

## Week 1 (2012.6.11-2012.6.15)

Key Words: [pSB1C3](#)

	<b>Monday 2012.6.11</b>	<b>Tuesday 2012.6.12</b>	<b>Wednesday 2012.6.13</b>	<b>Thursday 2012.6.14</b>	<b>Friday 2012.6.15</b>
Work done		<ul style="list-style-type: none"> <li>- Transformation of the <a href="#">plasmid (pCMV-BMP2)</a> into E.coli and <a href="#">BBa_J04450-pSB1C3</a></li> </ul>	<ul style="list-style-type: none"> <li>- Inoculation of <a href="#">BBa_J04450-pSB1C3</a></li> <li>- Repeat the transformation of the <a href="#">plasmid (pCMV-BMP2)</a></li> </ul>	<ul style="list-style-type: none"> <li>- Inoculation of <a href="#">plasmid (pCMV-BMP2)</a></li> <li>- Restreak the plate containing <a href="#">plasmid (pCMV-BMP2)</a></li> </ul>	
Result			<ul style="list-style-type: none"> <li>- Got plates with E.Coli carrying <a href="#">BBa_J04450-pSB1C3</a> (Confirmed by GFP screening)</li> </ul>	<ul style="list-style-type: none"> <li>- Got plates with E.Coli carrying <a href="#">plasmid (pCMV-BMP2)</a></li> </ul>	
Discussion					
Remark					

## Week 2 (2012.6.18-2012.6.22)

Key Words: RFP, BMP2, Pveg

	<b>Monday 2012.6.18</b>	<b>Tuesday 2012.6.19</b>	<b>Wednesday 2012.6.20</b>	<b>Thursday 2012.6.21</b>	<b>Friday 2012.6.22</b>
Work done	<ul style="list-style-type: none"> <li>- Digestion confirmation of plasmid (pCMV-BMP2)</li> <li>- PCR for front part and back part of BMP2 from the plasmid (pCMV-BMP2)</li> <li>- (Amplify the BMP2 from the plasmid, introduce mutation afterwards )</li> </ul>	<ul style="list-style-type: none"> <li>- PCR for P<sub>veg</sub>-R.B.S. (insert R.B.S. into the biobrick)</li> <li>- Gel check of PCR product P<sub>veg</sub>-R.B.S.</li> <li>- Gel check for PCR product (BMP2)</li> </ul>	<ul style="list-style-type: none"> <li>- Gel check of PCR product. P<sub>veg</sub>-R.B.S.</li> </ul>	<ul style="list-style-type: none"> <li>- gDNA extraction from <i>B. subtilis</i></li> <li>- Gel check of DNA from genomic extraction</li> <li>- PCR the front part of BMP2 from the plasmid (pCMV-BMP2)</li> </ul>	<ul style="list-style-type: none"> <li>- PCR for Pveg-R.B.S. (insert R.B.S. into the biobrick) (receive more products)</li> <li>- PCR the back part of BMP2 from the plasmid (pCMV-BMP2)</li> <li>- Digestion of backbone BBa_J04450 and insert Pveg-R.B.S using</li> </ul>

					EcoRI and SpeI. - Dephosphorylation of pSB1C3 in BBa_J04450
Result	<ul style="list-style-type: none"> <li>- The gel photo show the right bands of the plasmid (pCMV-BMP2)</li> <li>- after digestion</li> </ul>	<ul style="list-style-type: none"> <li>- Gel result cannot tell whether PCR P<sub>veg</sub>-R.B.S.is success</li> <li>- The gel photo shows the correct band for the front part of BMP2, but does not show the correct band for the back part of BMP2</li> </ul>	- Gel shows PCR product, PCR P <sub>veg</sub> -R.B.S success.	- No DNA found from gDNA extraction	<ul style="list-style-type: none"> <li>- Gel photo does not show the insert P<sub>veg</sub>-R.B.S and backbone pSB1C3. Save the product</li> </ul>
Discussion		No product for the back part of BMP2. Probably the primer is not correct. In addition, the template may not contain the complementary site as predicted, as the sequence of the template is not documented.		- Need to optimize the protocol for gDNA extraction	<ul style="list-style-type: none"> <li>- Still no solution solving the problem of PCR for back part of BMP2</li> </ul>
Remark					

## Week 3 (2012.6.25-2012.6.29)

Key Words: *P<sub>veg</sub>-R.B.S.* gDNA extraction, PCR mutagenesis

	<b>Monday 2012.6.25</b>	<b>Tuesday 2012.6.26</b>	<b>Wednesday 2012.6.27</b>	<b>Thursday 2012.6.28</b>	<b>Friday 2012.6.29</b>
Work done	- gDNA extraction from <i>B. subtilis</i>	- gDNA extraction from <i>B. subtilis</i>	- Ligation of insert <i>P<sub>veg</sub>-R.B.S</i> and backbone <i>pSB1C3</i> . - Transformation of <i>P<sub>veg</sub>-R.B.S-pSB1C3</i>  - PCR for signal peptide <i>YdjM</i> from <i>B. subtilis</i> gDNA - Gel check for the PCR <i>YdjM</i> result	- Gradient PCR the whole BMP2 from plasmid ( <i>pCMV-BMP2</i> ) - Gel check for PCR BMP2  - Check the plate after transformation of <i>P<sub>veg</sub>-R.B.S-pSB1C3</i>	
Result	- Got gDNA of <i>B. subtilis</i>	- Got gDNA of <i>B. subtilis</i>	- Got signal peptide <i>YdjM</i>	- No PCR product from gel photo  - Contamination of the transformed plate	
Discussion	-	-	-	- There is some problem	

				<p>on the back part of BMP2, or the reverse primer. We try to make a new template which is a BMP2*-PBS. The BMP2* would be extract from genomic DNA of mouse. And we use PCR to perform the mutagenesis which aims to remove the EcoRI cutting site in the gene sequence of BMP2.</p> <p>- Because of the contamination, we are going to prepare the plasmid P<sub>veg</sub>-R.B.S-pSB1C3 again</p>	
Remark					

## Week 4 (2012.7.2-2012.7.6)

- Key Words: YbdN PCR, P<sub>veg</sub>-R.B.S-PSB1C3

	<b>Monday 2012.7.2</b>	<b>Tuesday 2012.7.3</b>	<b>Wednesday 2012.7.4</b>	<b>Thursday 2012.7.5</b>	<b>Friday 2012.7.6</b>
Work done	-	<ul style="list-style-type: none"> <li>- Gradient PCR the whole BMP2 from plasmid (pCMV-BMP2) again</li> <li>- Gel check for PCR BMP2</li> </ul>	<ul style="list-style-type: none"> <li>- PCR the whole BMP2 from BMP2 extracted in mouse gDNA</li> <li>- Gel check of PCR product BMP2</li> </ul>	<ul style="list-style-type: none"> <li>- PCR YbdN from gDNA of <i>B. subtilis</i></li> <li>- Gel check for PCR product YbdN</li> <li>- Digestion of backbone BBa_J04450 and insert P<sub>veg</sub>-R.B.S using EcoRI and SpeI.</li> <li>- Dephosphorylation of pSB1C3 in BBa_J04450</li> <li>- Purification of backbone pSB1C3 and insert P<sub>veg</sub>-R.B.S</li> <li>- Ligation of P<sub>veg</sub>-R.B.S and</li> </ul>	<ul style="list-style-type: none"> <li>- Check the plate of transformation P<sub>veg</sub>-R.B.S-pSB1C3</li> </ul>

				PSB1C3 - Transformation of P <sub>veg</sub> -R.B.S-pSB1C3	
Result	-	- No PCR product	- Whole BMP2 is amplified from BMP2 in mouse gDNA	- no product YbdN in the gel photo	- Colonies found in P <sub>veg</sub> -R.B.S-pSB1C3
Discussion				Wrong YbdN primer is added.	
Remark					

## Week 5 (2012.7.9-2012.7.13)

Key Words: P<sub>veg</sub>-R.B.S-pSB1C3

	<b>Monday 2012.7.9</b>	<b>Tuesday 2012.7.10</b>	<b>Wednesday 2012.7.11</b>	<b>Thursday 2012.7.12</b>	<b>Friday 2012.7.13</b>
Work done	<ul style="list-style-type: none"> <li>- Inoculation 8 colonies from transformed P<sub>veg</sub>-R.B.S-pSB1C3</li> <li>- Digestion check for BMP2 template plasmid</li> </ul>	<ul style="list-style-type: none"> <li>- Digestion check of P<sub>veg</sub>-R.B.S-pSB1C3 from 8 colonies using XbaI and NcoI.</li> <li>- Gel check of the digestion product plasmid (pCMV-BMP2) using NotI-HF</li> </ul>	<ul style="list-style-type: none"> <li>- PCR YbdN from gDNA of <i>B. subtilis</i></li> <li>- Gel check for PCR product YbdN</li> </ul>		<ul style="list-style-type: none"> <li>- Sequence the plasmid P<sub>veg</sub>-R.B.S-pSB1C3</li> </ul>

	(pCMV-BMP2) using NotI-HF				
Result		<ul style="list-style-type: none"> <li>- Some plasmid <b>P<sub>veg</sub>-R.B.S-pSB1C3</b> showed the right band. The cloning success</li> <li>- Gel showed the right band of the digested plasmid (pCMV-BMP2).</li> </ul>	<ul style="list-style-type: none"> <li>- Gel showed the right band. Signal peptide <b>YbdN</b> is successfully extracted</li> </ul>		
Discussion		<ul style="list-style-type: none"> <li>- Success clone <b>P<sub>veg</sub>-R.B.S-pSB1C3</b></li> </ul>			
Remark					

## Week 6 (2012.7.16-2012.7.20)

### Key Words: **Overlapping PCR**

	<b>Monday 2012.7.16</b>	<b>Tuesday 2012.7.17</b>	<b>Wednesday 2012.7.18</b>	<b>Thursday 2012.7.19</b>	<b>Friday 2012.7.20</b>
Work done	- Gradient PCR the <b>YdjM-tag-BMP2</b>	- Gel Purify the PCR product	- Gradient PCR the <b>YbdN-tag-BMP2</b>	- Overlapping PCR using signal	- Digestion of <b>YdjM+BMP2</b> and



	(insert the complementary site of YdjM at the front site of DNA) - Gel check of PCR product YdjM-tag-BMP2	YdjM-tag-BMP2	(insert the complementary site of YdjM at the front site of DNA) - Gel check of PCR product YbdN-tag-BMP2 - Gel Purify the PCR product YbdN-tag-BMP2	peptide YdjM and DNA YdjM-tag-BMP2 to make YdjM+BMP2 - Gel check for PCR product YdjM+BMP2	BBa_J04450 using XbaI and PstI - Dephosphorylation of pSB1C3 in BBa_J04450 - Purification of backbone pSB1C3 and insert YdjM+BMP2 - Ligation of YdjM+BMP2 and PSB1C3 - Transformation of YdjM+BMP2-pSB1C3
Result	- Gel showed the correct band, PCR success		- Gel showed three bands with one correct.	- Gel showed the right band	- P <sub>veg</sub> -R.B.S-pSB1C3 has one point mutation on the promoter sequence
Discussion					
Remark					

Week 7 (2012.7.23-2012.7.27)

Key Words: EcoRI cutting site, PCR mutagenesis, BMP2\* (\*:mutated)-pBS

	<b>Monday 2012.7.23</b>	<b>Tuesday 2012.7.24</b>	<b>Wednesday 2012.7.25</b>	<b>Thursday 2012.7.26</b>	<b>Friday 2012.7.27</b>
Work done	<ul style="list-style-type: none"> <li>- PCR <b>BMP2</b> from mouse genomic DNA.</li> <li>- Gel check for PCR product <b>BMP2</b></li> <li>- Check the plate of transformation <b>YdjM+BMP2-pSB1C3</b></li> <li>- Inoculation of 3 colonies from transformed <b>YdjM+BMP2-pSB1C3</b></li> </ul>	<ul style="list-style-type: none"> <li>- Digestion check of the 3 colonies with <b>YdjM+BMP2-pSB1C3</b> using StyI</li> <li>- Gel check of the digested product <b>YdjM+BMP2-pSB1C3</b></li> <li>- Digestion of <b>BMP2</b> and <b>pBS</b> using XbaI and PstI</li> <li>- Purification of backbone <b>pBS</b> and insert <b>BMP2</b></li> <li>- Ligation of <b>pBS</b> and <b>BMP2</b></li> <li>- Transformation of <b>BMP2-PBS</b>.</li> <li>-</li> </ul>	<ul style="list-style-type: none"> <li>- Inoculation of 8 colonies from transformed <b>YdjM+BMP2-pSB1C3</b></li> <li>- Inoculation 8 colonies of <b>BMP2-pBS</b></li> </ul>	<ul style="list-style-type: none"> <li>- Transformation of the <b>Ba_E1010</b></li> <li>- Gel check the plasmid <b>BMP2-pBS</b></li> <li>- PCR <b>BMP2-pBS</b> which is a PCR mutagenesis.</li> <li>- Gel check the PCR product <b>BMP2*-pBS</b></li> </ul>	<ul style="list-style-type: none"> <li>- Digestion check of the 8 colonies with <b>YdjM+BMP2-pSB1C3</b> using StyI</li> <li>- Sequence the <b>BMP2*-pBS</b></li> <li>- Transformation of plasmid <b>BMP2*-pBS</b></li> </ul>
Result	<ul style="list-style-type: none"> <li>- <b>YdjM+BMP2-pSB1C3</b> transformation success. Colonies found on the</li> </ul>	<ul style="list-style-type: none"> <li>- Gel shows the right band of the all digested product.</li> </ul>	<ul style="list-style-type: none"> <li>- Transformation of <b>BMP2-pBS</b> success. We are going to do a mutation on it to</li> </ul>	<ul style="list-style-type: none"> <li>- Gel shows the right band of all products</li> </ul>	<ul style="list-style-type: none"> <li>- Gel shows the right band of the digested product.</li> </ul>

	plate. - Gel shows the correct band of PCR product <a href="#">BMP2</a>		remove EcoRI cutting site.		
Discussion		- Clone <a href="#">YdjM+BMP2-pSB1C3</a> is success. But there is a EcoRI cutting site in the BMP2 sequence. Wait the mutant BMP2 to make a standard biobrick			
Remark					

## Week 8 (2012.7.30-2012.8.3)

Key Words: [YdjM-tag-BMP2\\*](#) (\*:mutated), [YbdN-tag-BMP2\\*](#) (\*:mutated)

	<b>Monday 2012.7.30</b>	<b>Tuesday 2012.7.31</b>	<b>Wednesday 2012.8.1</b>	<b>Thursday 2012.8.2</b>	<b>Friday 2012.8.3</b>
Work done	- Gradient PCR the			- Digestion of insert	- Colony PCR of 3

	<p>YdjM-tag-BMP2* and YbdN-tag-BMP2* with the template which is BMP2*-PBS.</p> <ul style="list-style-type: none"> <li>- Gel check the PCR product</li> </ul> <p>YdjM-tag-BMP2* and YbdN-tag-BMP2*</p> <ul style="list-style-type: none"> <li>- Overlapping PCR YdjM+BMP2* from signal peptide YdjM and YdjM-tag-BMP2*</li> <li>- Overlapping PCR YbdN+BMP2* from signal peptide YbdN and YbdN-tag-BMP2*</li> </ul> <ul style="list-style-type: none"> <li>- Inoculation of BBa_E1010</li> </ul>			<p>YdjM+BMP2* and BBa_J04450 using XbaI and PstI</p> <ul style="list-style-type: none"> <li>- Dephosphorylation of pSB1C3 in BBa_J04450</li> <li>- Purification of backbone pSB1C3 and insert YdjM+BMP2*</li> <li>- Ligation of YdjM+BMP2* and pSB1C3</li> <li>- Transformation of YdjM+BMP2*-pSB1C3</li> <li>- Digestion of insert YbdN+BMP2* and BBa_J04450 using XbaI and PstI</li> <li>- Dephosphorylation of pSB1C3 in BBa_J04450</li> <li>- Purification of backbone pSB1C3</li> </ul>	<p>Colonies from YdjM+BMP2*-pSB1C3 and 3 colonies from YbdN+BMP2*-pSB1C3</p> <ul style="list-style-type: none"> <li>- Gel check for colony PCR product</li> <li>- Standard assembly of P<sub>veg</sub>-R.B.S-pSB1C3 to BBa_E1010</li> <li>- Digestion confirmation of plasmid BBa_B0015 with XhoI</li> </ul>
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				and insert YbdN+BMP2* - Ligation of YbdN+BMP2* and pSB1C3 - Transformation of YbdN+BMP2*-pSB 1C3	
Result	<ul style="list-style-type: none"> <li>- Successfully got the product YdjM+BMP2* and YbdN+BMP2*</li> <li>- Successfully got the plate BBa_E1010</li> <li>- Sequence of BMP2*-pBS is correct</li> </ul>				<ul style="list-style-type: none"> <li>- YbdN+BMP2* and YdjM+BMP2* are successfully transformed</li> <li>- Gel showed the correct band of all YdjM+BMP2*-pSB1C3 and YbdN+BMP2*-pSB1C3</li> <li>- Confirm the right clone BBa_B0015</li> </ul>
Discussion					
Remark					

## Week 9 (2012.8.6-2012.8.10)

Key Words: [BBa\\_E1010](#) (RFP)

	<b>Monday 2012.8.6</b>	<b>Tuesday 2012.8.7</b>	<b>Wednesday 2012.8.8</b>	<b>Thursday 2012.8.9</b>	<b>Friday 2012.8.10</b>
Work done	<ul style="list-style-type: none"> <li>- Ligation of <a href="#">P<sub>veg</sub>-R.B.S</a> and <a href="#">BBa_E1010</a></li> <li>- Transformation of <a href="#">P<sub>veg</sub>-R.B.S</a> -<a href="#">BBa_E1010</a></li> </ul>				
Result		<ul style="list-style-type: none"> <li>- Observe the red colonies with the clone <a href="#">P<sub>veg</sub>-R.B.S</a> -<a href="#">BBa_E1010</a>. Confirm the function of promoter Pveg*(one point mutation) in E.coli</li> </ul>			
Discussion					
Remark					

## Week 10 (2012.8.13-2012.8.17)

Key Words: Sequencing YbdN+BMP2\* and YdjM+BMP2\*  
 Protein Assays and Filter Sterilization of Supernatant

	<b>Monday 2012.8.13</b>	<b>Tuesday 2012.8.14</b>	<b>Wednesday 2012.8.15</b>	<b>Thursday 2012.8.16</b>	<b>Friday 2012.8.17</b>
Work done	- Digestion confirmation of PDG 1661 using EcoRI and BamHI.	- Inoculation of Bacillus subtilis with SpC culture	- Sequencing of constructs YbdN+BMP2* and YdjM+BMP2* in pSB1C3	- Filter sterilization of the supernatant of bacterial culture with 0.22um and 0.45um filter - Bradford assay of both sterilized and non-sterilized supernatant - Spread plate of supernatant of non-sterilized and two kinds of filter	- Count the colonies
Result			- Colonies with correct sequences are found	- Protein amount: - Non-sterilized: 45 ng/ul - 0.45 um filter: 39	- Colonies: Non-sterilized: Full of colonies - 0.45 um filter:

				ng/ml - 0.22 um filter: 35 ng/ml	3 colonies - 0.22 um filter: No colony
Discussion					
Remark					

## Week 11 (2012.8.20-2012.8.24)

Key Words: [Constructions of Pveg+RBS+YdjM+BMP2\\*+BBa\\_0015](#) and  
[Pveg+RBS+YbdN+BMP2\\*+BBa\\_0015](#)

	<b>Monday 2012.8.20</b>	<b>Tuesday 2012.8.21</b>	<b>Wednesday 2012.8.22</b>	<b>Thursday 2012.8.23</b>	<b>Friday 2012.8.24</b>
Work done	<ul style="list-style-type: none"> <li>- Digestion of YbdN+BMP2* in pSB1C3 with XbaI and PstI</li> <li>- Digestion of YdjM+BMP2* in pSB1C3 with XbaI and PstI</li> <li>- Digestion and dephosphorylation of Pveg-RBS in pSB1C3</li> </ul>	<ul style="list-style-type: none"> <li>- Gel Purification of digested YbdN+BMP2*</li> <li>- Gel Purification of digested YdjM+BMP2*</li> <li>- Ligation of Pveg-RBS in pSB1C3 with YdjM+BMP2*</li> <li>- Ligation of</li> </ul>	<ul style="list-style-type: none"> <li>- Digestion of BBa_0015 in pSB1AK3 with EcoRI and XbaI</li> <li>- Colony PCR for screening of Pveg+RBS+YbdN+BMP2* in pSB1C3 and Pveg-RBS-YdjM+BMP2* in pSB1C3</li> <li>- Ligation of Pveg-RBS+YbdN+BMP2* in pSB1C3 and Pveg-RBS-YdjM+BMP2* in pSB1C3</li> </ul>	<ul style="list-style-type: none"> <li>- Plasmid extraction from Pveg+RBS+YbdN+BMP2* in pSB1C3 and Pveg-RBS-YdjM+BMP2* in pSB1C3 in <i>E. coli</i></li> <li>- Digestion of Pveg+RBS+YbdN+BMP2* in pSB1C3 with EcoRI and SpeI</li> </ul>	<ul style="list-style-type: none"> <li>- Colony PCR for screening of Pveg+RBS+YdjM+BMP2*+BBa_0015 in pSB1AK3 and Pveg+RBS+YbdN+BMP2*+BBa_0015 in pSB1AK3</li> <li>- Inoculation of Pveg+RBS+YdjM</li> </ul>



	<p>with SpeI and PstI</p> <ul style="list-style-type: none"> <li>- Cleanup of Digested Pveg-RBS in pSB1C3 using column</li> </ul>	<p>Pveg-RBS in pSB1C3 with YbdN+BMP2*</p> <ul style="list-style-type: none"> <li>- Transformation of Pveg+RBS+YbdN+BMP2* in pSB1C3 and Pveg-RBS-YdjM+BMP2* in pSB1C3 into <i>E. coli</i></li> </ul>	<ul style="list-style-type: none"> <li>- Inoculation of Pveg+RBS+YbdN+BMP2* in pSB1C3 and Pveg-RBS-YdjM+BMP2* in pSB1C3 in <i>E. coli</i></li> </ul>	<ul style="list-style-type: none"> <li>- Digestion of Pveg-RBS-YdjM+BMP2* in pSB1C3 with EcoRI and SpeI</li> <li>- Gel purification of digested Pveg-RBS-YdjM+BMP2* and Pveg+RBS+YbdN+BMP2*</li> <li>- Ligation of Pveg-RBS-YdjM+BMP2* with BBa_0015 in pSB1AK3</li> <li>- Ligation of Pveg+RBS+YbdN+BMP2* with BBa_0015 in pSB1AK3</li> <li>- Transformation of Pveg+RBS+YdjM+BMP2*+BBa_0015 in pSB1AK3 and Pveg+RBS+YbdN+BMP2*+BBa_0015 in pSB1AK3 into <i>E. coli</i></li> </ul>	<p>+BMP2*+BBa_0015 in pSB1AK3 and Pveg+RBS+YbdN+BMP2*+BBa_0015 in pSB1AK3 in <i>E. coli</i></p>
Result	- Cleanup of digestion	- Cleanup of	- Pveg+RBS+YbdN	- Plasmid extraction of	- Gel

	product Pveg-RBS in pSB1C3 has good concentration and purity	digestion products YbdN+BMP2* and YdjM+BMP2* have good concentration and purity	+BMP2* in pSB1C3 and Pveg-RBS-YdjM+BMP2* in pSB1C3 are successfully transformed into <i>E. coli</i> . - Cleanup of digestion product BBa_0015 in pSB1AK3 has good concentration and purity	the constructs has good concentration and purity. - Gel electrophoresis of digestion products shows correct bands to be cut. - Gel purification products have good concentration.	electrophoresis of colony PCR products shows expected bands
Discussion					

## Week 12 (2012.8.27-2012.8.31)

Key Words: Transfer Pveg+RBS+YdjM+BMP2\*+BBa\_0015 and Pveg+RBS+YbdN+BMP2\*+BBa\_0015 to pDG1661, Transformation of Pveg+RBS+YbdN+BMP2\*-BBa\_0015 into *B. subtilis*

	<b>Monday 2012.8.27</b>	<b>Tuesday 2012.8.28</b>	<b>Wednesday 2012.8.29</b>	<b>Thursday 2012.8.30</b>	<b>Friday 2012.8.31</b>
Work done	<ul style="list-style-type: none"> <li>- Plasmid extraction from Pveg+RBS+YbdN+BMP2*+BBa_0015 in pSB1AK3 and Pveg+RBS+YdjM+BMP2*+BBa_0015 in pSB1AK3 from <i>E. coli</i></li> <li>- Digestion of Pveg+RBS+YbdN+BMP2*+BBa_0015 in pSB1AK3 and Pveg+RBS+YdjM+BMP2*+BBa_0015 in pSB1AK3 with EcoRI and PstI</li> <li>- Digestion of pBS with EcoRI and PstI</li> <li>- Gel purification of digested Pveg+RBS+YbdN+BMP2*+BBa_0015 and Pveg+RBS+YdjM+B</li> </ul>	<ul style="list-style-type: none"> <li>- Colony PCR for screening of constructs Pveg+RBS+YbdN+BMP2*+BBa_0015 in pBS and Pveg+RBS+YdjM+BMP2*+BBa_0015 in pSB1AK3</li> <li>- Inoculation of Pveg+RBS+YdjM+BMP2*+BBa_0015 in pSB1AK3 and Pveg+RBS+YbdN+BMP2*+BBa_0015 in pBS</li> </ul>	<ul style="list-style-type: none"> <li>- Plasmid extraction of Pveg+RBS+YdjM+BMP2*+BBa_0015 in pSB1AK3 and Pveg+RBS+YbdN+BMP2*+BBa_0015 in pBS from <i>E. coli</i></li> <li>- Digestion of Pveg+RBS+YdjM+BMP2*+BBa_0015 in pSB1AK3 with EcoRI and PstI</li> <li>- Digestion of Pveg+RBS+YbdN+BMP2*+BBa_0015 in pBS with EcoRI and BamHI</li> <li>- Digestion of backbone pDG1661 with EcoRI and BamHI</li> <li>- Clean up the digestion products</li> </ul>	<ul style="list-style-type: none"> <li>- Colony PCR for screening of constructs Pveg+RBS+YdjM+BMP2*+BBa_0015 in pBS and Pveg+RBS+YbdN+BMP2*+BBa_0015 in pDG1661</li> <li>- Inoculation of Pveg+RBS+YdjM+BMP2*+BBa_0015 in pBS and Pveg+RBS+YbdN+BMP2*+BBa_0015 in pDG1661</li> </ul>	<ul style="list-style-type: none"> <li>- Digestion Check for construct Pveg+RBS+YbdN+BMP2*+BBa_0015 in pDG1661 using XbaI</li> <li>- Digestion of Pveg+RBS+YdjM+BMP2*+BBa_0015 in pBS with EcoRI and BamHI</li> <li>- Ligation of Pveg+RBS+YdjM+BMP2*+BBa_0015 with pDG1661</li> <li>- Transformation of Pveg+RBS+YdjM+BMP2*+BBa_0015 in pDG1661 into <i>E. coli</i></li> <li>- Transformation of Pveg+RBS+YbdN+BMP2*+BBa_0015 in pDG1661</li> </ul>

	<ul style="list-style-type: none"> <li>- Ligation of Pveg+RBS+YbdN+BMP2*+BBa_0015 with pBluescript (pBS)</li> <li>- Transformation of Pveg+RBS+YbdN+BMP2*+BBa_0015 in pBS into <i>E. coli</i></li> <li>- Ligation of Pveg-RBS-YdjM+BMP2* with BBa_0015 in pSB1AK3</li> <li>- Transformation of Pveg+RBS+YdjM+BMP2*+BBa_0015 in pSB1AK3 into <i>E. coli</i></li> </ul>		<ul style="list-style-type: none"> <li>- Ligation of Pveg+RBS+YdjM+BMP2*+BBa_0015 with pBS</li> <li>- Ligation of Pveg+RBS+YbdN+BMP2*+BBa_0015 with pDG1661</li> <li>- Transformation of Pveg+RBS+YdjM+BMP2*+BBa_0015 in pBS and Pveg+RBS+YbdN+BMP2*+BBa_0015 in pDG1661 into <i>E. coli</i></li> </ul>		<ul style="list-style-type: none"> <li>- 15 in pDG1661 into <i>B. subtilis</i></li> </ul>
Result	<ul style="list-style-type: none"> <li>- Contamination on subculture and plates of Pveg+RBS+YdjM+BMP2*+BBa_0015 in pSB1AK3</li> <li>- Gel electrophoresis of digestion product</li> </ul>	<ul style="list-style-type: none"> <li>- Pveg+RBS+YbdN+BMP2*+BBa_0015 in pBS and Pveg+RBS+YdjM+BMP2*+BBa_0015 in pSB1AK3 are successfully transformed into</li> </ul>	<ul style="list-style-type: none"> <li>- Plasmid extraction of the constructs has good concentration and purity.</li> <li>- Gel electrophoresis of digestion products shows correct bands.</li> <li>- Gel purification</li> </ul>	<ul style="list-style-type: none"> <li>- Pveg+RBS+YdjM+BMP2*+BBa_0015 in pBS and Pveg+RBS+YbdN+BMP2*+BBa_0015 in pDG1661 are successfully transformed into <i>E.</i></li> </ul>	<ul style="list-style-type: none"> <li>- Plasmid extraction of the constructs has good concentration and purity.</li> <li>- Gel electrophoresis of digestion products</li> </ul>

	<p>insert  <a href="#">Pveg+RBS+YbdN+BM P2*+BBa_0015</a>  shows expected bands.</p> <p>- Gel electrophoresis of digestion product insert  <a href="#">Pveg+RBS+YdjM+BM P2*+BBa_0015</a> does not show any bands.</p>	<p><i>E. coli</i></p> <p>- Gel electrophoresis of colony PCR products shows expected bands</p>	<p>products have good concentration.</p>	<p><i>coli.</i></p> <p>- Gel electrophoresis of Colony PCR products shows correct bands.</p>	<p>shows correct bands.</p> <p>- Gel purification products have good concentration</p>
Discussion					
Remark					

## Week 13 (2012.9.3-2012.9.7)

Key Words: [Transformation of Pveg+RBS+YdjM+BMP2\\*+BBa\\_0015 into \*B. subtilis\*](#)

	<b>Monday 2012.9.3</b>	<b>Tuesday 2012.9.4</b>	<b>Wednesday 2012.9.5</b>	<b>Thursday 2012.9.6</b>	<b>Friday 2012.9.7</b>
Work done	<p>- Digestion of <a href="#">Pveg+RBS+YdjM+BM P2*+BBa_0015</a> in</p>	<p>- Colony PCR for screening of <a href="#">Pveg+RBS+YdjM+BM</a></p>	<p>- Plasmid Extraction of <a href="#">Pveg+RBS+YdjM+BM P2*+BBa_0015</a> in</p>		

	<p>pSB1AK3 and Pveg+RBS+YbdN+BM P2*+BBa_0015 in pSB1AK3 with XbaI and PstI</p> <ul style="list-style-type: none"> <li>- Cleanup of Digestion products by gel purification</li> <li>- Ligation of Pveg+RBS+YdjM+BM P2*+BBa_0015 with pSB1C3</li> <li>- Ligation of Pveg+RBS+YbdN+BM P2*+BBa_0015 with pSB1C3</li> <li>- Ligation of Pveg+RBS+YdjM+BM P2*+BBa_0015 with pDG1661</li> <li>- Transformation of Pveg+RBS+YdjM+BM P2*+BBa_0015 in pSB1C3 and Pveg+RBS+YbdN+BM P2*+BBa_0015 in pSB1C3 into <i>E. coli</i></li> </ul>	<p>P2*+BBa_0015 in pSB1C3, Pveg+RBS+YbdN+BM P2*+BBa_0015 in pSB1C3 and Pveg+RBS+YdjM+BM P2*+BBa_0015 in pDG1661</p> <ul style="list-style-type: none"> <li>- Inoculation of Pveg+RBS+YdjM+BM P2*+BBa_0015 in pSB1C3, Pveg+RBS+YbdN+BM P2*+BBa_0015 in pSB1C3 and Pveg-RBS-YdjM+BMP2-BBa_0015 in pDG1661</li> </ul>	<p>pSB1C3, Pveg+RBS+YbdN+BM P2*+BBa_0015 in pSB1C3 and Pveg+RBS+YdjM+BM P2*+BBa_0015 in pDG1661 from <i>E. coli</i></p> <ul style="list-style-type: none"> <li>- Transformation of Pveg+RBS+YdjM+BM P2*+BBa_0015 in pDG1661 into <i>B. subtilis</i></li> </ul>		
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	- Transformation of Pveg+RBS+YdjM+BM P2*+BBa_0015 in pDG1661 into <i>E.coli</i>				
Result	- Contamination on plates of Pveg+RBS+YdjM+BM P2*+BBa_0015 in pDG1661 - Pveg+RBS+YbdN+BM P2*+BBa_0015 in pDG1661 is successfully transformed into <i>B. subtilis</i>	- Pveg+RBS+YdjM+BM P2*+BBa_0015 in pSB1C3 and Pveg+RBS+YbdN+BM P2*+BBa_0015 in pSB1C3 are successfully transformed into <i>E. coli</i> - Pveg+RBS+YdjM+BM P2*+BBa_0015 in pDG1661 is successfully transformed into <i>E.coli</i> . - Gel electrophoresis of colony PCR products shows expected bands	- Plasmid extraction products have good concentration and purity	- Pveg+RBS+YdjM+BM P2*+BBa_0015 in pDG1661 is successfully transformed into <i>B. subtilis</i> .	
Discussion					
Remark					

Week 14 (2012.9.10-2012.9.14)

Key Words:

	<b>Monday 2012.9.10</b>	<b>Tuesday 2012.9.11</b>	<b>Wednesday 2012.9.12</b>	<b>Thursday 2012.9.13</b>	<b>Friday 2012.9.14</b>
Work done					
Result					
Discussion					
Remark					

Week 15 (2012.9.17-2012.9.21)

Key Words:

	<b>Monday 2012.9.17</b>	<b>Tuesday 2012.9.18</b>	<b>Wednesday 2012.9.19</b>	<b>Thursday 2012.9.20</b>	<b>Friday 2012.9.21</b>
Work done					
Result					
Discussion					
Remark					