**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



**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 |

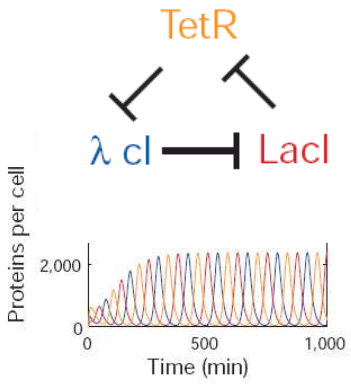
and so on...

**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**



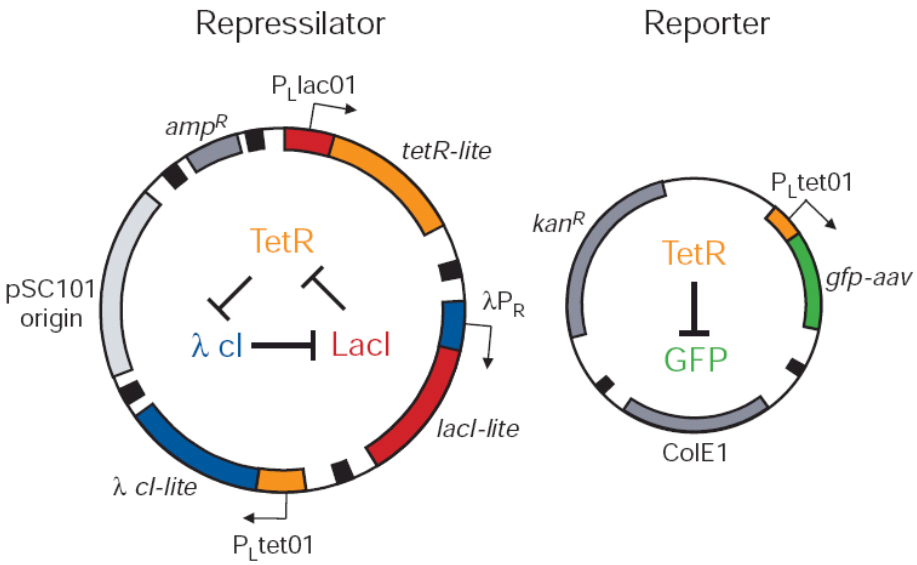


**Green fluorescent protein**

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

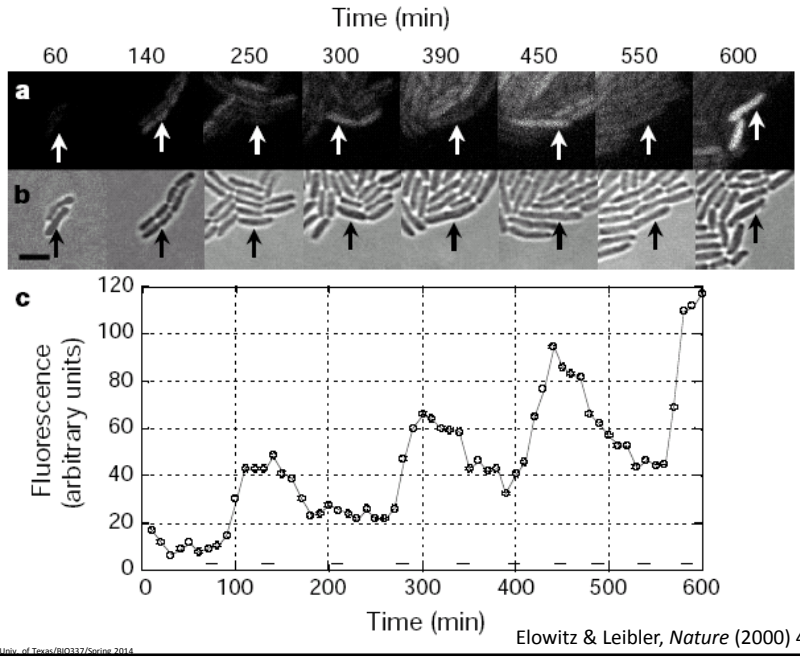


**The Repressilator** = engineered genetic circuit designed to make bacteria glow in a oscillatory fashion




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

## The repressilator in action...




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



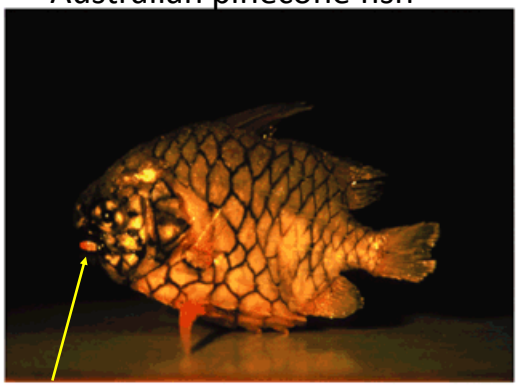
## What other kinds of circuits can be built? First, we need some more parts!

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

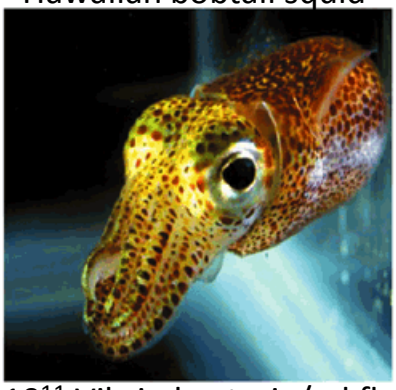
Australian pinecone fish



$\sim 10^{10}$ 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

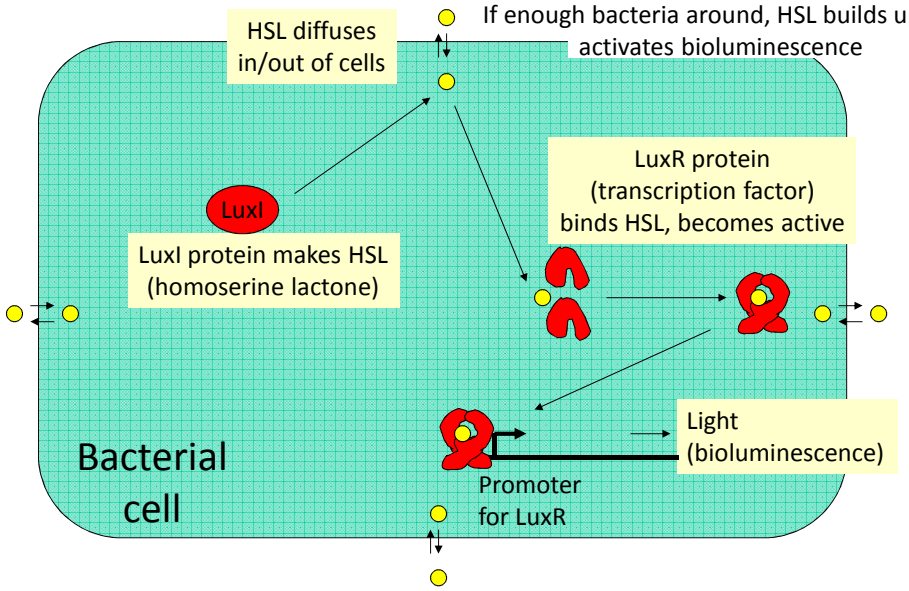
Squid uses for disguise (light shines downward, looks like moonlight)

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

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

## Quorum sensing: chemical-based bacterial communication

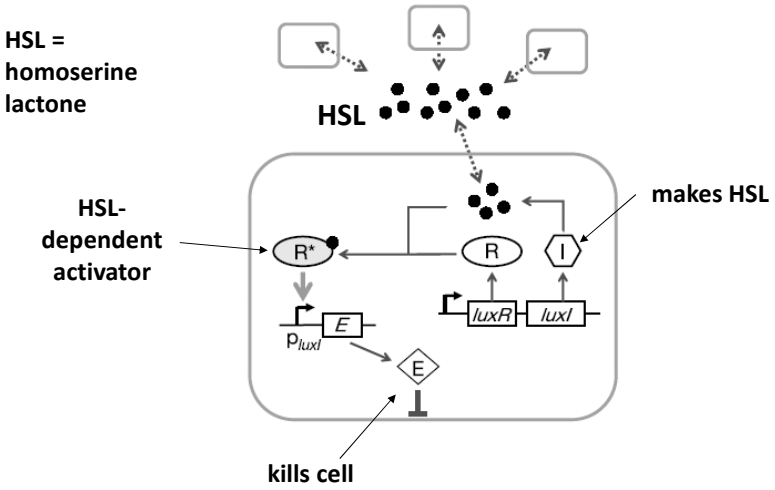
Neighboring bacteria produce HSL also
If enough bacteria around, HSL builds up, activates bioluminescence




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

## An application of quorum sensing

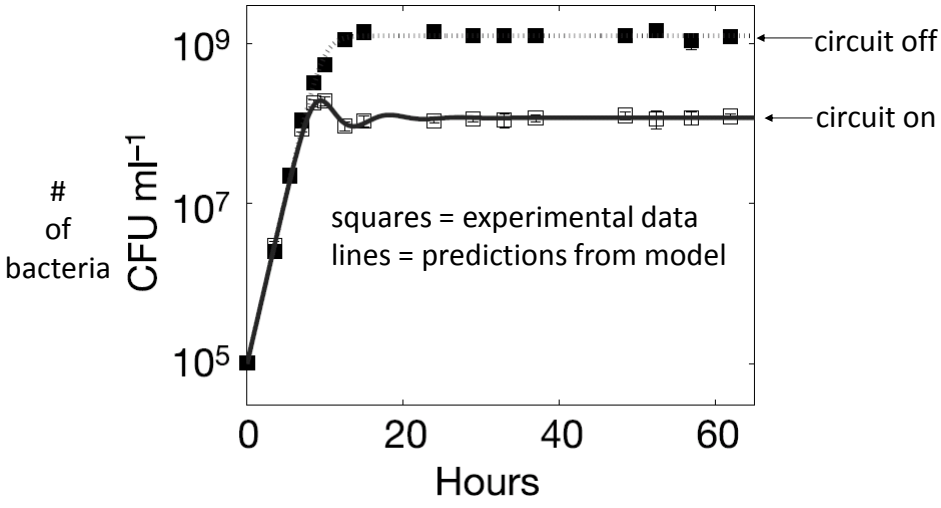
Programming population control into bacteria with a simple designed circuit




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

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

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

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

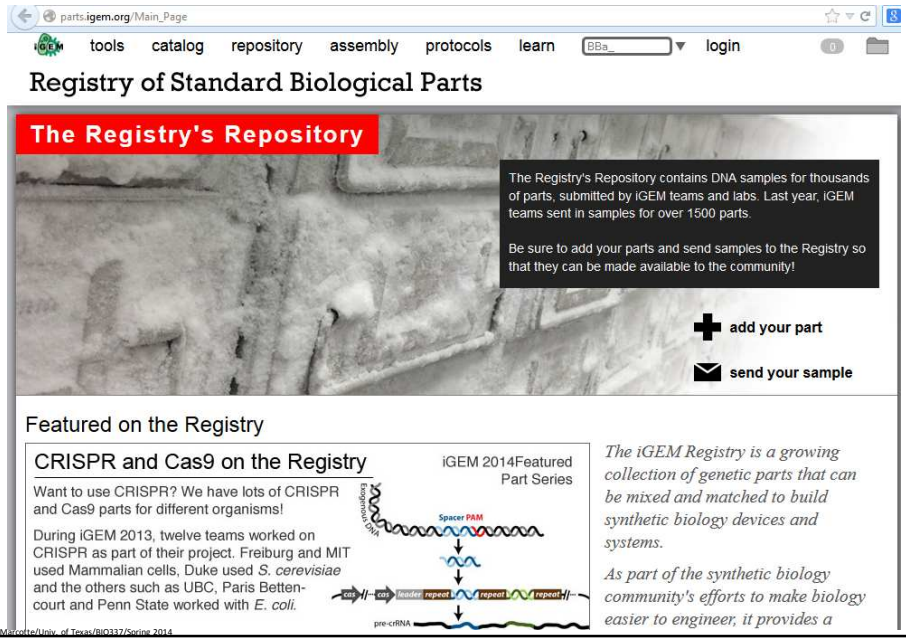
HSL synthesis rate

HSL degradation rate

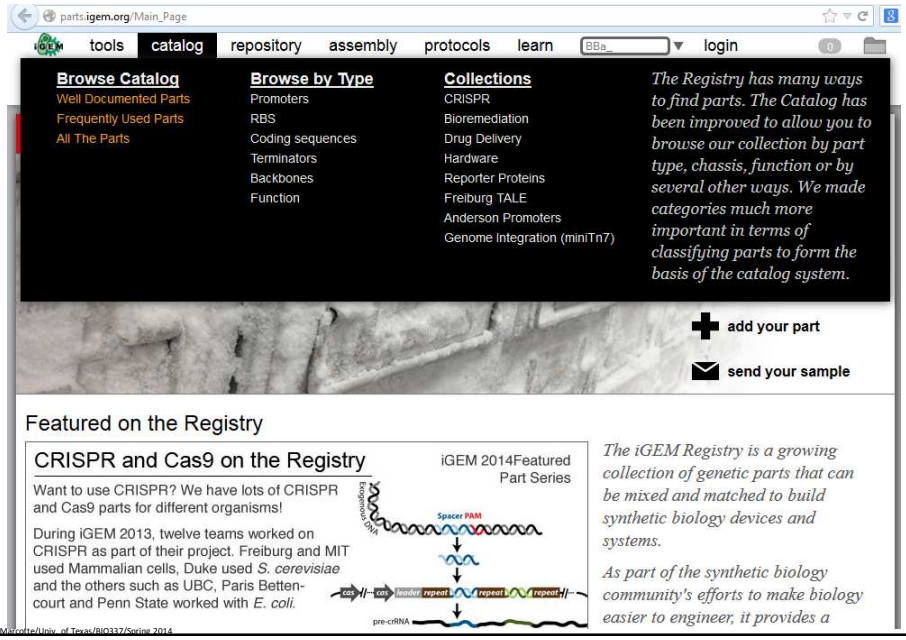
You, Cox, Weiss, Arnold, *Nature* (2004)



## Standardization of parts: the iGEM "BioBricks" project

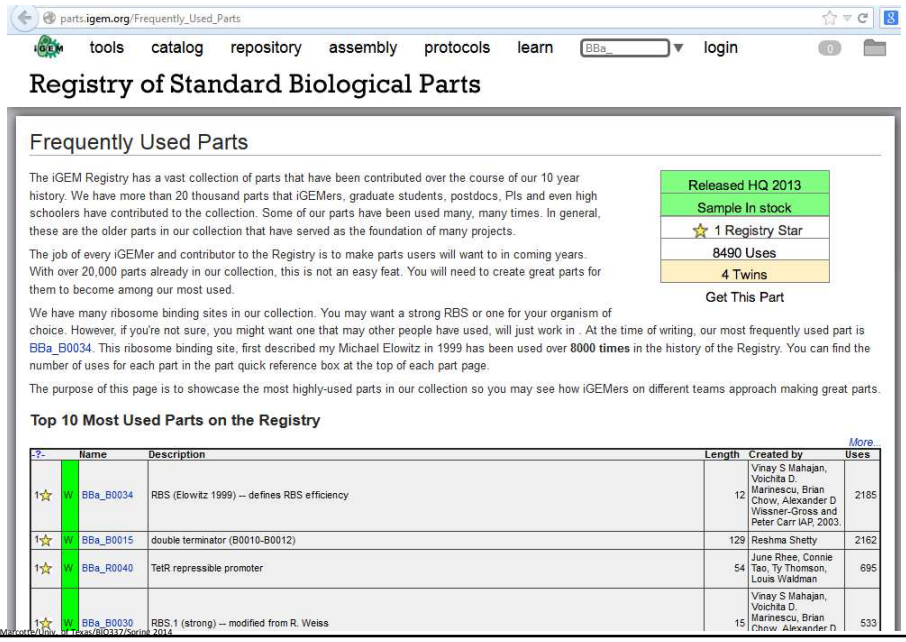


## Standardization of parts: the iGEM “BioBricks” project






## Standardization of parts: the iGEM “BioBricks” project



## 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:

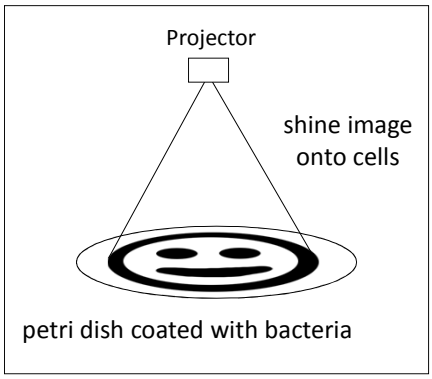
- To enable systematic engineering of biology
- To promote open & transparent development of tools for engineering biology
- To help construct a society that can productively apply biological technology

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



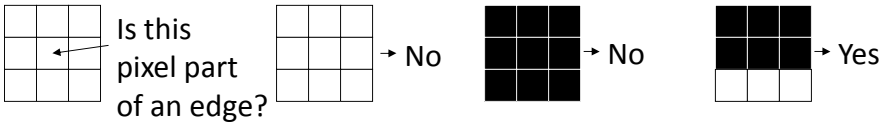

Original image

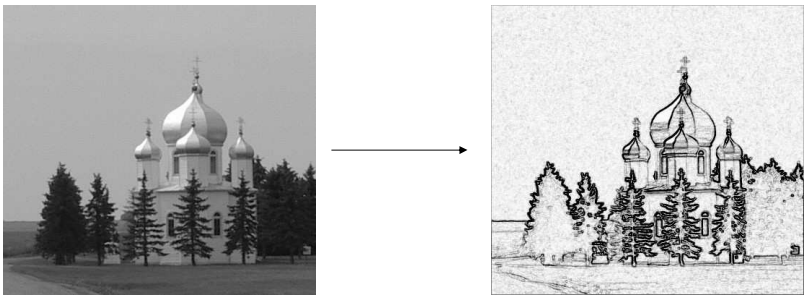
Cells luminesce along the light/dark boundaries

Adapted from Zack Simpson

## How does edge detection work in principle?

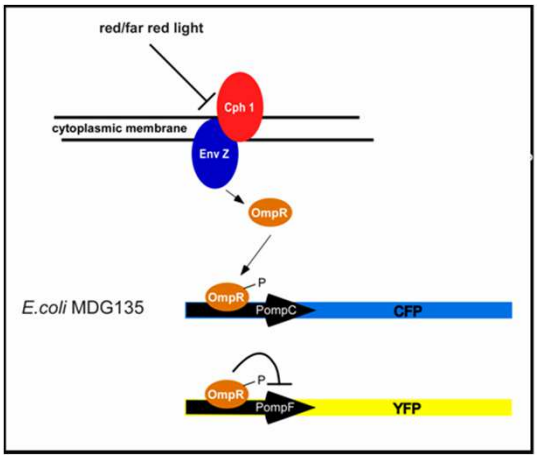
A computer might visit each pixel in turn, and check to see if it is bordered by both black & white pixels. If yes, highlight the pixel.

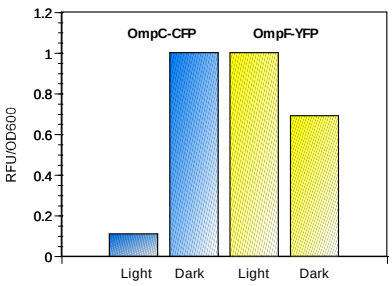




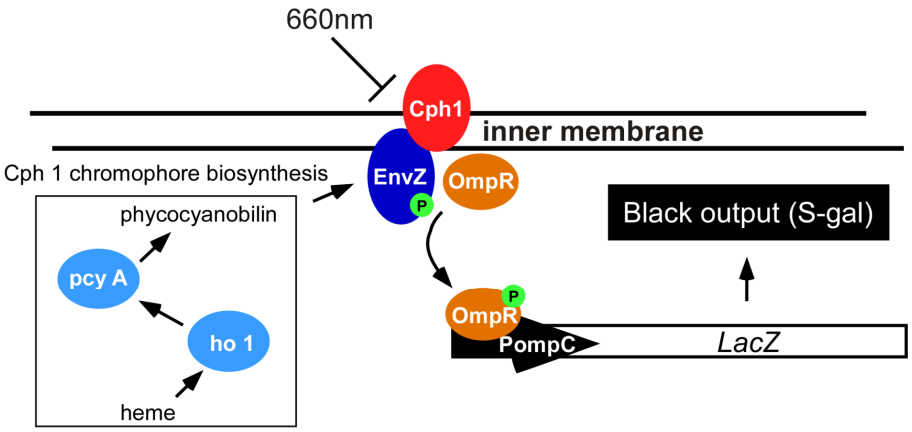
## Light-dependent gene expression



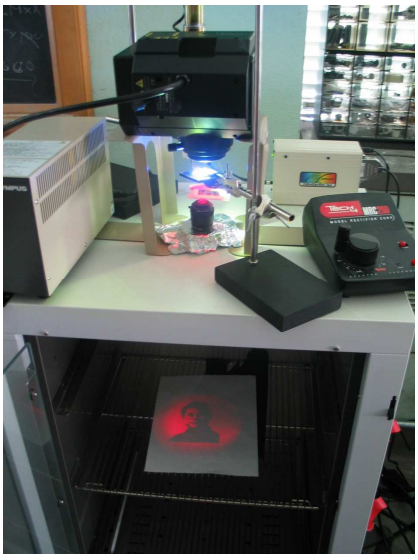

Levskaya *et al. Nature*, 438(7067):441-2 (2005)

# Bacterial photography

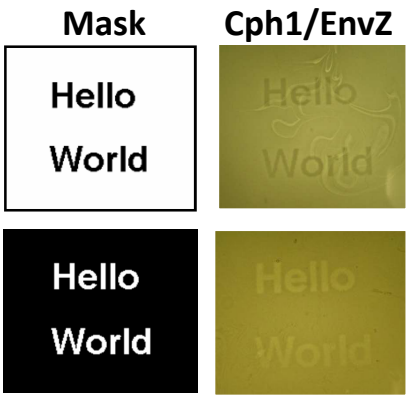



Levskaya *et al. Nature*, 438(7067):441-2 (2005)





"Light cannon" developed by Aaron Chevalier, UT undergraduate




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

## The first bacterial photograph (coliroid?)...

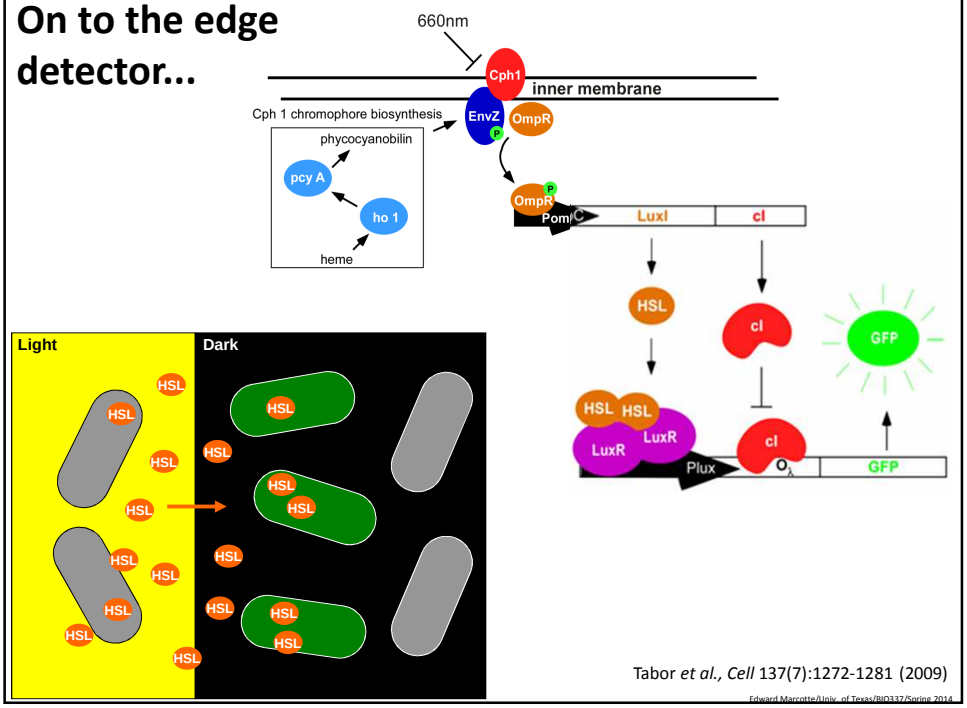
Levskaya *et al. Nature*, 438(7067):441-2 (2005)



*Escherichia darwinia*

Image: Aaron Chevalier

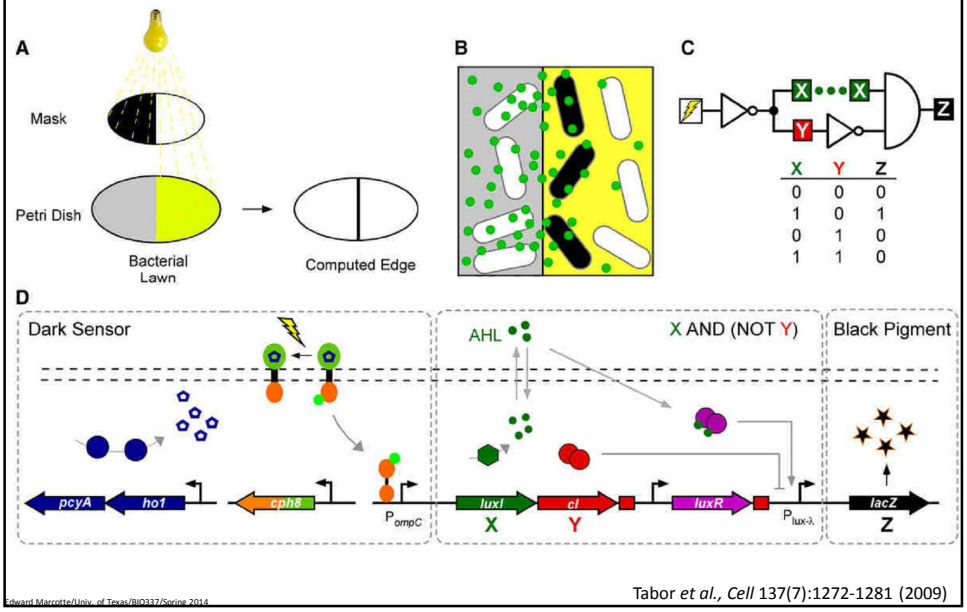
# On to the edge detector...




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



# The edge detector circuit in more detail




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

# It works!

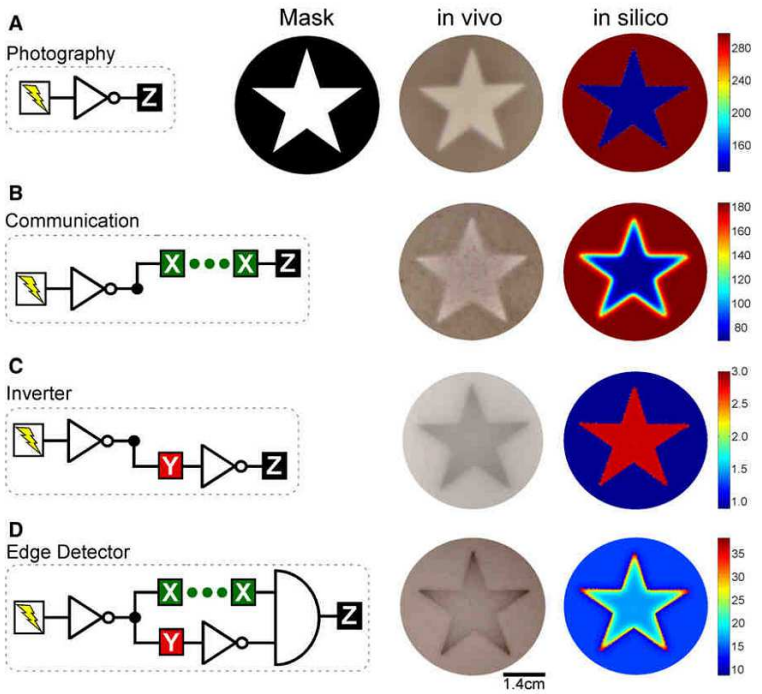
Projected Mask

Photo strain

Edge detector strain

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






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

# UT's 2012 iGEM project – build caffeine biosensor

ACS SyntheticBiology — Letter — pubs.acs.org/synthbio

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

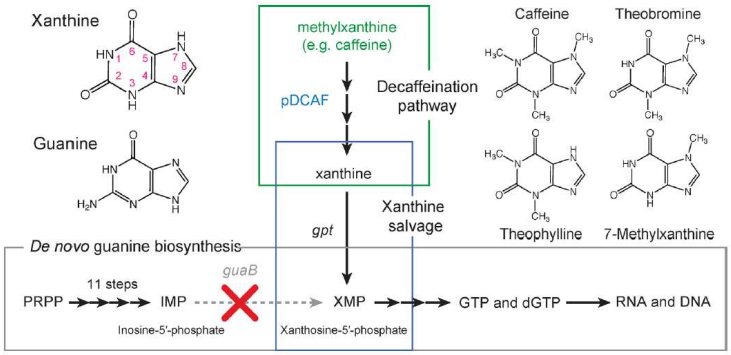
Erik M. Quandt,[†] Michael J. Hammerling,[†] Ryan M. Summers,[‡] Peter B. Otoupal,[†] Ben Slater,[†] Razan N. Alnahhas,[†] Aurko Dasgupta,[†] James L. Bachman,[†] Mani V. Subramanian,[‡] and Jeffrey E. Barrick*[,†]

Basic idea

Block *de novo* guanine synthesis

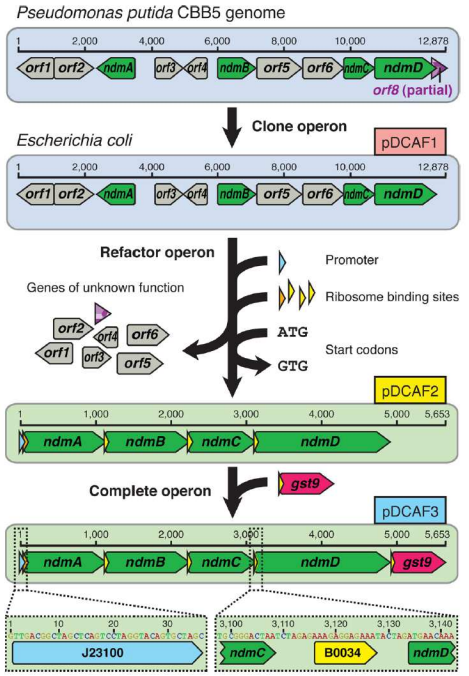
Convert caffeine to xanthine

Addict *E. coli* bacteria to caffeine





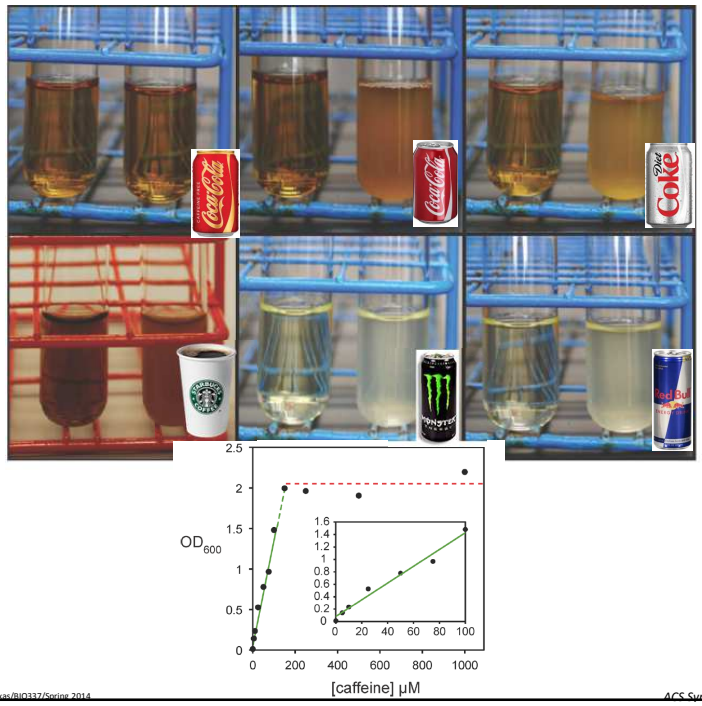

ACS Synth. Biol. 2013, 2, 301–307







ACS Synth. Biol. 2013, 2, 301–307




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

*ACS Synth. Biol.* 2013, 2, 301−307