- Announcements
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- Pre-lab Lecture
  - ❖ Where we are/going
  - ❖ Today in Lab (M2D5)

Announcements

- Office hours (OH) next week
  - Two days: M 2-2:30, T 4-4:30
  - I will stay longer if there is demand!
  - You can already be working on: figures, results, methods, introduction, reading literature
- OH just before report is due (M 11.12)
  - Friday and/or Saturday? Share preferred times

Where are we/going?

Discuss goal of each phase/experiment with a partner

- Construction phase:

  Make Cph8 library (NK) and put into cells (109)

  improve dynamic range of BP system using T3Y1X (K+P- hoped for)

- Evaluation phase:

  DNA Sequencing what is the mutant? ✱ other mutations?

  protein
  - β-gal assay does mutation seem to affect relative (β-gal) in light and/or dark?
  - Western is (Cph8) itself changed? ✱ potential also size dbl-check for artifacts
  - Other that? etc. ✱ controlling conditions

Chance for design/investigation

start on D6 (grow cells, etc.)

- Day 7 = Western = lots of long incubations
- Chance to design and carry out an additional experiment that could shed light on your results
- Start thinking about it now (or rather as soon as you have your first batch of mutant data)
- Also a day to share, collaborate with other groups

Today in Lab: M2D5

- Miniprep each mutant candidate (light *or* dark)
- Resuspend in sterile water, mix an aliquot with sequencing primer → ideally done by 3 pm!
- β-gal assay: WT, HK dead, and your mutant
- Leslie will discuss elements of a successful research article @ 4 pm

4D in L

and D/NS for [Cpl8]

★ two [SPS] - don't mix up!

reference { WT = NB466 D 1.34 L 1.3 OD600's
H537A D 1.25 L 1.3 ★ vortex ★ before use