Vector Linearization
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1 Vector Linearization through PCR

Estimated bench time: 45 minutes
Estimated total time: 5-7 hours (depends on the vector)
Purpose: Preparing a linear vector which can be used in the Gibson Assembly reaction.

When linearizing a vector, you are working with DNA. It is essential to work with gloves at all times to protect your vector from DNase activity.

1.1 Materials

- Autoclaved H$_2$O
- Bucket with ice
- Pair of primers which yield the necessary overlaps for the insert
- PCR tubes
- Pipettes and tips
- Q5 High-Fidelity 2X Master Mix (high-fidelity polymerase to linearize the vector)
- Thermal cycler
- Vector which is to be linearized
- Vortex

1.2 Setup & Protocol

- Thaw the Q5-HF 2X master mix on ice. If the master mix contains a pellet, briefly vortex or flick the tube until the pellet disappears.
- Set up a PCR with the following reaction components for the vector to be amplified. Add the Q5-HF 2X master mix lastly. Quickly transfer the PCR tube to the thermocycler after adding the polymerase:

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock concentration</th>
<th>In the PCR tube</th>
<th>Volume to be pipetted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid</td>
<td>1 ng/ul</td>
<td>1 ng</td>
<td>1</td>
</tr>
<tr>
<td>Forward primer</td>
<td>10 uM</td>
<td>0.5 uM</td>
<td>2.5</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>10 uM</td>
<td>0.5 uM</td>
<td>2.5</td>
</tr>
<tr>
<td>Q5 High-Fidelity 2X Master Mix</td>
<td>2X</td>
<td>1X</td>
<td>25</td>
</tr>
<tr>
<td>H$_2$O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Run the following thermal cycling program:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp (°C)</th>
<th>Time (sec.)</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>98</td>
<td>120 (2 min.)</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>98</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>Annealing</td>
<td>X¹</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>30/kb</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72</td>
<td>120 (2 min.)</td>
<td>1</td>
</tr>
<tr>
<td>Hold</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2 (Optional) DpnI digestion

Estimated bench time: 5 minutes + 1 minute per sample
Estimated total time: 1.5 hours
Purpose: Digestion of the template vector from the PCR product mixture. This will remove the number of background colonies which do not carry the desired insert after Gibson Assembly.

2.1 Materials

- 10X CutSmart buffer from New England Biolabs
- Bucket with ice
- DpnI restriction enzyme
- PCR Product
- Thermal cycler

2.2 Setup & Protocol

- Thaw the 10X CutSmart buffer at room temperature and thaw the DpnI restriction enzyme on ice. Setup the following reaction:

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock concentration</th>
<th>In the PCR tube</th>
<th>Volume in ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Product</td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>10X CutSmart buffer</td>
<td>10X</td>
<td>1X</td>
<td>5.7</td>
</tr>
<tr>
<td>DpnI</td>
<td>20U/ul</td>
<td>20U</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>2X</td>
<td>1X</td>
<td>56.7</td>
</tr>
</tbody>
</table>

- Digest the vector for 1 hour at 37°C. Heat inactivate DpnI for 20 minutes at 80°C

3 (Optional) PCR Purification

Estimated bench time: 45 minutes
Estimated total time: 45 minutes

¹ The annealing temperature can be calculated for the set of primers using New England Biolabs Tm calculator. An annealing temperature of 3°C lower than the lowest melting temperature was used to increase yields.
**Purpose:** If the PCR product is <90% pure, large volumes of unpurified PCR product could significantly inhibit the Gibson Assembly [1]. PCR purification may be performed to increase the efficiency.

For more information, see our general PCR purification protocol.

## 4 NanoDrop

**Estimated bench time:** 5 minutes start-up and 2 minutes per sample  
**Estimated total time:** 5 minutes start-up and 2 minutes per sample  
**Purpose:** Measuring the concentration of the PCR product which is necessary to set up the Gibson Assembly reaction.

For more information, see our general NanoDrop protocol.

## 5 (Optional) Gel Electrophoresis

**Estimated bench time:** 40 minutes  
**Estimated total time:** 1.5 hours  
**Purpose:** Agarose gel electrophoresis may be used to verify the purity of your PCR product. If the product is pure, a single bond will show up during the gel electrophoresis.

For more information, see our general Gel Electrophoresis protocol.

## 6 References & Acknowledgements

This protocol was based on information from New England Biolabs NEBuilder HiFi DNA Assembly Cloning Kit manual as well as on Integrated DNA Technologies’ gBlocks Gene Fragments Cloning Protocols.