

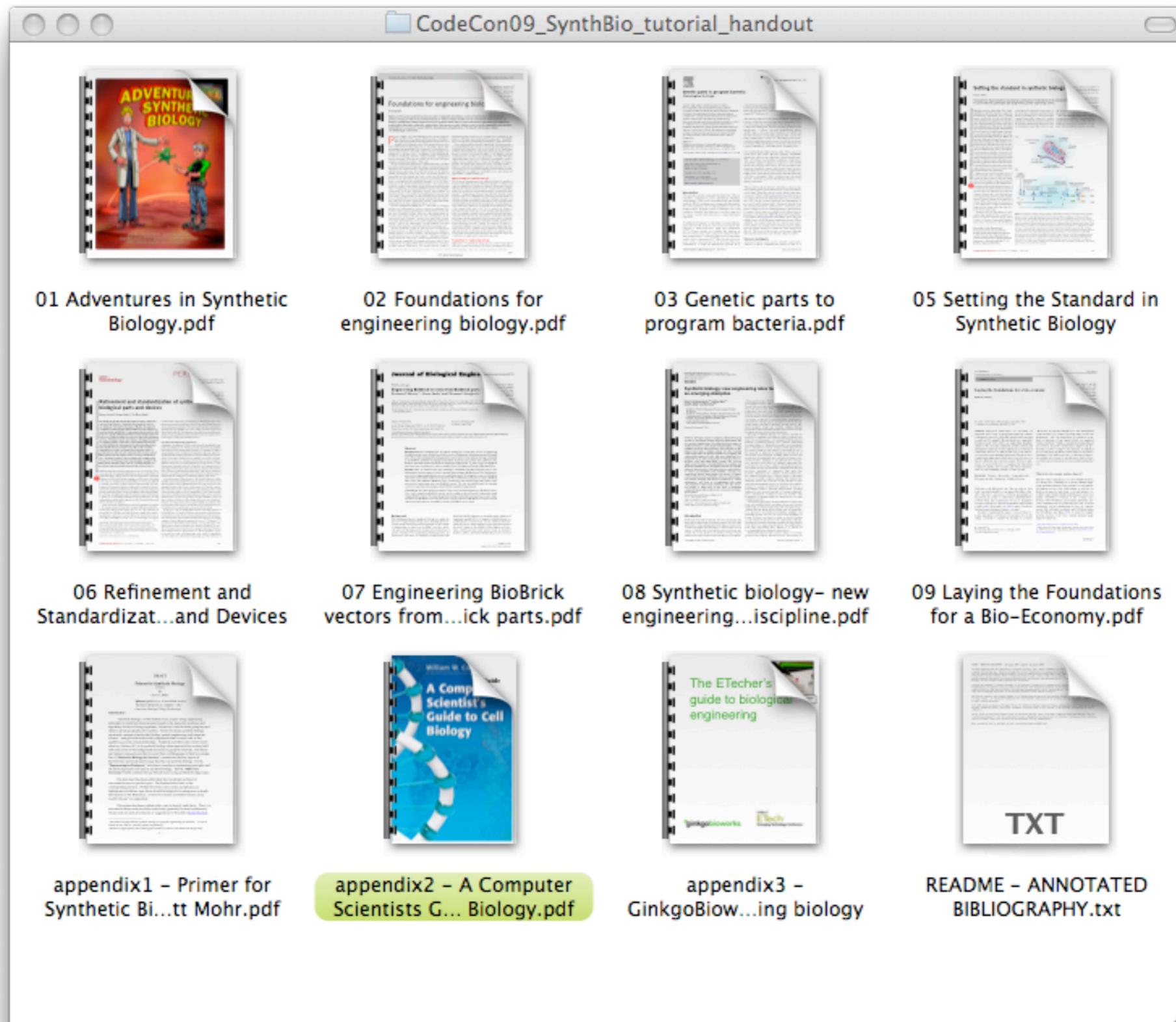
# DIY Synthetic Biology @ Maker Revolution

26 Apr 2009



<http://digg.com/d1nuSY>

# <http://bit.ly/diybioecc>



FREEMAN J. DYSON

THE SUN, THE GENOME,  
AND

AND

THE INTERNET



TOOLS OF SCIENTIFIC REVOLUTION

and pour into overcrowded cities. There are now ten megacities in the world with populations twice as large as New York City. Soon there will be more. Mexico City is one of them. The increase of human population is one of the causes of the migration. The other cause is the poverty and lack of jobs in the villages. Both the population explosion and the poverty must be reversed if we are to have a decent future. Many experts on population say that if we can mitigate the poverty, the population will stabilize itself, as it has done in Europe and Japan.

I am not an expert on population, so I will say no more about that. I am saying that the poverty can be reduced by a combination of solar energy, genetic engineering, and the internet. And perhaps when the poverty stops increasing, the population will stop exploding.

I have seen with my own eyes what happens to a

area that I know  
wealth came to the  
poor people seek

the flow of people  
the world, to stop  
into unmanage-  
can be reversed by  
it is happening in  
possible, the vil-  
of wealth. How

can a godforsaken Mexican village become a source of wealth? Three facts can make it possible. First, solar energy is distributed equitably over the earth. Second, genetic engineering can make solar energy usable everywhere for the local creation of wealth. Third, the internet can provide people in every village with the information and skills they need to develop their talents. The sun, the genome, and the internet can work together to bring wealth to the villages of Mexico, just as the older technology of electricity and automobiles brought wealth to the villages of England. Each of the three new technologies has essential gifts to offer.

# Towards a Biotech Society

BIOSECURITY AND BIOTERRORISM: BIODEFENSE STRATEGY, PRACTICE, AND SCIENCE  
Volume 1, Number 3, 2003  
© Mary Ann Liebert, Inc.

## The Pace and Proliferation of Biological Technologies

ROBERT CARLSON

THE ADVENT OF THE home molecular biology laboratory is not far off. While there is no *Star Trek* "Tricorder" in sight, the physical infrastructure of molecular biology is becoming more sophisticated and less expensive every day. Automated commercial instrumentation handles an increasing fraction of laboratory tasks that were once the sole province of doctoral level researchers, reducing labor costs and increasing productivity. This technology is gradually moving into the broader marketplace as laboratories upgrade to new equipment. Older, still very powerful instruments are finding their way into wide distribution, as any cursory tour of eBay will reveal.<sup>1</sup> These factors are contributing to a proliferation that will soon put highly capable tools in the hands of both professionals and amateurs worldwide. There are obvious short term risks from increased access to DNA synthesis and sequencing technologies, and the general improvement of technologies used in measuring and manipulating molecules will soon enable a broad and distributed enhancement in the ability to alter biological systems. The resulting potential for mischief or mistake causes understandable concern—there are already public calls by scientists and politicians alike to restrict access to certain technologies, to regulate the direction of biological research, and to censor publication of some new techniques and data. It is questionable, however, whether such efforts will increase security or benefit the public good. Proscription of information and artifacts generally leads directly to a black market that is difficult to monitor and therefore difficult to police. A superior alternative is the deliberate creation of an open and expansive research community, which may be better able to respond to crises and better able to keep track of research whether in the university or in the garage.

### FACTORS DRIVING THE BIOTECH REVOLUTION

The development of powerful laboratory tools is enabling ever more sophisticated measurement of biology at the molecular level. Beyond its own experimental utility, every new measurement technique creates a new

mode of interaction with biological systems. Moreover, new measurement techniques can swiftly become means to manipulate biological systems. Estimating the pace of improvement of representative technologies is one way to illustrate the rate at which our ability to interact with and manipulate biological systems is changing.

For example, chemically synthesized DNA fragments, or oligonucleotides, can be used in DNA computation, in the fabrication of gene expression arrays ("gene chips"), and to make larger constructs for genetic manipulation. Mail-order oligonucleotides were with much fanfare recently used to build a functional poliovirus genome from constituent molecules for the first time.<sup>2</sup> The rate at which DNA synthesis capacity is changing is thus a measure of the improvement in our ability to manipulate biological systems and biological information. Similarly, improvements in DNA sequencing capabilities are a measure of our ability to read biological information; in particular the ability to proofread the results of DNA synthesis. Here I refer to such technology, whether instrument or molecule, as "biological technology."

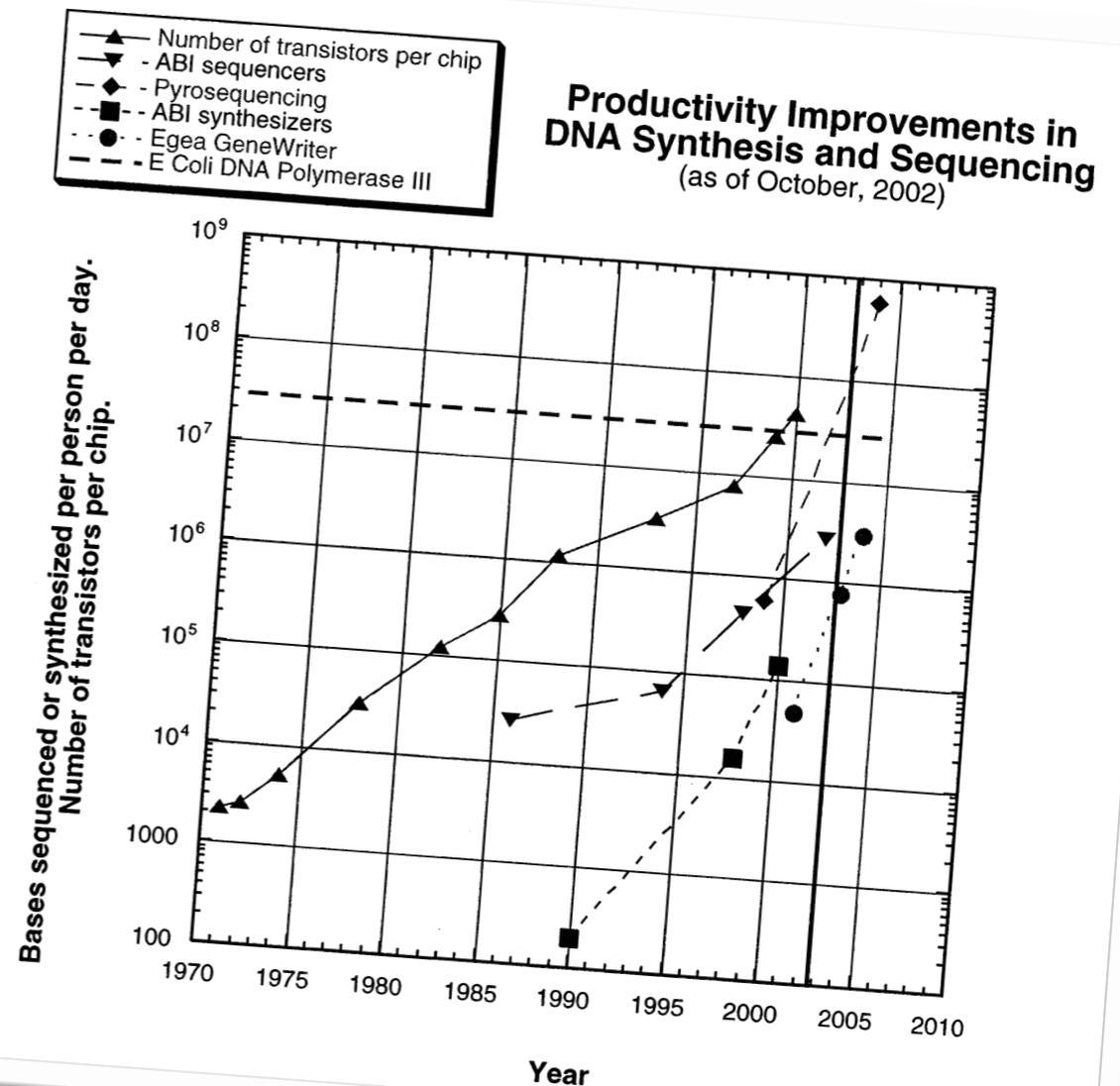
### THE PACE OF TECHNOLOGICAL CHANGE THROUGH THE PRISM OF MOORE'S LAW

Figure 1 contains estimates of potential daily productivity of DNA synthesis and sequencing based on commercially available instruments, including the time necessary to prepare samples. There have been only a few generations of instruments—there is thus a limited amount of data for examination. These estimates are not intended to absolutely quantify a rate of change, but rather to capture the essence of the trends. Several tech-

<sup>1</sup>See <http://listings.ebay.com/pool1/listings/list/all/category/11811/index.html>.

<sup>2</sup>Cello J, Paul AV, Wimmer E. Chemical Synthesis of Poliovirus cDNA: Generation of Infectious Virus in the Absence of Natural Template. *Science* 2002. 297(5583): p. 1016–1018.

Robert Carlson, PhD, is a Research Scientist in the Department of Electrical Engineering at the University of Washington and an Adjunct Research Fellow at the Molecular Sciences Institute in Berkeley, California.

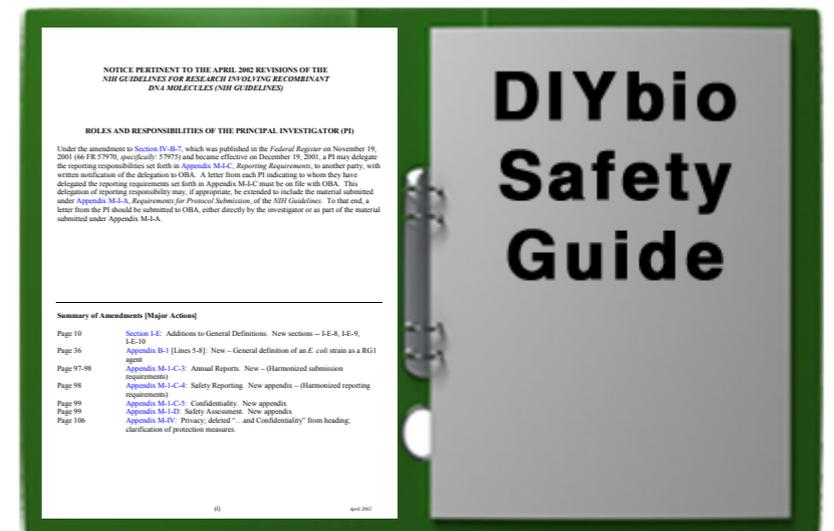
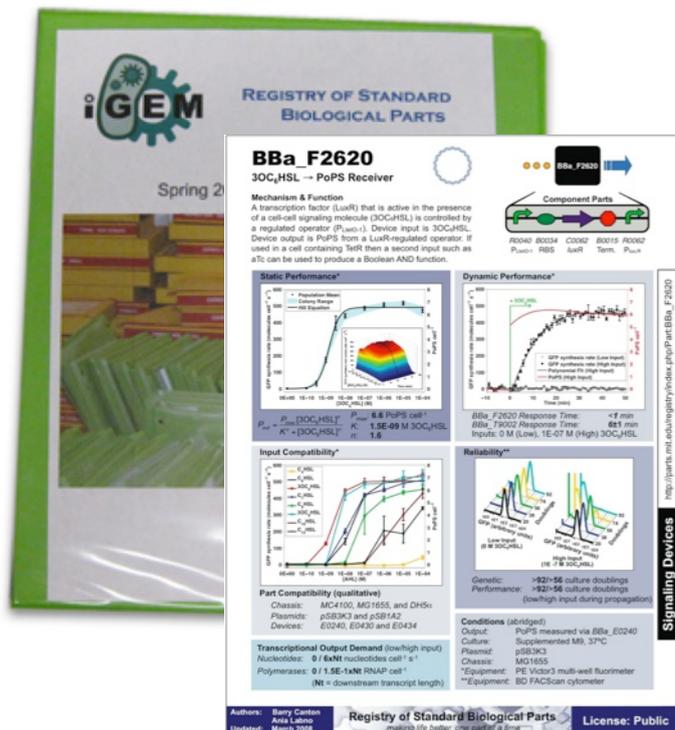


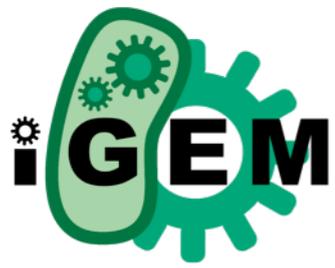
# Long Tail Life Sciences

Standardization &  
abstraction; SB tools;  
Part Collections

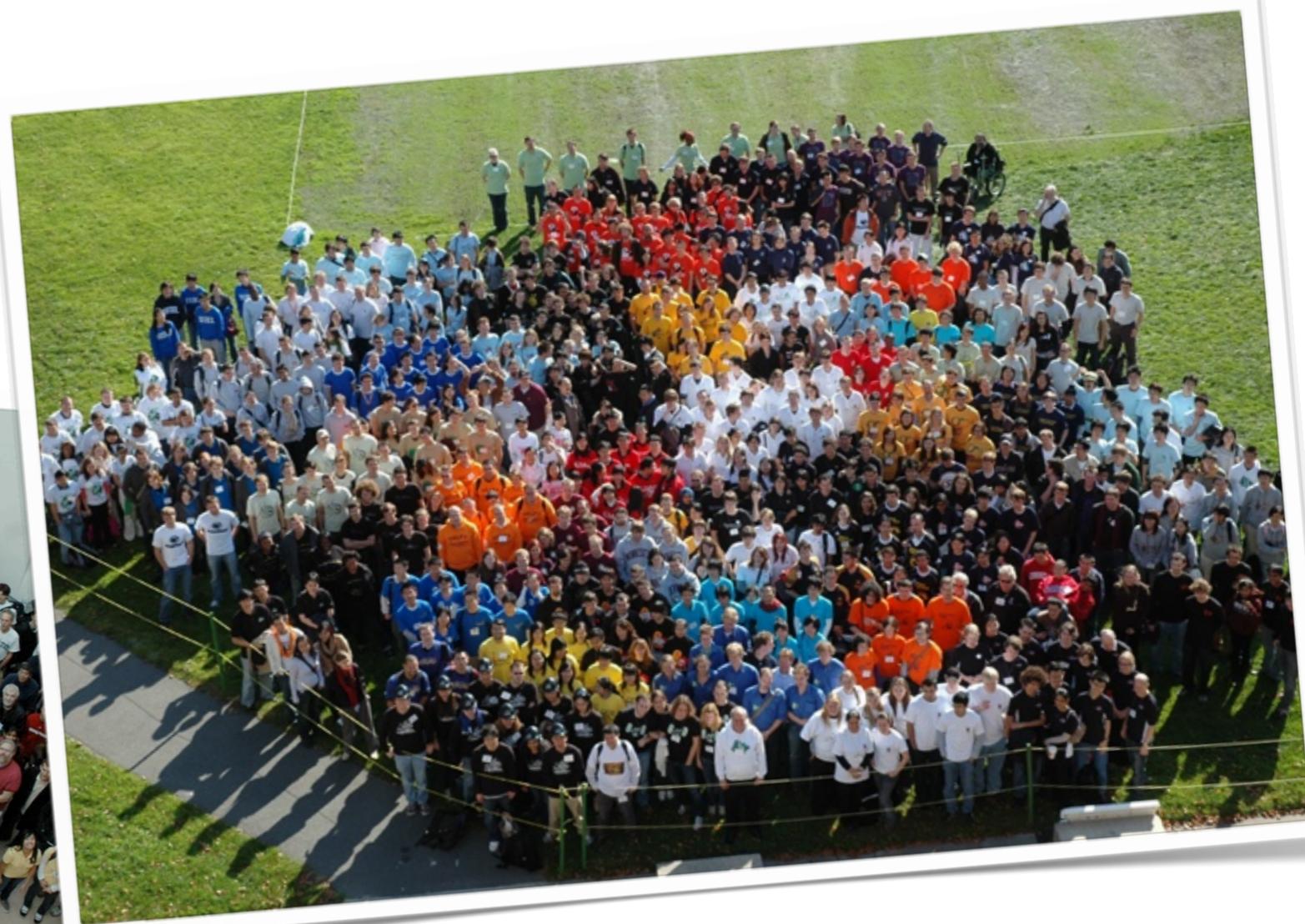
DIY and off-the-shelf  
equipment and services

framework that minimizes  
safety risks and  
repercussions for society  
and garge biohackers





# international Genetically Engineered Machine Competition **2002 - 2008**





## Teams Registered for iGEM 2008

[Return to the iGEM Registration Page](#)

### These 85 teams are registered for iGEM 2008

<a href="#">Alberta_NINT</a>	<a href="#">Bay_Area_RSI</a>	<a href="#">BCCS-Bristol</a>	<a href="#">Beijing_Normal</a>
<a href="#">Bologna</a>	<a href="#">Brown</a>	<a href="#">BrownTwo</a>	<a href="#">Calgary_Ethics</a>
<a href="#">Calgary_Software</a>	<a href="#">Calgary_Wetware</a>	<a href="#">Caltech</a>	<a href="#">Cambridge</a>
<a href="#">Chiba</a>	<a href="#">Colombia</a>	<a href="#">CPU-NanJing</a>	<a href="#">Davidson-Missouri_Western</a>
<a href="#">Duke</a>	<a href="#">Edinburgh</a>	<a href="#">EPF-Lausanne</a>	<a href="#">ESBS-Strasbourg</a>
<a href="#">ETH_Zurich</a>	<a href="#">Freiburg</a>	<a href="#">Groningen</a>	<a href="#">Guelph</a>
<a href="#">Harvard</a>	<a href="#">Hawaii</a>	<a href="#">Heidelberg</a>	<a href="#">HKUSTers</a>
<a href="#">iHKU</a>	<a href="#">IIT_Madras</a>	<a href="#">Illinois</a>	<a href="#">Imperial_College</a>
<a href="#">Istanbul</a>	<a href="#">Johns_Hopkins</a>	<a href="#">KULeuven</a>	<a href="#">Kyoto</a>
<a href="#">LCG-UNAM-Mexico</a>	<a href="#">Lethbridge_CCS</a>	<a href="#">Melbourne</a>	<a href="#">METU_Turkey</a>
<a href="#">Mexico-UNAM-IPN</a>	<a href="#">Michigan</a>	<a href="#">Minnesota</a>	<a href="#">Mississippi_State</a>
<a href="#">Missouri_Miners</a>	<a href="#">MIT</a>	<a href="#">Montreal</a>	<a href="#">Newcastle_University</a>
<a href="#">NTU-Singapore</a>	<a href="#">NYMU-Taipei</a>	<a href="#">Paris</a>	<a href="#">Peking_University</a>
<a href="#">PennState</a>	<a href="#">Peru</a>	<a href="#">Prairie_View</a>	<a href="#">Princeton</a>
<a href="#">Purdue</a>	<a href="#">Rensselaer</a>	<a href="#">Rice_University</a>	<a href="#">Slovenia</a>
<a href="#">Tianjin</a>	<a href="#">Tokyo_Tech</a>	<a href="#">Toronto_Bluegenes</a>	<a href="#">Tsinghua</a>
<a href="#">TUDelft</a>	<a href="#">TU_Munchen</a>	<a href="#">UCSF</a>	<a href="#">UC_Berkeley</a>
<a href="#">UC_Berkeley_Tools</a>	<a href="#">UNIPV-Pavia</a>	<a href="#">University_of_Alberta</a>	<a href="#">University_of_Chicago</a>
<a href="#">University_of_Lethbridge</a>	<a href="#">University_of_Ottawa</a>	<a href="#">University_of_Sheffield</a>	<a href="#">University_of_Washington</a>
<a href="#">USTC</a>	<a href="#">Utah_State</a>	<a href="#">Valencia</a>	<a href="#">Virginia</a>
<a href="#">Warsaw</a>	<a href="#">Waterloo</a>	<a href="#">WEGO_Taipei</a>	<a href="#">Wisconsin</a>
<a href="#">Example</a>			

Applications for these 4 teams are pending approval by iGEM Headquarters

## Teams Registered for iGEM 2009

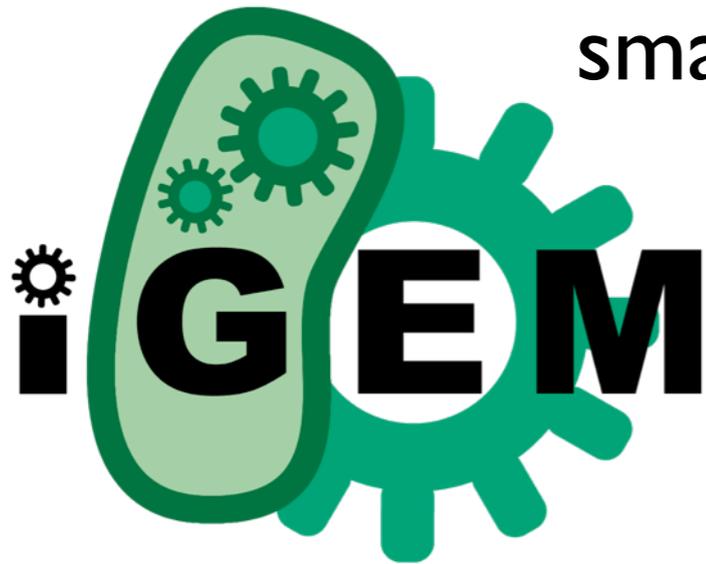
[Return to the iGEM Registration Page](#)

These 107 teams are registered for iGEM 2009

<a href="#">Aberdeen_Scotland</a>	<a href="#">Alberta</a>	<a href="#">Amsterdam</a>	<a href="#">ArtScienceBangalore</a>
<a href="#">Bay_Area_RSI</a>	<a href="#">BCCS-Bristol</a>	<a href="#">Berkeley_Software</a>	<a href="#">Berkeley_Wetlab</a>
<a href="#">BIOTEC_Dresden</a>	<a href="#">Bologna</a>	<a href="#">British_Columbia</a>	<a href="#">Brown</a>
<a href="#">BrownTwo</a>	<a href="#">Calgary</a>	<a href="#">Cambridge</a>	<a href="#">CBNU-Korea</a>
<a href="#">Chiba</a>	<a href="#">CityColSanFrancisco</a>	<a href="#">Cornell</a>	<a href="#">DTU_Denmark</a>
<a href="#">Duke</a>	<a href="#">Edinburgh</a>	<a href="#">EPF-Lausanne</a>	<a href="#">ESBS-Strasbourg</a>
<a href="#">Freiburg_bioware</a>	<a href="#">Freiburg_software</a>	<a href="#">Gaston_Day_School</a>	<a href="#">Groningen</a>
<a href="#">Harvard</a>	<a href="#">Heidelberg</a>	<a href="#">HKU-HKBU</a>	<a href="#">HKUST</a>
<a href="#">IBB_Pune</a>	<a href="#">IGIB-Delhi</a>	<a href="#">IIT_Bombay_India</a>	<a href="#">IIT_Madras</a>
<a href="#">Illinois</a>	<a href="#">Illinois-Tools</a>	<a href="#">Imperial College London</a>	<a href="#">Indiana</a>
<a href="#">IPN-UNAM-Mexico</a>	<a href="#">Johns_Hopkins</a>	<a href="#">Johns_Hopkins-BAG</a>	<a href="#">KULeuven</a>
<a href="#">KU_Seoul</a>	<a href="#">Kyoto</a>	<a href="#">LCG-UNAM-Mexico</a>	<a href="#">Lethbridge</a>
<a href="#">METU-Gene</a>	<a href="#">Michigan</a>	<a href="#">Minnesota</a>	<a href="#">Missouri_Miners</a>
<a href="#">MIT</a>	<a href="#">MoWestern_Davidson</a>	<a href="#">NCTU_Formosa</a>	<a href="#">Nevada</a>
<a href="#">Newcastle</a>	<a href="#">NTU-Singapore</a>	<a href="#">NYMU-Taipei</a>	<a href="#">NYU</a>
<a href="#">Osaka</a>	<a href="#">Paris</a>	<a href="#">PKU_Beijing</a>	<a href="#">Purdue</a>
<a href="#">Queens</a>	<a href="#">Rice</a>	<a href="#">SDU-Denmark</a>	<a href="#">Sheffield</a>
<a href="#">SJTU-BioX-Shanghai</a>	<a href="#">Slovenia</a>	<a href="#">Southampton</a>	<a href="#">Stanford</a>
<a href="#">Sweden</a>	<a href="#">Todai-Tokyo</a>	<a href="#">Tokyo-Nokogen</a>	<a href="#">Tokyo_Tech</a>
<a href="#">TorontoMaRSDiscovery</a>	<a href="#">Tsinghua</a>	<a href="#">TUDelft</a>	<a href="#">TzuChiU_Formosa</a>
<a href="#">UAB-Barcelona</a>	<a href="#">UChicago</a>	<a href="#">UCL_London</a>	<a href="#">UCSF</a>
<a href="#">UC_Davis</a>	<a href="#">ULB-Brussels</a>	<a href="#">UNC_Chapel_Hill</a>	<a href="#">UNICAMP-Brazil</a>
<a href="#">UNIPV-Pavia</a>	<a href="#">uOttawa</a>	<a href="#">Uppsala-Sweden</a>	<a href="#">UQ-Australia</a>
<a href="#">USTC</a>	<a href="#">USTC_Software</a>	<a href="#">Utah_State</a>	<a href="#">Valencia</a>
<a href="#">VictoriaBC</a>	<a href="#">Victoria_Australia</a>	<a href="#">Virginia</a>	<a href="#">Virginia_Commonwealth</a>
<a href="#">Warsaw</a>	<a href="#">Washington</a>	<a href="#">Washington-Software</a>	<a href="#">Wash_U</a>
<a href="#">Waterloo</a>	<a href="#">Wisconsin-Madison</a>	<a href="#">Yeshiva_NYC</a>	

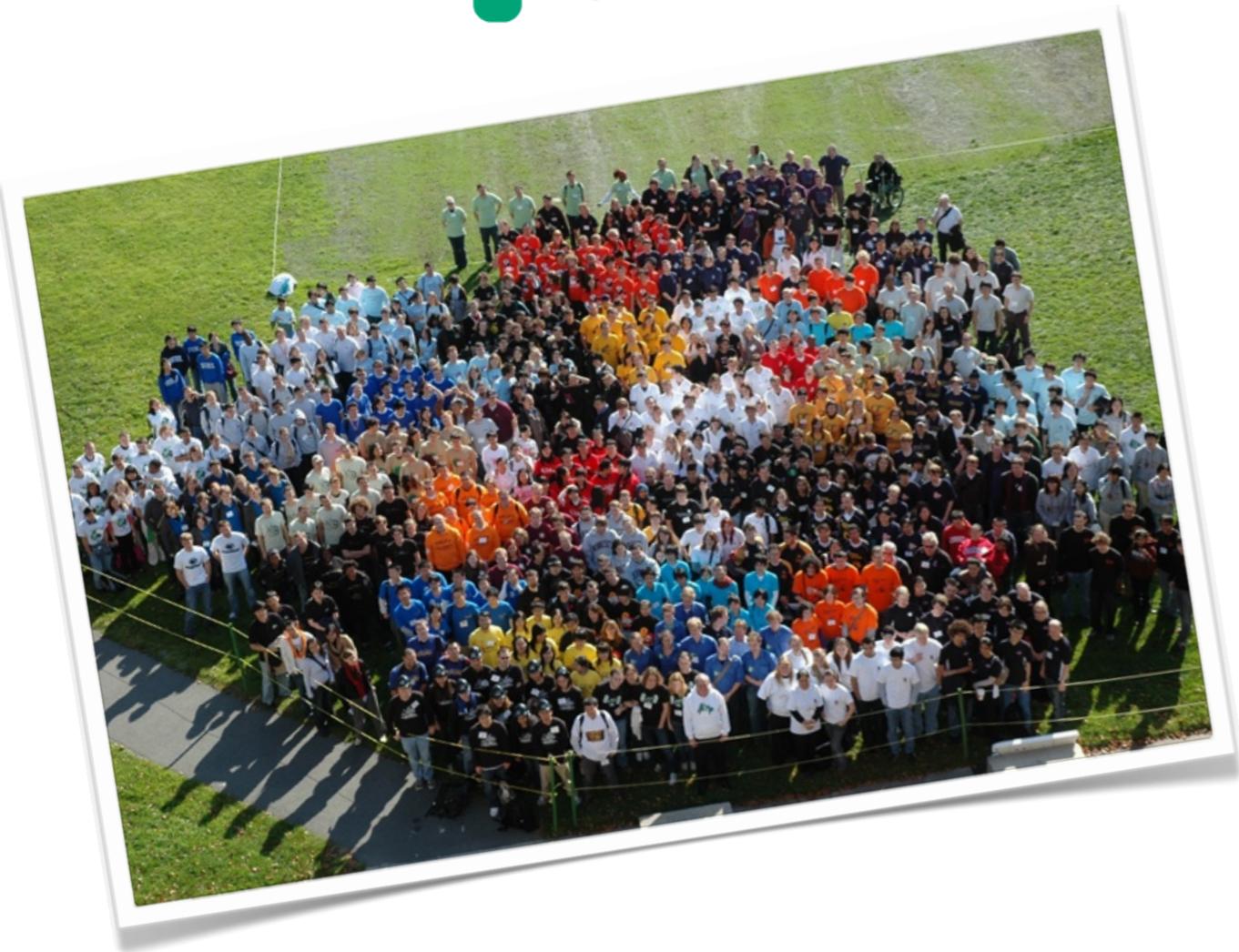
# iGEM

an existence proof for  
small team-based biotech innovation



- Resveratrol Beer
- Bacterial Photography
- odorant synthesis (banana!)
- arsenic & lead biosensors
- H. pylori vaccine

Opportunity for DIYbio teams  
to compete in 2010





HMM... ARE YOU SURE YOU UNDERSTAND ENOUGH ABOUT WHAT YOU WANT TO DO?

YOU DON'T WANT TO MAKE THINGS WORSE.

WE'LL ONLY FIND OUT BY TRYING!

LET'S GRAB ONE!

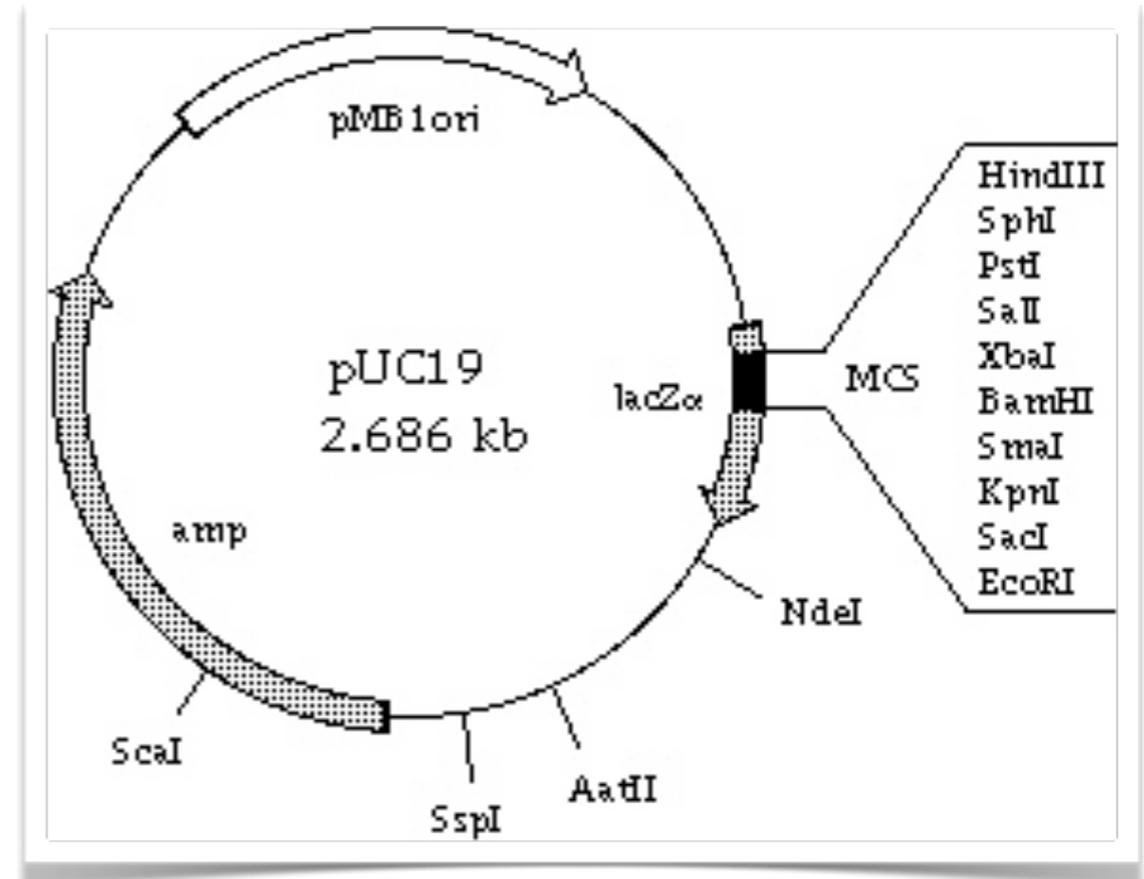
HI THERE, BUDDY.

# Case Study: pUC19

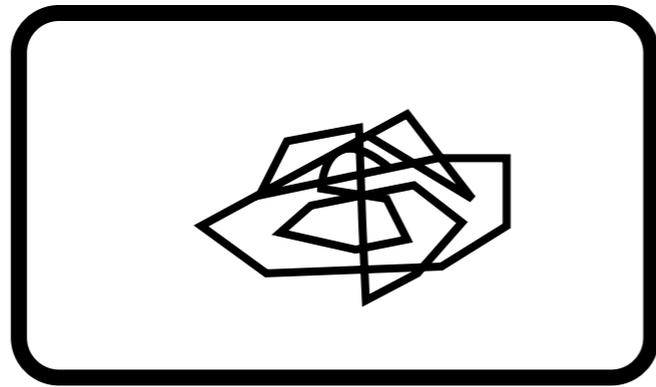
One of the most popular genetic engineering tools

It's a cloning vector - built to grab and carry DNA

Where did it come from?

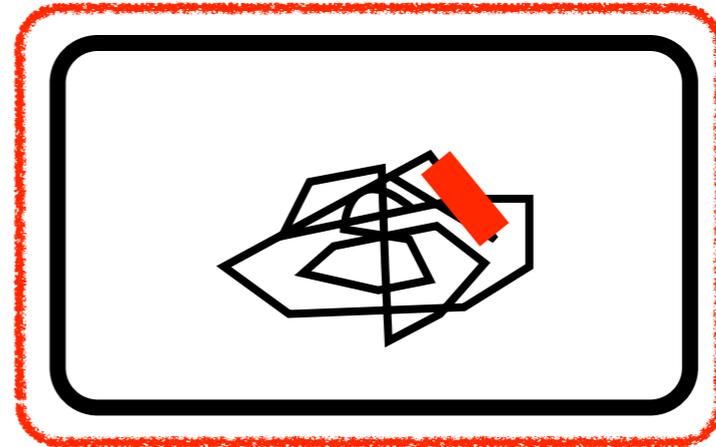


# Finding resistance genes



sensitive

vs.



resistant



live sensitive cells + resistant DNA = rare survivors

# Mini-genomes as tools

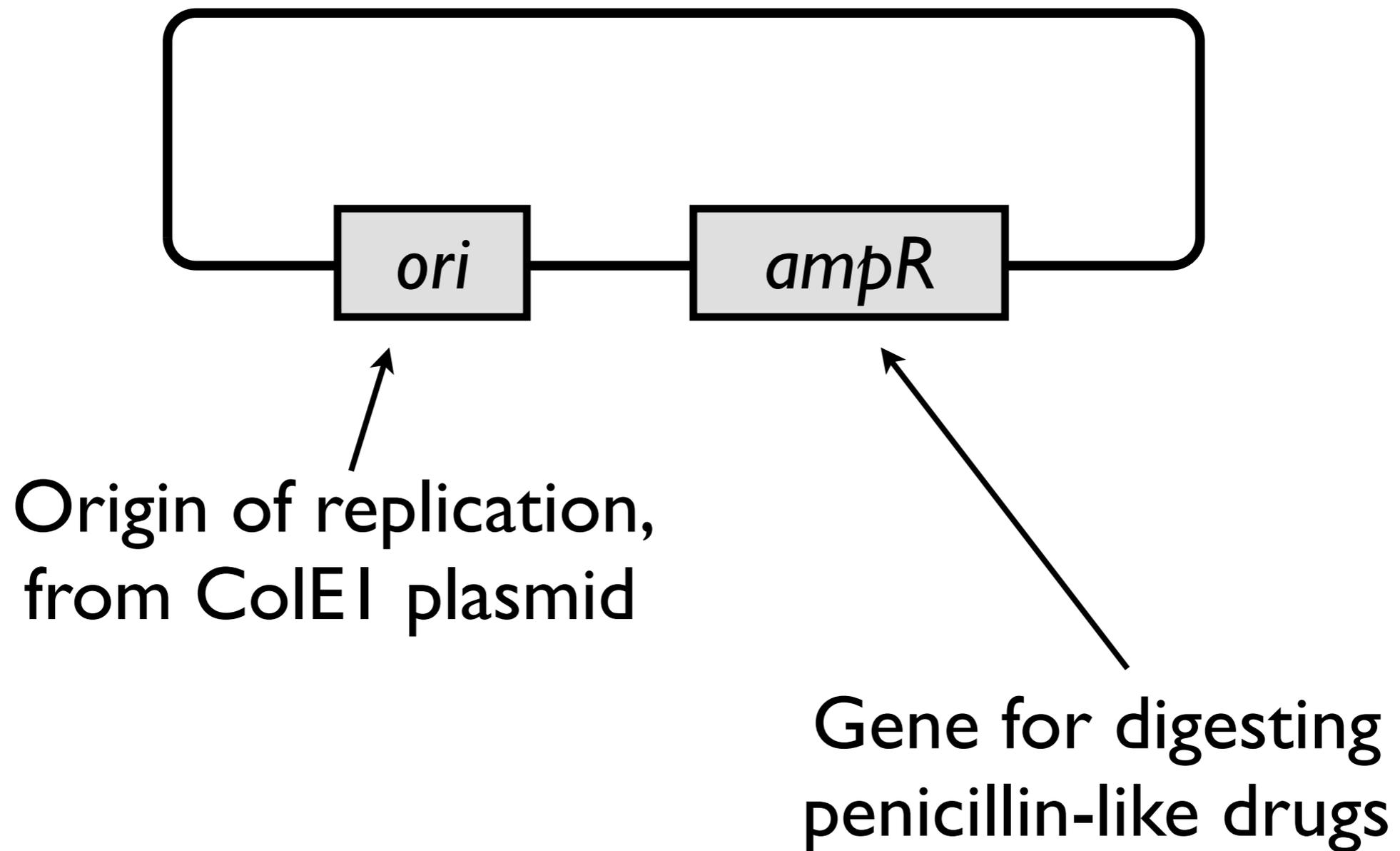
How do I recover the DNA?

Put it on a plasmid - a small, self-replicating genetic device.

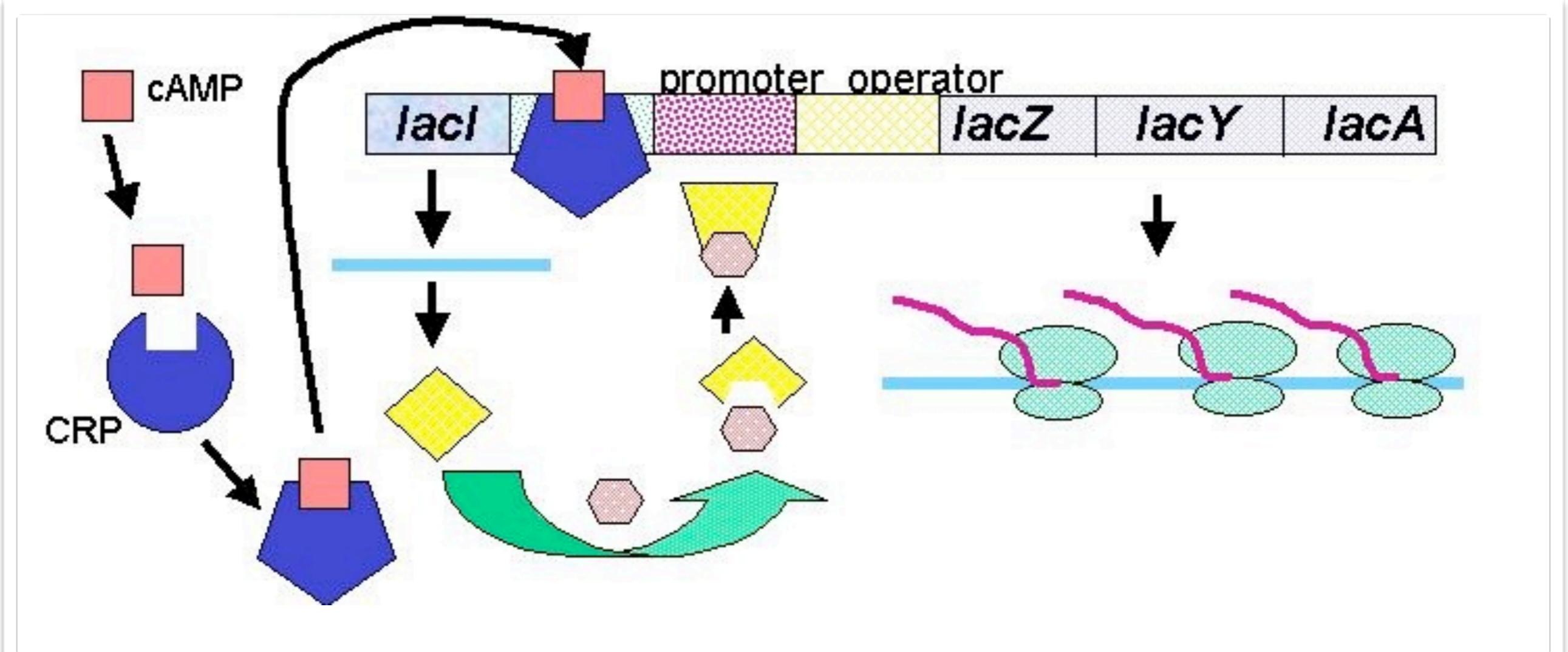
Modularity is your friend.



# Synthetic resistance vector



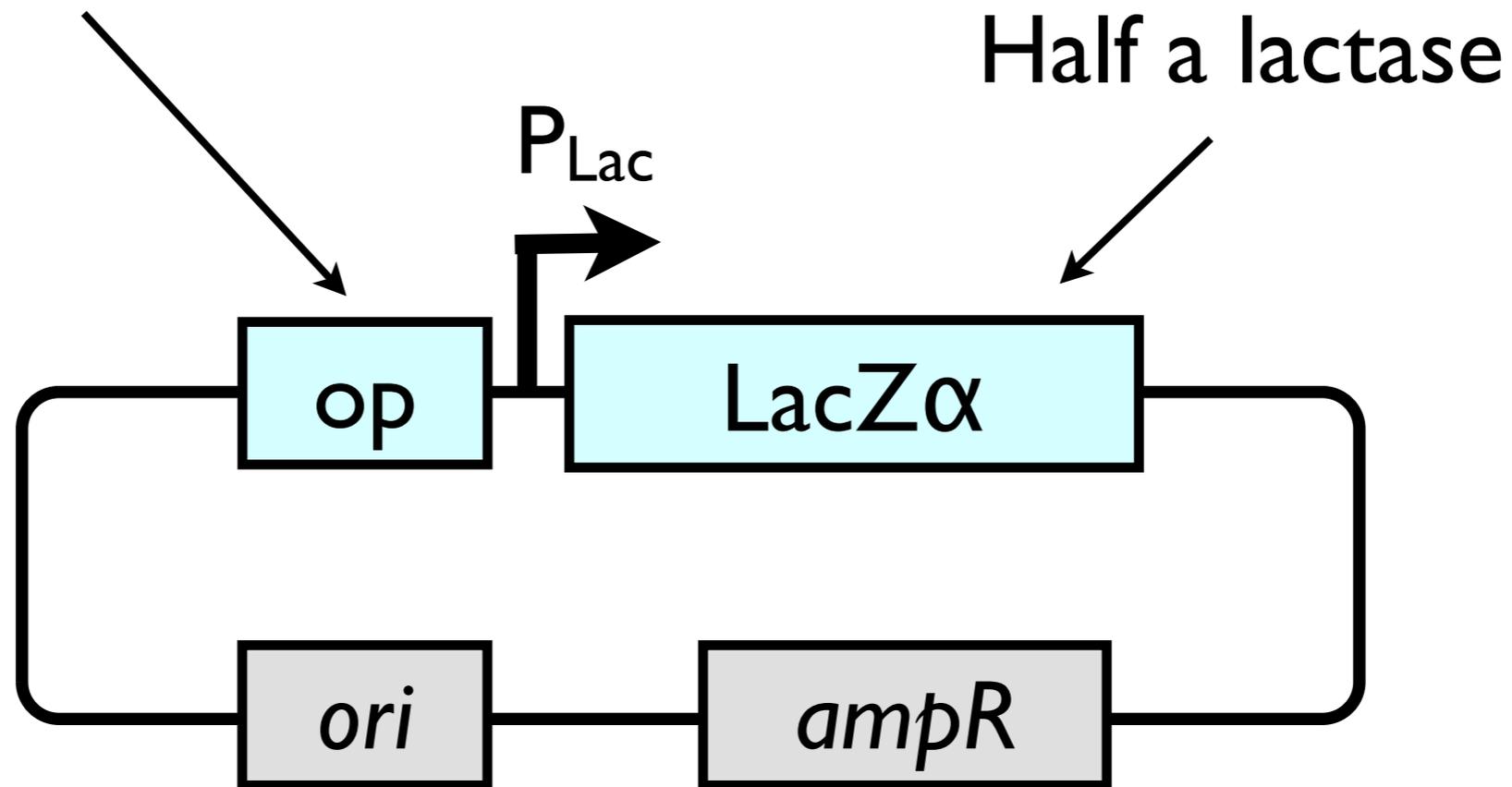
# Lactose operon



If lactose and no glucose...then eat lactose

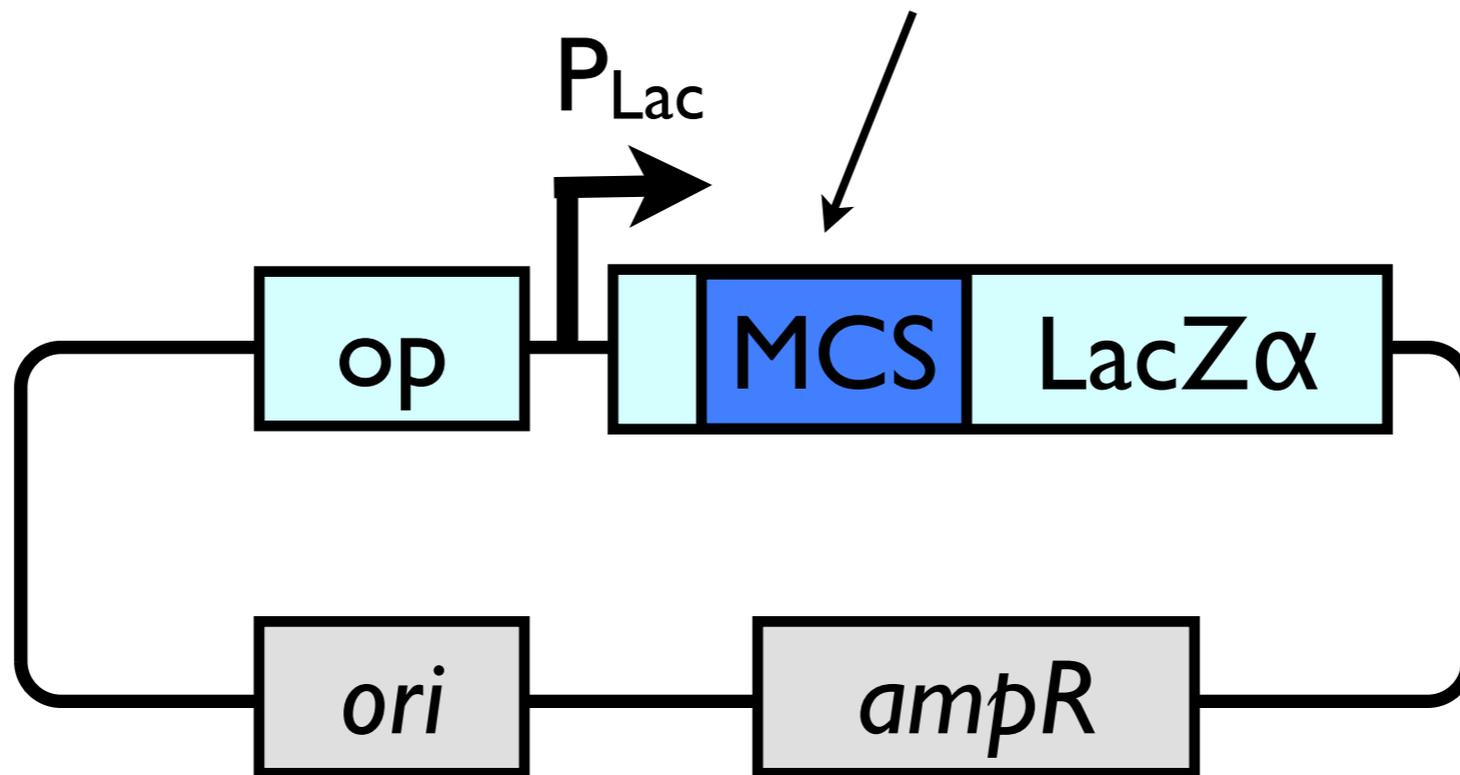
# Test kit for simplified *lac*

LacI binding site



# Hack it for cloning (add restriction sites)

*EcoRI* *Ecl136II* *Acc65I* *Bsp68I* *Mph1103I* *XbaI* *EcoRV* *BamHI* *Eco88I* *ApaI* *Sall* *PstI* *Eco147I* *PaeI* *HindIII* 476  
*XapI* *SacI* *KpnI* *MvaI* *XbaI* *EcoRV* *BamHI* *SmaI* *Bsp120I* *XmiI* *PstI* *Eco147I* *PaeI* *HindIII* |  
ATT CGA GCT CGG TAC CTC GCG AAT GCA TCT AGA TAT CGG ATC CCG GGC CCG TCG ACT GCA GAG GCC TGC ATG CAA GCT TGG  
TAA GCT CGA GCC ATG GAG CGC TTA CGT AGA TCT ATA GCC TAG GGC CCG GGC AGC TGA CGT CTC CGG ACG TAC GTT CGA ACC  
Asn Ser Ser Pro Val Glu Arg Ile Cys Arg Ser Ile Pro Asp Arg Ala Arg Arg Ser Cys Leu Gly Ala His Leu Ser Pro



# How can I reuse this?

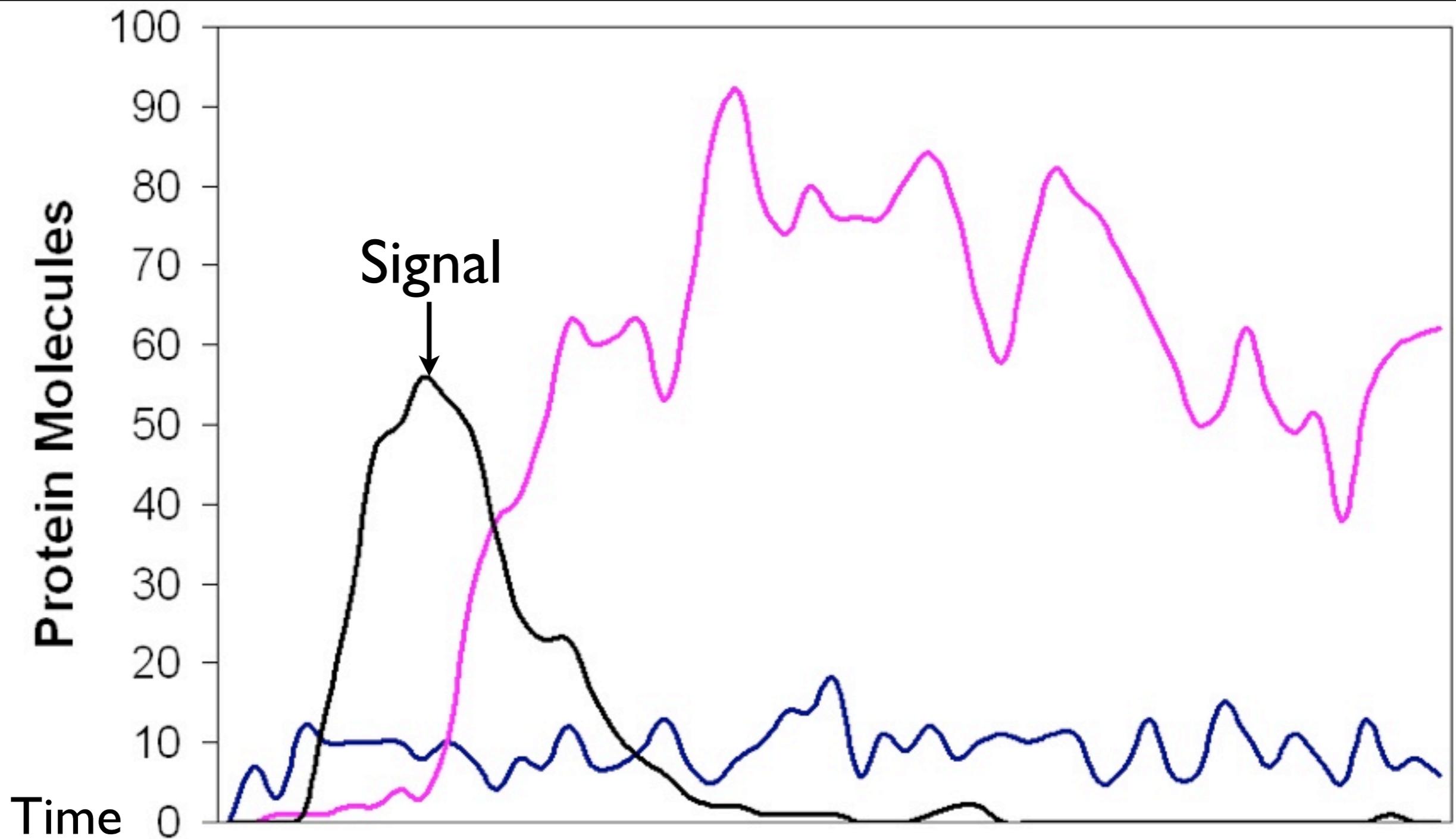
We need abstractions.

And documentation.

Otherwise, we're stuck doing all that work again.



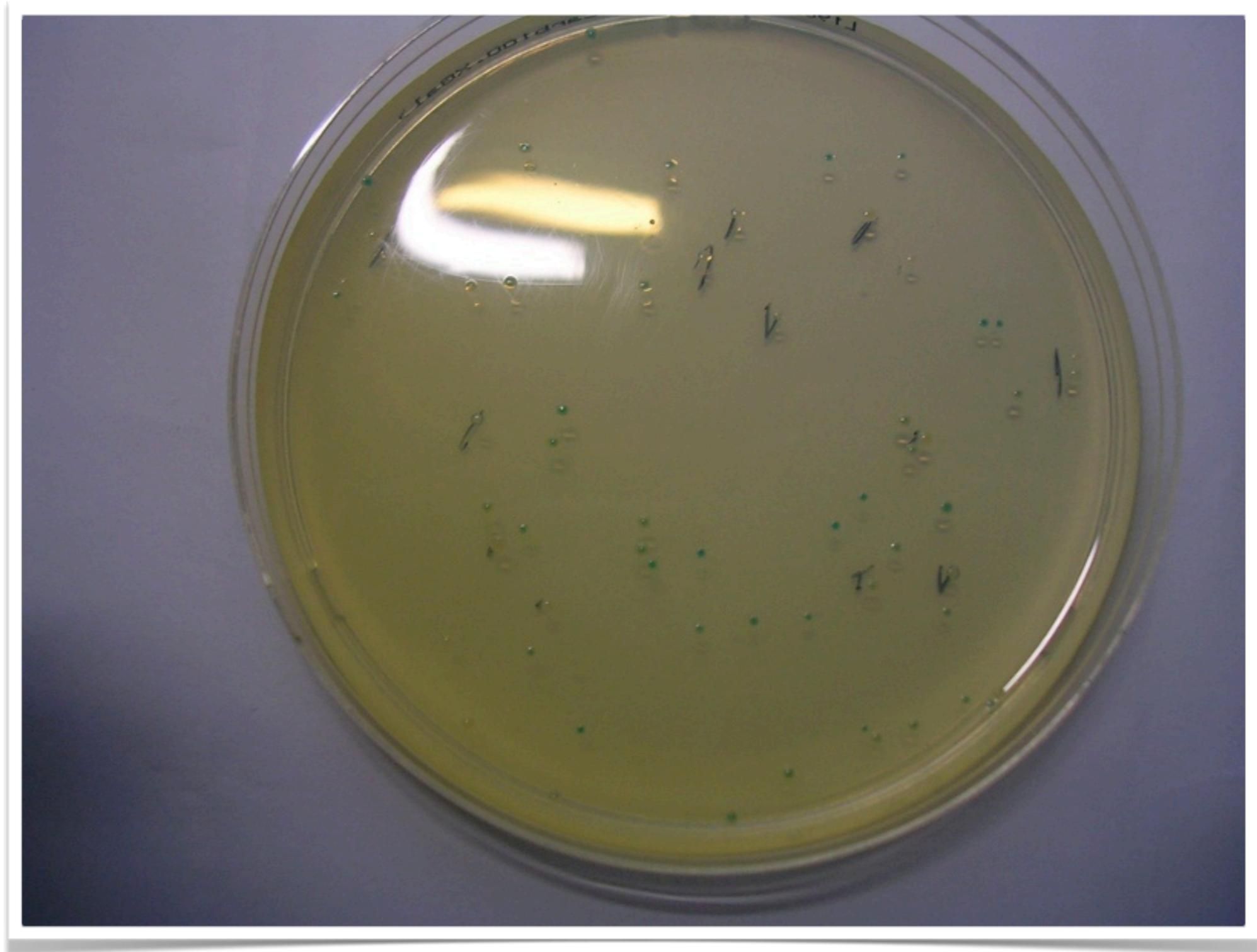




**Figure 3. Bio-SPIKE simulation of “carry” detection.**

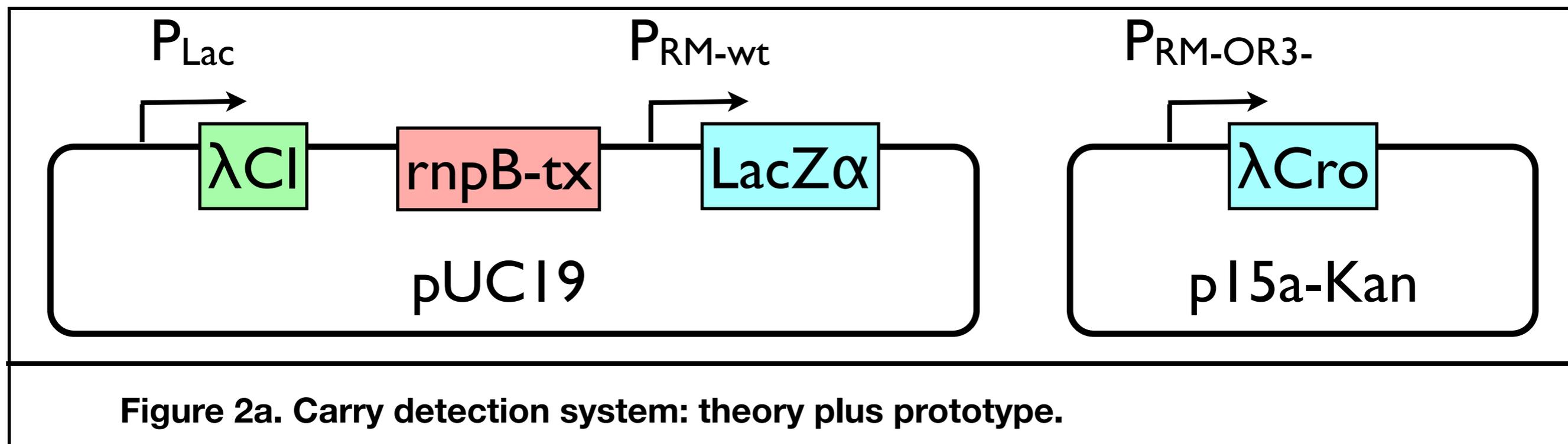
Blue line: CI activator. Purple line: Cro repressor. Black line: carry signal. Simulation was performed on a representation of the carry detector, using 15 mass action equations.

# Actually not vaporware

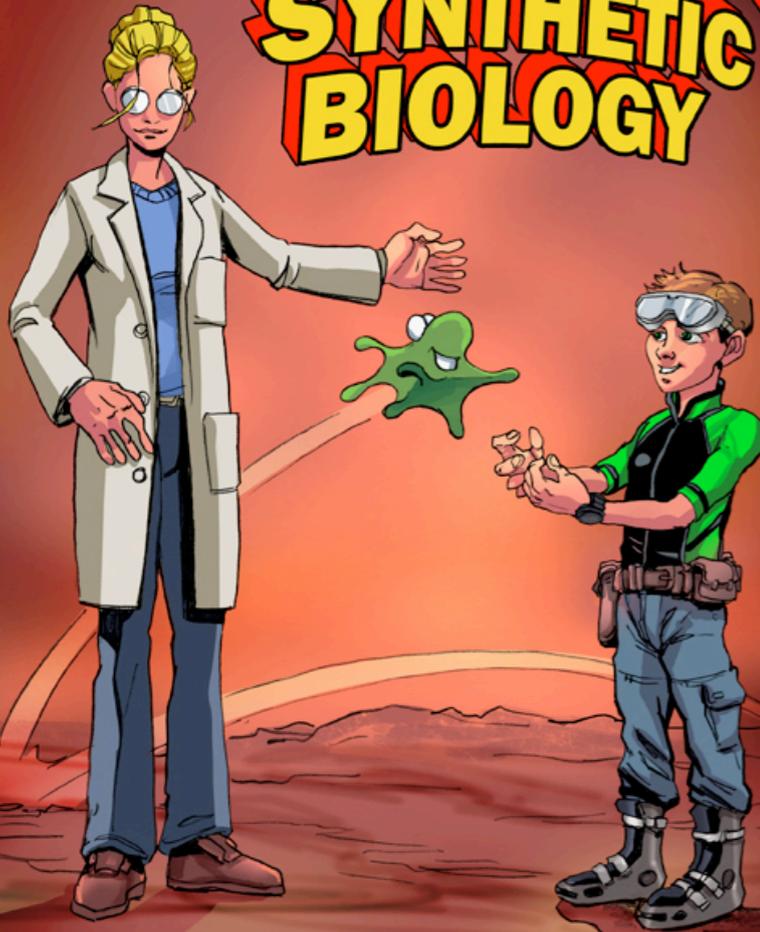


# Benchmarking: what *is* possible?

- One geek, one closet, one month's rent  
(and roughly two months' time)



# ADVENTURES IN SYNTHETIC BIOLOGY



STORY: DREW ENDY ISADORA DEESE  
THE MIT SYNTHETIC BIOLOGY WORKING GROUP  
ART: CHUCK WADEY [WWW.CHUCKWADEY.COM](http://WWW.CHUCKWADEY.COM)



WANNA BET?

ALL I NEED TO DO IS MAKE THEM FORM A CLOSED FILM, -



FIRST YOU NEED TO ASSEMBLE THE DNA PARTS THAT ENCODE YOUR PROGRAM.



DNA'S READY TO GO.

HELP ME CATCH HIM!



MEEP!



HE'S CHANGING!



P.O.P P.O.P



GET THEM FROM THE CATALOG.

ONE BALLOON-O-GENESIS-



-AND ONE GAS-O-MATIC MODULE.

COOL, HERE THEY ARE!

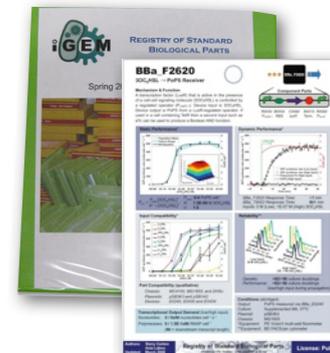
NOW WHAT?



LOAD THE DNA INTO OUR LITTLE FRIEND HERE.

WHAT DO YOU THINK, BUDDY?





# SB 101

- Abstraction
- Standards
- Synthesis

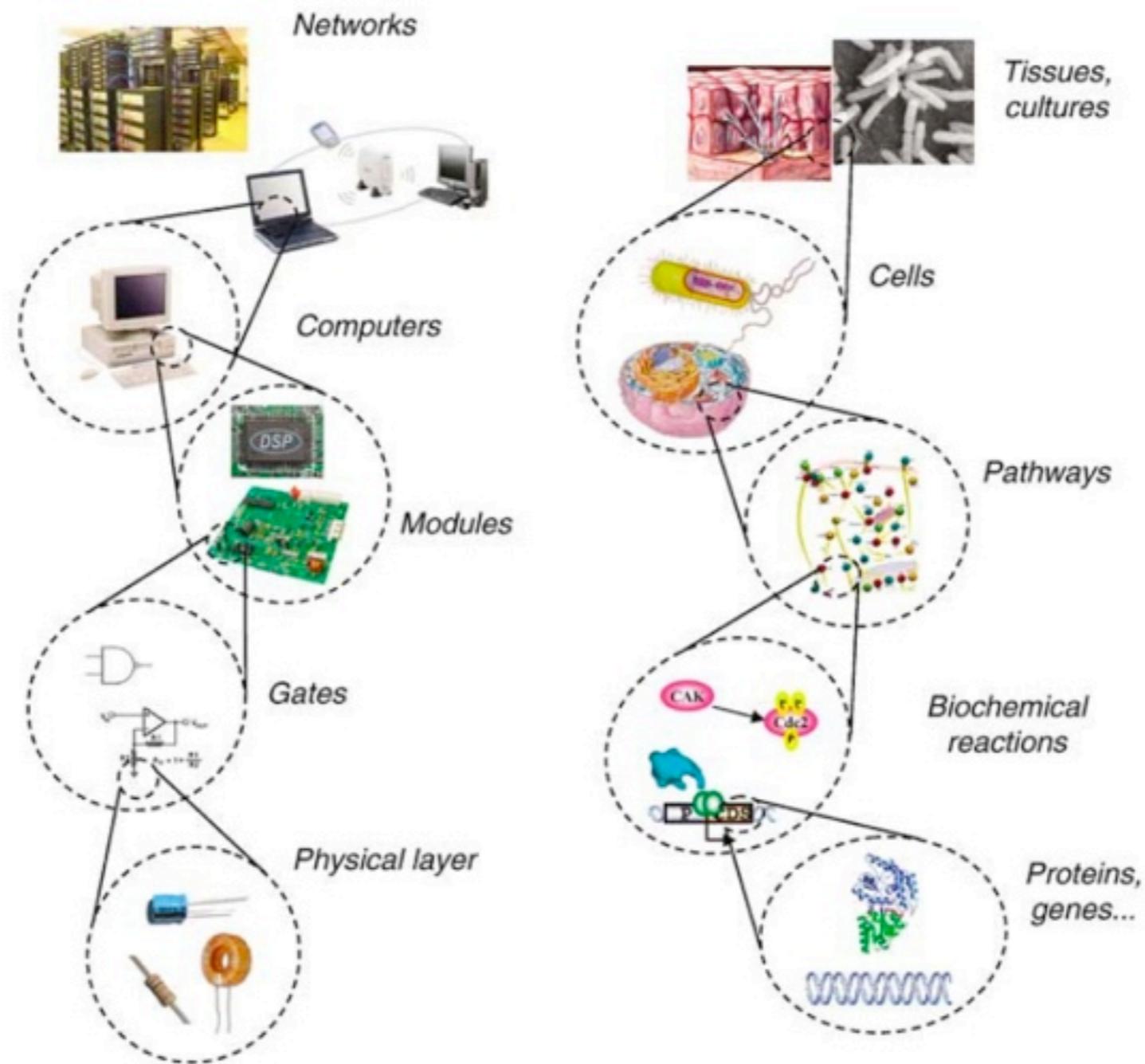
R0080

gcgtaacaaa  
cacggcagaaa  
attattg  
cacacttgctatg  
tttatccataagatt  
tctacctgacgctt  
aactctctactgttct

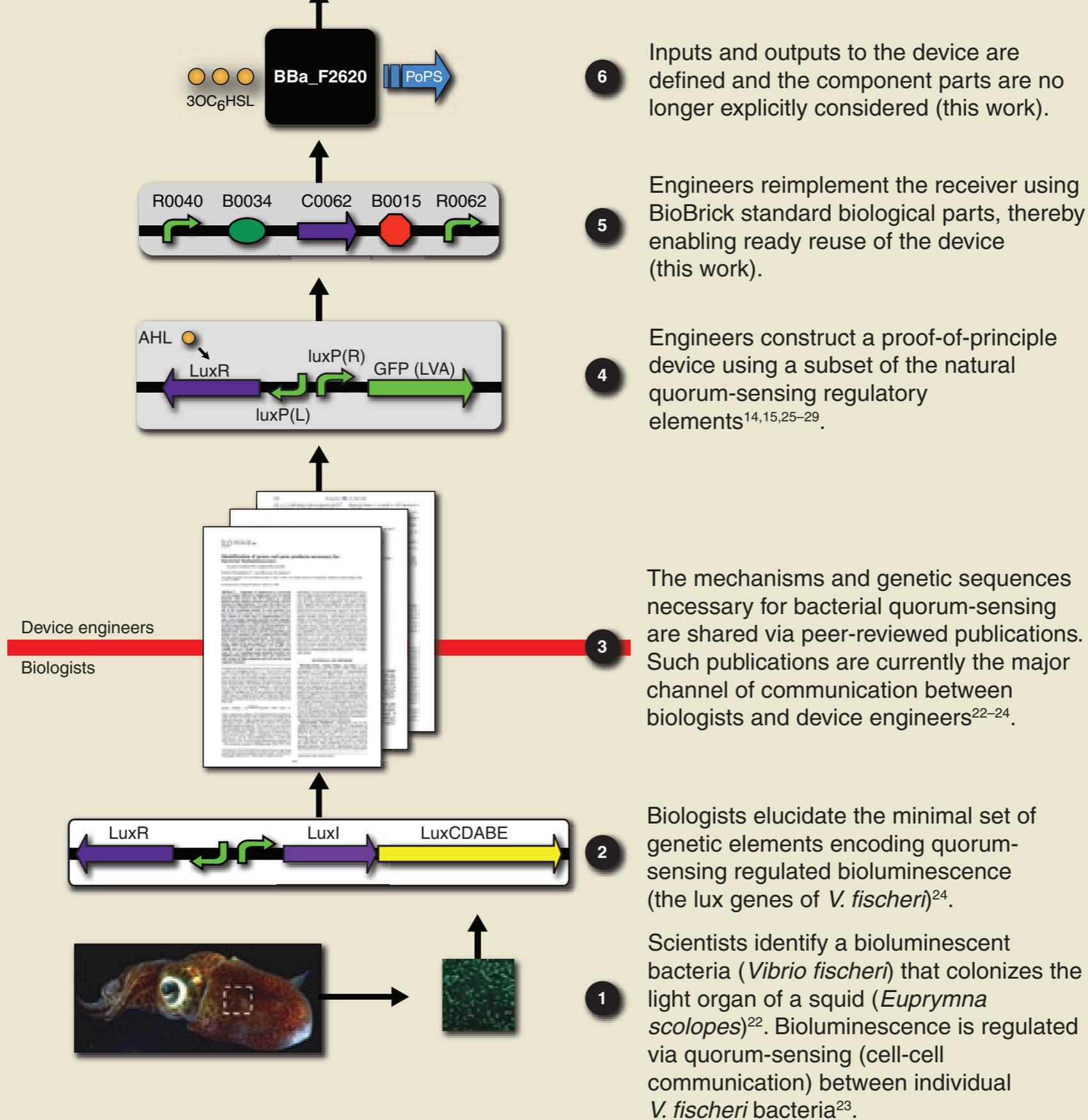


“A good device standard defines sufficient information about discrete parts to allow the design of predictable complex composite systems. It also provides guidelines for the minimal characterization and manufacturing tolerances of new elements.”

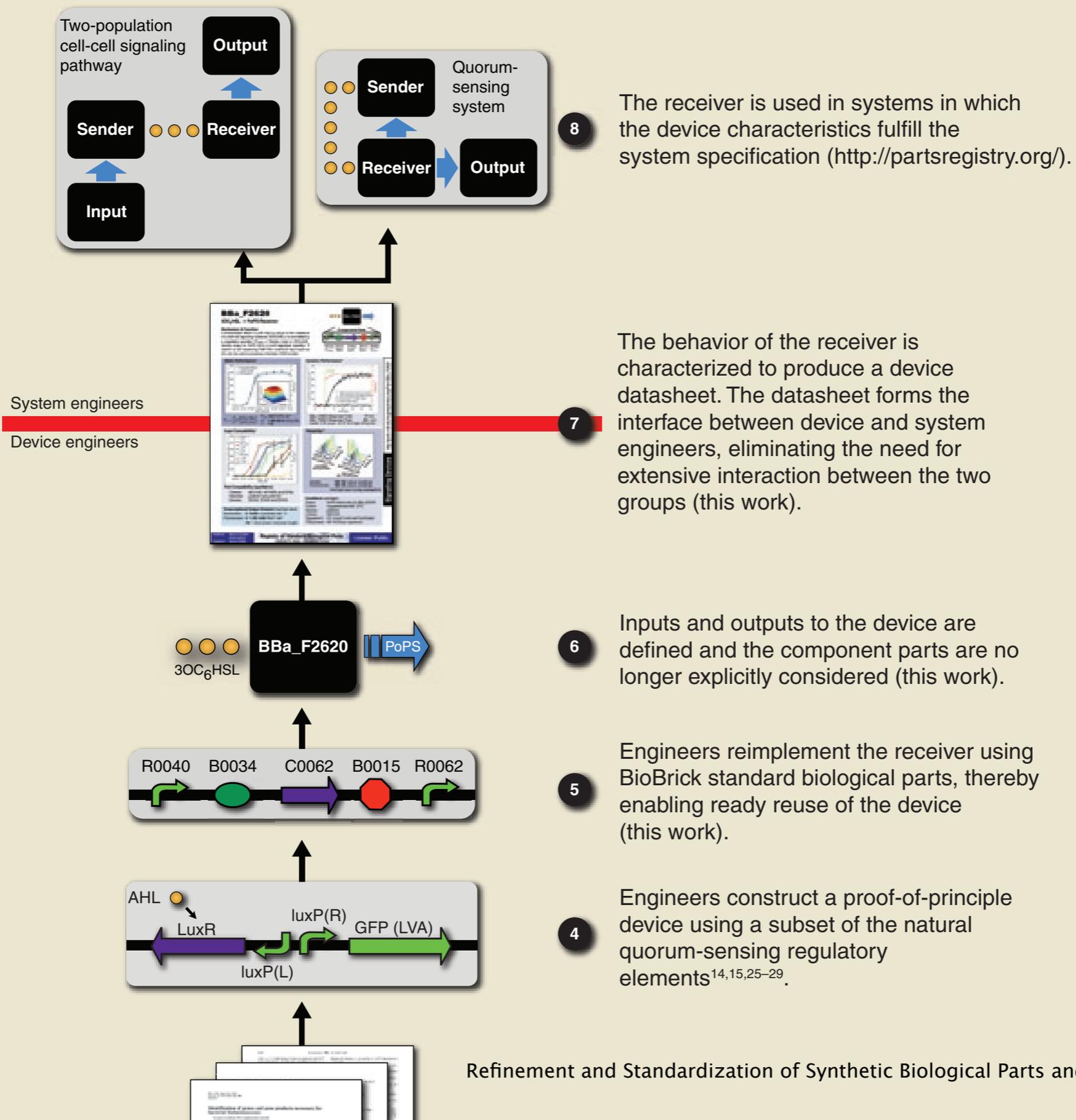
- Arkin, Setting the Standard.



**Figure 1** A possible hierarchy for synthetic biology is inspired by computer engineering.

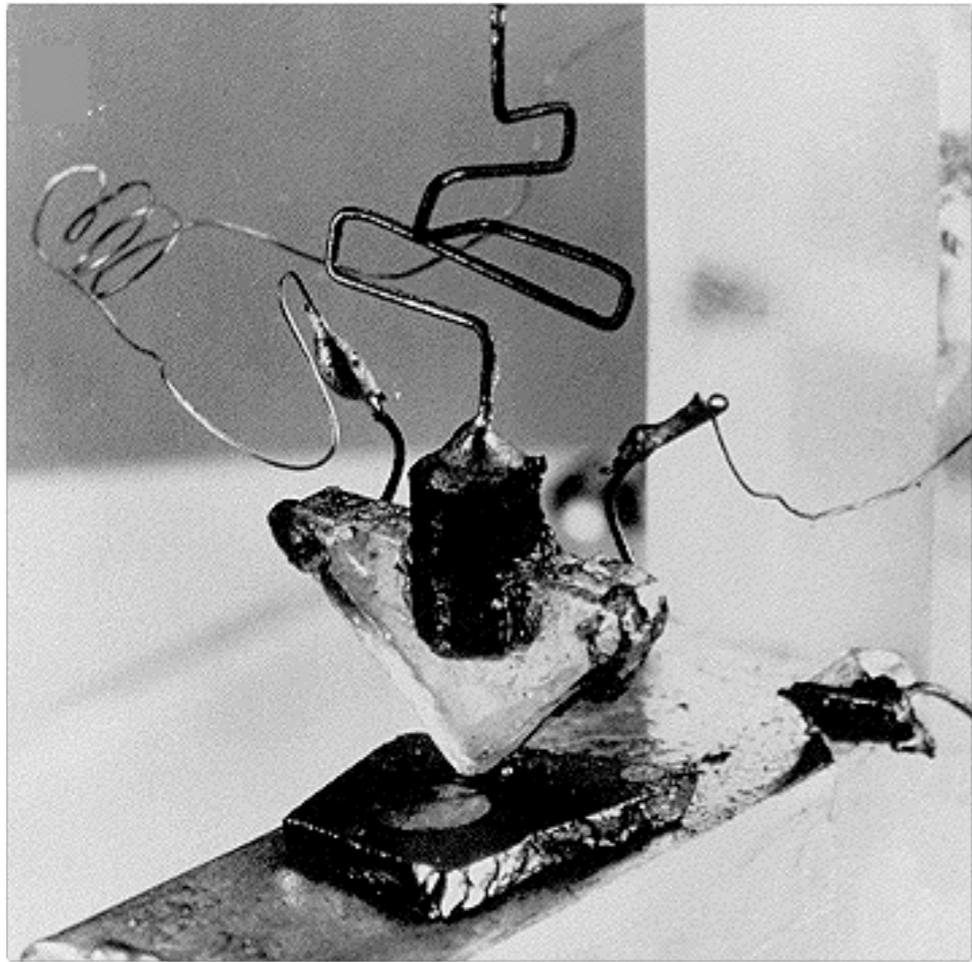


*Euprymna scolopes* copyright 2006 Chun *et al.*; licensee BioMed Central (doi:10.1186/1471-2164-7-154).  
*Vibrio fischeri* plate copyright 2006 Dr. Alan J. Cann (http://www.flickr.com/photos/ajc1/252308050).



# The Problem

We Build This



*transistor, v1*

We Expect This

## MOSPEC

### NPN SILICON HIGH-VOLTAGE TRANSISTORS

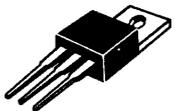
... designed for use general-purpose, high voltage applications requiring high  $f_T$

#### FEATURES:

- \*Collector-Emitter Sustaining Voltage-  
 $V_{CE(sus)} = 350 \text{ V (Min.) @ } I_C = 2.5 \text{ mA}$
- \* DC Current Gain-  
 $h_{FE} = 40 \text{ (Min.) @ } I_C = 100 \text{ mA- MJE2361T}$
- \* Current Gain-Bandwidth Product  
 $f_T = 10 \text{ MHz (Typ) @ } I_C = 50 \text{ mA}$

**NPN**  
**MJE2360T**  
**MJE2361T**

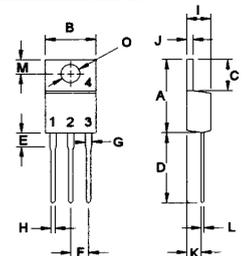
**0.5 AMPERE**  
**POWER**  
**TRANSISTORS**  
**350 VOLTS**  
**30 WATTS**



**TO-220**

#### MAXIMUM RATINGS

Characteristic	Symbol	Rating	Unit
Collector-Emitter Voltage	$V_{CEO}$	350	V
Collector-Emitter Voltage	$V_{CEV}$	375	V
Emitter-Base Voltage	$V_{EBO}$	6.0	V
Collector Current - Continuous - Peak	$I_C$ $I_{CM}$	0.5 1.0	A
Base current	$I_B$	0.25	A
Total Power Dissipation @ $T_C = 25^\circ\text{C}$ Derate above $25^\circ\text{C}$	$P_D$	30 0.24	W W/ $^\circ\text{C}$
Operating and Storage Junction Temperature Range	$T_J, T_{STG}$	-65 to +150	$^\circ\text{C}$

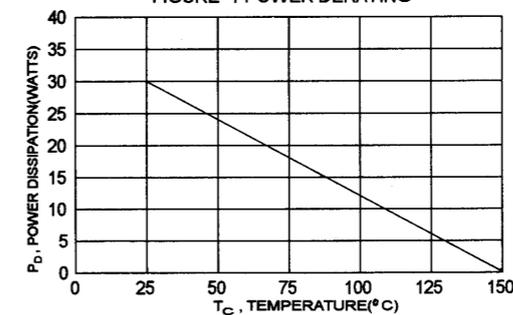


PIN 1.BASE  
2.COLLECTOR  
3.EMITTER  
4.COLLECTOR(CASE)

#### THERMAL CHARACTERISTICS

Characteristic	Symbol	Max	Unit
Thermal Resistance Junction to Case	$R_{\theta jc}$	4.167	$^\circ\text{C/W}$

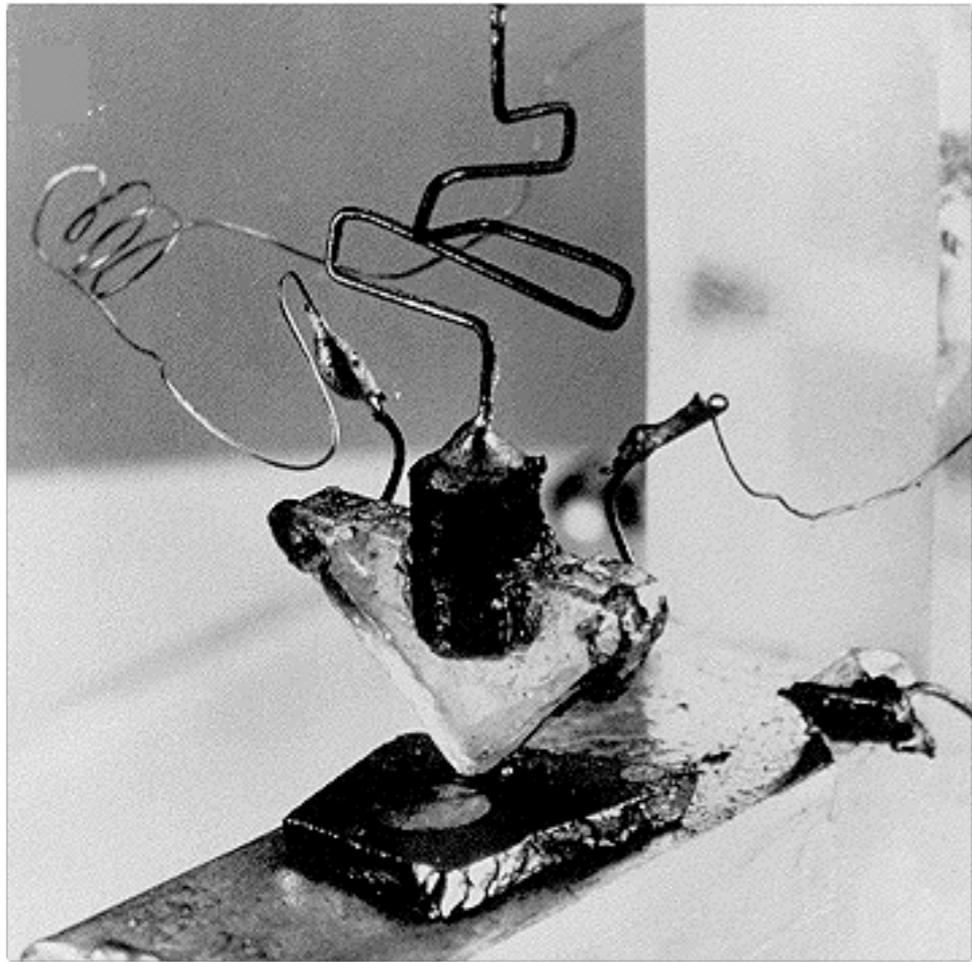
FIGURE -1 POWER DERATING



DIM	MILLIMETERS	
	MIN	MAX
A	14.68	15.31
B	9.78	10.42
C	5.01	6.52
D	13.06	14.62
E	3.57	4.07
F	2.42	3.66
G	1.12	1.36
H	0.72	0.96
I	4.22	4.98
J	1.14	1.38
K	2.20	2.97
L	0.33	0.55
M	2.48	2.98
O	3.70	3.90

# The Problem

We Build This



*transistor, v1*



We Expect This

## MOSPEC

### NPN SILICON HIGH-VOLTAGE TRANSISTORS

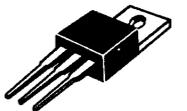
... designed for use general-purpose, high voltage applications requiring high  $f_T$

#### FEATURES:

- \*Collector-Emitter Sustaining Voltage-  
 $V_{CE(sus)} = 350 \text{ V (Min.) @ } I_C = 2.5 \text{ mA}$
- \*DC Current Gain-  
 $hFE = 40 \text{ (Min.) @ } I_C = 100 \text{ mA- MJE2361T}$
- \*Current Gain-Bandwidth Product  
 $f_T = 10 \text{ MHz (Typ) @ } I_C = 50 \text{ mA}$

**NPN**  
**MJE2360T**  
**MJE2361T**

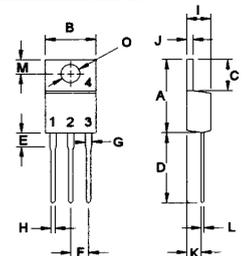
**0.5 AMPERE**  
**POWER**  
**TRANSISTORS**  
**350 VOLTS**  
**30 WATTS**



**TO-220**

#### MAXIMUM RATINGS

Characteristic	Symbol	Rating	Unit
Collector-Emitter Voltage	$V_{CEO}$	350	V
Collector-Emitter Voltage	$V_{CEV}$	375	V
Emitter-Base Voltage	$V_{EBO}$	6.0	V
Collector Current - Continuous - Peak	$I_C$ $I_{CM}$	0.5 1.0	A
Base current	$I_B$	0.25	A
Total Power Dissipation @ $T_C = 25^\circ\text{C}$ Derate above $25^\circ\text{C}$	$P_D$	30 0.24	W W/ $^\circ\text{C}$
Operating and Storage Junction Temperature Range	$T_J, T_{STG}$	-65 to +150	$^\circ\text{C}$



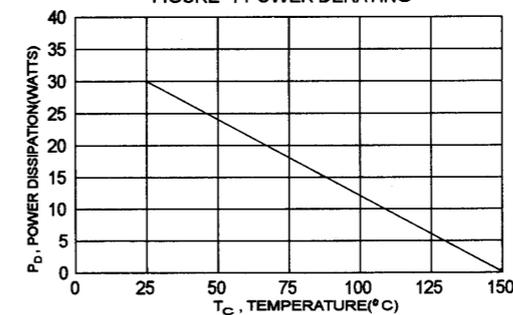
PIN 1.BASE  
2.COLLECTOR  
3.EMITTER  
4.COLLECTOR(CASE)

#### THERMAL CHARACTERISTICS

Characteristic	Symbol	Max	Unit
Thermal Resistance Junction to Case	$R_{\theta jc}$	4.167	$^\circ\text{C/W}$

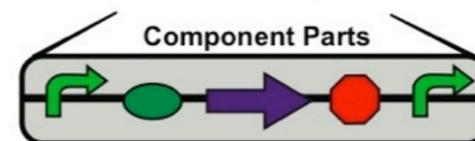
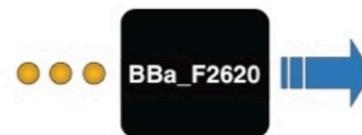
DIM	MILLIMETERS	
	MIN	MAX
A	14.68	15.31
B	9.78	10.42
C	5.01	6.52
D	13.06	14.62
E	3.57	4.07
F	2.42	3.66
G	1.12	1.36
H	0.72	0.96
I	4.22	4.98
J	1.14	1.38
K	2.20	2.97
L	0.33	0.55
M	2.48	2.98
O	3.70	3.90

FIGURE -1 POWER DERATING



# BBa\_F2620

3OC<sub>6</sub>HSL → PoPS Receiver

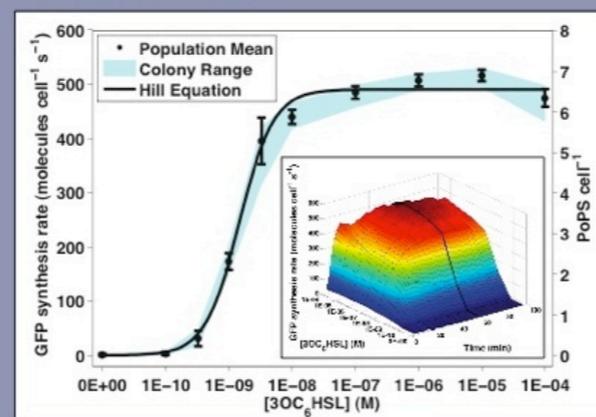


R0040 B0034 C0062 B0015 R0062  
P<sub>LtetO-1</sub> RBS luxR Term. P<sub>lux,R</sub>

## Mechanism & Function

A transcription factor (LuxR) that is active in the presence of a cell-cell signaling molecule (3OC<sub>6</sub>HSL) is controlled by a regulated operator (P<sub>LtetO-1</sub>). Device input is 3OC<sub>6</sub>HSL. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input such as a Tc can be used to produce a Boolean AND function.

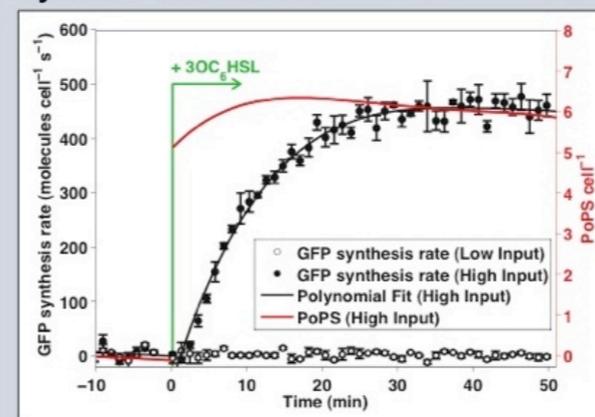
### Static Performance\*



$$P_{out} = \frac{P_{max} [3OC_6HSL]^n}{K^n + [3OC_6HSL]^n}$$

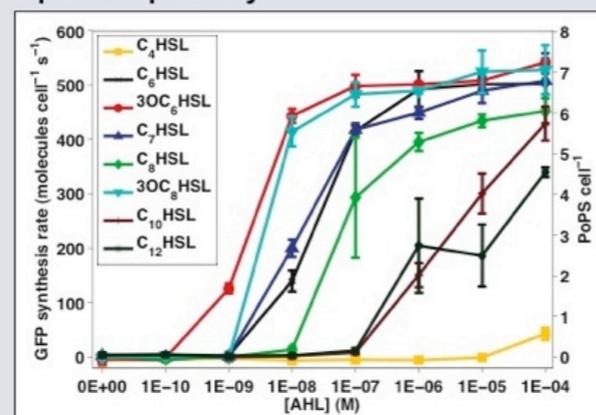
$P_{max}$ : 6.6 PoPS cell<sup>-1</sup>  
 $K$ : 1.5E-09 M 3OC<sub>6</sub>HSL  
 $n$ : 1.6

### Dynamic Performance\*



BBa\_F2620 Response Time: <1 min  
BBa\_T9002 Response Time: 6±1 min  
Inputs: 0 M (Low), 1E-07 M (High) 3OC<sub>6</sub>HSL

### Input Compatibility\*



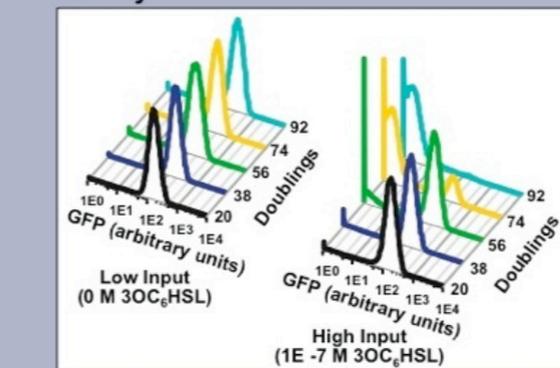
### Part Compatibility (qualitative)

Chassis: MC4100, MG1655, and DH5α  
Plasmids: pSB3K3 and pSB1A2  
Devices: E0240, E0430 and E0434

### Transcriptional Output Demand (low/high input)

Nucleotides: 0 / 6xNt nucleotides cell<sup>-1</sup> s<sup>-1</sup>  
Polymerases: 0 / 1.5E-1xNt RNAP cell<sup>-1</sup>  
(Nt = downstream transcript length)

### Reliability\*\*



Genetic: >92/>56 culture doublings  
Performance: >92/>56 culture doublings  
(low/high input during propagation)

### Conditions (abridged)

Output: PoPS measured via BBa\_E0240  
Culture: Supplemented M9, 37°C  
Plasmid: pSB3K3  
Chassis: MG1655  
\*Equipment: PE Victor3 multi-well fluorimeter  
\*\*Equipment: BD FACScan cytometer

[http://parts.mit.edu/registry/index.php/Part:BBa\\_F2620](http://parts.mit.edu/registry/index.php/Part:BBa_F2620)

Signaling Devices

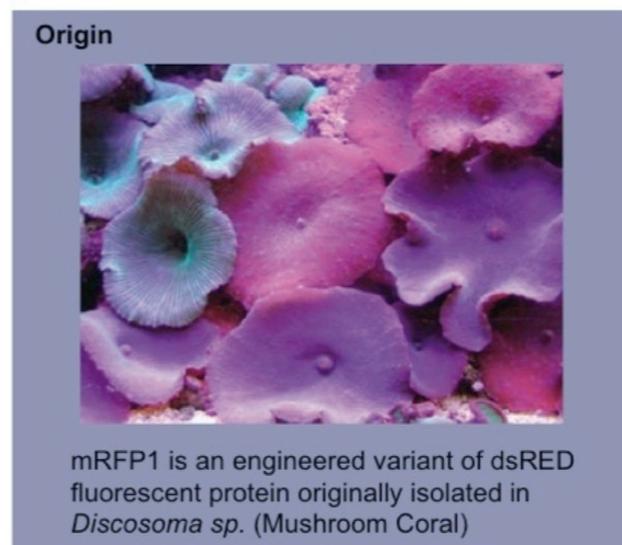
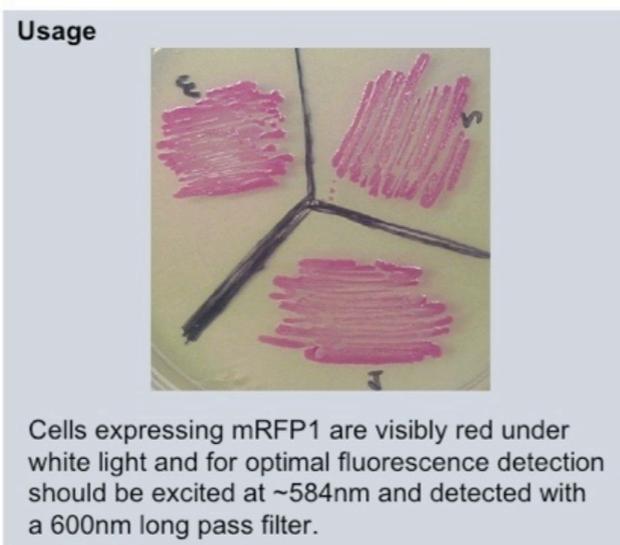
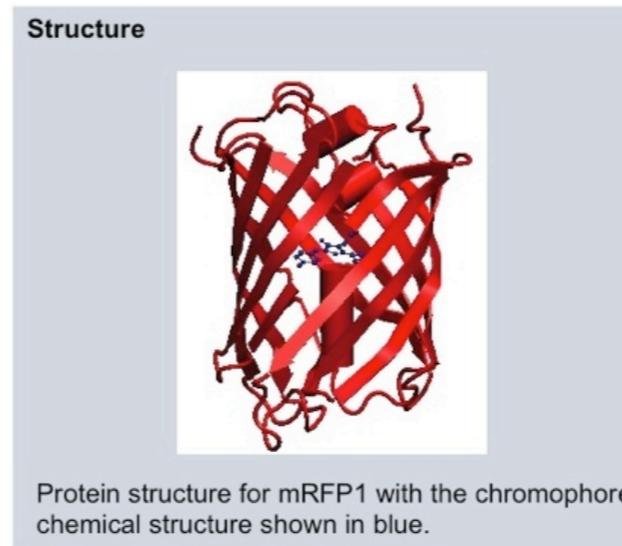
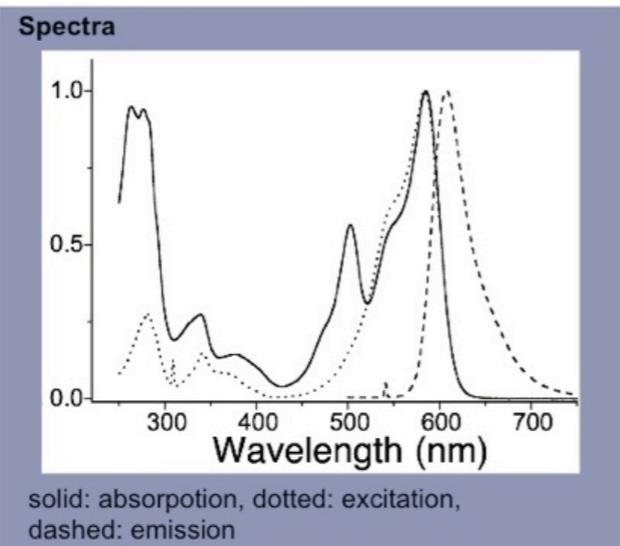
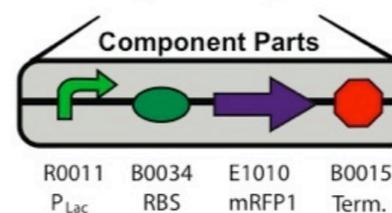
# BBa\_J04450

## Red Fluorescent Protein Generator



### Mechanism & Function

The expression of the gene for mRFP1 is controlled by a regulated operator (PLac). If used in a cell containing lac repressor (LacI) then an input such as IPTG can be used to modulate the expression of mRFP1.



[http://partsregistry.org/Part:BBa\\_J04450](http://partsregistry.org/Part:BBa_J04450)  
**Reporter Devices**

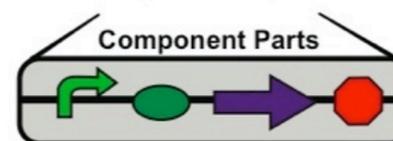
# BBa\_J45200

## Banana Odor Generator



DNA

BBa\_J45200

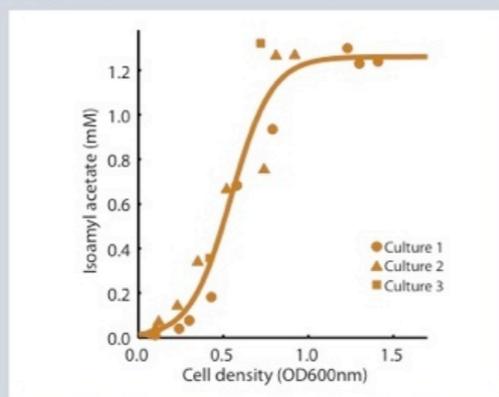


R0040 P<sub>tetR</sub> B0030 RBS J45014 ATF1 B0015 Term.

### Mechanism & Function

The banana odor generator (BBa\_J45200) catalyzes the conversion of the precursor isoamyl alcohol to the odor isoamyl acetate that has a banana smell. The biosynthetic device is composed of two transcriptional devices: a constitutive transcription source (BBa\_R0040) and an odor enzyme generator (BBa\_J45199). Odor enzyme generators produce as output an enzyme that catalyzes production of an odor from a chemical precursor.

### Activity



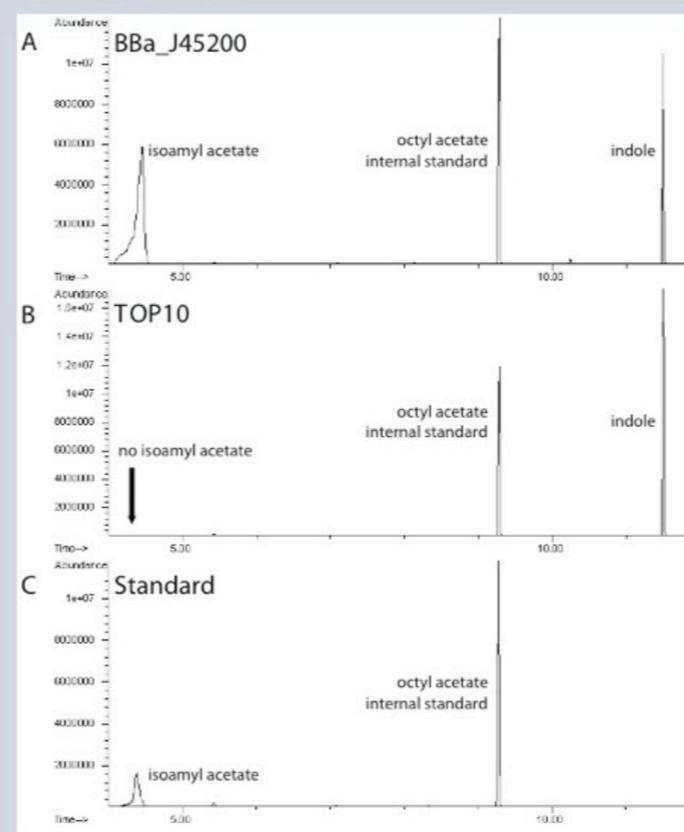
Isoamyl acetate concentration increases with cell density

### Usage



Cells expressing ATF1 should be grown in the presence of isoamyl alcohol (5mM).

### Chromatography



High levels of isoamyl acetate are produced when the precursor isoamyl alcohol is added to the culture medium (A), unless the cellular chassis (E. coli strain TOP10) does not contain J45200 (B). The retention time of the isoamyl acetate peak from J45200 is identical to that of the pure isoamyl acetate standard (C). Most E. coli strains produce indole. Octyl acetate was used as an internal standard for all samples containing isoamyl acetate

[http://partsregistry.org/Part:BBa\\_J45200](http://partsregistry.org/Part:BBa_J45200)

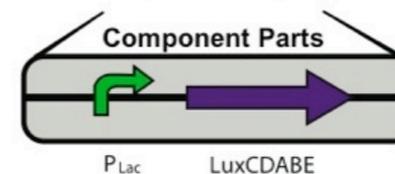
Reporter Devices

# BBa\_G10001

## Visible Light Generator



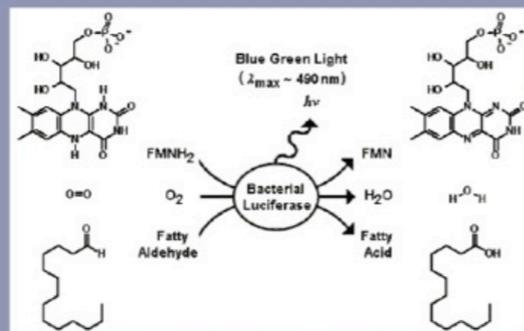
DNA



### Mechanism & Function

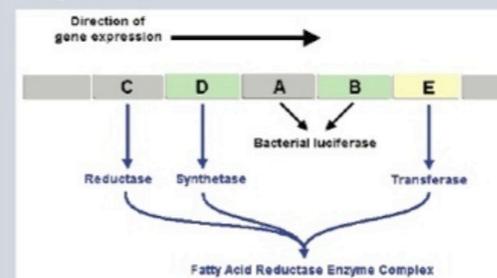
The expression of the Lux operon is controlled by a regulated operator (PLac). If used in a cell containing lac repressor (LacI) then an input such as IPTG can be used to modulate the expression of the Lux operon. When expressed this operon produces the necessary enzymes to generate the fatty aldehyde substrates as well as the luciferase enzyme that converts the fatty aldehyde substrates as well as the luciferase enzyme that converts luciferin to visible light.

### Reaction Mechanism



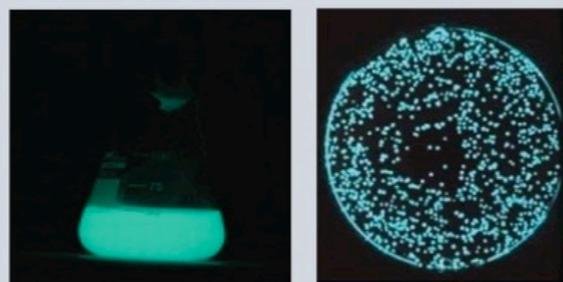
Bacterial luciferin is a reduced riboflavin phosphate (FMNH<sub>2</sub>, above) which is oxidized in association with a long-chain aldehyde, oxygen, and a luciferase to produce visible light.

### Lux Operon



The fatty acid reductase enzyme complex is needed to recycle the fatty aldehyde substrate in the reaction and luciferase is required to catalyze the reaction. No other exogenous enzymes are necessary since FMNH<sub>2</sub> is provided by the native electron transport chain in *E. coli*.

### Usage



Cells expressing the lux operon are visible in low light in liquid culture or as colonies.

### Origin

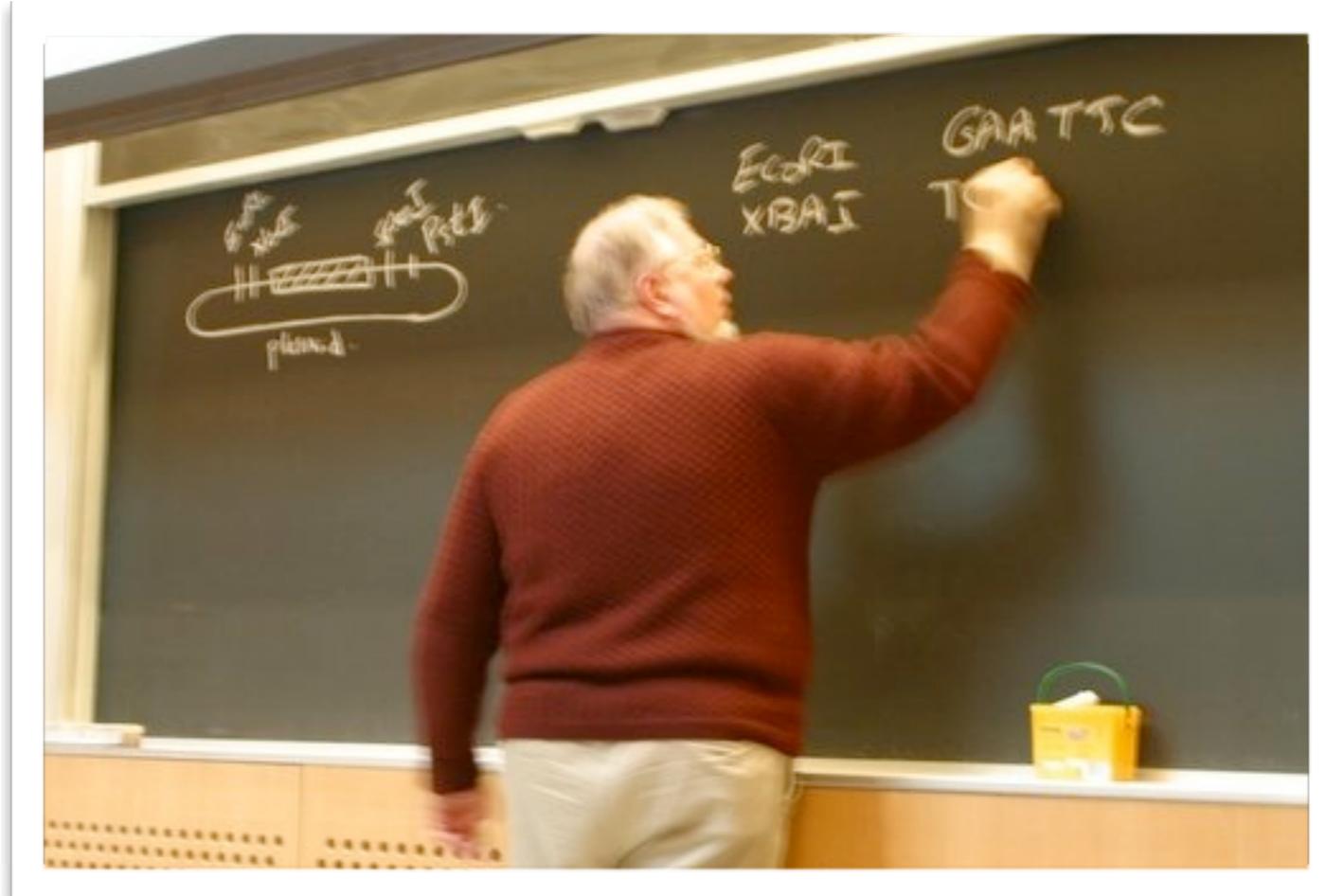


The Lux operon was isolated from *Vibrio fischeri* a bacteria found predominantly in symbiosis with marine animals such as the bobtail squid (above).

[http://partsregistry.org/Part:BBa\\_G10001](http://partsregistry.org/Part:BBa_G10001)

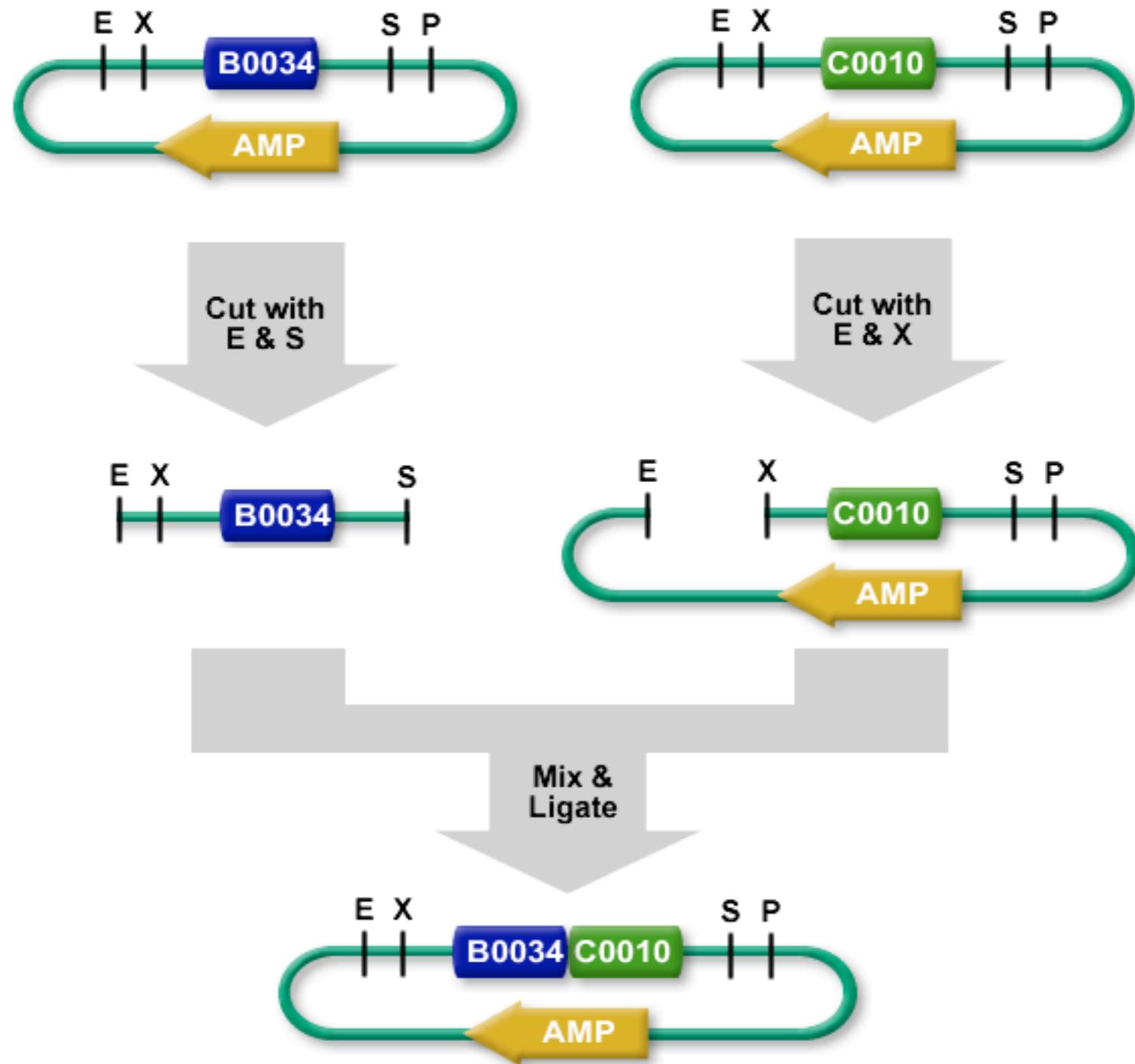
Reporter Devices

# BioBricks

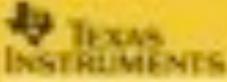


*“I was surprised to find that molecular biologists were spending something like 50% of their time at the bench just on manipulating DNA to build particular constructs”*      *-Tom Knight*

# BioBrick Standard Assembly (BBa)



# A Catalog


  
**TTL Logic**  
 Standard TTL, Schottky,  
 Low-Power Schottky

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Data Book

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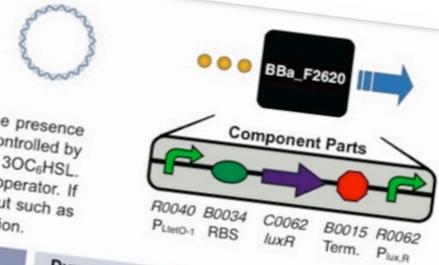
1988

## BBa\_F2620

3OC<sub>6</sub>HSL → PoPS Receiver

**Mechanism & Function**

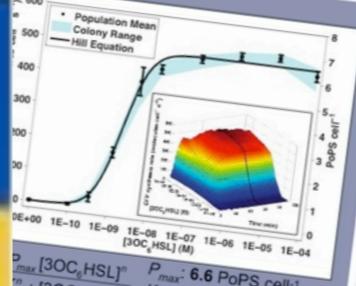
The transcription factor (LuxR) that is active in the presence of a cell-cell signaling molecule (3OC<sub>6</sub>HSL) is controlled by a regulated operator (P<sub>LuxO-1</sub>). Device input is 3OC<sub>6</sub>HSL. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input such as TetR can be used to produce a Boolean AND function.



**Component Parts**

R0040 P<sub>LuxO-1</sub> B0034 RBS C0062 luxR B0015 Term. R0062 P<sub>Lux,R</sub>

**Static Performance\***



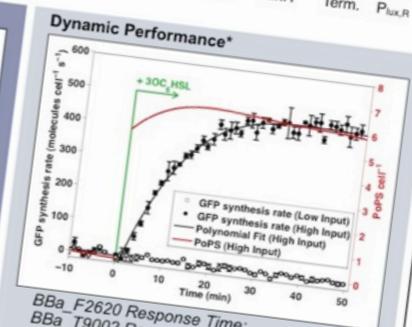
$$P_{max} \frac{[3OC_6HSL]^n}{K + [3OC_6HSL]^n}$$

$$P_{max}: 6.6 \text{ PoPS cell}^{-1}$$

$$K: 1.5E-09 \text{ M } 3OC_6HSL$$

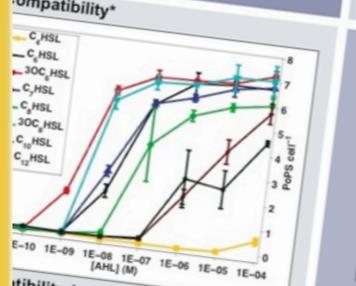
$$n: 1.6$$

**Dynamic Performance\***



**BBa\_F2620 Response Time:** <1 min  
**BBa\_T9002 Response Time:** 6±1 min  
 Inputs: 0 M (Low), 1E-07 M (High) 3OC<sub>6</sub>HSL

**Compatibility\***



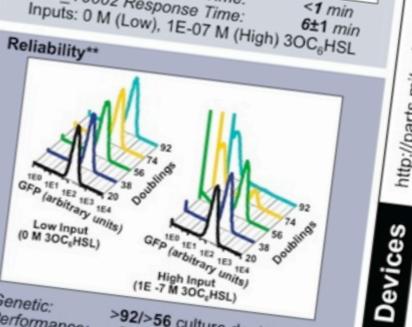
**Compatibility (qualitative)**

MC4100, MG1655, and DH5α  
 pSB3K3 and pSB1A2  
 E0240, E0430 and E0434

**Minimal Output Demand (low/high input)**

100 / 6xNt nucleotides cell<sup>-1</sup> s<sup>-1</sup>  
 100 / 1.5E-1xNt RNAP cell<sup>-1</sup>  
 Nt = downstream transcript length)

**Reliability\*\***



**Genetic:** >92/>56 culture doublings  
**Performance:** >92/>56 culture doublings (low/high input during propagation)

**Conditions (abridged)**

Output: PoPS measured via BBa\_E0240  
 Culture: Supplemented M9, 37°C  
 Plasmid: pSB3K3  
 Chassis: MG1655  
 \*Equipment: PE Victor3 multi-well fluorimeter  
 \*\*Equipment: BD FACScan cytometer

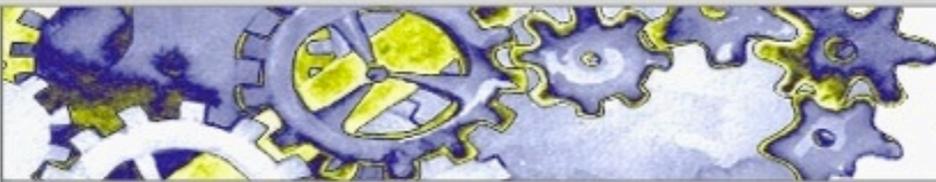
http://parts.mit.edu/registry/index.php/Part:BBa\_F2620

Signaling Devices

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**Registry of Standard Biological Parts**  
*making life better, one part at a time*

License: Public



# Registry of Standard Biological Parts

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## Part Types

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- To discuss how to measure the functions of these parts visit [Characterization of Parts](#)

- 6491 parts defined
- 1880 physically available

-July 2008


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# Parts Registry kits



# NEWSLETTER

## Homebrew Computer Club

Robert Reiling, Editor □ Post Office Box 626, Mountain View, CA 94042 □ Joel Miller, Staff Writer  
Typesetting, graphics and editorial services donated by Laurel Publications, 17235 Laurel Rd., Los Gatos, CA 95030 (408) 353-3609

### RANDOM DATA

By Robert Reiling

Computer clubs continue to form around the country...E. Brooner would like to have material to help him get started with the "Flathead Computer Society" in the Kalispell area. His Address is P.O. Box 236, Lakeside, Montana 59922.

Did you see the SOL terminal demonstrated by Bob Marsh at the Sept. 1st meeting? An excellent design that will interest hobbyists and commercial users alike. It's available from Processor Technology, 6200 Hollis St., Emeryville, CA 94608. Write them for prices and specifications.

The OSI Systems Journal has been sent to all OSI customers (free—at least for the time being). It's a bi-monthly magazine with plans to go monthly in the future. There are 28 pages in the first issue (August 1976, Vol. 1, No. 1) with a hardware feature covering the OSI 440 Video Graphics System and software, features concerning Tiny BASIC for the 6800 and a Graphics Editor for the 6502. It also includes OSI product and software catalog data. The BASIC is, of course, the 2K Tiny BASIC developed by Tom Pittman. Many of you have met Tom at the Homebrew computer Club meetings. The OSI Systems Journal is a good way to learn more about the OSI computer hardware and software along with helpful user information. The contact address is: The OSI Systems Journal, P.O. Box 134, Hiram, Ohio 44234.

KIM-1 users now have a newsletter. Eric Rehnke is producing the newsletter every 5-8 weeks, MOS Technology, Inc. helped get it started by sending copies to all known KIM owners. The user group, however, is independent of MOS Technology, Inc. The newsletter is devoted to KIM-1 support. Subscriptions are \$5.00 for the next six issues. Contact "KIM-1 User Notes," c/o Eric C. Rehnke, Apt. 207, 7656 Broadview Rd., Parma, Ohio 44134.

The BAMUG club has a new contact address. It is BAMUG, c/o Timothy O'Hare, 1211 Santa Clara Ave., Alameda, CA 94501. Write Timothy for club information. I suggest you include a stamped, self-addressed envelope.

Beware of board snatchers! Glenn Ewing reports 11 boards were taken out of his IMSAI computer. The boards are: MPU, 4 RAM-4's, SIO-2, P10-4, PIC-8, PROM-4, IFM and FIB. Glenn suggests you consider providing good security for your computer and associated equipment. In his case the computer was in a locked office which was burglarized. In the event you

have information on the above boards, write Lt. Glenn Ewing, Code 62EI, Naval Post Graduate School, Monterey, CA 93940.

For family and friends of people who always wanted to know about computers, but didn't want to ask them, four easy-going classes are available starting Oct. 19th on Tuesdays from 7 to 9 p.m. You can learn how computers work and what they can and can't do. You will also have some of the jargon deciphered, see what you can do with a computer, play some games and learn to program. The cost is \$25. Contact the Community Computer Center, 1919 Menalto Ave., Menlo Park, CA 94025, phone (415) 325-4444.

A call for papers in personal computing has been issued by the 1977 National Computer Conference. The conference is scheduled for June 13-16, 1977. I have a few copies of the guidelines if you would like to submit a paper.

The First West Coast Computer Faire will be held April 16 and 17, 1977 at the San Francisco Civic Auditorium. This faire is shaping up rapidly. If you would like to lead a conference or participate in a conference session, please contact me. More information about the Faire is in the accompanying article. □

### THE FIRST WEST COAST COMPUTER FAIRE

*A Call For Papers And Participation*

The San Francisco Bay Area is finally going to have a major conference and exhibition exclusively concerned with personal and home computing—The First West Coast Computer Faire. And, it promises to be a massive one! It will take place in the largest convention facility in Northern California: The Civic Auditorium in San Francisco. It will be a two-and-a-half day affair, starting on Friday evening and running through Sunday evening, April 15-17.

It is being sponsored by a number of local and regional hobbyist clubs, educational organizations and professional groups. These include:

- The two largest amateur computer organizations in the United States—the Homebrew Computer Club and the Southern California Computer Society
- Both of the Bay Area chapters of the Association Of Computing Machinery—the San Francisco Chapter and the Golden Gate Chapter
- Stanford University's Electrical Engineering Department





# DIYbio

## Local Groups

There are DIYbioers all over the globe! See if there is a meetup near you on the map below. If there is not, add your location and your contact information to the map, so others can get in touch with you - just don't forget to update it once you start a regular meetup!



View a larger map, or to add yourself or your group to the map. You'll need to sign into your Google account in order to add a new point. It's a little unclear, so here's a [screenshot of adding a new point](#)

### about us

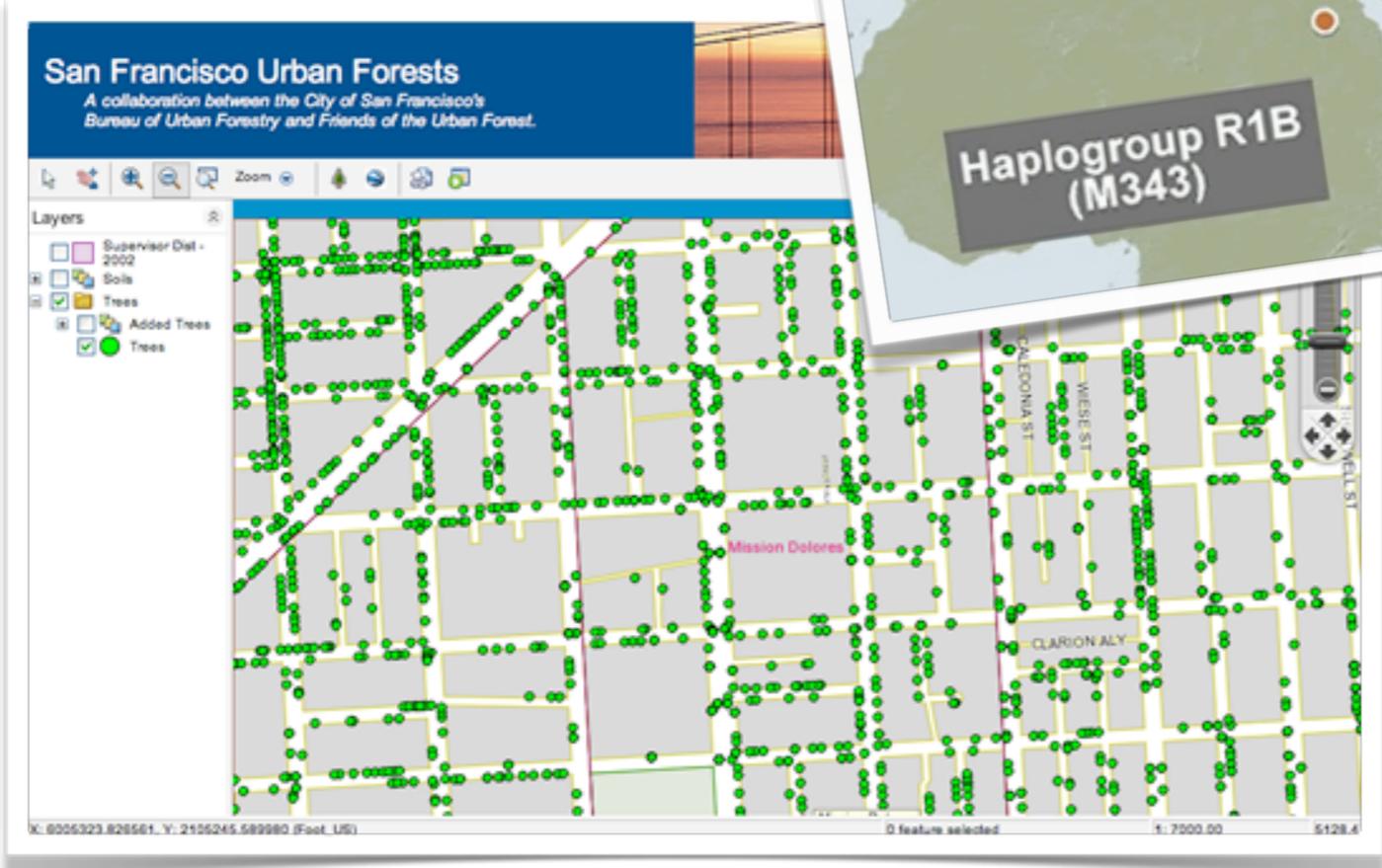
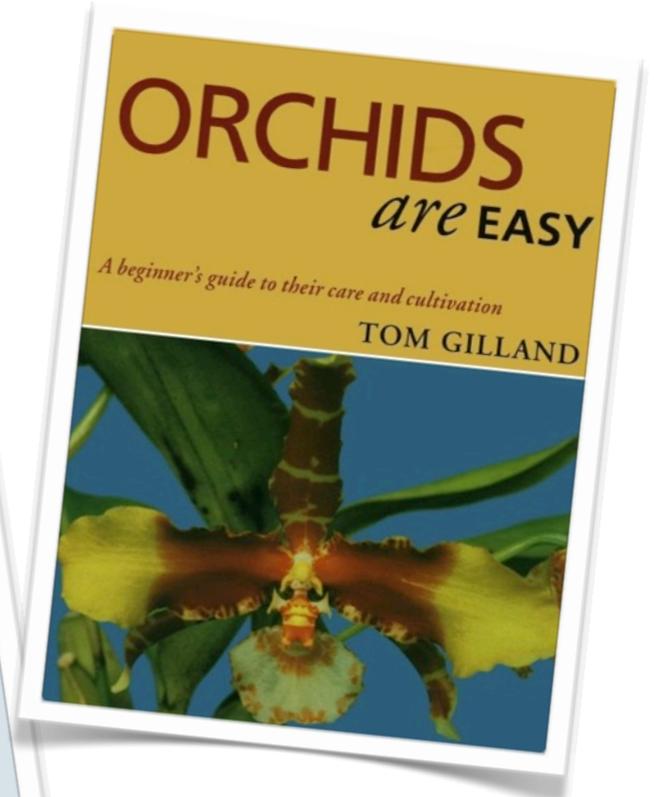
DIYbio is an organization that aims to help make biology a worthwhile pursuit for citizen scientists, amateur biologists, and DIY biological engineers who value openness and safety. This will require mechanisms for amateurs to increase their knowledge and skills, access to a community of experts, the development of a code of ethics, responsible oversight, and leadership on issues that are unique to doing biology outside of traditional professional settings.

### recent comments

- Ana (Quo):** Hola Fernando, Soy una redactora de la revista Quo y estoy ...
- Nick See Weinberg:** Would someone please add CodeCon to the DIYbio G-Cal? Thanks...
- Charles Stone:** Hey everyone!

# diybio is naturalism

macroscopic  
to  
microscopic



# diybio is engineering

- graft a hybrid cranberry-apple tree
- or
- add resveratrol production to yeast (healthier beer!)



Ben Harris-Roxis / Flickr

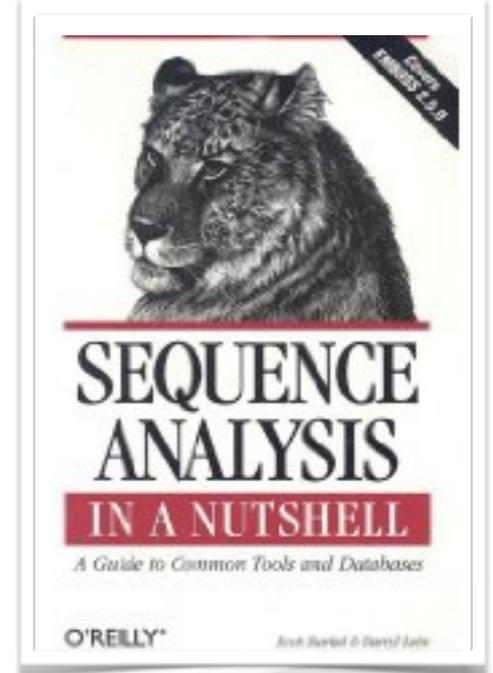


# diybio is more

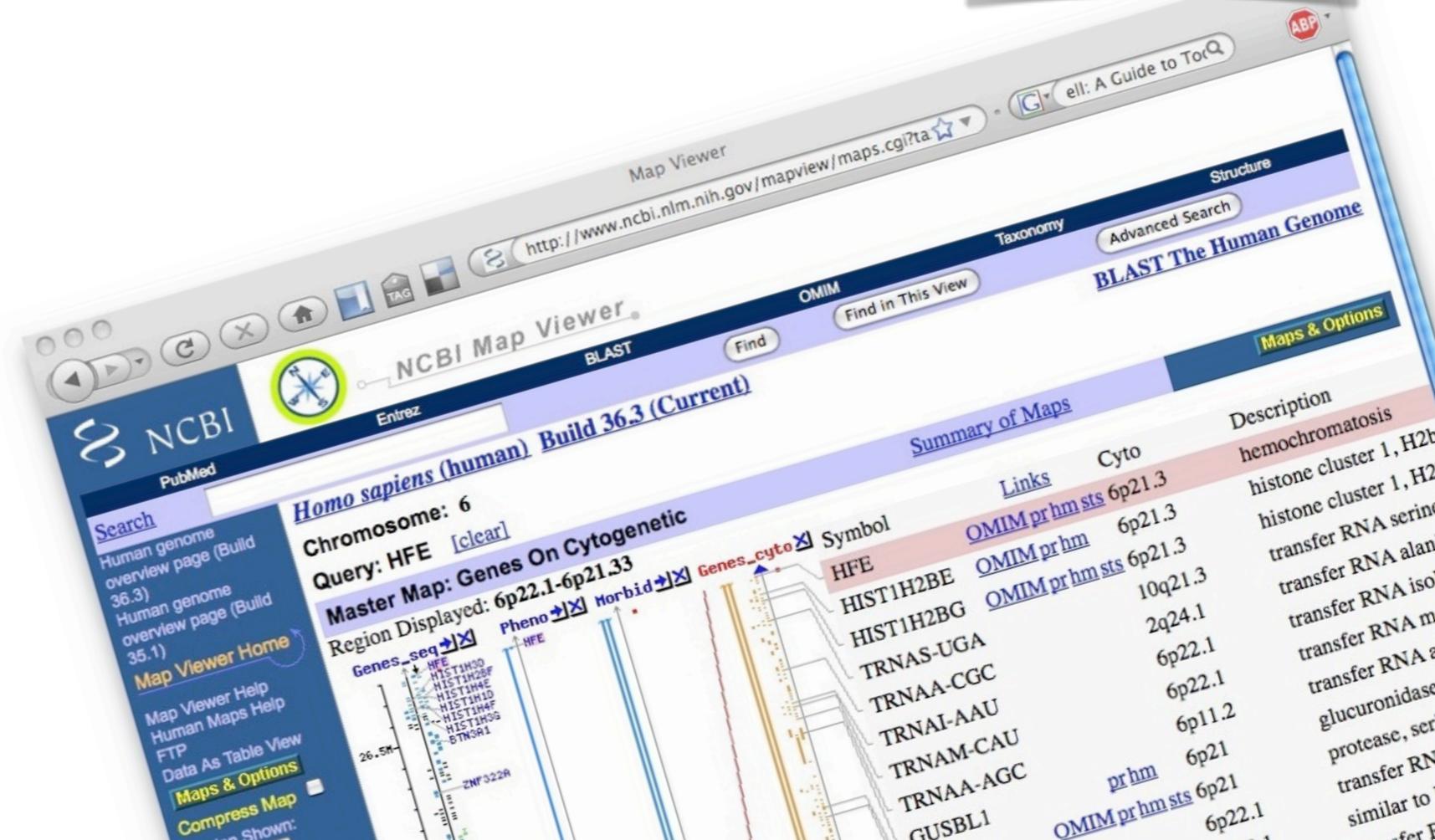
- hardware
- informatics
- art



working on the SmartLab table, Dec 08

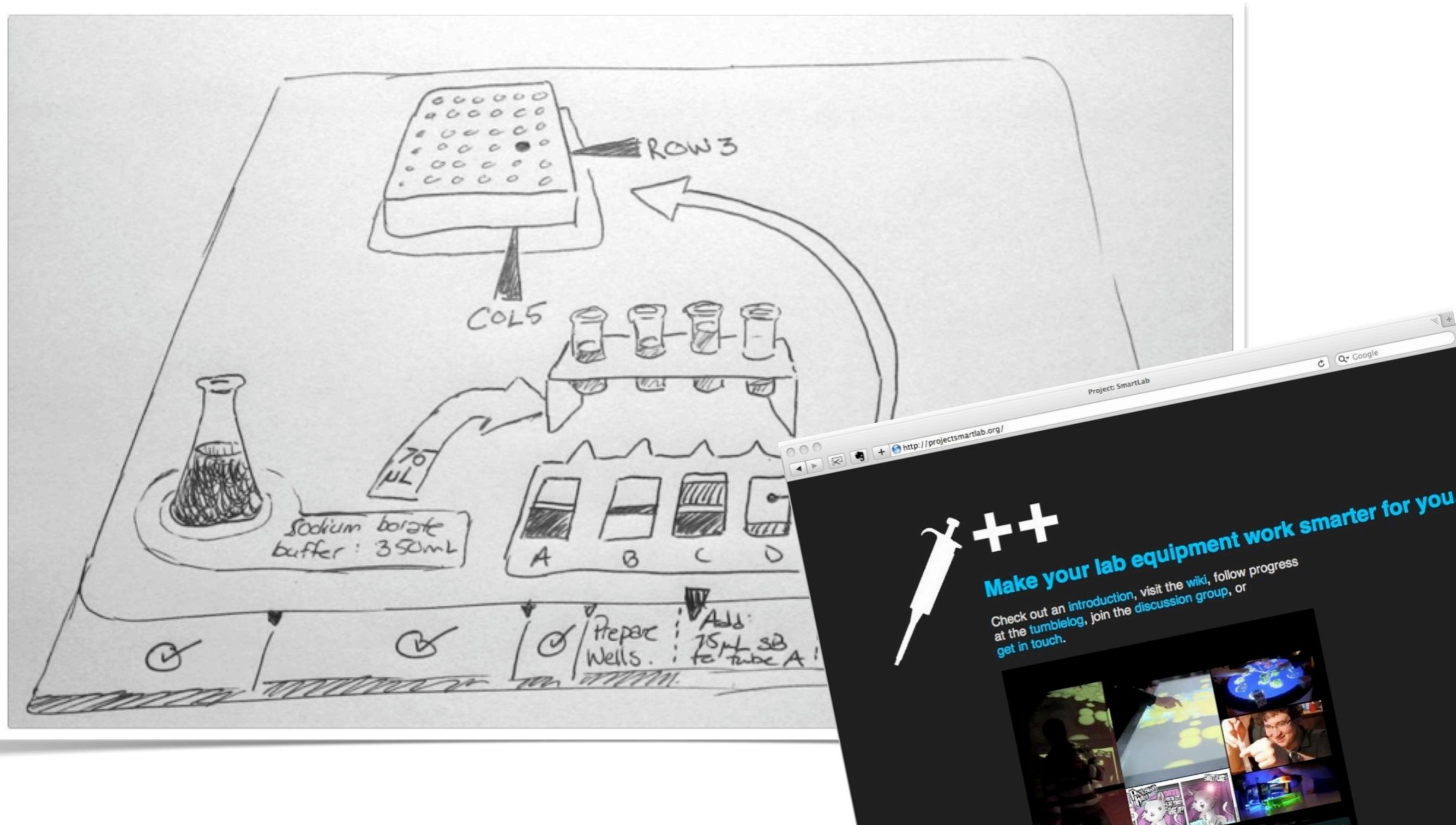


Alba, the fluorescent bunny (Eduardo Kac, 2000)



# SmartLab Project

multitouch augmented reality lab bench for recording and teaching molecular biology

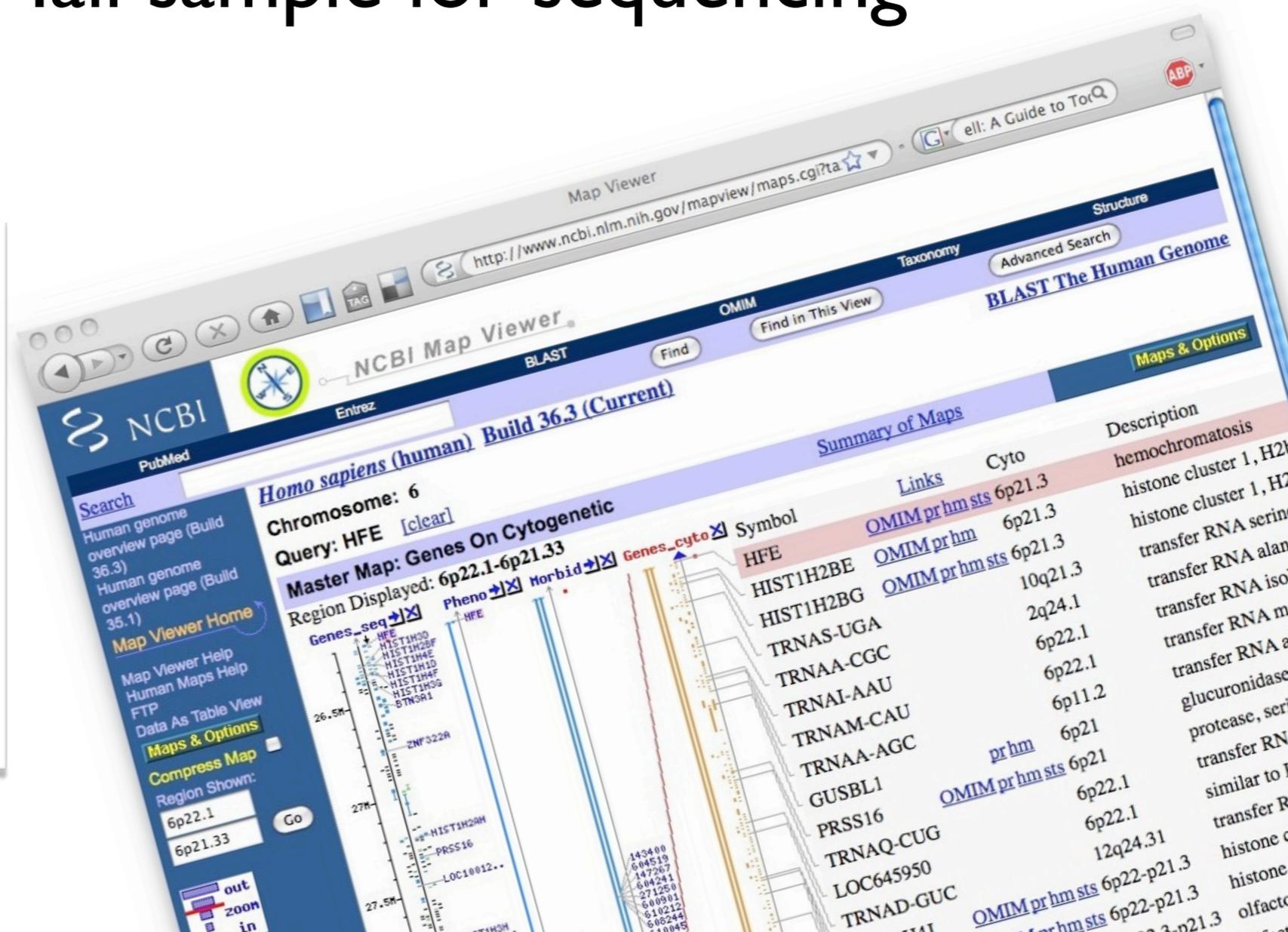
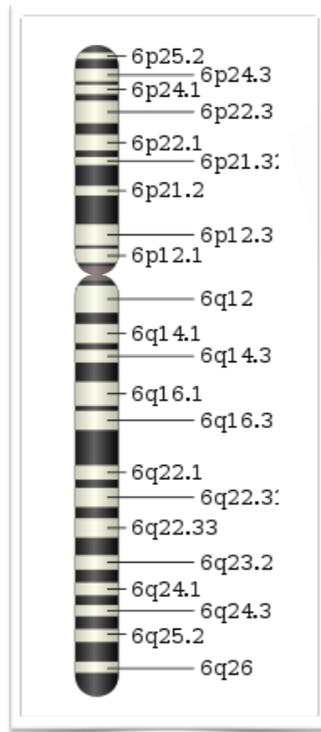
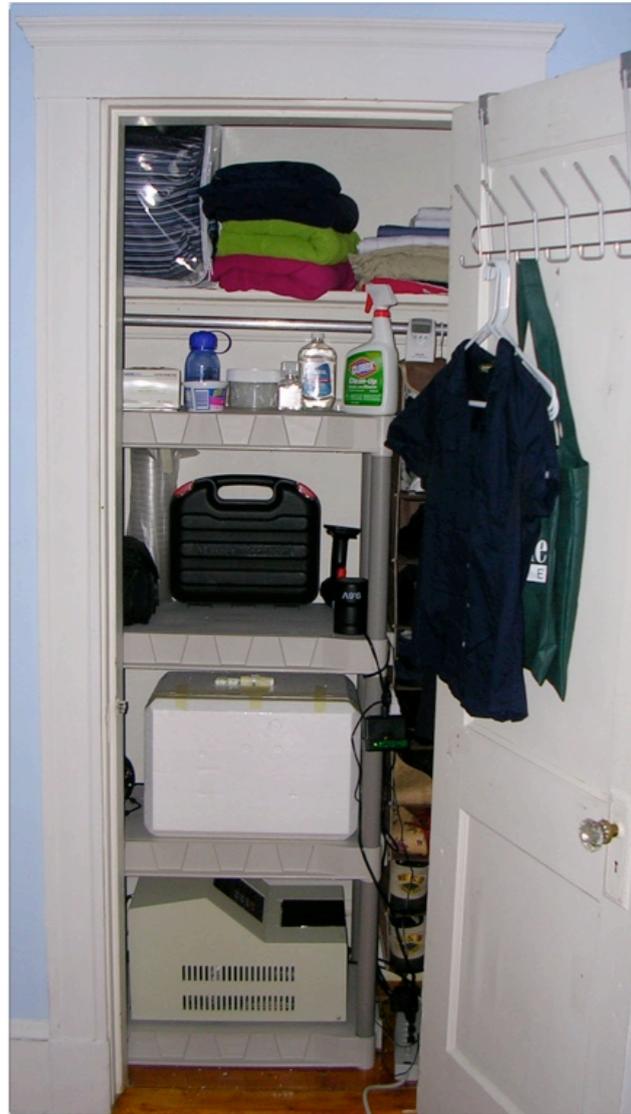


# self-genotyping



Is Kay a carrier of hemochromatosis on her 6th chromosome?

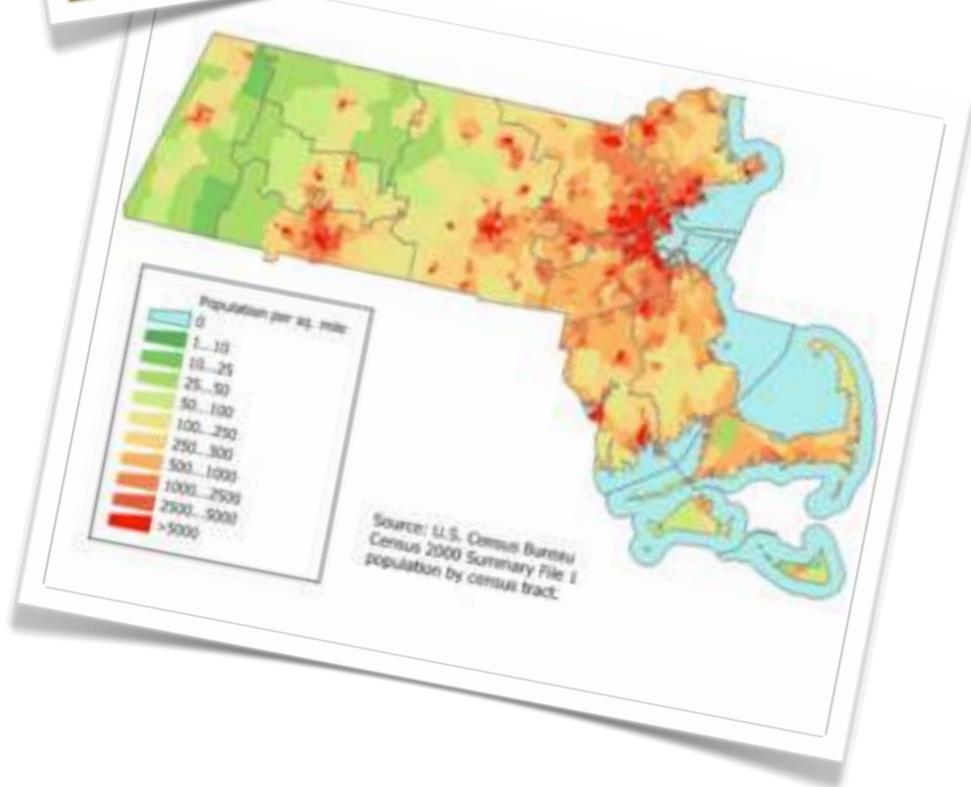
1. Allele-specific PCR at home
2. Mail sample for sequencing



# bioweathermaps

\$3000  
Lab-in-a-box:  
Affordable tools,  
equipment &  
supplies

flashmob + science =  
distributing tracking of bacterial  
populations across cities



## Welcome

Ever wonder how the microbial communities living on cross-walk buttons in Boston compare or Manhattan, or the cross-walk nearest your home? We're going to find out and you can go!

Meet up at points around the city, swab crosswalk buttons with Q-tips, and bring the same samples back home. The samples will be sent for DNA sequencing. A few weeks later, receive analysis results that were living on the crosswalk button they swabbed. The data will be published on a map so that crosswalk buttons can be compared.

Check out the [Instructable](#) for more details, or come visit us at [DIY for CHI](#).

Join our project. Sign-up for email updates at [bioweathermap.org](#) with any questions.

# Gel Box 2.0

for sorting dna by size



the best commercial boxes cost > \$1200.  
build an open source alternative for ~\$100

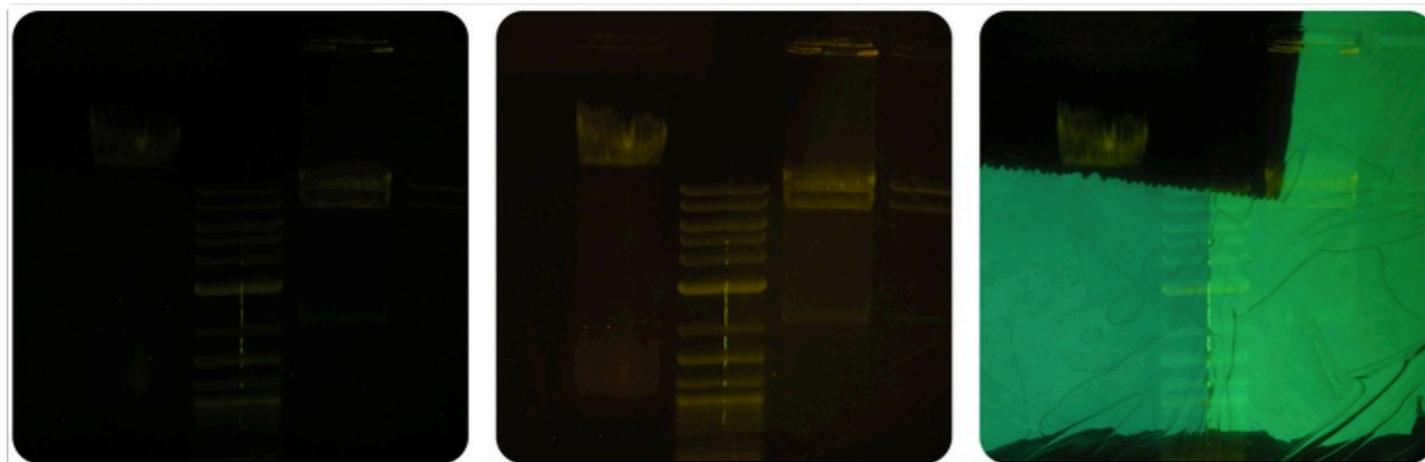
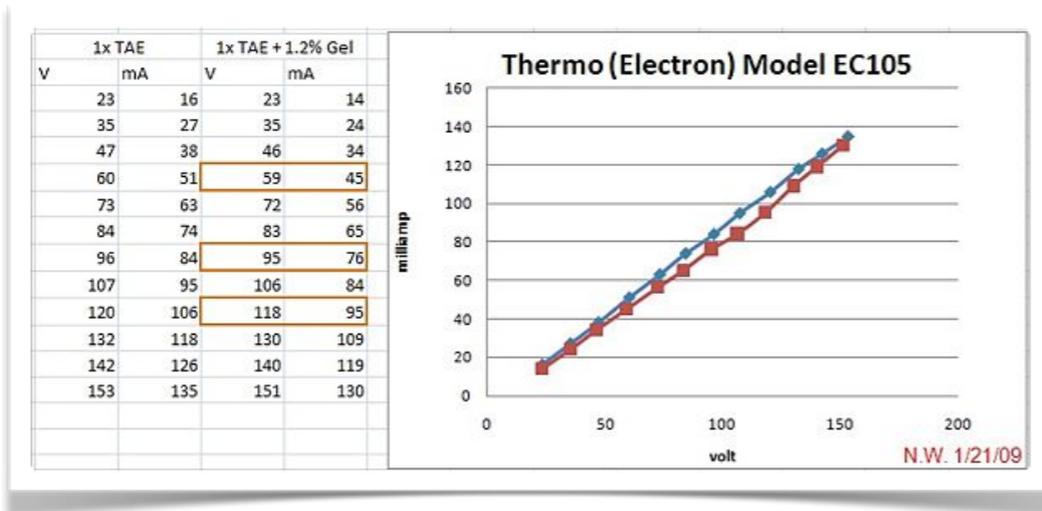
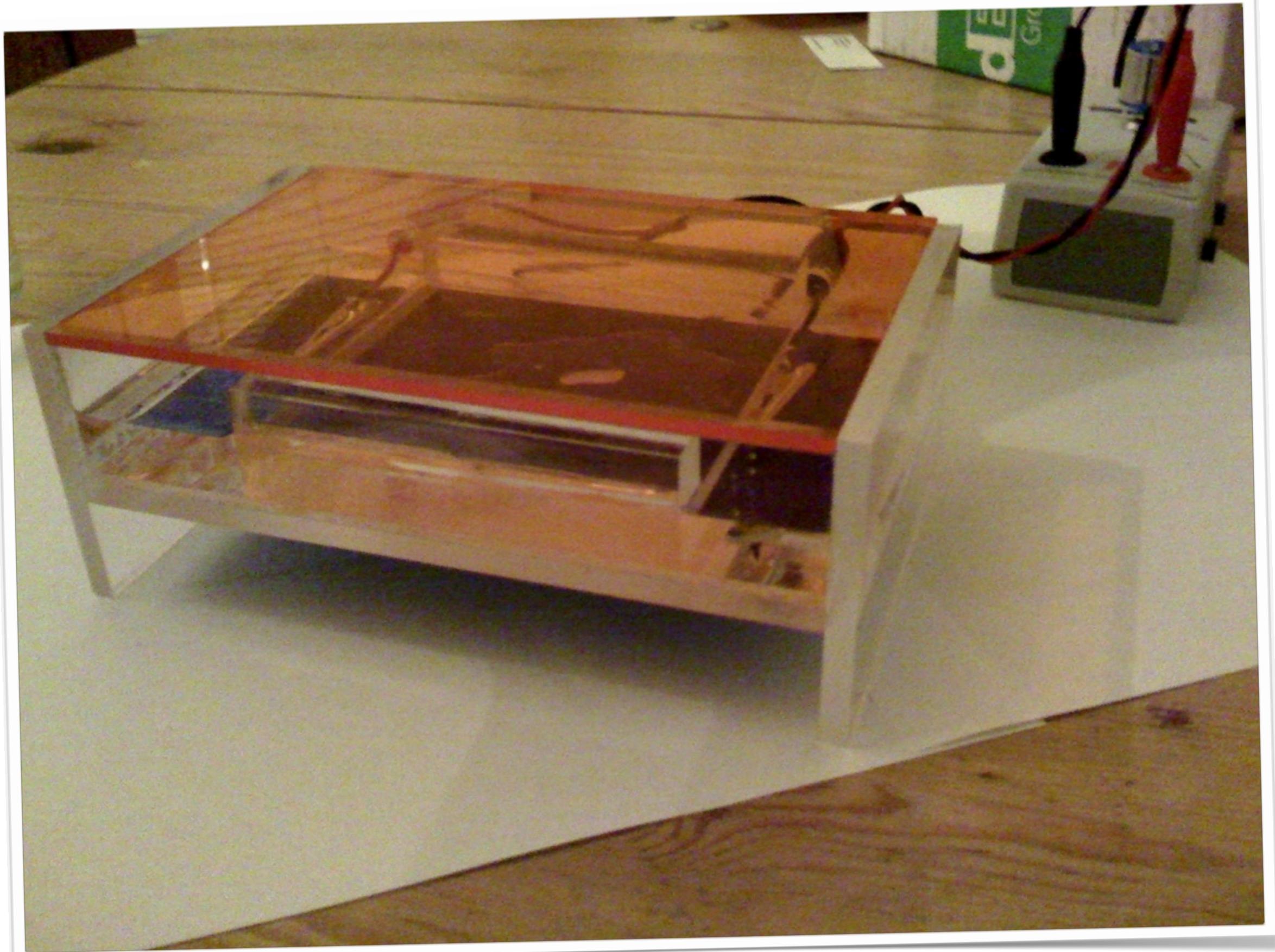


Image: Norman Wang - [http://bit.ly/GelBox2-transilluminator\\_image](http://bit.ly/GelBox2-transilluminator_image)

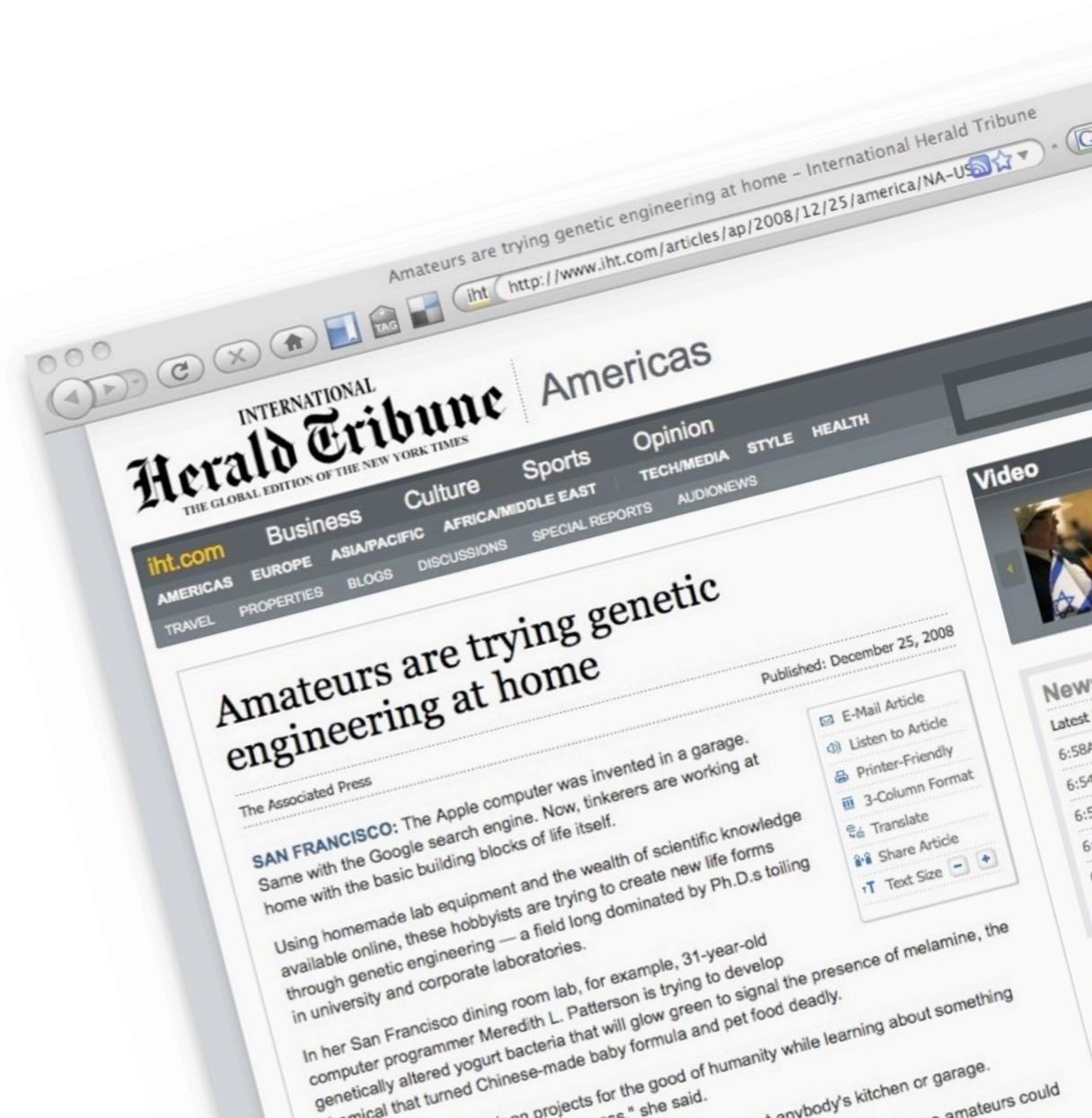
Gel Box 2.0 v0.1 circa feb 09 (\$150)



# GloGurt & Melaminometer

\$3000  
Lab-in-a-box:  
Affordable tools,  
equipment &  
supplies

*Lactobacillus* “hello world” +  
biosensing melamine



# \$3000 lab



at a boston-based coworking space



testing diy hardware  
testing diy protocols

(DNA extraction, transformation, culturing, gels, PCR)

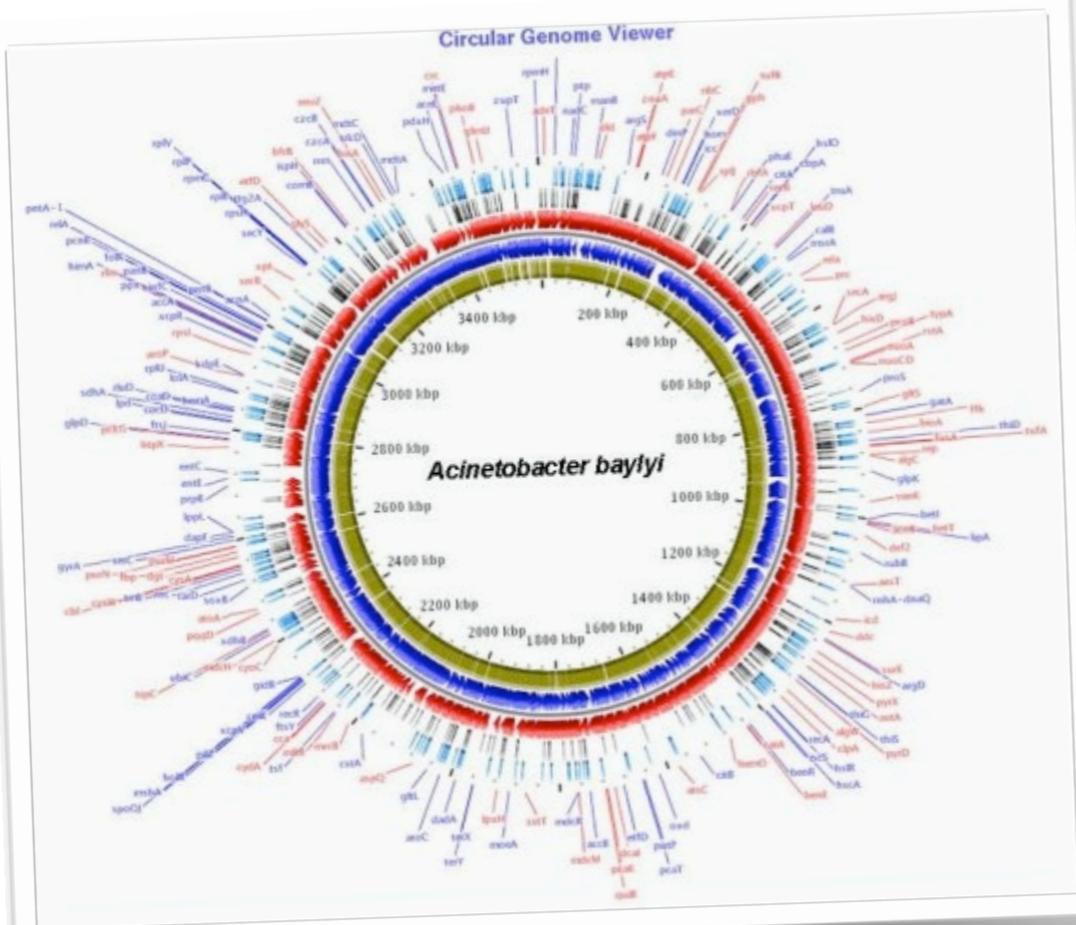
**demonstrating it works**



# Acinetobacter Baylyi

## ADP1

- gram-negative
- genome sequenced
- naturally competent!



5780-5790 *Nucleic Acids Research*, 2004, Vol. 32, No. 19  
doi:10.1093/nar/gkh881

### *Acinetobacter* sp. ADP1: an ideal model organism for genetic analysis and genome engineering

David Metzgar<sup>1</sup>, Jamie M. Bacher<sup>1</sup>, Valérie Pezo<sup>1,2</sup>, John Reader<sup>1</sup>, Volker Döring<sup>2</sup>, Paul Schimmel<sup>1</sup>, Philippe Marlière<sup>2</sup> and Valérie de Crécy-Lagard<sup>1,\*</sup>

<sup>1</sup>The Scripps Research Institute, BCC-379, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA and  
<sup>2</sup>Evologic SA, 2 rue Gaston Crémieux, 91000 Evry, France

Received July 1, 2004; Revised August 30, 2004; Accepted September 21, 2004

#### ABSTRACT

*Acinetobacter* sp. strain ADP1 is a naturally transformable gram-negative bacterium with simple culture requirements, a prototrophic metabolism and a compact genome of 3.7 Mb which has recently been sequenced. Wild-type ADP1 can be genetically manipulated by the direct addition of linear DNA constructs to log-phase cultures. This makes it an ideal organism for the automation of complex strain construction. Here, we demonstrate the flexibility and versatility of ADP1 as a genetic model through the construction of a broad variety of mutants. These include marked and unmarked insertions and deletions, complementary replacements, chromosomal expression tags and complex combinations thereof. In the process of these constructions, we demonstrate that ADP1 can effectively express a wide variety of foreign genes including antibiotic resistance cassettes, essential metabolic genes, negatively selectable catabolic genes and even intact operons from highly divergent bacteria. All of the described mutations were achieved by the same process of splicing PCR, direct transformation of growing cultures and plating on selective media. The simplicity of these tools make genetic analysis and engineering with *Acinetobacter* ADP1 accessible to laboratories with minimal microbial genetics expertise and very little automation of genetic analysis and engineering protocols.

protein requires the addition of sequences coding for binding tags to chromosomal genes. The development of new biochemical pathways for biomedical and biotechnological industries requires highly reiterative genetic manipulation, including insertion and deletion of many genes in the same strain, and often alteration of those genes in the process. These uses of manipulative genetics are essential to the current progress of biological research, and often determine the cost and efficiency of the experimental process.

Many fields of biology have either chosen or happened upon primary model organisms for which there are straightforward, user-friendly methods for genetic manipulation. *Caenorhabditis elegans* and *Drosophila* are relatively challenging, but the complexity of animal development and metabolism makes increased difficulties in these organisms inevitable. The *Agrobacterium/Arabidopsis* system provides a reasonably simple way to test genetic hypotheses in plants. *Saccharomyces cerevisiae* offers the same to mycologists, and bacteria, the primary gram-positive model *Bacillus subtilis* offers a relatively easy target for genetic manipulation. However, the primary gram-negative model organism, the archetypal model organism for all genetics, *Escherichia coli*, is relatively resistant to genetic manipulation.

*E. coli* has been the primary genetic model since the first functional description of a mapped genetic locus, the lac operon (1). Since then, researchers have struggled to overcome the genetic obstacles presented by this model, obstacles created by two specific traits of this bacterium. Due to a lack of natural competence, *E. coli* must be manipulated to allow transformation. The second obstacle is a lack of natural recombination capabilities. This must be overcome by the addition of recombination functions from other organisms and the simultaneous deletion or inhibition of native nuclease activities prevent recombination through direct destruction of introduced DNA construct (2,3). The manipulations needed to achieve recombination are deleterious and have considerable antagonistic effects, necessitating their reversal after the desired genetic manipulation has been achieved (4). All of these steps have been achieved (4). All of these steps have been achieved (4). All of these steps have been achieved (4).

# Acinetobacter Baylyi ADP1

- gram-negative
- genome sequenced
- naturally competent!

5780-5790 Nucleic Acids Research, 2004, Vol. 32, No. 19  
doi:10.1093/nar/gkh881

## Acinetobacter sp. ADP1: an ideal model organism for genetic analysis and genome engineering

David Metzgar<sup>1</sup>, Jamie M. Bacher<sup>1</sup>, Valérie Pezo<sup>1,2</sup>, John Reader<sup>1</sup>, Volker Döring<sup>2</sup>, Paul Schimmel<sup>1</sup>, Philippe Marlière<sup>2</sup> and Valérie de Crécy-Lagard<sup>1,\*</sup>

<sup>1</sup>The Scripps Research Institute, BCC-379, 10550 N. Torrey Pines Road, La Jolla,  
<sup>2</sup>Evologic SA, 2 rue Gaston Crémieux, 91000 Evry, France

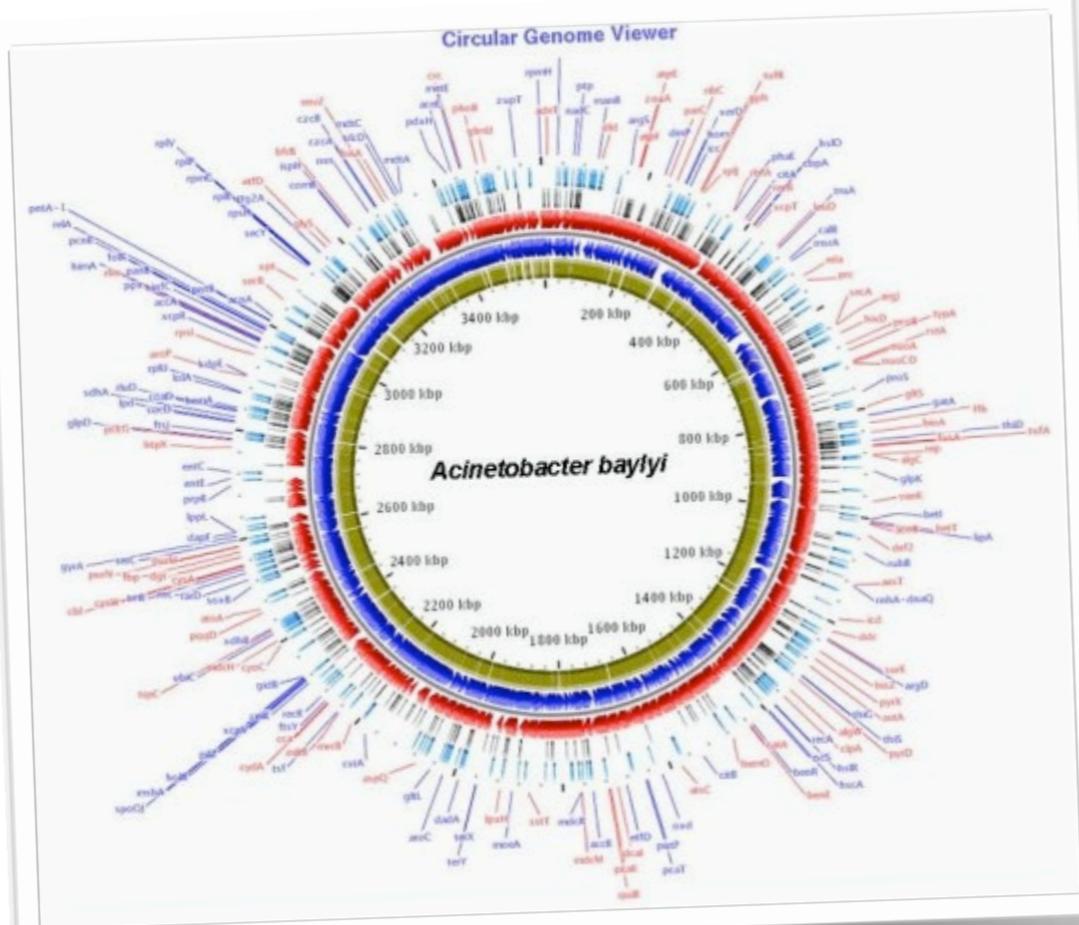
Received July 1, 2004; Revised August 30, 2004; Accepted September 21, 2004

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protein requires the tags to chromosomal biochemical pathways industries requires including insertion strain, and often alternative uses of manipulating efficiency of the

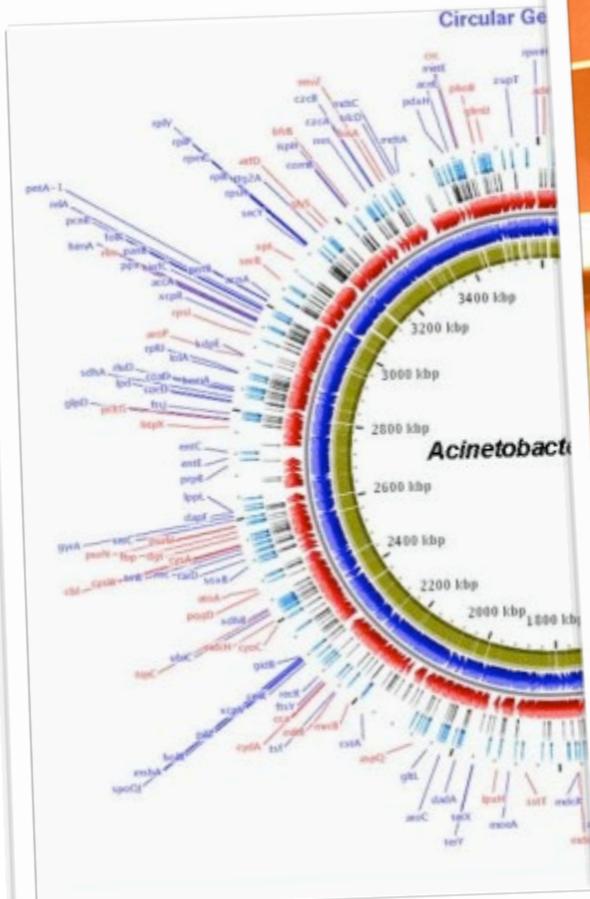
Many fields of upon primary forward, user-friendly *Caenorhabditis* challenging, but metabolism manipulation inevitable. The a reasonably simple *Saccharomyces* serves as the bacteria, the offers a relatively archetypal *E. coli* is relatively functional operon (1) the genetic material by the natural transformation recombination recombination prevent introduction achieve recombination antagonistic effects, necessitating their removal. All of these steps have been achieved (4). All of these steps have been achieved (4). All of these steps have been achieved (4).



# Acinetobacter

# Baylyi

- gram-negative
- genome seq
- naturally com



## An ideal model organism for genome engineering

by Pezo<sup>1,2</sup>, John Reader<sup>1</sup>, Volker Döring<sup>2</sup>, and Valérie de Crécy-Lagard<sup>1,\*</sup>

<sup>1</sup> Torrey Pines Road, La Jolla, California, USA

September 21, 2004

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tools make *Acinetobacter* ADP1 accessible to minimal microbial genetics expertise and very little equipment. They are also compatible with complete automation of genetic analysis and engineering protocols.

achieve recombination... necessitating their... have been achieved (4). All of these step... subject to unpredictable fai...

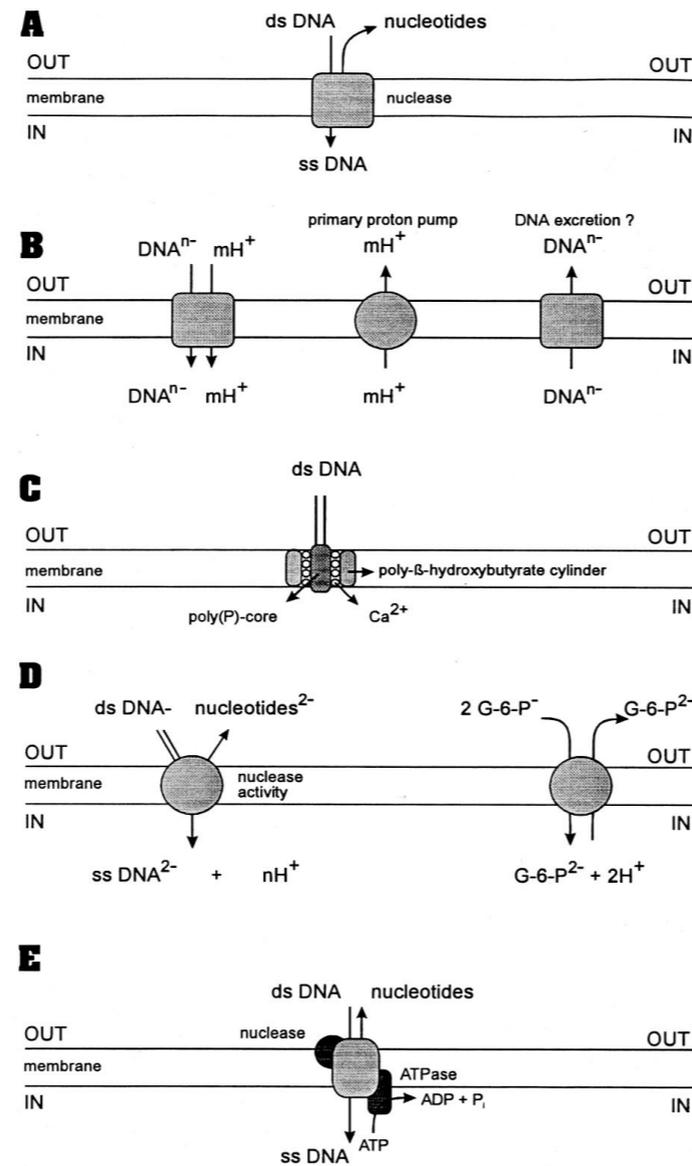


Fig. 2. Schematic representation of different models proposed to explain the mechanism and/or energisation of DNA uptake. (A) The hydrolysis of one strand provides the energy to drive the uptake of the complementary strand. (B) DNA is taken up electroneutrally in symport with protons and is thus driven by the transmembrane pH gradient. Hypothetically, DNA may be excreted electrogenically using the existing transmembrane electrical potential. (C) A pHB cylinder filled with a polyphosphate core [poly(P)-core] and stabilised by Ca<sup>2+</sup> may constitute a DNA transporter. The DNA is bound to the polyphosphate core, which is subsequently degraded allowing the DNA to be internalised. (D) Analogous to glucose-6-phosphate (G-6-P) transport by the *E. coli* UhpT transporter, negatively charged ds DNA is transported towards the cytoplasm, encounters an endonuclease and is hydrolysed, resulting in monovalent negatively charged ss DNA and nt. The monovalently charged ss DNA and nt liberate a proton into the more alkaline cytoplasm and the now divalent negatively charged nt are expelled. This results in an anion exchange reaction dependent on proton cycling. (E) ds DNA is hydrolysed by an endonuclease, the nt are liberated, and the ss DNA is internalised via an ATP-dependent uptake system.

# Acinetobacter sp. ADP1: an ideal model organism for genetic analysis and genome engineering

David Pazouk<sup>1,2</sup>, John Reader<sup>1</sup>, Volker Döring<sup>2</sup>, and David M. Karp<sup>1,\*</sup>

Nucleic Acids Research, 2004, Vol. 32, No. 19 5789

expression of the *tdk* gene, together with supplemental thymidine, is essential in the absence of *thyA* activity as *Acinetobacter* ADP1 does not contain an endogenous *tdk* allele.

## DISCUSSION

*Acinetobacter* ADP1 provides a remarkably simple, inexpensive and robust model system for genetic manipulation. Most of the existing antibiotics and antibiotic resistance cassettes tested here were functional in ADP1. These markers were used to build positive/negative selection cassettes, which were in turn used to efficiently construct a wide variety of mutations including gene disruptions and deletions, expressed chromosomal insertions, tagged chromosomal genes and various combinations of these types of mutations. Moving mutations from one strain to another was as straightforward as amplifying the mutation from the donor strain and inoculating a growing culture of the recipient strain with the raw PCR product, or even simply transforming with purified genomic DNA from the donor strain. All constructions shown here utilized approximately 1 kb flanking regions to specifically integrate constructs into the ADP1 genome. Attempts to use shorter flanks were generally unsuccessful. Splicing PCRs with shorter flanks resulted in high yields of product, but no transformants were recovered in selection. This limitation may be due to the minimal volume of our transformations.

The techniques used in this paper were reiterative and highly similar. All manipulations aside from the initial cassette constructions were performed using splicing PCR and selective plating only. Primers were chosen using very simple rules based on melting temperature, GC content, potential primer misextension and position with regard to the affected ORF. All PCRs were performed in identical conditions. Furthermore, all direct manipulations of ADP1 cells were performed in minimal volumes similar to those found in standard 96-well plate formats. The high rate of success under these conditions suggests that this system could be readily adapted to an automated platform, allowing for all steps to be achieved robotically. Similarly, the simplicity of the ADP1 genetic engineering protocols developed here should allow this system to be adopted by both training institutions and laboratories that have a need for an inexpensive and user-friendly method for generating genetically manipulated strains. The *Acinetobacter* constructions described here required only a PCR machine, incubators and access to oligonucleotide synthesis. It is notable that the majority of all constructions, including the cassette constructions, antibiotic tests, resistance allele tests and associated design efforts, were achieved by one researcher (D.Metzgar), with very little previous genetic manipulation experience in the course of one year. Attempts by other researchers to use the same system were generally equally successful, but it was noted that success was dependent on careful and consistent choice of primer sequences (see Materials and Methods).

Together, the paired traits of natural competence and recombination allow for rapid production of genetically engineered strains. Replacement of existing genetic models with *Acinetobacter* ADP1 should be straightforward, as ADP1 presented no particular challenges with respect to culturing conditions, bioinformatic prediction of metabolic pathways, or

mutational stability in culture, even in conditions that were optimized for *E.coli* rather than *Acinetobacter*. In the short time during which this model has been under development in our laboratory, it has allowed us to test a number of biological questions (27) in a much more efficient manner than would have been possible with previously utilized model organisms.

## ACKNOWLEDGEMENTS

We thank L. Nicholas Ornston for critical reading of this work, and Integrated Genomics for access to ERGO. This work was supported by National Institute of Health Grant GM23562, National Science Foundation Grant MCB-0128901 and a fellowship from the National Foundation for Cancer Research.

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# ADP1:

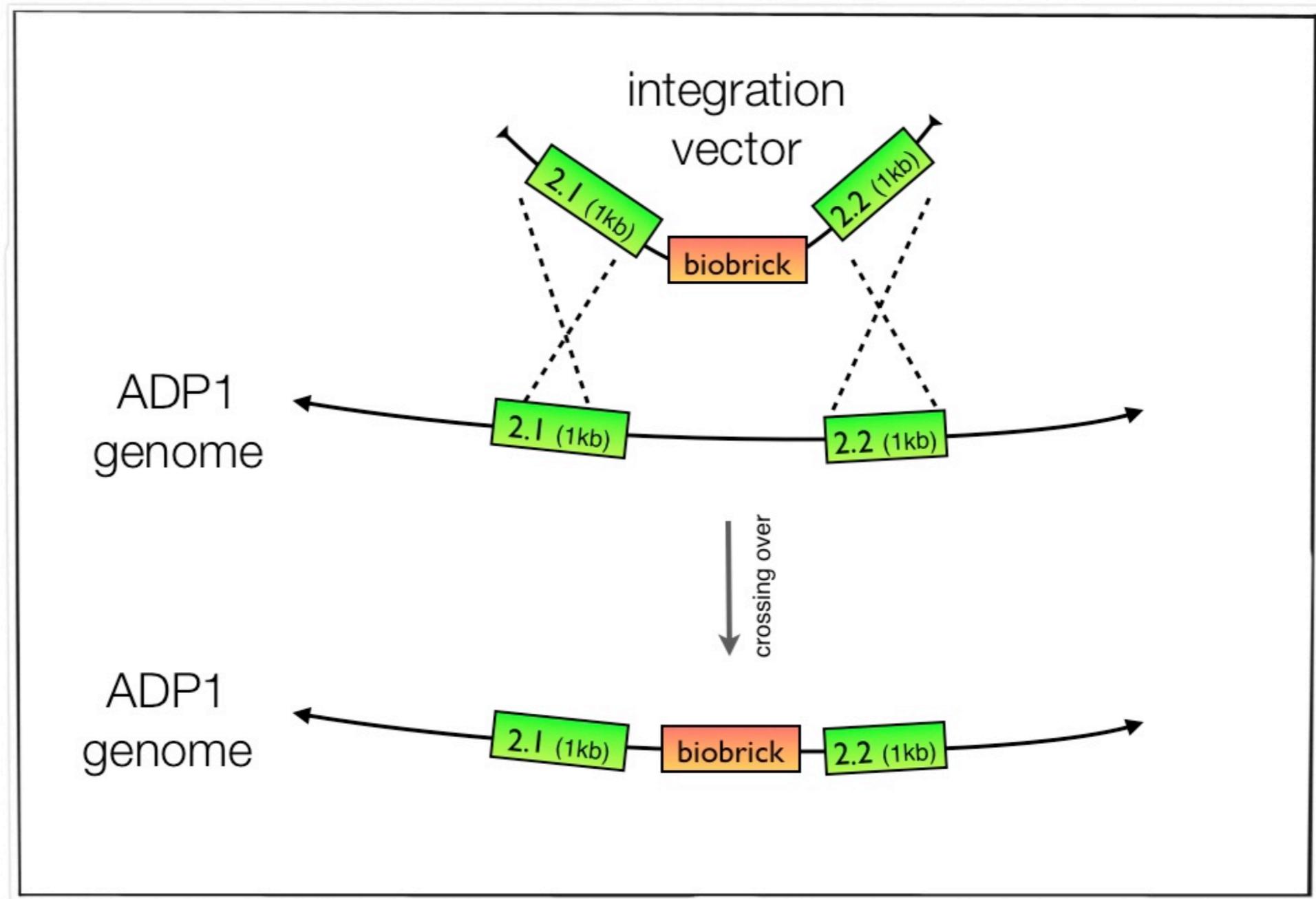
- simple, inexpensive, robust model system
- transferring parts is easy:
  - amplify donor genome, add to growing culture
- Techniques are reiterative and pseudo-idempotent; good for automation
- Equipment needed:
  - PCR
  - incubators
  - oligo synthesis
- All accomplished by one researcher over one year with minimal prior experience w/ genetic engineering

- 2.1 - j04550 - 2.2: fail (RFP)
- 2.1 - p1003 - 2.2 : worked (Kan resistance)

not all E. coli promoters compatible?



Samantha Burke



# Registry of Standard Biological Parts

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[BBa J04450 Main Page](#) [Part Design](#) [Physical DNA](#) [Hard Information](#) [Experience](#)

## Part:BBa\_J04450



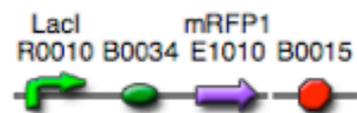
DNA Available

Experience: Works

Designed by Tamar Odle Group: iGEM\_Davidson

Entered: 2005-06-09

### RFP Coding Device switched on by IPTG



Constitutive RFP device. The colonies are clearly red in color under natural light after about 18 hours. Smaller colonies are visibly red under UV. The RFP part does not contain a degradation tag and the RBS is strong.

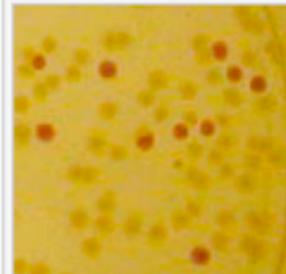
### Pictures



BBa\_J04450 visualized under non-UV lightbox



BBa\_J04450 visualized under 254nm wavelength UV lightbox



BBa\_J04450 Colonies

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# Registry of Standard Biological Parts

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[BBa P1003 Main Page](#) [Part Design](#) [Physical DNA](#) [Hard Information](#) [Experience](#)

## Part:BBa\_P1003



**DNA Available**  
**Experience: Works**

Designed by Reshma Shetty Group: Knight Lab, MIT

Entered: 2006-01-23

### kanamycin resistance cassette

Kanamycin resistance cassette including promoter and coding sequence. It lacks a terminator.

### Usage and Biology

- Used for modular BioBrick vector construction using BioBrick base vector [BBa\\_I51020](#).

### References

#### Engineering BioBrick vectors from BioBrick parts

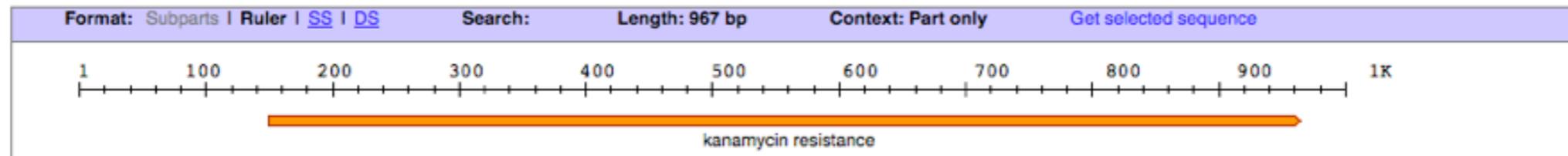
*Journal of Biological Engineering*, 2008 Apr 14;2:5

Reshma Shetty, Drew Endy, Tom Knight

[URL](#)

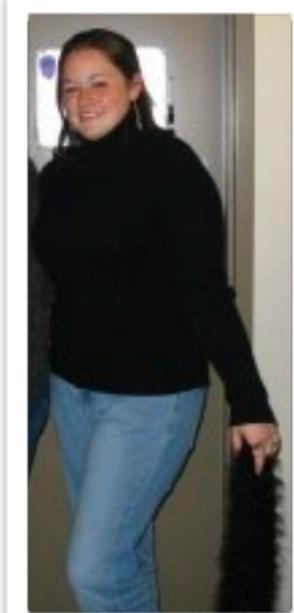
[GenBank EU496093](#)

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Samantha Burke

- 2.1 - j04550 - 2.2: fail (RFP)
- 2.1 - p1003 - 2.2 : worked (Kan resistance)

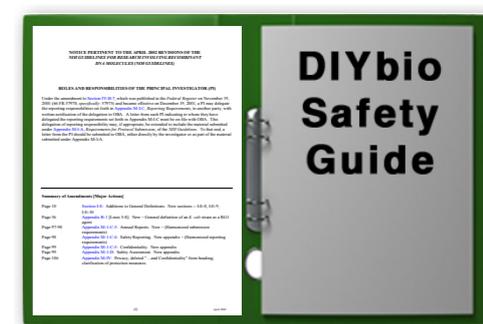
Genes work;  
Promoters didn't?

## NEXT

---

- RFP ADP1 !
- ID promoters that work in ADP1
- minimize 2.1 & 2.2
  - 1000bp -> 100bp
- or find ways of using plasmids
  - don't integrate into genome
  - circular, not linear
  - easier to isolate (miniprep)

# Safety



“

Dear DIY bio people,  
Do you think people might be receptive to some measure  
of absolute prohibition, along the lines of:



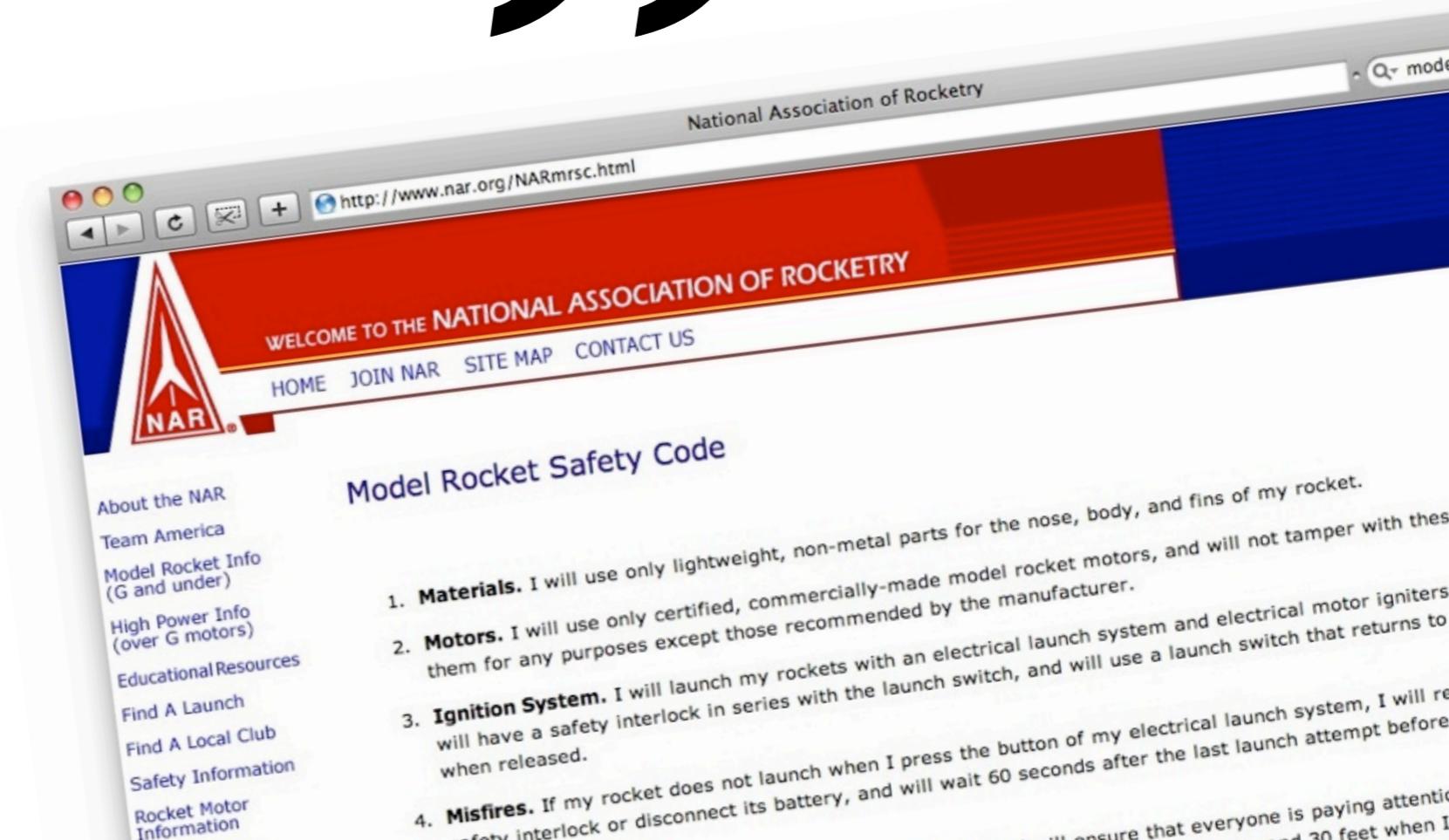
*"Thou shalt not design, nor build, nor isolate, nor  
modify, nor grow, nor release any self replicating  
organism, with the intent of causing harm?"*

”

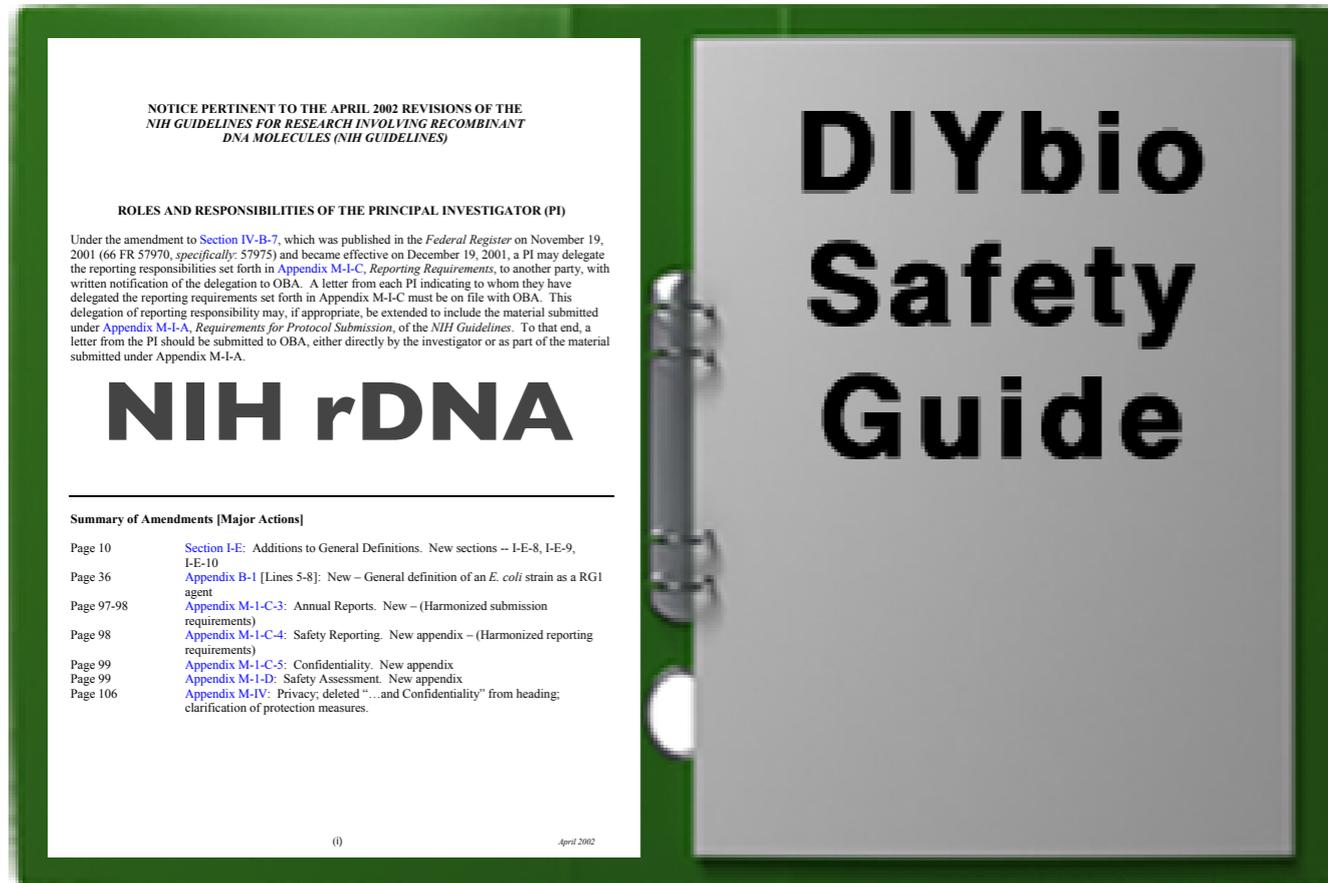
-Roger Brent

## DIYbio creed:

Safe as an undergrad lab  
*or better:*  
safe enough to eat



# safety@diybio.org



Need safety norms  
before we can expect  
broad-scale innovation  
must preempt stupidity

**social hack:** what is the 1-5  
year strategy for DIYbio as a  
movement to be successful?

**safety working group:** safety@diybio.org

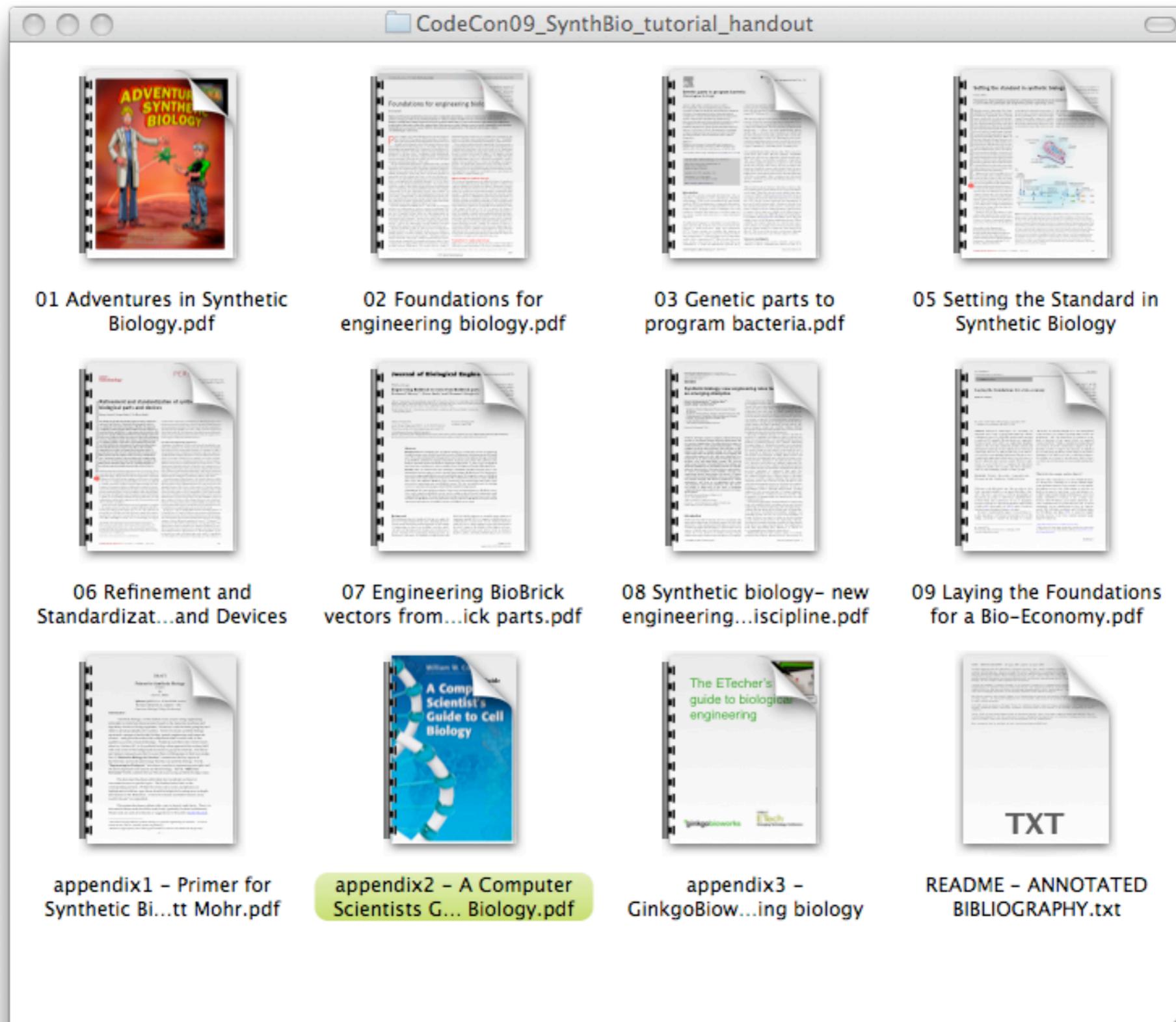
# get involved?

Periodic meetups in San Francisco, Boston, NYC, Seattle and Chicago - email [diybio@googlegroups.com](mailto:diybio@googlegroups.com)

visit [diybio.org](http://diybio.org) for more info



# <http://bit.ly/diybioecc>



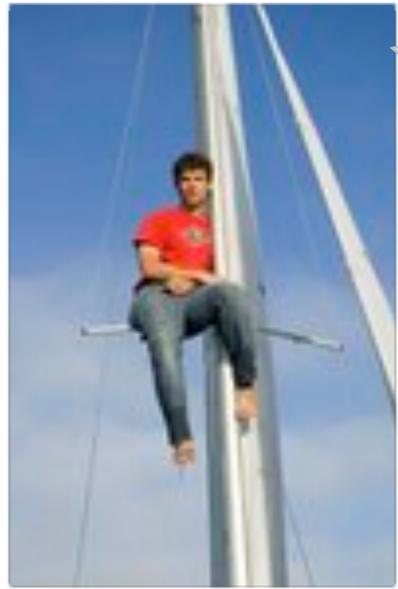
# 5-min dna extraction in a shot glass

just add:  
saliva + soap + salt +  
160 proof rum



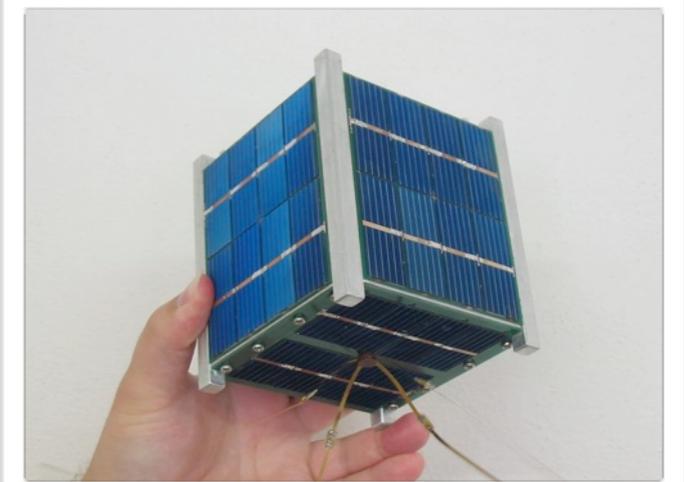
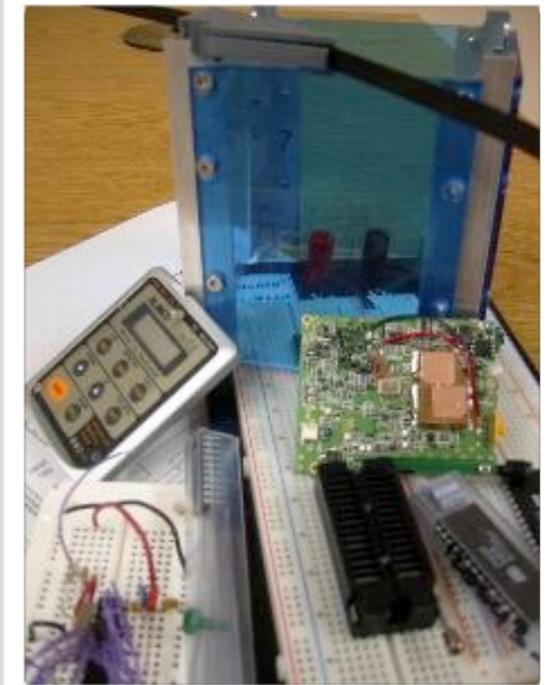
A screenshot of the Instructables website. The page title is "5 minute DNA Extraction in a Shot Glass". The page features a navigation bar with categories like Home, Explore, Community, Submit, Home, Kids, Life, Music, Offbeat, Outdoors, Pets, Ride, Science, Sports, Tech. The main content area includes an "Intro" section with a description of DNA extraction, a "Materials &amp; Set Up" section, and a series of step-by-step images. On the right side, there is a sidebar with "INFO" (4.03 rating, 19 ratings), "SUBSCRIBERS" (29,346 views), and "RELATED" articles like "Mapping Microbes" and "The Science of DNA!".

# DIYbio in space



Eric Stackpole

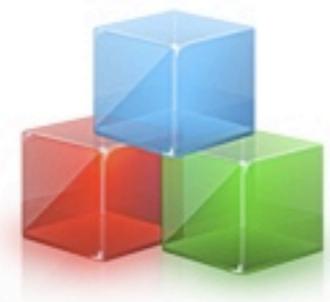
- \$1000 DIY cubesat
- launching in 6 mo
- altoids-size DIYbio payload
- 100-200g
- 5v, 100 mA
- -80c to 100c
- DTMF downlink



<http://bit.ly/ReadySatGo>

**Software interchange  
formats for  
libraries and modeling.**

# PoBoL

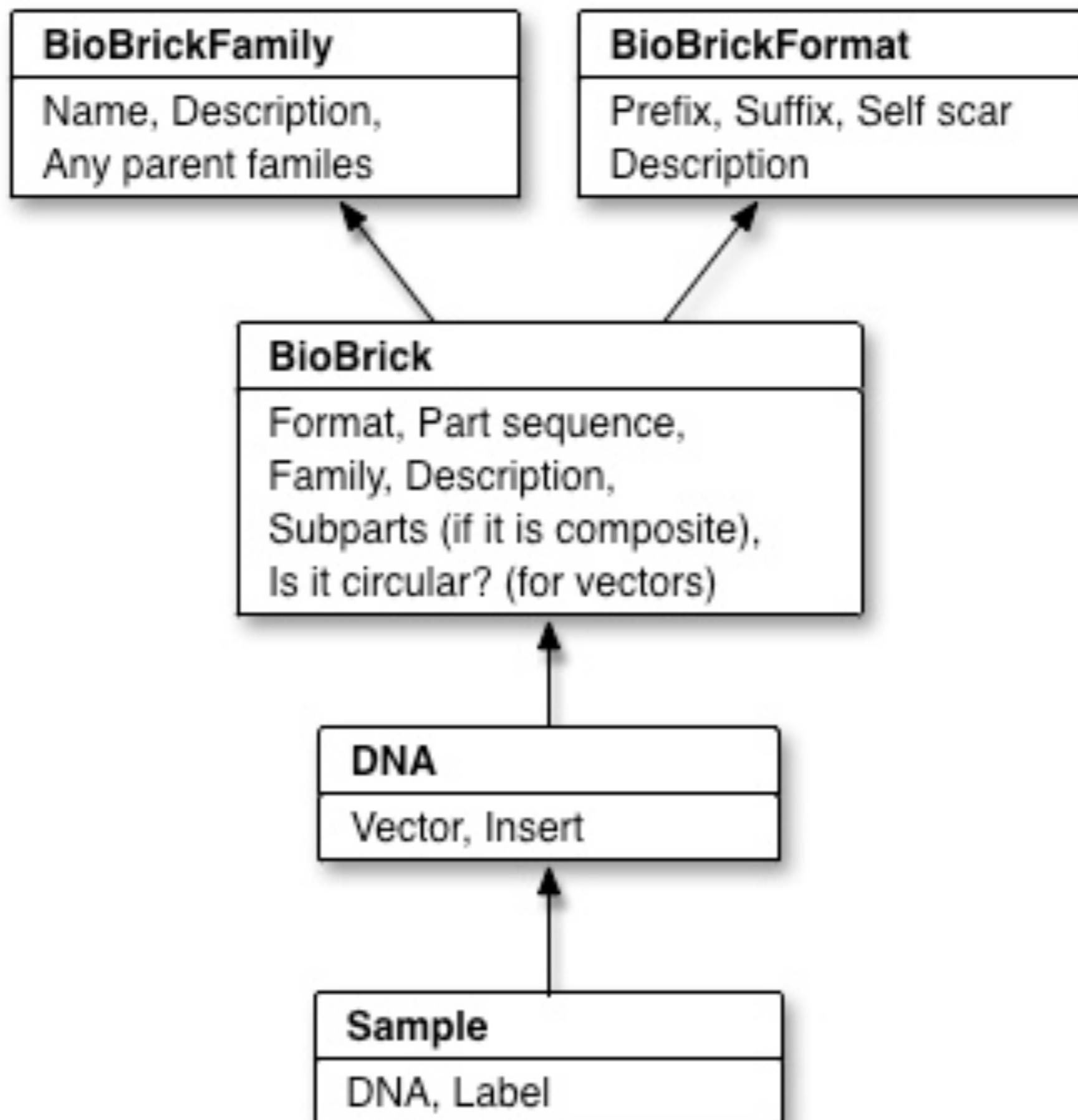


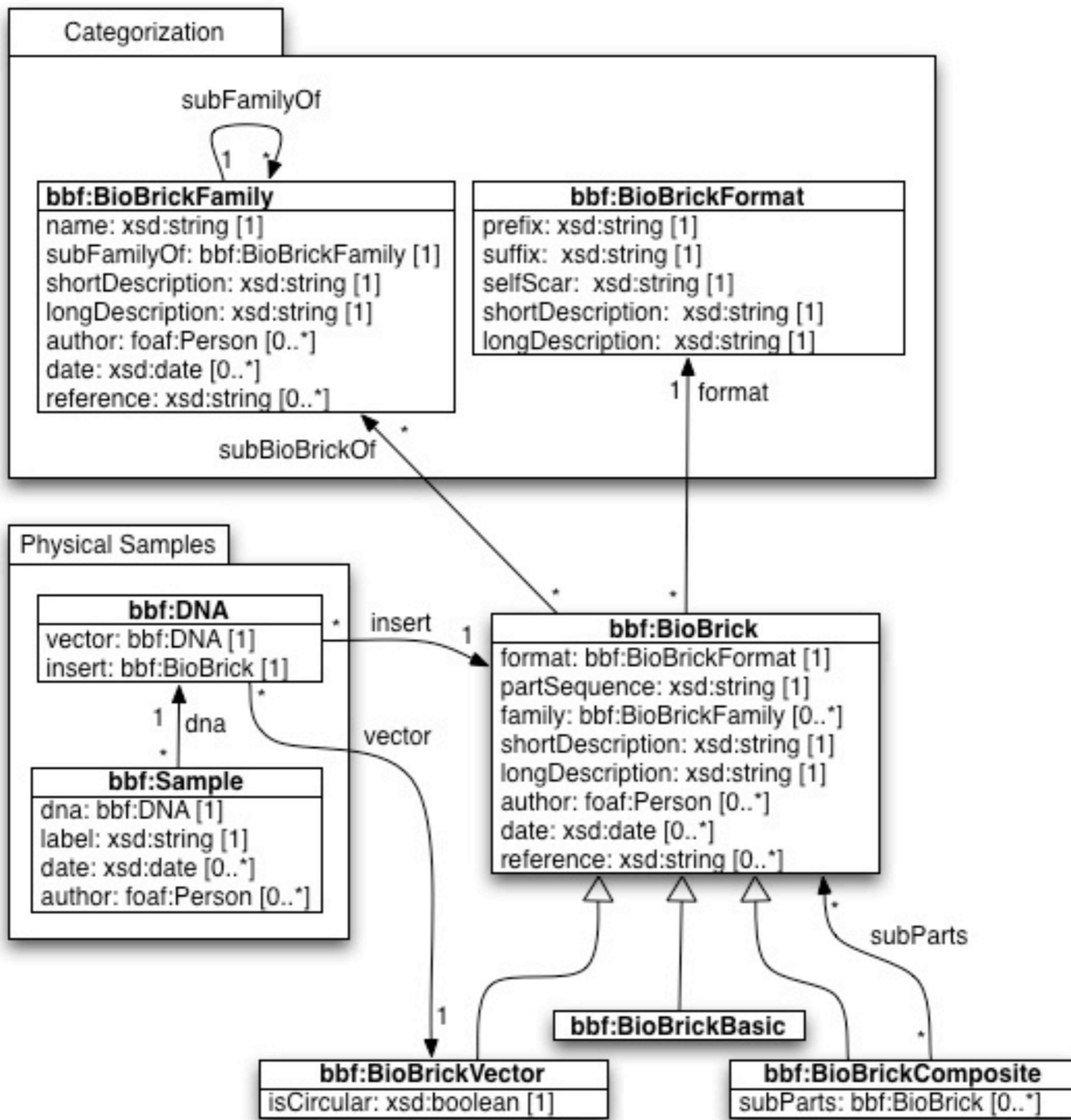
The PoBoL project aims to define an RDF-based data exchange standard for standard biological parts, [BioBricks](#). The goals are to:

- Capture the minimal information needed to describe a BioBrick.
- Allow the connection of additional data to BioBricks.
- Remain open for extension and interlinking.

“PoBoL” stands for Provisional BioBrick Language. “Pobol” is also Welsh for “people”, which reflects our desire for a community-driven format. Find out more at our:

- [OpenWetWare Wiki page](#)
- [Google Group](#) for discussion
- [Google Code project](#) for specifications and code





@prefix xsd: <<http://www.w3.org/2001/XMLSchema#>> .  
@prefix rdfs: <<http://www.w3.org/2000/01/rdf-schema#>> .  
@prefix rdf: <<http://www.w3.org/1999/02/22-rdf-syntax-ns#>> .  
@prefix owl: <<http://www.w3.org/2002/07/owl#>> .  
@prefix foaf: <<http://xmlns.com/foaf/0.1#>> .  
@prefix bbf: <<http://www.biobricks.org/rdf/0.1#>> .  
@prefix bbx: <<http://www.biobricks.org/rdf/formats#>> .  
@prefix usr: <<http://www.partsregistry.org/users#>> .

:BBa\_P1010

rdf:type bbf:BiobrickBasic ;  
bbf:author usr:Leon\_Chan ;  
bbf:date "2008-05-31"^^xsd:date ;  
bbf:format bbx:BBa ;  
bbf:longDescription "negative selection marker for construction plasmids. Only certain E.coli strains (DB3.1) can survive the expression of this marker."^^xsd:string ;  
bbf:partSequence "actggctgtgtata.....atccacgcgt"^^xsd:string ;  
bbf:shortDescription "cccdB death cassette"^^xsd:string .

**Athena**

# Basic Features

The screenshot shows the Athena software interface with several callout boxes pointing to specific features:

- Construct a module from the selected items**: Points to the 'Make Module' menu item.
- Simulate a model or visualize rate equations**: Points to the 'Simulation ...' menu item.
- Get a list of properties for the selected item(s)**: Points to the 'Properties ...' menu item.
- Automatic transcription rate derivation**: Points to the 'Transcription' menu item.
- View sequence of selected item(s)**: Points to the 'Sequence View' menu item.
- Interface to the R language**: Points to the 'Script Console' menu item.
- Use this to connect two modules by indicating where they overlapping**: Points to the 'Statistics/Coding' menu item.
- Add a modifier o a reaction or a transcription factor to a promoter**: Points to the 'Add Modifier' icon in the toolbar.
- Make reactions with reactants being converted to products**: Points to the 'Add Reaction' icon in the toolbar.
- Insert genes, promoters/ operator, terminators, rbs, molecular species, etc..**: Points to the 'Insert' icon in the toolbar.

The right-hand side of the interface shows the 'Module Viewer' panel with the following sections:

- Summary** / **Properties** tabs
- Reactions** table:

ID	Rate
----	------

- Molecular Species** table:

ID	Concentration	Constant
----	---------------	----------

- DNA Parts** table:

ID	PoPS
----	------

- Parameters** table:

ID	Value
----	-------

New Document

# Looking at the complete model

The screenshot displays the Athena software interface. The main workspace shows a biological model diagram with components: t1, lacI, c1, pCL1, pCL2, c2, and t2. Above these are molecules LacI, CL1, and CL2. Reactions J0 through J5 are indicated with arrows and inhibition symbols. A red box highlights the diagram. The Module Viewer panel on the right shows the following data:

Module ID: M0

Reactions

ID	Rate
J0	d2
J1	$k0 \cdot CL2$
J2	d1
J3	$k0 \cdot CL1$
J4	lacI

Molecular Species

ID	Concentration	Constant	Exposed
CL1	0	<input type="checkbox"/>	<input type="checkbox"/>
CL2	0	<input type="checkbox"/>	<input type="checkbox"/>
LacI	0	<input type="checkbox"/>	<input type="checkbox"/>

DNA Parts

ID	PoPS	Exposed
d1	0	<input type="checkbox"/>
d2	$k1 / (1 + CL1^h)$	<input type="checkbox"/>
lacI	0	<input type="checkbox"/>

Parameters

ID	Value
h	2
k0	1
k1	5

The Module Viewer shows the rates of each reaction, concentration of all molecules, PoPS across each part, and parameters of the module.

The module viewer updates when you select different modules. If you select nothing, everything will be shown.

# Property Viewers

The screenshot shows the Athena software interface. The main window displays a metabolic pathway diagram with components: t1, lacl, cl1, pCL1, pCL2, cl2, and t2. Above the pathway are regulatory elements: Lacl, CL1, and CL2. Reactions are labeled J1 through J5. The pCL2 component is highlighted with a red box. On the right, the Part Viewer panel is open, showing the properties for the selected part pCL2. The Activity is  $k1/(1+CL1^h)$ . The Modifiers table shows CL1 with a stoichiometry of 1. The Parameters table shows h with a value of 2 and k1 with a value of 5.

Selected item influences the properties shown on the right

As you select different items on the screen, the viewer will be updated.

For example, when a part is selected, the Part Viewer replaces the Module Viewer

ID	Stoichiometry
CL1	1

ID	Value
h	2
k1	5

# Connecting Parts (DNA stands)

The screenshot shows the Athena software interface. The main workspace contains two DNA parts: a Promoter\_0 (represented by a green arrow) and a Terminator\_0 (represented by a red octagon). A red line connects the two parts, indicating they are linked. A text box in the foreground provides instructions on how to connect parts:

Connecting two parts together:

- Option 1: bring one part close enough to the other, so that they touch
- Option 2: Click and drag the red circle so that the red line meets the other part

The Part Viewer panel on the right shows the properties for the selected part, Promoter\_0. It includes fields for Part ID (Promoter\_0) and Activity (10). There are also sections for Available Rates, Modifiers, and Parameters.

ID	Stoichiometry
----	---------------

ID	Value
----	-------

# Making a Module

The screenshot displays the Athena software interface. At the top, the menu bar includes File, Edit, Plugins, Simulators, SBW, Selected, and Help. The toolbar contains various icons, and the 'Make Module' option is highlighted. The main workspace shows a reaction network diagram with nodes X0, S1, S2, S3, S4, and X1, connected by transitions J0, J1, J2, J3, and J4. A text box labeled 'Selected items' points to the nodes. A callout box provides instructions on how to create a module:

Select all the items you want to construct the module with, and click "Make Module". Other options:

- Option 1) Ctrl+M
- Option 2) Right click on selected items, and select "Make Module" from Module Tool menu

The bottom panel shows the Simulation Window with tabs for Simulation, Vector field, and Rate Functions. The Simulation tab is active, showing a graph of Concentration vs. time. The graph has a y-axis labeled 'Concentration' ranging from 0.0 to 4.0 and an x-axis labeled 'time' ranging from 0 to 60. The simulation parameters are: Start Time: 0, End Time: 50, Num Points: 1000, and X-axis: (dropdown menu). The status bar at the bottom indicates 'Moving Selected'.

# Simulating a Module

The screenshot shows the Athena software interface. The main window displays a simulation graph with concentration on the y-axis (0.0 to 4.0) and time on the x-axis (0 to 60). The graph shows four oscillating curves: S1 (blue), S4 (green), S3 (magenta), and S2 (cyan). The S1 curve has the highest amplitude, peaking at approximately 3.5. The S4 curve peaks at about 2.5, S3 at 1.5, and S2 at 1.0. The graph is titled 'Simulation Window' and has tabs for 'Simulation', 'Vector field', and 'Rate Functions'. The 'Rate Functions' tab is active, showing a table of reaction rates.

On the right side, the 'Module Viewer' is open, showing the 'Properties' tab for a module named 'feedback'. It includes a 'Reactions' table and a 'Molecular Species' table.

At the top, a 'Simulation Window' button is highlighted with a callout box that says 'Opens the Simulation window'. Below it, a diagram shows a module with two nodes, Node0 and Node1, connected by reactions J0 and J1. A callout box points to this diagram with the text 'Only the selected Module is simulated so that you can analyze different modules independently'.

At the bottom left, a callout box points to the 'Rate Functions' tab with the text 'Use the Rate Functions tab to see how different variables affect the different rates'.

Opens the Simulation window

Only the selected Module is simulated so that you can analyze different modules independently

Use the Rate Functions tab to see how different variables affect the different rates

Module Viewer

Summary Properties

Module ID: feedback

Schematic View  Encapsulate

Reactions

ID	Rate
J0	$J0\_VM1 * (X0 - S1 / J0\_Keq1) / (1 + X0 + S1 + pow(10 * S1 - 2 * S2, 2))$
J1	$(10 * S1 - 2 * S2) / (1 + S1 + S2)$
J2	$(10 * S2 - 2 * S3) / (1 + S2 + S3)$
J3	$(10 * S3 - 2 * S4) / (1 + S3 + S4)$

Molecular Species

ID	Concentration	Constant	Exposed
S1	0	<input type="checkbox"/>	<input type="checkbox"/>
S2	0	<input type="checkbox"/>	<input type="checkbox"/>
S3	0	<input type="checkbox"/>	<input type="checkbox"/>

DNA Parts

ID	PoPS	Exposed

Parameters

ID	Value
J0_h	10
J0_Keq1	10
J0_VM1	10
J4_KS4	0.5
J4_V4	2.5

# Connecting two or more modules

The screenshot displays the Athena software interface with the following components:

- Simulation Window:** Shows a network diagram with nodes X0, S1, S2, S3, S4, and X1. Reactions J0, J1, J2, and J3 are indicated between nodes. A red dashed arrow points from S4 to Node0 in the Module Viewer.
- Module Viewer:** Shows the 'equilib' module with a summary of reactions and molecular species.
 

ID	Rate
feedback_J0	$feedback\_J0\_VM1 * (X0 - S1 / feedback\_J0\_Keq1)$
feedback_J1	$(10 * S1 - 2 * S2) / (1 + S1 + S2)$
J2	$(10 * S2 - 2 * S3) / (1 + S2 + S3)$
J3	$(10 * S3 - 2 * S4) / (1 + S3 + S4)$

ID	Concentration	Constant
Node1	0	<input type="checkbox"/>
S1	0	<input type="checkbox"/>
S2	0	<input type="checkbox"/>
- Simulation Graph:** A plot of Concentration(s) vs time. The legend includes S1 (blue), Node0 (green), S4 (purple), S3 (cyan), and S2 (magenta). The graph shows oscillatory behavior for S1, S2, S3, and S4, and a steady increase for Node0.
- Simulation Window Controls:** Includes 'Reset' and 'Simulate' buttons, and a 'simulation graph' section with 'Start Time: 0', 'End Time: 50', and 'Num Points: 1000'.

**Annotations:**

- Link two modules together using this button:** Points to the connection button in the Simulation Window toolbar.
- S4 and Node0 are now the same molecule, but the separate modules have not been altered:** Points to the red dashed arrow connecting S4 to Node0.
- Observe that there is no Node0 in the module viewer:** Points to the Module Viewer window.

**BioJADE**

Library default

Part BBa\_9999

Aspects: Schematic

FunctionalNetwork

DNA

Simulation

Icon

Select

Stamp

Wire

Cut

Copy

Paste

Zoom In

Zoom Out

Save

Revert

Optimize

Compile

Wire

## Templates

nand2

not

reporter

source

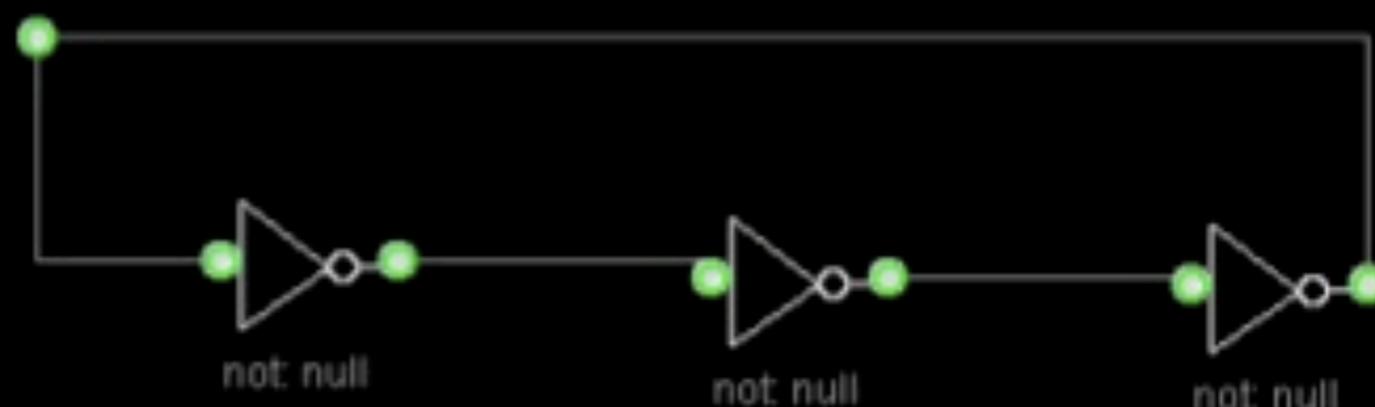
or2

sender

Endy.1

Reverse Terminators

BBa\_C0082



Used Parts

## Help!

Wire Mode: Click mouse to start a wire. Click again to stop the wire, or ALT-click to set a waypoint. CTL-Click on terminals and press w to automatically wire together terminals.

## Part Information

Part Type : Other

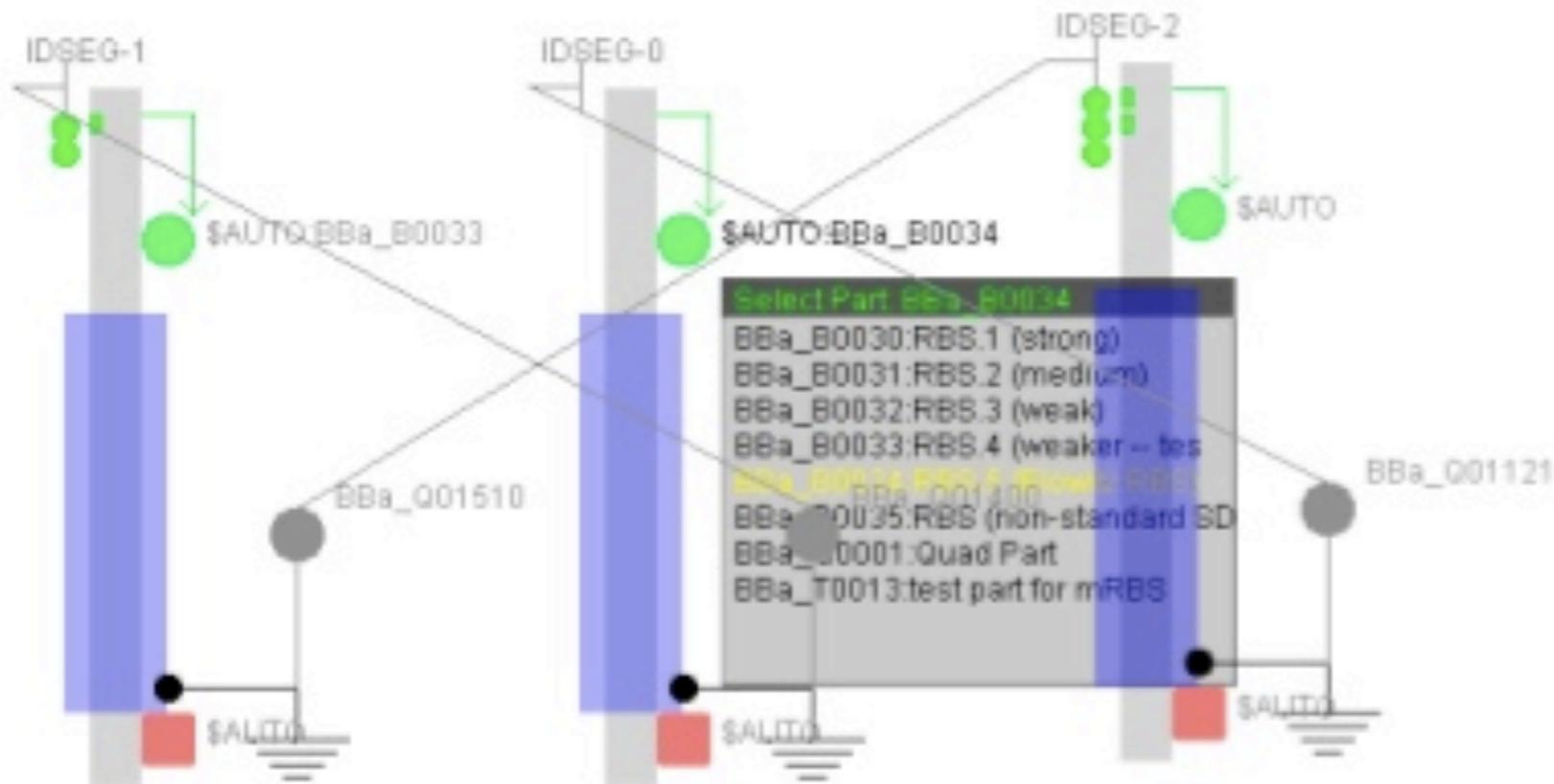
Designer : Jonathan Goler

Description : demo repressilator

Notes :



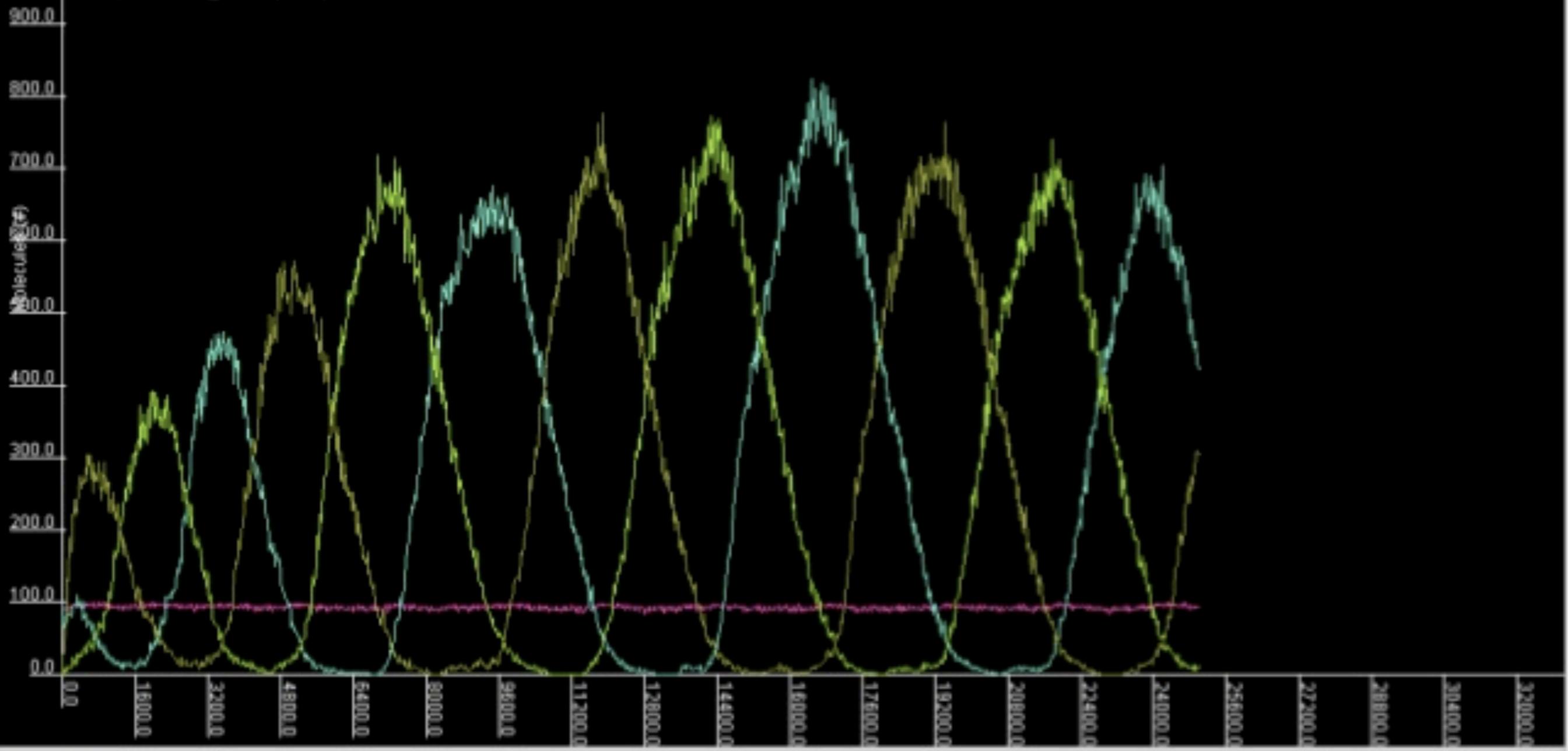
BBa\_9999



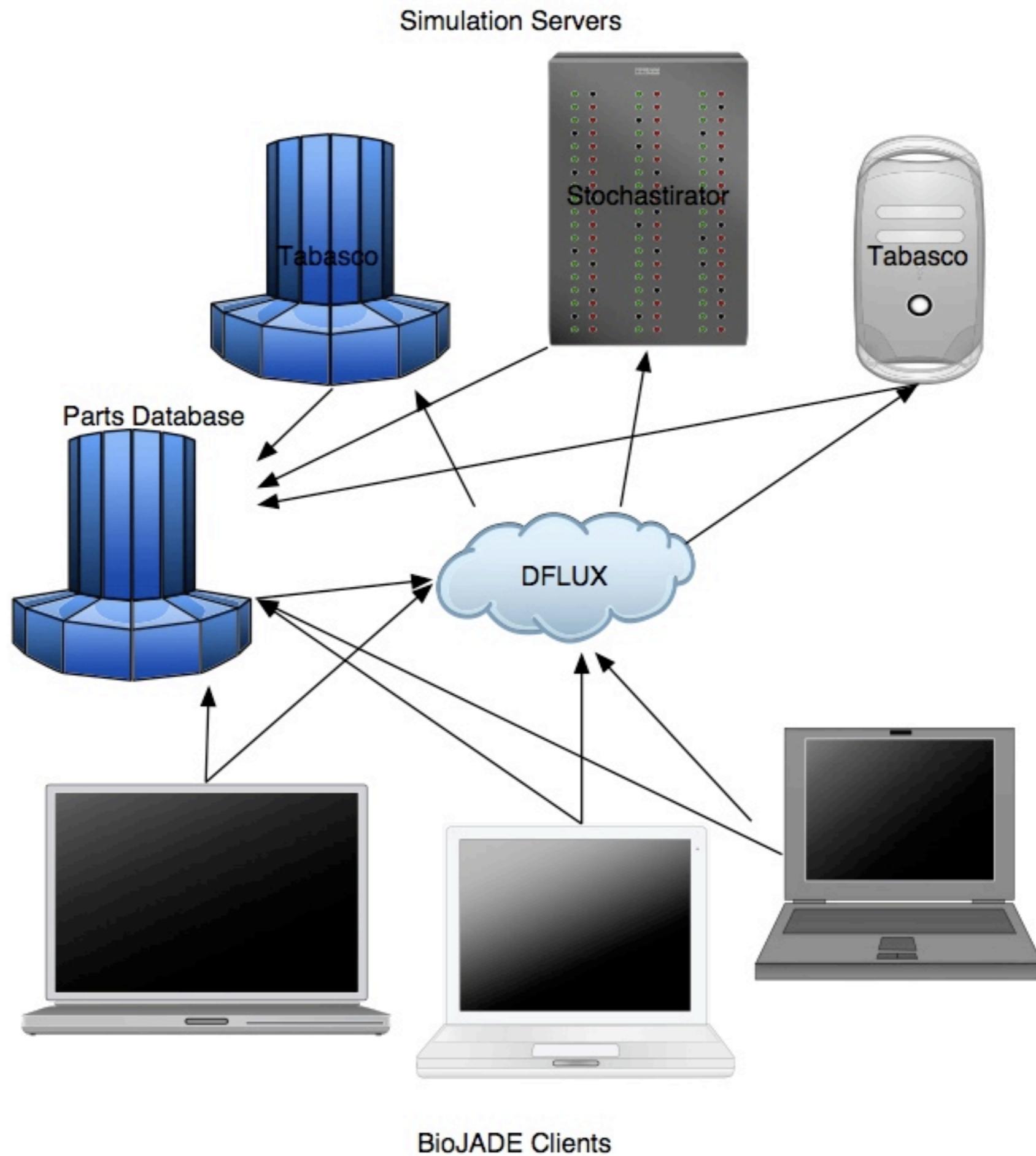
FunctionalNetwork

Data Points Zoom In (0) Zoom Out (0) Zoom In (r) Zoom Out (r) Colors View Parameters Print Close

- poly(100.0)
- species-BBa\_D0001(824.0)
- species-BBa\_D0002(773.0)
- species-BBa\_D0003(776.0)







Simulation Servers

Tabasco

Stochastirator

Tabasco

Parts Database

DFLUX

BioJADE Clients

## How to use this site:

GenoCAD™ is an experimental tool allowing you to build and verify complex genetic constructs derived from a library of standard genetic parts.

© 2007 Virginia Bioinformatics Institute  
contact information

## Design

- 1 Think of a construct
- 2 Build its structure
- 3 Select its parts
- 4 Download your sequence

Start a Design ▶

## Validate

- 1 Upload your sequence
- 2 Click validate
- 3 View structure

Validate ▶

