- Announcements, Review HW
- Lab Quiz
- Pre-lab Lecture
  - Where we are-going
  - DNA Ligation, part 2
  - Bacterial Transformation
  - Controls, Expected Outcomes
  - Safety + Technical Tips
Enzyme activity measure in arbitrary units U
1 U = amount of enzyme to digest \( \text{1 mg} \) DNA within \( \text{1 hr} \) at \( 37^\circ \text{C} \) in total of \( 50 \text{ mL} \)

Will 75% Xbal and 100% EcoRI activity always give a 3:1 ratio of double-cut to single-cut product?

*extreme cases*
- \( \text{u. little DNA (} \leq \text{1 mg}) \) ~ all double-cut
- \( \text{a lot DNA} \) ~ not even all single-cut
Old HW, problem 1

PCX - NNX

4791 bp

XhoI 2260

XhoI 2015

Lost

XbaI

~100 bp

EcoRI

ΔSEKΔP

100 bp

5346 bp

XbaI

n 640 bp

lost caps

EcoRI
Where we are/going

- make the desired clone (ligation)
- amplify and select the clone in E. coli
- next time, test candidate clones
DNA Ligation

Reaction creates a new phosphodiester bond.

Reaction requires ATP.

What factors affect yield?
- conc. of DNA
- ratio of DNA:ligating
- enzyme (conc., T, age)

How do we assess if it worked?
* diagnostic digest
(sequencing)
Bacterial transformation

- Chemical treatment
- Heat shock
- Competent 2-step
- Other methods (1-step)
  - Electro poration
  - Ballistics
DNA Amplification in Bacteria

Plate bacteria + DNA mixture

- Die if no plasmid
- Took up plasmid, grow into multi-cell colonies

Why grow/test multiple candidates? Not all are correct
## Ligation Controls + Outcomes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Expectation</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCX-EGFP</td>
<td>lots (of colonies)</td>
<td>positive control</td>
</tr>
<tr>
<td>no DNA</td>
<td>none</td>
<td>neg. control</td>
</tr>
<tr>
<td>bkb + ins, no ligase</td>
<td>some</td>
<td>control for uncut plasmid efficiency</td>
</tr>
<tr>
<td>bkb + ligase</td>
<td>some</td>
<td>control for single-cut plasmid</td>
</tr>
<tr>
<td>bkb + ins, + ligase</td>
<td>some - many</td>
<td>experiment</td>
</tr>
</tbody>
</table>
# Ligation Controls + Outcomes

<table>
<thead>
<tr>
<th>Sample</th>
<th>What if?</th>
<th>In general, keep in mind:</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCX-EGFP</td>
<td>none</td>
<td>* consider all outcomes together</td>
</tr>
<tr>
<td></td>
<td></td>
<td>* runs, do not go to completion</td>
</tr>
<tr>
<td>no DNA</td>
<td>some</td>
<td>wrong cell line</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bkb + ins, no ligase</td>
<td>many</td>
<td>wrong DNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bad digestion, enzymes bad</td>
</tr>
<tr>
<td>bkb + ligase</td>
<td>many</td>
<td></td>
</tr>
<tr>
<td>bkb + ins, + ligase</td>
<td>none</td>
<td>exp. stuff</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or new plasmid kills bacteria (not in our case)</td>
</tr>
</tbody>
</table>
Today in Lab

• Keep ligase and ligase buffer (ATP) cold
• DNA precipitation after ligation reaction
  – Yeast tRNA ➔ "carrier" to visualize
  – Ethanol ➔ precipitates DNA

• Be gentle with competent cells
  keep cold, don’t vortex
• Sterile technique for transformations – demo