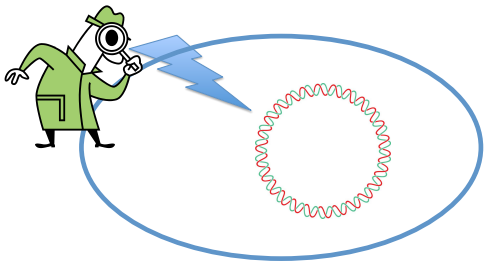
System Engineering

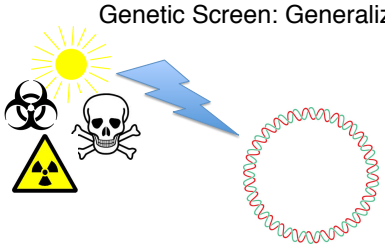
20.109(F14)
M2D5 lecture
10.23.14

Genetic Screen: Generalized



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

Genetic Screen: Generalized



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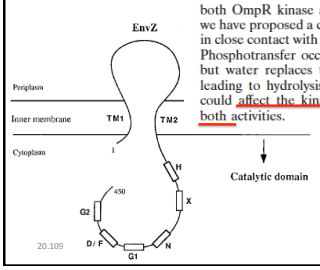
JOURNAL OF BACTERIOLOGY, Sept. 1998, p. 4538–4546
0021-9193/98/$04.00+0

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.



K+P- Library Variations: oligo design

EnvZ wt seq	CAC	H243	GAC	D244	TTG	L245	CGC	R246	Thr = ACG	CCG	P248
CpxR	HS37		D538		L539		R540	T541		P542	

Kinase Dead mutant
GCC = Ala

	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

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

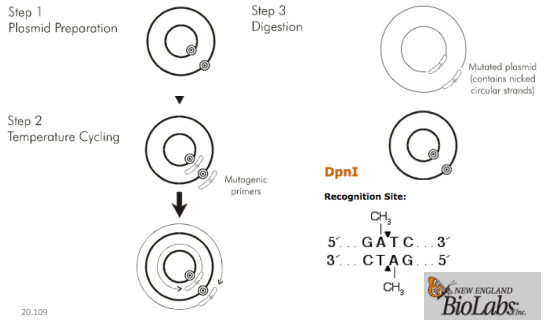
N = G A T C
Y = C T

15 possible amino acids
No stops

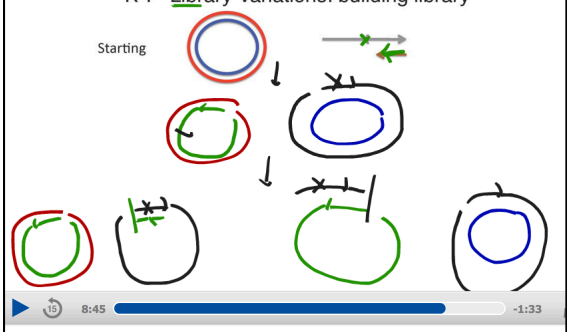
again

Mutagenesis

based on Stratagene's "QuickChange"



K^+P^- Library Variations: building library



https://www.educreations.com/lesson/view/cph8-mutagenesis-revisited/2301653/?s=Gm5so3&ref=appemail

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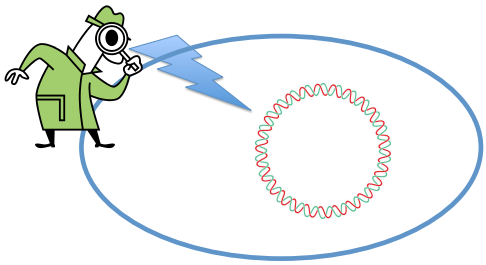
Genetic Screen: Generalized



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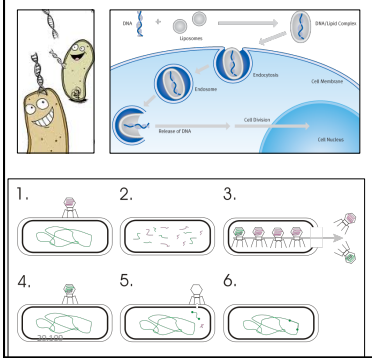
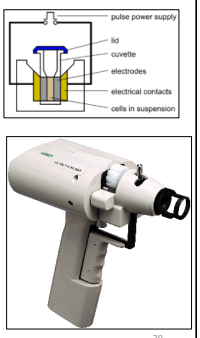
Ways to move DNA into cells





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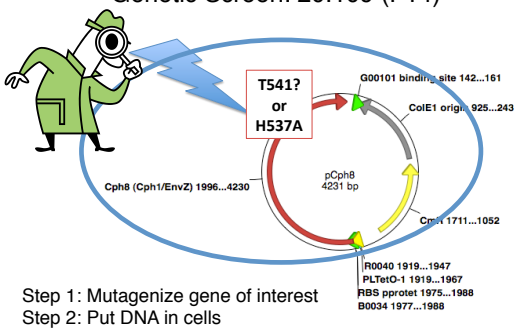
Genetic Screen: 20.109 (F14)



Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells
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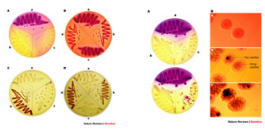
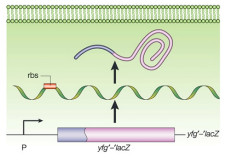
"The art and design of genetic screens: E. coli"

SELECTION	SCREEN
e.g. growth on a particular sugar e.g. resistance to a virus or an antibiotic	e.g. fermentation of a particular sugar e.g. chromogenic substrate e.g. observable (luciferase or GFP)





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Nature Reviews Genetics (2003) 4, 419-431

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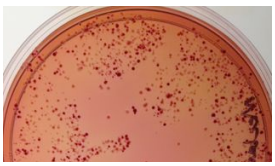

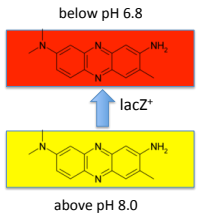

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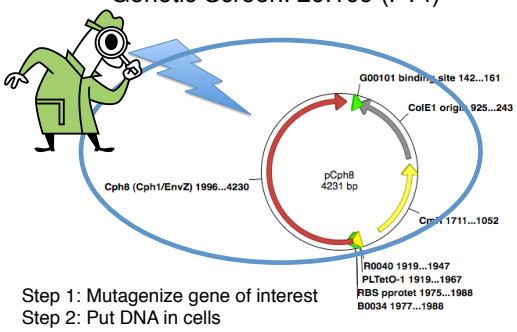
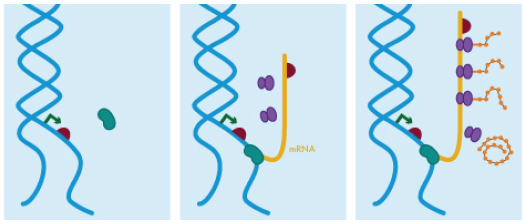
Genetic Screen: 20.109 (F14)



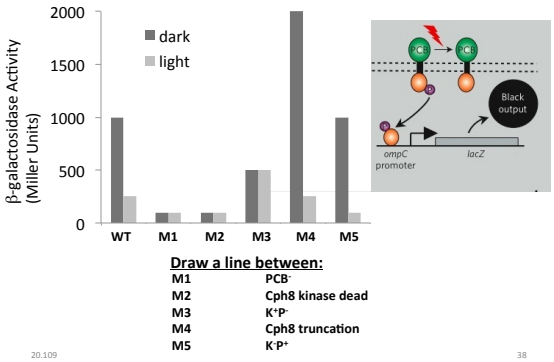
Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells
Step 3: Look for mutant phenotype
Step 4: Study the behavior of the mutant cells

Central Dogma



DNA sequence → amino acid sequence of protein

Possible mutant "phenotypes"



Draw a line between:

M1	PCB^-
M2	Cph8 kinase dead
M3	K^+P^-
M4	Cph8 truncation
M5	K^-P^+

Summary

Mutagenesis Strategies
- general
- site directed
- building our library

Mutated DNA in cells
- Putting it there
- Looking for phenotypes

Bacterial Photography K^+P^-