DNA Engineering: 
M1D4 Lab Talk

20.109 (F12)
09.25.12
About ligation...

Ligase requires ATP

What affects efficiency?

Temp: RT / 16°C

[DNA]: blotting insert [1:4]
Your Data

~100 ng

4.6 Kb

5X

200

--- --- --- --- --- --- (1:6:1)

--- --- --- --- --- --- (1:2:1)
# Ligation Reactions

Controls for:  

<table>
<thead>
<tr>
<th>Control</th>
<th>bkb + insert, no ligase</th>
<th>bkb only, plus ligase</th>
<th>bkb + insert, plus ligase</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCX-NNX bkb</td>
<td>? µl</td>
<td>? µl</td>
<td>? µl</td>
</tr>
<tr>
<td>PCR insert</td>
<td>? µl</td>
<td>xxx</td>
<td>? µl</td>
</tr>
<tr>
<td>10X Ligation Buffer (^\wedge)</td>
<td>1.5 µl</td>
<td>1.5 µl</td>
<td>1.5 µl</td>
</tr>
<tr>
<td>T4 DNA Ligase</td>
<td>xxx</td>
<td>0.5 µl</td>
<td>0.5 µl</td>
</tr>
<tr>
<td>Water</td>
<td>To 15 µl not including volume of enzyme</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Room temp 10 minutes, then clean up again!
## Transformation Reactions

<table>
<thead>
<tr>
<th>Tube</th>
<th>Transformation</th>
<th>Expectation</th>
<th>What if…?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not done</td>
<td>Nothing</td>
<td></td>
<td>What if…?</td>
</tr>
<tr>
<td>1</td>
<td>pCX-EGFP 5 ng</td>
<td>10+5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Bkb+insert, no ligase</td>
<td>2</td>
<td>10+5</td>
</tr>
<tr>
<td>3</td>
<td>Bkb, + ligase</td>
<td>5/6 few</td>
<td>10+5</td>
</tr>
<tr>
<td>4</td>
<td>Bkb + insert + ligase</td>
<td>smiley</td>
<td></td>
</tr>
</tbody>
</table>

- Transformation efficiency = 10+5 ng
- plated all
About transformations...

Technical Notes:
• Treat competent cells gently
• Use sterile technique to plate
Today and next week in lab....

1. Ligate/TA in/EHS
   - Optional HW Assignment

2. Min prep / gel + intro to T
   - Optional HW

3. Lab certification + lipofect
   - R21 lab

4. FACS