

# System Engineering

20.109(F14)  
M2D3 lecture  
10.16.14

## Synthetic Biologist's toolkit

**SYNTHETIC BIOLOGY**

Standardization .....

Abstraction .....

Synthesis ..... **A+A+C+T+T...**

**GENETIC ENGINEERING**

rDNA .....

Sequencing ..... **A A C T T**

PCR .....

### Registry of Standard Biological Parts

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. 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.

**Registry tools**

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

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

### "Bb" standard biological part

Any DNA-encoded biological function

P ..... S

"prefix" ..... "suffix"

EcoRI, XbaI ..... SpeI, PstI

### Physical Composition of Standard Biological Parts

**Legend:**

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

**Assembly:**

- Upstream Part (E X S P) + Downstream Part (E X S P) → Cut E + S, Cut X + P
- Upstream Part (E X S P) + Destination Plasmid (E X S P) → Cut E + P
- Final assembly: E X M S P → Composite part in Destination Plasmid

### Alternative: Standard Physical Composition "Gibson Assembly"

Overlap (at least 40 bp)

Chew-back at 37°C with T4 pol

Anneal at 75°C → 60°C

Repair at 45°C with T4 pol and T4 ligase

Methods in Enzymology (2011) 498: 349-361

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

Ginkgo's Part Design Tool  
<http://austinch.name/cgi/primer.cgi>

Registry of Standard Biological Parts

Part:BBa\_I15010:Design

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  
 0021-9193/98/044538-09  
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### Mutations That Alter the Kinase and Phosphatase Activities of the Two-Component Sensor EnvZ

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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

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	
	GGC = Ala				CIV = Leu	
					CCV = Pro	
					CAY = His	
					CGV = Arg*	
					TTY = Phe	
					TCV = Ser	
					TAY = Tyr	
					TGY = Cys	
					ATV = Ile	
					ACV = Thr	
					AAV = Asn	
					ACV = Ser	
					again	
					GTV = Val	
					GCY = Ala	
					GAY = Asp	
					GGY = Gly	
					N = G A T C	
					Y = C T	
					15 possible amino acids	
					No stops	

### 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

