

Synthetic Biology = design and engineering of biological systems that aren't found in nature

Why would we want to do this?

- Want to understand natural systems. One of the best ways to understand a system is to change it or make new, related ones
- To fully “understand” a system, we should be able to predict the outcome when we change the system
- For molecular biology, this means:
 - designing new gene circuits and networks
 - modeling the designed systems & predicting their properties
 - making & testing the designs
 - updating our understanding from the model/test agreement

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Engineers often look at biological systems & think that the systems are equivalent to electronic circuits

e.g,

fluorescent proteins	light bulbs or LEDs
transcription factors	transistors or logic gates
repressors	NOT gates
activators	OR/AND gates
polymerases	
(transcriptional machinery)	batteries

and so on...

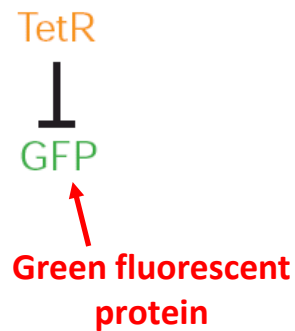
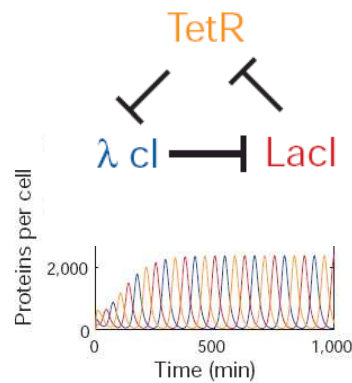
Are they right?

→ raises the possibility that biological parts (genes, proteins, etc.) could be combined using the rules established for analog/digital circuits

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The Repressilator = engineered genetic circuit designed to make bacteria glow in a oscillatory fashion
= “repressor” + “oscillator”

Transcriptional repressors

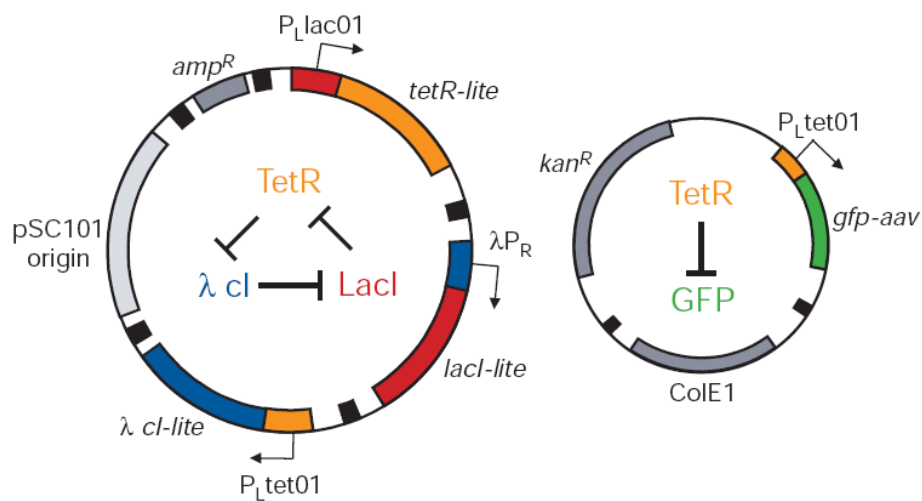


Elowitz & Leibler, *Nature* (2000) 403:335-8

The Repressilator = engineered genetic circuit designed to make bacteria glow in a oscillatory fashion

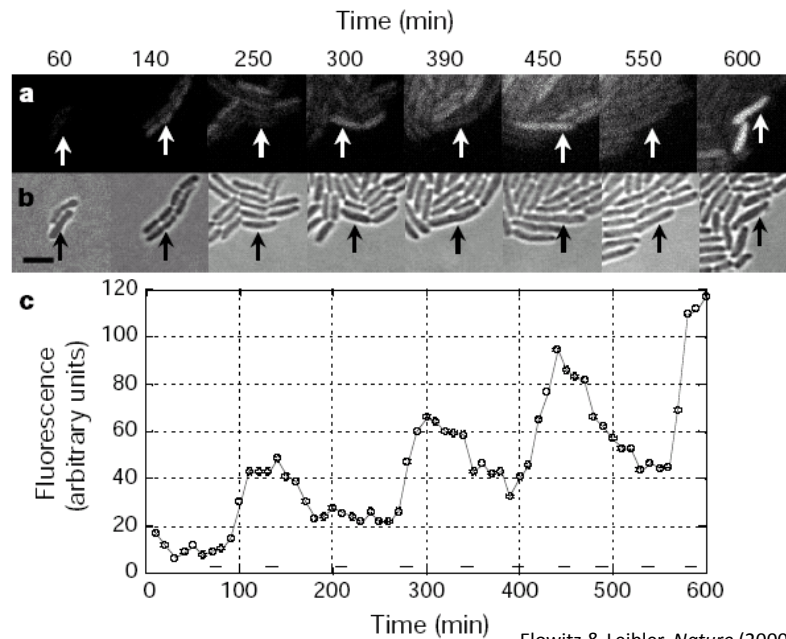
Repressilator

Reporter



Elowitz & Leibler, *Nature* (2000) 403:335-8

The repressilator in action...



What other kinds of circuits can be built?

First, we need some more parts!

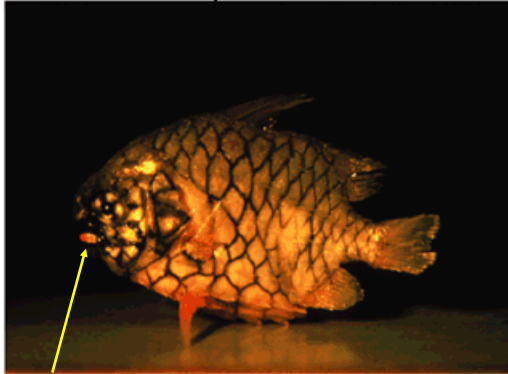
Some of the other parts available include:

- various sensors
 - light, dark, heat, cold
- more switches, logic gates
 - more repressors, activators
- parts for intracellular communication
 - helpful if cells could tell each what condition they're in
 - **quorum sensing**
- parts for signaling the output of circuits
 - fluorescent & luminescent proteins

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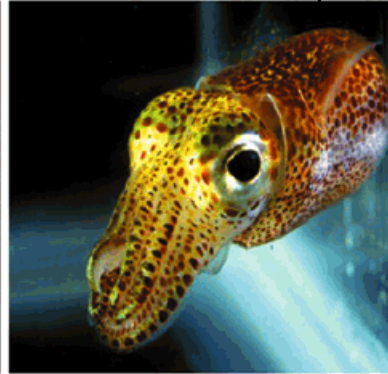
Bioluminescence – occurs when bacteria are at high density
→ bacteria communicate in order to establish their density

Australian pinecone fish



$\sim 10^{10}$ *Vibrio* bacteria/ml fluid
 Fish uses to hunt for prey

Hawaiian bobtail squid

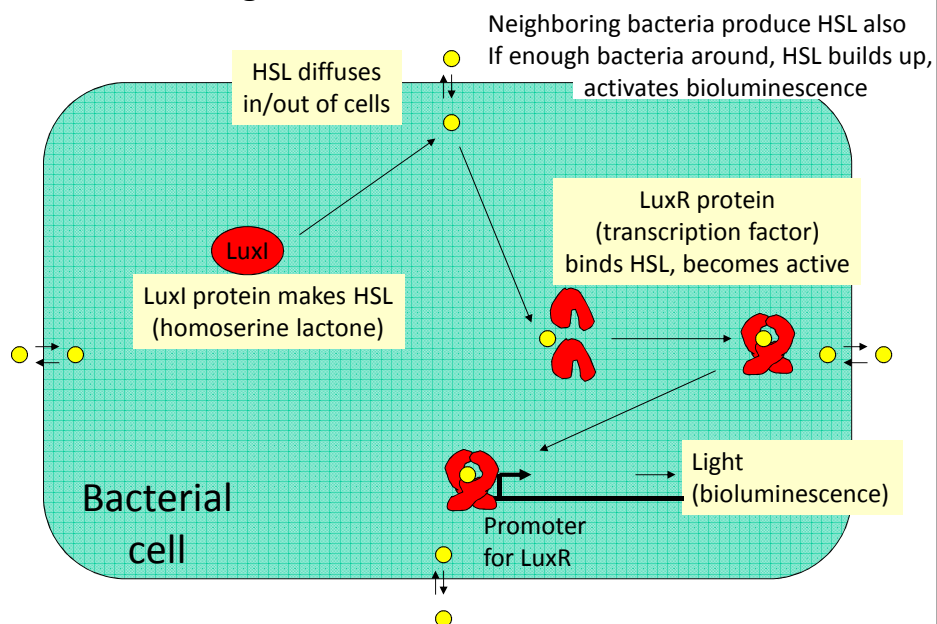


$\sim 10^{11}$ *Vibrio* bacteria/ml fluid
 in light organ in squid mantle
 Squid uses for disguise (light
 shines downward, looks like
 moonlight)

Nature Reviews Molecular Cell Biology 3; 685-695 (2002)

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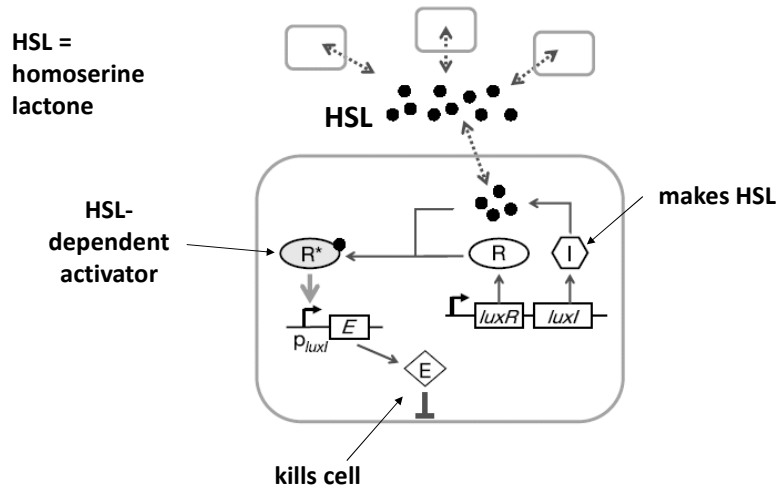
Quorum sensing: chemical-based bacterial communication



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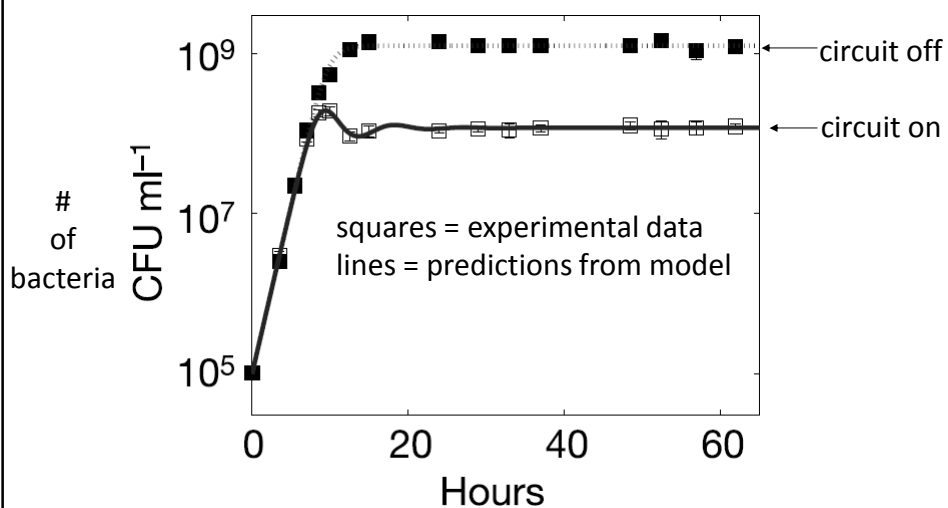
An application of quorum sensing

Programming population control into bacteria with a simple designed circuit



You, Cox, Weiss, Arnold, *Nature* (2004)

& the engineered circuit works ...



You, Cox, Weiss, Arnold, *Nature* (2004)

The behaviour can be predicted with a simple model

rate of
cell
growth

$$\frac{dN}{dt} = \overset{\text{cell growth rate}}{kN(1 - N/N_m)} - \overset{\text{cell death rate}}{dEN}$$

amount of killer
protein

rate of
killer protein
production

$$\frac{dE}{dt} = \overset{\text{amount of HSL}}{k_E A} - \overset{\text{killer protein}}{d_E E}$$

killer protein
synthesis rate killer protein
degradation rate

rate of HSL
production

$$\frac{dA}{dt} = \overset{\text{HSL}}{v_A N} - \overset{\text{HSL}}{d_A A}$$

synthesis rate degradation rate

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You, Cox, Weiss, Arnold, *Nature* (2004)

Standardization of parts: the iGEM "BioBricks" project



Registry of Standard Biological Parts

The Registry's Repository

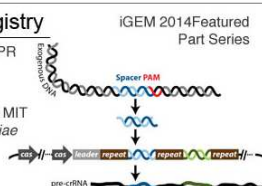


Featured on the Registry

CRISPR and Cas9 on the Registry

Want to use CRISPR? We have lots of CRISPR and Cas9 parts for different organisms!

During iGEM 2013, twelve teams worked on CRISPR as part of their project. Freiburg and MIT used Mammalian cells, Duke used *S. cerevisiae* and the others such as UBC, Paris Bettencourt and Penn State worked with *E. coli*.



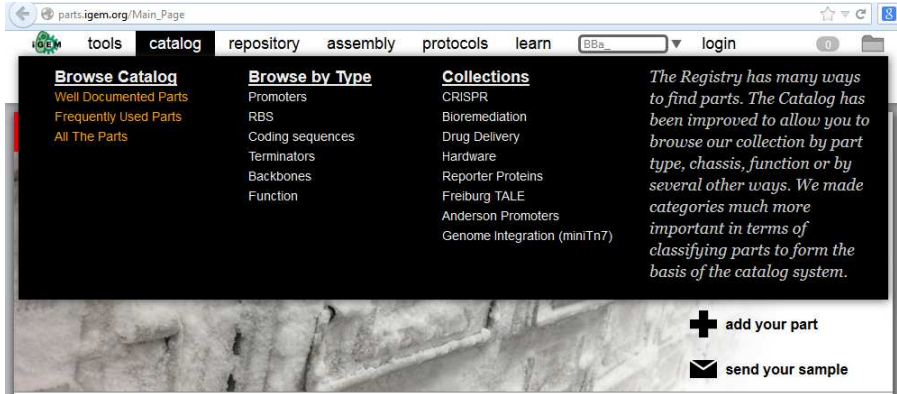
iGEM 2014 Featured Part Series

The iGEM Registry is a growing collection of genetic parts that can be mixed and matched to build synthetic biology devices and systems.

As part of the synthetic biology community's efforts to make biology easier to engineer, it provides a

Edward Marcotte/Univ. of Texas/BIO337/Spring 2014

Standardization of parts: the iGEM “BioBricks” project



The screenshot shows the iGEM Registry Main Page. The navigation bar includes links for tools, catalog, repository, assembly, protocols, learn, a search bar, and a login button. The main content area is divided into several sections:

- Browse Catalog:** Well Documented Parts, Frequently Used Parts, All The Parts.
- Browse by Type:** Promoters, RBS, Coding sequences, Terminators, Backbones, Function.
- Collections:** CRISPR, Bioremediation, Drug Delivery, Hardware, Reporter Proteins, Freiburg TALE, Anderson Promoters, Genome Integration (miniTn7).

A text box on the right states: "The Registry has many ways to find parts. The Catalog has been improved to allow you to browse our collection by part type, chassis, function or by several other ways. We made categories much more important in terms of classifying parts to form the basis of the catalog system."

Below the navigation bar, there are two buttons: "add your part" and "send your sample".

Featured on the Registry

CRISPR and Cas9 on the Registry

Want to use CRISPR? We have lots of CRISPR and Cas9 parts for different organisms!

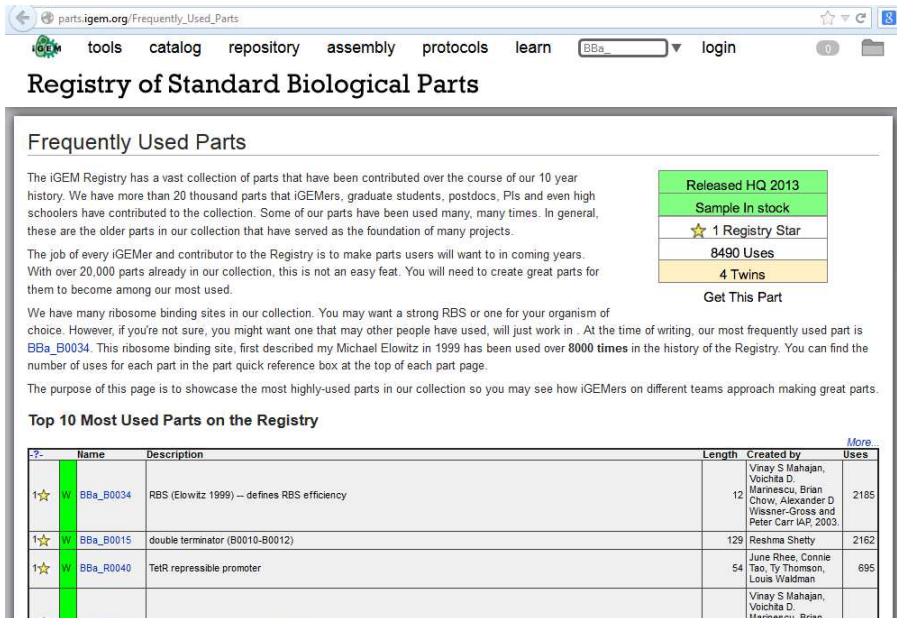
During iGEM 2013, twelve teams worked on CRISPR as part of their project. Freiburg and MIT used Mammalian cells, Duke used *S. cerevisiae* and the others such as UBC, Paris Bettencourt and Penn State worked with *E. coli*.

The diagram illustrates the CRISPR-Cas9 system. It shows a DNA sequence with a "Spacer PAM" region. Below this, a "pre-crRNA" is transcribed, which is then processed into "crRNA" and "tracrRNA". The crRNA is then used by the Cas9 protein to target and cut the DNA at the PAM site.

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Standardization of parts: the iGEM “BioBricks” project



The screenshot shows the iGEM Registry Frequently Used Parts page. The navigation bar is the same as the previous page. The main content area is titled "Frequently Used Parts" and includes the following text:

The iGEM Registry has a vast collection of parts that have been contributed over the course of our 10 year history. We have more than 20 thousand parts that iGEMers, graduate students, postdocs, PIs and even high schoolers have contributed to the collection. Some of our parts have been used many, many times. In general, these are the older parts in our collection that have served as the foundation of many projects.

The job of every iGEMer and contributor to the Registry is to make parts users will want to in coming years. With over 20,000 parts already in our collection, this is not an easy feat. You will need to create great parts for them to become among our most used.

We have many ribosome binding sites in our collection. You may want a strong RBS or one for your organism of choice. However, if you're not sure, you might want one that may other people have used, will just work in . At the time of writing, our most frequently used part is BBa_B0034. This ribosome binding site, first described by Michael Elowitz in 1999 has been used over 8000 times in the history of the Registry. You can find the number of uses for each part in the part quick reference box at the top of each part page.

The purpose of this page is to showcase the most highly-used parts in our collection so you may see how iGEMers on different teams approach making great parts.

Top 10 Most Used Parts on the Registry

Rank	Name	Description	Length	Created by	Uses
1	BBa_B0034	RBS (Elowitz 1999) -- defines RBS efficiency	12	Vinay S Mahajan, Voichita D. Marinescu, Brian Chow, Alexander D Wiesner-Gross and Peter Carr IAP, 2003.	2185
2	BBa_B0015	double terminator (B0010-B0012)	129	Reshma Shetty	2162
3	BBa_R0040	TetR repressible promoter	54	June Rhee, Connie Tao, Ty Thomson, Louis Waldman	695
4	BBa_B0030	RBS.1 (strong) -- modified from R. Weiss	15	Vinay S Mahajan, Voichita D. Marinescu, Brian Chow, Alexander D	533

On the right side of the page, there is a box with the following information:

- Released HQ 2013
- Sample In stock
- 1 Registry Star
- 8490 Uses
- 4 Twins
- Get This Part

iGEM: A synthetic biology contest

(from iGEM's web site)

Can simple biological systems be built from standard, interchangeable parts and operated in living cells? Or is biology simply too complicated to be engineered in this way?

iGEM's broader goals include:

- To enable systematic engineering of biology
- To promote open & transparent development of tools for engineering biology
- To help construct a society that can productively apply biological technology

2004: MIT, UT, Princeton, Boston University, Cornell

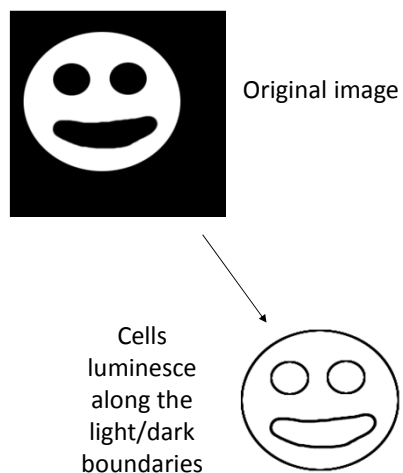
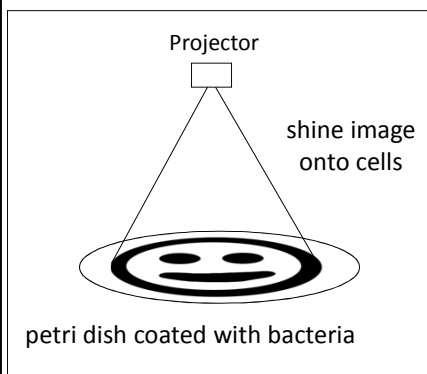
2005: 13 teams (the above + UK, Germany, more...)

2006: 32 teams, incl. Japan/Latin America/Korea/India/more Europe

54 teams in 2007, 84 teams in 2008, 112 teams in 2009, 130 teams in 2010, 165 teams in 2011, and 245 teams in 2012 and 2013...

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UT's 2004/2005 iGEM project – build bacterial edge detector

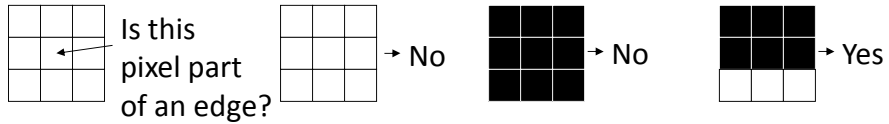


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Adapted from Zack Simpson

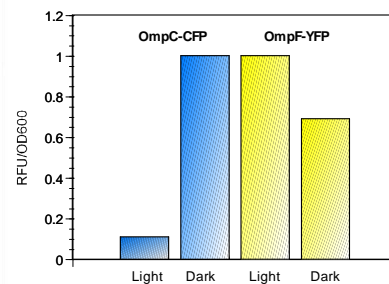
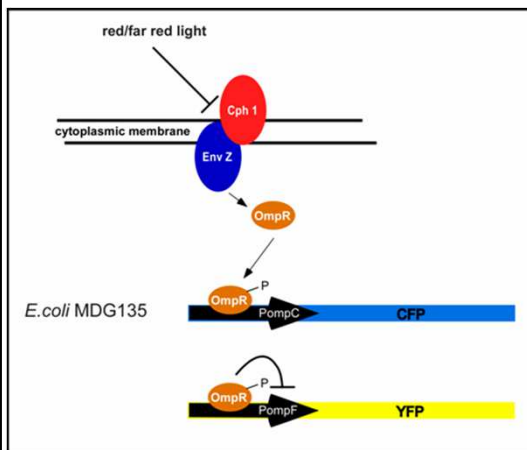
How does edge detection work in principle?

A computer might visit each pixel in turn, and check to see if it is bordered by both black & white pixels. If yes, highlight the pixel.



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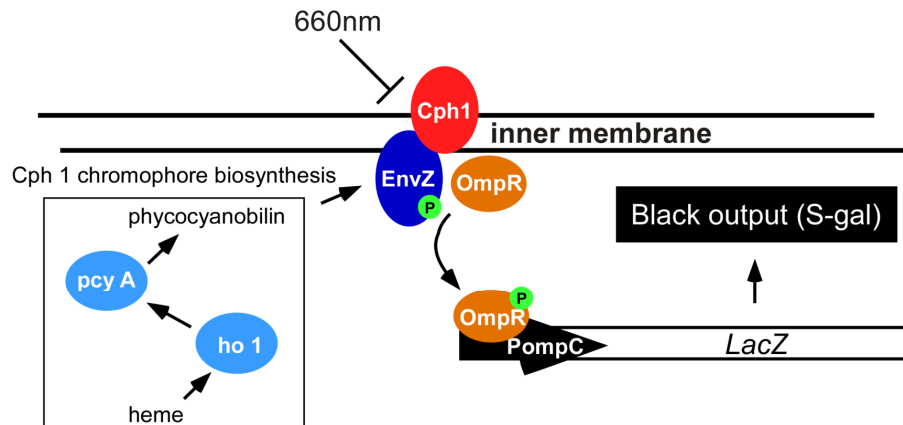
Light-dependent gene expression



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Levskaya et al. *Nature*, 438(7067):441-2 (2005)

Bacterial photography



Levskaya *et al. Nature*, 438(7067):441-2 (2005)

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Mask

Cph1/EnvZ

Hello
World



Hello
World



"Light cannon" developed by Aaron Chevalier,
UT undergraduate

Levskaya *et al. Nature*, 438(7067):441-2 (2005)

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The first bacterial photograph (coliroid?)...



Levskaya et al. *Nature*, 438(7067):441-2 (2005)

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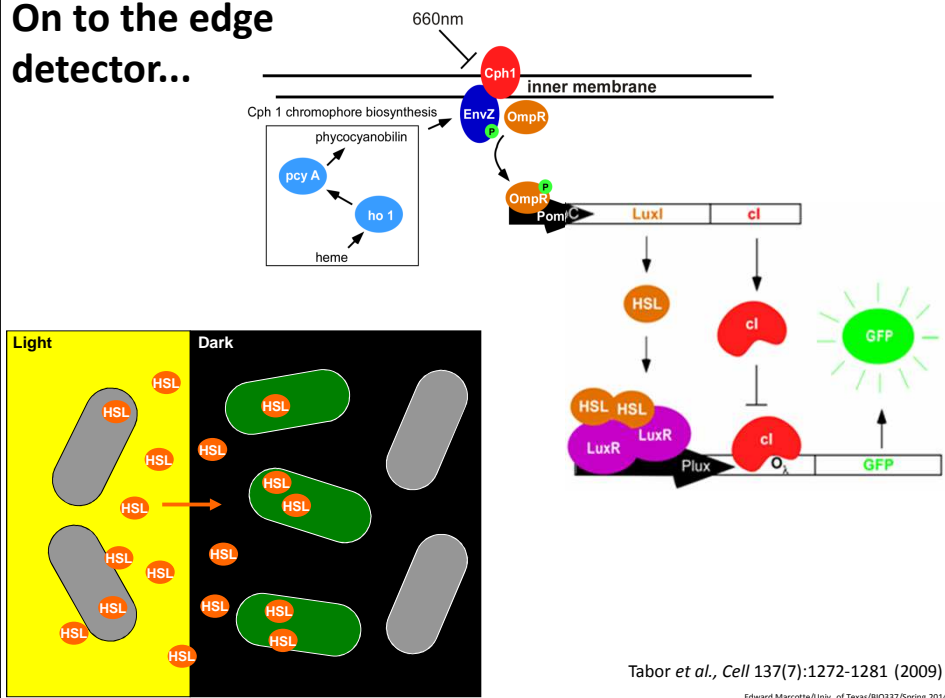
*Escherichia
darwinia*



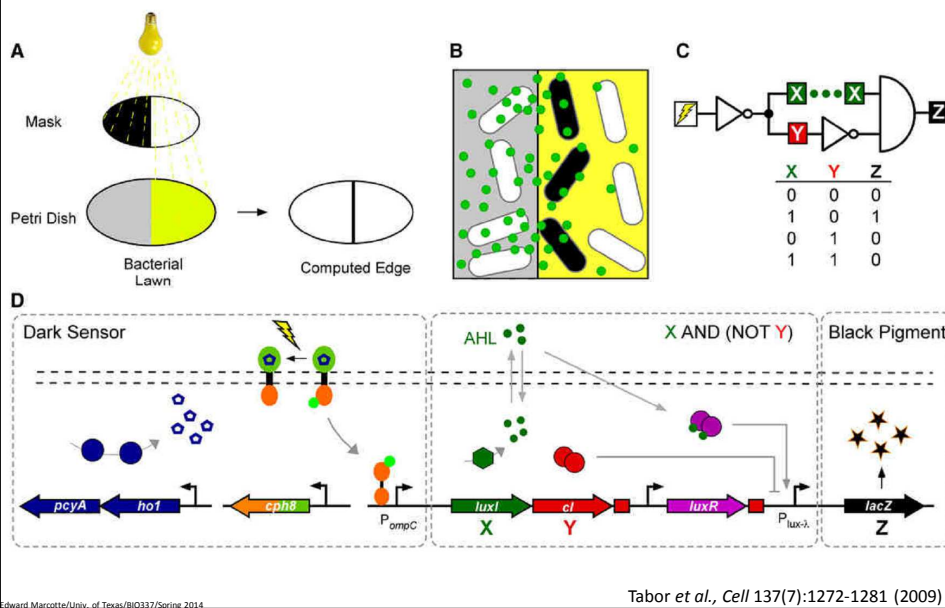
Image: Aaron Chevalier

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On to the edge detector...



The edge detector circuit in more detail



It works!

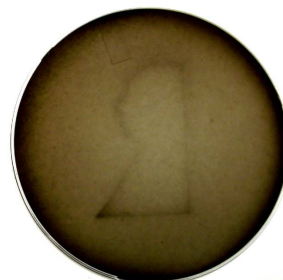
Projected Mask



Photo strain

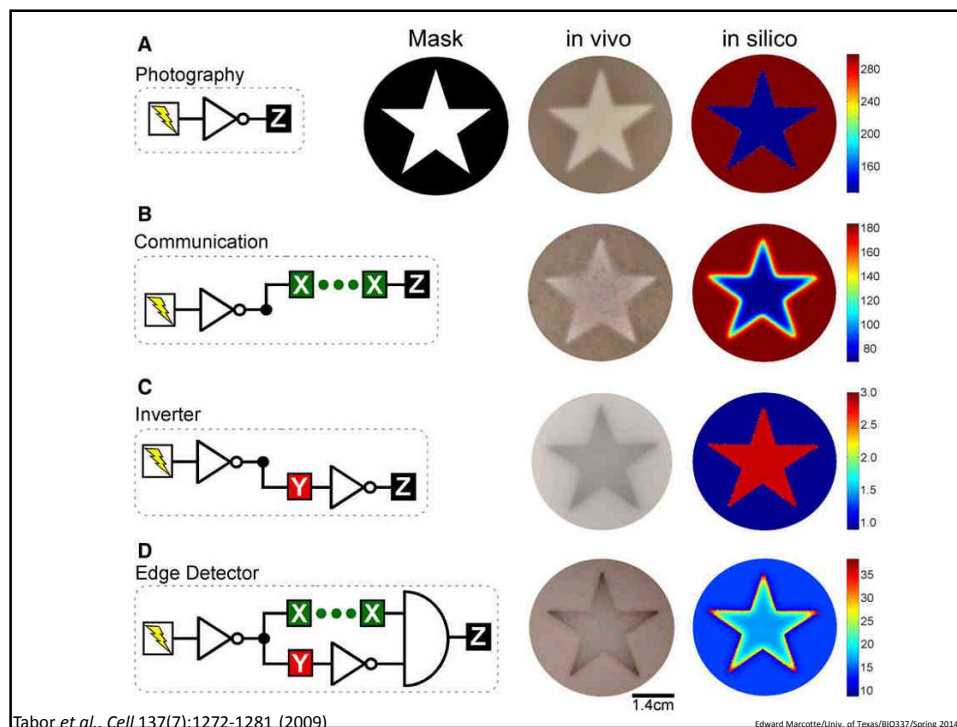


Edge detector strain



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Tabor *et al.*, *Cell* 137(7):1272-1281 (2009)



Tabor *et al.*, *Cell* 137(7):1272-1281 (2009)

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UT's 2012 iGEM project – build caffeine biosensor

ACS
SyntheticBiology

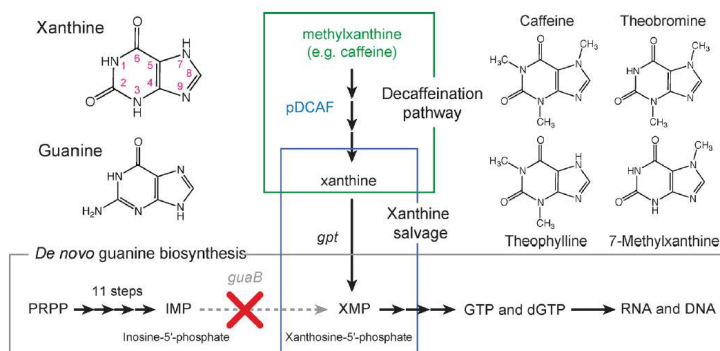
Letter
pubs.acs.org/synbio

Decaffeination and Measurement of Caffeine Content by Addicted *Escherichia coli* with a Refactored *N*-Demethylation Operon from *Pseudomonas putida* CBB5

Erik M. Quandt,[‡] Michael J. Hammerling,[‡] Ryan M. Summers,[‡] Peter B. Otoupal,[‡] Ben Slater,[‡] Razan N. Alnahhas,[‡] Aurko Dasgupta,[‡] James L. Bachman,[‡] Mani V. Subramanian,[‡] and Jeffrey E. Barrick^{*,‡}

Basic idea

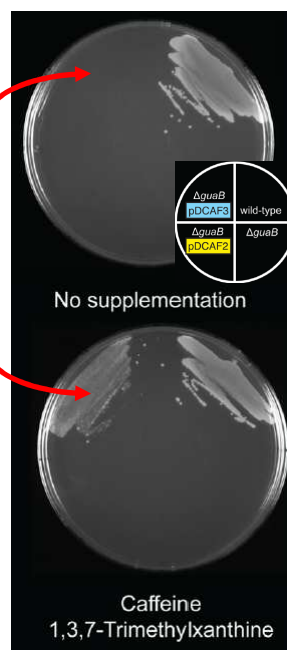
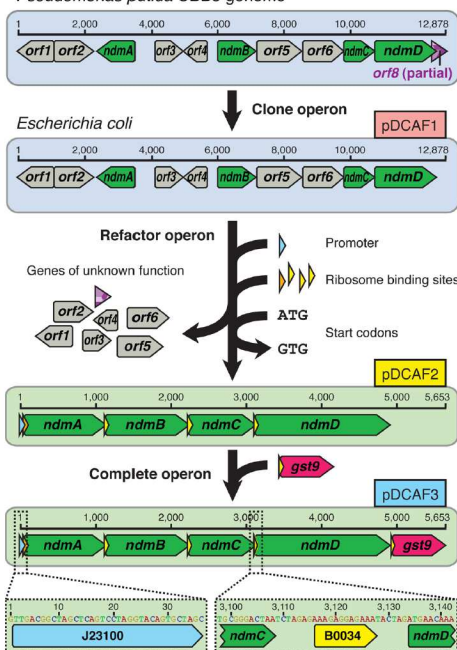
Block *de novo* guanine synthesis
Convert caffeine to xanthine
Addict *E. coli* bacteria to caffeine



Edward Marcotte/Univ. of Texas/Bio337/Spring 2014

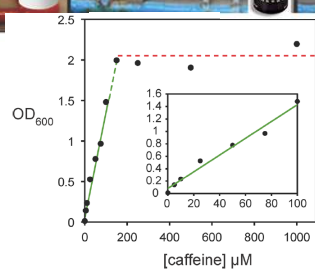
ACS Synth. Biol. 2013, 2, 301–307

Pseudomonas putida CBB5 genome



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ACS Synth. Biol. 2013, 2, 301–307



Edward Marcotte/Univ. of Texas/BIO337/Spring 2014

ACS Synth. Biol. 2013, 2, 301-307