3A Assembly (Digestion and Ligation)

Aim

To assemble two BioBricks into a new vector.

Procedure

Digestion

1. Prepare following Master Mixes

<table>
<thead>
<tr>
<th>Enzyme Master Mix for Plasmid Backbone (25µl total, for 5 runs)</th>
<th>Enzyme Master Mix for Part A (BioBrick on the 5′ end)</th>
<th>Enzyme Master Mix for Part B (BioBrick on the 3′ end)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 µl NEB Buffer 2</td>
<td>5 µl NEB Buffer 2</td>
<td>5 µl NEB Buffer 2</td>
</tr>
<tr>
<td>0.5 µl BSA</td>
<td>0.5 µl BSA</td>
<td>0.5 µl BSA</td>
</tr>
<tr>
<td>0.5 µl EcoRI-HF</td>
<td>0.5 µl EcoRI-HF</td>
<td>0.5 µl XbaI</td>
</tr>
<tr>
<td>0.5 µl PstI</td>
<td>0.5 µl SpeI</td>
<td>0.5 µl PstI</td>
</tr>
<tr>
<td>18 µl dH₂O</td>
<td>18.5 µl dH₂O</td>
<td>18.5 µl dH₂O</td>
</tr>
</tbody>
</table>

2. Digest Plasmid Backbone
   - Add 4 µl linearized plasmid backbone (25 ng/µl for 100 ng total)
   - Add 4 µl of Enzyme Master Mix

3. Digest Part A
   - Add 4 µl Part A (25 ng/µl for 100 ng total)
   - Add 4 µl of Enzyme Master Mix

4. Digest Part B
   - Add 4 µl Part B (25 ng/µl for 100 ng total)
   - Add 4 µl of Enzyme Master Mix

5. Digest all three reactions at 37 °C for 60 min, heat kill 80 °C at 20 min

Ligation

1. Add 2 µl of digested Plasmid Backbone (25 ng)
2. Add equimolar amount of Part A (EcoRI-HF SpeI digested) fragment (< 3 µl)*
3. Add equimolar amount of Part B (XbaI PstI digested fragment) (< 3 µl)*
4. Add 1 µl T4 DNA ligase buffer.
5. Add 0.5 µl T4 DNA ligase
6. Add water to 10 µl
7. Ligate at RT for 10 min, heat kill 80 °C/20 min

8. Transform with 1-2 µl of product

* Easiest to calculate using [http://www.insilico.uni-duesseldorf.de/Lig_Input.html](http://www.insilico.uni-duesseldorf.de/Lig_Input.html)

Use a 1:3 vector:insert ratio

Sources

This protocol is modified from the 3A Assembly protocol at igem.org:
[http://parts.igem.org/Help:Assembly/3A_Assembly](http://parts.igem.org/Help:Assembly/3A_Assembly)

N.B. We used exceedingly more DNA than iGEM did - DNA volumes of 500 - 1000 µl.