pBioBrickator construction

Saturday 25/08/2018
Benchling tool: Primer design for the Gibson assembly of the pBioBrickator vector.
For the mRFP TU insert:
5' ggccgcttctagagggagtgagacgtttacggctagctcagtcc 3'
5' cggccgctactagttgactgagacgtataaacgcagaaaggcccacc 3'
For the pUD2 backbone:
5' tttctgcgttttatagcttcagtcaactagtagcgccgctgcagtcc 3'
5' gagctagccgtaaaccgtctcactcttctagaagcggccggaattccag 3'

Friday 31/08/2018
pBioBrickator construction
- Q5 PCR pSB1C3 backbone. Normal conditions, GC Enhancer and DMSO. Annealing Tª 72°C
PCR conditions: 37 cycles
98 ºC - 10 segs
70 ºC - 30 segs
72 ºC - 1:20 segs
Extra ext. 5 min
Electrophoresis gel (1%): correct amplification

Monday 3/09/2018
DpnI digestion of PCR products:
21 uL PCR product
2.4 uL CutSmart Buffer
1uL DpnI Enzime
Incubate 30' @ 37ºC and deactivate 20' @ 80ºC

PCR Clean-up and DNA concentration

<table>
<thead>
<tr>
<th>Name</th>
<th>260/280</th>
<th>ng/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRFP Gibson</td>
<td>1.775</td>
<td>35.446</td>
</tr>
<tr>
<td>pSB1C3 Gibson</td>
<td>1.89</td>
<td>31.598</td>
</tr>
</tbody>
</table>

Gibson Assembly

<table>
<thead>
<tr>
<th></th>
<th>Amount (ng)</th>
<th>conc.</th>
<th>uL</th>
<th>uL</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSB1C3 Gibson Vector</td>
<td>50</td>
<td>31.59</td>
<td>1.58</td>
<td>1.59</td>
</tr>
<tr>
<td>mRFP Gibson Insert</td>
<td>67.96</td>
<td>35.44</td>
<td>1.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>35.45</td>
<td>1.27</td>
<td></td>
</tr>
</tbody>
</table>
**Gibson Master Mix**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibson Master Mix</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>H2O</td>
<td>6.5</td>
<td>7.15</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Transformation in 10G electrocompetent cells. Plating in kanamycin solid medium and incubate overnight.

**Tuesday 4/08/18**
There are colonies in all the plates, but PCR was made with a pBS1C3 from Part Registry that has a BsmbI site within the Cm resistance. Thus, we repeat the Gibson assembly with the pUD2 vector (where BsmbI recognition site is removed).

**Friday 7/08/2018**
Miniprep protocol (NucleoSpin® Plasmid EasyPure).

<table>
<thead>
<tr>
<th>Name</th>
<th>260/280</th>
<th>ng/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBioBrickator v2 c.1</td>
<td>1,987</td>
<td>96,089</td>
</tr>
<tr>
<td>pBioBrickator v2 c.2</td>
<td>1,94</td>
<td>84,285</td>
</tr>
</tbody>
</table>

**Wednesday 12/08/2018**
Restriction analysis of pBioBrickator vector. Digestion with BsmbI and EcoRI/PstI. 1h 37°C. Deactivation 80°C 20 min
Run electrophoresis gel (1%): correct digestion of both colonies

*ladder; BsmbI digestion BioBrickator c.1 v.2; E/P digestion BioBrickator c.1 v.2; BioBrickator c.1 v.2, BsmbI digestion BioBrickator c.2 v.2; BioBrickator c.2 v.2*
Transcriptional units assembly into pBioBrickator vector

Tuesday 18/09/2018
BsmBI GB reaction with pBiobrickator as destination plasmid to insert our composite parts. Electroporation with 10G electrocompetent cells. Plating in Cm solid medium and incubation overnight.

Pick 2 colonies of each to do Miniprep+stock

Monday 24/08/18

Friday 28/09/2018
BsmBI GB reaction with pBiobrickator as destination plasmid to insert the next 10 composite parts. Electroporation with 10G electrocompetent cells. Plating in Cm solid medium and incubation overnight.

Tuesday 2/10/2018

Wednesday 3/10/2018
TUs in pBioBrickator. Last Minipreps (NucleoSpin® Plasmid EasyPure).