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Date: 6/18/19

Goal:
1. Simulate gel for restriction digest on Snapgene
2. Run a gel on the Restriction Digest for pCB302 samples A and B from 6/17/19 and verify if we have the correct parts.
3. Colony PCR on ligations from previous day
4. Overnight culture on ligations as well as the pCB302 plasmid
5. Create Primers for pCB302 partial sequence on snapgene

Protocol

Colony PCR Protocol

Prepare 24 PCR tubes.

For 20 μL Reaction
1. Prepare a PCR concentration cocktail with the following proportions: 200μL of diH2O, 250 μL PCR Mastermix(2x), 25 μL of the forward primer, and 25 μL of the reverse primer. (Total 500μL PCR cocktail)
2. Add 20 μL of the concentration cocktail into each PCR tube.
3. Using a 10 μL micropipette, touch the tip onto the selected colony and swirl around in the PCR tube.
4. Place PCR tube in the thermocycler at the following generic settings:
   1. 95° C for 3:00 minutes
   2. 95° C for 1:00 minute
   3. 52° C for 1:00 minute *Annealing temperature varies depending on primer
   4. 72° C for 1:00 minute
   5. 30X (Go to Step 2)
   6. 72° C for 5:00 minutes
   Lid Temperature: 105° C

For the samples leaving in the machine, its label are as follows:
1 1:ligation 1 100 μL colony 1,1 2:ligation 1 100 μL colony 2,…,to 1 6
1’ 1:ligation 1 150 μL colony1,…,to 1’6
2 1:ligation 2 100 μL colony 1, … to 2 6
2’1: ligation 2 150 μL colony 1, … to 2’6

Overnight Cultures
1. Add about 6 mL of LB (chloro added already) to a 15 mL Falcon tube
2. Dip a p10 tip into your selected colony and drop into the tube
3. Incubate in the water bath at 37° C at 220 rpm for 16-18 hours

Results

S = sample

Gel 1: A samples

<table>
<thead>
<tr>
<th>Lane 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<th>10</th>
<th>11</th>
<th>12</th>
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<tbody>
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<td>Gene</td>
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</tbody>
</table>

(run 20 min. longer)
### Gel 2: B samples

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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene Ruler 1 Kb plus</td>
<td>empty</td>
<td>B1 s 1</td>
<td>A1s 2</td>
<td>A2 s 1</td>
<td>A2 s 2</td>
<td>A3s 1</td>
<td>A3s 2</td>
<td>A4 s 1</td>
<td>A4 s 2</td>
<td>B5 s 1</td>
<td>B5 s 2</td>
<td>Ladder</td>
<td>empty</td>
</tr>
</tbody>
</table>

(Run 20 min. longer)

### Expected Results
MW: 1 kb DNA Ladder

1. pCM802 GFP LIC
   April
   1. 4473 bp
   2. 1283 bp