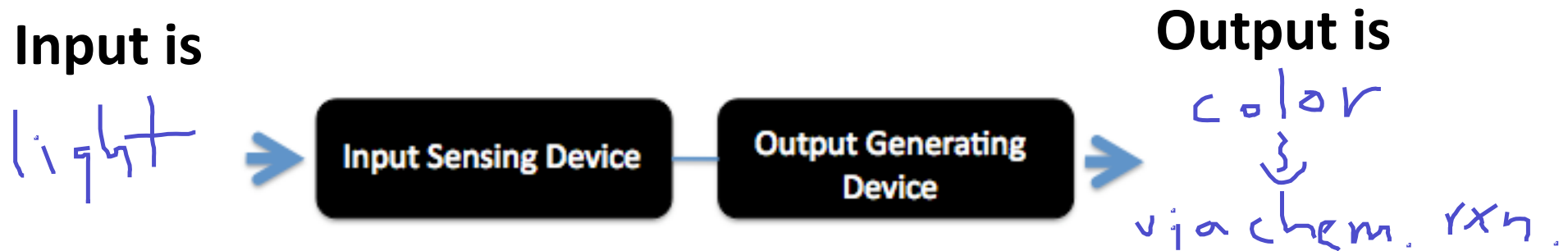


- Announcements
- Pre-lab Lecture
  - ❖ Intro to “*coliroid*” system
  - ❖  $\beta$ -gal assays
  - ❖ Today in Lab (M2D1)

# Announcements

- Introducing... Eric, TA for Module 2
- Lab certification
  - great job overall: average 83.9
  - a few common crucial gaps in understanding
- Module 2 heads-up
  - journal club presentations W 10/24, W 11/7
  - please choose paper ASAP *by Mon*
  - *everyone's* draft data+sum. slides due F 10/19 (12 am), whether oral (D4 or D8) or written conc.  
*+ orals w/ Bevin today*  
*+ all one day Mon T, TBP*

# Bacterial photography abstracted view



## System states:

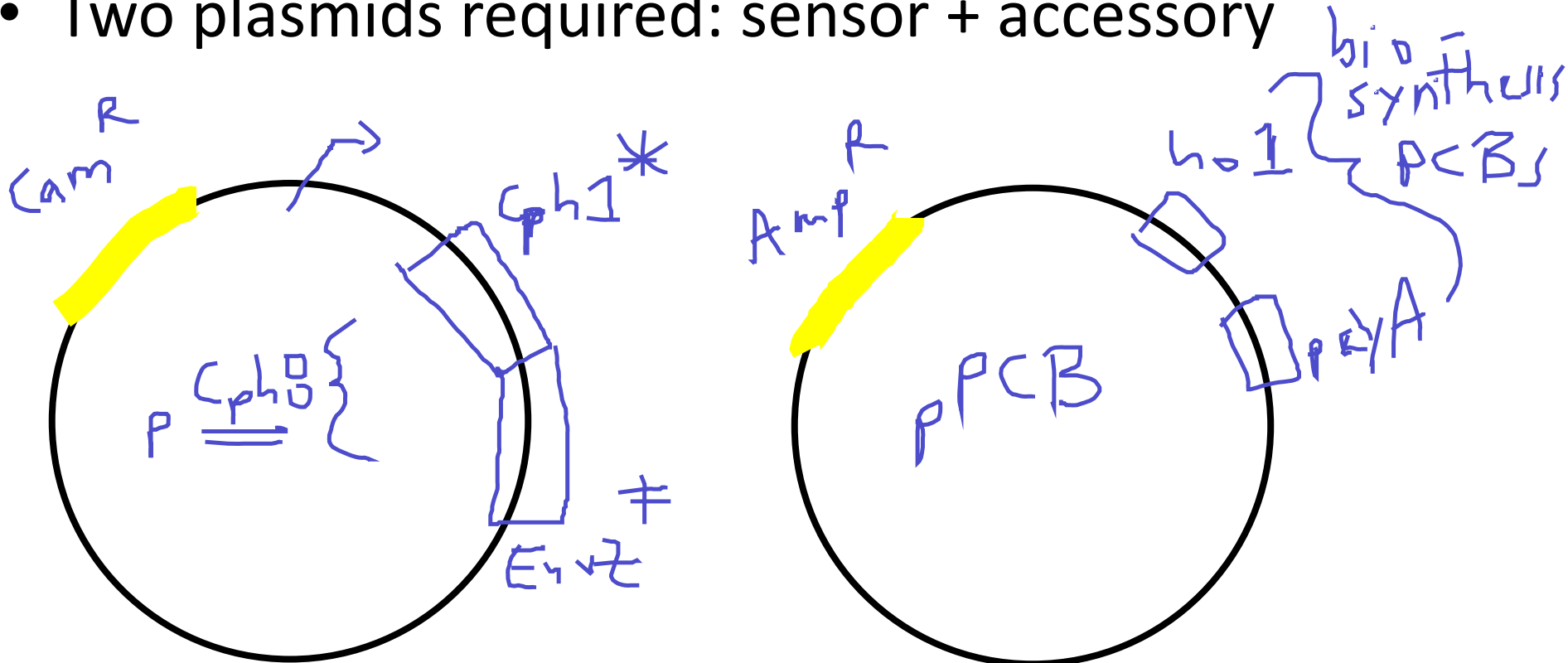
- 1) light off, color on  
→ black
- 2) light on, color off  
→ yellowish

**Design goal:** improve contrast

**Method:** genetic screen

# Sensor details

- Two plasmids required: sensor + accessory



\*cyanobacteria-  
≠ E.coli-derived

heme → phyco-  
cyanobilins

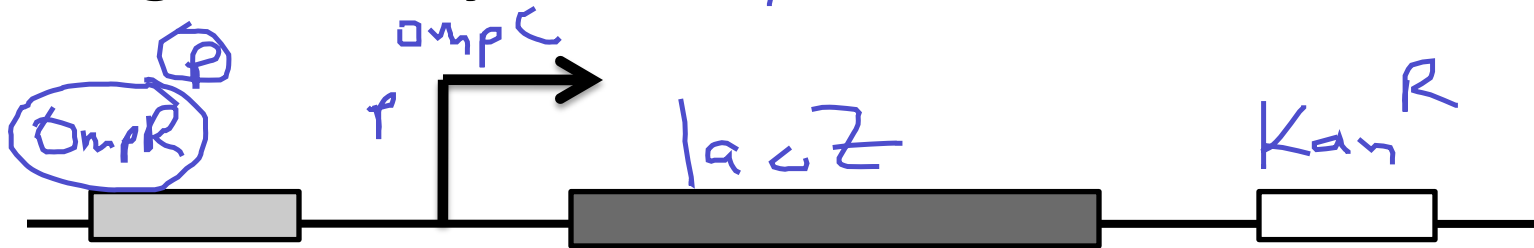
# Regulation details

**Natural 2-component system:** osmoregulation

Sensor  $EnvZ$       Responder  $OmpR$   $\rightarrow$  porins

Stimulus osmotic shock

**Engineered system:** light  $\rightarrow$  color  $CphB \rightarrow$  lacZ



OmpR-dependent promoter

OmpR binds when  $\textcircled{P}$

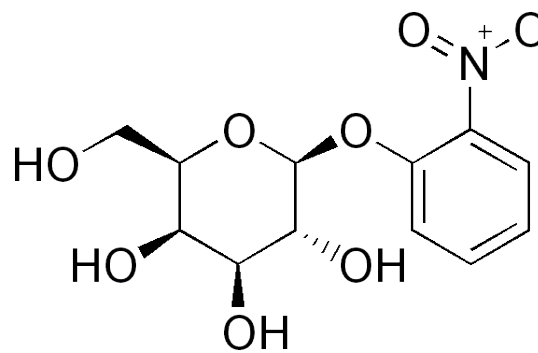
$\therefore \textcircled{P} \rightarrow$  lacZ ON

★ this part  
in genome

★ must use  
 $\Delta EnvZ$  strain

# $\beta$ -gal assay: background

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



galactose

ONP = yellow

measure on spec.

saturation

Wikimedia Commons, public domain image

- Useful range of assay

target

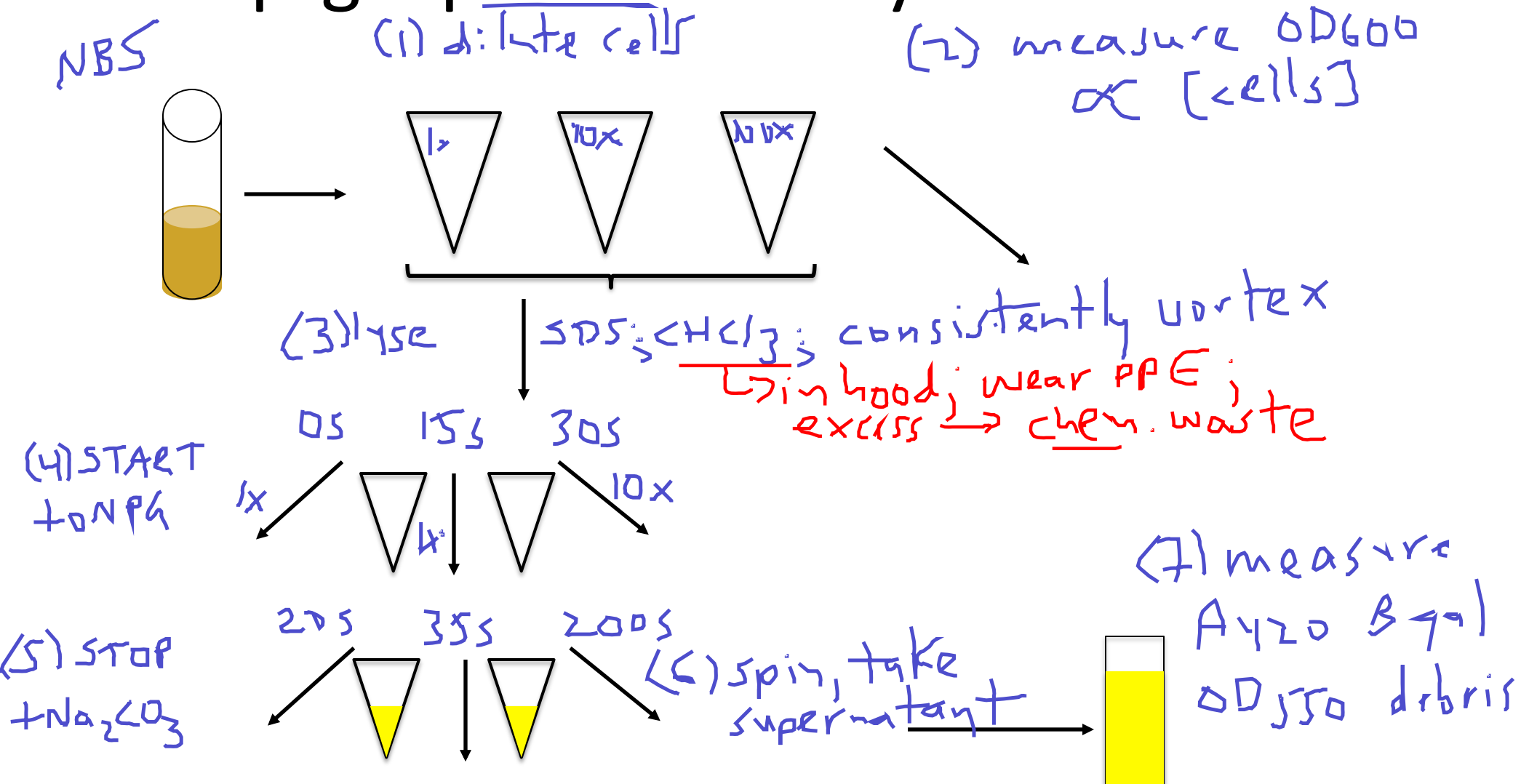
Abs 0.1-1

ABS @ 420nm

below detection limit



# $\beta$ -gal practice assay: workflow



★ target color: "pipet tip yellow" before stop ★

# Today in Lab: M2D1

- Set up bacterial plates in light and dark

- here S-gal, *not* ONP, makes the color

- purpose? learn original dynamic range of BP system

- Set up liquid cultures in light and dark

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

NBS,  $\beta$ -gal over expression

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