- Announcements
- Pre-lab Lecture
  - Intro to "*coli*roid" system
  - β-gal assays
  - Today in Lab (M2D1)

Announcements

- Introducing… Jingjing, TA for Module 2
- Lab practical announcements recap
- Module 2 heads-up
  - Journal club presentations W 10/26, W 11/9
  - About half should sign up for each day

Bacterial photography abstracted view

Input is light

Output is color ? via chem. rxn.

System states:

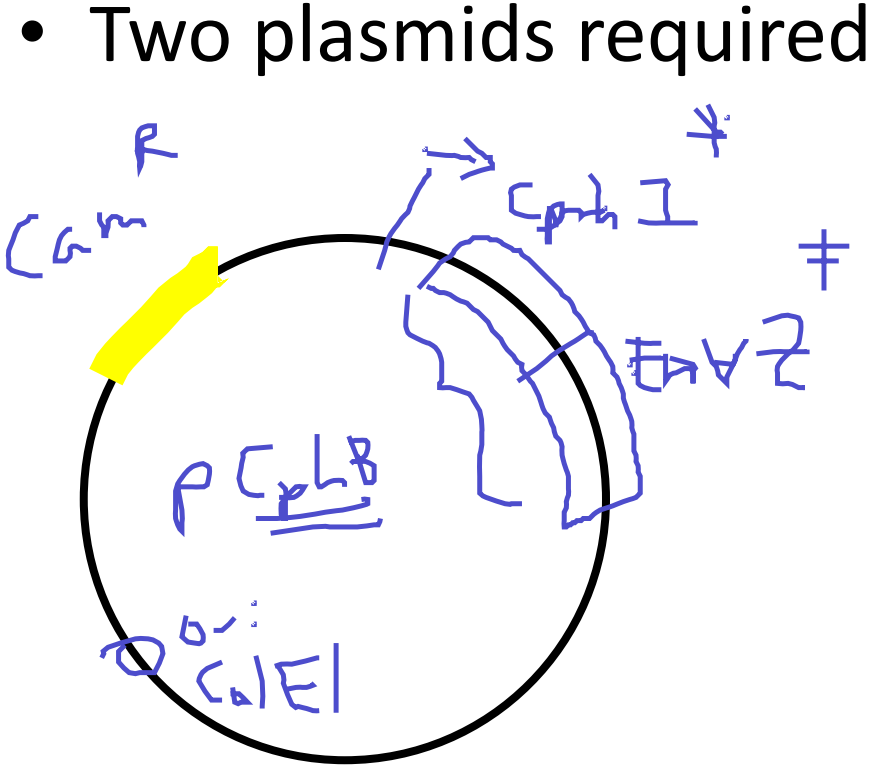
1) light off, color on → black
2) light on, color off → yellow

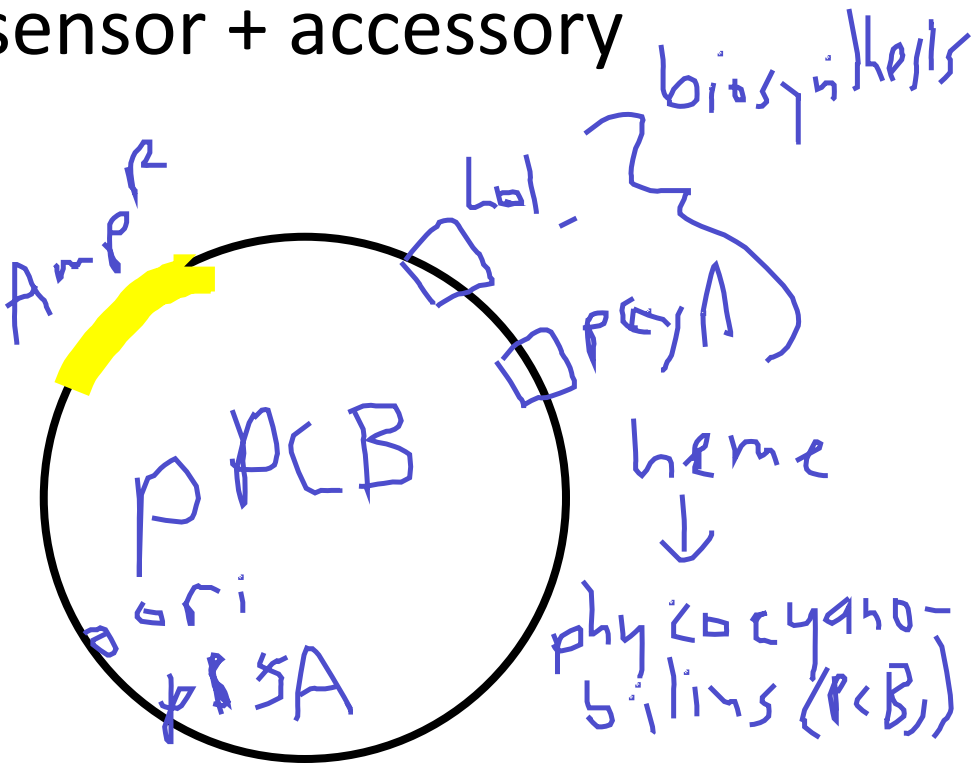
Design goal: improve contrast

Method: genetic screen

Sensor details

- Two plasmids required: sensor + accessory





* cyanobacteria-
≠ E. coli-derived

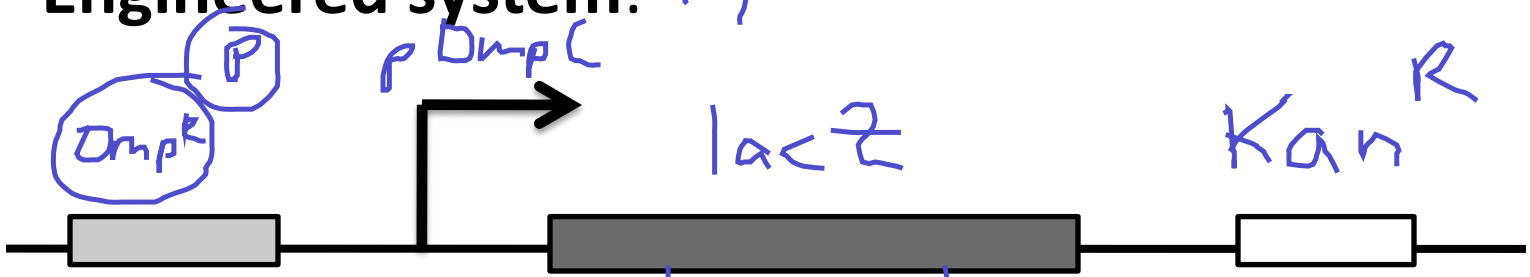
Regulation details

Natural 2-component system: osmoregulation

Sensor EnvZ Responder $OmpR$

Stimulus osmotic shock

Engineered system: light → color



OmpR dependent promoter

$OmpR$ binds with P

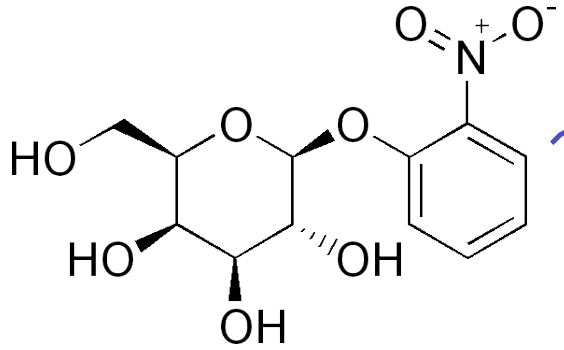
∴ P → lacZ ON

★ in genome

★ ΔEnvZ strain

β-gal assay: background

- β-gal is protein encoded by lacZ
- ONPG is used to detect β-gal. How?



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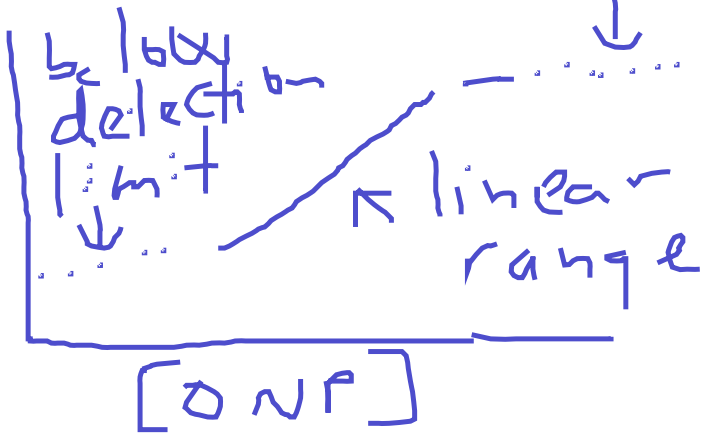
galactose

ONP = yellow → measure on spec.

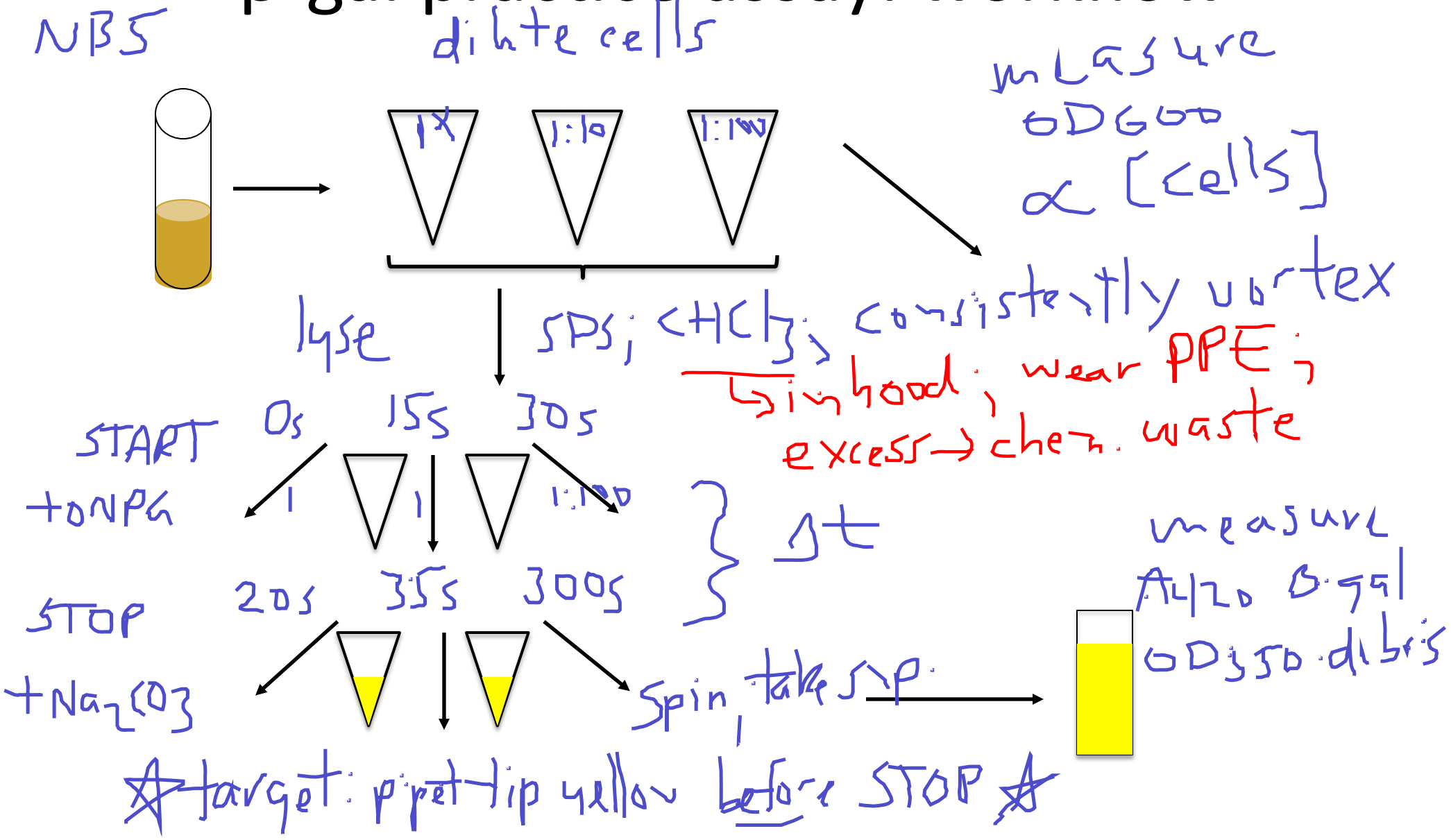
- Useful range of assay

target Abs 0.1–1

Abs @ 420 nm



β-gal practice assay: workflow



Today in Lab: M2D1

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

BP system → learn original dynamic range

↳ not BPS; NBS overexpresses β-gal

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