Expression of Endo-β-Galactosidase from Biobrick

Background
Endo-β-Galactosidase was also designed into a gBlock with the registry compatible prefix and suffix with an added histag that was lacking in the source plasmid. The gBlock for the Endo-β-Galactosidase will also be ligated to a the backbone pSB1C3.

Assembly of gBlock with pSB1C3 backbone

Aim
The gBlock contains Endo-β-Galactosidase with a Histag and has to be inserted into pSB1C3 before it can be expressed.

Procedure
Digestion
The backbone pSB1C3 provided in the iGEM kit and the gBlock containing Endo-β-Galactosidase were digested using the following digestion mastermix.

Digestion mastermix for five runs
2 µl Tango Buffer
1.1 µl EcoRI
1.1 µl PstI
15.8 µl H₂O

A total of 4 µl of the mastermix was mixed with a total of 100 ng of DNA and incubated at 37 °C for 60 minutes. Heat kill was performed at 80 °C for 20 minutes.

Ligation
A service¹ was used to calculate how much vector DNA (pSB1C3) should be added to insert DNA (gBlock of Endo-β-Galactosidase) for ratios of 1:1, 1:2 and 1:3 moles. Total reaction volume for ligation reactions were 10 µl.
<table>
<thead>
<tr>
<th>Ratio</th>
<th>Plasmid backbone (µl)</th>
<th>EBG Insert (µl)</th>
<th>Ligase (µl)</th>
<th>H₂O (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>2</td>
<td>1,7</td>
<td>1,5</td>
<td>4,8</td>
</tr>
<tr>
<td>1:2</td>
<td>2</td>
<td>3,4*</td>
<td>1,5</td>
<td>3,1</td>
</tr>
<tr>
<td>1:3</td>
<td>2</td>
<td>5,1</td>
<td>1,5</td>
<td>1,4</td>
</tr>
</tbody>
</table>

*Not enough insert DNA for 3,4 µl.

**Transformation with the Endo-β-Galactosidase plasmid**

**Aim**
To transform Top10 cells with the Endo-β-Galactosidase assembled plasmid to make plasmid stocks.

**Procedure**
The protocol for transformations was used with no modifications except for the use of 25 µl of competent cells instead of 50 µl. Top10 cells were used and three plates were made, one for each ratio between the vector and the insert DNA.

**Results**
Colonies had grown on plates with ratios 1:1 and 1:2.

**Cultivation and plasmid purification**

**Aim**
To cultivate the transformed cells for plasmid purification.

**Procedure**
Four colonies were picked from plates 1:1 and 1:2 and cultivated at 37 °C in four flasks of 10 ml with nutrient broth and final concentration of 20 µg/µl of chloramphenicol was added.

The cultivation was followed by a plasmid purification performed with a Qiagen plasmid miniprep kit. A Nanodrop device was used to measure the concentrations.

**Results**
There were four flask but the fourth one was divided into two samples for the plasmid purification. These are number 4 and 5 in the list below.
Sample concentrations:
1: 43.4 ng/µl
2: 100.4 ng/µl
3: 39.7 ng/µl
4: 47.7 ng/µl
5: 45.3 ng/µl

Blank: Elution buffer

**Digestion of Endo-β-Galactosidase plasmid**

**Aim**
To digest the plasmid with Endo-β-Galactosidase and visualize the digestion on a gel to make sure the gBlock was inserted into the backbone.

**Procedure**
Protocol from BioLabs used for digestion.

1 µg DNA
5 µl 10x Cutsmart buffer
1 µl EcoRI-HF
1 µl SpeI
42 µl dH₂O

**Results**
The gel shows that the plasmid pSB1C3 with Endo-β-Galactosidase was cut as expected in all samples.
Transformation of Endo-β-Galactosidase into BL21(DE3)

Aim
To transform BL21(DE3) with the pSB1C3 plasmid with the Endo-β-Galactosidase insert to produce the enzyme.

Procedure
Protocol for transformation used without modification except 25 µl of bacteria used and 2,5 µl of plasmid added. Three transformations were made where one was used as a control. BL21(DE3) was used for the transformation.

Results
The transformations were successful.

Cultivation of BL21(DE3) transformed with Endo-β-Galactosidase

Aim
To cultivate BL21(DE3) transformed with Endo-β-Galactosidase plasmid.

Procedure
Six flask were cultivated with cells containing EBG and one flask was cultivated for control. Three colonies were taken from one plate and three from the other. The volume of the cultures were 10 mL and chloramphenicol with a final volume of 20 µl/mL was added.