1. SOPs in use

iGEM2017 SOP03 v03 EG Gel_purification
iGEM2017 SOP04 v02 FN Colony_PCR_with_MyTaq
iGEM2017 SOP05 v03 EG Plasmid_Miniprep
iGEM2017 SOP06 v02 SJ TSB_transformation
iGEM2017 SOP07 v03 SJ Fast_digest
iGEM2017 SOP09 v03 JB Ligation
iGEM2017 SOP13 v02 EG Agarose_gel_DNA

2. Purpose

To create BioBricks with enzymes required for the Calvin cycle and carboxysome. The parts assembled is originally created by the Bielefeld 2014 iGEM team. The purpose of the created BioBricks is to perform an in-depth characterisation.
3. Overview

3.a BioBricks containing the Calvin cycle enzymes

<table>
<thead>
<tr>
<th>Date</th>
<th>SOPs</th>
<th>Persons</th>
<th>Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.08.17</td>
<td>SOP05</td>
<td>SJ</td>
<td>Plasmid Miniprep</td>
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<td>17.08.30</td>
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3.b BioBricks containing the carboxysome - all from kits

<table>
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<tr>
<th>Date (YY.MM.DD)</th>
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<th>Experiments</th>
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<td>SOP05</td>
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</tr>
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<tr>
<td>17.09.07</td>
<td>SOP03</td>
<td>EG, SJ</td>
<td>Gel purification</td>
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### 3.c BioBricks containing the carboxysome - partly from synthesis

<table>
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<tr>
<th>Date (YY.MM.DD)</th>
<th>SOPs</th>
<th>Persons</th>
<th>Experiments</th>
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<td>17.10.02</td>
<td>SOP06</td>
<td>EG, SJ</td>
<td>Transformation</td>
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</table>

### 4. Experiment history

#### 4.a BioBricks containing the Calvin cycle enzymes

<table>
<thead>
<tr>
<th>Date (YY.MM.DD)</th>
<th>SOPs</th>
<th>Alterations to SOPs and remarks to experiments</th>
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</thead>
<tbody>
<tr>
<td>17.08.17</td>
<td>SOP05</td>
<td>Plasmid Miniprep Plasmid Miniprep on K1465214-pSB1C3 and K1465228-pSB1C3</td>
</tr>
<tr>
<td>17.08.17</td>
<td>SOP07</td>
<td>Fast digest (B91 is cut with BccI+PstI and added AP, B114 is cut with XbaI and PstI) 45 min incubation</td>
</tr>
<tr>
<td>17.04.22</td>
<td>SOP13</td>
<td>Gel electrophoresis 1% gel, small+thick combs. 2.5 µL 1kb ladder, 2.5 µL 6x loading dye, 20 µL sample loaded</td>
</tr>
<tr>
<td>Date</td>
<td>SOP</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
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<tr>
<td>17.04.23</td>
<td>SOP03</td>
<td>Gel purification</td>
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</tbody>
</table>
| 17.04.23   | SOP09 | Ligation  
Plasmid:  
$p_{lac}$ promoter with RuBisCo and phosphoribulokinase: R111 (17 ng/µL, ~2000bp)  
Insert:  
Sedoheptulose-1,7-bisphosphatase: R110 (2 ng/µL, ~1000bp)  
1:0, 1:1, 1:2, 1:4 and 1:5 were made. Ligated overnight at 16°C. |
| 17.04.23   | SOP13 | Transformation  
RuBisCo and phosphoribulokinase ligated with Sedoheptulose-1,7-bisphosphatase in MG1655.  
$OD_600$:0.34  
20 µL plasmid from ligation used |
| 17.08.19   | SOP04 | Colony PCR  
One colony from the control plate, and 2 colonies from each of the four other plates were used.  
1 min 15 s elongation  
5 min extra elongation |
| 17.08.19   | SOP13 | Gel electrophoresis  
1% agarose gel, small-thick comb, 2.5 µL 6x loading dye, 2.5 µL 1 kb ladder.  
Loading order:  
Ladder, 1-9. |
| 17.08.21   | SOP05 | Miniprep  
5 mL ONC made from the restreaks of colony 2, 5, and 6. |
| 17.08.21   | SOP07 | Fast digest  
EcoRI and PstI digestion for 45 min.  
Double volume made for no 2 due to low concentration. |
| 17.08.21   | SOP13 | Gel electrophoresis  
1% agarose gel, broad-thick comb, 2.5 µL 1 kb ladder, 2.5 µL 6x loading dye in 5 and 6, 4 µL 6x loading dye in 2.  
Loading order:  
Ladder, 2, 5, 6  
80V, 25 min. |
| 17.08.23   | SOP07 | Fast digest  
K1465214 is cut with EcoR1 and Xbal  
k1465228 is cut with EcoR1 and Bcul  
45 min incubation |
| 17.08.23   | SOP13 | Gel electrophoresis  
1% gel, broad-thick combs.  
2.5 µL 1 kb ladder, 2.5 µL 6x loading dye, 20 µL sample loaded  
Run gels: 75 V, ~40 minutes. |
<p>| 17.08.23   | SOP03 | Gel purification |</p>
<table>
<thead>
<tr>
<th>Date</th>
<th>SOP</th>
<th>Protocol</th>
<th>Details</th>
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<tbody>
<tr>
<td>17.08.24</td>
<td>SOP09</td>
<td>Ligation</td>
<td>K1465214 insert, 4000 BP and K1465228 backbone, 3000 BP. 1:0, 1:1, 1:MAX and 1:MAX were placed at 16°C overnight.</td>
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<tr>
<td>17.08.25</td>
<td>SOP06</td>
<td>Transformation</td>
<td>RuBisCo and phosphoribulokinase (K1465214) ligated with Sedoheptulose-1,7-bisphosphatase (K1465228) in TOP10.</td>
</tr>
<tr>
<td>17.08.28</td>
<td>SOP04</td>
<td>Colony PCR</td>
<td>4 samples from each plate (1:0, 1:1, 1:MAX and 1:MAX) were used. 1 min 45 sec elongation, 5 min extra elongation.</td>
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<tr>
<td>17.08.28</td>
<td>SOP13</td>
<td>Gel electrophoresis</td>
<td>1% gel, broad-thick combs. 2.5 µL 1 kb ladder, 2.5 µL 6x loading dye, 20 µL sample loaded Run gels: 75 V, ~40 minutes.</td>
</tr>
<tr>
<td>17.08.31</td>
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<td>Ligation</td>
<td>K1465214 insert, 4000 BP and K1465228 backbone, 3000 BP. 1:0, 1:1, 1:MAX and 1:MAX were placed at 16°C overnight.</td>
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<td>17.09.01</td>
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<td>Transformation</td>
<td>RuBisCo and phosphoribulokinase (K1465214) ligated with Sedoheptulose-1,7-bisphosphatase (K1465228) in TOP10.</td>
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<td>17.09.04</td>
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<td>Fast digest</td>
<td>K1465214 is cut with EcoR1 and Xbal k1465228 is cut with EcoR1 and Bcul 45 min incubation</td>
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<td>17.09.04</td>
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<td>Gel electrophoresis</td>
<td>1% agarose gel, small-thick comb, 2.5 µL 6x loading dye, 2.5 µL 1 kb ladder.</td>
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<td>K1465214 is cut with EcoR1 and Xbal k1465228 is cut with EcoR1 and Bcul 45 min incubation</td>
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<td>Gel electrophoresis</td>
<td>1% agarose gel, small-thick comb, 2.5 µL 6x loading dye, 2.5 µL 1 kb ladder.</td>
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<td>17.09.08</td>
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<td>Fast digest</td>
<td>K1465214 is cut with EcoR1 and Xbal k1465228 is cut with EcoR1 and Bcul 45 min incubation Sendt to sequencing</td>
</tr>
<tr>
<td>17.09.14</td>
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<td>Fast digest</td>
<td>W88, W89, W90, W91, W92, all cut with EcoR1 and Pstl Sendt to sequencing. Reason: Possible mismatch had happened in freeze stock.</td>
</tr>
<tr>
<td>17.08.19</td>
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<td>Fast digest</td>
<td>K1465214 is cut with EcoR1 and Xbal k1465228 is cut with EcoR1 and Bcul</td>
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4. BioBricks containing the carboxysome - all from kits

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>17.08.22</td>
<td>SOP5</td>
<td>Miniprep</td>
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<tr>
<td></td>
<td></td>
<td>5 mL ONC of W45 used.</td>
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<tr>
<td>Date</td>
<td>SOP</td>
<td>Description</td>
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<tr>
<td>17.08.22</td>
<td>SOP07</td>
<td>Fast digest</td>
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<td></td>
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<td>B94 digested with Spel+Pstl and AP.</td>
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<tr>
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<td>B115 digested with Xbal+Pstl.</td>
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<tr>
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<td>45 min digestion time.</td>
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<td>17.08.22</td>
<td>SOP13</td>
<td>Gel electrophoresis</td>
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<tr>
<td></td>
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<td>1 % agarose gel, broad-thick comb, 2.5 µL DNA ladder mix, 2.5 µL 6x loading dye.</td>
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<td>Loading order:</td>
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<td>Ladder, B94, B115</td>
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<td>75 V, 35 min.</td>
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<td>Gel purification</td>
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<td>60 °C melting temperature.</td>
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<td>SOP09</td>
<td>Ligation</td>
</tr>
<tr>
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<td>R118 ligated with R119. 1:0, 1:1, 1:2, and 1:4 ligations were made and placed at RT for 2 hr.</td>
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<td>17.08.22</td>
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<td>Transformation</td>
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<td>OD measured by eye.</td>
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<tr>
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<td>All ligation product transformed into TOP10 as well as the ptac promoter from 2017 kit plate 1 well 17H. 10 µL water added, from which 5 µL was used.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phenotypical expression for 1 hr 10 min.</td>
</tr>
<tr>
<td>17.08.23</td>
<td>SOP04</td>
<td>Colony PCR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Two ptac colonies used.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>One colony from the 1:0 plate, two from each of the 1:1 and 1:2 and three colonies from the 1:4 plate of the carboxysome ligation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ptac: 15 s elongation and 2.5 min extra elongation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carboxysome: 1 min 15 s elongation, 5 min extra elongation.</td>
</tr>
<tr>
<td>17.08.23</td>
<td>SOP13</td>
<td>Gel electrophoresis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 % agarose gel, small-thick comb, 2.5 µL 1 kb ladder, 2.5 µL 6x loading dye.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loading order:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gel 1: Ladder, C1-8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gel 2: Ladder, P1-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75 V, 20 min.</td>
</tr>
<tr>
<td>17.08.24</td>
<td>SOP05</td>
<td>Plasmid Miniprep</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 mL ONC of P1 (ptac promoter) and C1 (all the carboxysome genes) was used.</td>
</tr>
<tr>
<td>17.08.24</td>
<td>SOP07</td>
<td>Fast digest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ptac promoter digested with Spel+Pstl and AP.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1 digested with Xbal+Pstl.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45 min digestion time.</td>
</tr>
<tr>
<td>17.08.24</td>
<td>SOP13</td>
<td>Gel electrophoresis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 % agarose gel, small-thik comb, 2.5 µL 1 kb ladder mix, 2.5 µL 6x loading dye.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loading order:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ladder, ptac promoter, carboxysome</td>
</tr>
<tr>
<td>Date</td>
<td>SOP</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>17.08.24</td>
<td>SOP03</td>
<td>Gel purification 60 °C melting temperature. Washed with Wash Buffer 2 times.</td>
</tr>
<tr>
<td>17.08.24</td>
<td>SOP09</td>
<td>Ligation ptac ligated with the carboxysome. 1:0, 1:1 and 1:2 ligations were made. Because of low DNA concentrations 1:2 had an end-volumen at 40 µL. Ligated overnight at 16°C.</td>
</tr>
<tr>
<td>17.08.25</td>
<td>SOP06</td>
<td>Transformation ptac promoter ligated with carboxysome in MG1655. OD_{600}: 0.46 20 µL plasmid from 1:0 and 1:1 ligation and 40 µL plasmid from 1:2 ligation used.</td>
</tr>
<tr>
<td>17.08.26</td>
<td>SOP04</td>
<td>Colony PCR One colony from the 1:0 plate, two from of the 1:1 and seven from the 1:2 plate and three colonies. 35 s elongation and 3 min extra elongation</td>
</tr>
<tr>
<td>17.08.26</td>
<td>SOP13</td>
<td>Gel electrophoresis 1 % agarose gel, small-thick comb, 2.5 µL 1 kb ladder, 2.5 µL 6x loading dye. Loading order: Gel 1: Ladder, colony 1-10 75 V, 30 min.</td>
</tr>
<tr>
<td>17.08.28</td>
<td>SOP05</td>
<td>Miniprep ptac-carboxysome 5 from 30 mL exponential phase</td>
</tr>
<tr>
<td>17.08.28</td>
<td>SOP07</td>
<td>Fast digest ptac-carboxysome with EcoRI and Pst. 35 min digestion time</td>
</tr>
<tr>
<td>17.08.28</td>
<td>SOP13</td>
<td>Gel electrophoresis 1 % agarose gel, 2.5 µL 1 kb ladder, 2.5 µL 6x loading dye Loading order: Ladder, digestion product 80V, 30 min</td>
</tr>
<tr>
<td>17.08.30</td>
<td>SOP07</td>
<td>Fast digest K1465204 digested with Spel, Pstl and AP for 1.5 hr. K1465209 digested with Xbal and Pstl for 45 min.</td>
</tr>
<tr>
<td>17.08.30</td>
<td>SOP13</td>
<td>Gel electrophoresis 1 % agarose gel, small-thick comb, 2.5 µL 100 bp ladder, 2.5 µL 6x loading dye. Loading order: Ladder, K1465204, K1465209 75V, 40 min.</td>
</tr>
<tr>
<td>17.08.30</td>
<td>SOP03</td>
<td>Gel purification 60 °C melting temperature</td>
</tr>
<tr>
<td>17.08.30</td>
<td>SOP09</td>
<td>Ligation K1465209 ligated behind K1465204.</td>
</tr>
<tr>
<td>Date</td>
<td>SOP</td>
<td>Procedure</td>
</tr>
<tr>
<td>------------</td>
<td>-------</td>
<td>----------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| 17.08.31   | SOP06 | Transformation Top10 OD<sub>600</sub>=0.37
All ligation product used.
45 min phenotypical expression at 300 rpm.
Plated on CML plates. |
| 17.09.01   | SOP04 | Colony PCR
There were colonies on all plates. 9 colonies were used.
1:0 plate: no. 1, 1:1 plate: no. 2-5, 1:2 plate: no. 6, 1:4 plate: no. 7, 1:5 plate: no. 8-9.
1 min 45 s elongation.
5 min extra elongation. |
| 17.09.03   | SOP05 | Plasmid Miniprep
5 mL ONC of C3, C4, and C5 (all the carboxysome genes) was used. |
| 17.09.03   | SOP07 | Fast digest
C3, C4, and C5 digested with XbaI+PstI.
45 min digestion time. |
| 17.09.03   | SOP13 | Gel electrophoresis
1 % agarose gel, small-thik comb, 2.5 µL 1 kb ladder mix, 2.5 µL 6x loading dye.
Loading order:
Ladder, C3, C4, C5
75 V, 45 min. |
| 17.09.03   | SOP03 | Gel purification
No gel slice was put into the C5 tube and therefore C5 was not extracted from the gel.
60 °C melting temperature.
Washed with Wash Buffer 2 times. |
| 17.09.04   | SOP07 | Fast digest
ptac promoter digested with SpeI and PstI for 45 min. Heat inactivated for 5 min at 80 °C. Treated with AP for 2 hr. |
| 17.09.04   | SOP13 | Gel electrophoresis
1% agarose gel, small-thick comb. |
| 17.09.04   | SOP03 | Gel purification
60 °C melting temperature |
| 17.09.04   | SOP09 | Ligation
ptac promoter with carboxysome 3 and 4.
1:0, 1:1, 1:2, and 1:4 ligations were made |
| 17.09.05   | SOP06 | Transformation MG1655 OD<sub>600</sub>=0.37
All ligation product used
Phenotypical expression for 45 min at 300 rpm. |
| 17.09.07   | SOP07 | Fast digest
ptac promoter digested with SpeI + PstI for 45 min.
5 min heat inactivation at 80 °C.
AP treatment for 2 hr. |
<table>
<thead>
<tr>
<th>Date</th>
<th>SOP</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.09.07</td>
<td>SOP13</td>
<td>Gel electrophoresis 1% agarose gel, small-thick comb, 2.5 µL loading dye, 2.5 µL DNA ladder mix. Loading order: Ladder, ptac, carboxysome, (other)</td>
</tr>
<tr>
<td>17.09.07</td>
<td>SOP03</td>
<td>Gel purification 60 °C melting temperature</td>
</tr>
<tr>
<td>17.09.07</td>
<td>SOP09</td>
<td>Ligation ptac promoter with carboxysome #5. 1:0, 1:1, 1:2, and 1:4 ligations were made and placed O.N.</td>
</tr>
<tr>
<td>17.09.08</td>
<td>SOP06</td>
<td>Transformation All ligation product into MG1655. OD$_{600}$ = 0.3. 45 min phenotypical expression at 300 rpm.</td>
</tr>
<tr>
<td>17.09.09</td>
<td>SOP04</td>
<td>Colony PCR There were colonies on all plates. 8 colonies were used. 1 min 45 s elongation. 5 min extra elongation.</td>
</tr>
<tr>
<td>17.09.09</td>
<td>SOP13</td>
<td>Gel electrophoresis 1% agarose gel, small-thick comb, 2.5 µL DNA ladder mix, 2.5 µL 6x loading dye. Loading order: Ladder, 2x other, no. 1-8. 80 V, 30 min.</td>
</tr>
<tr>
<td>17.09.11</td>
<td>SOP05</td>
<td>Miniprep 5 mL ONC of ptac-carboxysome no. 6 was used.</td>
</tr>
<tr>
<td>17.09.14</td>
<td>SOP07</td>
<td>Fast digest ptac-carboxysome digested with EcoRI and PstI to check the length. ptac digested with Spel, Pstl, and AP for 2 hr. Carboxysom digested with Xbal and Pstl for 45 min.</td>
</tr>
<tr>
<td>17.09.14</td>
<td>SOP13</td>
<td>Gel electrophoresis 1% agarose gel, small-thick comb, 2.5 µL 6x loading dye, 6 µL 1 kb ladder. Loading order: Gel 1: ptac-carboxysom, other, ladder Gel 2: ladder, ptac S+P, ptac S, ptac P, carboxysome, other x2. Only 2 µL of all three ptac promoter loaded.</td>
</tr>
<tr>
<td>17.09.14</td>
<td>SOP03</td>
<td>Gel purification ptac S+P rest purified as if it had been run on a gel. Carboxysome purified from gel. 60 °C melting temperature</td>
</tr>
<tr>
<td>17.09.14</td>
<td>SOP09</td>
<td>Ligation 1:0, 1:1, 1:2, and 1:3 ligations were made and placed O.N. at 16 °C.</td>
</tr>
<tr>
<td>17.09.15</td>
<td>SOP06</td>
<td>Transformation All ligation product into TOP10. OD$_{600}$ = 0.4</td>
</tr>
</tbody>
</table>
### 4.c BioBricks containing the carboxysome - partly from synthesis

<table>
<thead>
<tr>
<th>Date (YY.MM.DD)</th>
<th>SOPs</th>
<th>Alterations to SOPs and remarks to experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.10.02</td>
<td>SOP07</td>
<td>Fast digest 500 ng ptac-csoS2 from synthesis digested with EcoRI and PstI for 45 min.</td>
</tr>
<tr>
<td>17.10.02</td>
<td>SOP13</td>
<td>Gel electrophoresis 1% agarose gel, small-thick comb, 2.5 μL 6x loading dye, 2.5 μL DNA ladder mix Loading order: Ladder, sample</td>
</tr>
<tr>
<td>17.10.02</td>
<td>SOP03</td>
<td>Gel purification Melting Temperature was 60 °C.</td>
</tr>
<tr>
<td>17.10.02</td>
<td>SOP09</td>
<td>Ligation ptac-csoS2 with pSB1C3 backbone from R44. 1:0, 1:1, 1:2, and 1:4 were made. Placed at RT for two hours.</td>
</tr>
<tr>
<td>17.10.02</td>
<td>SOP06</td>
<td>Transformation TOP10 OD&lt;sub&gt;600&lt;/sub&gt; = 45 min phenotypical expression at 300 rpm. Plated on CML plates. NB: overgrown plates</td>
</tr>
</tbody>
</table>
5. Results and conclusions

5.a BioBricks containing the Calvin cycle enzymes
17.08.23 colony PCR and gel electrophoresis

![Image of gel electrophoresis with bands]

1 kb ladder, 1-9.
Religation would be at 4 kbp, and succeeded ligation at 5 kbp.
We chose 2, 5, and 6 for ONC, miniprep and fast digest to check whether these are alright, since colony PCR can be a bit faulty.

17.08.21 fast digest

![Image of gel electrophoresis with bands]

1kb ladder, 2, 5, 6

W88 to W92 sequenece: No mitchmatch had happened. But showed promoter missing on K1465214.
5.b BioBricks containing the carboxysome - all from kits

17.08.23 colony pcr

Ladder, C1-8  Ladder, P1-2
17.08.26 colony pcr of ptac-carboxysome

1 kb ladder, C1, C2, C3, C4, C5, C6, C7, C8. We chose to work with C5

17.08.28 fast digest of C5 with EcoRI and PstI.

1 kb, C5.

17.09.01 colony PCR

1 kb ladder, 1-9
5.c BioBricks containing the carboxysome - partly from synthesis