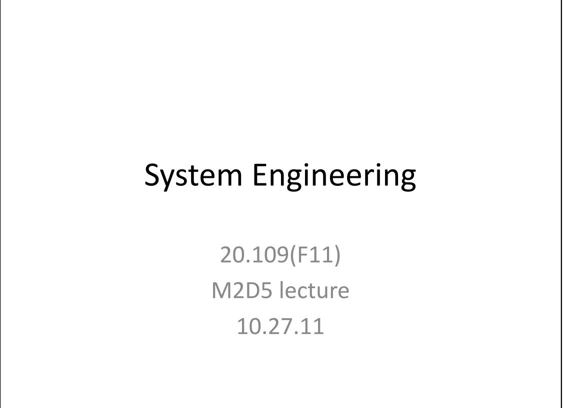
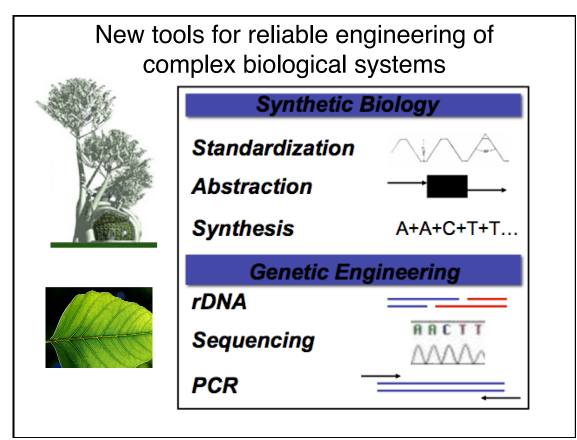
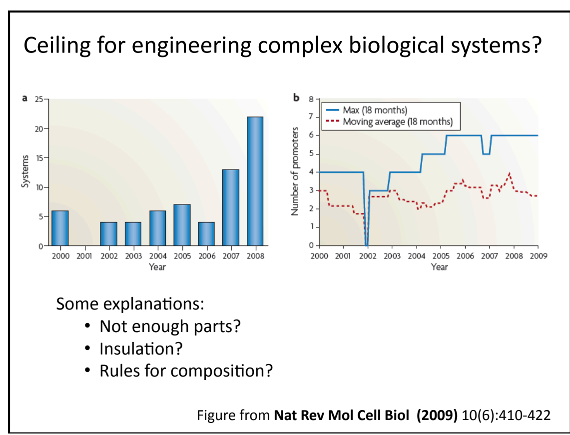
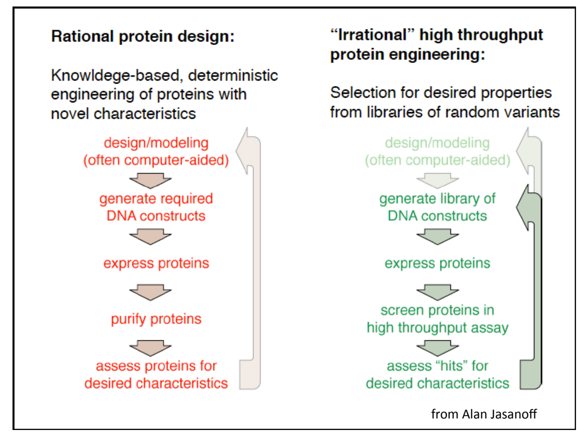
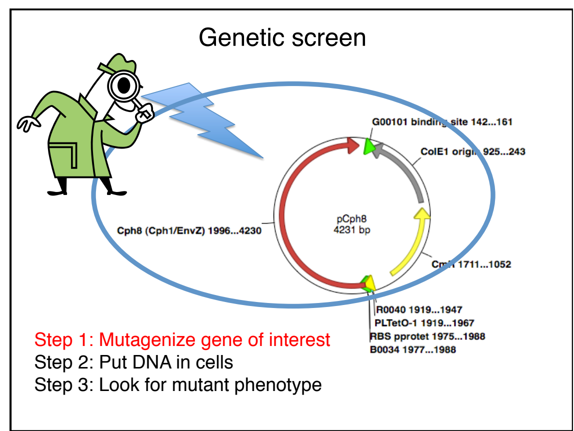
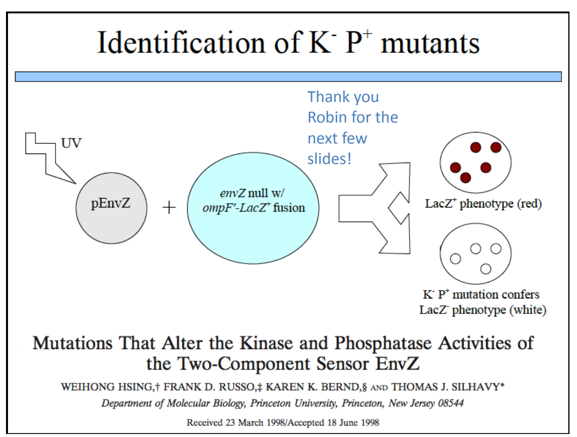

System Engineering
20.109(F11)
M2D5 lecture
10.27.11

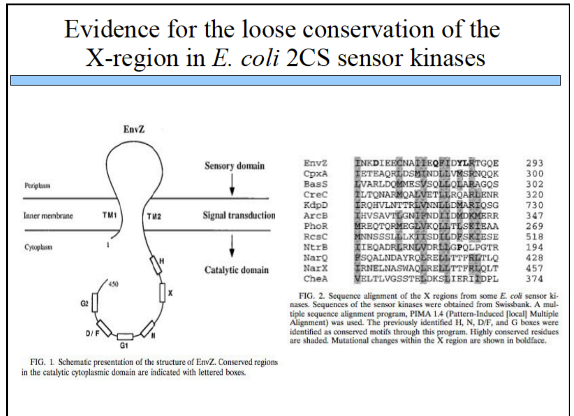

New tools for reliable engineering of complex biological systems
Synthetic Biology
Standardization
Abstraction
Synthesis
A+A+C+T+T…
Genetic Engineering
rDNA
Sequencing
PCR

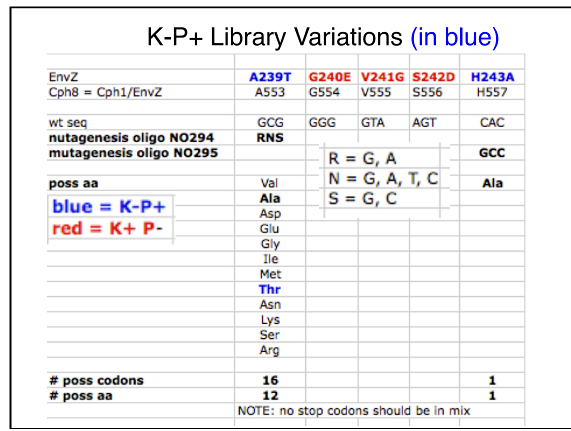

Ceiling for engineering complex biological systems?
Systems
Year
Number of promoters
Max (18 months)
Moving average (18 months)
Some explanations:
• Not enough parts?
• Insulation?
• Rules for composition?
Figure from Nat Rev Mol Cell Biol (2009) 10(6):410-422


Rational protein design:
Knowldege-based, deterministic engineering of proteins with novel characteristics
design/modeling (often computer-aided)
generate required DNA constructs
express proteins
purify proteins
assess proteins for desired characteristics
"Irrational" high throughput protein engineering:
Selection for desired properties from libraries of random variants
design/modeling (often computer-aided)
generate library of DNA constructs
express proteins
screen proteins in high throughput assay
assess "hits" for desired characteristics
from Alan Jasanoff


Genetic screen
G00101 binding site 142...161
ColE1 origin 925...243
pCph8
4231 bp
Cph8 (Cph1/EnvZ) 1996...4230
R0040 1919...1947
PLTetO-1 1919...1967
RBS pprotet 1975...1988
B0034 1977...1988
Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells
Step 3: Look for mutant phenotype


Identification of K- P+ mutants
Thank you Robin for the next few slides!
UV
pEnvZ
envZ null w/ ompF+-LacZ+ fusion
LacZ+ phenotype (red)
K- P+ mutation confers LacZ- phenotype (white)
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*
Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544
Received 23 March 1998/Accepted 18 June 1998



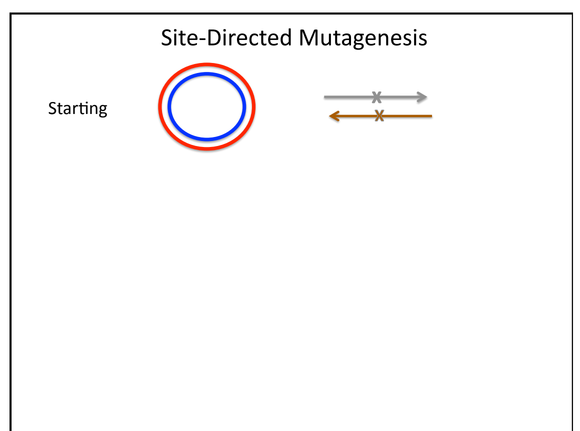


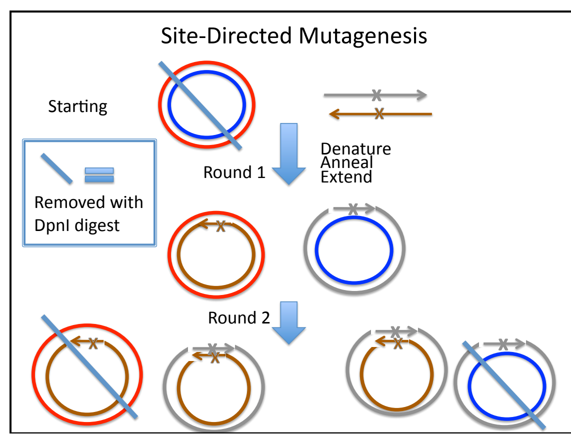
K-P+ Library Variations (in blue)

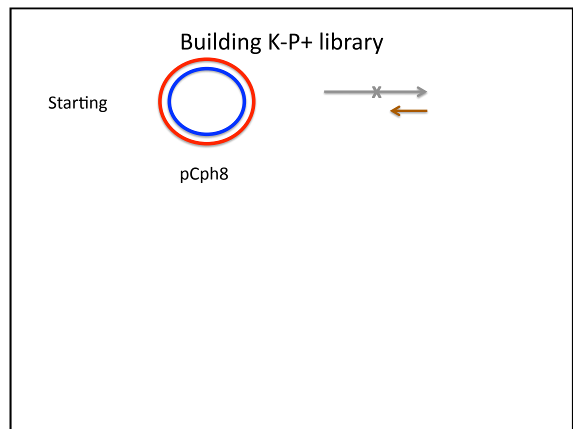
EnvZ	A239T	G240E	V241G	S242D	H243A
Cph8 = Cph1/EnvZ	A553	G554	V555	S556	H557
wt seq	GCG	GGG	GTA	AGT	CAC
nutagenesis oligo NO294	**RNS**				
mutagenesis oligo NO295					**GCC**
poss aa	Val				**Ala**
	Ala				
	Asp				
	Glu				
	Gly				
	Ile				
	Met				
	Thr				
	Asn				
	Lys				
	Ser				
	Arg				
# poss codons	**16**				**1**
# poss aa	**12**				**1**
	NOTE: no stop codons should be in mix				

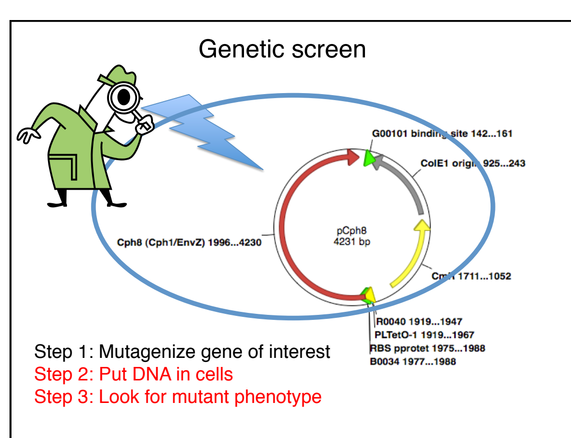
R = G, A
N = G, A, T, C
S = G, C

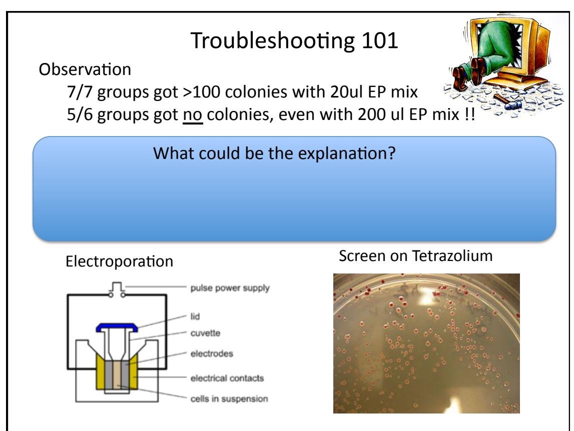
blue = K-P+
red = K+ P-

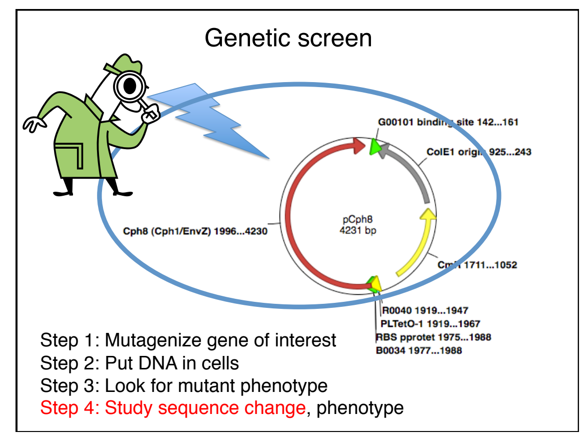


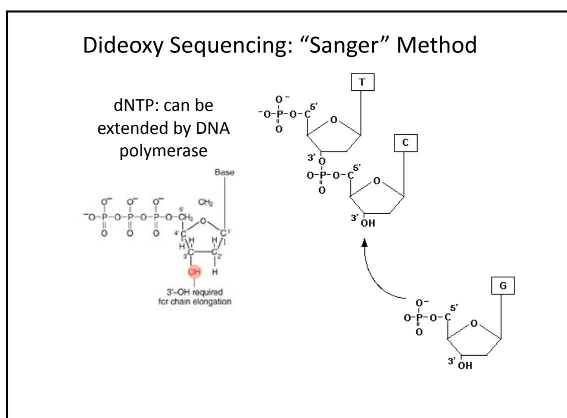


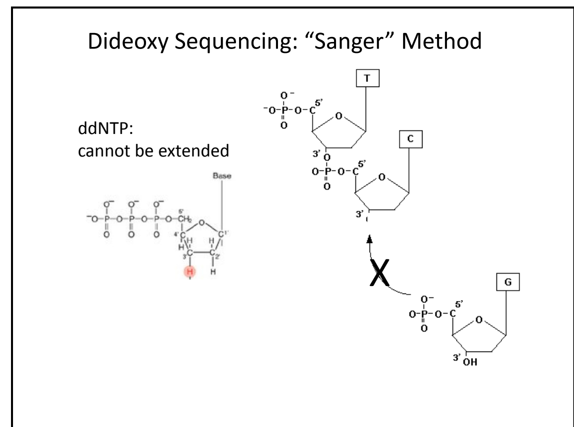


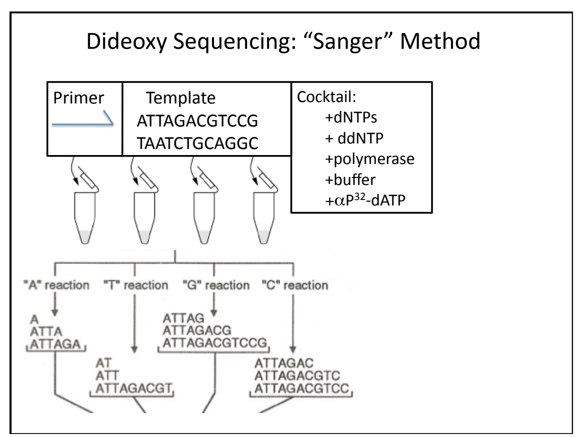


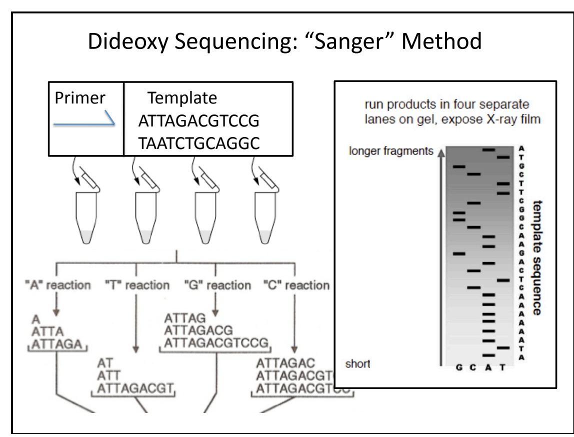

Troubleshooting 101
Observation
7/7 groups got >100 colonies with 20ul EP mix
5/6 groups got no colonies, even with 200 ul EP mix !!
What could be the explanation?
Electroporation
Screen on Tetrazolium
pulse power supply
lid
cuvette
electrodes
electrical contacts
cells in suspension

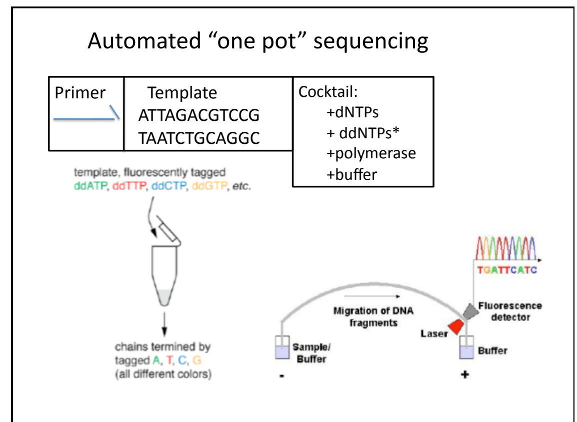

Genetic screen
pCph8
4231 bp
Cph8 (Cph1/EnvZ) 1996...4230
ColE1 origin 925...243
Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells
Step 3: Look for mutant phenotype
Step 4: Study sequence change, phenotype

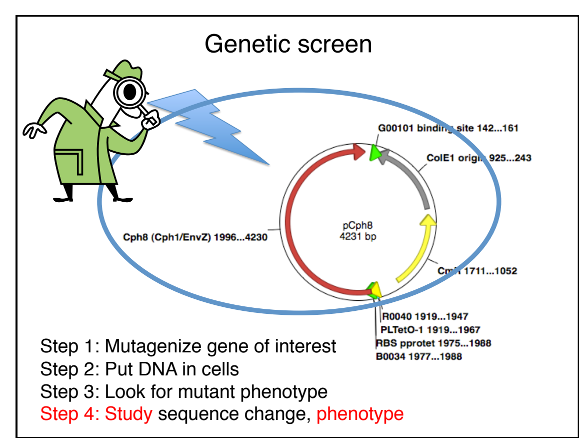

Dideoxy Sequencing: "Sanger" Method
dNTP: can be extended by DNA polymerase
3'-OH required for chain elongation


Dideoxy Sequencing: "Sanger" Method
ddNTP:
cannot be extended


Dideoxy Sequencing: "Sanger" Method
Primer
Template
ATTAGACGTCCG
TAATCTGCAGGC
Cocktail:
+dNTPs
+ ddNTP
+polymerase
+buffer
+αP32-dATP
"A" reaction
"T" reaction
"G" reaction
"C" reaction


Dideoxy Sequencing: "Sanger" Method
Primer
Template
ATTAGACGTCCG
TAATCTGCAGGC
run products in four separate lanes on gel, expose X-ray film
longer fragments
short
template sequence
"A" reaction
"T" reaction
"G" reaction
"C" reaction


Automated "one pot" sequencing
Primer
Template
ATTAGACGTCCG
TAATCTGCAGGC
Cocktail:
+dNTPs
+ ddNTPs*
+polymerase
+buffer
template, fluorescently tagged
ddATP, ddTTP, ddCTP, ddGTP, etc.
chains terminated by
tagged A, T, C, G
(all different colors)
Migration of DNA fragments
Sample/ Buffer
Laser
Fluorescence detector
Buffer
TGATTCATC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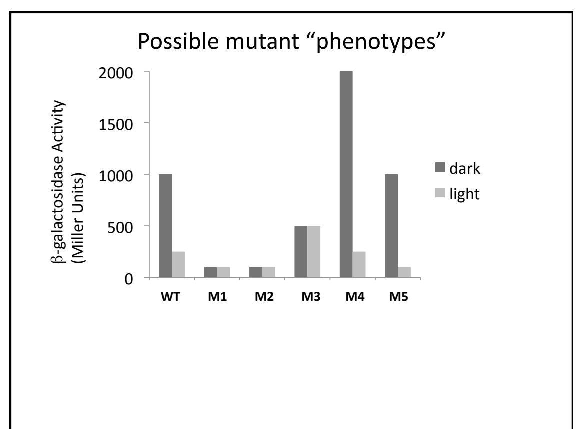
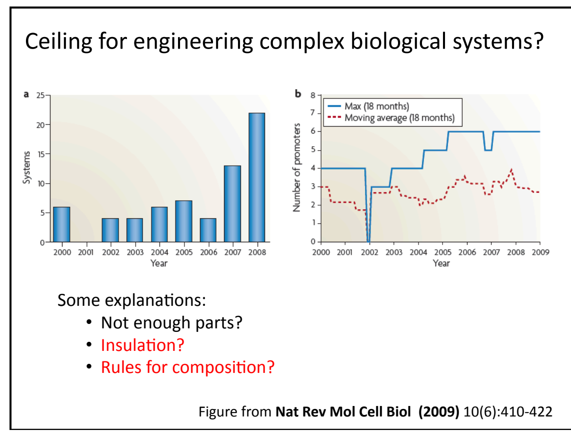

Possible mutant "phenotypes"
β-galactosidase Activity (Miller Units)
2000
1500
1000
500
0
WT
M1
M2
M3
M4
M5
dark
light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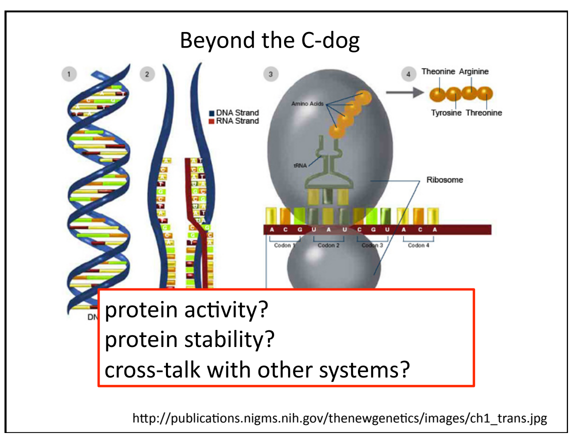
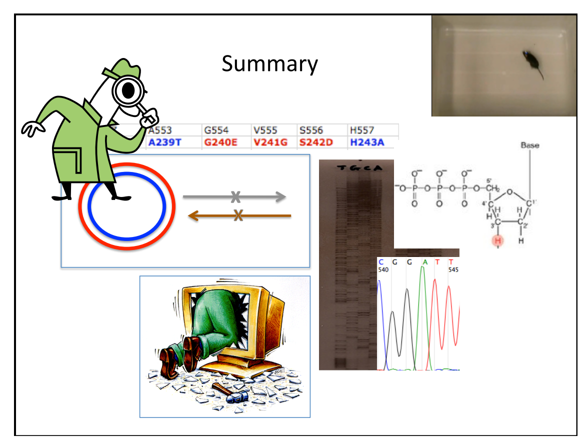

Beyond the C-dog
DNA Strand
RNA Strand
Ribosome
protein activity?
protein stability?
cross-talk with other systems?
http://publications.nigms.nih.gov/thenewgenetics/images/ch1_trans.jpg


Summary
A239T
G240E
V241G
S242D
H243A