- Announcements, Review HW
- Lab Quiz
- Pre-lab Lecture
  - DNA Extraction (Miniprep)
  - Diagnostic Gel Review
  - Safety + Technical Tips
Announcements

• Your progress report is due in one week
  – OH (room TBA) on: Sun 4-5:30pm
    Natalie 5th 3-5pm (The 3-4)

• Please post your colony counts in talk page table before leaving – we’ll discuss them next time

  • read NCB paper for next time

  * use 20109.talk@gmail.com
Previous HW comments

- Analyzing error in linearized gel analysis
  - Error measurement? 
    - No error, not absolute difference
  - Sources of error?
    - Human measurement \rightarrow band thickness \rightarrow gel loading \rightarrow error

- Comments on Figures
  - Titles: Great overall! Hone in on key details.
  - Caption:
    - Introduce the players, use consistent naming scheme.
    - Avoid explaining minor methods details.
    - Stick to facts (e.g., expected band sizes), not interpretation.
  - Labeling: often insufficient. Label a few bands on ladder.
## DNA Extraction from Bacteria

<table>
<thead>
<tr>
<th>Step</th>
<th>Contains</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soln. I</td>
<td>EDTA, buffer, glucose</td>
<td>→ weakens cell envelope</td>
</tr>
<tr>
<td></td>
<td></td>
<td>→ otherwise stable</td>
</tr>
<tr>
<td>Soln. II</td>
<td>SDS, DNA+</td>
<td>→ disrupts, solubilizes lipid membrane (proteins)</td>
</tr>
<tr>
<td></td>
<td>NaOH</td>
<td>→ denatures dsDNA → ssDNA</td>
</tr>
<tr>
<td>Soln. III</td>
<td>acetic acid, KAc</td>
<td>neutralize pH, precipitate SDS</td>
</tr>
<tr>
<td>Transfer</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Final steps</td>
<td>EtOH, H2O</td>
<td>→ precipitates DNA, wash away salt, etc.</td>
</tr>
<tr>
<td></td>
<td>→ renature</td>
<td></td>
</tr>
<tr>
<td></td>
<td>quronic DNA &quot;cracks&quot;</td>
<td></td>
</tr>
</tbody>
</table>
Diagnostic DNA Gels

Choosing restriction sites for digest

* consider cause one site unique to insert, one on tkb
* consider results of partial digestion
Today in Lab

• Miniprep three Δ5-EGFP candidates, and bacteria transformed with pCX-NNX

• Count and post colony #s

• Visit from Christine Tafoya, EHS: biosafety talk

• Set up digests
  – We will add loading dye if lab runs late