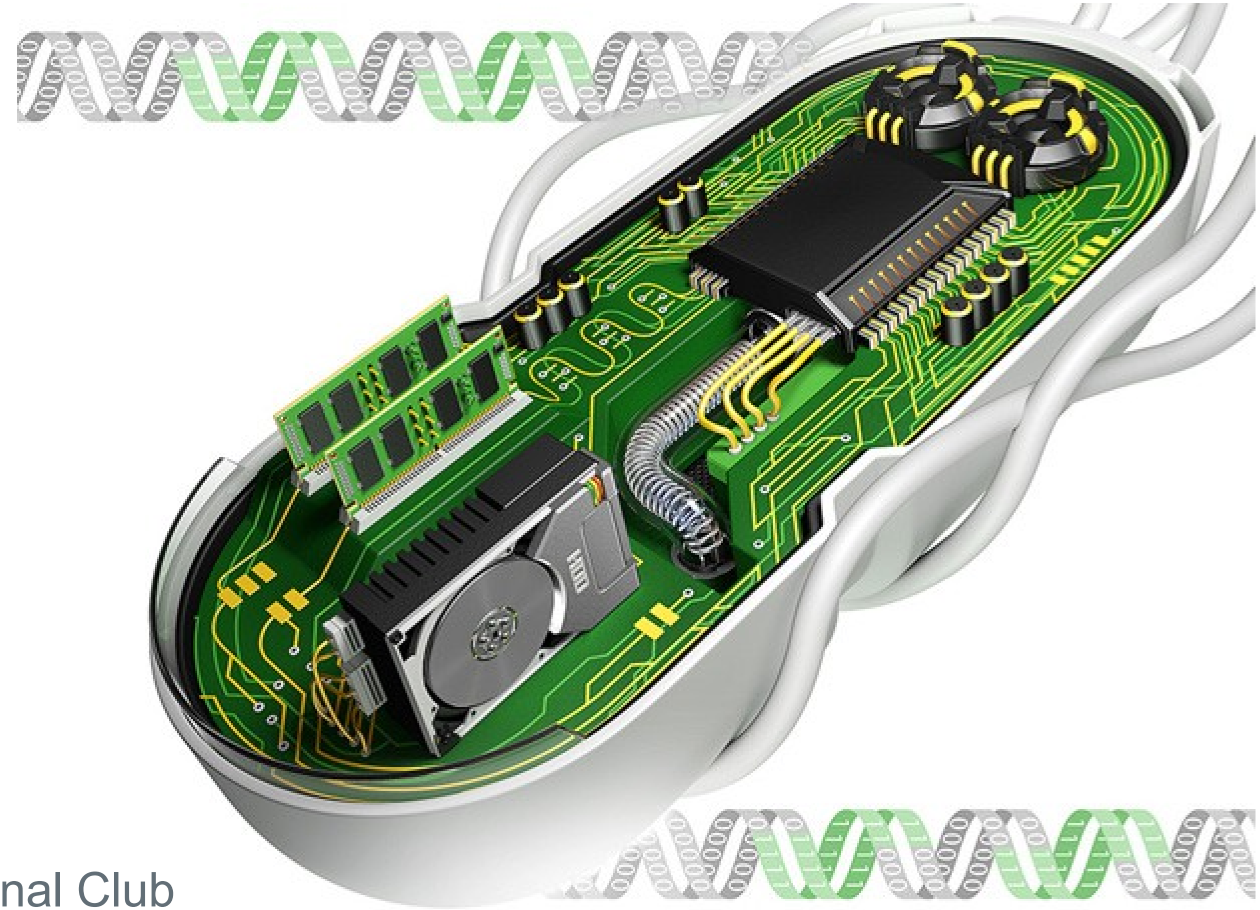
Biosensors and Genetic Circuits
Programmable Cells



Technical Journal Club
24.06.2014
Rahel Gerosa

http://www.engadget.com/2013/02/12/mit-crafts-genetic-circuits-that-remember-their-work-through-dna/

Synthetic Biology

Synthetic Biology is
A) the design and construction of new biological parts, devices, and systems, and
B) the re-design of existing, natural biological systems for useful purposes.

- It is an area that combines biology and engineering

- Approach to create new biological systems from different perspectives → finding how life works (the origin of life) or how to use it to benefit society

- Difference of synthetic biology and genetic engineering:
    - rather than altering an already existent DNA strand, build an entirely new strand of DNA from scratch which is then placed into an empty living cell

Synthetic Biology

Examples:

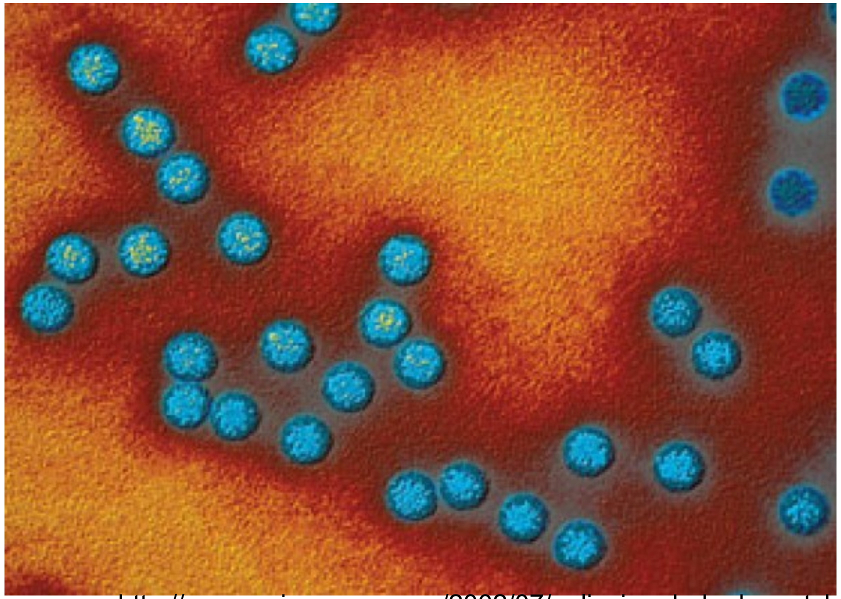
- Synthetic DNA
- Synthetic Cells
- Genome Editing
- Information Storage
- Synthetic circuits
- Biosensors

Synthetic Biology

Examples:

- **Synthetic DNA**
- Synthetic Cells
- Genome Editing
- Information Storage
- Synthetic circuits
- Biosensors

In 2002 researchers succeeded in synthesizing the 7741 base poliovirus genome from its published sequence (Couzin J. et al., Science, 2002)



http://news.sciencemag.org/2002/07/poliovirus-baked-scratch

Poliovirus reconstructed from its genetic sequence is indistinguishable from the original

Synthetic Biology

Examples:

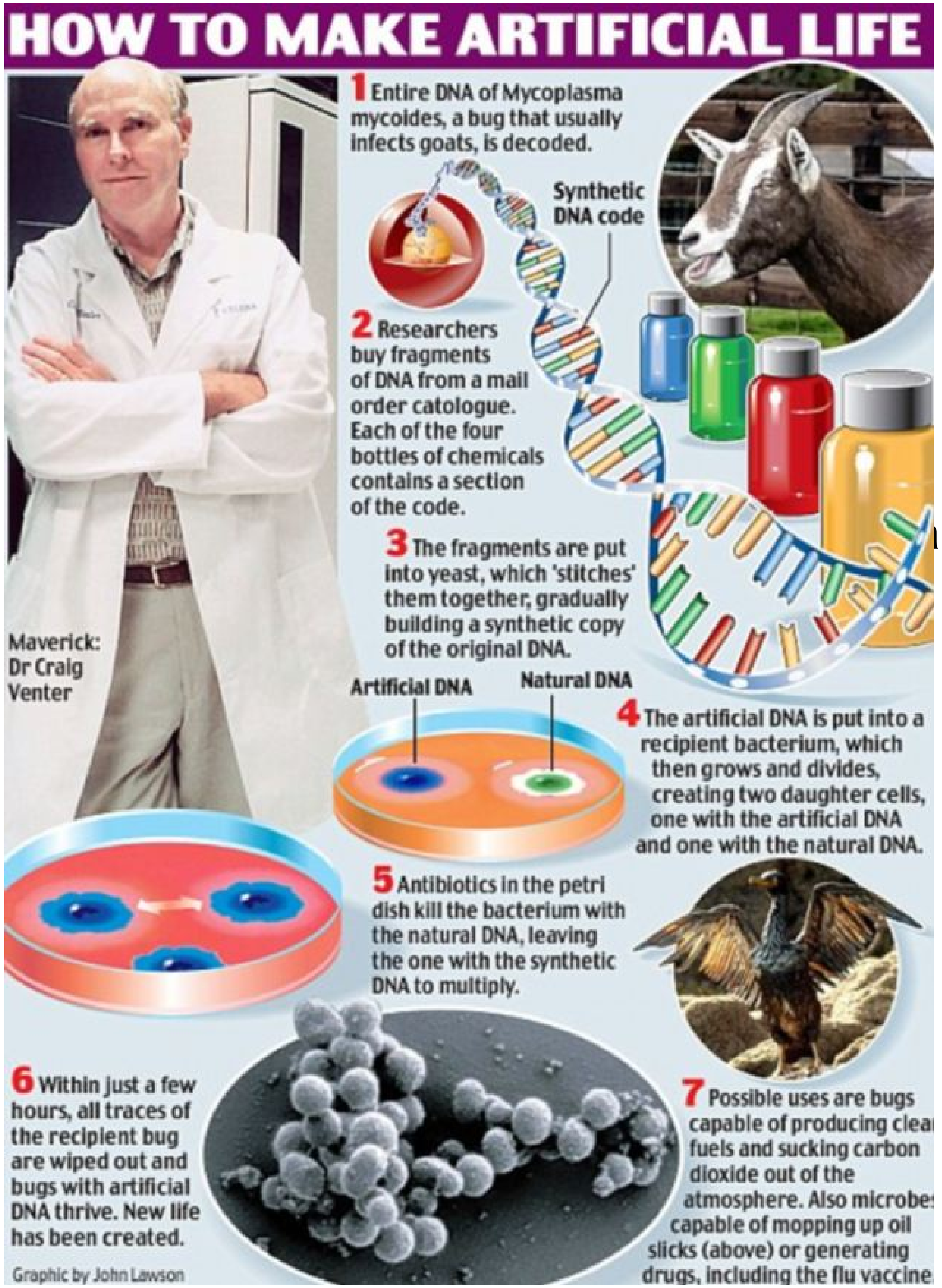
- Synthetic DNA
- **Synthetic Cells**
- Genome Editing
- Information Storage
- Synthetic circuits
- Biosensors

In May 2010, Craig Venter's group announced they had been able to assemble a complete genome of millions of base pairs, insert it into a cell, and cause that cell to start replicating
(Gibson DG, Science, 2010)

Synth

Examples:

- Synthetic DNA
- **Synthetic Cells**
- Genome Editing
- Information Storage
- Synthetic circuits
- Biosensors



HOW TO MAKE ARTIFICIAL LIFE

1 Entire DNA of Mycoplasma mycoides, a bug that usually infects goats, is decoded.

2 Researchers buy fragments of DNA from a mail order catalogue. Each of the four bottles of chemicals contains a section of the code.

3 The fragments are put into yeast, which 'stitches' them together, gradually building a synthetic copy of the original DNA.

4 The artificial DNA is put into a recipient bacterium, which then grows and divides, creating two daughter cells, one with the artificial DNA and one with the natural DNA.

5 Antibiotics in the petri dish kill the bacterium with the natural DNA, leaving the one with the synthetic DNA to multiply.

6 Within just a few hours, all traces of the recipient bug are wiped out and bugs with artificial DNA thrive. New life has been created.

7 Possible uses are bugs capable of producing clea fuels and sucking carbon dioxide out of the atmosphere. Also microbe capable of mopping up oil slicks (above) or generating drugs, including the flu vaccine

Graphic by John Lawson

http://www.dailymail.co.uk/sciencetech/article-2029158/Life-Mars-closer-Craig-Venter-creates-artificial-life-form.html

Synthetic Biology

Examples:

- Synthetic DNA
- Synthetic Cells
- **Genome Editing**
- Information Storage
- Synthetic circuits
- Biosensors

The **Sleeping Beauty transposon system** is an example of an engineered enzyme for inserting precise DNA sequences into genomes of vertebrate animals

Synthetic Biology

Examples:

- Synthetic DNA
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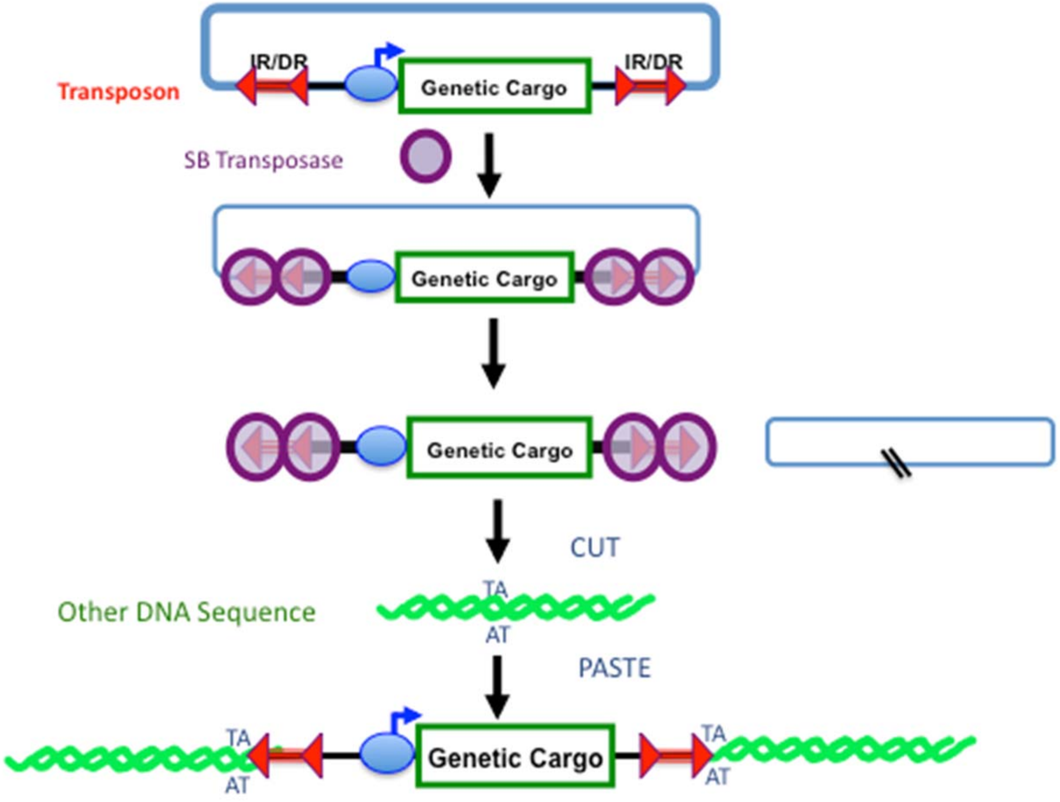
- **Genome Editing**
- Information Storage
- Synthetic circuits
- Biosensors

Mechanism of Transposition



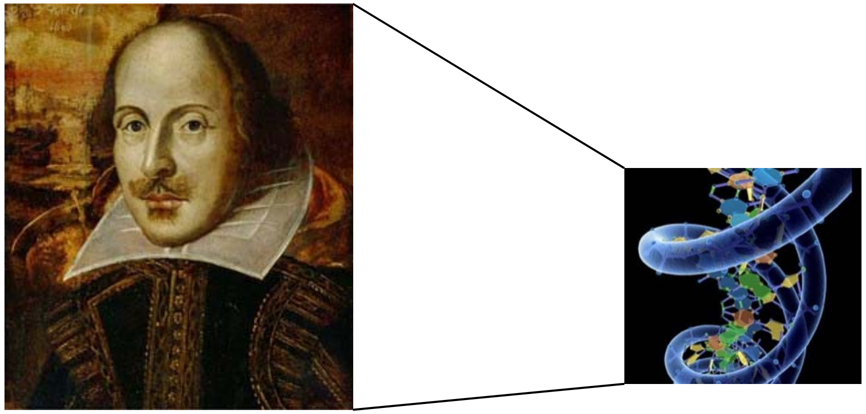
http://en.wikipedia.org/wiki/Sleeping_Beauty_transposon_system#mediaviewer/File:SBTS1.png

Synthetic Biology

Examples:

- Synthetic DNA
- Synthetic Cells
- Genome Editing
- **Information Storage**
- Synthetic circuits
- Biosensors

- In 2012, George M. Church encoded one of his books about synthetic biology in DNA
- A similar project had encoded the complete sonnets of William Shakespeare in DNA → Just as a computer stores digital files as a unique code of 'ones' and 'zeros', scientists wrote information into a strand of synthetic DNA made from a sequence of four chemical 'letters‘ (Goldman N. et al., Nature, 2013)



Synthetic Biology

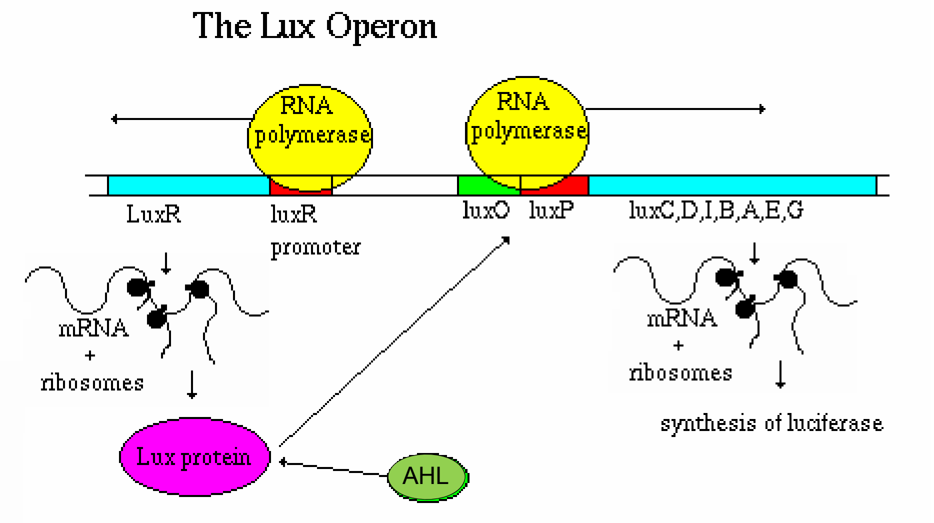
Examples:

- Synthetic DNA
- Synthetic Cells
- Genome Editing
- Information Storage
- **Synthetic circuits**
- **Biosensors**

Biosensors

- A biosensor refers to an engineered organism (usually a bacterium) that is capable of reporting some environmental phenomenon (presence of heavy metals or toxins)
- A very widely used system is the Lux operon of A. fischeri
- AHL = *N*-Acyl homoserine lactone

→ AHL is a natural biological signal secreted by Gram-negative bacteria as a means of coordinating cellular activity with the **cell population density** (Quorum sensing)



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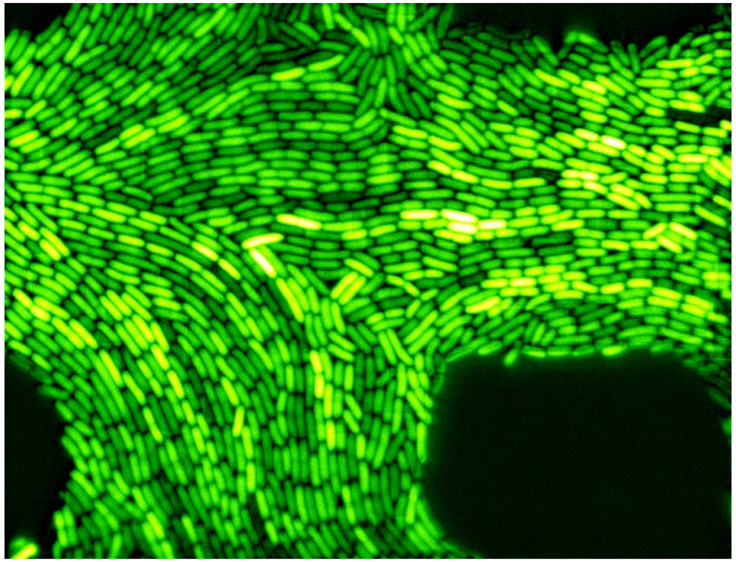
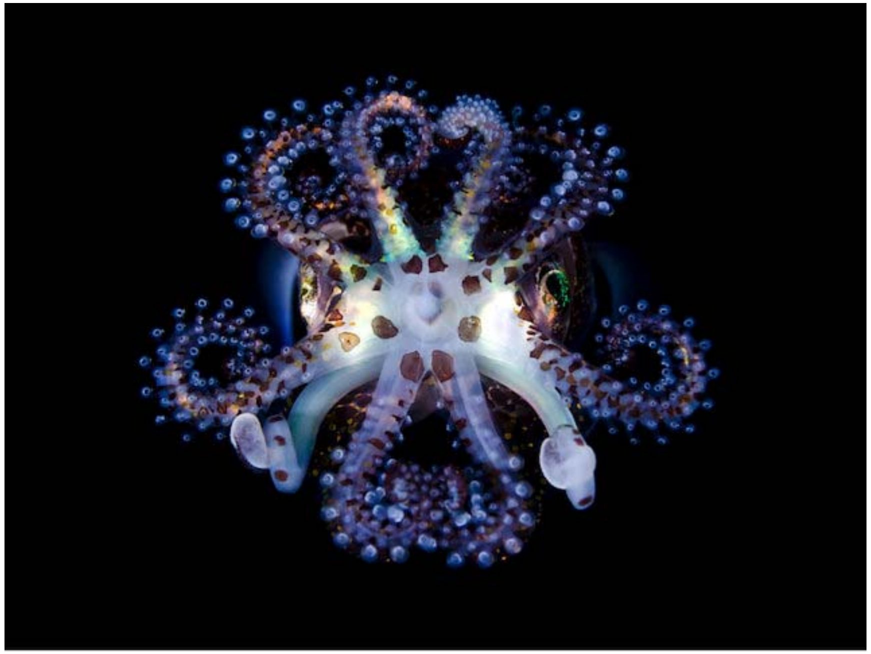


http://spectregroup.wordpress.com/2010/10/page/2/

Biosensors

- Density of bacteria is very high → inside a light organ of a squid → has to produce luciferase

http://somfblog.wordpress.com/tag/aliivibrio-fischeri/



http://somfblog.wordpress.com/tag/aliivibrio-fischeri/

Synthetic circuits

- Creating a system where some gene of interest can be expressed under prescribed conditions
- The lac operon is a natural example that is often emulated

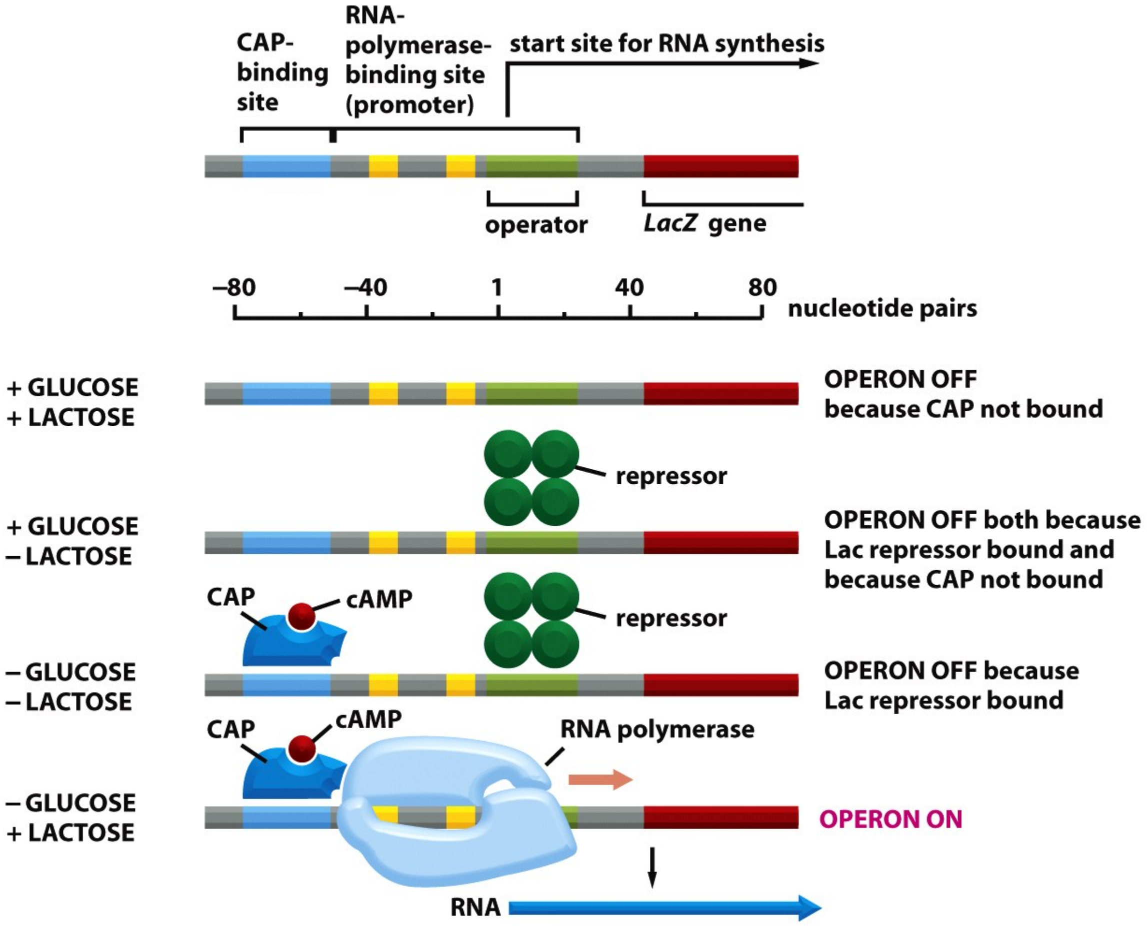
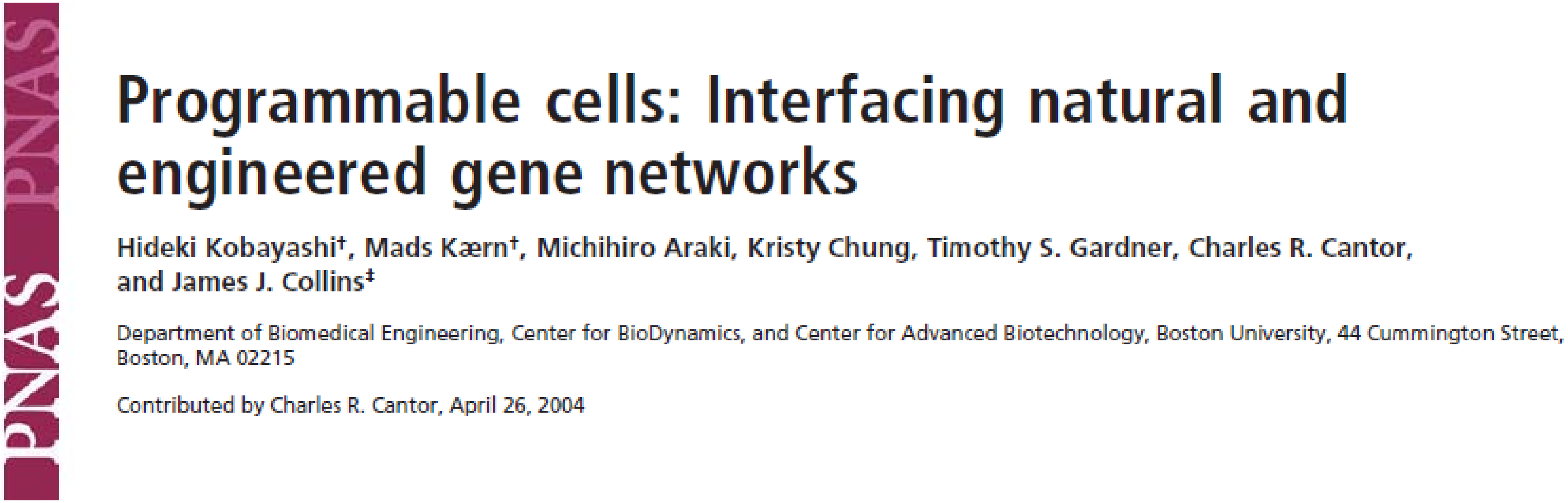


Figure 7-39 Molecular Biology of the Cell 5/e (© Garland Science 2008)



Programmable cells: Interfacing natural and engineered gene networks

Hideki Kobayashi[†], Mads Kærn[†], Michihiro Araki, Kristy Chung, Timothy S. Gardner, Charles R. Cantor,
and James J. Collins[‡]

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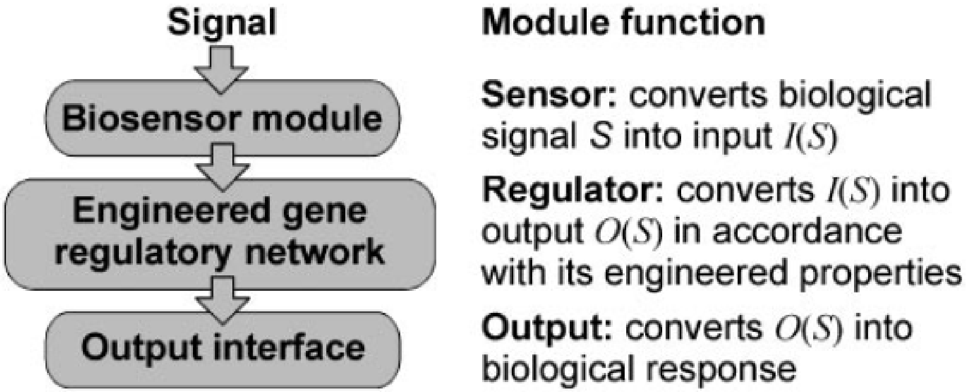
Boston, MA 02215

Contributed by Charles R. Cantor, April 26, 2004

Couple engineered gene networks to the regulatory circuitry of the cell → novel cellular behaviors and characteristics

The modular structure of simple programmable cell



Module function

Sensor: converts biological signal S into input $I(S)$

Regulator: converts $I(S)$ into output $O(S)$ in accordance with its engineered properties

Output: converts $O(S)$ into biological response

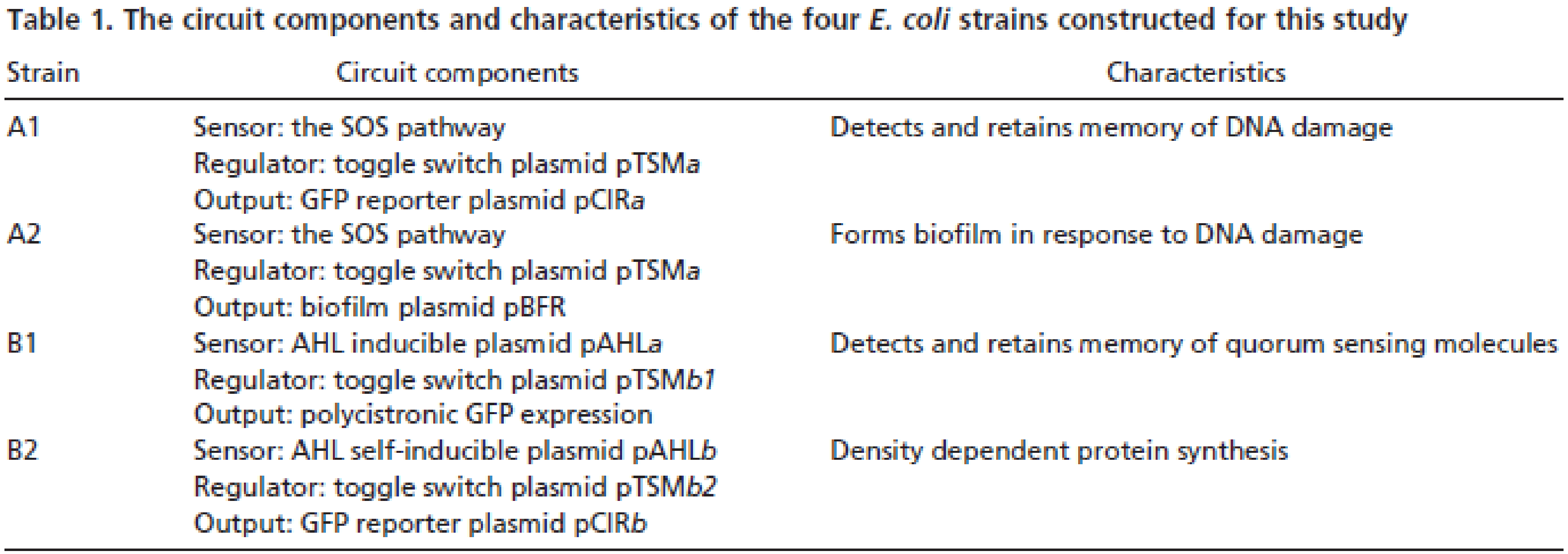
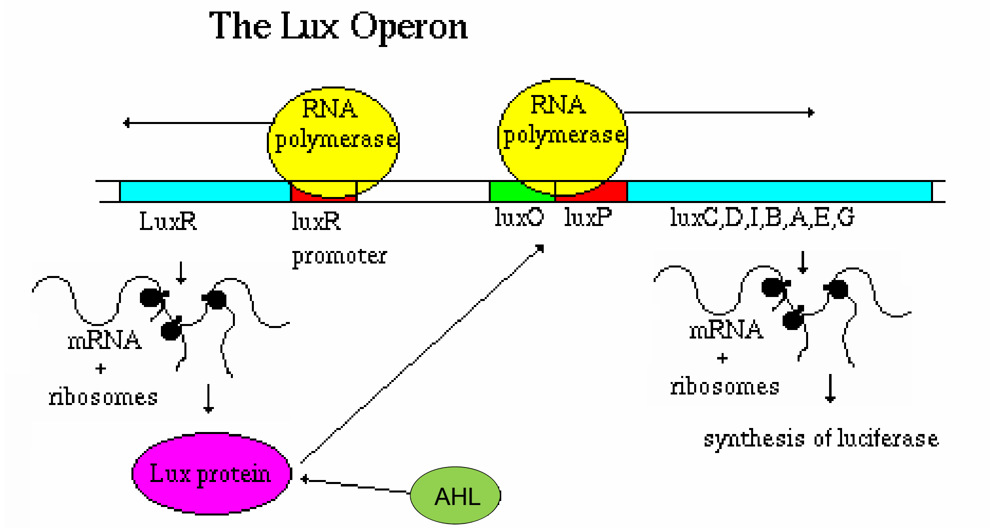
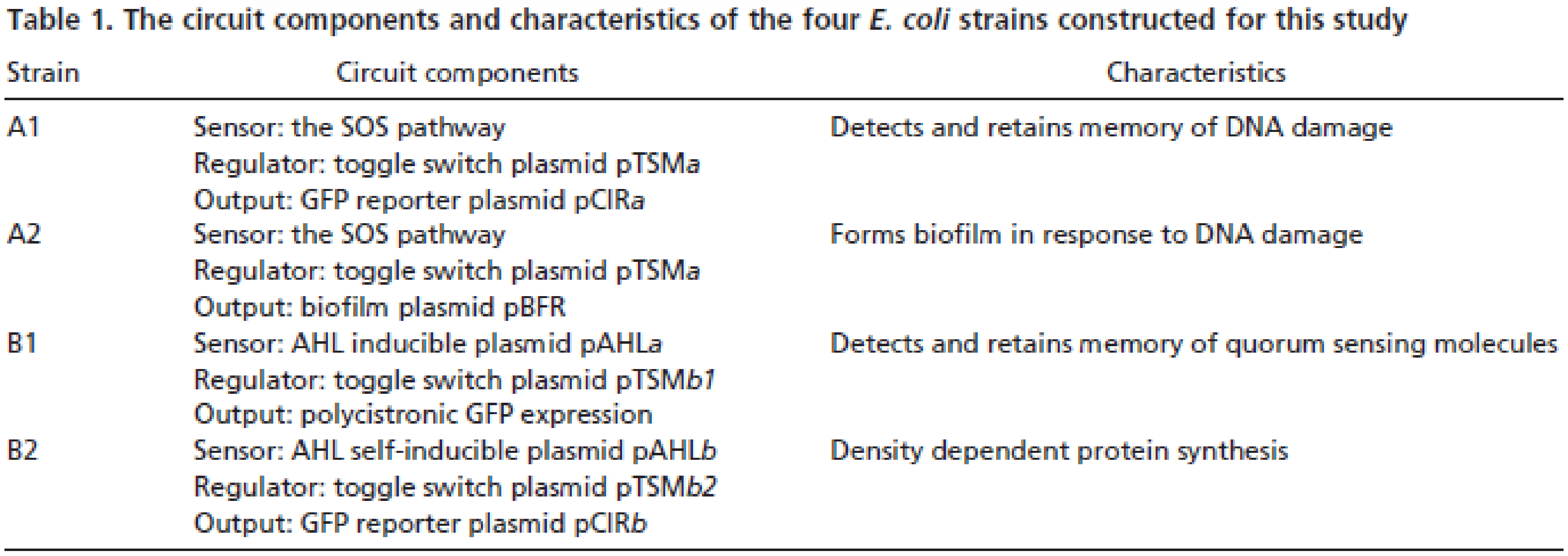


Table 1. The circuit components and characteristics of the four *E. coli* strains constructed for this study

Strain	Circuit components	Characteristics
A1	Sensor: the SOS pathway Regulator: toggle switch plasmid pTSM*a* Output: GFP reporter plasmid pCIR*a*	Detects and retains memory of DNA damage
A2	Sensor: the SOS pathway Regulator: toggle switch plasmid pTSM*a* Output: biofilm plasmid pBFR	Forms biofilm in response to DNA damage
B1	Sensor: AHL inducible plasmid pAHL*a* Regulator: toggle switch plasmid pTSM*b1* Output: polycistronic GFP expression	Detects and retains memory of quorum sensing molecules
B2	Sensor: AHL self-inducible plasmid pAHL*b* Regulator: toggle switch plasmid pTSM*b2* Output: GFP reporter plasmid pCIR*b*	Density dependent protein synthesis

The modular structure of simple programmable cell





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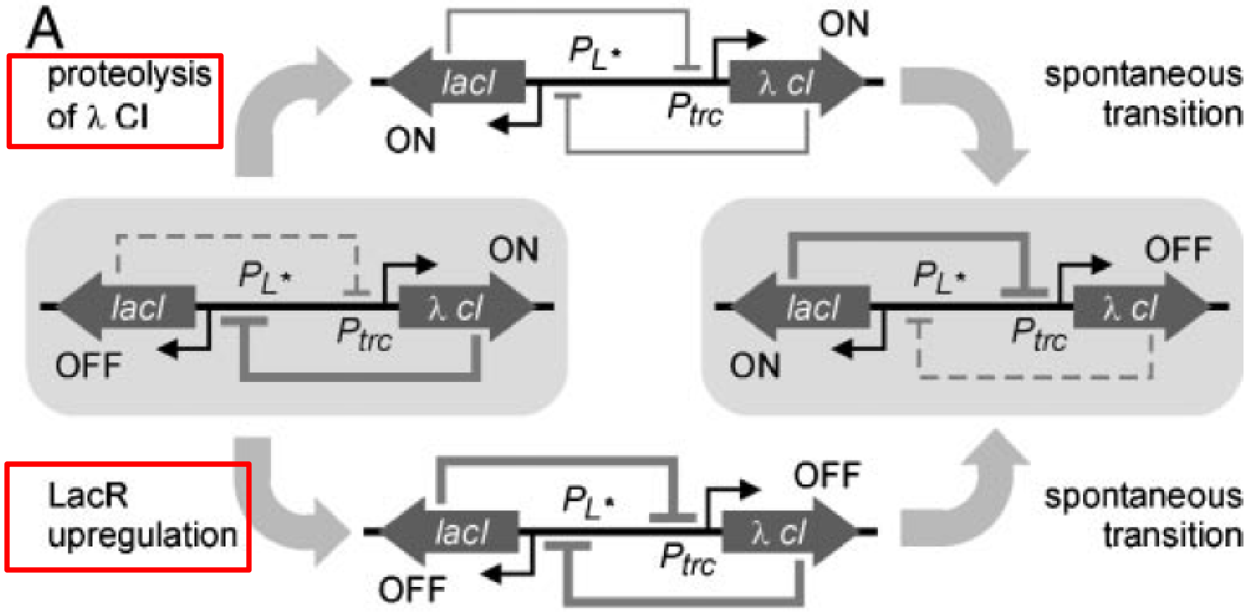
Transitions in the genetic toggle switch



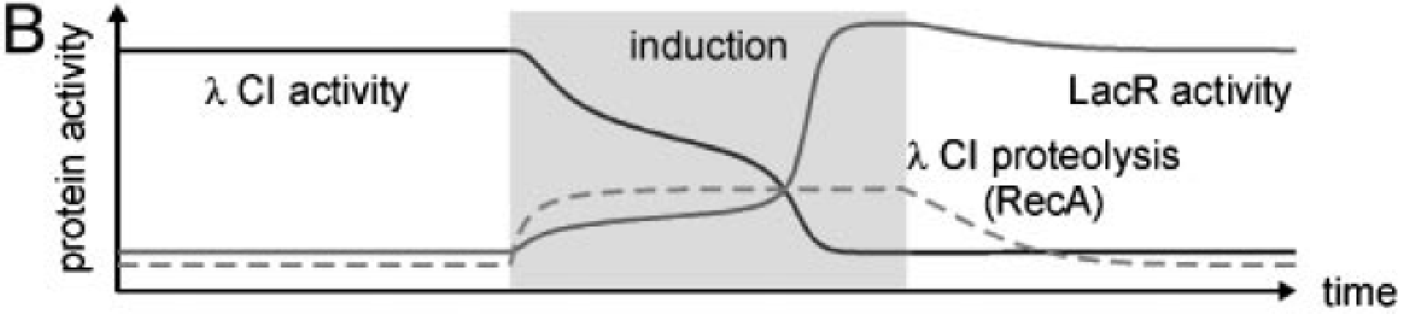
stable
expression
states

(i) The activity of the protein that is highly expressed can be decreased
(ii) The activity of the protein whose expression is repressed can be increased

- Perturbation must be sufficient large to bring the system across a certain treshold from one stable state to the other

Transitions in the genetic toggle switch

Simulated response of a single cell



- Transitions from one stable state to the other can be induced by a signal that temporarily brings the system out of the region of bistability

- Intermediate signals will give rise to bimodal population distributions because individual cells have slightly different threshold values, due to variability in plasmid copy number

Transitions in the genetic toggle switch

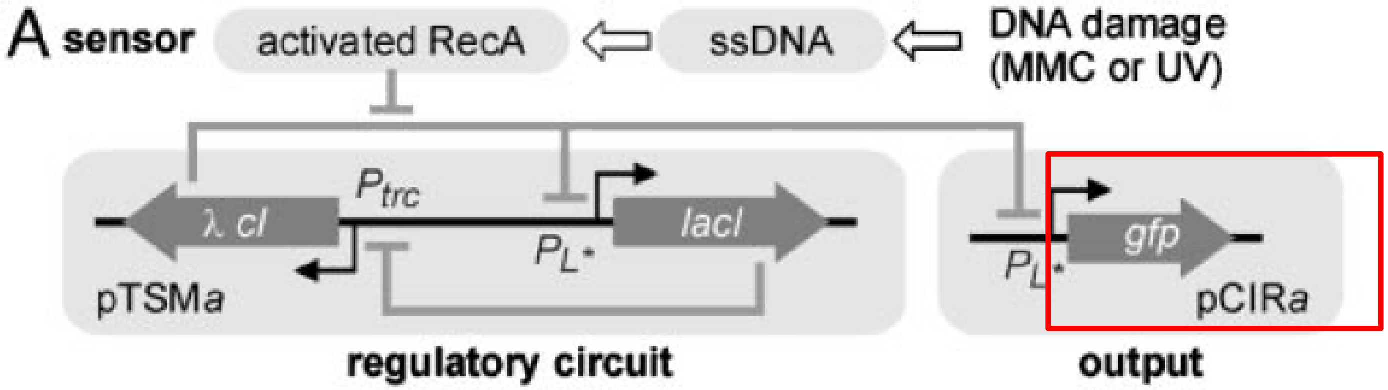
Strains A1 and A2

- The signaling pathway that **degrades CI naturally** in strains A1 and A2 is the **SOS-response pathway**, where the RecA coprotease is activated in the presence of **single-stranded DNA**
- Activated RecA cleaves the CI repressor protein, causing derepression of the *PL* promoter

Strains B1 and B2

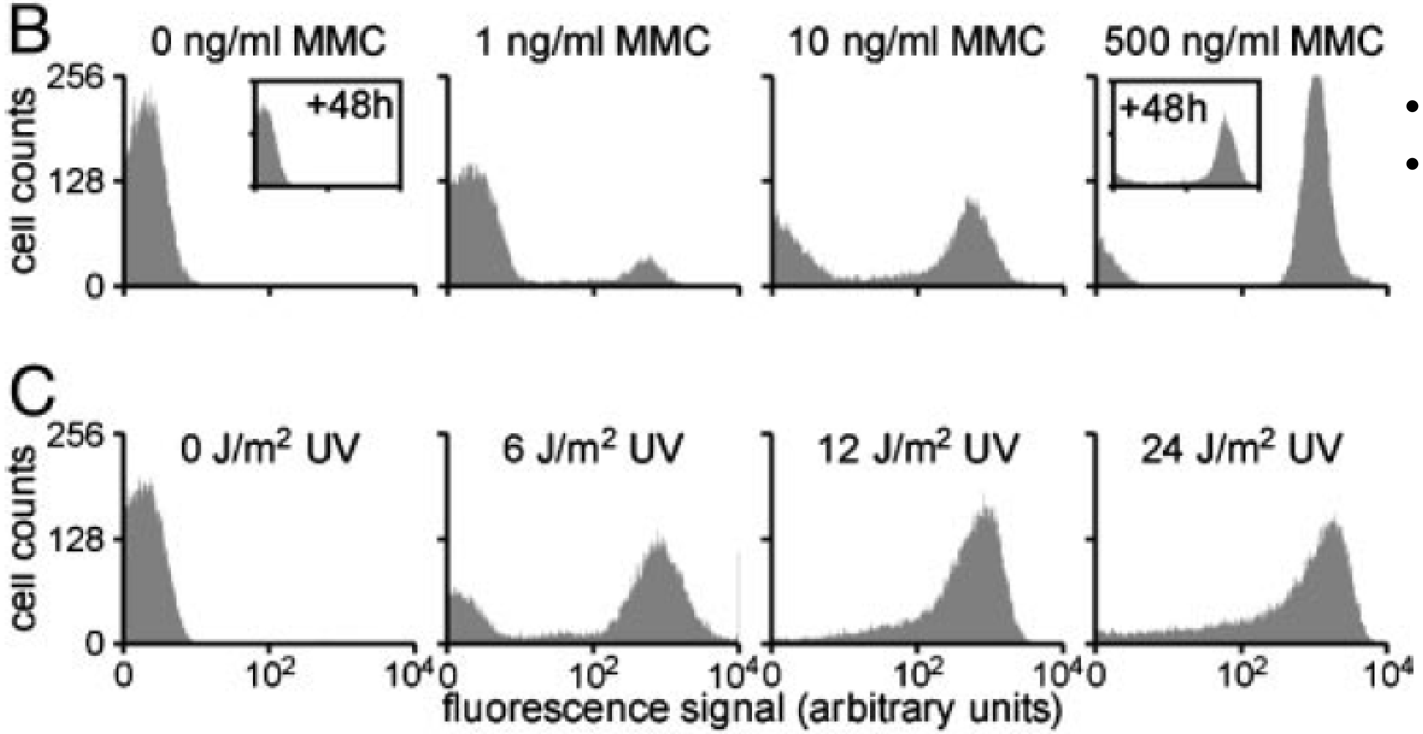
- The engineered signaling pathway that **increases** the basal expression of the ***lacI* gene** in strains B1 and B2 is based on the **quorum sensing** pathway *V. fischeri*
- The regulator protein of the *lux* operon, LuxR, is induced **by AHL**, and the induced LuxR protein activates expression from the *lux* promoter, *PluxI*

Interfacing the SOS Pathway



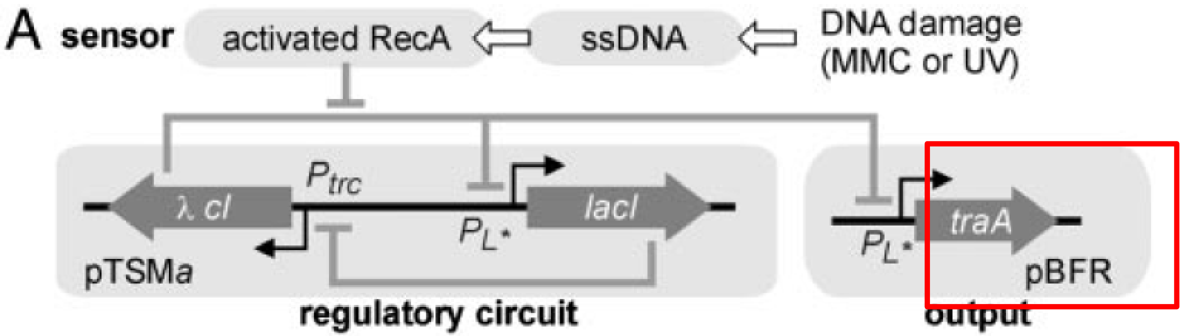
toggle switch plasmid

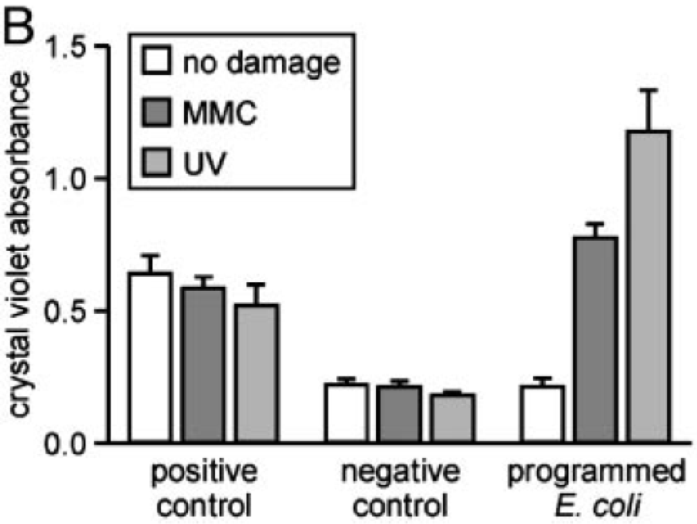
bimodal populations



- After 15h
- MMC = mitomycin C → crosslinking agent

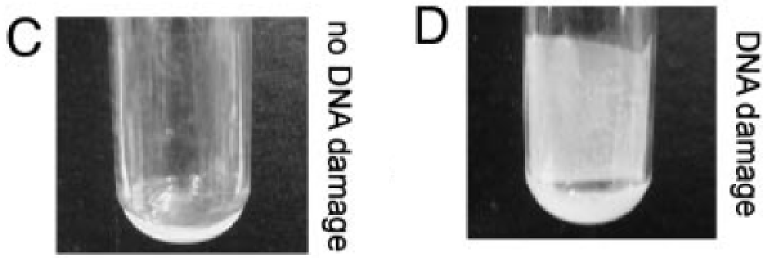
Programmed Phenotype in Strain A2





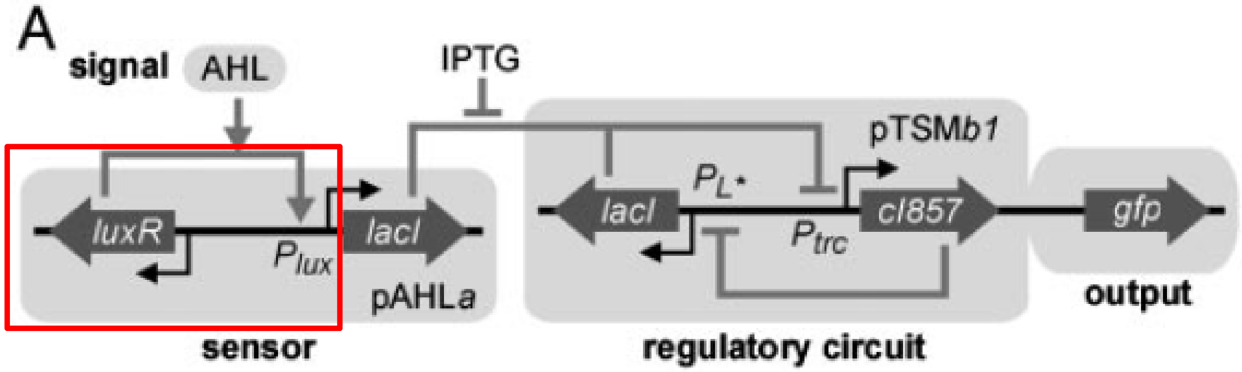
strain lacking the *traA* gene (the negative control) and strain with the *traA* gene (the positive control)

level of biofilm was measured quantitatively by using a crystal violet microtiter absorbance assay



confirmed this observation by using microfermentor experiments where the biofilm formed after MMC treatment can be detected visually

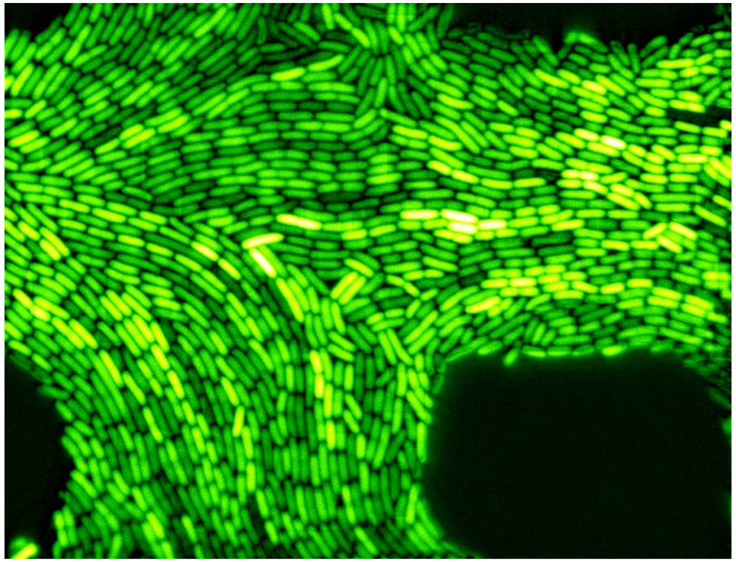
Interfacing General Input Signals



AHL = *N*-Acyl homoserine lactone
→ AHL is a natural biological signal secreted by Gram-negative bacteria as a means of coordinating cellular activity with the cell population density

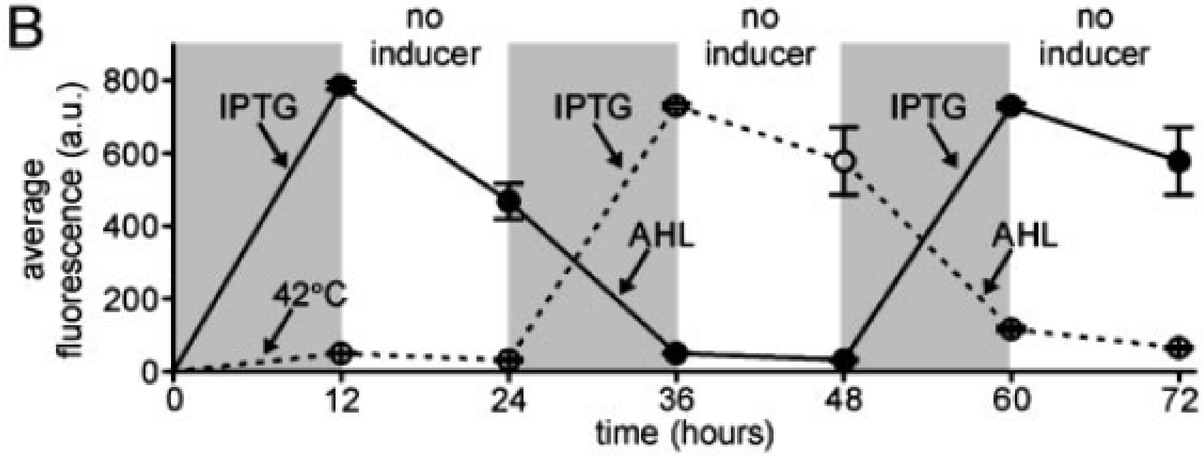
GFP expression activated by transient treatment with IPTG and deactivated by transient exposure to AHL

Lux operon from the V. fischeri



http://spectregroup.wordpress.com/2010/10/page/2/

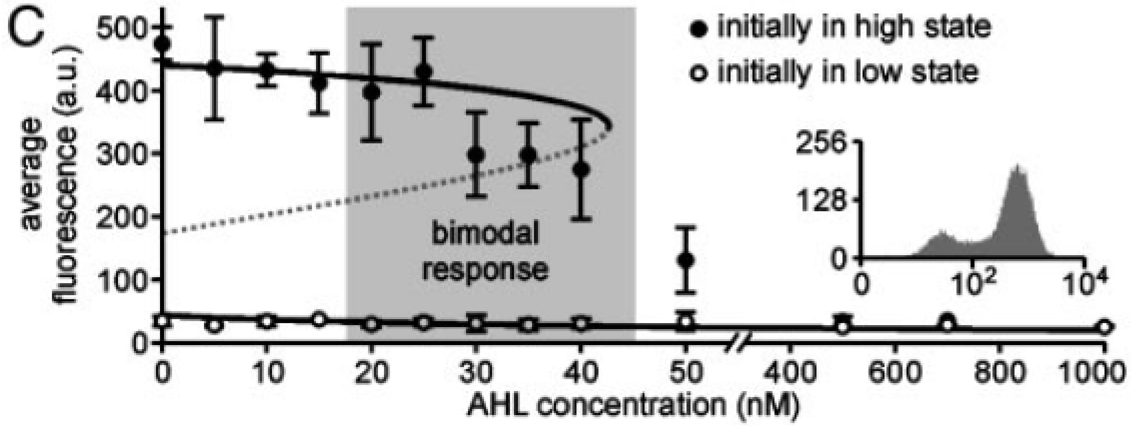
Interfacing General Input Signals



Partial decrease in fluorescence observed 12 h after removal of IPTG reflects the relaxation from a state where LacR is completely inactive to a stable state where CI is the dominant repressor, but LacR still has some basal activity

The stability of the distinct expression states was confirmed in a separate control experiment where stable expression was observed for up to 50 h (corresponding to 50–60 generations) after the removal of the inducing factor

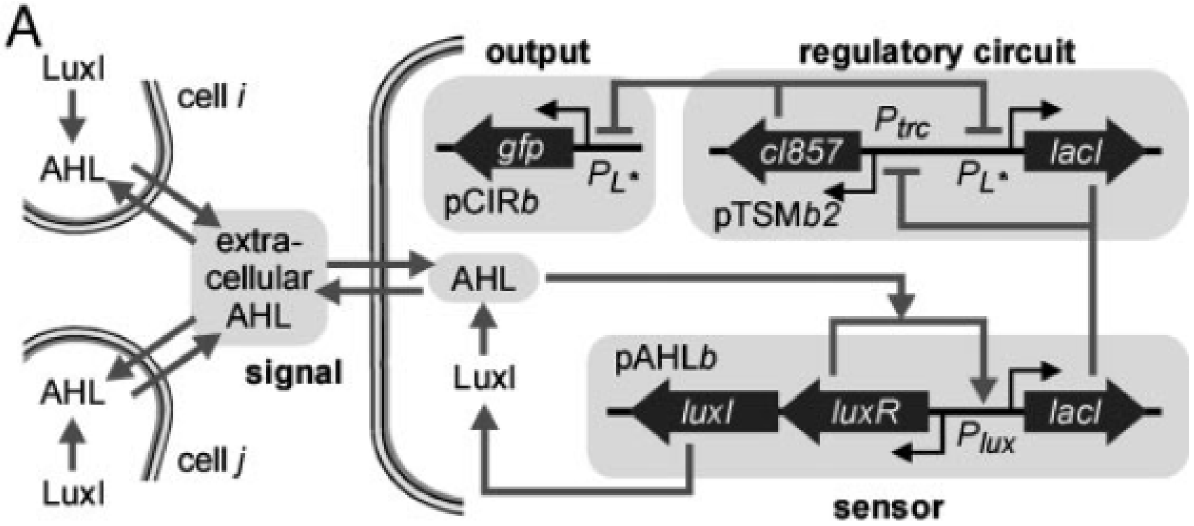
Interfacing General Input Signals



Cells initially in the high or low GFP expression states were exposed to AHL at various concentrations for 24 h

- Cells that were initially in the high LacR state (low GFP expression) remained in this state
- Cells initially in the high CI state (high GFP expression) remained in that state at AHL concentrations 20 nM
- All cells switched to the low GFP state when treated with AHL at 50 nM concentration or higher
- Bimodal population distributions were observed at AHL concentrations between 20 and 50 nM

Density-Dependent Gene Activation

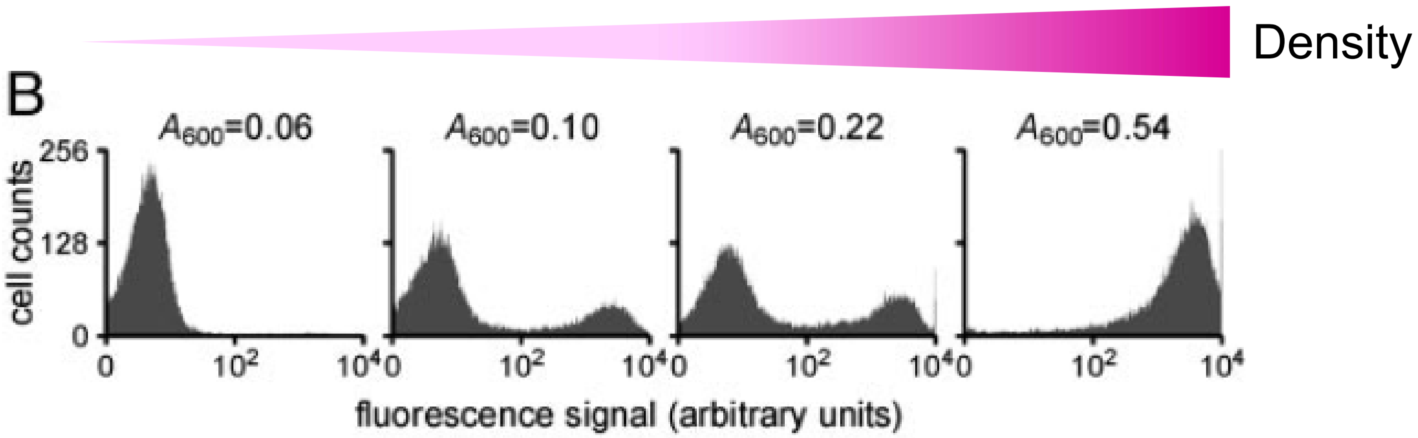


cotransformed three different plasmids to create the B2 strain

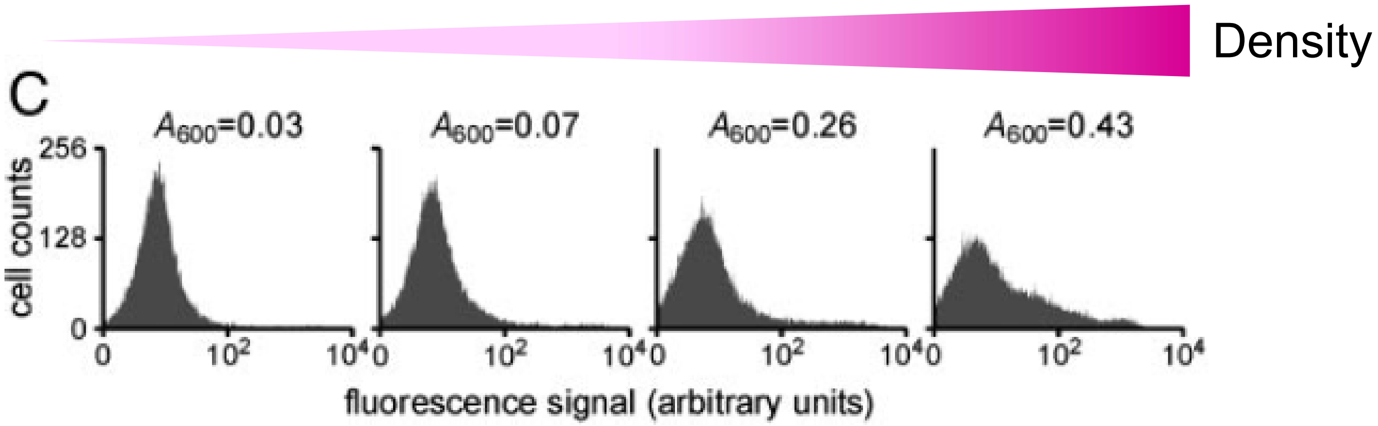
To enable the *E. coli* population **<u>to measure its own density</u>** through AHL, created a plasmid where the *luxI* gene from *V. fischeri* is expressed polycistronically with the *luxR* gene and *lacI* is expressed from the *Plux* promoter

The protein encoded by *luxI* is a synthetase that converts common precursor metabolites into AHL signaling molecules

Density-Dependent Gene Activation



Inoculated cultures with different numbers of cells, assayed after 14 h of growth



A strain lacking the *luxI* gene was used as a negative control

Conclusion

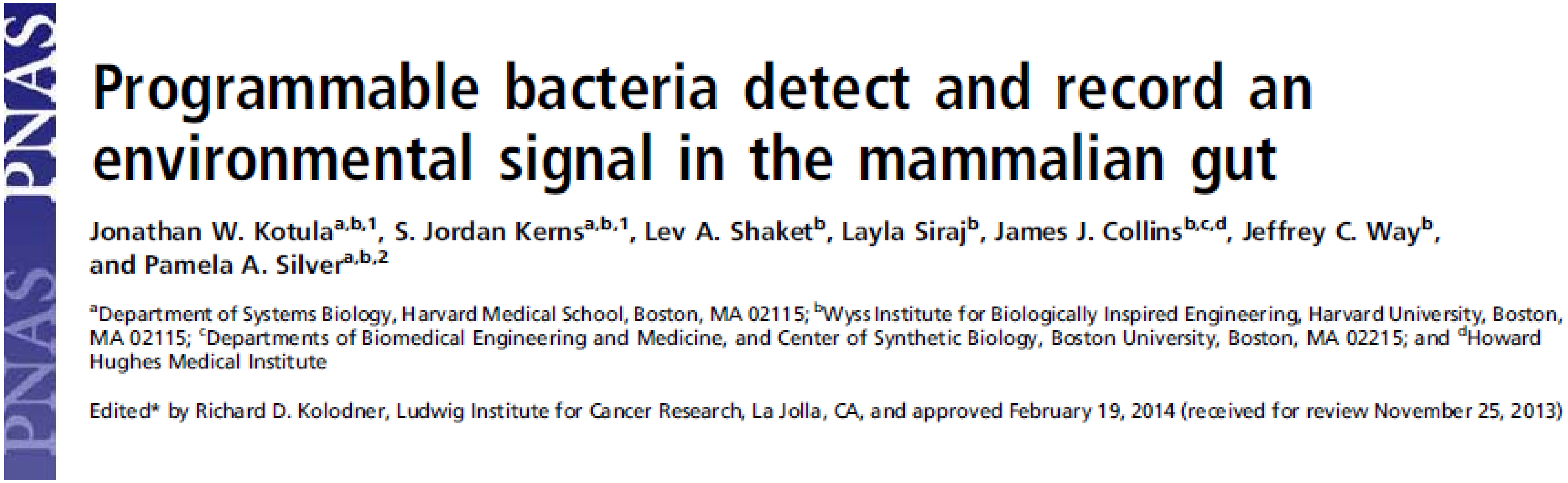
- Programmable cells can be constructed by designing appropriate interfaces **that couple natural signaling pathways with an engineered gene network**

- Engineered genetic toggle switch used to construct strains with binary switching responses

- Cells could change gene expression patterns to biological signals

- Signals (e.g., activating or repressing transcription factors) are **appropriately adjusted** to allow effective information transmission between circuit modules

Genetic Memory



Programmable bacteria detect and record an environmental signal in the mammalian gut

Jonathan W. Kotula[a,b,1], S. Jordan Kerns[a,b,1], Lev A. Shaket[b], Layla Siraj[b], James J. Collins[b,c,d], Jeffrey C. Way[b], and Pamela A. Silver[a,b,2]

[a]Department of Systems Biology, Harvard Medical School, Boston, MA 02115; [b]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115; [c]Departments of Biomedical Engineering and Medicine, and Center of Synthetic Biology, Boston University, Boston, MA 02215; and [d]Howard Hughes Medical Institute

Edited* by Richard D. Kolodner, Ludwig Institute for Cancer Research, La Jolla, CA, and approved February 19, 2014 (received for review November 25, 2013)

Develop tools to effectively monitor the gut microbiota and ultimately help in disease diagnosis

Diagnostics capable of nondestructively probing the mammalian gut

Fantastic Voyage/ Innerspace

1966

http://www.cadolphmoores.com/reviews/2009/12/23/fantastic-voyage-1966.html

1987

http://www.unitedcypher.com/uc/tag/innerspace

- Traveling through the body in a shrunken submarine
- Save a comatose scientist from a blood clot
- After curing the clot, they escape through a teardrop, and the scientist survives

- **Half a century later, sending in submarines is not yet a realistic option, instead recruited bacteria, this time for adventures in the mouse gut**

The Idea

- Human microbiota: trillions of bacteria that live on the skin, in the oral and nasal cavities, and throughout the gastrointestinal tract

- The gut microbiota closely interact with host cells and have a profound impact on health, disease, and metabolism

- Changes in its behavior can lead to liver disease, inflammatory/autoimmune disease, transfer of antibiotic resistance, obesity and diabetes, inflammatory bowel disease, pathogenic infections, and cancer

- Engineered E. coli to sense, remember and report environmental stimuli to develop tools to effectively monitor the gut microbiota and ultimately help in disease diagnosis

Engineered Bacteria

Engineer a bacterium to record an environmental signal in the mammalian gut:

Criterias	
“Nonmemory” state should be highly stable	
“Memory” state should be highly stable	
Engineered elements integrated into the chromosome (to minimize the chance of loss)	
Engineered elements should not impose a large fitness burden on the host	

Engineered Bacteria

Engineer a bacterium to record an environmental signal in the mammalian gut:

Used the well-characterized cI/cro genetic switch from bacteriophage lambda to construct a memory element for the circuit

Criterias	Bacteriophage Lambda
"Nonmemory" state should be highly stable	Repressed cI state stable due to natural selection
"Memory" state should be highly stable	Cro state is stable for many cell divisions
Engineered elements integrated into the chromosome (to minimize the chance of loss)	Chose a correspondent strain construction method
Engineered elements should not impose a large fitness burden on the host	Little burden on host as only 100-200 cI copies and later on < 1000 cro copies per cell

Bacteriophage Lambda

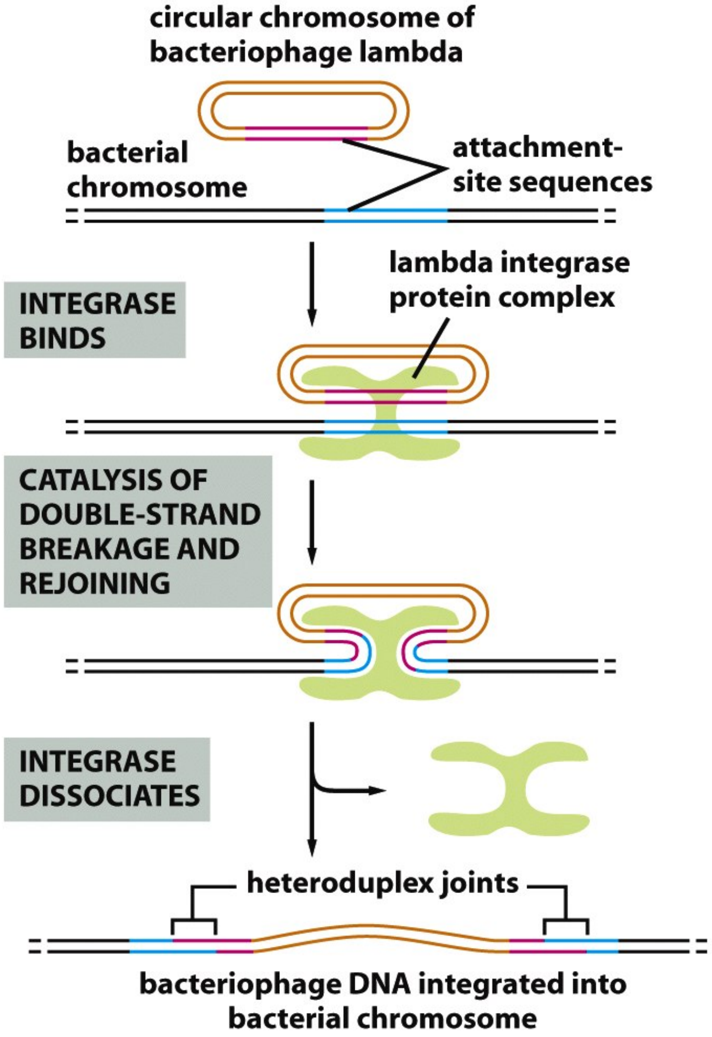


Figure 5-77 Molecular Biology of the Cell 5/e (© Garland Science 2008)

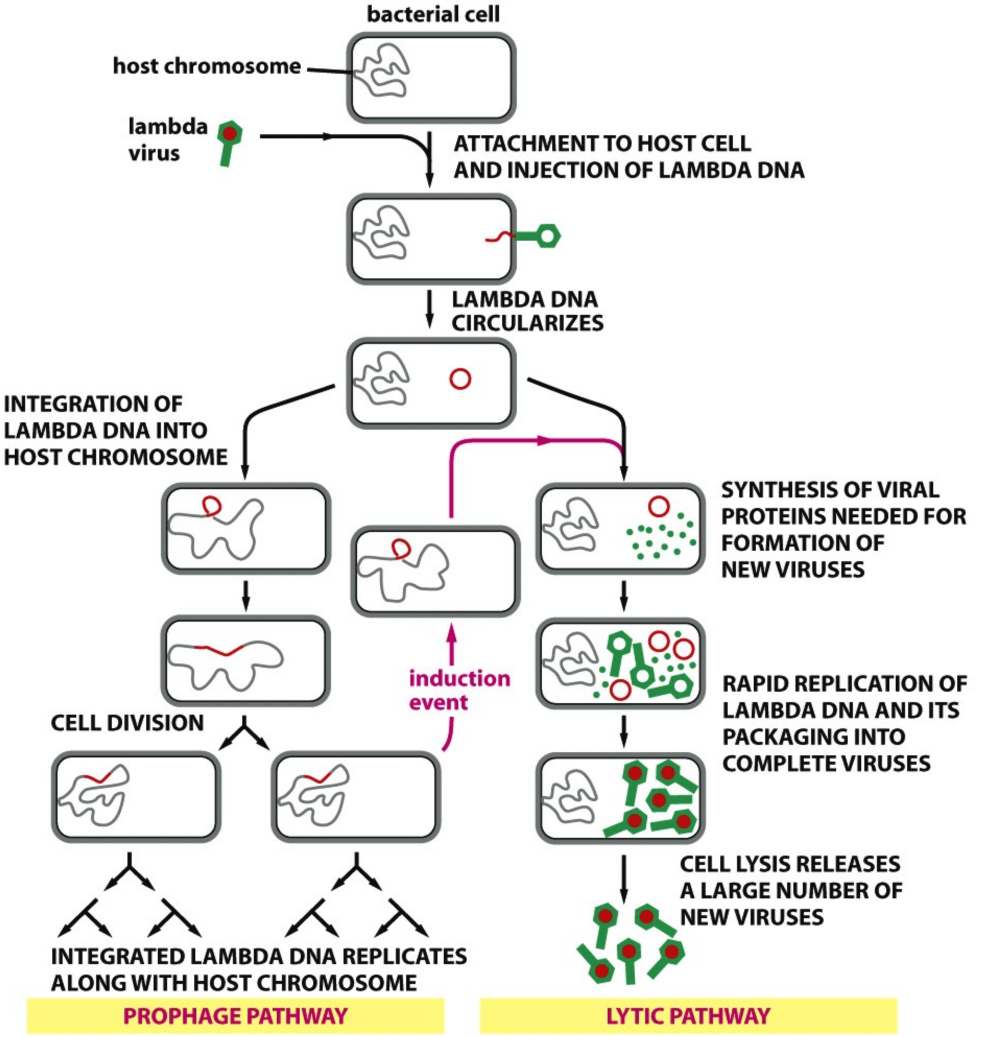
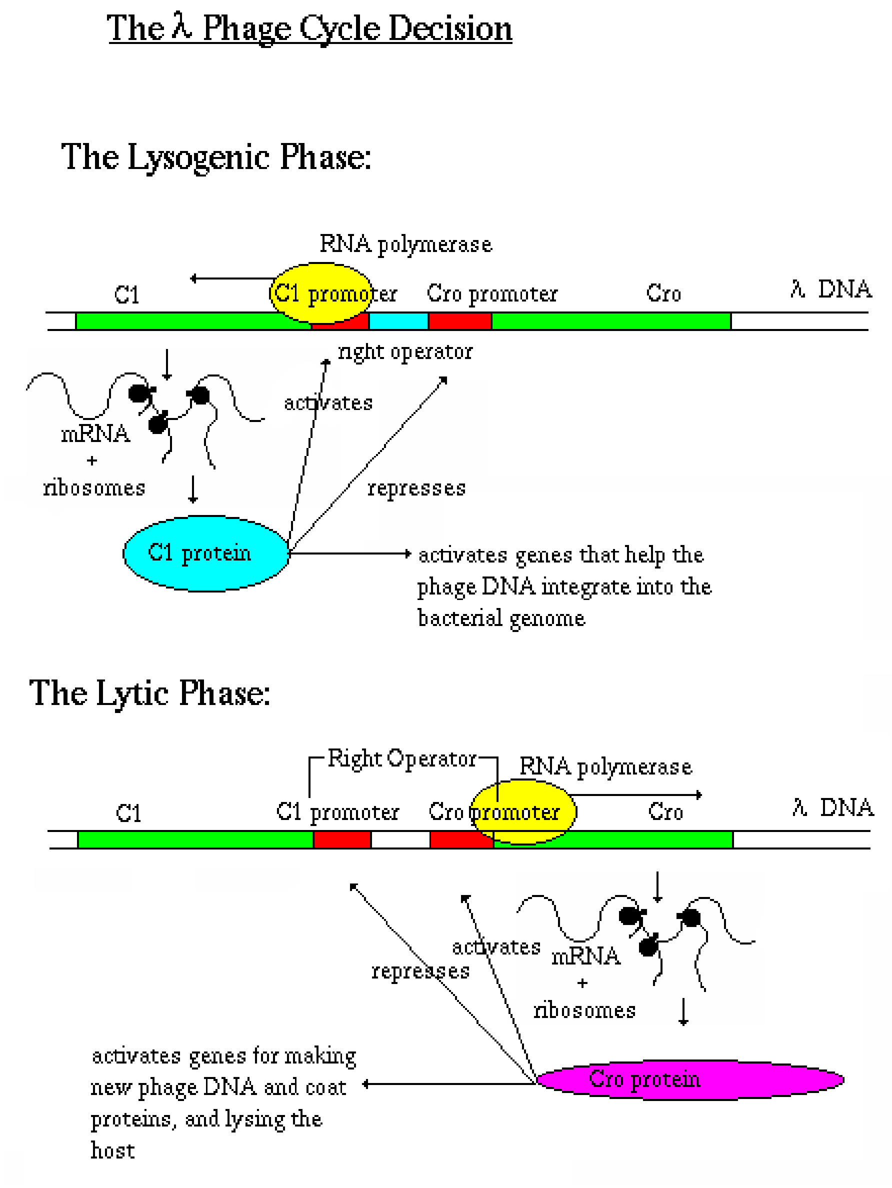


Figure 5-78 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Bacteriophage Lambda Operon

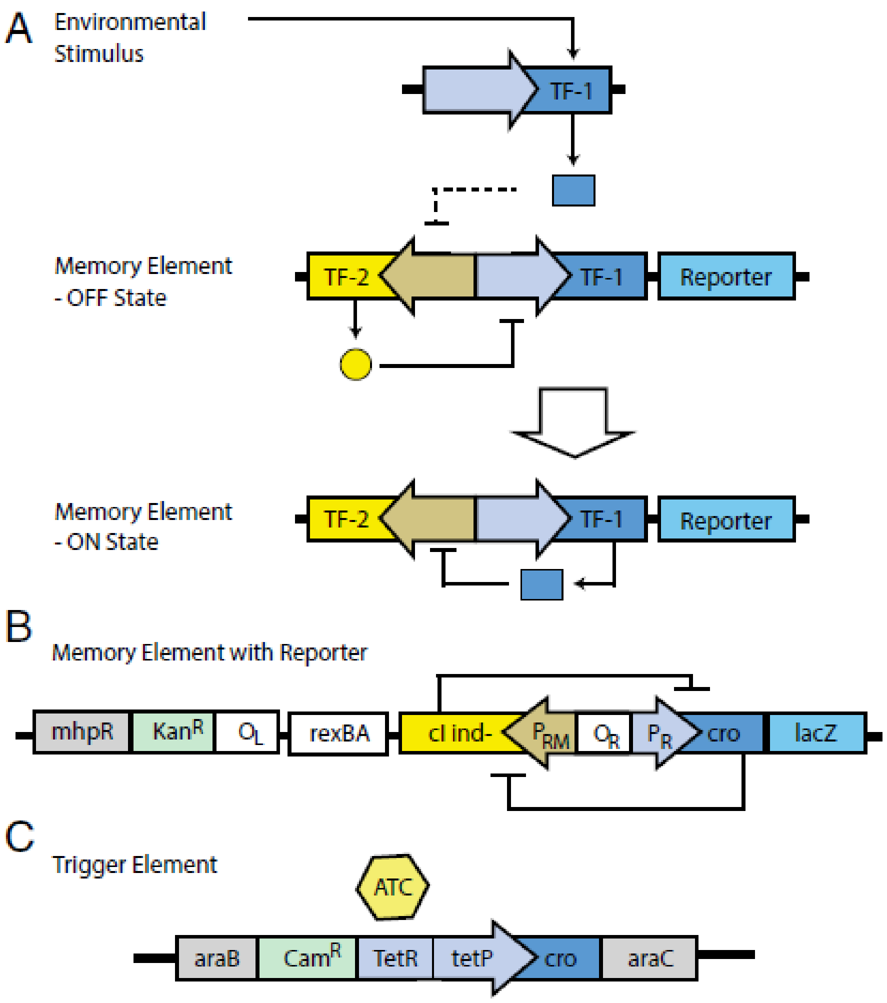


When the concentration of cI falls below about 10% of its steady-state value in a lysogen, lambda switches from the lysogenic to lytic state, which leads to derepression of the PR promoter and the expression of Cro

When Cro levels reach about 100 molecules per cell, the activity of the PRM promoter decreases

http://202.114.65.51/fzjx/wsw/website/mit/pge/pgeother.html

Engineered Bacteria (E.coli K12) Memory circuit



Environmental stimulus = ATC

(anhydrotetracycline → tetracycline derivate without antibiotic activity)

TF1 = cro
TF2 = cI
Reporter = lacZ → β-galctosidase

mhpR = DNA binding transcriptional activator
Kan^R = kanamycin-resistance cassette
O_L, O_R = operator left and right
rexBA = genes of the B. lambda operon
cI ind- = induction-deficient cI
P_{RM}, P_R = promoter right, middle and right

araB, araC = elements of the arabinose operon
Cam^R = chloramphenicol-resistance cassette
TetR, tetP = tetracyclin repressor and promoter

Engineered bacteria strains

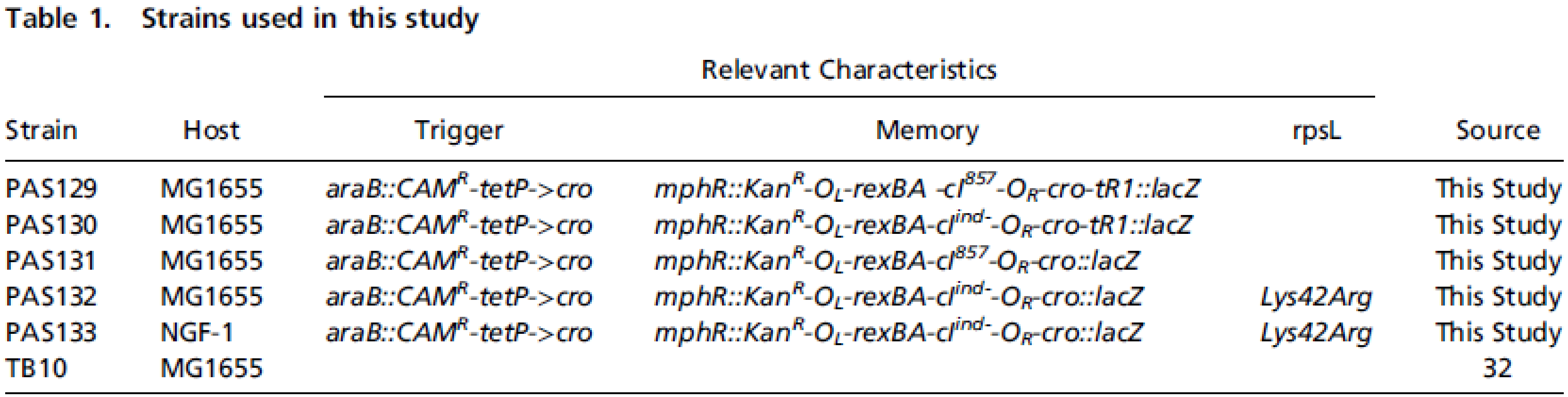
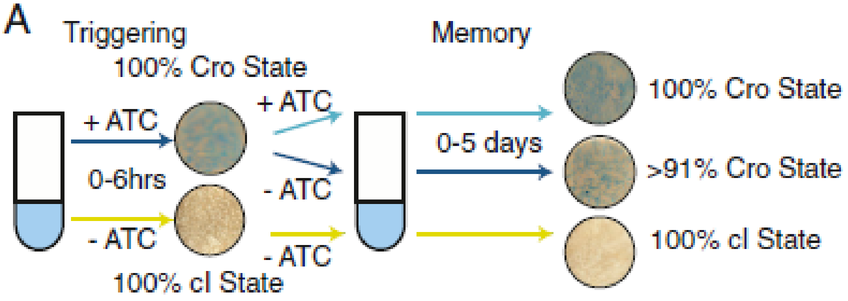


Table 1. Strains used in this study

Strain	Host	Relevant Characteristics			Source
		Trigger	Memory	rpsL	
PAS129	MG1655	*araB::CAM*^{R}*-tetP->cro*	*mphR::Kan*^{R}*-*O_L*-rexBA -cI*^{857}*-*O_R*-cro-tR1::lacZ*		This Study
PAS130	MG1655	*araB::CAM*^{R}*-tetP->cro*	*mphR::Kan*^{R}*-*O_L*-rexBA-cI*$^{ind-}$*-*O_R*-cro-tR1::lacZ*		This Study
PAS131	MG1655	*araB::CAM*^{R}*-tetP->cro*	*mphR::Kan*^{R}*-*O_L*-rexBA-cI*^{857}*-*O_R*-cro::lacZ*		This Study
PAS132	MG1655	*araB::CAM*^{R}*-tetP->cro*	*mphR::Kan*^{R}*-*O_L*-rexBA-cI*$^{ind-}$*-*O_R*-cro::lacZ*	*Lys42Arg*	This Study
PAS133	NGF-1	*araB::CAM*^{R}*-tetP->cro*	*mphR::Kan*^{R}*-*O_L*-rexBA-cI*$^{ind-}$*-*O_R*-cro::lacZ*	*Lys42Arg*	This Study
TB10	MG1655				32

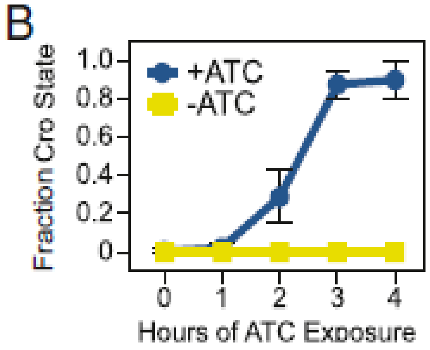
MG1655 = E.coli K12 derived
NGF-1 = natural gut flora 1
cI^{ind-} = induction deficient
cI^{857}= temperature-sensitive repressor
tR1 = cro gene terminator
rpsL = mutation in rpsL gives rise to a resistance to > 300 ug/ml streptomycin

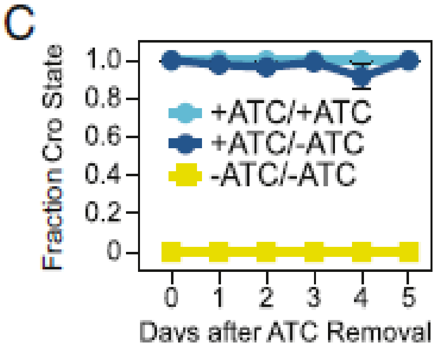
Engineered bacteria sense and remember ATC exposure in vitro

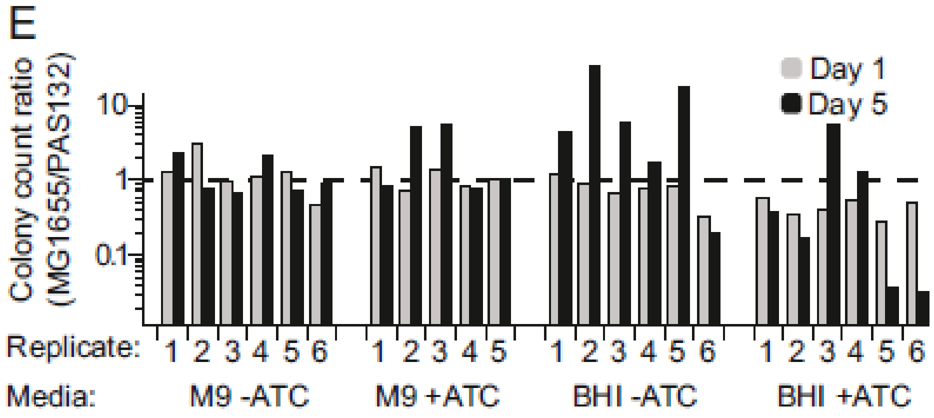


- low dose of ATC (100ng/ml) is enough
- M9 glucose X-gal plates

after ATC removal, Cro state remained for at least 5 d of subculturing (about 150 cell divisions)

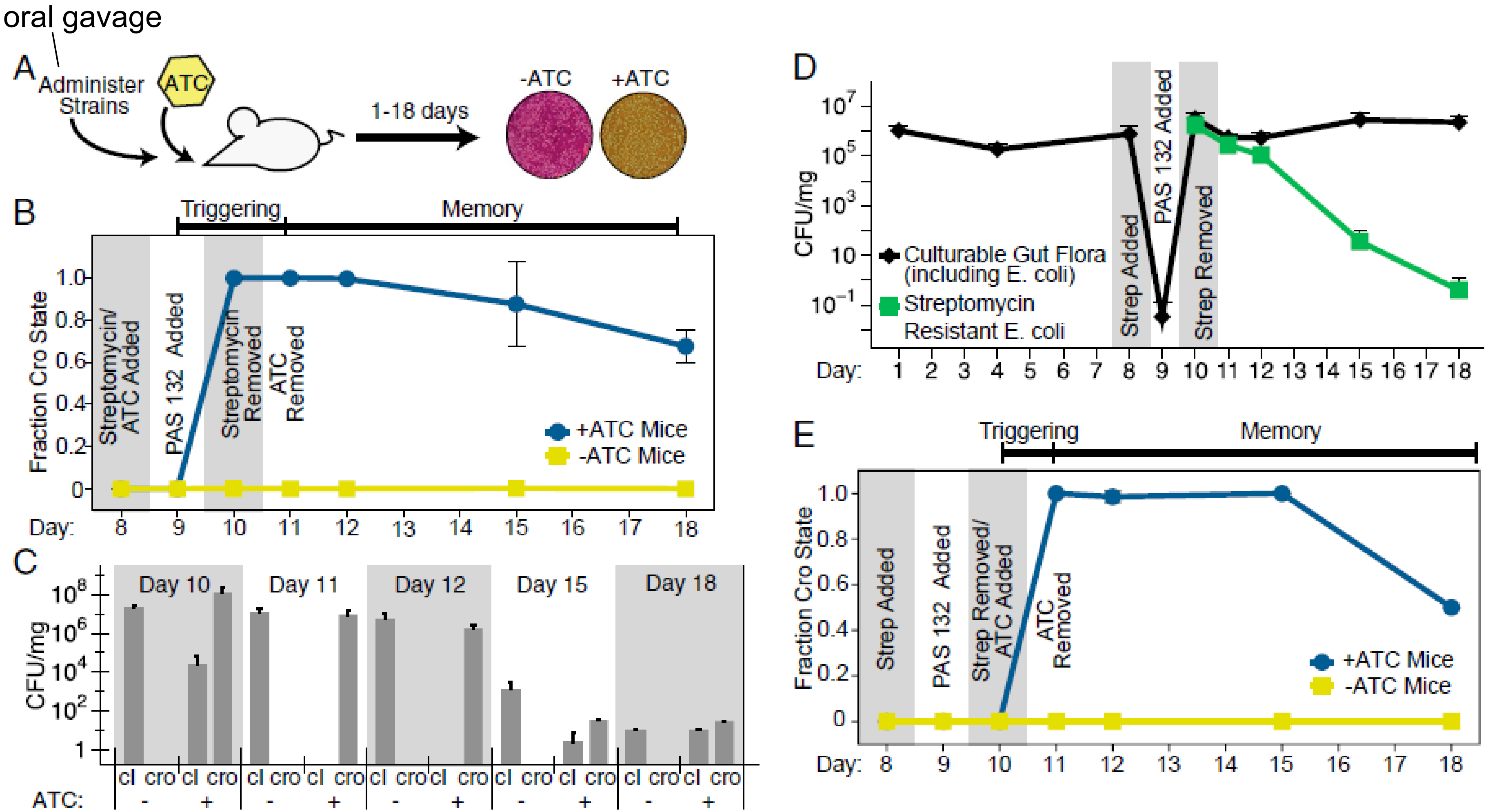






- competitive growth experiments in mixed cultures with the parental strain
- mixed cultures with initial ratio of about 1:1 E. coli MG1655 and PAS132 were subcultured with and without ATC for about 50 cell divisions
- change in ratios of parent cells to engineered cells varied but did not show a consistent overgrowth of parental cells

Engineered bacteria record, remember and report ATC exposure from the mammalian gut

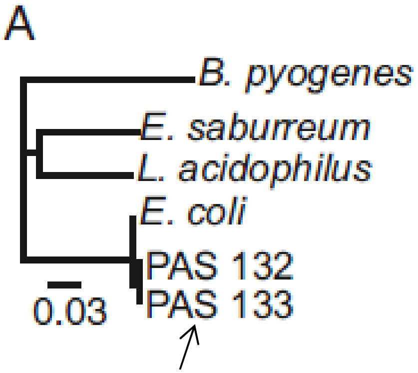


- streptomycin → to allow colonization by PAS132
- 50% of survivors stayed in the Cro stateafter more than one week
- titer of engineered bacteria decreased slowly after strep. removal

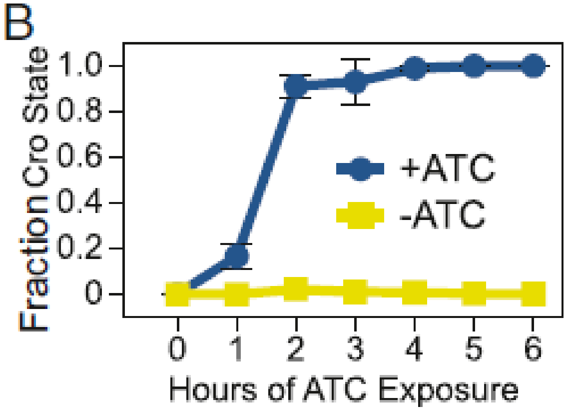
- endogenous gut flora began recolonizing the gut as soon as the streptomycin treatment ended
- ATC administration after strain administration

Memory behavior of an endogenous murine E. coli strain engineered to contain the memory circuit

Compartion of 16S ribosomal subunits

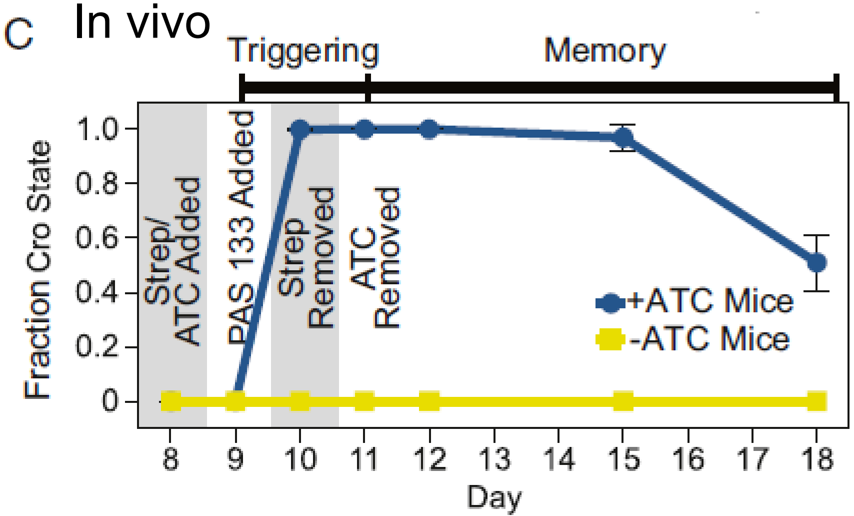


newly isolated murine E.coli strain

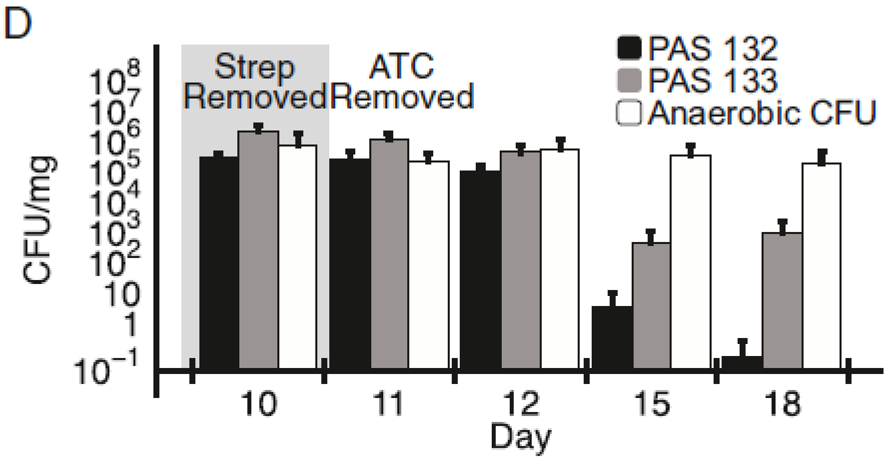


In vitro

In vivo



- memory circuit functioned essentially identically in an uncharacterized coliform bacterium from the mouse gut
- PAS133 stably colonized the mouse gut longer than PAS132



Anaerobic CFU → coliform titers in pretreated mice

Conclusion

- **cI and Cro states** were **stable** both in bacterial **cultures** and when the bacteria were passaged through the **mouse gut**

- **Switching** from the cI to the Cro state occurred **efficiently** upon exposure to ATC in E. coli in laboratory **culture** or in the **mouse gut**

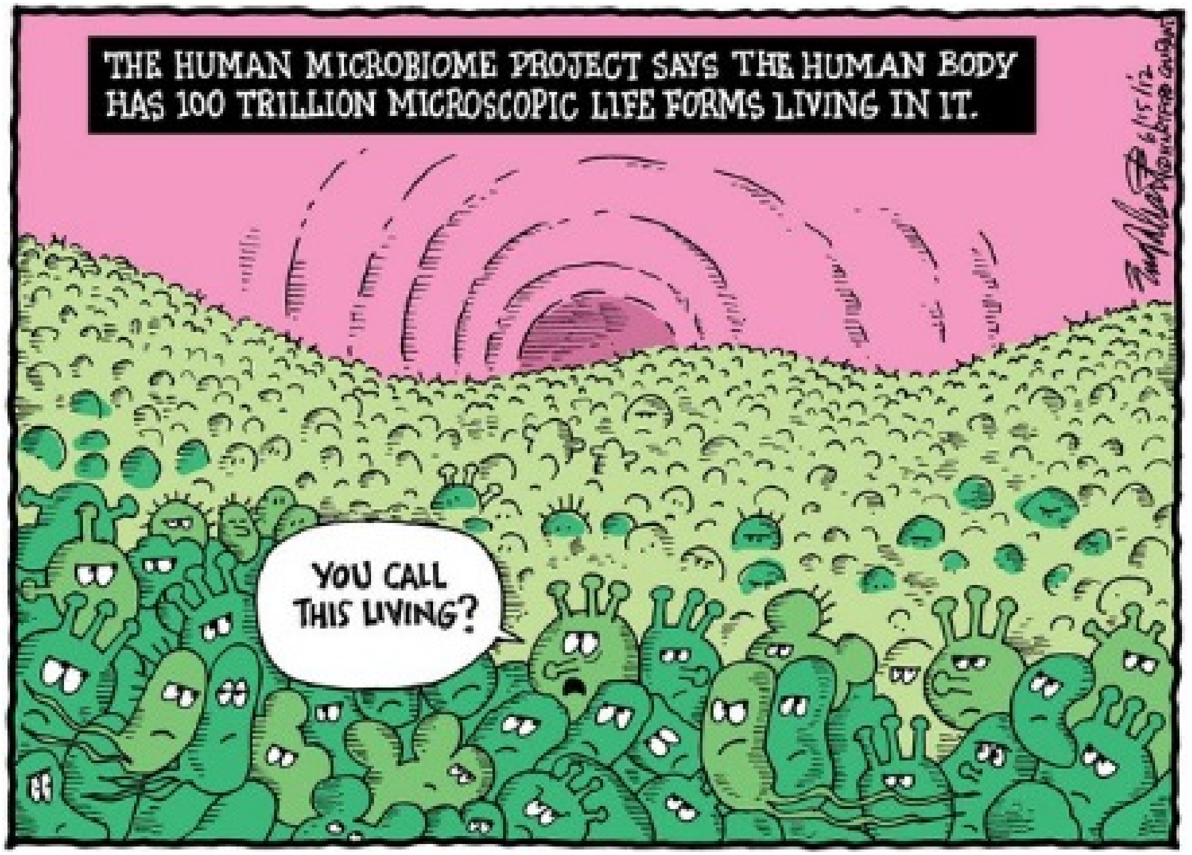
- Circuit was transferred from E. coli K12 to an uncharacterized **murine E. coli** strain → **system behavior** was virtually **identica**l, but the engineered **murine strain** was more **stably established** in the mouse gut

- Indicate that artificial genetic circuits can be **designed and characterized** in well-understood but attenuated **laboratory strains**, and then **transferred** to a related isolate from the environment of interest

- Lambda cI/Cro system as a memory element had low **burden on the host** →
  - cI protein only present in 100–200 copies per cell
  - Cro protein present in <1,000 copies per cell

- **Microbe-based recording systems** have the potential to be used as **diagnostics** in health care, environmental monitoring, and other applications

Thanks for your attention



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