System Engineering

20.109(F10)
M2D5 lecture
10.28.10

New tools for reliable engineering of complex biological systems

Synthetic Biology

Standardization

Abstraction

Synthesis A+A+C+T+T…

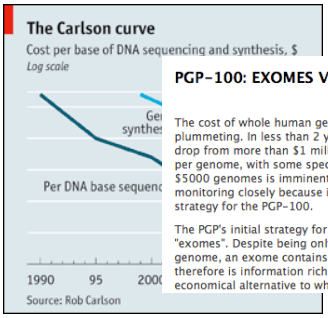
Genetic Engineering

rDNA

Sequencing

PCR

Personal Genomics



Personal Genome Project

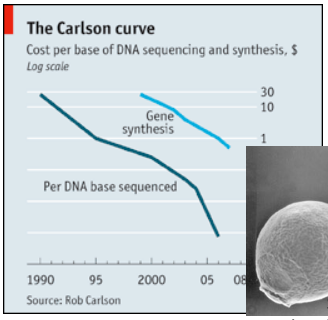
PGP–100: EXOMES VS. WHOLE GENOMES

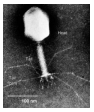
The cost of whole human genome sequencing is plummeting. In less than 2 years, we have witnessed prices drop from more than $1 million to now less than $50,000 per genome, with some speculating that the arrival of $5000 genomes is imminent. This is a trend that the PGP is monitoring closely because it will impact our sequencing strategy for the PGP–100.

The PGP's initial strategy for sequencing was to focus on "exomes". Despite being only a fraction of a human genome, an exome contains all 20,500+ genes and therefore is information rich and (in theory) a more economical alternative to whole genome sequencing.

PGP Newsletter #2, Oct 2009

Synthetic Genomics





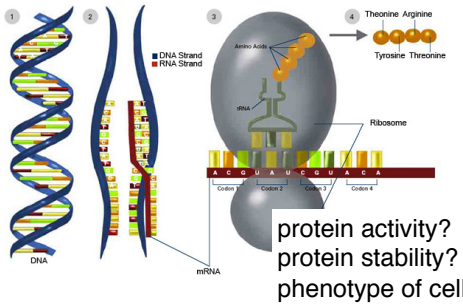
T7 ~ 40K bp

Simple prok. ~1 million bp

S. cerevisiae

Mitochondrial DNA <100K
Chromosome 1 ~250K

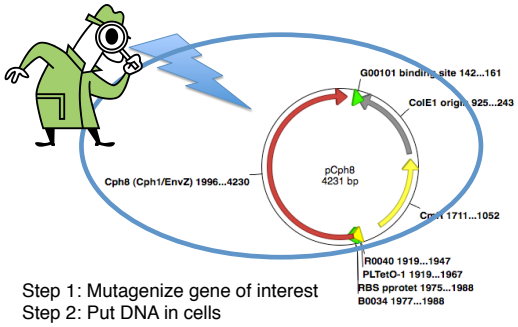
Beyond the C-dog



protein activity?
protein stability?
phenotype of cell?

http://publications.nigms.nih.gov/thenewgenetics/images/ch1_trans.jpg

Genetic screen



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

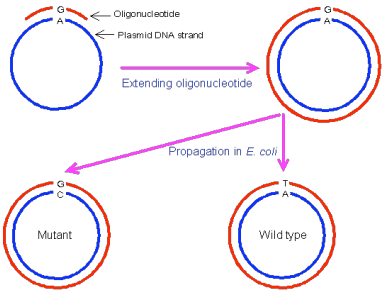
K+ Library Variations (in red)

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

Site-Directed Mutagenesis

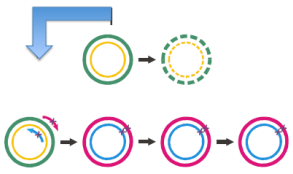
Simplest = one oligo



http://www.web-books.com/MoBio/Free/Ch9G.htm

Site-Directed Mutagenesis

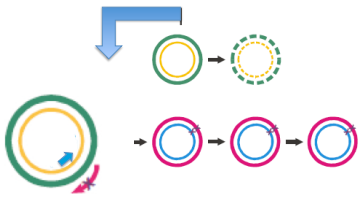
v2.0= two oligos, both mutated



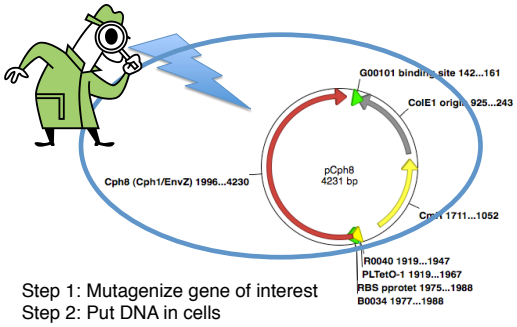
http://stanxterm.aecom.yu.edu/wiki/data/Product_manuals_attach/quikchange2xl.pdf

Building K+ library

v2.1 = site-directed mutagenesis, two oligos, one mutated

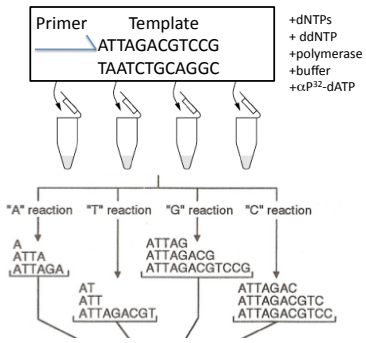


Genetic screen



Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells
Step 3: Look for mutant phenotype
Step 4: Study sequence change, phenotype

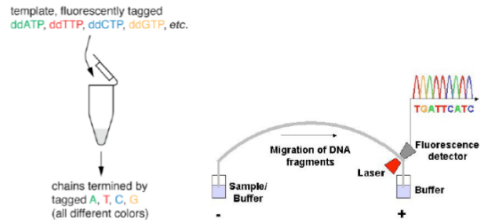
Dideoxy Sequencing: "Sanger" Method



Automated "one pot" sequencing

Primer Template
ATTAGACGTCCG
TAATCTGCAGGC

+dNTPs
+ ddNTP
+polymerase
+buffer



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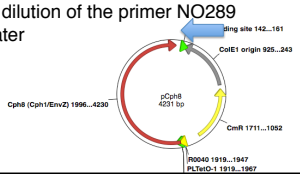
(1) Sequencing K+ candidates
Miniprep DNA as you did in Module 1
(Soln I, Soln II, Soln III, EtOH, wash, dry)

Resuspend pellets in 40 ul of water

For sequencing, mix:
- 2 ul plasmid DNA
- 6.4 ul of a 1:100 dilution of the primer NO289
- 15.6 ul sterile water



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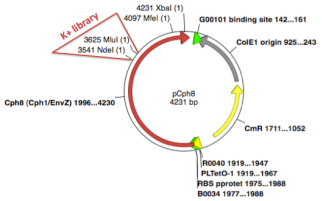
(2) Also check your DNA by digest
- Provide 10 ul of each
- Teaching faculty will cut with

Nde I
Xba I

to run on agarose gel and post



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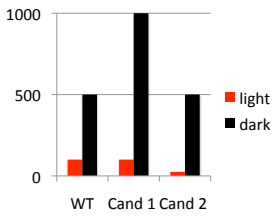
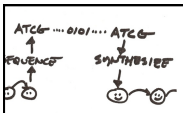
(3) Grow wt and 2 candidates in light/dark for β-gal assay next week



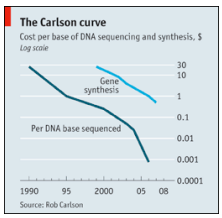
What if Mark gave a quiz asking:

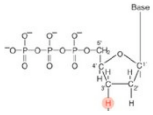
What kind of mutant
is Cand 1?
is Cand 2?

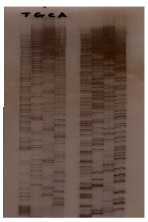
Summary



Cph1/EnvZ	A53	G54	V55	S56	H57
EnvZ	A239T	G240E	V241G	S242D	H243A







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