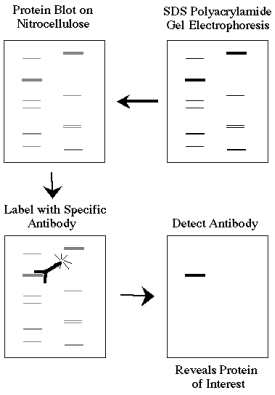
System Engineering

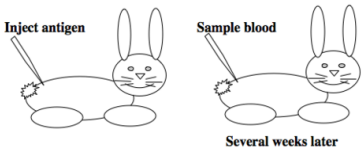
20.109(F11)
M2D7 lecture
11.03.11

Western blot

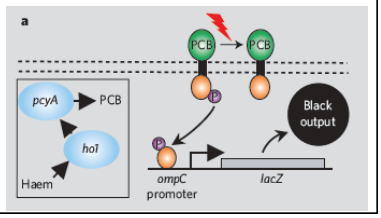


http://www.bio.davidson.edu/courses/genomics/method/Westernblot.html

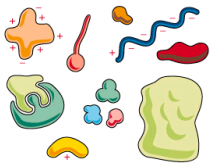
Polyclonal Antibody Production



Which protein would we like to have an antibody recognize?



Affinity Purification

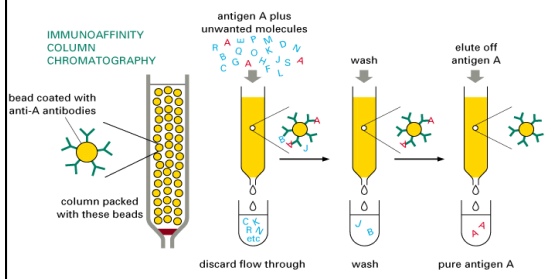


Mixture contains proteins of various
- size,
- shape,
- charge,
- hydrophobicity,
- affinity for different molecules

These properties can be exploited to separate individual protein from mixture

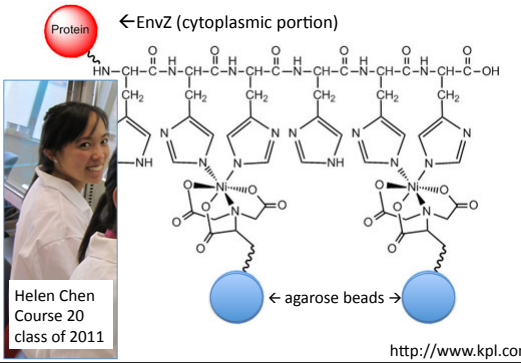
Alberts "Essential Cell Biology"

Affinity Chromatography



Alberts "Essential Cell Biology"

His_6-tag for Affinity Purification



Helen Chen
Course 20
class of 2011

http://www.kpl.com

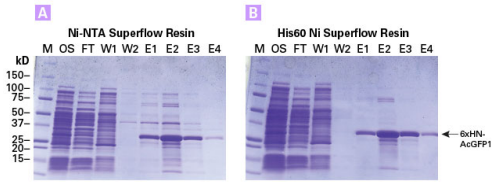
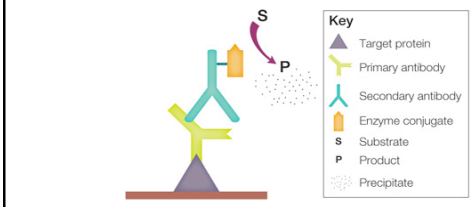


Figure 3. Superior yields and better purity were obtained when using His60 Ni Superflow for purification of recombinant his-tagged protein—as compared to Ni-NTA Superflow resin. Clontech's pEcoli Linear Expression System was used to express 6xHN-AcGFP1 in *E. coli.* Equivalent amounts of the same sample 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

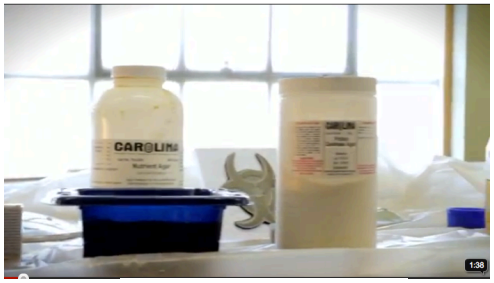
Colorimetric Detection



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

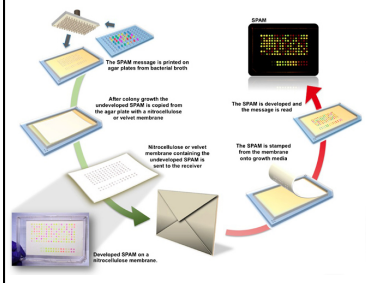
A module in review….

Science? Art? Education? Marketing?

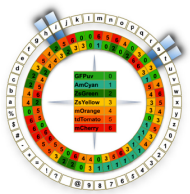


Contagion - Bacteria Billboard

007 meets *E. coli*



Steganography by Printed Arrays of Microbes (= SPAM)

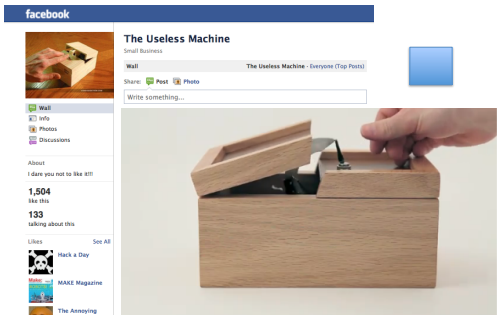


WIRED SCIENCE

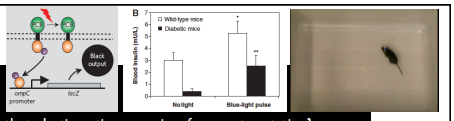
nature news

PNAS (2011) 108(40):16510

Not all machines are useful



Concerns



What stops that plastic-eating organism from not mutating?

I am worried about self replication.

They say they can put in these stop buttons...but I'd like to see more guarantees (more research) because, ...you can't control nature.

I would be concerned about who was running this, and how far they go to re-engineer a certain species.

How do you regulate that?

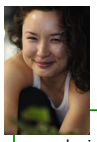
I think the ramifications are not going to be short term.

I am adamantly against any sort of genetic modification. I think it is a very fine line to play God.

Forbes

Citizen Science Takes Off: Could Community Labs Hatch the Next Generation of Bio Innovators?

"I hate to sound elitist," Tom Knight says, "but you hear a lot of people talking in a naive way about doing very complex and difficult things that are challenging projects for the very best scientists and engineers in the world."



"There's an elitism around science," Eri Gentry says. "I want to rid American culture of that fear of science by giving the community ways to have fun with it."

GINKGOBIOWORKS

bioCURIOUS

A story of ownership and sharing

edge detection strain

Get Mail · Write · Address Book · Tag · reply · forward · junk

from Rena Hill
subject **Re: edge detection strain** 4/29/10 9:16 PM
to You other actions

Hi Natalie
I'm almost ready to send you the plasmids to build the edge detector.
Unfortunately we cannot send you the strain.

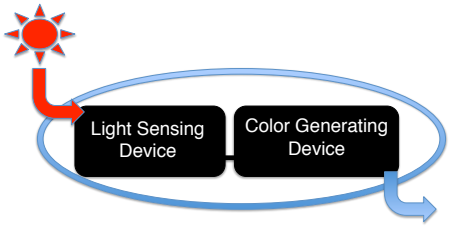
You will need to request the pPLPCB plasmid from the Lagarias Lab at UC Davis.

Email: jclagarias@ucdavis.edu
http://www.mcb.ucdavis.edu/faculty-labs/lagarias/

Sorry for the delay.

Rena

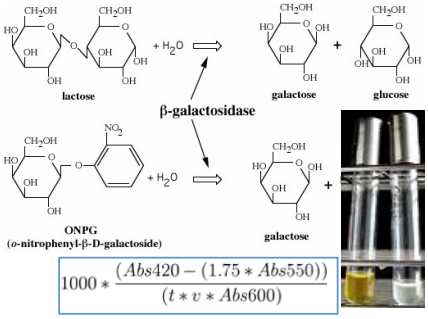
Abstracted View of Bacterial Photography



Photons	Color
1 (= cells are in the light)	0
0 (= cells are in the dark)	1

β-gal activity reflected with indicator compounds

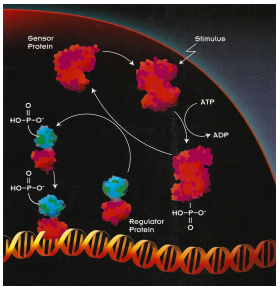
Color Generating Device



$$1000 * \frac{(Abs420 - (1.75 * Abs550))}{(t * v * Abs600)}$$

The 2CS paradigm: in general

Light Sensing Device



Stimulus

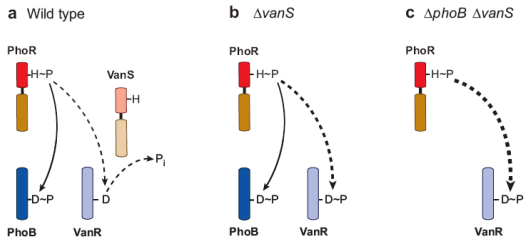
Sensor protein (HK)

±P

Response regulator (RR)

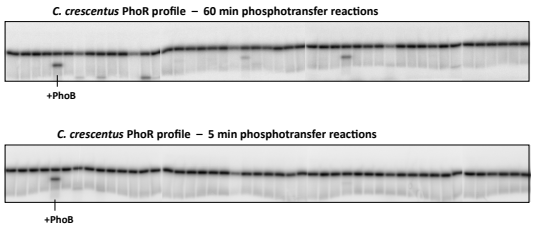
Output

Preventing cross-talk: phosphatase activity



Ann Rev Genetics, 2007 Laud and Goulian

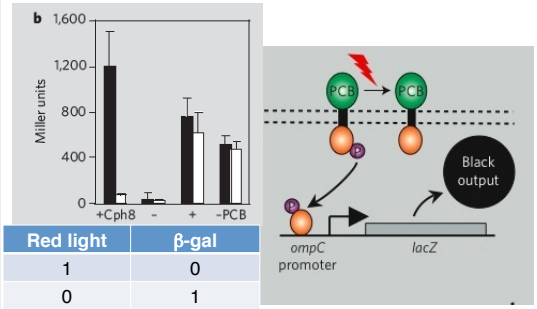
Assessing Specificity: Phosphotransfer Profiling



- histidine kinases exhibit a strong kinetic preference *in vitro* for their *in vivo* cognate substrate
- specificity based on molecular recognition

Slide from Mike Laub

Circuitry and Measurement



Red light	β-gal
1	0
0	1

Nature (2005) **438**, 441-442

New tools for reliable engineering of complex biological systems

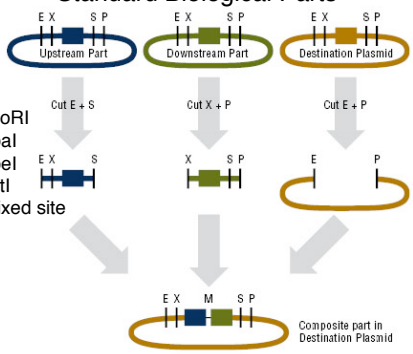
Synthetic Biology

- ***Standardization***
- ***Abstraction***
- ***Synthesis*** A+A+C+T+T…

Genetic Engineering

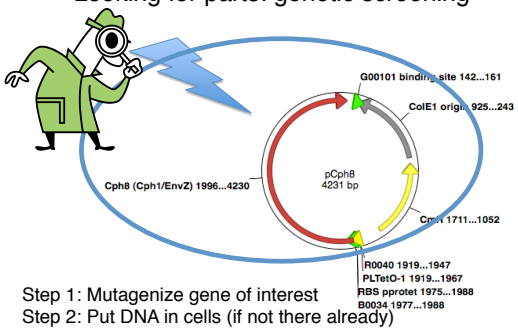
- ***rDNA***
- ***Sequencing***
- ***PCR***

Physical Composition of Standard Biological Parts



E = EcoRI
X = XbaI
S = SpeI
P = PstI
M = Mixed site

Looking for parts: genetic screening



Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells (if not there already)
Step 3: Look for mutant phenotype

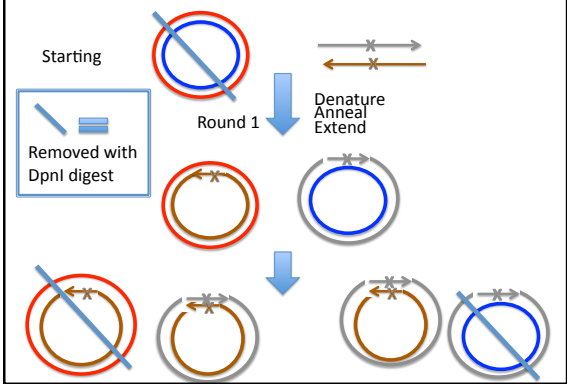
K-P+ Library Variations (in blue)

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**
blue = K-P+	**Ala**				
red = K+ P-	Asp				
	Glu				
	Gly				
	Ile				
	Met				
	Thr				
	Asn				
	Lys				
	Ser				
	Arg				
# poss codons	**16**				**1**
# poss aa	**12**				**1**

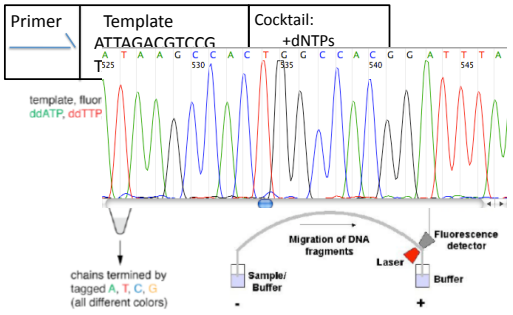
R = G, A
N = G, A, T, C
S = G, C

NOTE: no stop codons should be in mix

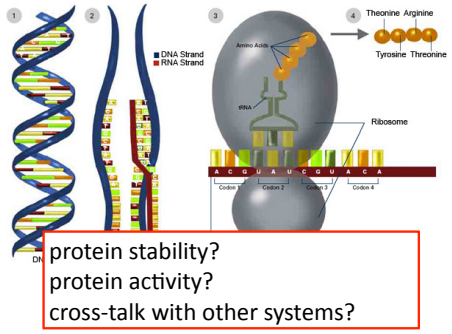
Site-Directed Mutagenesis



Automated "one pot" sequencing



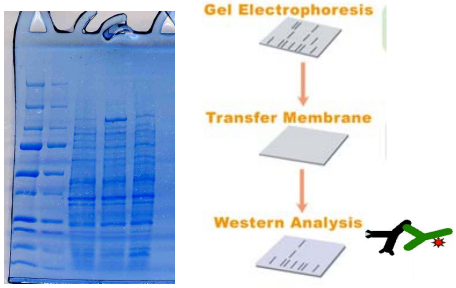
Beyond the C-dog



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

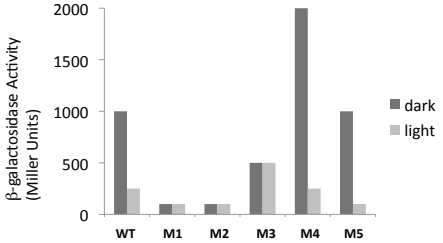
http://publications.nigms.nih.gov/thenewgenetics/images/ch1_trans.jpg

Detection through Western Analysis



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

Possible mutant "phenotypes"



Research Article Module 2 Cap

Linda L. Sutliff, BA, MA, MBA
Writing across the Curriculum Lecturer
Presentation to Laboratory Fundamentals of Biological Engineering
October 27/28, 2011

Instructors: Angela Belcher, Bevin Engelward, Natalie Kuldell and Agi Stachowiak
Communications Coordinator: Atissa Banuazizi

From Howard Silver, MIT Libraries

BE 20.109 Laboratory Fundamentals of Biological Engineering
Thursday, October 27, 2011 & Friday, October 28, 2011, 56-322
Instructors: Angela Belcher, Bevin Engelward, Natalie Kuldell and Agi Stachowiak
Writing Instructor: Linda Sutliff , **Oral Presentation Instructor:** Atissa Banuazizi

Setup: Science and engineering are about discovery and problem solving, but that activity is meaningless without effective communication. When communicating we not only share our discoveries, we also acknowledge the foundational efforts that our work is built on. Just as we expect accurate and careful work in the lab by ourselves and our colleagues. We have equally demanding expectations for precision and accuracy in acknowledging the work of others.

Traditionally the process of citing work in publications is one of the most tedious and frustrating activities that researchers and academics engage in. You are fortunate to be entering this field in an era where there are tools to support and simplify the citation and acknowledgement process and that's what we'll cover in today's session.

This session:
- Show how a citation management tool, in this case –RefWorks, can help support the writing process.
- Generate a manuscript
- Create an account
- Populate an account with your research using library resources.
- Create your own manuscript

Creating Your 20.109 Presentation

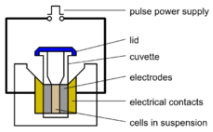
Atissa Banuazizi
Lecturer, Writing Across the Curriculum
atissa@mit.edu

Troubleshooting 101

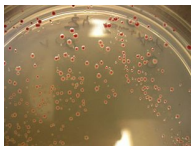
Observation
7/7 groups got >100 colonies with 20ul EP mix
5/6 groups got no colonies, even with 200 ul EP mix !!

What could be the explanation?
- User error
- Materials
- Scientific

Electroporation



Screen on Tetrazolium



Some of my hopes…