System Engineering

20.109(F09)
M2D3 lecture
10.22.09

Roadmap for System Engineering Module
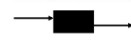
	Day	Lab	Lecture
✔	1	Testing v1.0	System Eng: Bact. Photography
✔	2	Measuring	Two Component Signaling
	3	Tools	Synthetic Biology + Genetics
	4	Journal Club	Group Meeting
	5	Re-tune	Examining the C-dog
	6	Readout v2.0	System Eng: revisited
	7	Journal Club	Group Meeting

New tools for reliable engineering of complex biological systems

Synthetic Biology

Standardization

Abstraction

Synthesis A+A+C+T+T…

Genetic Engineering

rDNA

Sequencing AACTT

PCR

Registry of Standard Biological Parts

Welcome to the Registry of Standard Biological Parts.

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

E = EcoRI
X = XbaI
S = SpeI
P = PstI
M = Mixed site
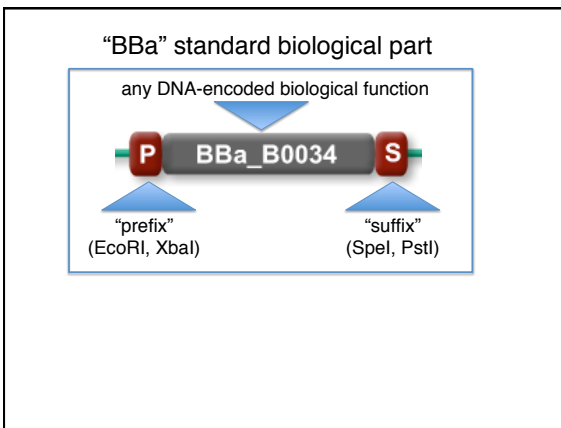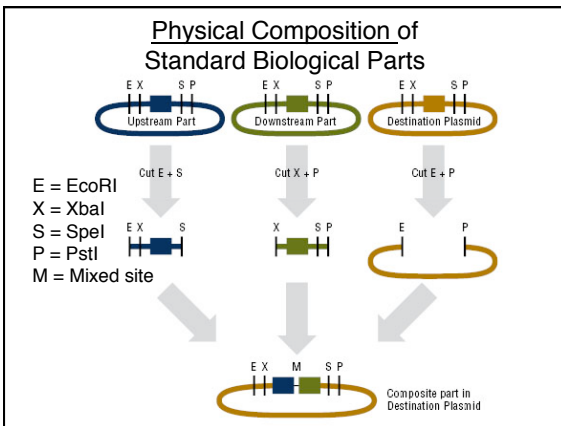
Registry of Standard Biological Parts

Part:BBa_I15010

cph8 (Cph1/EnvZ fusion)
Chimeric Cph1 light receptor/EnvZ protein.

Usage and Biology
Requires PCB biosynthetic genes (BBa_I15008 and BBa_I15009) for chromophore formation. The light responsive domain (Cph1) has maximal response to light near 660nm. Exposure to red light inhibits the activity of the EnvZ histidine kinase domain. When active (in the dark) EnvZ phosphorylates endogenous OmpR, a transcription factor which activates transcription from the OmpC promoter and represses transcription from the OmpF promoter. Must be used in E.coli deficient in natural EnvZ.

New tools for reliable engineering of complex biological systems

Does the Registry have Cph8 K^+ or P^+ 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

Mutagenesis: Library Construction

1. Fwd primer
2. Library seq
   (K+ and P+)
3. Rev primer

1630 MluI (1)
1646 NdeI (1)

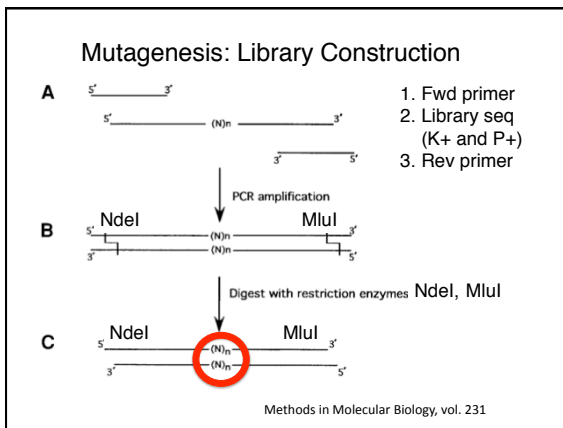New DNA from 1 to 2235   84 bp

Cph8 (Cph1/EnvZ)
(1…2235)

Mutagenesis: Library Construction

A

1. Fwd primer
2. Library seq
   (K+ and P+)
3. Rev primer

PCR amplification

B   NdeI   MluI

Digest with restriction enzymes NdeI, MluI

C   NdeI   MluI

Methods in Molecular Biology, vol. 231
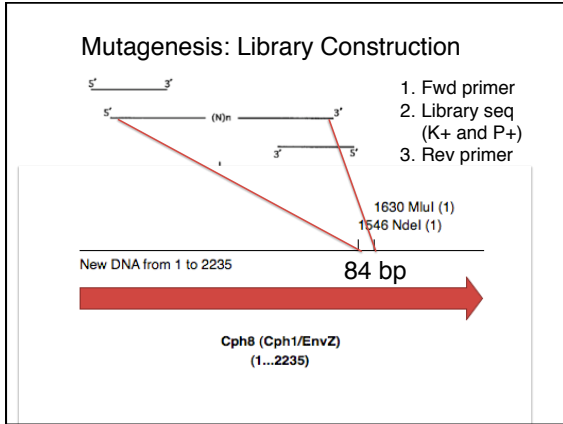
Library Variations

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**
poss aa	Val	Val	**Val**	Val	Val
	Ala	Ala	Ala	Ala	**Ala**
blue = K-P+	Asp	Asp	Asp	**Asp**	Asp
red = K+ P-	Glu	**Glu**	Glu	Glu	Glu
	Gly	**Gly**	**Gly**	Gly	Gly
	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					

Library Construction

1. Digest pCph8 with Nde/Mlu	2. Add "Stuffer frag" = 1 KB
3. Digest with Nde/Mlu again	4. Clone in library fragments

Variations in color from variations in activity

Strains 1, 2, 3   Strains 4, 5, 6, 7

MacConkey (maltose)

Tetrazolium (maltose)

Nature Reviews | Genetics

One more chromogenic indicator for LacZ: Methylumbelliferyl-β-D-galactoside (MUG)

S-gal

X-gal