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

• Methods sections
  - see general comments, apply to many of you
  - review writing guidelines
  - breakdown a published journal article as a model
  - meet with me or WAC for help revising

• Plus one more iPad experiment
  - Plus music. Oops
Where we are going

- Each colony you chose was re-streaked, then grown O/N in light and dark. Now what?

1. β-gal assay – to compare mutant to original
   - perform as similarly as possible (OD)
2. Sequence DNA – what is each mutant?
   - are there other mutations?
3. is [cp18] protein itself changed?
   - potential for artifacts
4. new photo – compare to original
   - ideally, would run same day, with same light/media conditions,rell & D
   + later
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
Slide from N. Kuldell

β-gal assay of K-P+ candidates

β-galactosidase Activity (Miller Units)

<table>
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<tr>
<th>Cph1/EnvZ</th>
<th>A553</th>
<th>G554</th>
<th>V555</th>
<th>S556</th>
<th>H557</th>
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<tbody>
<tr>
<td>EnvZ</td>
<td>A239T</td>
<td>G240E</td>
<td>V241G</td>
<td>S242D</td>
<td>H243A</td>
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</tbody>
</table>

Our goal
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!
• Linda will discuss paper structure @ 4 pm