

- **Announcements**
- **Pre-lab Lecture**
  - ❖ **Library screen**
  - ❖ **Engineering tools/analogues**
  - ❖ **Today in Lab (M2D3)**

# Announcements

- M2D4 planning
  - brief stint in lab to take quiz and check plates – that's it!
  - Tue: commitment to attend some 2CS jclubs
    - please tell me in advance which 1 hr+ of jclub time you plan to attend (broken up is okay), likely b/w 1:30-3:30 pm
    - don't walk in *during* a talk!
- Module 2 report due date now Wed 11/12 at noon
  - shift in Veteran's Day break this year
- Error: Kan<sup>R</sup> is for the EnvZ deletion, not the integration (see strain description)
  - should've reviewed last year's complete pre-labs!

# Measuring LacZ: original system

Team Color	B-gal units (dark)	B-gal units (light)
Green	~1200	~300
Blue	~800	~160
Pinkle	450	400

~4-fold difference (bracketed next to Green and Blue rows)

\* Known lab issues (next to Pinkle row)

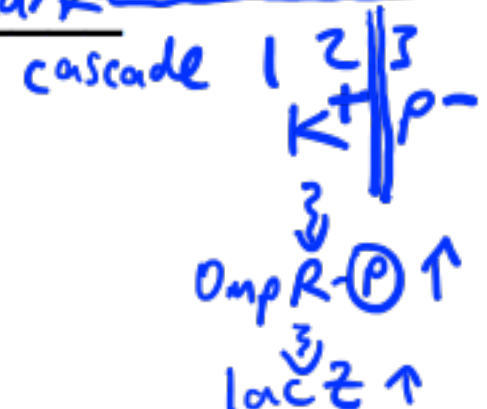
$$1 \text{ Miller Unit} = 1000 * \frac{(Abs_{420} - (1.75 * Abs_{550}))}{(t * v * Abs_{600})}$$

Slide modified from N. Kuldell

# Genetic library screen: concepts

- Goal: improve contrast of bact. photographs
- Specifically, make plates dark er in the dark
- Which mutations should have this effect?
- Why randomize at or near those sites?
- Control/reference mutation:  $H^{S37} \rightarrow A$

⊕ How might this strategy benefit? contrast vs. absolute




F11:	EnvZ		<b>A239T</b>	<b>G240E</b>	<b>V241G</b>	<b>S242D</b>	<b>H243A</b>	blue = K-P+
F12:	<u>EnvZ</u>	<b>H243</b>	D244	L245	R246	<b>T247R (K+P-)</b>	P248	
	wt seq	CAC	GAC	TTG	CGC	Thr = ACG	CCG	
	<u>Cph8</u>	<b>H537</b>	D538	L539	R540	<b>T541</b>	P542	
	to test:	<b>Kinase Dead mutant</b>				<b>NNY mutagenesis</b>		

F14

Images from F11 and F12 wikis

# Genetic library screen: methods

- Electroporation of library into cells
  - modified bacterial photography strain:  $\Delta$ Cph8
  - library of T541 randomized to NNY

mutants of Cph8 

but has other plasmids

NNY → avoid stop codons

equimolar AT, G, C @ that synthesizes skp

- Selective media:

## MacConkey MUG agar

- contains lactose and red pH/metabolic indicator
- high [B-gal] leads to darker red colonies (cleavage)

vs. paler red \* in a sensitive range \*

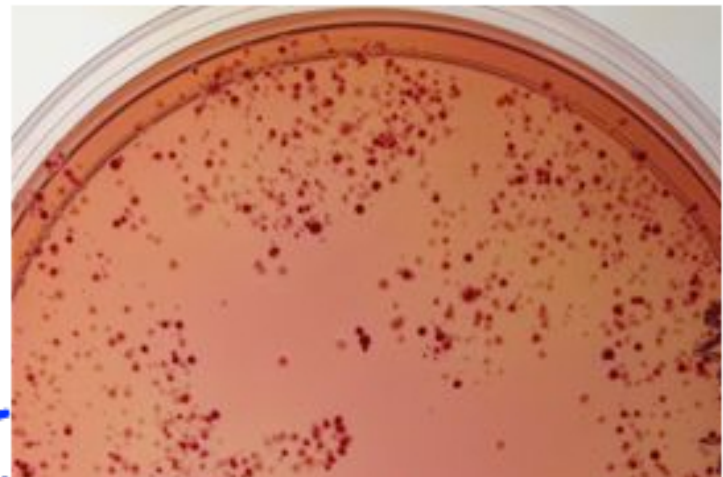


Image from wiki (M2D4 "Talk" page)

# Registry of Std Bio Parts

levels of abstraction

**Goal: design protein generator that functions in *E. coli***

## Browse parts by type

Catalog

List



Promoters (?): A promoter is of the downstream DNA seq



Ribosome Binding Sites (?) can bind and initiate translati



Protein domains (?): Protein up a protein coding sequence target the protein for cleavag



Protein coding sequences ORF Note that some protein codin protein from start codon to st also included here.



Translational units (?): Trar They begin at the site of tran

transcription

translation

ORF

## Browse parts and devices by function

*This section replaces the previous **Featured parts** pages.*



**Biosynthesis:** Parts involved in the producti



**Cell-cell signaling and quorum sensing:** f



**Cell death:** Parts involved in killing cells.



**Colloid:** Parts involved in taking a bacterial

EX-SP

## Browse parts and devices by standard

*Unless otherwise specified, most parts in the Registr*

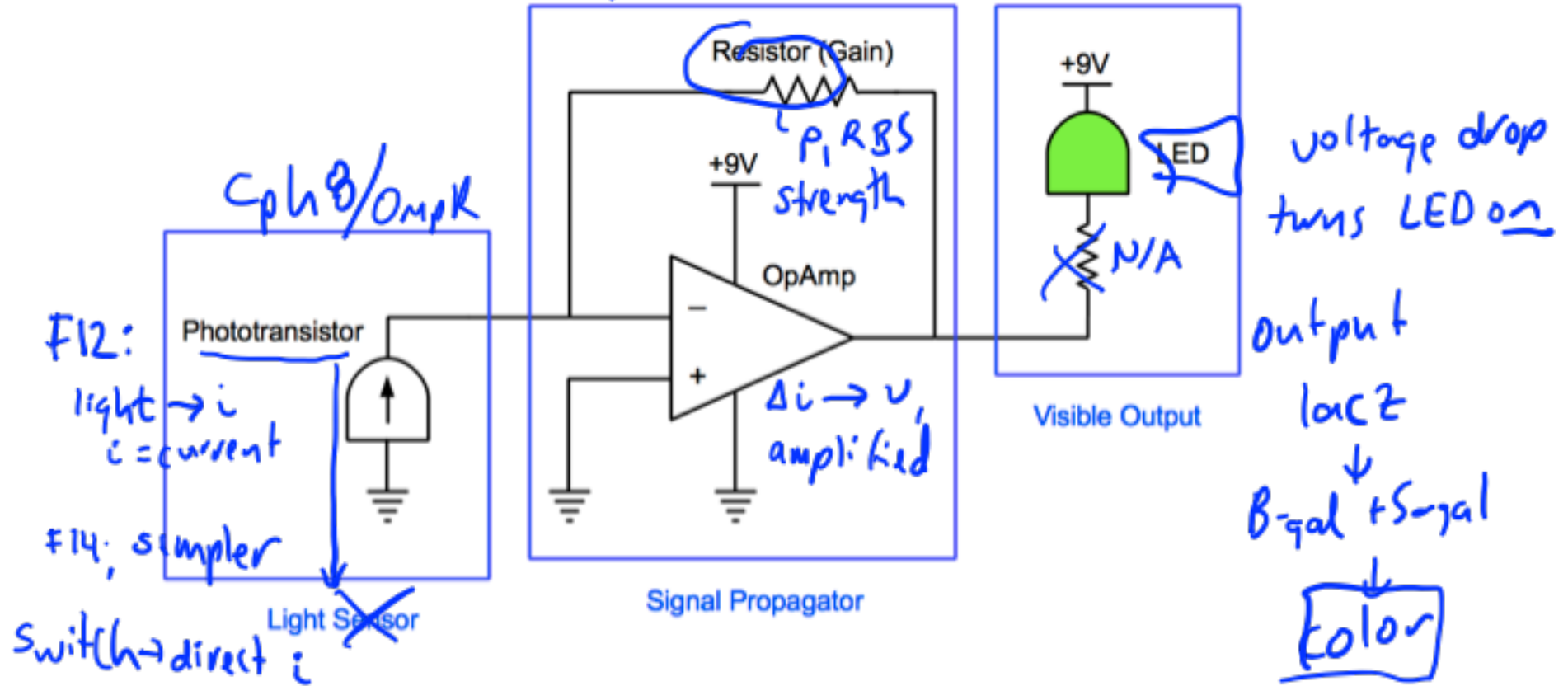
**Assembly standard 10** (?): Assembly standard 10, i comply with this assembly standard.

**Assembly standard 23** (?): Assembly standard 23, i

Images from parts.mit.edu

# EE analogue to BP system

*Omp R → lac & logic/signaling*



**Goal: vary R and observe outcomes**

*(limit cases:  $R=0, R=\infty$ )*

# Today in Lab: M2D3

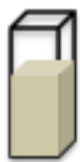
Observe/document *coli*roid from last time



Set up library screen (Start FNW...)

1. electroporate

2. plate cells (BPΔCph8)



incubate 1 hr\*

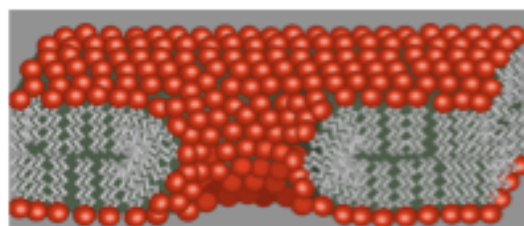
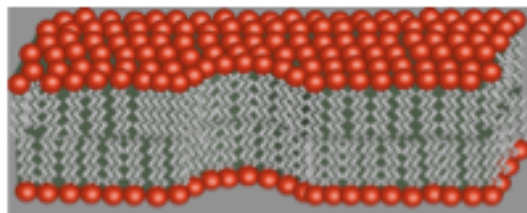


Explore EE analogue

Explore [parts.mit.edu](http://parts.mit.edu)

Explore TinkerCell

\*transfer to SOC medium  
(has extra sugar, etc.)



Membrane model from Wikimedia Commons, public domain image





“Modular” Lego fun

Combining Star Wars  
Legos and Heartlake  
Stables Lego Friends