- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Where we are-going
  - Today in Lab (M2D5)
• 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 $T541X$ (K+P- hoped for)

• Evaluation phase:
  DNA Sequencing
  what is the mutant? * other mutations?
  β-gal assay
  does mutation seem to affect relative (β-gal) in light and/or dark?
  Western
  is (Cph8) itself changed? * potential artifacts
  Other
  what? etc. * controlling conditions
Chance for design/investigation

- 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

Two [Sps] - don’t mix up!

\[ \text{WT=NB466 D 1.34 L 1.3 OD600's} \]
\[ \text{H537A D 1.25 L 1.3 \# vortex before use} \]