Announcements

- Pre-lab Lecture
  - Mod 2 overview
  - Intro to "coliroid" system
  - β-gal assays
  - Today in Lab (Mod 2 Day 1)
Announcements

• Introducing... Tyler, TA for Module 2
• Module 2 heads-up
  – Journal club presentations next week
  – 7 of you on M2D4, 3 of you on M2D7
  – Sign up on M2D1 Talk page
Module 2 Goals + Overview

- Starting point: bacterial photography system
- Design goal: improve dynamic range
  \[ \text{Contrast} \leq \text{light to dark} \]
- Method: screen DNA library
- Big picture: programmability of biology
Bacterial photography abstracted view

Input is:

light

\[ \Rightarrow \quad \text{Input Sensing Device} \quad \Rightarrow \quad \text{Output Generating Device} \quad \Rightarrow \]

Output is:

\[
\text{color} \downarrow \\
\text{via chem. rxn.}
\]

System states:

1) light off, color on (black)

2) light on, color off (yellow)
Sensor details

- Two plasmids required: sensor + accessory

* cyanobacteria
† from E. coli
Regulation details

Natural 2-component system:
Sensor $EnvZ$  Responder $OmpR$
Stimulus osmotic shock

Engineered system:
- Ompr-dependent promoter
- Ompr binds only when phosphorylated
- $Po_4 \rightarrow lacZ$ ON

$\text{osmoregulation}$

$\text{OmpR}^R$  $\text{lacZ}$  $\text{Kan}^R$

$\Delta EnvZ$ strain
β-gal assay: background

- β-gal is protein encoded by \( \text{lac}^Z \)
- ONPG is used to detect β-gal. How?

![ONPG structure]

Wikimedia Commons, public domain image

- Useful range of assay

\[
\text{Abs.} \quad (\text{405 nm})
\]

\[
\text{Spectrophotometric measurement}
\]

Target: \( \text{Abs} = 0.1-1 \)
β-gal practice assay: workflow

- Dilute cells
- Measure OD (600nm)

1. Lyse
2. SDS, CHCl₃; vortex consistently in hood in PPE excess → chem. waste

START:
- ONPG
- + Na₂CO₃

STOP:
- 30s
- 200s

Spin

Δt = 2 sec.

Measure
- A₄₂₀ - yellow
- A₅₅₀ - debris
Today in Lab

• Set up bacterial plates in light and dark
• Set up liquid cultures in light and dark
• Practice β-gal assay (calculations FNT)

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