

- Announcements
- Pre-lab Lecture
  - ❖ Intro to “*coliroid*” system
  - ❖  $\beta$ -gal assays
  - ❖ Today in Lab (M2D1)

# Announcements

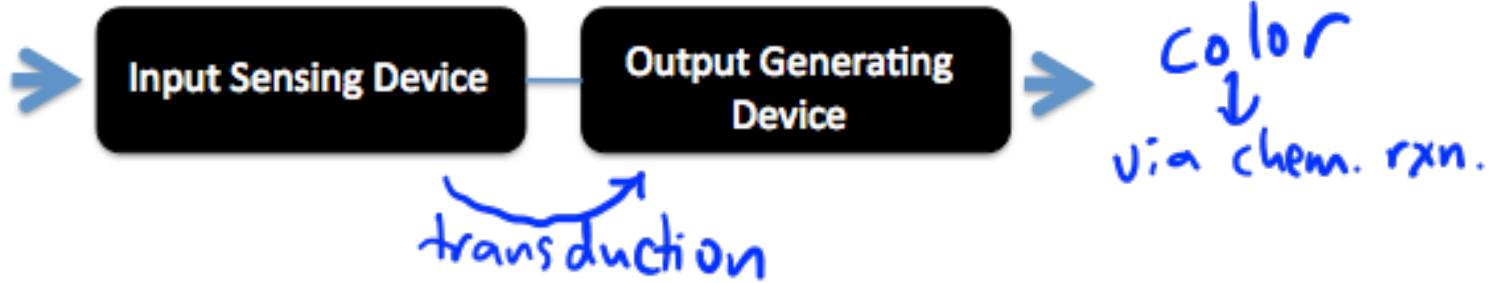
- Introducing... Yongjin, TA for Module 2
  - Evernote sharing: yjpark352 (gmail)
- Wrapping up Mod 1
  - summary due Sat 5 pm
  - blog post due Sun 5 pm
  - OH R4-5:30 (ANS), R 7-8:30 and F 10am (SKH)
- Module 2 heads-up
  - journal club presentations
    - W 10/22 on 2CSignaling, W 11/5 on SynthBio
  - please choose paper by M2D3

+ quiz next time  
+ our emails = required  
class reading

# Bacterial photography abstracted view

**Input is**

light



**Output is**

color  
↓  
via chem. rxn.

**System states:**

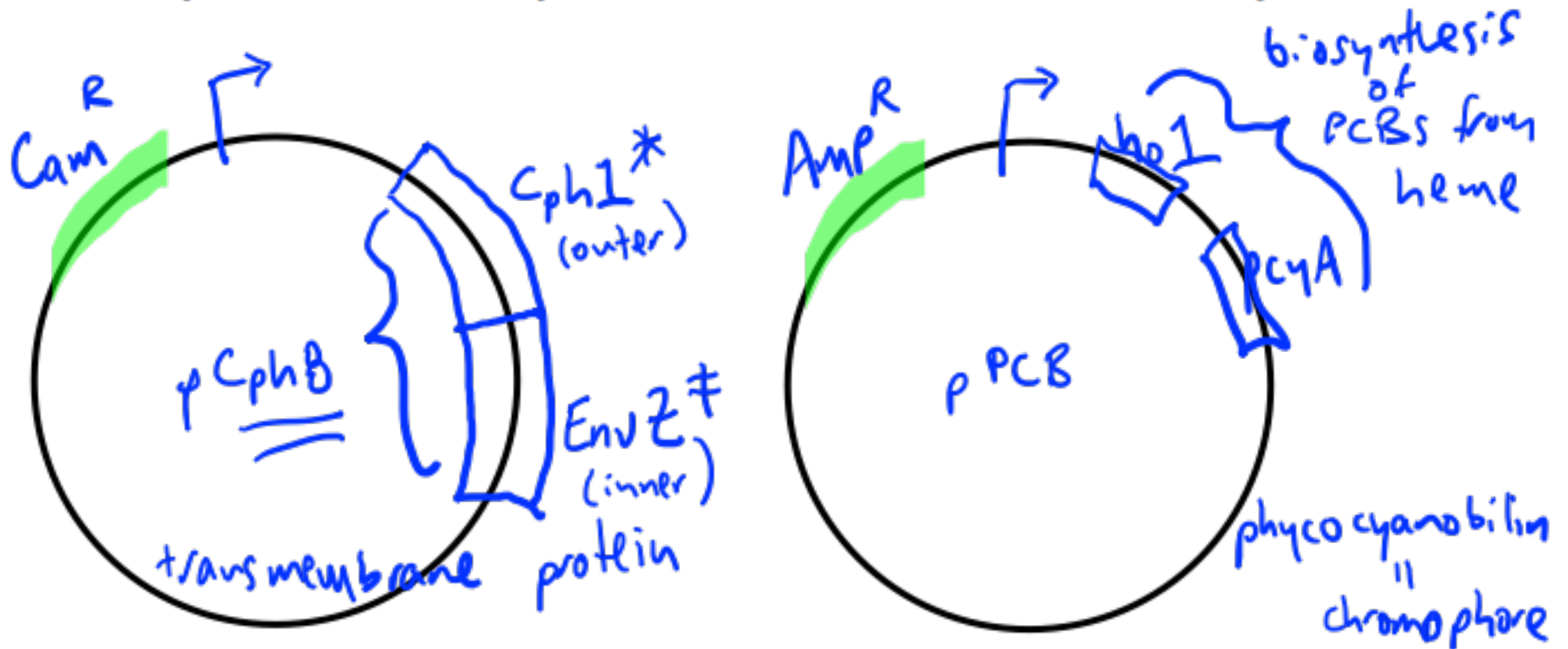
- 1) light off, color on  
→ black
- 2) light on, color off  
→ yellowish

**Design goal:** improve contrast

**Method:** genetic screen

# Light Sensor details

- Two plasmids required: sensor + accessory



\* cyanobacteria  
‡ E. coli derived

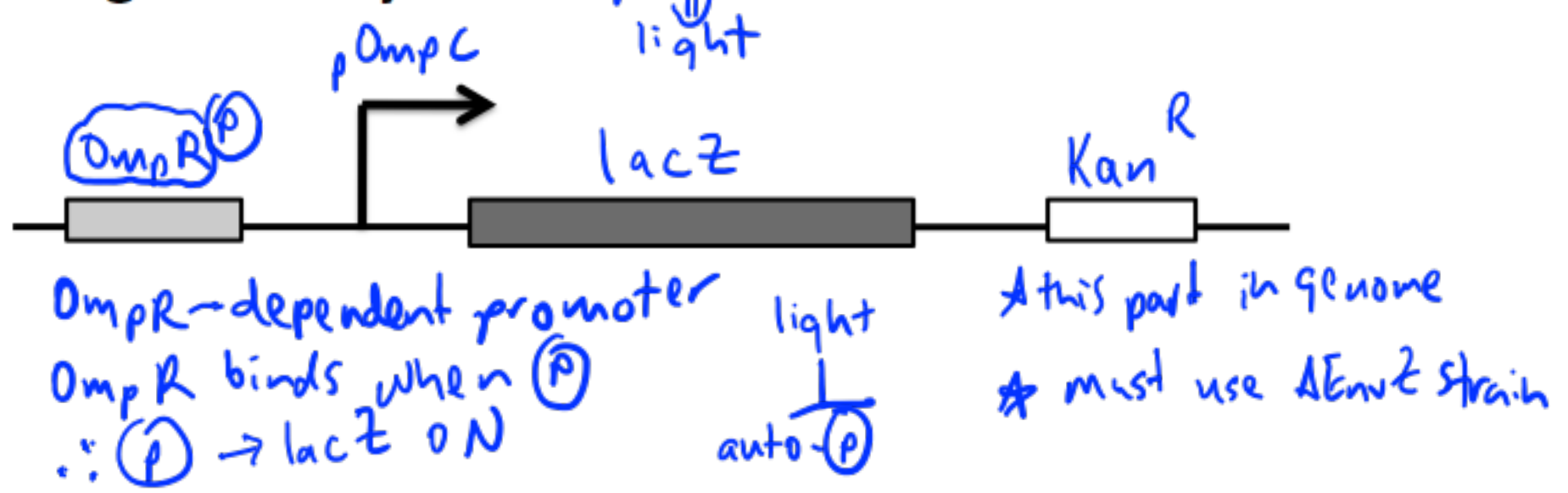
$\text{Cph1}$  "apo" protein  
 $\text{Cph1} + \text{PCBs}$  "holo"

# Regulation details

## Natural 2-component system:

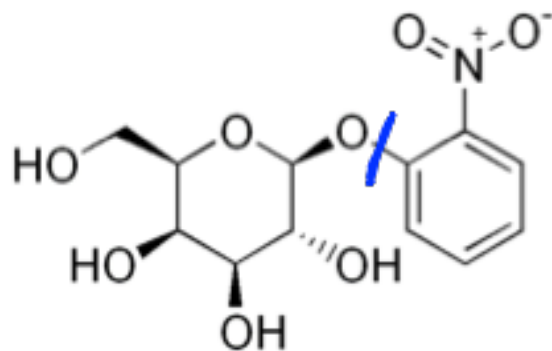
Sensor  $EnvZ$       Responder  $OmpR \rightarrow \Delta$  porins  
Stimulus osmotic shock

Engineered system:  $CphB$        $OmpR \rightarrow lacZ$   
light



# $\beta$ -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 = yellow  
(instead of glucose)

measure  
on spec.

saturation (spec. or cpm)

- Useful range of assay

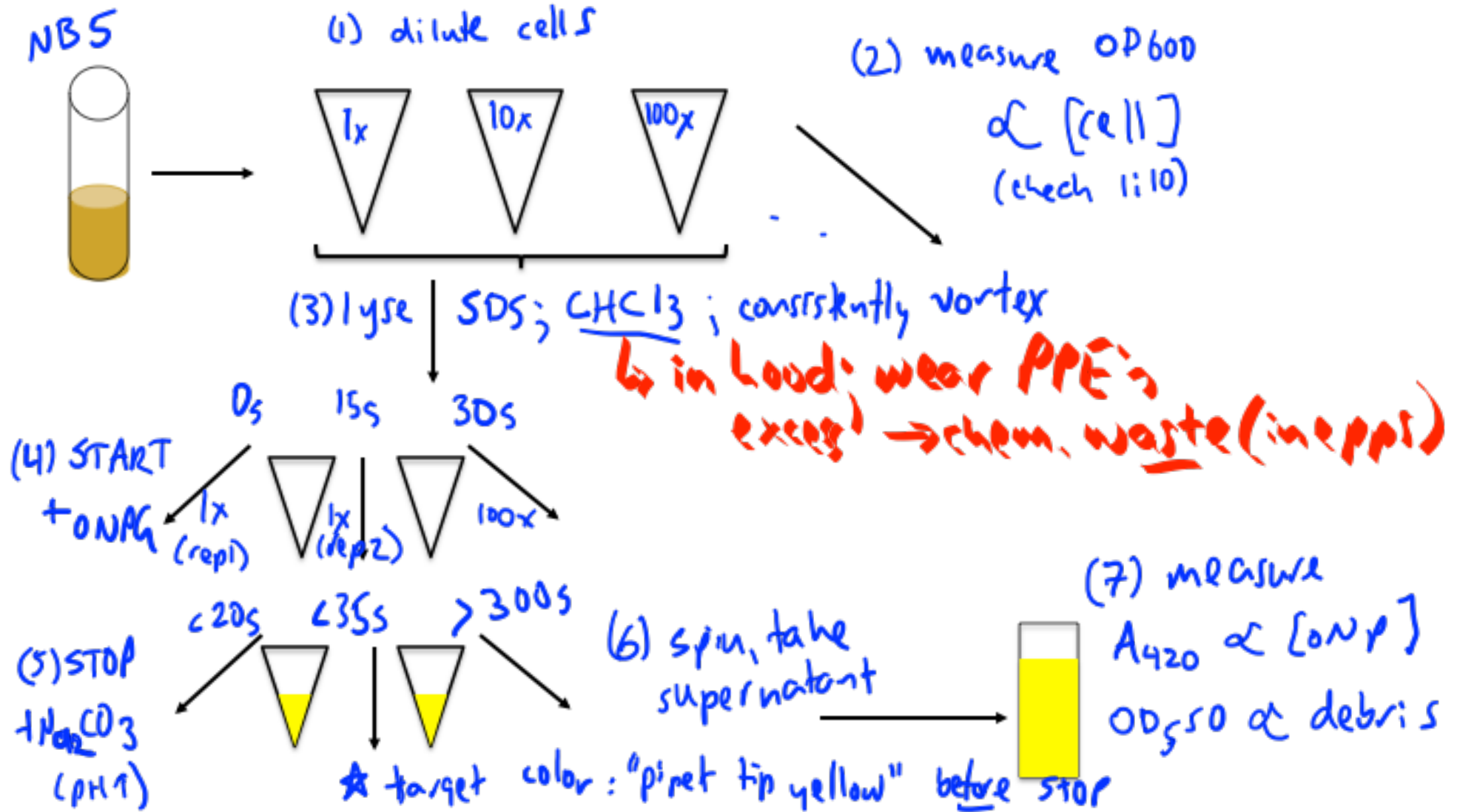
target  
Abs 0.1-1.0

A<sub>420</sub>

below  
detection  
limit



# $\beta$ -gal practice assay: workflow





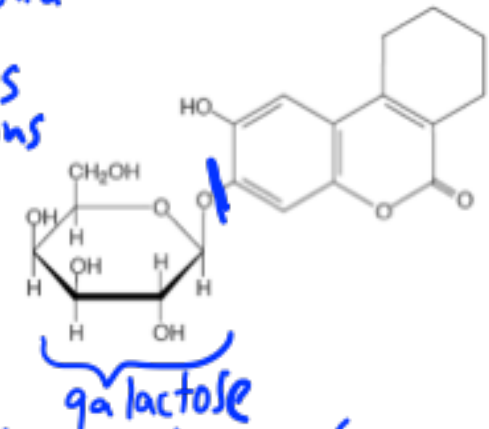
# Today in Lab: M2D1

- Set up bacterial plates in light and dark

– here S-gal, *not* ONP, makes the color *chelates ferric ions*

– purpose? *establish original dynamic range of BP system*

*solid - NB 466*



- Set up liquid cultures in light and dark

*light - NB 466*

- Practice  $\beta$ -gal assay  $\rightarrow$  calculations

*Get BP strain; NB5 = over-expresses  $\beta$ -gal  
vortex cells for uniform [cell]*

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

*You may stagger order*