| Week 1 (2012.6.11-2012.6.15) | | | | | |
|---|---|---|---|---|---|
| Key Words: pSB1C3 | | | | | |
| | **Monday 2012.6.11** | **Tuesday 2012.6.12** | **Wednesday 2012.6.13** | **Thursday 2012.6.14** | **Friday 2012.6.15** |
| Work done | | . Transformation of the plasmid (pCMV-BMP-2) into E.coli and BBa_J04450-pSB1C3 | . Inoculation of BBa_J04450-pSB 1C3<br>- Repeat the transformation of the plasmid (pCMV-BMP-2) | . Inoculation of plasmid (pCMV-BMP-2)<br>- Restreak the plate containing plasmid (pCMV-BMP-2) | |
| Result | | | . Got plates with E.Coli carrying BBa_J04450-pSB 1C3 (Confirmed by GFP screening) | - Got plates with E.Coli carrying plasmid (pCMV-BMP-2) | |
| Discussion | | | | | |
| Remark | | | | | |

| Week 2 (2012.6.18-2012.6.22) | | | | | |
|---|---|---|---|---|---|
| Key Words: RFP, BMP-2, Pveg | | | | | |
| | **Monday 2012.6.18** | **Tuesday 2012.6.19** | **Wednesday 2012.6.20** | **Thursday 2012.6.21** | **Friday 2012.6.22** |
| Work done | . Digestion confirmation of plasmid (pCMV-BMP-2)<br><br>. PCR for front part and back part of BMP-2 from the plasmid (pCMV-BMP-2) (Amplify the BMP-2 from the plasmid, introduce mutation afterwards ) | . PCR for $P_{veg}$-R.B.S. (insert R.B.S. into the biobrick)<br>. Gel check of PCR product $P_{veg}$-R.B.S.<br><br>- Gel check for PCR product (BMP-2) | . Gel check of PCR product. $P_{veg}$-R.B.S. | . gDNA extraction from B. *subtilis*<br>. Gel check of DNA from genomic extraction<br>- PCR the front part of BMP-2 from the plasmid (pCMV-BMP-2) | . PCR for $P_{veg}$-R.B.S. (insert R.B.S. into the biobrick) (receive more products)<br><br>- PCR the back part of BMP-2 from the plasmid (pCMV-BMP-2 )<br><br>- Digestion of backbone BBa_J04450 |

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| | | | | | and insert $P_{veg}$-R.B.S using EcoRI and SpeI.<br>- . Dephosphorylation of PSB1C3 in BBa_J04450 |
| Result | . The gel photo show the right bands of the plasmid (pCMV-BMP-2) after digestion<br><br>- | - Gel result cannot tell whether PCR Pveg-R.B.S. is success<br>- The gel photo shows the correct band for the front part of BMP-2, but does not show the correct band for the back part of BMP-2 | - Gel shows PCR product, PCR $P_{veg}$-R.B.S success. | - No DNA found from gDNA extraction | - Gel photo does not show the insert $P_{veg}$-R.B.S and backbone PSB1C3. Save the product |
| Discussion | | No product for the back part of BMP-2. Probably the primer is not correct. In addition, the template may not contain the complementary site as predicted, as the | | - Optimize the protocol for gDNA extraction | - Still no solution solving the problem of PCR for back part of BMP-2 |

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| | | sequence of the template is not documented. | | | |
| Remark | | | | | |

| Week 3 (2012.6.25-2012.6.29) | | | | | |
|---|---|---|---|---|---|
| Key Words: Pveg-R.B.S. gDNA extraction | | | | | |
| | **Monday 2012.6.25** | **Tuesday 2012.6.26** | **Wednesday 2012.6.27** | **Thursday 2012.6.28** | **Friday 2012.6.29** |
| Work done | - gDNA extraction from B. *subtilis* | . gDNA extraction from B. *subtilis* | - Ligation of insert $P_{veg}$-R.B.S and backbone PSB1C3.<br>- Transformation of $P_{veg}$-R.B.S-PSB1C3<br>- PCR for signal peptide YdjM from B. *subtilis* gDNA<br>- Gel check for the PCR YdjM result | - Gradient PCR the whole BMP-2 from plasmid (pCMV-BMP-2)<br>- Gel check for PCR BMP-2<br>- Check the plate after transformation of $P_{veg}$-R.B.S-PSB1C3 | |
| Result | . Got gDNA of B. | . Got gDNA of B. *subtilis* | . Got signal peptide | . No PCR product | |

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| | *subtilis* | | YdjM | from gel photo<br><br>- Contamination of the transformed plate | |
| Discussion | | | | - There is some problem on the back part of BMP-2, or the reverse primer<br>- Prepare the plasmid $P_{veg}$-R.B.S-PSB1C3 again | |
| Remark | | | | | |

| Week 4 (2012.7.2-2012.7.6) | | | | | |
|---|---|---|---|---|---|
| Key Words: | | | | | |
| | **Monday 2012.7.2** | **Tuesday 2012.7.3** | **Wednesday 2012.7.4** | **Thursday 2012.7.5** | **Friday 2012.7.6** |
| Work done | | - Gradient PCR the whole BMP-2 from plasmid | - PCR the whole BMP-2 from BMP-2 extracted in mouse | . PCR YbdN from gDNA of B. *subtilis*<br>- Gel check for PCR | - Check the plate of transformation $P_{veg}$-R.B.S-PSB1C |

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| | | (pCMV-BMP-2) again<br>- Gel check for PCR BMP-2 | gDNA<br>- Gel check of PCR product BMP-2<br>- | product YbdN<br>- Digestion of backbone BBa_J04450 and insert $P_{veg}$-R.B.S using EcoRI and SpeI.<br>- Dephosphorylation of PSB1C3 in BBa_J04450<br>- Purification of backbone PSB1C3 and insert $P_{veg}$-R.B.S<br>- Ligation of $P_{veg}$-R.B.S and PSB1C3<br>- Transformation of $P_{veg}$-R.B.S-PSB1C3 | 3<br>- |
| Result | - | - No PCR product | - Whole BMP-2 is amplified from BMP-2 in mouse gDNA | . no product YbdN in the gel photo | - Colonies found in $P_{veg}$-R.B.S-PSB1C3 |
| Discussion | | | | Wrong YbdN primer is added. | |

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| Remark | | | | | |

| Week 5 (2012.7.9-2012.7.13) | | | | | |
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| Key Words: $P_{veg}$-R.B.S-PSB1C3 | | | | | |
| | **Monday 2012.7.9** | **Tuesday 2012.7.10** | **Wednesday 2012.7.11** | **Thursday 2012.7.12** | **Friday 2012.7.13** |
| Work done | . Inoculation 8 colonies from transformed $P_{veg}$-R.B.S-PSB1C3<br><br>- Digestion check for BMP-2 template plasmid (pCMV-BMP-2) using NotI-HF | - Digestion check of $P_{veg}$-R.B.S-PSB1C3 from 8 colonies using XbaI and NcoI.<br>- Gel check of the digestion product plasmid (pCMV-BMP-2) using NotI-HF | . PCR YbdN from gDNA of B. *subtilis*<br>- Gel check for PCR product YbdN | | - Sequence the plasmid $P_{veg}$-R.B.S-PSB1C3 |
| Result | | - Some plasmid $P_{veg}$-R.B.S-PSB1C3 showed the right band. The cloning success | - Gel showed the right band. Signal peptide YbdN is successfully extracted | | |

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| | | - Gel showed the right band of the digested plasmid (pCMV-BMP-2). | | | |
| Discussion | | Success clone $P_{veg}$-R.B.S-PSB1C3 | | | |
| Remark | | | | | |

| Week 6 (2012.7.16-2012.7.20) | | | | | |
|---|---|---|---|---|---|
| Key Words: Overlapping PCR | | | | | |
| | **Monday 2012.7.16** | **Tuesday 2012.7.17** | **Wednesday 2012.7.18** | **Thursday 2012.7.19** | **Friday 2012.7.20** |
| Work done | · Gradient PCR the YdjM-tag-BMP-2 (insert the complementary site of YdjM at the front site of DNA)<br>- Gel check of PCR product YdjM-tag-BMP-2 | - Gel Purify the PCR product YdjM-tag-BMP-2 | - Gradient PCR the YbdN-tag-BMP-2 (insert the complementary site of YdjM at the front site of DNA)<br>- Gel check of PCR product YbdN-tag-BMP-2<br>- Gel Purify the PCR | - Overlapping PCR using signal peptide YdjM and DNA YdjM-tag-BMP-2 to make YdjM-BMP-2<br>- Gel check for PCR product YdjM-BMP-2 | - Digestion of YdjM-BMP-2 and BBa_J04450 using XbaI and PstI<br>- Dephosphorylation of PSB1C3 in BBa_J04450<br>- Purfication of backbone PSB1C3 and insert |

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| | | | product YbdN-tag-BMP-2 | | YdjM-BMP-2<br>- Ligation of YdjM-BMP-2 and PSB1C3<br>- Transformation of YdjM-BMP-2-PSB1C3<br>- |
| Result | - Gel showed the correct band, PCR success | | - Gel showed three bands with one correct. | - Gel showed the right band | - $P_{veg}$-R.B.S-PSB1C3 has one point mutation on the promoter sequence |
| Discussion | | | | | |
| Remark | | | | | |

| Week 7 (2012.7.23-2012.7.27) | | | | | |
|---|---|---|---|---|---|
| Key Words: | | | | | |
| | **Monday 2012.7.23** | **Tuesday 2012.7.24** | **Wednesday 2012.7.25** | **Thursday 2012.7.26** | **Friday 2012.7.27** |
| Work done | - Check the plate of transformation YdjM-BMP-2-PSB1C3 | - Digestion check of the 3 colonies with YdjM-BMP-2-PSB1C3 using StyI | - Inoculation of 8 colonies from transformed YdjM-BMP-2-PSB1C3 | - Transformation of the BBa_E1010 | - Digestion check of the 8 colonies with YdjM-BMP-2-PSB1C3 using StyI |

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| | - Inoculation of 3 colonies from transformed YdjM-BMP-2-PSB 1C3 | - Gel check of the digested product YdjM-BMP-2-PSB 1C3 | | | |
| Result | - YdjM-BMP-2-PSB 1C3Transformation success. Colonies found on the plate. | - Gel shows the right band of the digested product. | | | - Gel shows the right band of the digested product. |
| Discussion | | - Clone YdjM-BMP-2-PSB 1C3 is success. But there is a EcoRI cutting site in the BMP-2 sequence. Wait the mutant BMP-2 to make a standard biobrick | | | |
| Remark | | | | | |

# Week 8 (2012.7.30-2012.8.3)

| Key Words: YdjM-tag-BMP-2* (*:mutated), YbdN-tag-BMP-2* (*:mutated) | | | | | |
|---|---|---|---|---|---|
| | **Monday 2012.7.30** | **Tuesday 2012.7.31** | **Wednesday 2012.8.1** | **Thursday 2012.8.2** | **Friday 2012.8.3** |
| Work done | . Gradient PCR the YdjM-tag-BMP-2* with the template which is mutated BMP-2<br>- Gel check the PCR product YdjM-tag-BMP-2*<br>- Overlapping PCR YdjM-BMP-2* from signal peptide YdjM and YdjM-tag-BMP-2*<br>- Inoculation of BBa_E1010 | | - Gradient PCR the YbdN-tag-BMP-2* with the template which is mutated BMP-2<br>- Gel check the PCR product YbdN-tag-BMP-2*<br>- Overlapping PCR YbdN-BMP-2* from signal peptide YdjM and YbdN-tag-BMP-2* | - Digestion of insert YdjM-BMP-2* and BBa_J04450 using XbaI and PstI<br>- Dephosphorylation of PSB1C3 in BBa_J04450<br>- Purfication of backbone PSB1C3 and insert YdjM-BMP-2*<br>- Ligation of YdjM-BMP-2* and PSB1C3<br>- Transformation of YdjM-BMP-2*-PSB1C3 | - Colony PCR of 3 Colonies from YdjM-BMP-2*-PSB1C3 and 3 colonies from YbdN-BMP-2*-PSB1C3<br>- Gel check for colony PCR product<br>- Standard assembly of $P_{veg}$-R.B.S-PSB1C3 to BBa_E1010<br>- Digestion confirmation of plasmid BBa_B0015 with XhoI |

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| | | | | - Digestion of insert YbdN-BMP-2* and BBa_J04450 using XbaI and PstI<br>- Dephosphorylation of PSB1C3 in BBa_J04450<br>- Purfication of backbone PSB1C3 and insert YbdN-BMP-2*<br>- Ligation of YbdN-BMP-2* and PSB1C3<br>- Transformation of YbdN-BMP-2*-PSB1C3 | |
| Result | Successfully got the product YdjM-BMP-2*<br>Successfully got the | | Successfully get the product YbdN-BMP-2* | | - Gel showed the correct band of all YdjM-BMP-2*-PSB1C |

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| | plate BBa_E1010 | | | | 3 and YbdN-BMP-2*-PSB1C 3<br><br>- Confirm the right clone BBa_B0015 |
| Discussion | | | | | |
| Remark | | | | | |

| Week 9 (2012.8.6-2012.8.10) | | | | | |
|---|---|---|---|---|---|
| Key Words: BBa_E1010 (RFP) | | | | | |
| | **Monday 2012.8.6** | **Tuesday 2012.8.7** | **Wednesday 2012.8.8** | **Thursday 2012.8.9** | **Friday 2012.8.10** |
| Work done | - Ligation of $P_{veg}$-R.B.S and BBa_E1010<br>- Transformation of $P_{veg}$-R.B.S -BBa_E1010 | | | | |
| Result | | - Observe the red | | | |

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| | | colonies with the clone $P_{veg}$-R.B.S-BBa_E1010. Confirm the function of promoter Pveg*(one point mutation) in E.coli | | | |
| Discussion | | | | | |
| Remark | | | | | |

| Week 10 (2012.8.13-2012.8.17) | | | | | |
|---|---|---|---|---|---|
| Key Words: | | | | | |
| | **Monday 2012.8.13** | **Tuesday 2012.8.14** | **Wednesday 2012.8.15** | **Thursday 2012.8.16** | **Friday 2012.8.17** |
| Work done | - Digestion confirmation of PDG 1661 using EcoRI and BamHI. | | | | |
| Result | | | | | |
| Discussion | | | | | |
| Remark | | | | | |

| Week 11 (2012.8.20-2012.8.24) | | | | | |
|---|---|---|---|---|---|
| Key Words: | | | | | |
| | **Monday 2012.8.20** | **Tuesday 2012.8.21** | **Wednesday 2012.8.22** | **Thursday 2012.8.23** | **Friday 2012.8.24** |
| Work done | | | | | |
| Result | | | | | |
| Discussion | | | | | |
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| Week 12 (2012.8.27-2012.8.31) | | | | | |
|---|---|---|---|---|---|
| Key Words: | | | | | |
| | **Monday 2012.8.27** | **Tuesday 2012.8.28** | **Wednesday 2012.8.29** | **Thursday 2012.8.30** | **Friday 2012.8.31** |
| Work done | | | | | |
| Result | | | | | |
| Discussion | | | | | |
| Remark | | | | | |

## Week 13 (2012.9.3-2012.9.7)

### Key Words:

| | **Monday 2012.9.3** | **Tuesday 2012.9.4** | **Wednesday 2012.9.5** | **Thursday 2012.9.6** | **Friday 2012.9.7** |
|---|---|---|---|---|---|
| Work done | | | | | |
| Result | | | | | |
| Discussion | | | | | |
| Remark | | | | | |

## Week 14 (2012.9.10-2012.9.14)

### Key Words: pTms characterization; Biobrick shipment

| | **Monday 2012.9.10** | **Tuesday 2012.9.11** | **Wednesday 2012.9.12** | **Thursday 2012.9.13** | **Friday 2012.9.14** |
|---|---|---|---|---|---|
| Work done | | | | | |
| Result | | | | | |
| Discussion | | | | | |
| Remark | | | | | |

## Week 15 (2012.9.17-2012.9.21)

| Key Words: $P_{xylA}$ characterization; Biobrick shipment | | | | | |
|---|---|---|---|---|---|
| | **Monday 2012.9.17** | **Tuesday 2012.9.18** | **Wednesday 2012.9.19** | **Thursday 2012.9.20** | **Friday 2012.9.21** |
| Work done | | | | | |
| Result | | | | | |
| Discussion | | | | | |
| Remark | | | | | |