
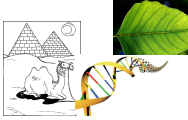


System Engineering

20.109(F12)
M2D3 lecture
10.18.12



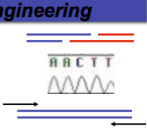


Genetic Engineering


rDNA

Sequencing


PCR




New tools for reliable engineering of complex biological systems




Synthetic Biology

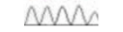
Standardization 


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 ~2000 genetic parts that can be mixed and matched to build synthetic biology devices and systems. For the Registry is part of the Synthetic Biology community's efforts to make biology easier to engineer. It provides a resource of available 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

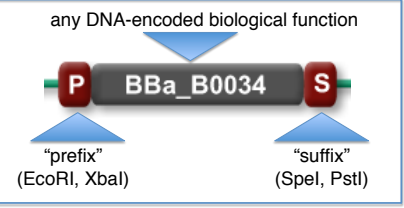
- Search parts (?)
- Add a part
- Request a part
- Send parts to the Registry
- Sequence analysis



<http://bbf.openwetware.org/>

"BBa" standard biological part

any DNA-encoded biological function




"prefix" (EcoRI, XbaI)

"suffix" (SpeI, PstI)

Physical Composition of Standard Biological Parts






BioBrick™ Assembly Help

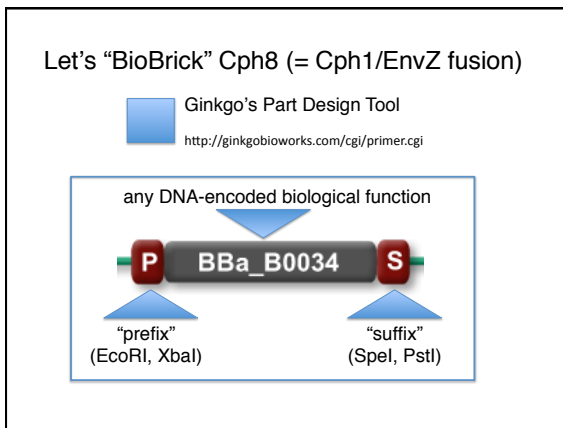
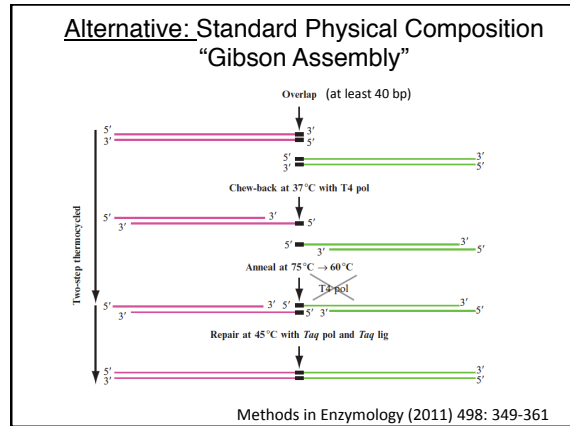
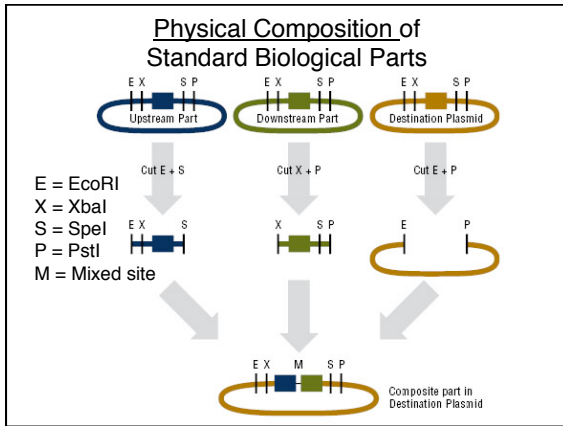


Using the kit

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.



Registry of Standard Biological Parts

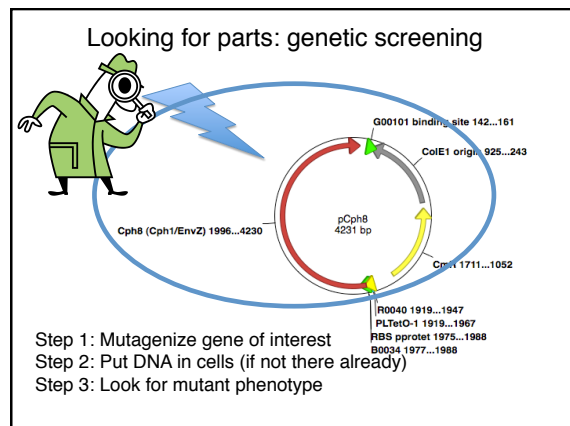
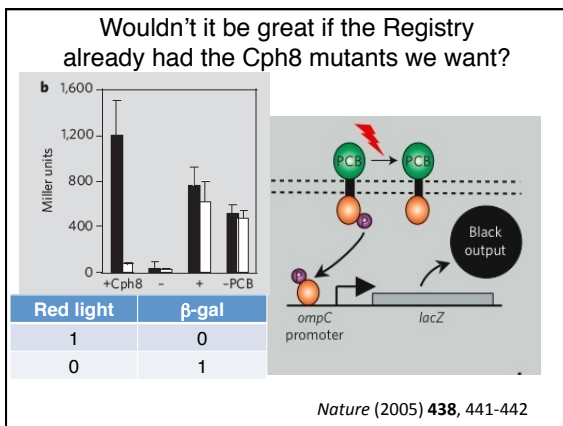
Part:BBa_I15010:Design
Designed by Jeff Taylor Group (UTAustin) (2004-09-20)

cph8 (Cph1/EnvZ fusion)
Format: SynGene | Ruler | G1 | G2 Search: Length: 2338 bp Context: Part only Get selected sequence

Assembly Compatibility: 10 28 28 28

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

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



JOURNAL OF BACTERIOLOGY, Sept. 1998, p. 4538-4546
 0021-9193/98/040538-09
 Copyright © 1998, American Society for Microbiology. All Rights Reserved.

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.

EnvZ

Periplasm

Inner membrane

Cytoplasm

Catalytic domain

Candidate Variations from Silhavy and Laub

EnvZ	H243	D244	L245	R246	T247R (K+P-)	P248
wt seq	CAC	GAC	TTG	CGC	Thr = ACG	CCG
Cph8	H537	D538	L539	R540	T541	P542
	Kinase Dead mutant			NNY mutagenesis		

"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

EnvZ	H243	D244	L245	R246	T247R (K+P-)	P248
wt seq	CAC	GAC	TTG	CGC	Thr = ACG	CCG
Cph8	H537	D538	L539	R540	T541	P542
	Kinase Dead mutant			NNY mutagenesis		
	GCC = Ala					

U	C	A	G	U
Phe	Ser	Tyr	Cys	Leu
Phe	Ser	Tyr	Cys	Leu
Leu	Ser	STOP	STOP	Leu
Leu	Ser	STOP	Trp	Leu
Leu	Pro	His	Arg	Leu
Leu	Pro	Gln	Arg	Leu
Leu	Pro	Gln	Arg	Leu
Ile	Thr	Asn	Ser	Ile
Ile	Thr	Asn	Ser	Ile
Ile	Thr	Lys	Arg	Met
Met	Thr	Lys	Arg	Met
Val	Ala	Asp	Gly	Val
Val	Ala	Asp	Gly	Val
Val	Ala	Glu	Gly	Val
Val	Ala	Glu	Gly	Val

CTY = Leu
 TTY = Phe
 TCY = Ser
 TAY = Tyr
 TGY = Cys
 ATY = Ile
 ACY = Thr
 AAY = Asn
 AGY = Ser
 GTY = Val
 GAY = Asp
 GGY = Gly
 N = G A T C
 Y = C T
 15 possible amino acids
 No stops

Mutagenesis based on Stratagene's "QuickChange"

Step 1 Plasmid Preparation

Step 2 Temperature Cycling

Step 3 Digestion

Mutagenic primers

Mutated plasmid (contains nicked circular strands)

Looking for parts: genetic screening

Cph8 (Cph1/EnvZ) 1996...4230

pCph8 4231 bp

Cph1 1711...1052

R0040 1919...1947

PLTetO-1 1919...1967

FBS pprtet 1975...1988

R0004 1977...1988

G00101 binding site 142...161

ColE1 origin 925...243

T541? or H537A

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

Summary

Tools for engineering biology

Standardization

Registry of Parts

physical assembly

functional assembly

Mutant Hunt

K+P-

Experimental approach

EnvZ	H243	D244	L245	R246	T247R (K+P-)	P248
wt seq	CAC	GAC	TTG	CGC	Thr = ACG	CCG
Cph8	H537	D538	L539	R540	T541	P542
	Kinase Dead mutant			NNY mutagenesis		