Announcements, Review HW

Lab Quiz

Pre-lab Lecture

- Where we are going
- DNA Ligation, part 2
- Bacterial Transformation
- Controls, Expected Outcomes
- Safety + Technical Tips

Mon. OH
1 2 4 5
5 6 6 5 5
1:30-2:30 pm
16-319
Mon.
Old HW, problem 1

PCX-NNX
4791 bp

1623
Xbal

~1006 bp
Ecor1

Lost

ΔSEGFP

1623
Xbal

+663 bp

-126 bp Cap

-100 bp

Xho1
2815
Old HW, problems 2+3

#3 Agarose gels have limited resolution/range.

Note: Enzyme activity measure in arbitrary units U
1 U = amount of enzyme to digest 1ug DNA
within 1hr. at 37°C in total of 50mL

* think of extreme cases
Where we are-going

D4: make the desired clone by liglation

D4.5: amplify and select the clone in E.coli

D5*: test candidate clones
DNA Ligation

Reaction creates new phospho diester bond

Reaction requires ATP

What factors affect yield?
- Temperature, pH
- Ratio of G/T: C/A
- Concentrations of DNA
- Concentration of ligase

How do we assess if it worked?
- Diagnostic digest
Bacterial transformation

1. chemical treatment

- plasmid
- "competent" cells

2. Heat shock

- other methods (1-step)
  - electroporation
  - ballistics
DNA Amplification in Bacteria

Plate bacteria + DNA mixture

Why grow/test multiple candidates?

not all correct

cells without plasmid grow into multicell colonies

Ampicillin

cells went plasmid die
<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>(+) control</td>
</tr>
<tr>
<td>no DNA</td>
<td>none</td>
<td>(-) control</td>
</tr>
<tr>
<td>bkb(+ ins), no ligase</td>
<td>few-some</td>
<td>control for intact plasmid</td>
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<tr>
<td></td>
<td></td>
<td>digestion efficiency</td>
</tr>
<tr>
<td>bkb + ligase</td>
<td>some</td>
<td>control for singly cut backbone</td>
</tr>
<tr>
<td>bkb + ins, + ligase</td>
<td>some-many</td>
<td>EXP</td>
</tr>
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</table>
### Ligation Controls + Outcomes

<table>
<thead>
<tr>
<th>Sample</th>
<th>What if?</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCX-EGFP</td>
<td>none</td>
<td>poor cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>too little, wrong DNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wrong plate (not Amp)</td>
</tr>
<tr>
<td>no DNA</td>
<td>some</td>
<td>contamination by other DNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wrong plate, etc.</td>
</tr>
<tr>
<td>bkb + ins, no ligase</td>
<td>many</td>
<td>poor digestion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>etc.</td>
</tr>
</tbody>
</table>
| bkb + ligase          | many     | exp, issue \(
|                       |          | inset\) design               |
| bkb + ins, + ligase   | none     | new plasmid is lethal (not now use!)|

In general, keep in mind:

* Consider all exprs, samples together
* rxns, do not go to completion
Today in Lab

• Keep ligase and ligase buffer (ATP) cold
• DNA precipitation after ligation reaction
  – Yeast tRNA – “carrier” to visualize DNA, improve yield
  – Ethanol – precipitates (along with salt)

  * get rid of liquid!

• Be gentle with competent cells

  * keep cold; don’t vortex

• Sterile technique for transformations – demo