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

Homework review

- HW comments for everyone
  - for figures, get *average* activity of duplicates
    - consider presentation: absolute or fold-change?
  - inherent cell activity or dilution variability?

ideal case outside of linear range

  - coupling of culture volume and OD600?
  - A420 vs. OD420?
  - meaning of OD550 < 0? less round to 0

Announcements

- 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
- Discuss tablet vs. iPad
- Error: Kan^R is for the EnvZ deletion, not the integration (see strain description)

Methods section tips

- Organizing sub-sections
  start w/an overview sentence → step-by-step details
  topical, not necessarily day-by-day in lab
- Methods should be concise and complete
  - Space-wise, avoid lists/tables when a sentence will do
  - Sentence-wise, avoid extra words
  - Content-wise, cover what's needed and only that needed to understand and replicate your work
- Concentrations are more useful than volumes; or you can state amounts, plus total volume.
- Read the guidelines!

Methods section exercise

- Consider the following passage: “Template DNA (5 ng) and primers were mixed with 20 uL of 2.5X Master Mix in a PCR tube. Water was added to 50 uL. A tube without template was prepared and labeled control. ”
  - What information is missing?
  - What information can be cut?

Measuring LacZ

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

Team Color	B-gal units (dark)	B-gal units (light)
Red	1203	538
Orange	1183	736
Yellow	546?	305?
Green	8249? 600?	2881? 200?
Blue	1196	594
Pink	1163	423

Slide from N. Kuldell

Genetic library screen: concepts

- Goal: improve contrast of bact. photographs
- Specifically, make plates light er in the light
- Which mutations should have this effect? blue
- Why randomize at those sites?

EnvZ	A239T	G240E	V241G	S242D	H243A		blue = K-P+
Cph8 = Cph1/EnvZ	A553	G554	V555	S556	H557		red = K+ P-

Image from wiki

Genetic library screen: methods

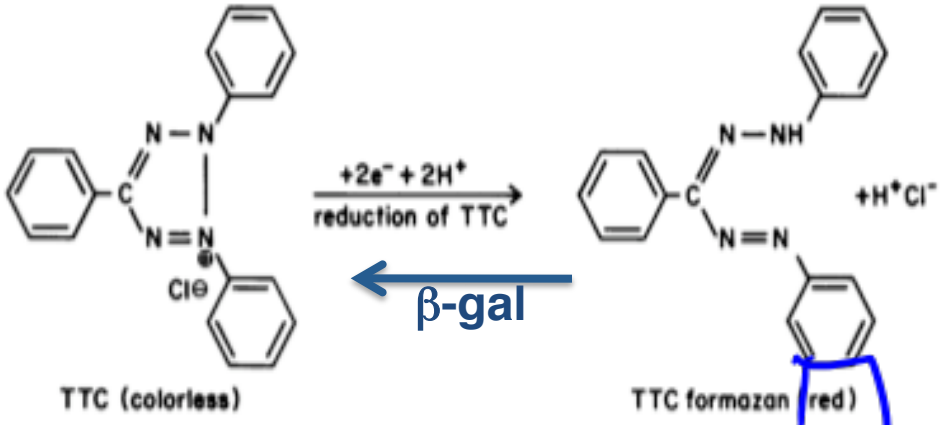
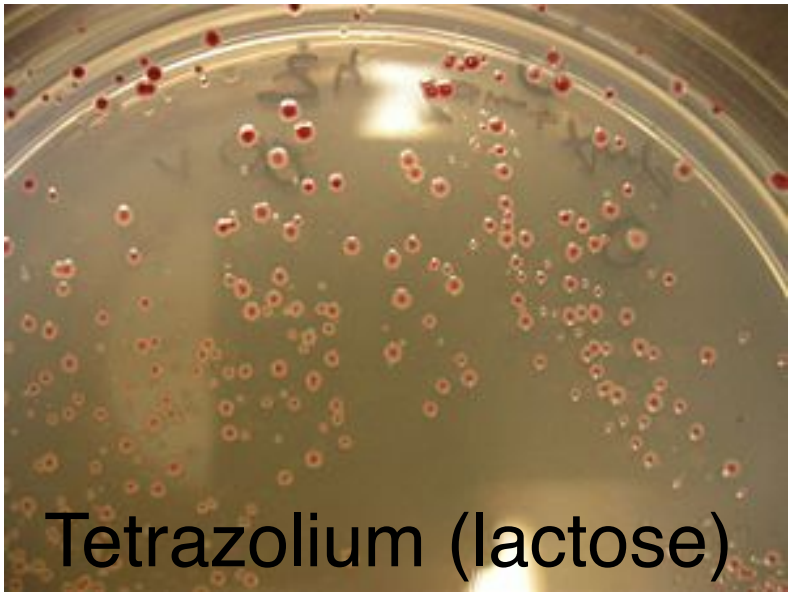


FIG. 1. *Structure and reduction of TTC.*



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Generalized Indicator Plate for Genetic, Metabolic, and Taxonomic Studies with Microorganisms

BARRY R. BOCHNER[1]* AND MICHAEL A. SAVAGEAU

Slide modified from N. Kuldell

Registry of Std Bio Parts

levels of abstraction

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

Browse parts by type

RBS

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: P

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

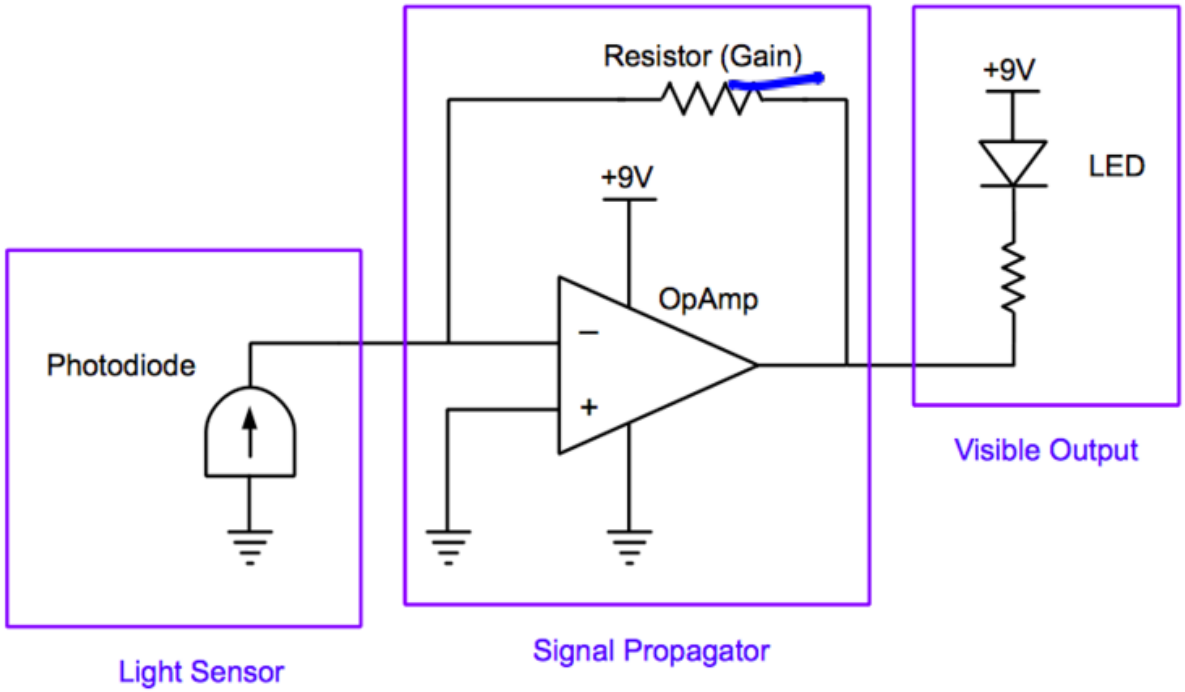
EXPSP

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



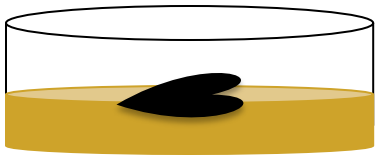
Goal: vary R and observe outcomes

limit cases: R = 0, ∞

digital vs. analog

Today in Lab: M2D3

Observe/document *coli*roid from last time

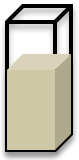


Set up library screen

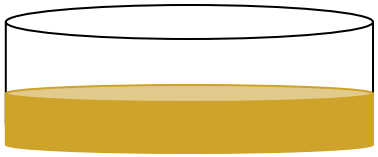
safety glasses

1. electroporate

2. plate cells (BPΔCph8)



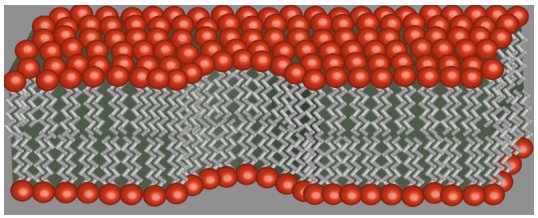
incubate 1 hr*

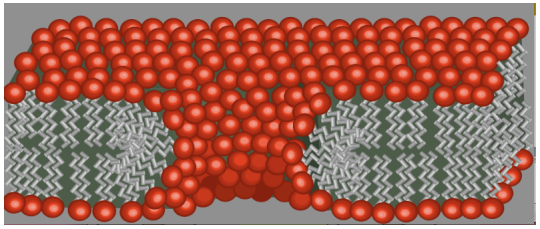


Explore EE analogue

Explore parts.mit.edu

Explore TinkerCell





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

Membrane model from Wikimedia Commons, public domain image