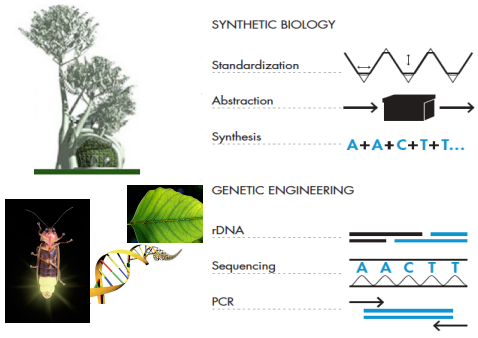
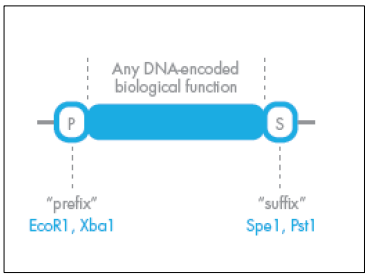
System Engineering

20.109(F14)
M2D3 lecture
10.16.14

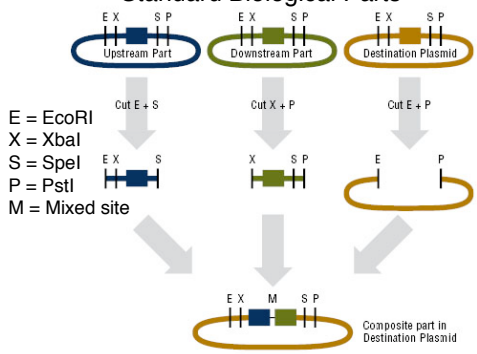
Synthetic Biologist's toolkit



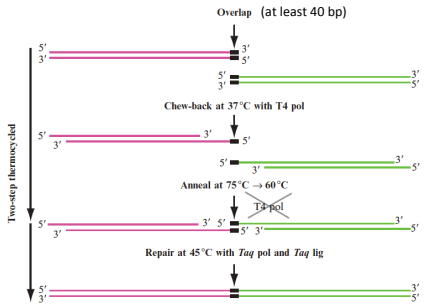
"BBa" standard biological part



Physical Composition of Standard Biological Parts



Alternative: Standard Physical Composition "Gibson Assembly"

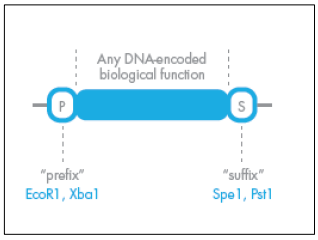


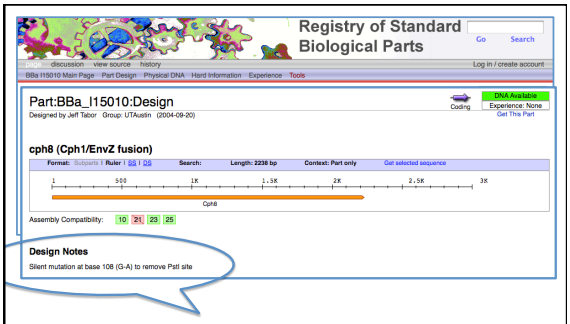
Methods in Enzymology (2011) 498: 349-361

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

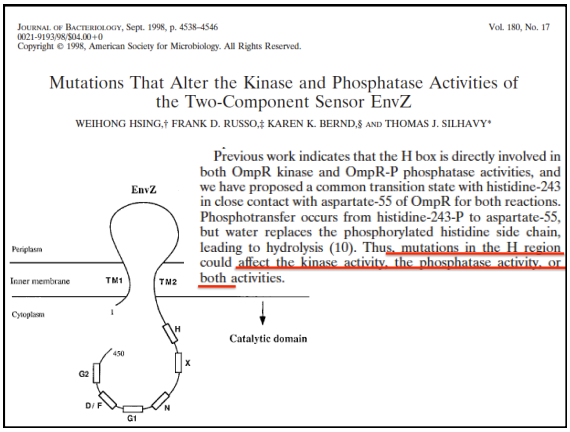
Ginkgo's Part Design Tool

http://austinche.name/cgi/primer.cgi





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



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"

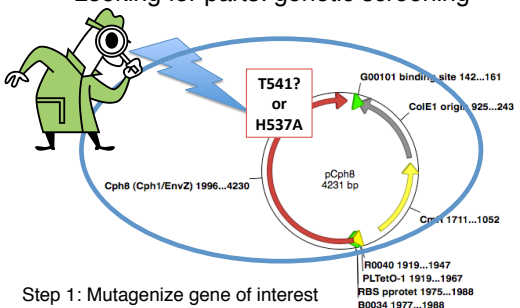
K+P- Library Variations

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				CTY = Leu	
					CCY = Pro	
					CAY = His	
					CGY = Arg*	
					TTY = Phe	
					TCY= Ser	
					TAY = Tyr	
					TGY = Cys	
					ATY = Ile	
					ACY = Thr	
					AAY = Asn	
					AGY = Ser	again
					GTY = Val	
					GCY = Ala	
					GAY = Asp	
					GGY = Gly	

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

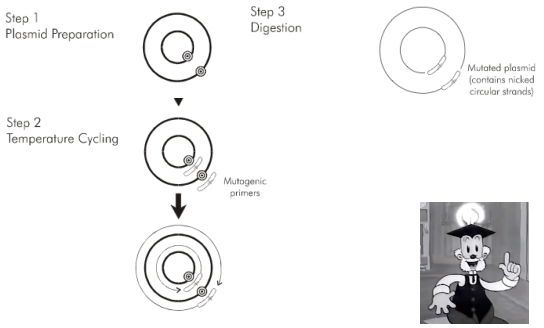
N = G A T C
Y = C T
15 possible amino acids
No stops

Looking for parts: genetic screening

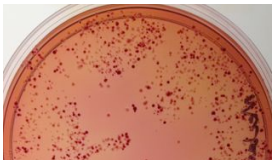


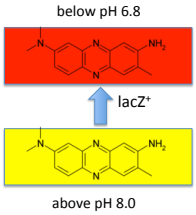
Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells (if not there already)
Step 3: Look for mutant phenotype

Mutagenesis
based on Stratagene's "QuickChange"

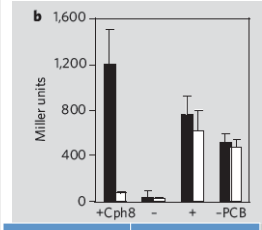


"The art and design of genetic screens: E. coli"

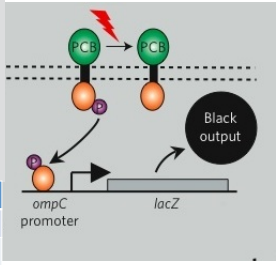




What behavior can we anticipate from the Cph8 mutants?



Red light	β-gal
1	0
0	1



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

Summary

Tools for engineering biology

Standardization
- Registry of Parts
- Physical assembly
- Competing standards

Mutant Hunt
- *K+P-*
- *color screen*