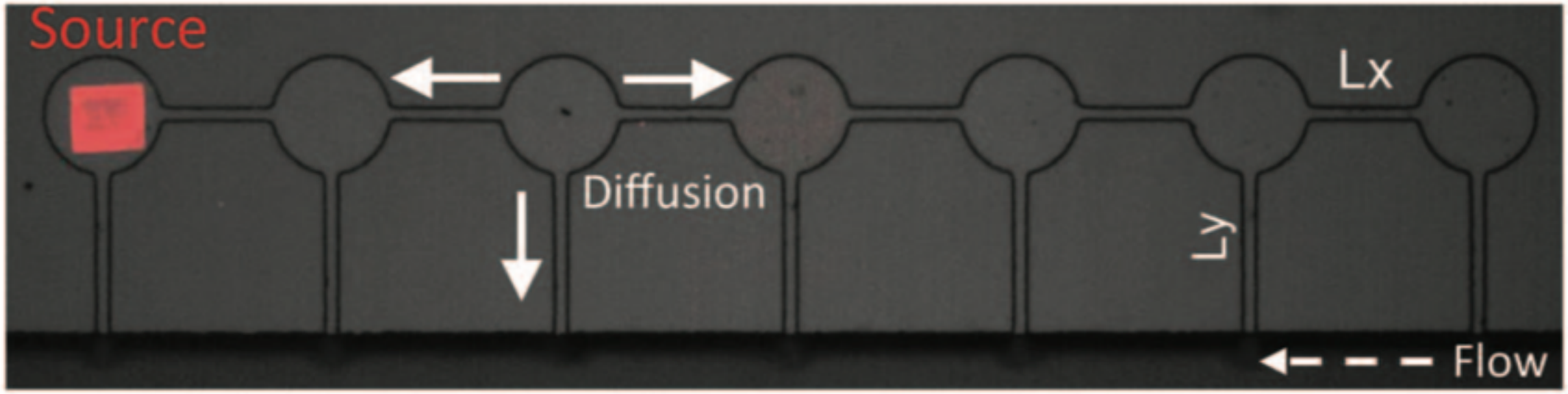

W
2R
DNA
GFP gradient
L
Flow

C


GFP
Fluorescence [AU]
0
10
20
0
100
200
300
400
x [μm]

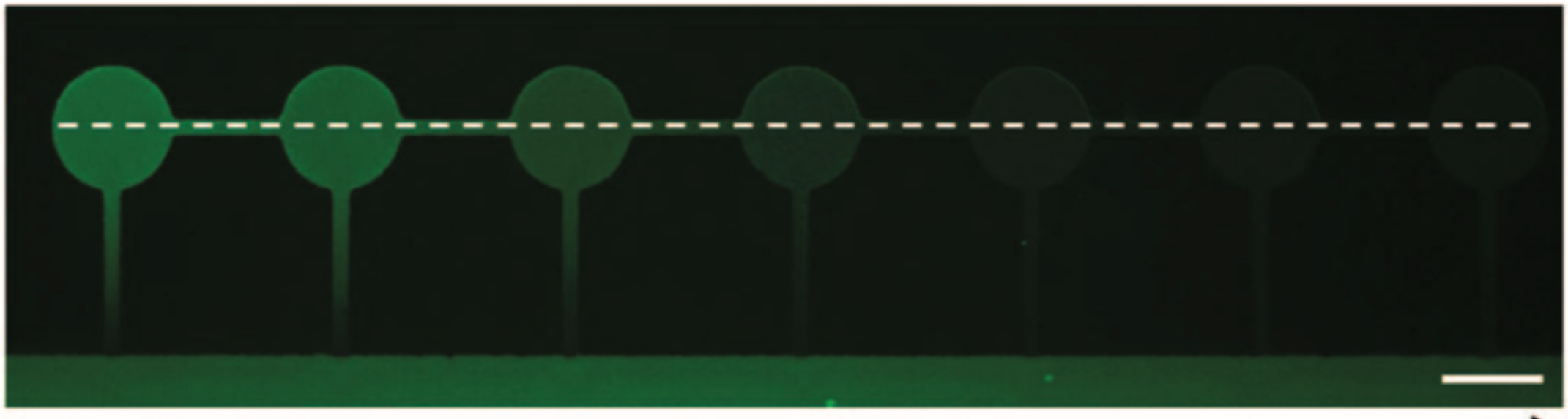
A

DNA

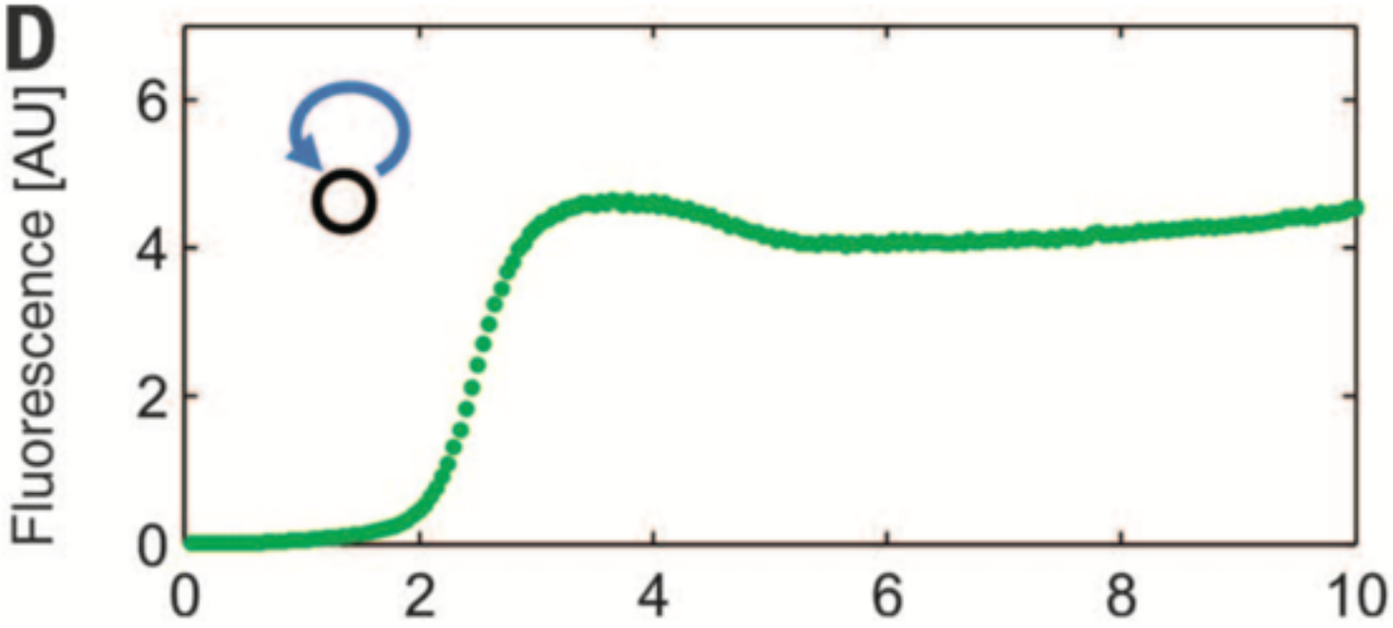


B

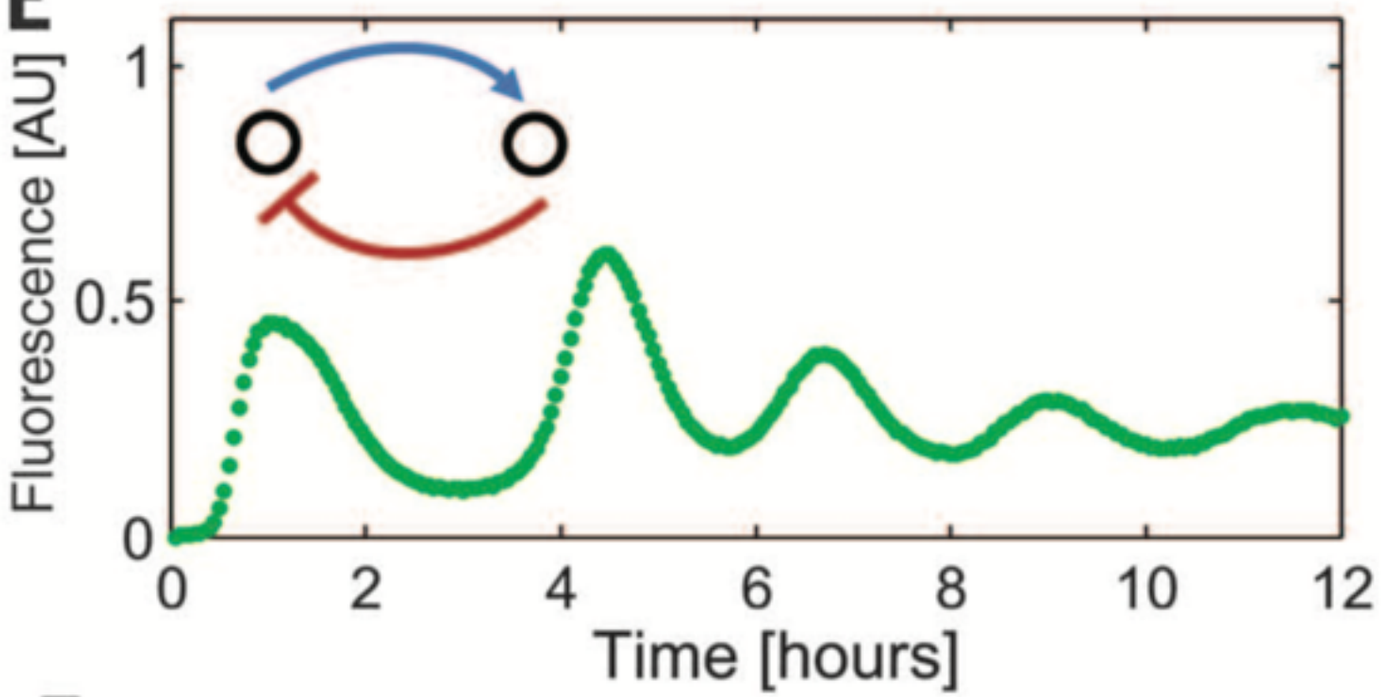
GFP



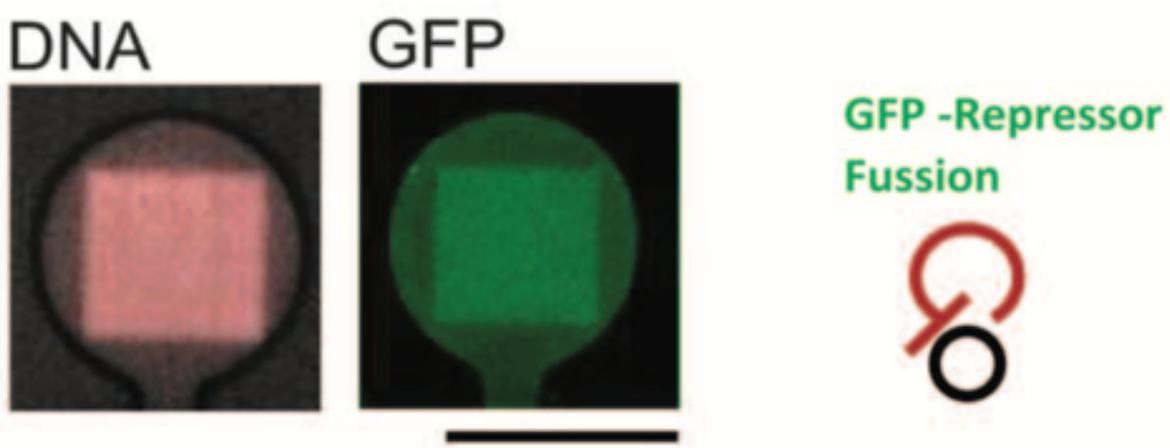
D


Fluorescence [AU]
0
2
4
6
0
2
4
6
8
10

E


Fluorescence [AU]
0
0.5
1
0
2
4
6
8
10
12
Time [hours]

F


DNA
GFP
GFP -Repressor
Fussion

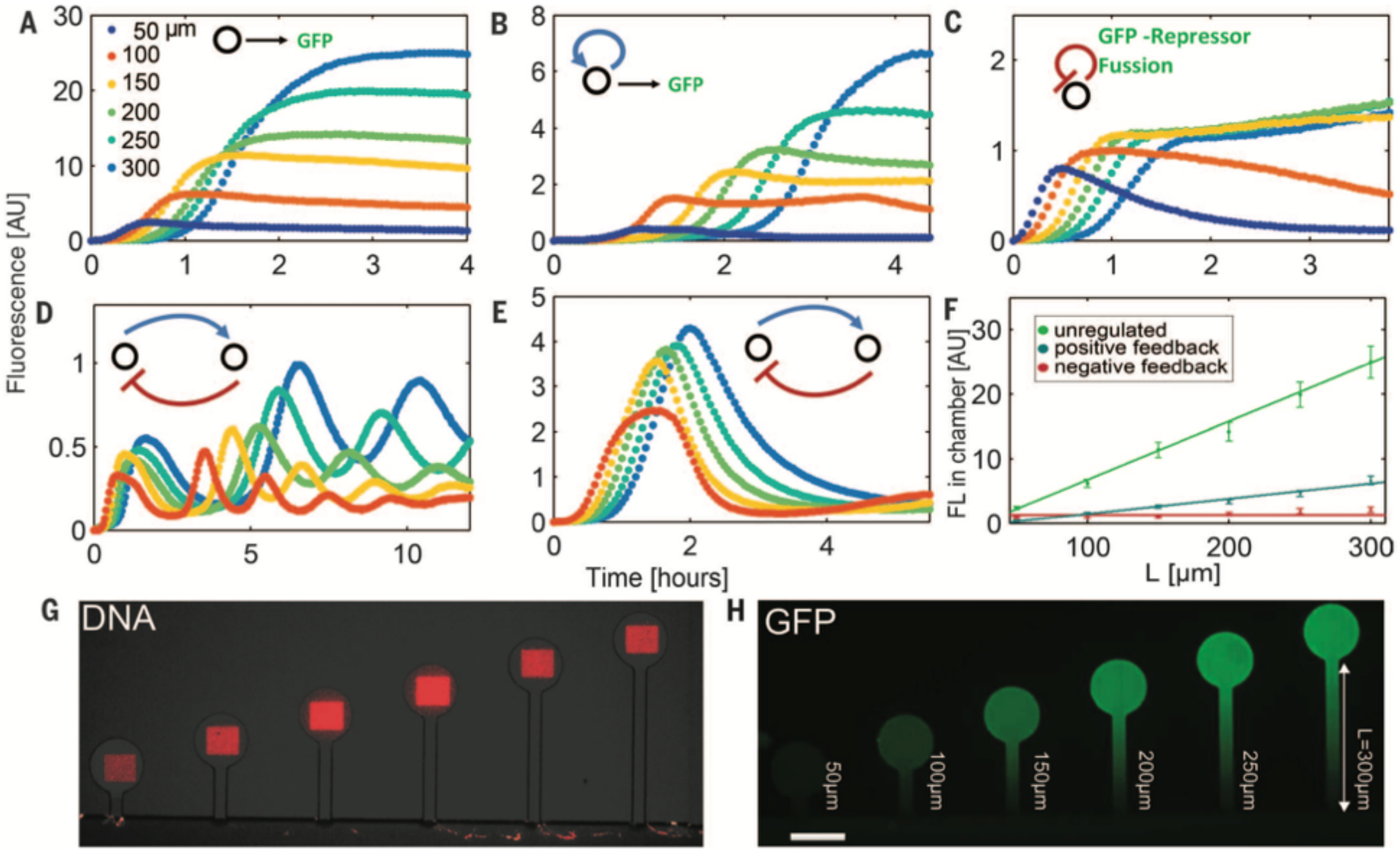


Fig. 2. Gene network dynamics regulated by geometry. Expression dynamics of GFP in the DNA compartment with L = 50 to 300 µm as denoted and for

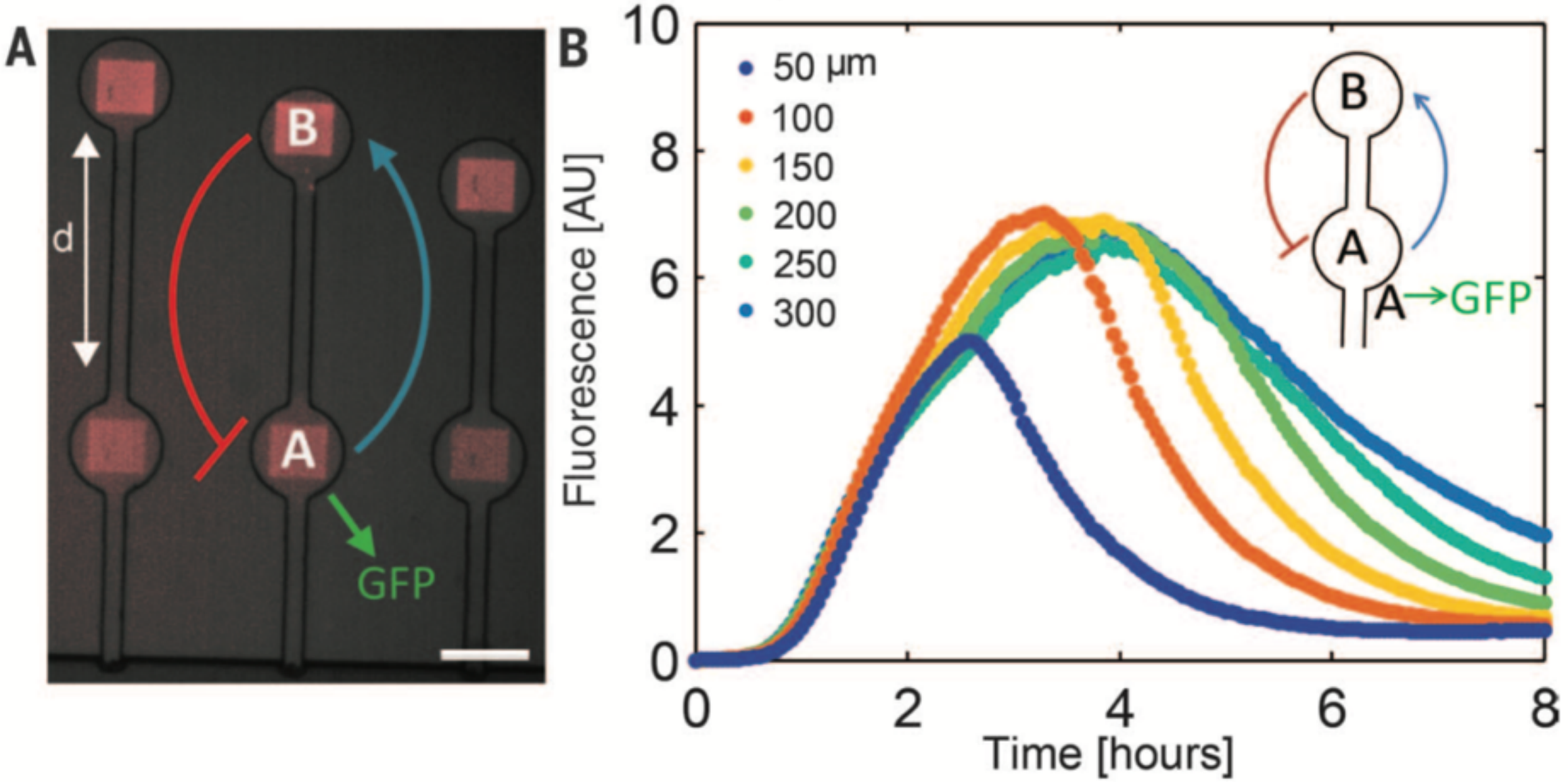
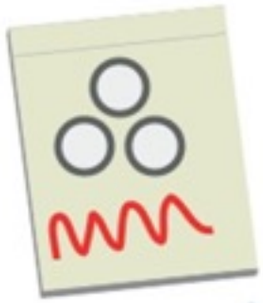
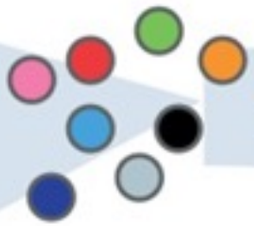


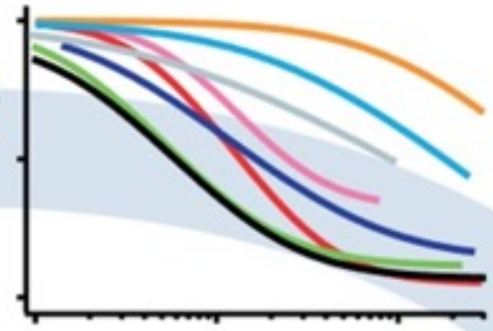
Fig. 3. Communication between DNA compartments. (**A**) Fluorescent image of DNA brush overlaid with the activator (denoted A)–repressor (denoted B) network scheme. The distance between compartments A and B varied, d = 50 to 300 µm. Scale bar, 100 µm. (**B**) GFP expression kinetics at compartment A for different distances between compartments, as denoted.

1. Design and **Model**:
circuit concept

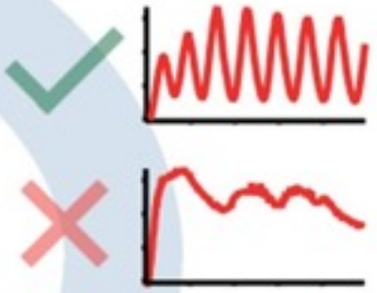


2. Build:
parts on linear DNA

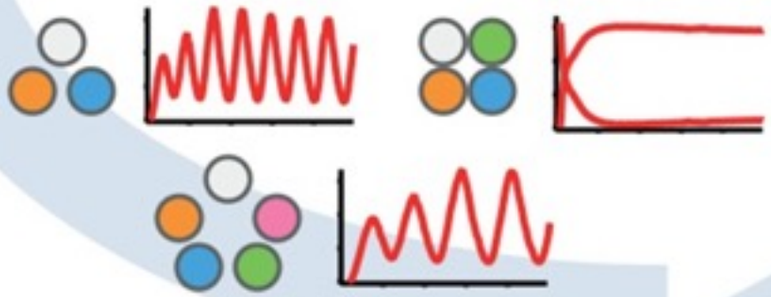




3. Test parts:
in vitro

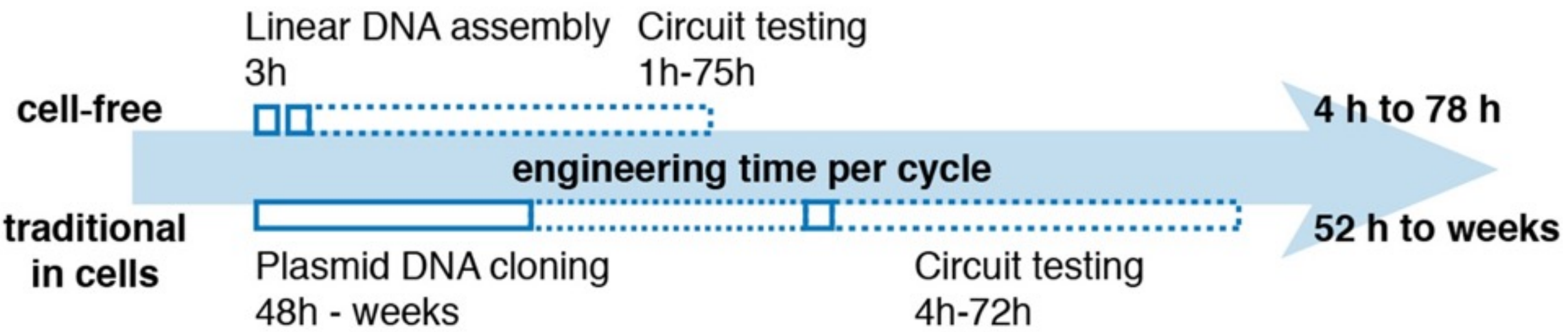


4. Test circuits:
multiple variants

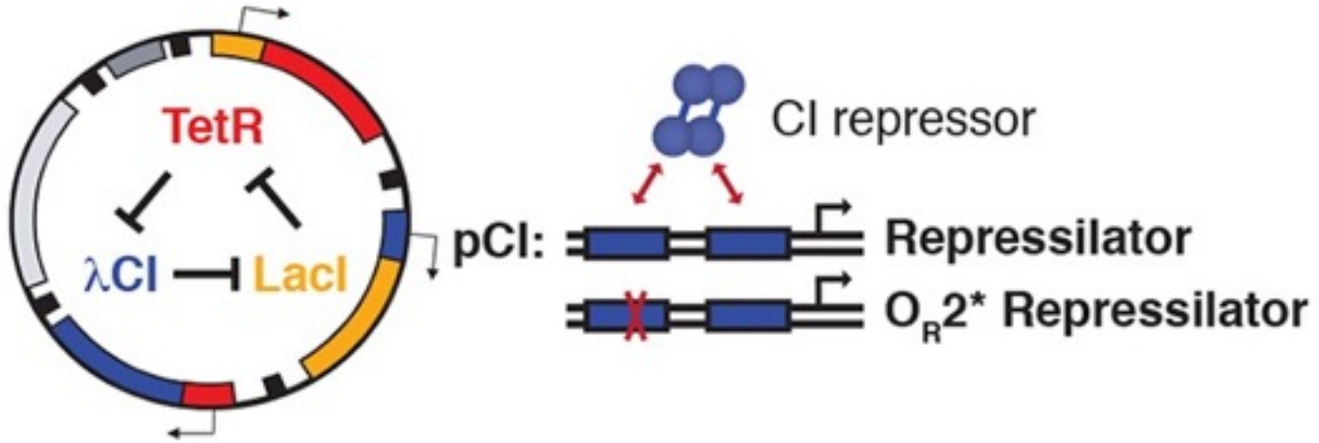


5. Characterize:
working circuits

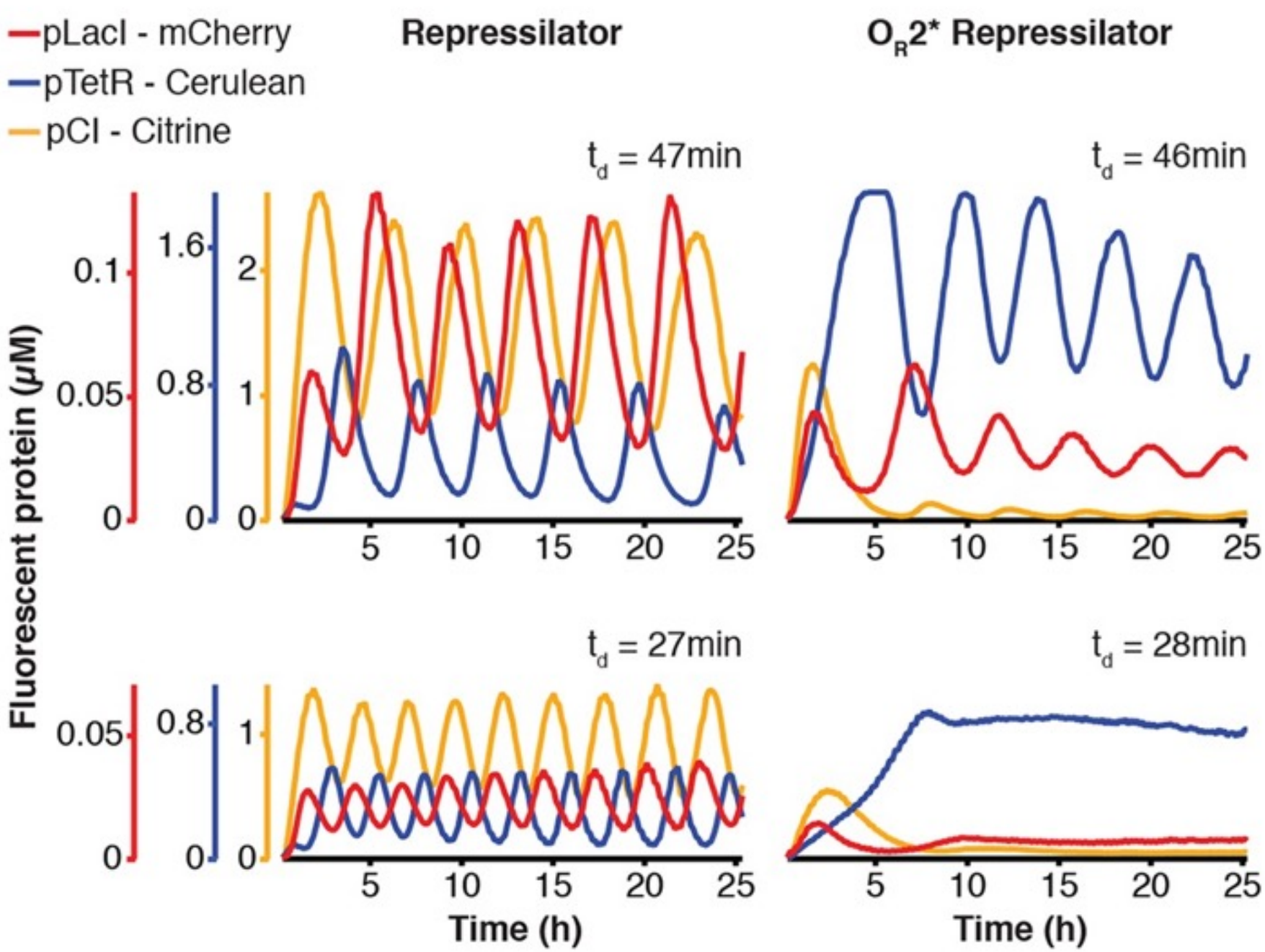
6. Clone and **Implement:**
in vivo



A


TetR
λCI
LacI
pCI:
CI repressor
Repressilator
O_R2* Repressilator

B


pLacI - mCherry
pTetR - Cerulean
pCI - Citrine
Repressilator
O_R2* Repressilator
t_d = 47min
t_d = 46min
t_d = 27min
t_d = 28min
Fluorescent protein (μM)
Time (h)

Composite
0 min

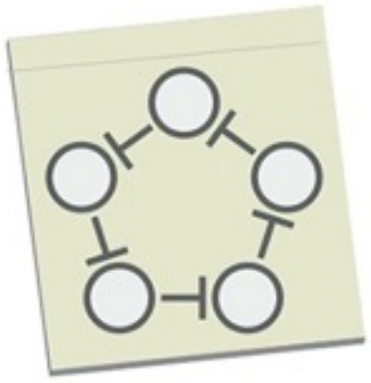
pCI

pTetR

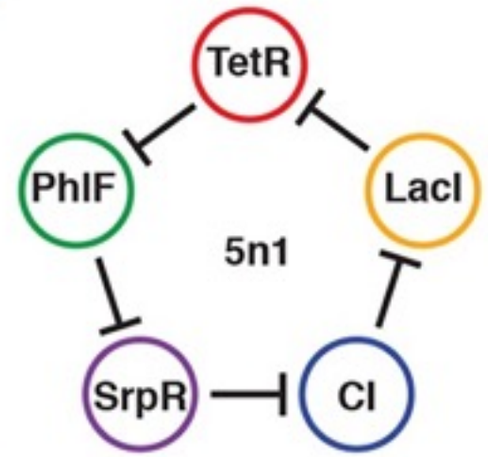
pLacI

50 µm

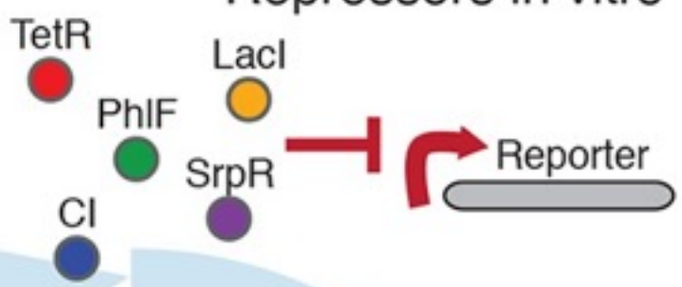
Design:
5-node
oscillator

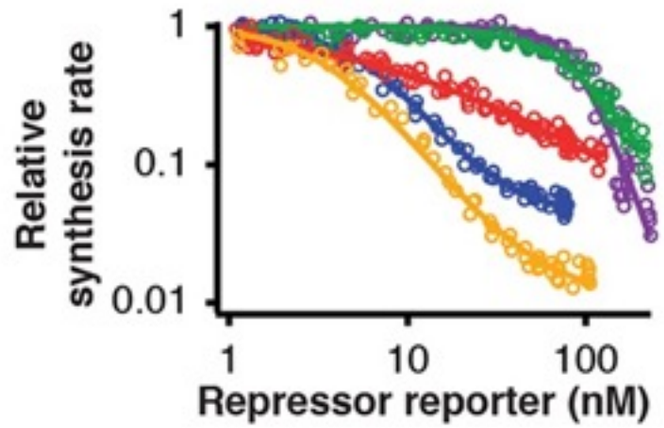


Model:
in silico

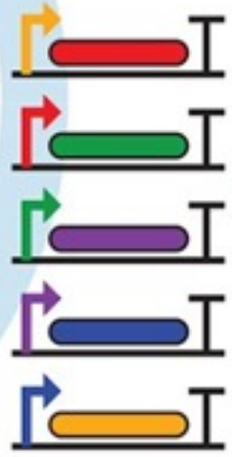


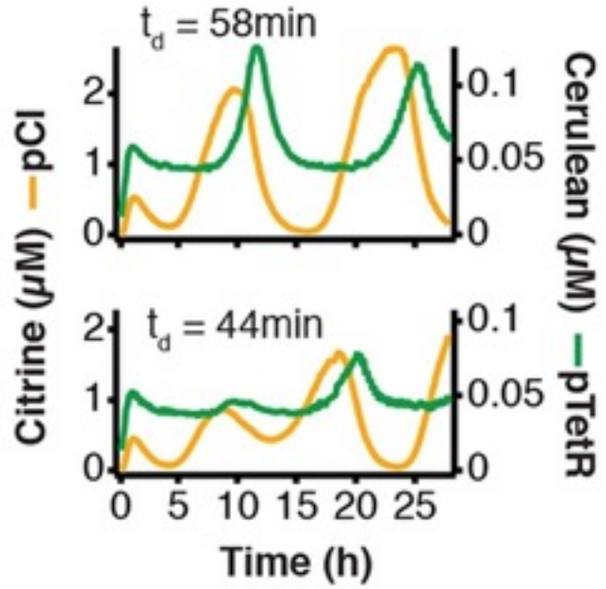
Test:
Repressors in vitro



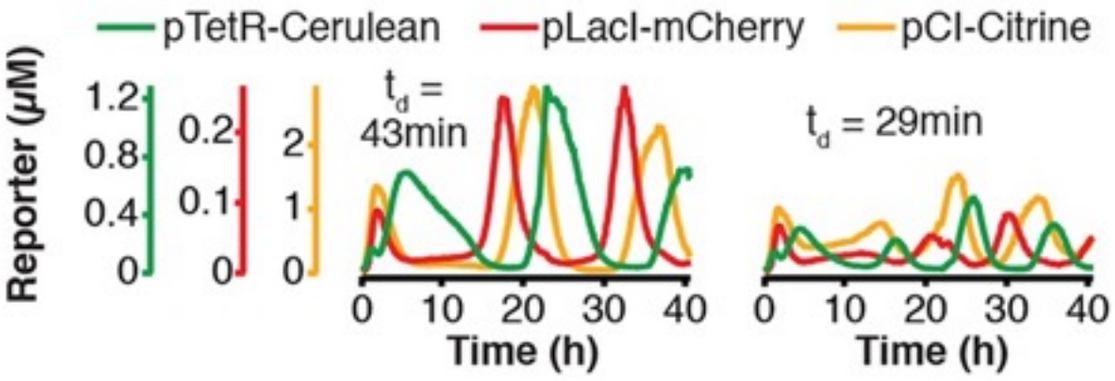


Test:
Circuit on linear DNA

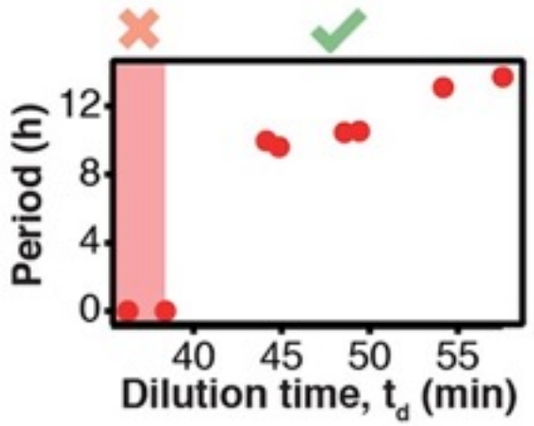


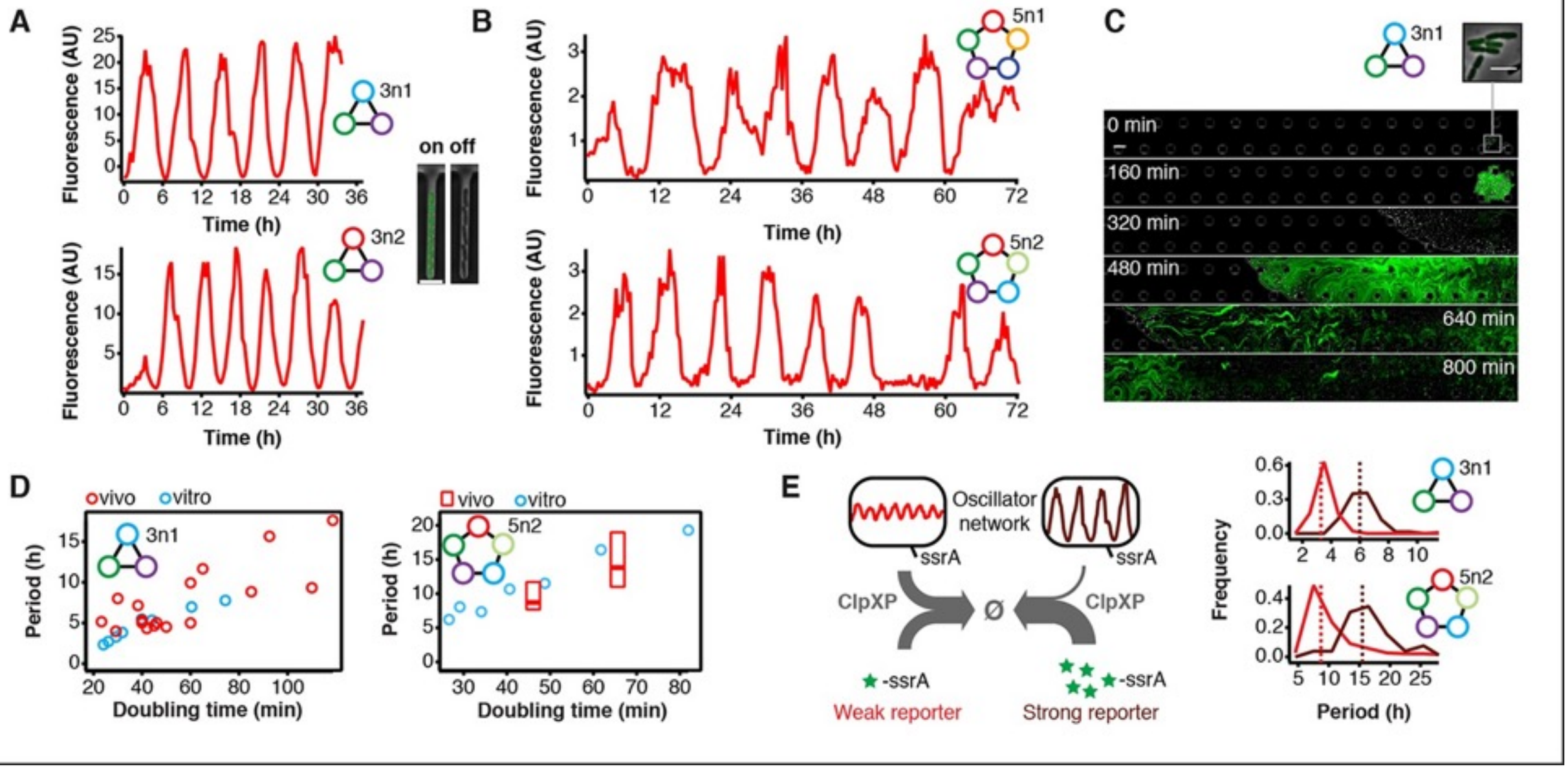


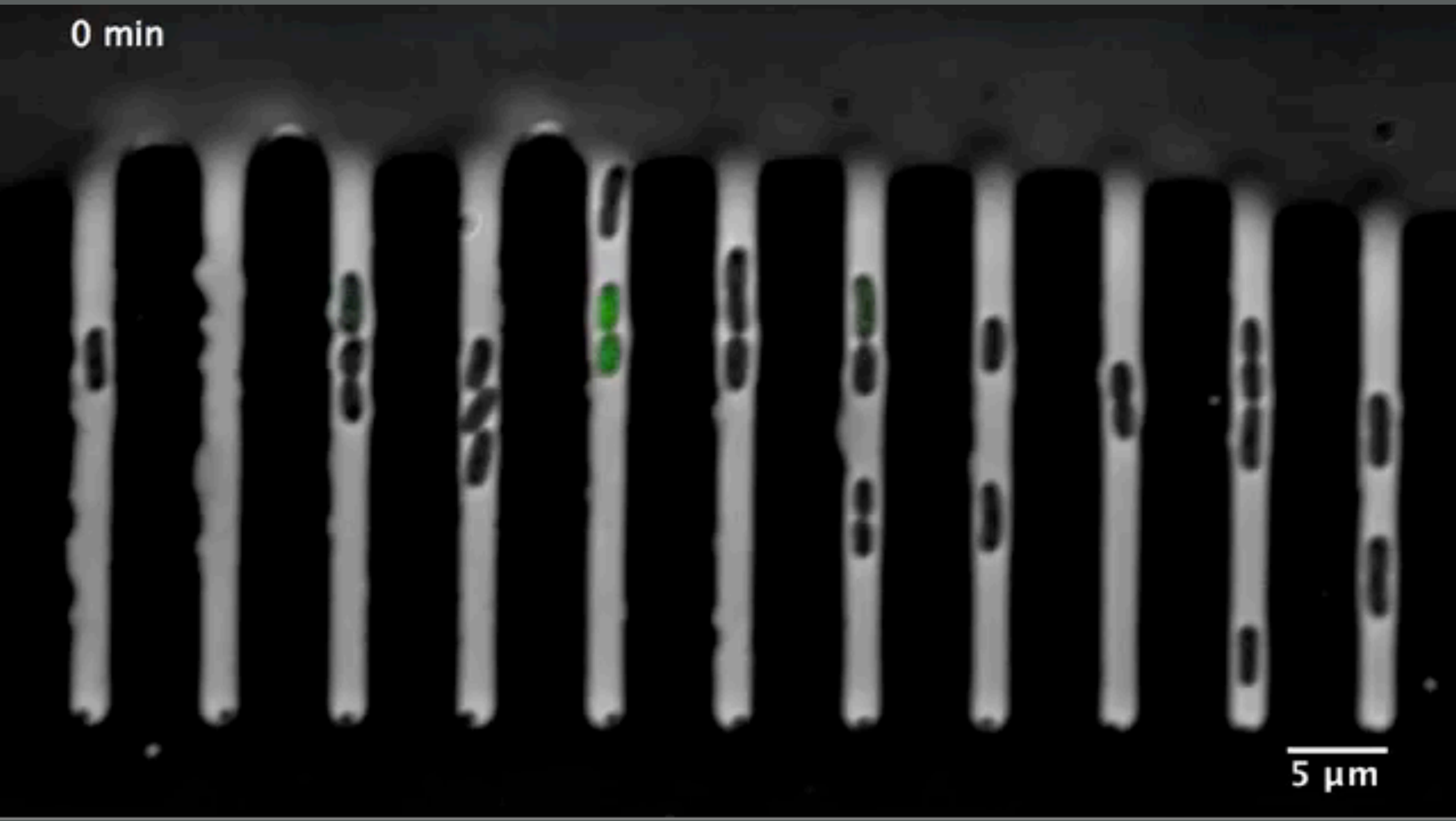
Verify:
Oscillator on plasmid
for in vivo implementation



Characterize:
Periods and
existence of oscillations




A
Fluorescence (AU)
Time (h)
3n1
3n2
on off
B
5n1
5n2
C
3n1
0 min
160 min
320 min
480 min
640 min
800 min
D
vivo
vitro
Period (h)
Doubling time (min)
3n1
5n2
E
Oscillator network
ssrA
ClpXP
Ø
-ssrA
Weak reporter
Strong reporter
Frequency
Period (h)

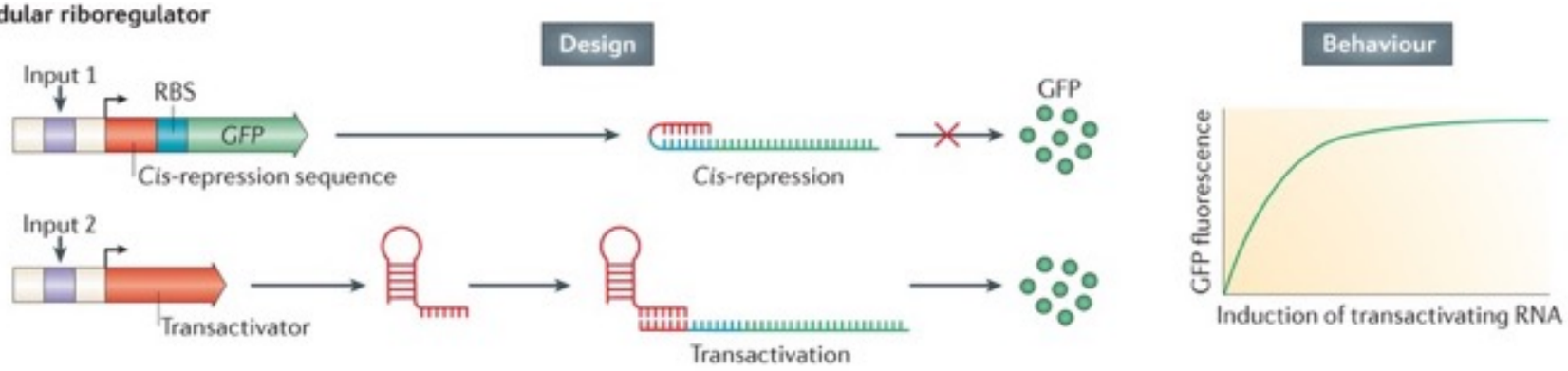

0 min
5 µm

Rational circuit design

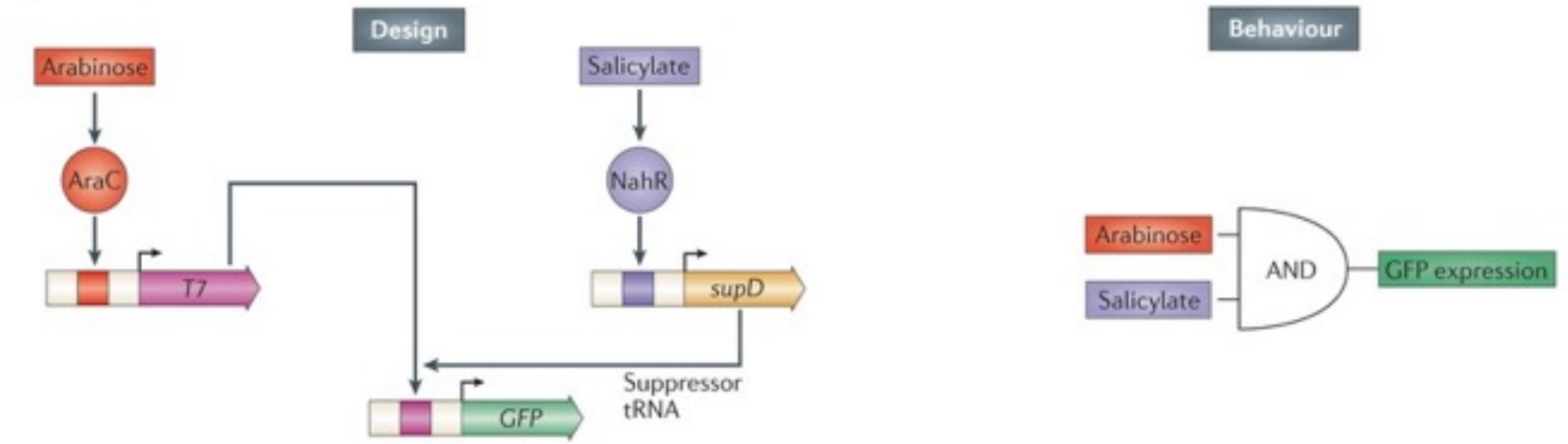
- Problem: to understand design principles of biological networks
- Approach: Design and construct synthetic network

- Knowledge gained from the design of synthetic networks should help understand real networks
- Could be used to engineer new cellular behaviours in plants

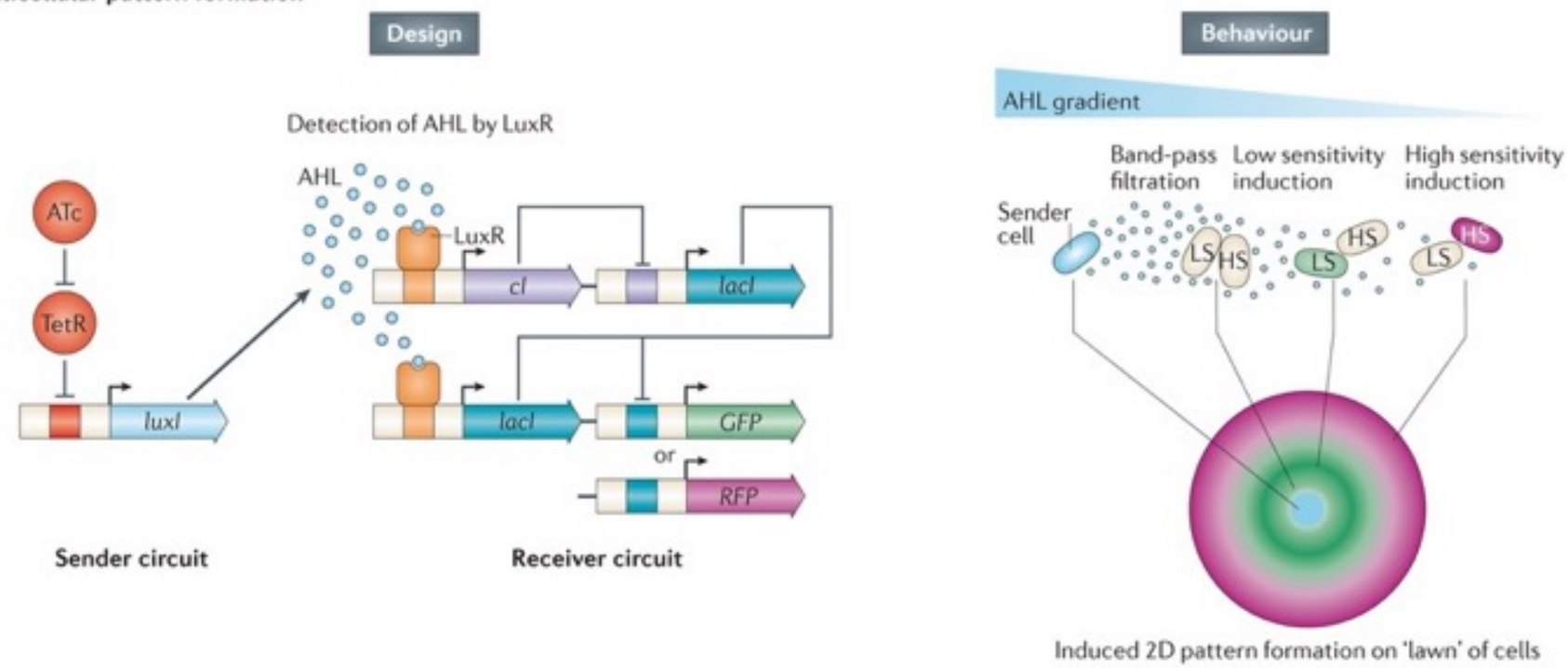
a Modular riboregulator



b Two-input AND gate



c Multicellular pattern formation



Lecture 1: Genetic modification in agriculture and the advent of Synthetic Biology.

Lecture 2: Genetic circuits and genome scale DNA engineering.

Lecture 3: Engineered logic and the control of gene expression.

1. **Cell autonomous genetic logic**
2. **Microbial test systems**
3. **Feedback regulation: toggle switches**
4. **Transcription networks: Genetic oscillators**
5. ***In vitro* systems for rapid testing**
6. **Complex circuit design**

Lecture 4: Self-organisation and reprogramming of multicellular systems.

Additional resources: **http://www.haseloff-lab.org** (Education)