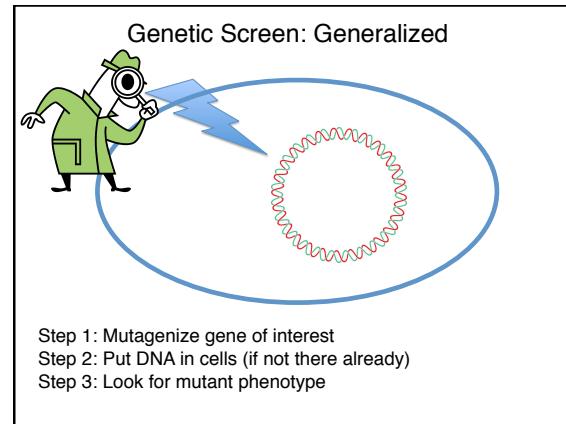
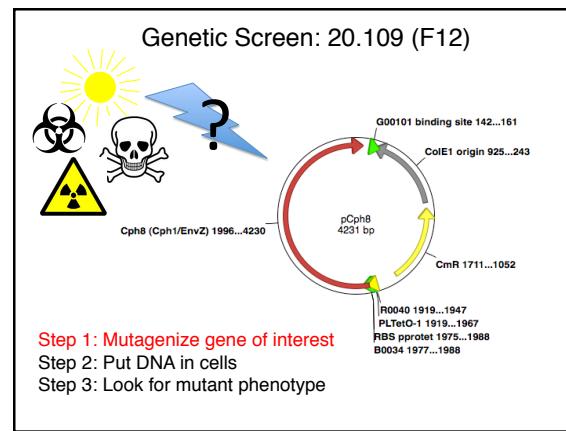


System Engineering

20.109(F12)
M2D5 lecture
10.25.12



A long time ago in a galaxy far, far away....



K⁺P⁻ Library Variations: oligo design

	U	C	A	G	
EnvZ wt seq	H243	D244	L245	R246	
	CAC	GAC	TTG	CGC	
Coo8	H537	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	
				C	
Leu	Ser	STOP	STOP	A	
				A	
Leu	Ser	STOP	Trp	G	
				G	
C	Leu	Pro	His	Arg	U
				C	
Leu	Pro	His	Arg	C	
				C	
Leu	Pro	Gln	Arg	A	
				A	
Leu	Pro	Gln	Arg	G	
				G	
A	Ile	Thr	Asn	Ser	U
				C	
Ile	Thr	Asn	Ser	C	
				C	
Ile	Thr	Lys	Arg	A	
				A	
Met	Thr	Lys	Arg	G	
				G	
G	Val	Ala	Asp	Gly	U
				C	
Val	Ala	Asp	Gly	C	
				C	
Val	Ala	Glu	Gly	A	
				A	
Val	Ala	Glu	Gly	G	
				G	

NNT mutagenesis
 CTY = Leu
 CCY = Pro
 CAY = His
 GTY = Thr*
 ATY = Ile
 ACY = Thr
 ANH = Alanine
 AGY = Ser again
 GTY = Val
 ATY = Ala
 ACY = Ala
 ANH = Asp
 AGY = Gly

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

