

- Announcements

- Pre-lab Lecture

- ❖ Mod 2 overview

- ❖ Intro to “*coliroid*” system

- ❖  $\beta$ -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

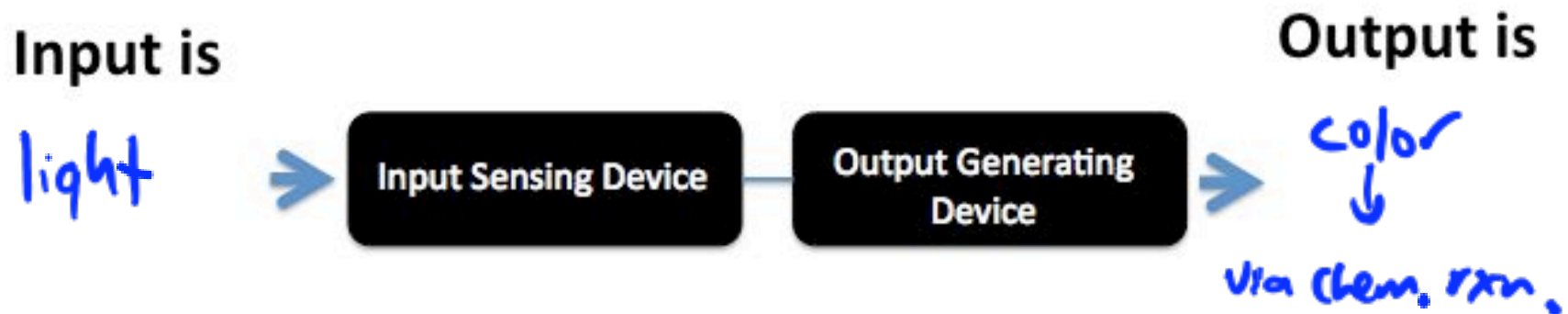
# Module 2 Goals + Overview

- Starting point: bacterial photography system
- Design goal: improve dynamic range

contrast ← light to dark

- Method: screen DNA library
- Big picture: programmability of biology

# Bacterial photography abstracted view

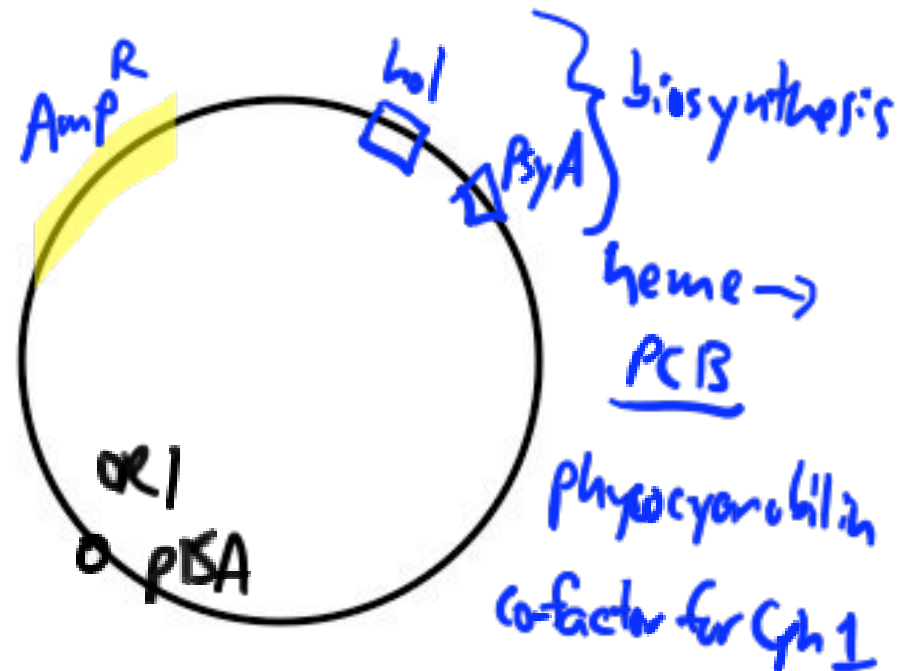


System states:

- 1) light off, color on (black)
- 2) light on, color off (yellow)

# Sensor details

- Two plasmids required: sensor + accessory



\* cyanobacteria  
† from E. coli

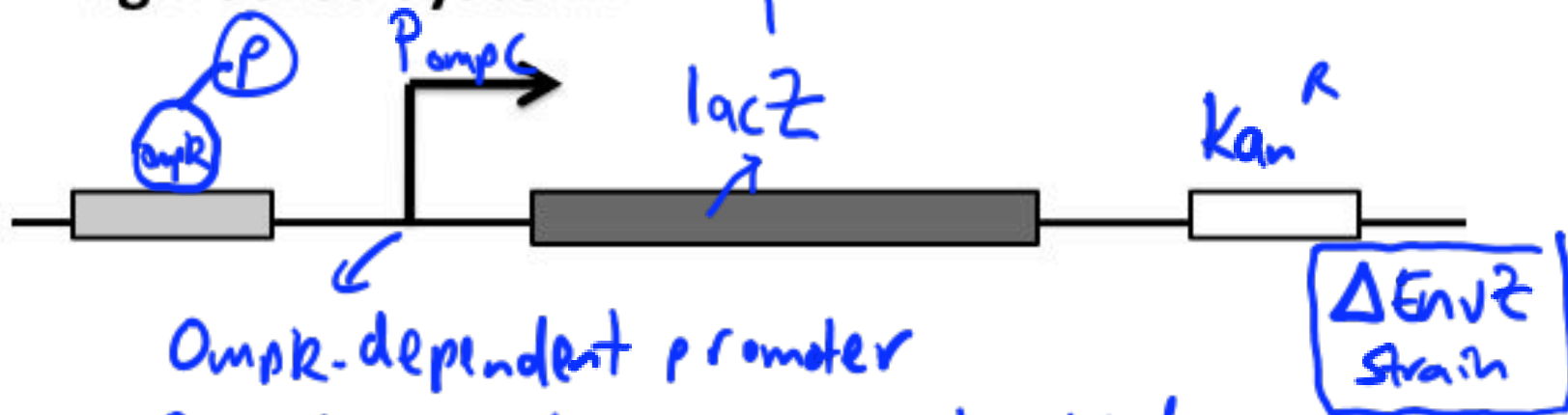
# Regulation details

Natural 2-component system: osmoregulation

Sensor EnvZ                      Responder OmpR

Stimulus osmotic shock

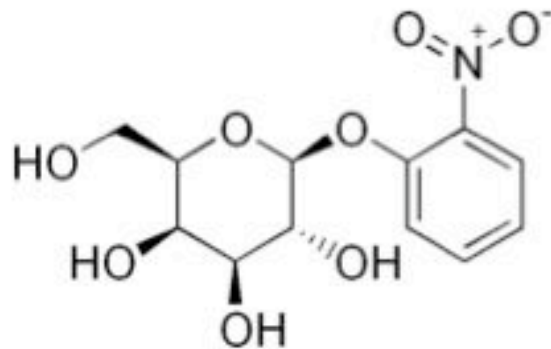
Engineered system: reporter reacts  $\rightarrow$  color formation



OmpR-dependent promoter  
OmpR binds only when phosphorylated  
 $\therefore P_0_4 \rightarrow lacZ$  ON

# $\beta$ -gal assay: background

- $\beta$ -gal is protein encoded by *lacZ*
- ONPG is used to detect  $\beta$ -gal. How?



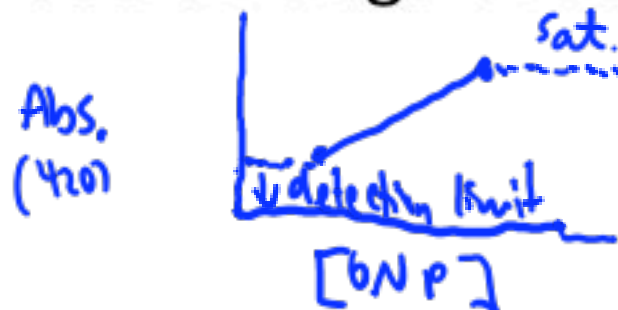
Wikimedia Commons, public domain image

galactose

ONP

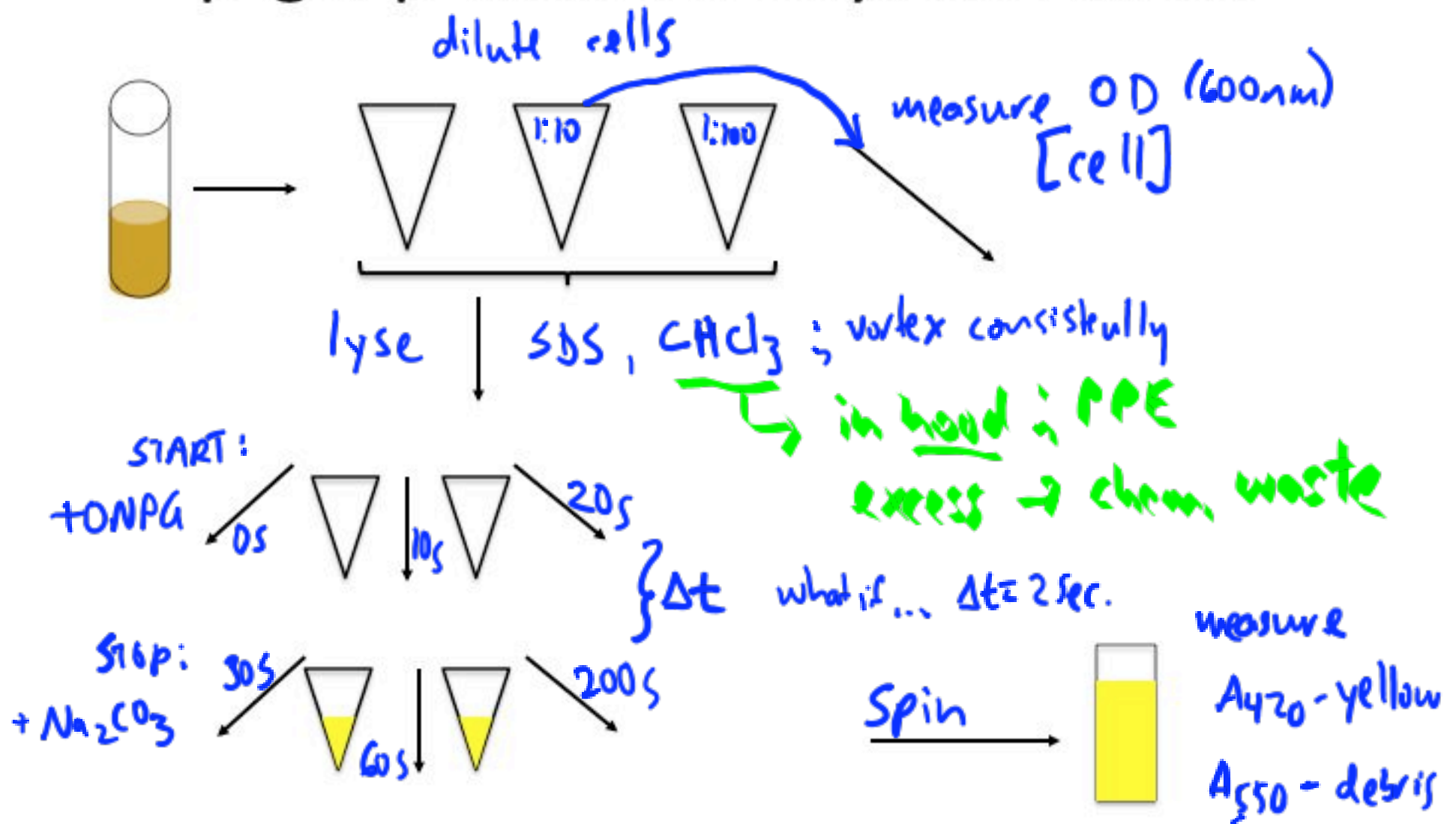
ortho nitrophenol  
yellow  
measure w/spec.!

- Useful range of assay



target: Abs = 0.1-1

# $\beta$ -gal practice assay: workflow





# Today in Lab

- Set up bacterial plates in light and dark
- Set up liquid cultures in light and dark
- Practice  $\beta$ -gal assay (calculations FNT)

↳ not BPS ; strain overproduces  $\beta$ -gal

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

} BP  
system  
see original  
dynamic  
range