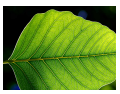
System Engineering

20.109(F11)
M2D3 lecture
10.20.11

New tools for reliable engineering of complex biological systems



Synthetic Biology

Standardization

Abstraction

Synthesis A+A+C+T+T…

Genetic Engineering

rDNA

Sequencing

PCR

Registry of Standard Biological Parts

page discussion view source history

Welcome to the Registry of Standard Biological Parts.

The Registry is a collection of ~3200 genetic parts that can be mixed and matched to build synthetic biology devices and systems. Fo the Registry is part of the Synthetic Biology community's efforts to make biology easier to engineer. It provides a resource of availab teams and academic labs.

The Registry is based on the principle of "get some, give some". Registry users benefit from using the parts and information available from the engineered biological systems. In exchange, the expectation is that Registry users will, in turn, contribute back information and data on existing they make to grow and improve this community resource.

Catalog of parts & devices | Help | Users & groups (Apply for an account) | DNA repositories

Registry tools
- Search parts (?)
- Add a part
- Request a part
- Send parts to the Registry
- Sequence analysis

BIO BRICKS foundation

http://bbf.openwetware.org/

"BBa" standard biological part

any DNA-encoded biological function

P BBa_B0034 S

"prefix" (EcoRI, XbaI)

"suffix" (SpeI, PstI)

Physical Composition of Standard Biological Parts

BioBrick™ Assembly Help

ginkgobioworks

NEW ENGLAND BioLabs Inc.

Using the kit

The BioBrick Assembly Manual provides step-by-step instructions for assembling BioBrick parts using the BioBrick Assembly Kit. To read more about the BioBrick system and browse the BioBrick collection, visit the Registry of Standard Biological Parts.

Get the kit from New England Biolabs. Download the manual or browse it below.

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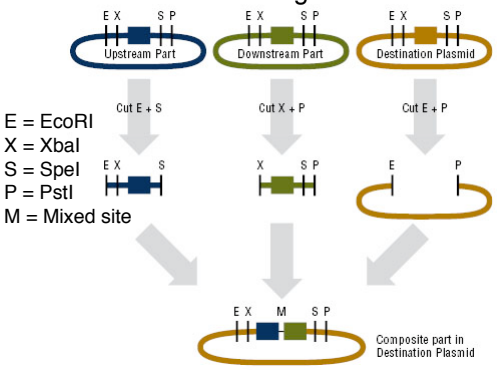
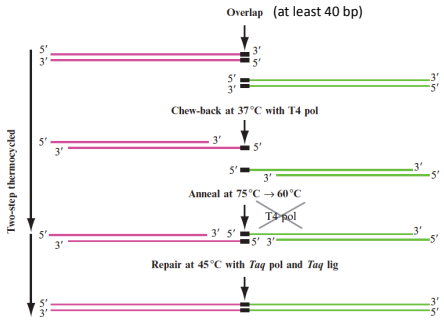
Physical Composition of Standard Biological Parts



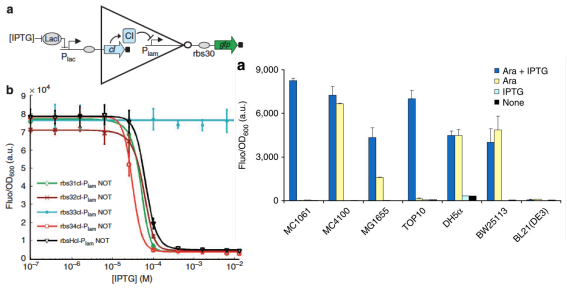
Alternative: Standard Physical Composition "Gibson Assembly"



Methods in Enzymology (2011) 498: 349-361

Engineering modular and orthogonal genetic logic gates for robust digital-like synthetic biology

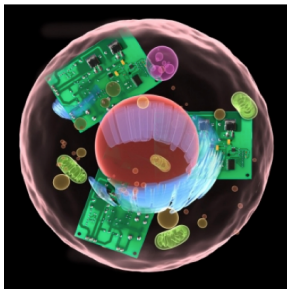
Baojun Wang, Richard I Kitney, Nicolas Joly & Martin Buck



Nature Communications **2**, Article number: 508 | doi:10.1038/ncomms1516
Received 21 April 2011 | Accepted 21 September 2011 | Published 18 October 2011

Mimicking cells with transistors

Analog — rather than digital — circuits could enable models of biological systems that are more efficient, more accurate and easier to build.



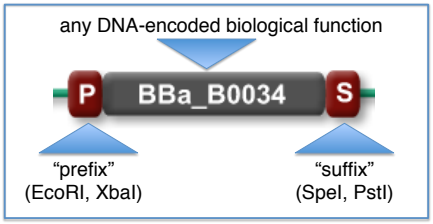
"The signals in cells are not ones or zeroes. That's an overly simplified abstraction that is kind of a first, crude, useful approximation for what cells do. But everybody knows that's really wrong." Prof. Rahul Sarpeshkar, MIT's Research Laboratory of Electronics (RLE)

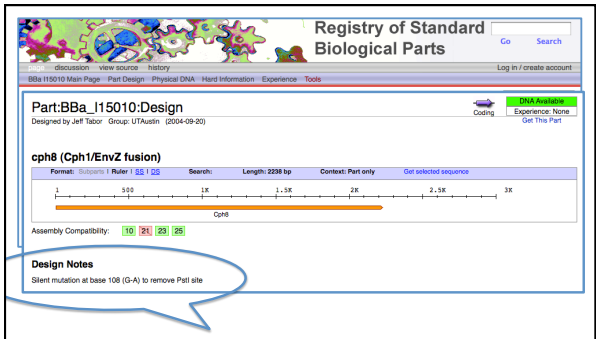
http://web.mit.edu/newsoffice/2011/analog-systems-biology-0928.html

Let's "BioBrick" Cph8 (= Cph1/EnvZ fusion)

Ginkgo's Part Design Tool

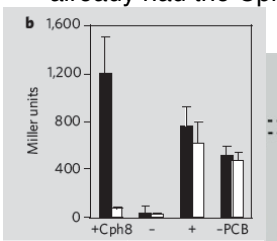
http://ginkgobioworks.com/cgi/primer.cgi



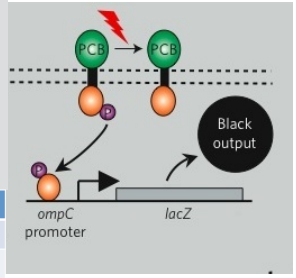


"Silent mutation at base 108 (G-A) to remove PstI site"

Wouldn't it be great if the Registry already had the Cph8 mutants we want?

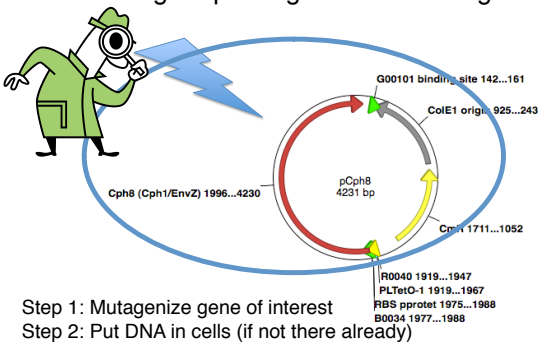


Red light	β-gal
1	0
0	1



Nature (2005) **438**, 441-442

Looking for parts: genetic screening



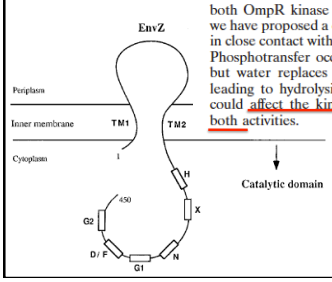
Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells (if not there already)
Step 3: Look for mutant phenotype

JOURNAL OF BACTERIOLOGY, Sept. 1998, p. 4538-4546
0021-9193/98/$04.00+0

Vol. 180, No. 17

Mutations That Alter the Kinase and Phosphatase Activities of the Two-Component Sensor EnvZ

WEIHONG HSING,† FRANK D. RUSSO,‡ KAREN K. BERND,§ AND THOMAS J. SILHAVY*



Previous work indicates that the H box is directly involved in both OmpR kinase and OmpR-P phosphatase activities, and we have proposed a common transition state with histidine-243 in close contact with aspartate-55 of OmpR for both reactions. Phosphotransfer occurs from histidine-243-P to aspartate-55, but water replaces the phosphorylated histidine side chain, leading to hydrolysis (10). Thus, mutations in the H region could affect the kinase activity, the phosphatase activity, or both activities.

Candidate Variations

from Silhavy and Laub

blue = K-P+
red = K+ P-

Cph1/EnvZ	A553	G554	V555	S556	H557
EnvZ	**A239T**	**G240E**	**V241G**	**S242D**	**H243A**

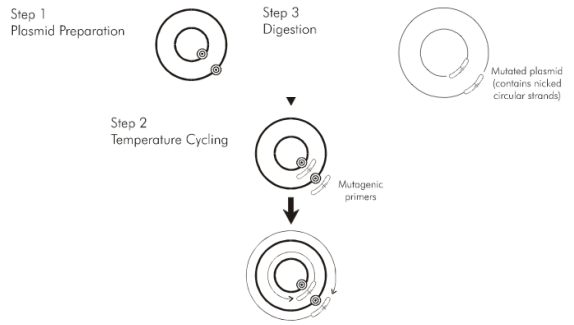
"*Keep in mind that K+P- really means a shift in the balance of kinase and phosphatase activities and similarly for the K-P+ alleles. None of them is perfectly "clean" in eliminating one of the activities"*

K-P+ Library Variations (in blue)

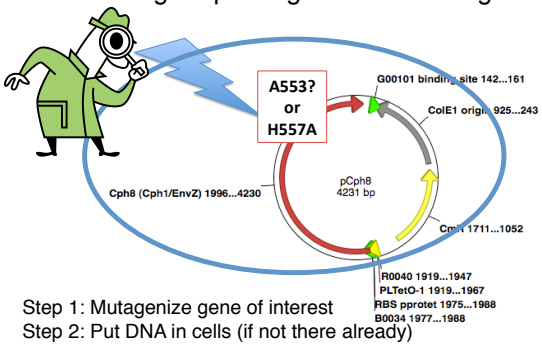
EnvZ	**A239T**	**G240E**	**V241G**	**S242D**	**H243A**
Cph8 = Cph1/EnvZ	A553	G554	V555	S556	H557
wt seq	GCG	GGG	GTA	AGT	CAC
nutagenesis oligo NO294	**RNS**				
mutagenesis oligo NO295					**GCC**
poss aa	Val	R = G, A			**Ala**
blue = K-P+	**Ala**	N = G, A, T, C			
red = K+ P-	Asp	S = G, C			
	Glu				
	Gly				
	Ile				
	Met				
	Thr				
	Asn				
	Lys				
	Ser				
	Arg				
# poss codons	**16**				**1**
# poss aa	**12**				**1**
	NOTE: no stop codons should be in mix				

Mutagenesis

based on Stratagene's "QuickChange"



Looking for parts: genetic screening



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Summary