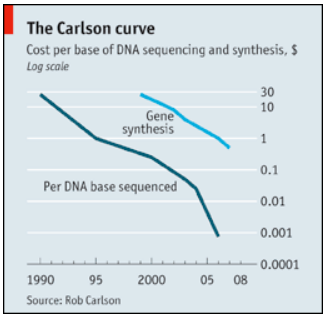
System Engineering

20.109(F09)
M2D5 lecture
10.29.09

Personal Genomics



One more acronym: GWAS

Height = 'model trait'
simple to measure and relatively constant

- in 2007, comparison made of 5000 genomes
- GWAS have revealed >40 loci involved in height

Editorial

Molecular Systems Biology **5** Article number: 273 doi:10.1038/msb.2009.32
Published online: 19 May 2009
Citation: *Molecular Systems Biology* 5:273

Personal phenotypes to go with personal genomes

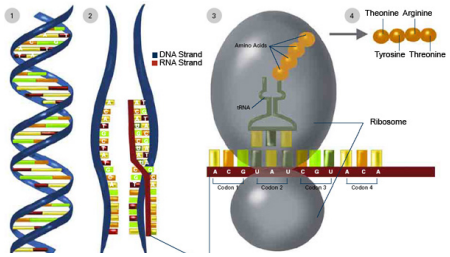
Michael Snyder[1,2], Sherman Weissman[3] & Mark Gerstein[2,4,5]

"the phenotyping of large numbers of individuals might well prove to be more expensive, complex and difficult to implement than the genomic sequencing."

What to phenotype?
- ✓ height, blood pressure, medical history…
- ○ behavioral (anxiety? Risk taking?)
- ○ cognative (intelligence?)

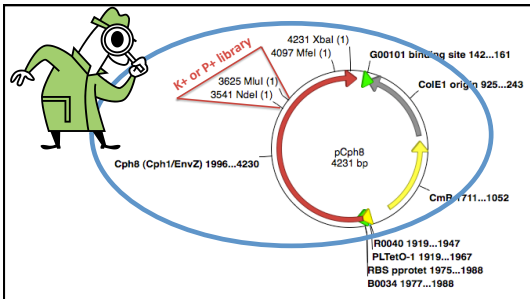
Standards for collection?
macroscopic VS molecular

The C-dog



DNA sequence → amino acid sequence of protein

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



Step 1: Mutagenize gene of interest
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Cph1/EnvZ	A553	G554	V555	S556	H557
EnvZ	A239T	G240E	V241G	S242D	H243A

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*

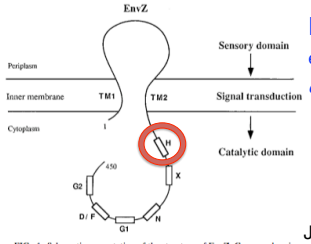

FIG. 1. Schematic presentation of the structure of EnvZ. Conserved regions in the catalytic cytoplasmic domain are indicated with lettered boxes.

K-P+ screen

envZ250 = P159S = TM2

envZ247 = A239T = H region

K+P- screen

Supp of envZ247

envZ971= V241G = H region

J. Mol. Biol. (1991) 222: 567

J. Bact. (1998) 180: 4538

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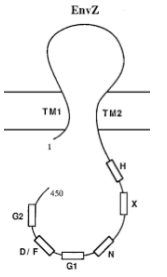

envZ allele	Residue change(s)	Location
envZ982	L23R	TM1
envZ972	L26R	TM1
envZ977	T30P	TM1
envZ973	L43P	TM1
envZ976	Q44P	TM1
envZ981	L230Q	H region
envZ975	V241G	H region
envZ974	Δ(A225-A239)	H region
envZ971	D273Y	X region
envZ978	Q283P	X region
envZ959	P248Q	H region
envZ954	P248L	H region
envZ966	Y287D	X region
envZ958	Y287N	X region
envZ965	Y287S	X region
envZ964	L288P	X region
envZ962	T402K	G2 region
envZ343	N343K	N region
envZ390	F390L	D/F region

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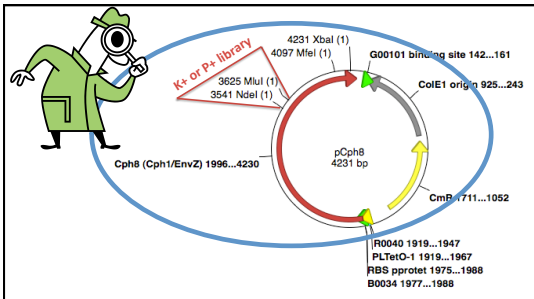

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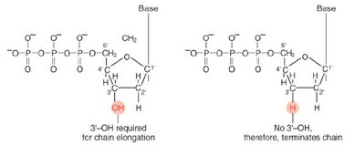
Dideoxy Sequencing Method aka Sanger Method

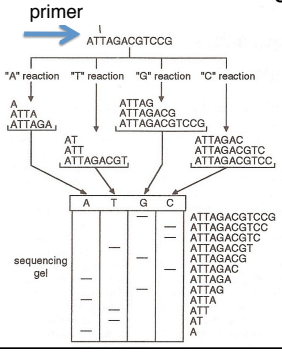
dNTP: can be extended by DNA polymerase

ddNTP: chain terminating!



Dideoxy Sequencing Method aka Sanger Method

primer



Elongation Reactions:
Template
Primer
DNA polymerase
dNTPs (limiting)
αP^{32}-dATP

4 Termination Reactions:
dNTPs (not limiting)
ddNTPs (either ddG, ddA, ddT or ddC)

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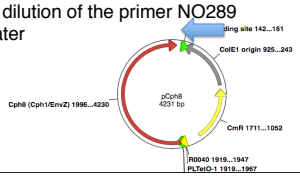
Sequencing K+ or P+ candidates

1. Miniprep DNA as you did in Module 1
   (Soln I, Soln II, Soln III, EtOH, wash, dry)

2. Resuspend pellets in 40 ul of water

3. 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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Also check your DNA by digest
- Provide 10 ul of each
- Teaching faculty will cut with

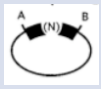
A= Nde
B= Mlu

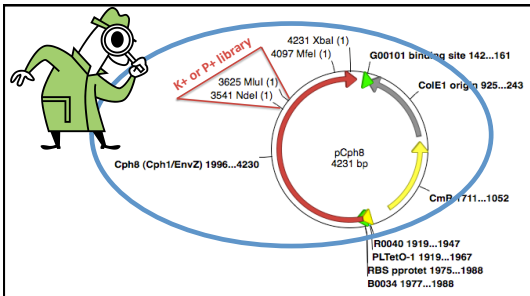
to run on agarose gel and post

With "stuffer" frag expect: _____
With library frag expect: _____

"Stuffer frag"

Library fragments





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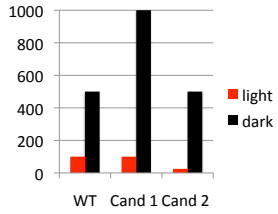
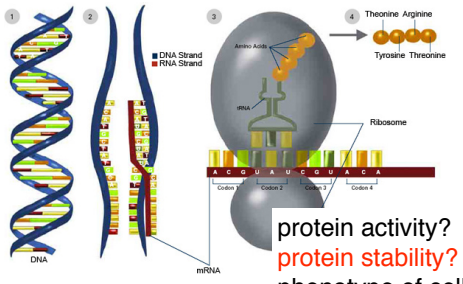
Grow 2 candidates in Light/Dark for β-gal next week



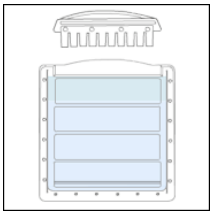
Beyond the C-dog



protein activity?
protein stability?
phenotype of cell?

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

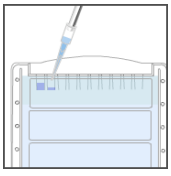
Part 1: SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

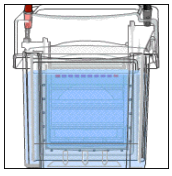


Loading dye has glycerol, SDS, reducing agent

Samples boiled before loading

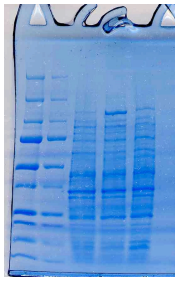
Part 1: SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

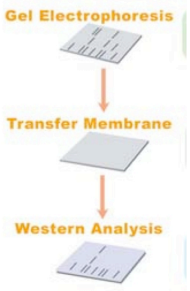




http://www.bme.gatech.edu/vcl/SDS_PAGE/Background/Introduction.htm

Part 2: Transfer for Western





Next week: probe membrane with an antibody

http://www.genscript.com/product_001/western_application/grp_id/60065/op/detail/Uvrag_Antibody_Analysis.html

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In lab you will:

- Measure OD600 of 1:10 of bacterial photography strain, Candidate 1, Candidate 2
- Harvest 4 OD
  e.g. if 0.5 OD, harvest 8 ml of 1:10 or 0.8 ml of undiluted
- Isolate protein with lysis kit (enzymatic lysis of cells, spin out debris)
- Mix supernatant with loading dye
- Boil
- Load for SDS-PAGE along with markers, + control lysate

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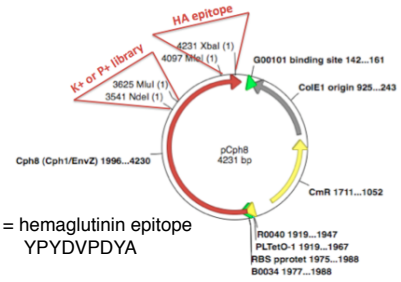

HA = hemaglutinin epitope
YPYDVPDYA