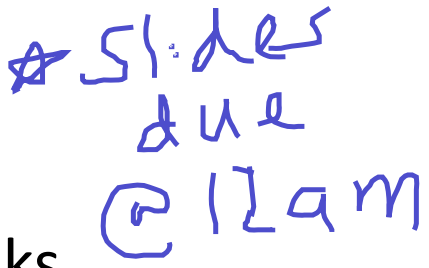
- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Library screen
  - Engineering tools/analogues
  - Today in Lab (Mod 2 Day 3)

Announcements

- Brief discussion of previous quiz
- Notebooks due at end of lab today
- Next time
  - come to *lab* first to set up experiment
  - no quiz!
  - we'll go together to 16-336 for j. club talks
- J. club grading rubric available up front
- Error: Kan^R is for the EnvZ deletion, not the integration (see strain description)



Measuring LacZ: original system

Team Color	B-gal units (dark)	B-gal units (light)
Green	358	173
Blue	1644	531
Pink	616	1319
Purple	1334	475

$$\text{1 Miller Unit} = 1000 * \frac{(Abs420 - (1.75 * Abs550))}{(t * v * Abs600)}$$

OD

OD

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: kinase dead, in II

red: K+P−

F11:

EnvZ	A239T	G240E	V241G	S242D	H243A	blue = K-P+

F12:

EnvZ	H243	D244	L245	R246	T247R (K+P-)	P248
wt seq	CAC	GAC	TTG	CGC	Thr = ACG	CCG
Cph8	H537	D538	L539	R540	T541	P542
to test:	Kinase Dead mutant				NNY mutagenesis	

H537A

Images from F11 and F12 wikis

Genetic library screen: methods

- Electroporation of library into cells
  - modified bacterial photography strain: ΔCph8
  - library of T247 randomized to NNY
- Selective media:
  MacConkey MUG agar
  - contains lactose and red pH/metabolic indicator
  - high [B-gal] leads to more red colonies (cleavage)

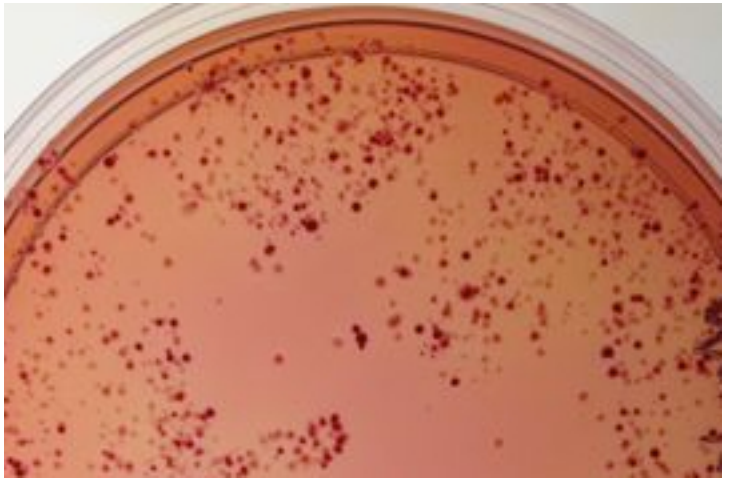


Image from wiki (M2D4 "Talk" page)

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 sequ

Ribosome Binding Sites (?
can bind and initiate translati

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

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

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

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.

Coliroid: Parts involved in taking a bacterial

Browse parts and devices by standard

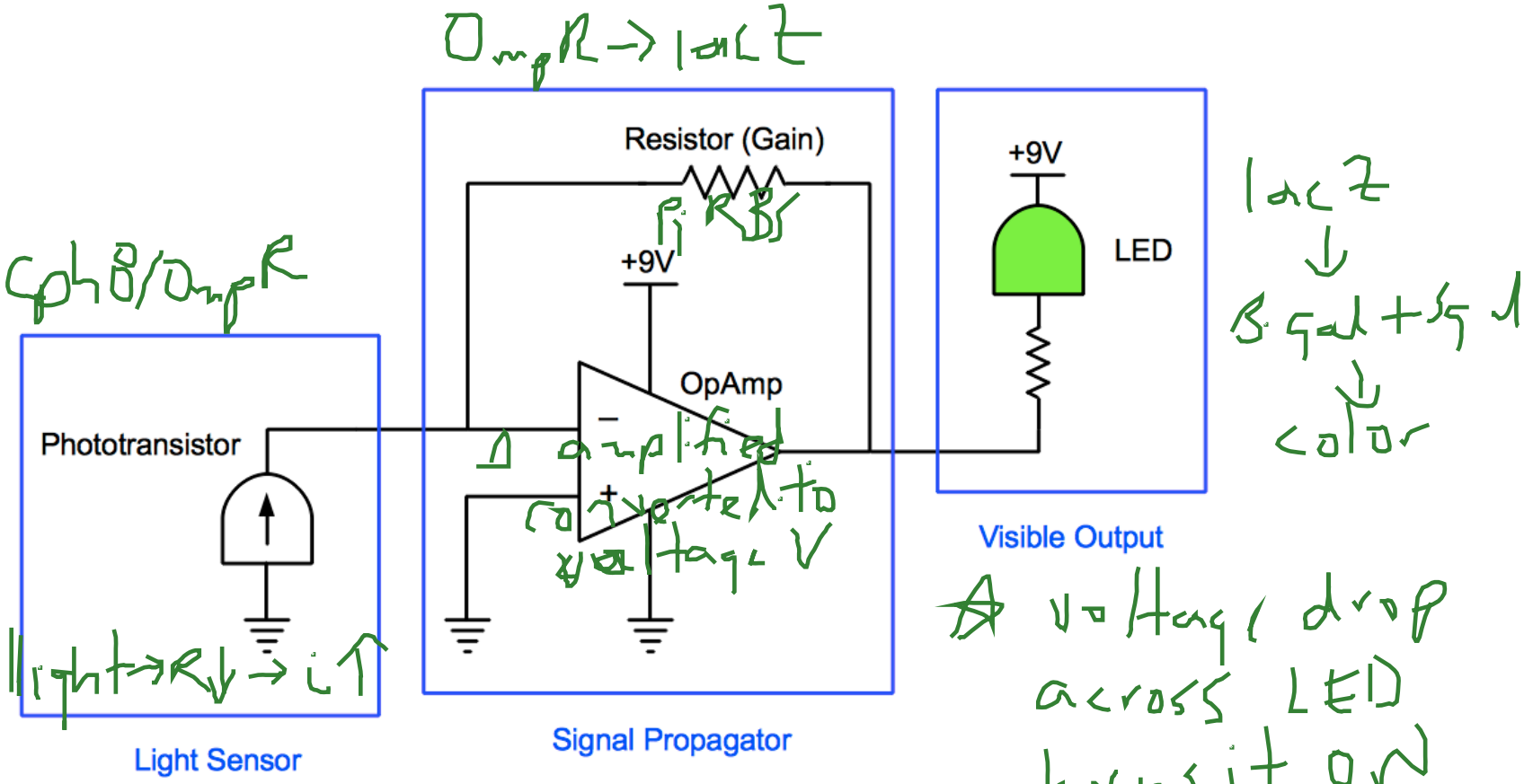
Unless otherwise specified, most parts in the Registr

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

Assembly standard 23 (?): Assembly standard 23,

Images from parts.mit.edu

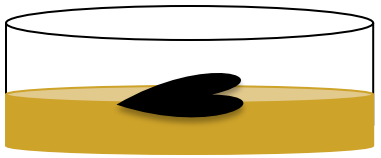
EE analogue to BP system



Goal: vary R and observe outcomes

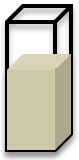
Today in Lab: M2D3

Observe/document *coli*roid from last time



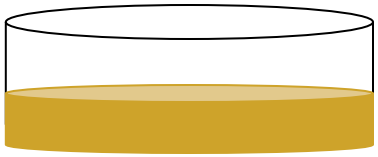
Set up library screen

1. electroporate



incubate 1 hr*

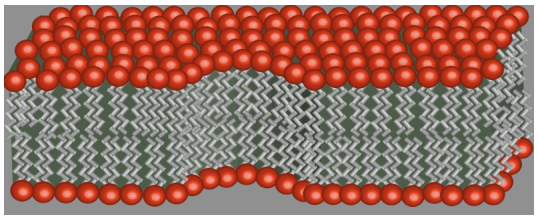
2. plate cells (BPΔCph8)

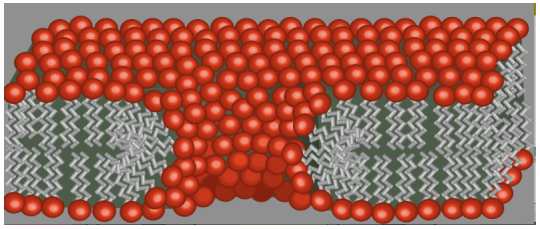


Explore EE analogue

Explore parts.mit.edu

Explore TinkerCell





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

Membrane model from Wikimedia Commons, public domain image