

System Engineering

20.109(F14)
 M2D7 lecture
 10.30.14 → 11.04.14

Analysis of mutant pCph8

protein activity?
protein stability?
cross-talk with other systems?

How Cph8 stability might affect β-gal activity

	Autophos events	Kinasing events	P-tase events	Amount OmpR+P
Wild type	5 "units"	3 "units"	2 "units"	1

Detection through Western Analysis

in lab!

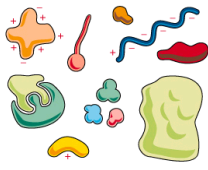
Western blot

<http://www.bio.davidson.edu/courses/genomics/method/Westernblot.html>

Polyclonal Antibody Production

Which protein would we like to have an antibody recognize?

Affinity Purification

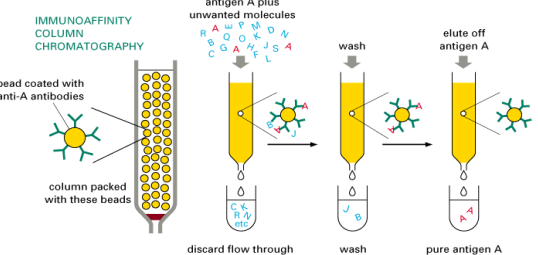


Mixture contains proteins of various size,
shape,
charge,
hydrophobicity,
affinity for different molecules

These properties can be exploited to separate individual protein from mixture

Alberts "Essential Cell Biology"

Affinity Chromatography

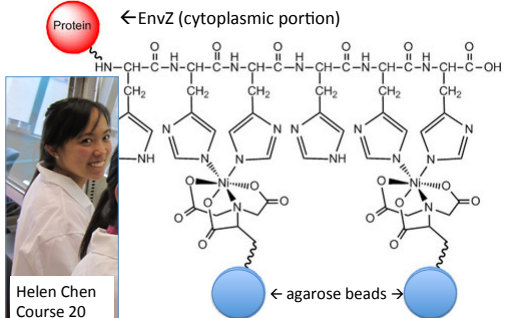


IMMUNOAFFINITY COLUMN CHROMATOGRAPHY

discard flow through wash elute off antigen A

Alberts "Essential Cell Biology"

His₆-tag for Affinity Purification



← EnvZ (cytoplasmic portion)

← agarose beads →

Helen Chen
Course 20
class of 2011

<http://www.kpl.com>

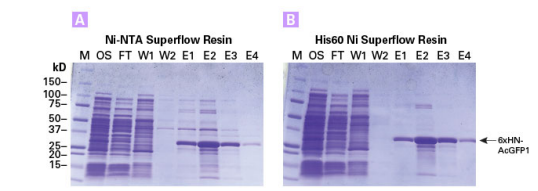
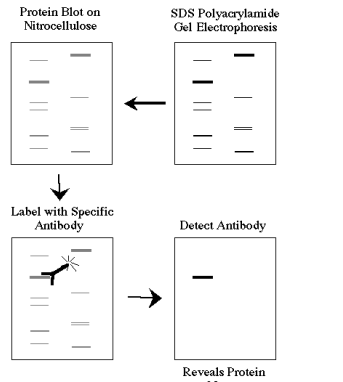


Figure 3. Superior yields and better purity were obtained when using His60 Ni Superflow for purification of recombinant his-tagged protein—as compared to Ni-NTA Superflow resin. Clontech's pEcoli Linear Expression System was used to express 6xHN-AcGFP1 in *E. coli*. Equivalent amounts of the same sample were used for comparing His60 Ni Superflow Resin purification (Panel B) with Competitor Q Ni Resin purification (Panel A). Lane M: Molecular weight marker. Lane OS: Original sample. Lane FT: Flowthrough. Lanes W1 and W2: Wash. Lane E1–E4: Eluted 6xHN-AcGFP1 fractions.

http://www.clontech.com/products/detail.asp?tabno=2&product_id=212698

Western blot

1. Block (TBS-T + milk)
2. Wash
3. Probe with primary antibody that recognizes protein of interest
4. Wash
5. Probe with secondary antibody that recognizes primary ("goat-antirabbit +AP")
6. Wash
7. Detect AP



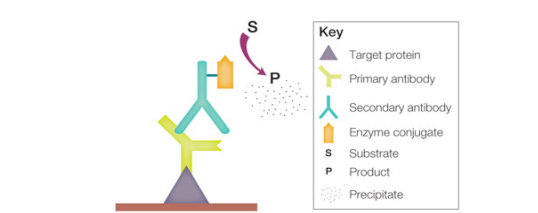
Protein Blot on Nitrocellulose SDS Polyacrylamide Gel Electrophoresis

Label with Specific Antibody Detect Antibody

Reveals Protein of Interest

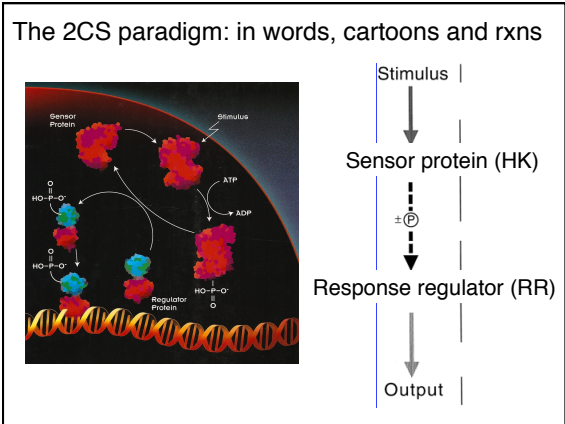
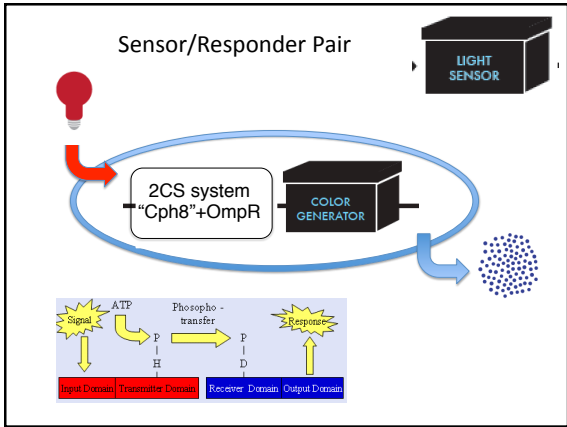
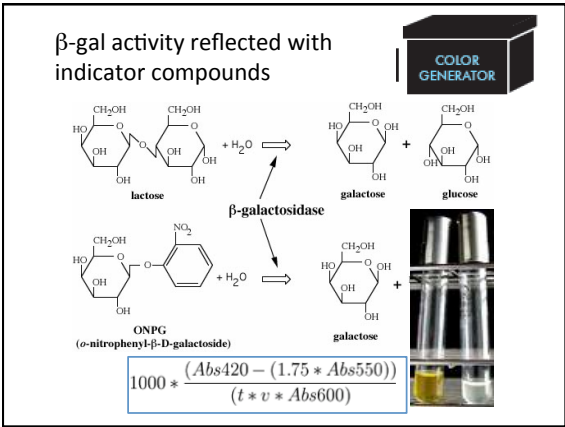
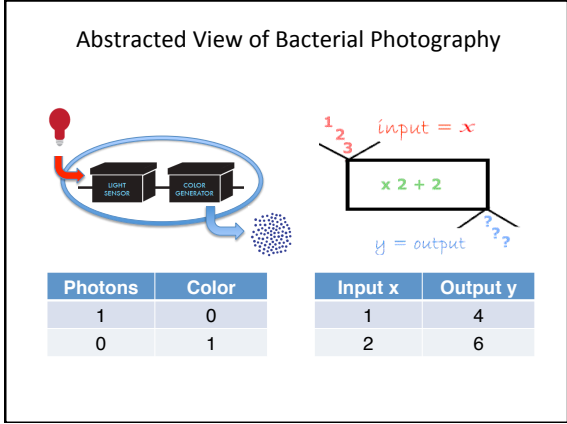
Colorimetric Detection

Colorimetric Chemistry

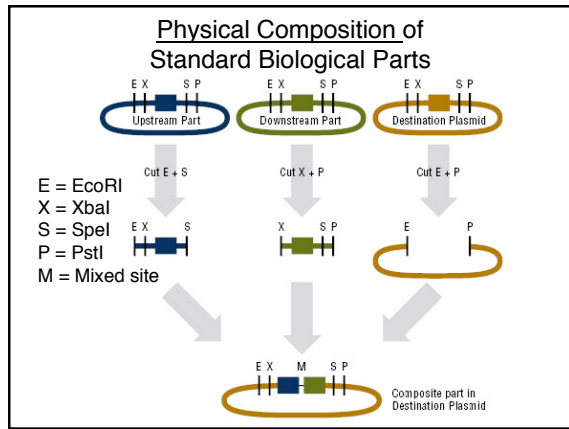
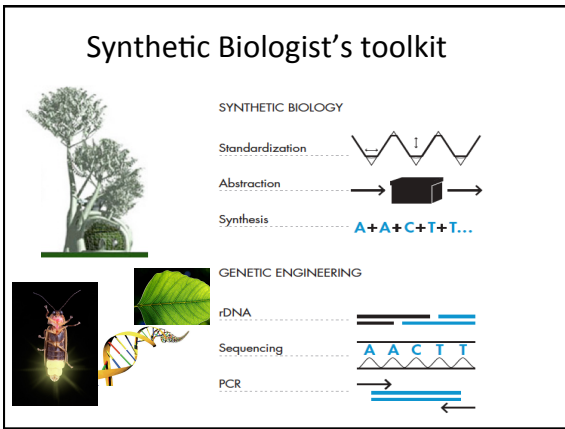
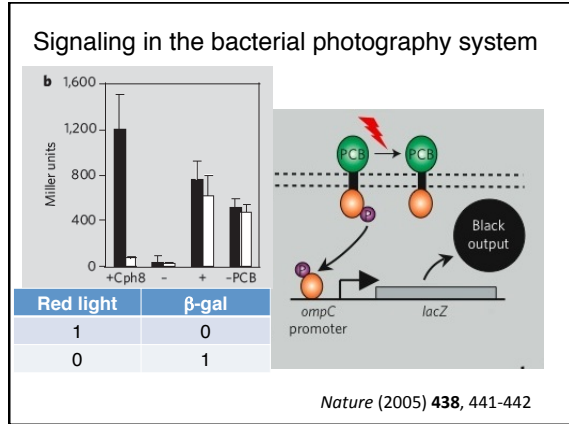
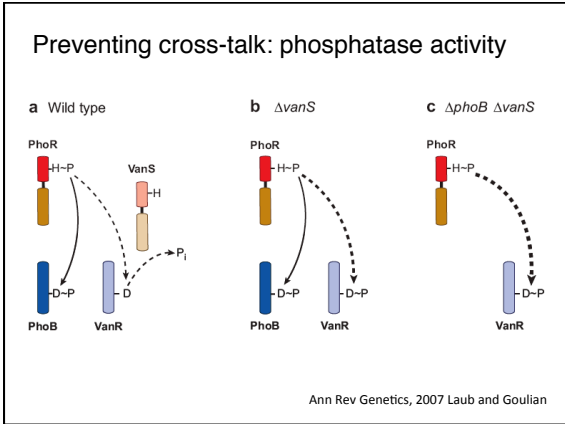


<http://www.bio-rad.com/>

A module in review....



(1) OmpR family	(2) Sporulation family	(3) CitB family	(4) LytT family
(5) AgrA family	(6) NarL family	(7) NtrC family	(8) Chemotaxis family
(9) Cell cycle family	(10) Lux family	(11) LuxR family	



Registry of Standard Biological Parts

Welcome to the Registry of Standard Biological Parts.

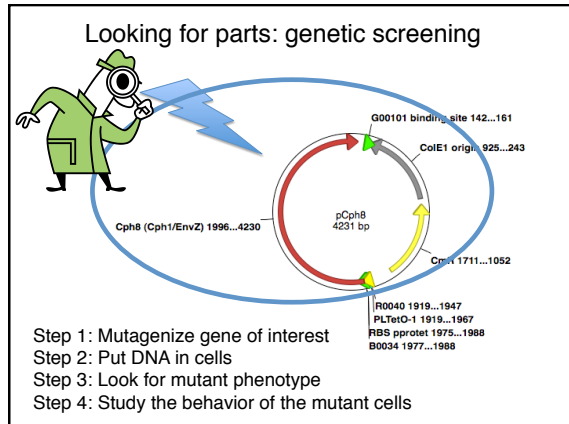
The Registry is a collection of ~3200 genetic parts that can be mixed and matched to build synthetic biology devices and systems. For the Registry is part of the Synthetic Biology community's efforts to make biology easier to engineer. It provides a resource of available teams and academic labs.

The Registry is based on the principle of "get some, give some". Registry users benefit from using the parts and information available from the engineered biological systems. In exchange, the expectation is that Registry users will, in turn, contribute back information and data on existing they make to grow and improve this community resource.

Registry tools

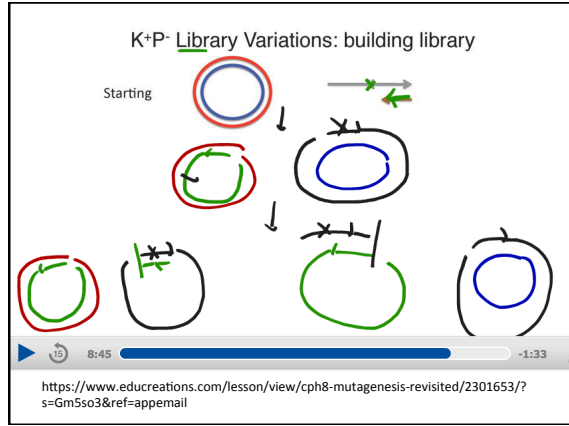
- Search parts (?)
- Add a part
- Request a part
- Send parts to the Registry
- Sequence analysis

<http://bbf.openwetware.org/>



K+P- Library Variations

Enz	H243	D244	L245	R246	T247R (K+P-)	P248
wt seq	CAC	GAC	TTG	CGC	Thr → ACG	CCG
Cph8	H537	D538	L539	R540	T541	P542
	Kinase Dead mutant GCC = Ala			NNY mutagenesis CTY = Leu CCY = Pro CAY = His CTY = Phe TCY = Ser TAY = Tyr TGY = Cys ATY = Ile ACY = Thr AAY = Asn ACY = Ser CTY = Val GCY = Ala GAY = Asp GGY = Gly		
	U C A G			N = G A T C Y = C T 15 possible amino acids No stops		
U	Phe	Ser	Tyr	Cys	U	Phe
Phe	Ser	Tyr	Cys	C	C	Ser
Leu	Ser	STOP	STOP	A	A	Tyr
Leu	Ser	STOP	Trp	G	G	Cys
C	Leu	Pro	His	Arg	U	Asn
Leu	Pro	His	Arg	C	C	Ser
Leu	Pro	Gln	Arg	A	A	Val
Leu	Pro	Gln	Arg	G	G	Ala
A	Ile	Thr	Asn	Ser	U	Asp
Ile	Thr	Asn	Ser	C	C	Gly
Ile	Thr	Lys	Arg	A	A	Gly
Met	Thr	Lys	Arg	G	G	Gly
G	Val	Ala	Asp	Gly	U	15 possible amino acids
Val	Ala	Ala	Asp	Gly	C	No stops
Val	Ala	Glu	Gly	A	A	
Val	Ala	Glu	Gly	G	G	

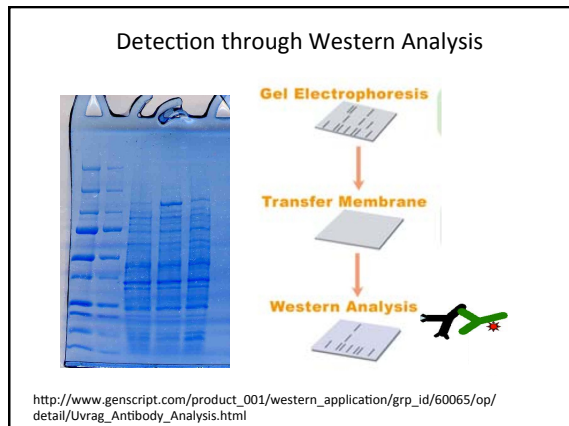
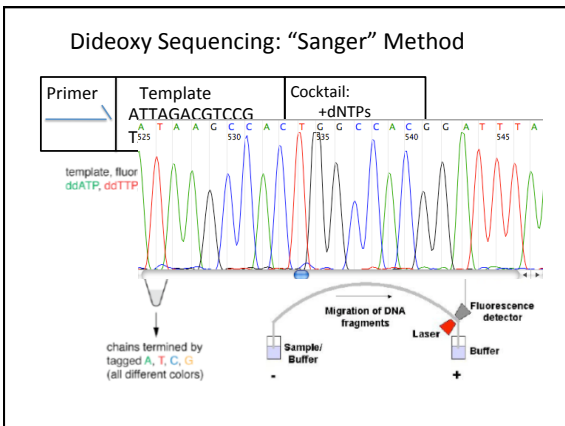
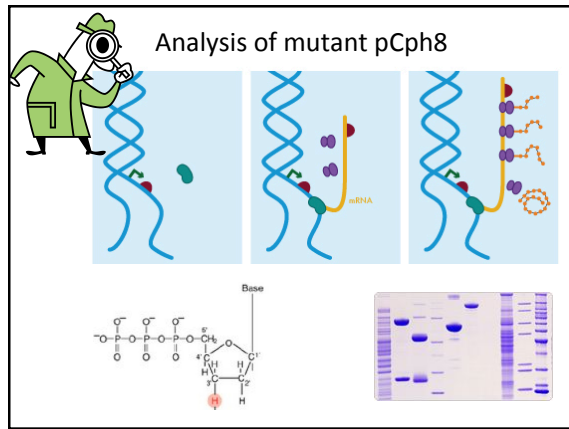


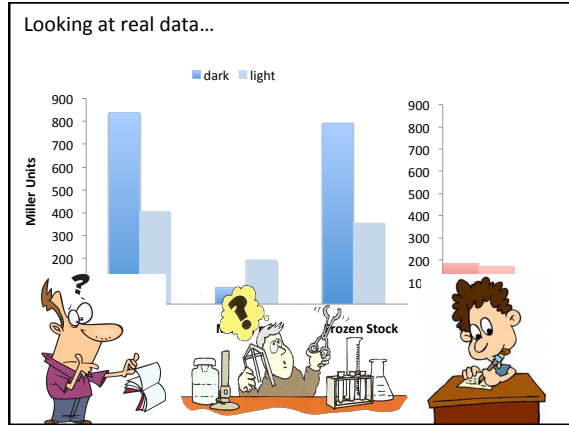
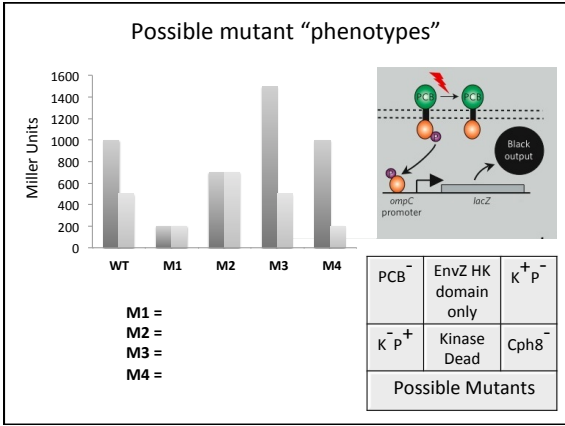
“The art and design of genetic screens: E. coli”

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

below pH 6.8

above pH 8.0





Scientific Writing

20.109
Fall 2012



:Leslie Ann Roldan



Creating Your 20.109 Presentation

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9-10 October 2014