Digression

costume day

just pretty much always awesome day
- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Recap SDS-PAGE
  - Sequence analysis preview
  - Today in Lab (M2D6)
Announcements

• Next time I’ll need to leave early and Shannon (T/R section leader for Mods 2 and 3) will come help out
• Please email us your experimental plans as you develop them
• Next journal club in 1 week
• Report due M 11.12.12 → fill out survey for extended OH
Mutant Miller assay data

<table>
<thead>
<tr>
<th>Mutant 1 B-Gal Average (Dark)</th>
<th>Mutant 1 B-Gal Average (Light)</th>
<th>Mutant 1 T541X; X =</th>
<th>Mutant 2 B-Gal Average (Dark)</th>
<th>Mutant 2 B-Gal Average (Light)</th>
<th>Mutant 2 T541X; X =</th>
</tr>
</thead>
<tbody>
<tr>
<td>2328.25</td>
<td>1928.66</td>
<td>1.2</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1417.88</td>
<td>1409.76</td>
<td>1.0</td>
<td>1450.07</td>
<td>454.78</td>
<td>3.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wild Type System B-Gal (Dark)</th>
<th>Wild Type System B-Gal (Light)</th>
<th>H243A Mutant B-Gal (Dark)</th>
<th>H243A Mutant B-Gal (Light)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1099.13</td>
<td>414.85</td>
<td>54.54</td>
<td>45.27</td>
</tr>
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<td>1099.13</td>
<td>414.85</td>
<td>54.54</td>
<td>45.27</td>
</tr>
</tbody>
</table>

Comparisons to make? Representing data?

Go, Team Pinkle!
SDS-PAGE preparation

- You will make whole cell extracts with equal cell #s
  - Based on OD_{600} reading, normalize
    \[
    \text{Goal: } 2 \text{ OD per sample} \quad (1) \quad 2 \text{ mL} \\
    (2) \quad 4 \text{ mL}
    \]

- Gel separates proteins based on size, shape, charge
- Sample preparation reduces analysis complexity
  - SDS: coat proteins w/(-) charge
  - β-Me: breaks S-S bonds \\ breaks in bold
  - Boiling: denature higher order
  - Sample Buffer has SDS, β-Me, plus glycerol, BPB dye
SDS-PAGE visualization, analysis

• Determine size in comparison to ladder (pre-stained)
• Visualize specific protein amount with antibody and staining
  – antibody conjugated to enzyme
  – enzyme catalyzes colorimetric rxn
  – more detail next time
• Goal: determine if mutation affected Cph8 production
Sequencing food for thought

- Where in the sequence do I expect a mutation?
- How do I interpret mutations occurring elsewhere?
- How do I interpret gaps (vs mismatches)?
- What resources may help me form a hypothesis about the behavior of a particular mutant?
Today in Lab (M2D6): Workflow

Check OD of cells

Lyse an aliquot and load onto SDS-PAGE

Also ladder & (+) control

Sequencing analysis (ApE or BLAST)

Mutant bacterial photograph

Meanwhile...

Let run 1 hour

Transfer to blot – run 1 hour

Store in milk solution until next time

Ideas/prep for experiments next time… from seq/β-gal repeats to ?your idea here?