

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

Edward M. Marcotte/Univ. of Texas @CH164C-3311/Spring 2015

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

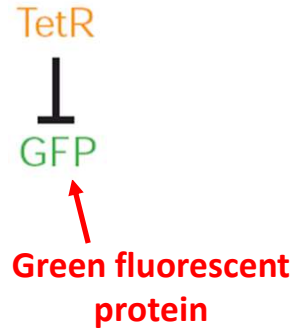
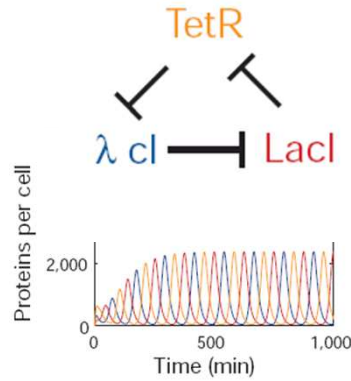
and so on...

Are they right?

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

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

Transcriptional repressors ↘

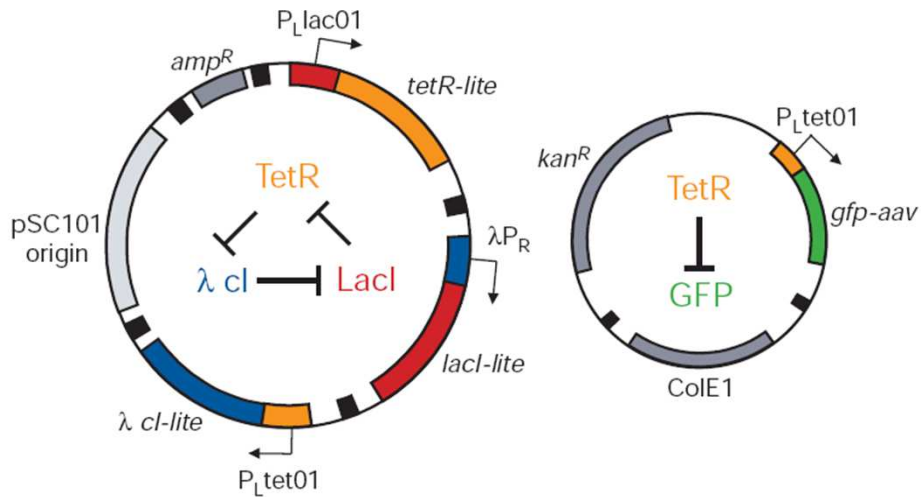


Elowitz & Leibler, *Nature* (2000) 403:335-8

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

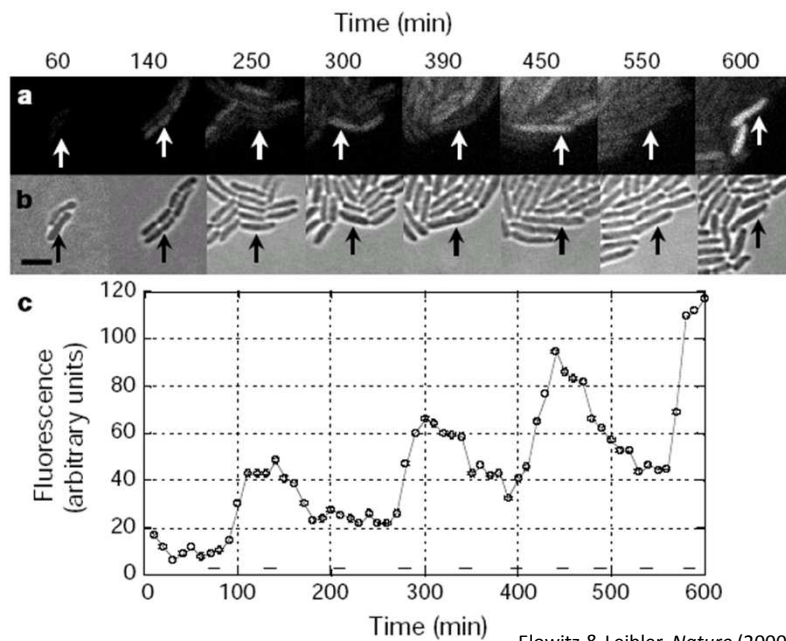
Repressilator

Reporter



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

The repressilator in action...



What other kinds of circuits can be built?

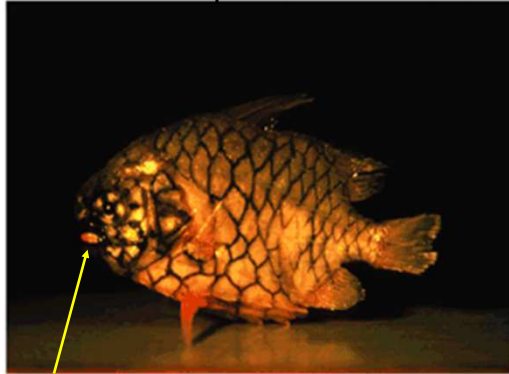
First, we need some more parts!

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

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

Australian pinecone fish



~10¹⁰ Vibrio bacteria/ml fluid
 Fish uses to hunt for prey

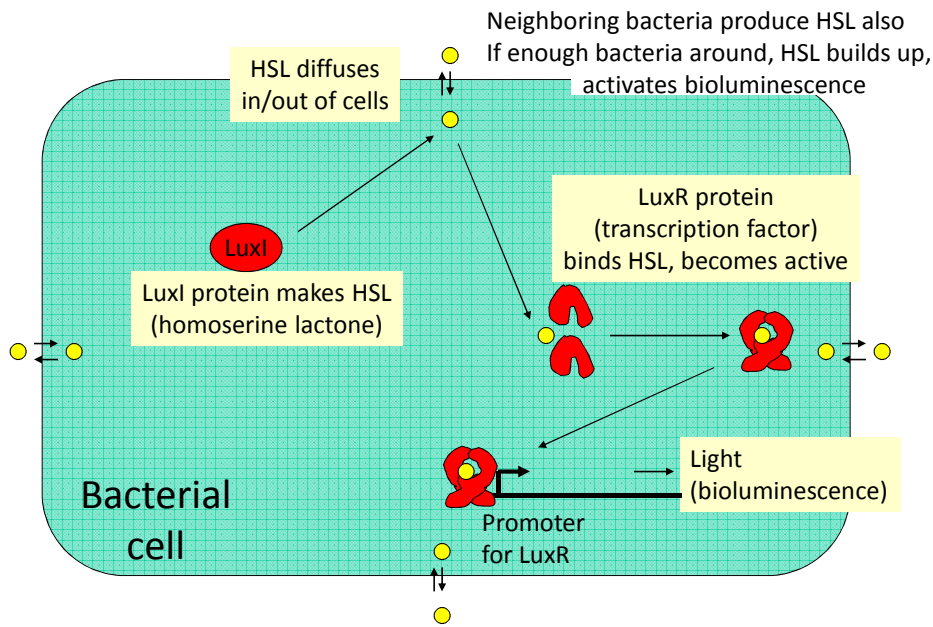
Hawaiian bobtail squid

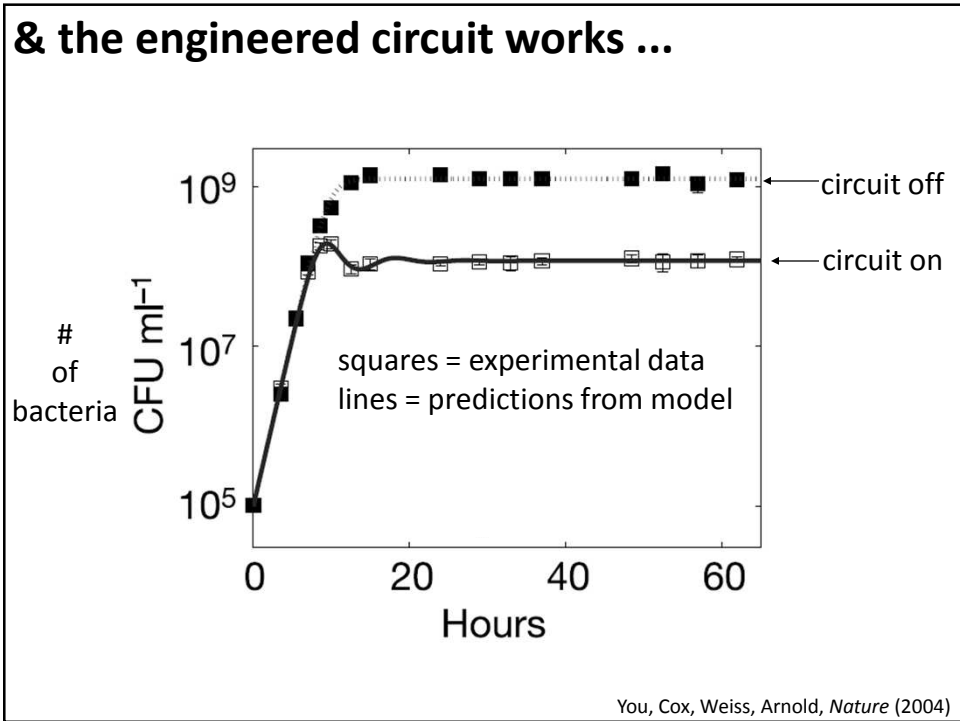
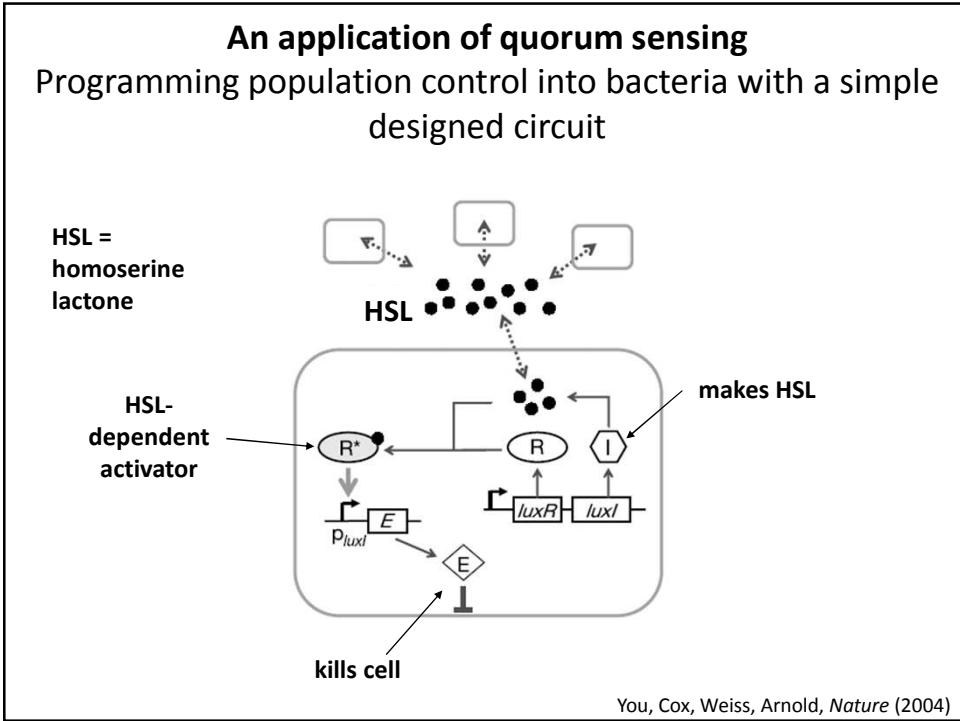


~10¹¹ Vibrio bacteria/ml fluid
 in light organ in squid mantle
 Squid uses for disguise (light
 shines downward, looks like
 moonlight)

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

Quorum sensing: chemical-based bacterial communication





The behaviour can be predicted with a simple model

rate of cell growth

$$\frac{dN}{dt} = kN(1 - N/N_m) - dEN$$

cell growth rate cell death rate amount of killer protein

rate of killer protein production

$$\frac{dE}{dt} = k_E A - d_E E$$

amount of HSL killer protein synthesis rate killer protein degradation rate

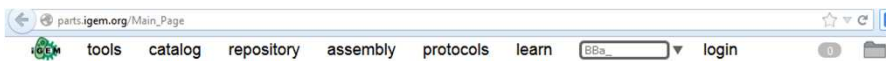
rate of HSL production

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

HSL synthesis rate HSL degradation rate

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

Standardization of parts: the iGEM "BioBricks" project



Registry of Standard Biological Parts

The Registry's Repository

The Registry's Repository contains DNA samples for thousands of parts, submitted by iGEM teams and labs. Last year, iGEM teams sent in samples for over 1500 parts.

Be sure to add your parts and send samples to the Registry so that they can be made available to the community!

+ add your part

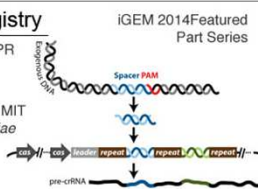
✉ send your sample

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*.



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

Standardization of parts: the iGEM "BioBricks" project

parts.igem.org/Main_Page

tools catalog repository assembly protocols learn BBa... login

- 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)

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.

+ add your part
✉ send your sample

Featured on the Registry

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parts.igem.org/Frequently_Used_Parts

tools catalog repository assembly protocols learn BBa... login

Registry of Standard Biological Parts

Frequently Used Parts

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.

Released HQ 2013

Sample In stock

★ 1 Registry Star

8490 Uses

4 Twins

Get This Part

Top 10 Most Used Parts on the Registry

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

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

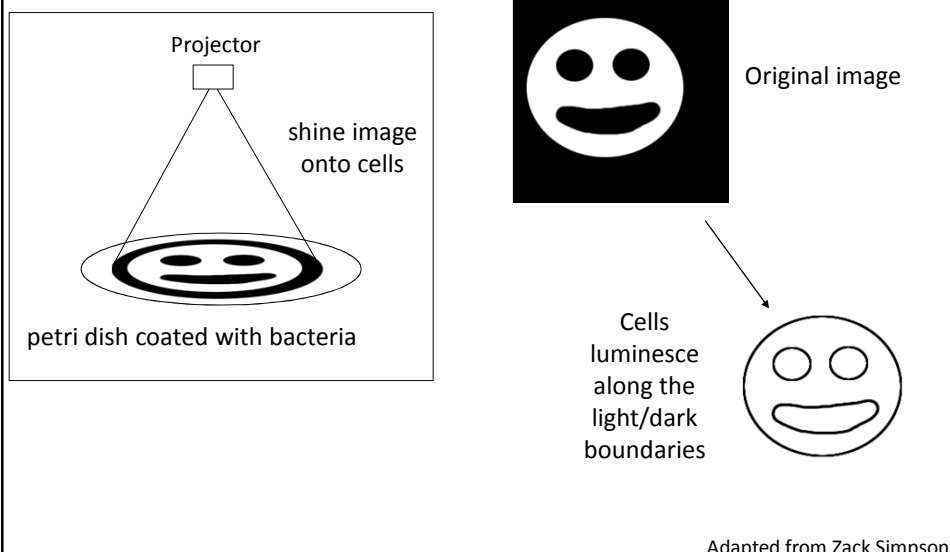
2004: MIT, UT, Princeton, Boston University, Cornell

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...

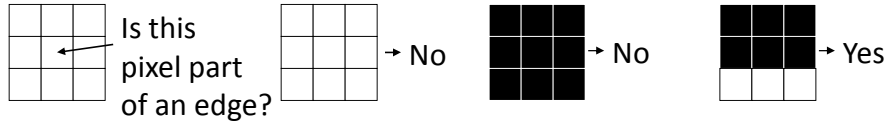
UT's 2004/2005 iGEM project – build bacterial edge detector



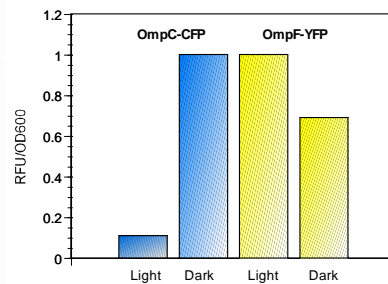
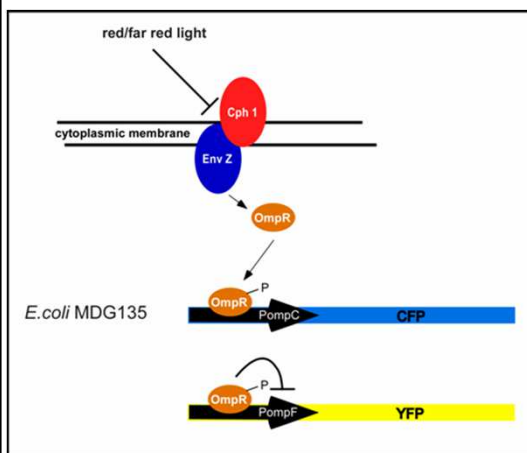
Adapted from Zack Simpson

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.

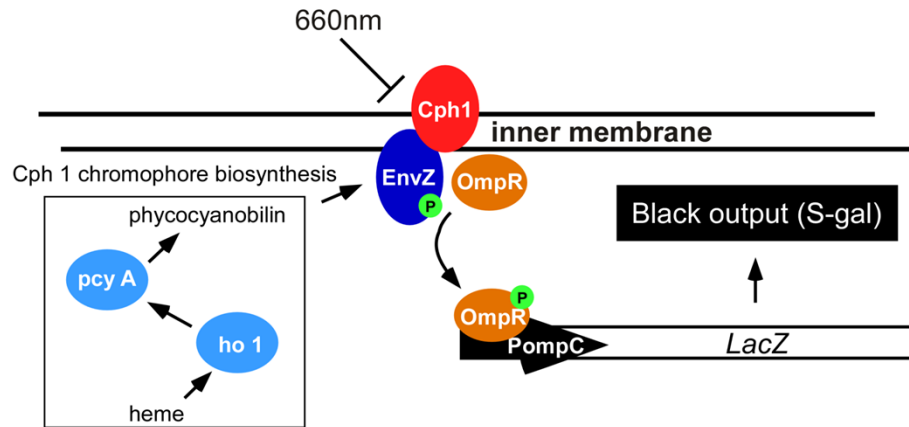


Light-dependent gene expression

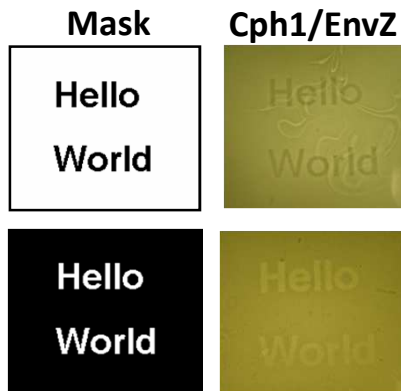


Levskaya *et al. Nature*, 438(7067):441-2 (2005)

Bacterial photography



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



"Light cannon" developed by Aaron Chevalier, UT undergraduate

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

The first bacterial photograph (coliroid?)...



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

*Escherichia
darwinia*



Image: Aaron Chevalier

It works!

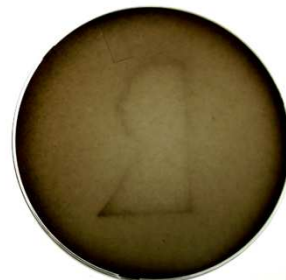
Projected Mask



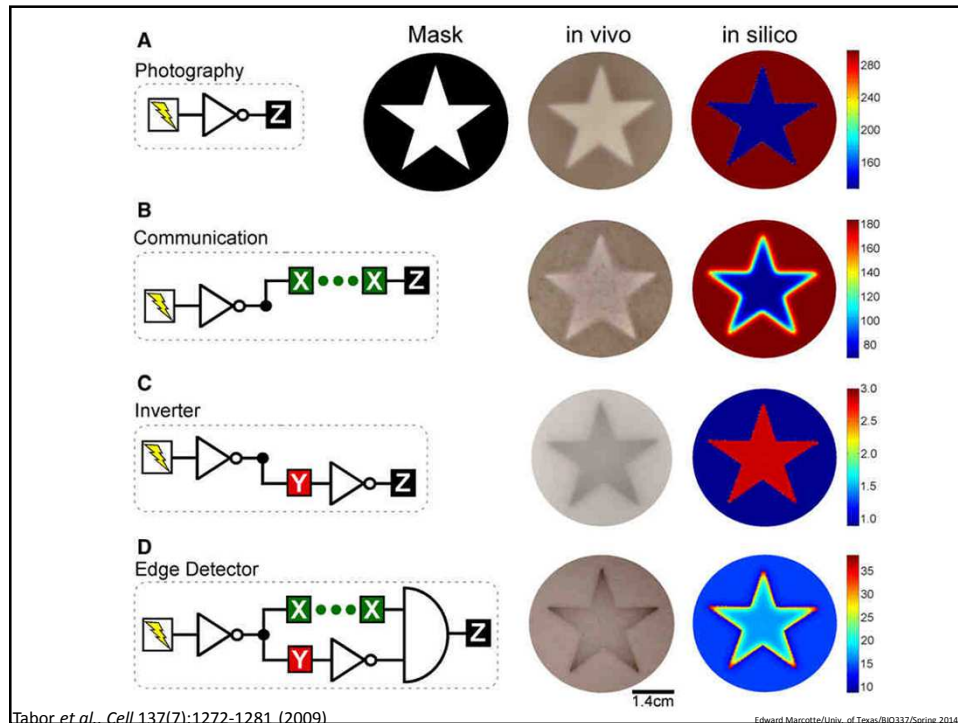
Photo strain



Edge detector strain



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



UT's 2012 iGEM project – build caffeine biosensor

ACS Synthetic Biology

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

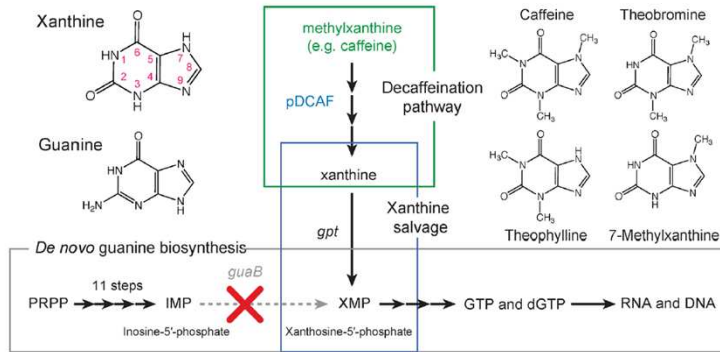
Erik M. Quandt,¹ Michael J. Hammerling,¹ Ryan M. Summers,¹ Peter B. Otoupal,¹ Ben Slater,¹ Razan N. Alnahhas,¹ Aurko Dasgupta,¹ James L. Bachman,¹ Mani V. Subramanian,² and Jeffrey E. Barrick^{1*}

Basic idea

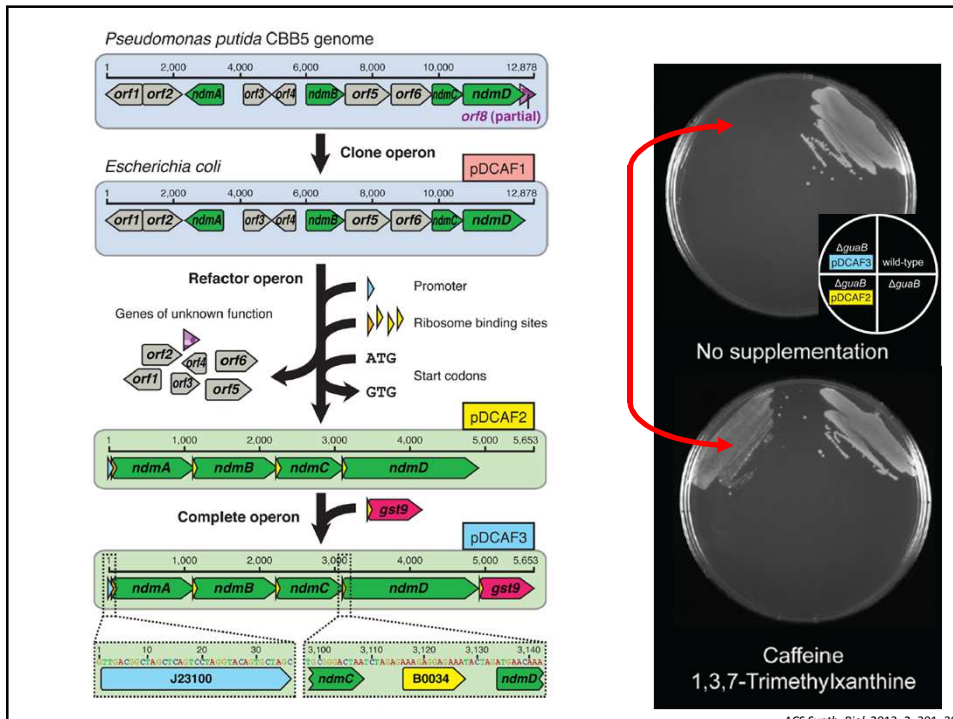
Block *de novo* guanine synthesis

Convert caffeine to xanthine

Addict *E. coli* bacteria to caffeine



ACS Synth. Biol. 2013, 2, 301–307



ACS Synth. Biol. 2013, 2, 301–307

