System Engineering

20.109(F10)
M2D3 lecture
10.21.10

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

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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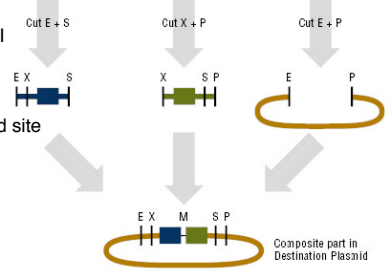
Physical Composition of Standard Biological Parts

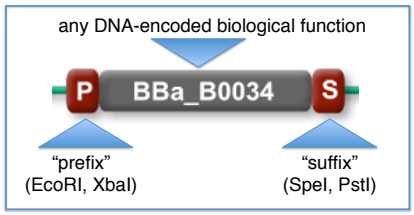
E = EcoRI
X = XbaI
S = SpeI
P = PstI
M = Mixed site

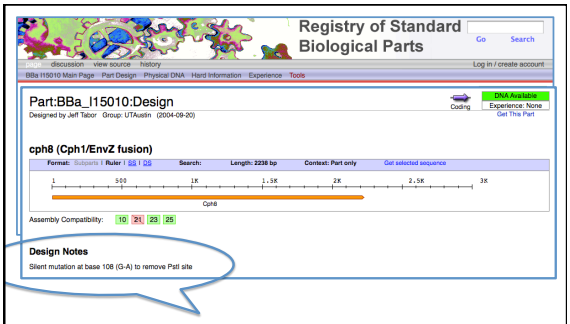


Let's "BioBrick" Cph8 (= Cph1/EnvZ fusion)

Ginkgo's Part Design Tool

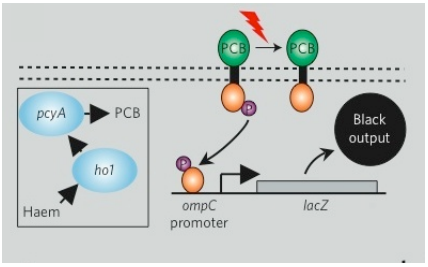
http://ginkgobioworks.com/cgi/primer.cgi





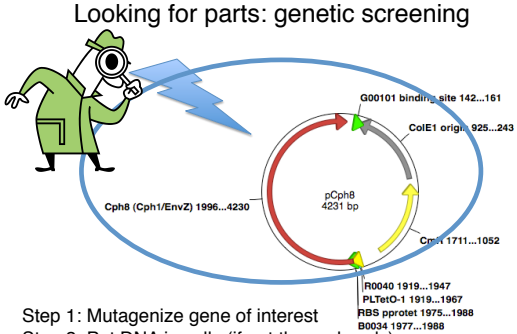
"Silent mutation at base 108 (G-A) to remove PstI site"

New tools for reliable engineering of complex biological systems

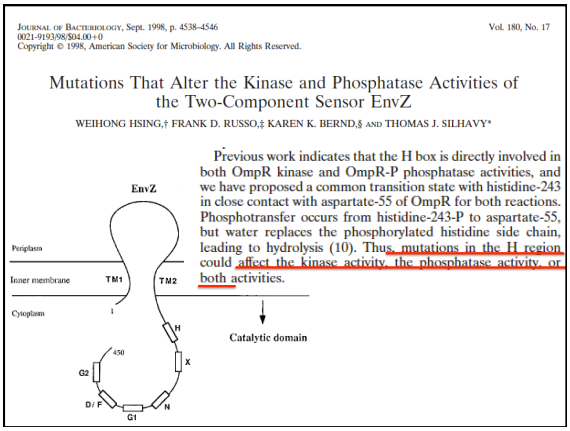


Does the Registry have Cph8 signaling mutants?

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



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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.

Library Variations

from Silhavy and Laub

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

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

If N = 3 aa = 9 bp
all possible seq variants = (4*4*4)^3
= 262,144 sequences !!!!!!

"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+ Library Variations (in red)

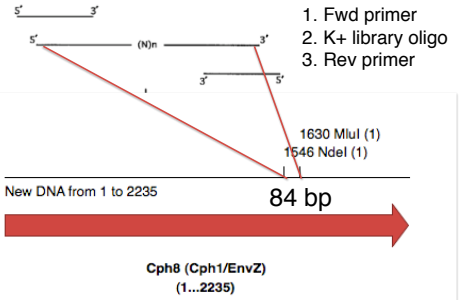
Cph1/EnvZ	A553	G554	V555	S556	H557
EnvZ	**A239T**	**G240E**	**V241G**	**S242D**	**H243A**
wt seq	GCG	GGG	GTA	AGT	CAC
oligo seq	**RNS**	**RNS**	**RNS**	**RNS**	**SNW**

If N = 3 aa = 9 bp
All seq variants = (2*4*2)^3
= 4, 096 DNA sequences !!

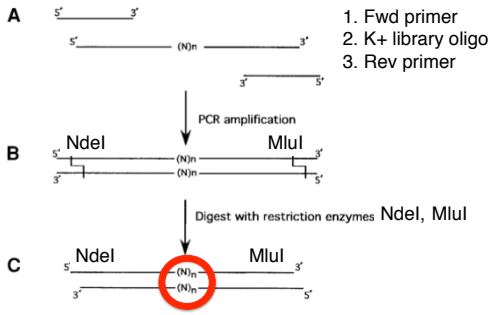
	Ile	Ile	Ile	Ile	Leu
	Met	Met	Met	Met	Pro
R = G, A	**Thr**	Thr	Thr	Thr	**His**
N = G, A, T, C	Asn	Asn	Asn	Asn	Gln
S = G, C	Lys	Lys	Lys	Lys	Arg
W = T, A	Ser	Ser	Ser	**Ser**	
	Arg	Arg	Arg	Arg	

NOTE: no stop codons should be in mix

Mutagenesis: Library Construction v1.0



Mutagenesis: Library Construction v1.0



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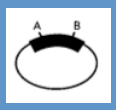
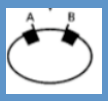
Mutagenesis: Library Construction v1.0

1. Digest pCph8 with Nde/Mlu

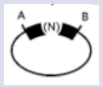


2. Add "Stuffer frag" = 1 KB

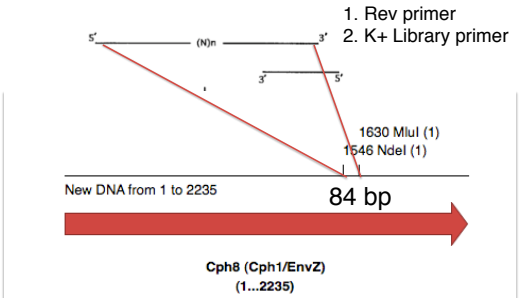


3. Digest with Nde/Mlu again

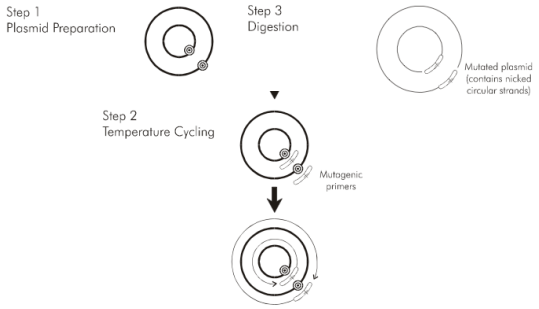
4. Clone in library fragments



Mutagenesis: Library Construction v2.0

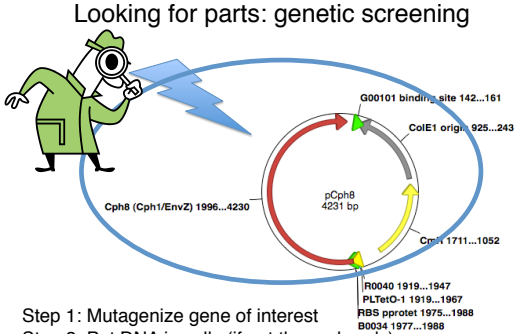


Based on Stratagene's "QuickChange" site-directed mutagenesis method



Library Sequencing Data

Looking for parts: genetic screening



Step 1: Mutagenize gene of interest
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Summary