

## 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>Resuspension of <a href="#">BBa_E0840-pSB1A2</a> and <a href="#">BBa_J04450-pSB1C3</a> from 2012 Distribution</li> <li>Transformation of <a href="#">BBa_E0840-pSB1A2</a> and <a href="#">BBa_J04450-pSB1C3</a> into E.Coli</li> </ul>	<ul style="list-style-type: none"> <li>Fluorescence screening of plates with <a href="#">BBa_E0840-pSB1A2</a> and <a href="#">BBa_J04450-pSB1C3</a> transformed E.Coli</li> <li>Inoculation of <a href="#">BBa_E0840-pSB1A2</a> and <a href="#">BBa_J04450-pSB1C3</a></li> </ul>	<ul style="list-style-type: none"> <li>Plasmid extraction of <a href="#">BBa_E0840-pSB1A2</a> and <a href="#">BBa_J04450-pSB1C3</a></li> </ul>	<ul style="list-style-type: none"> <li>Digestion of <a href="#">BBa_J04450-pSB1C3</a> with Xba I and Pst I</li> <li>Dephosphorylation of <a href="#">pSB1C3</a> digestion product</li> <li>Gel electrophoresis of digestion product</li> <li>Gel purification of <a href="#">pSB1C3</a></li> </ul>
Result			<ul style="list-style-type: none"> <li>Got plates with E.Coli carrying <a href="#">BBa_E0840-pSB1A2</a> and plates with E.Coli carrying <a href="#">BBa_J04450-pSB1C3</a> (Confirmed by GFP screening)</li> </ul>		<ul style="list-style-type: none"> <li>Little linear pSB1C3 purification product</li> </ul>
Discussion					The gel purification yields bad recovery as well as bad purity

Remark	<p>R.G.T stands for the abbreviation of RBS + GFP + Terminator Correct part BBa_E0840-pSB1A2</p>
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## Week 2 (2012.6.18-2012.6.22)

### Key Words: [Pveg-R.G.T-pSB1C3](#); [xyIR-P<sub>xyIA</sub>](#) mutagenesis

	<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>PCR mutagenesis of <a href="#">BBa_E0840</a> (to add B.Subtilis RBS)</li> <li>Gel electrophoresis check of <a href="#">R.G.T</a> PCR product</li> <li>PCR clean up of PCR product</li> <li>Digestion of <a href="#">R.G.T</a> with XbaI and PstI</li> <li>Digestion clean up of <a href="#">R.G.T</a></li> <li>Inoculation of shipped E.Coli carrying <a href="#">Pveg-pSB1C3</a></li> <li>Spreading of shipped E.Coli carrying <a href="#">Pveg-pSB1C3</a> onto plate</li> </ul>	<ul style="list-style-type: none"> <li>Primary PCR for <a href="#">xyIR-P<sub>xyIA</sub></a> mutagenesis (to eliminate the 1<sup>st</sup> illegal cutting site)</li> <li>Gel electrophoresis of primary PCR product</li> <li>Gel purification of primary PCR product</li> <li>Digestion of <a href="#">BBa_J04450-pSB1C3</a> with XbaI and PstI</li> <li>Dephosphorylation of <a href="#">pSB1C3</a> digestion product</li> <li>Gel electrophoresis of <a href="#">pSB1C3</a></li> <li>Gel purification of <a href="#">pSB1C3</a></li> </ul>	<ul style="list-style-type: none"> <li>Secondary PCR for <a href="#">xyIR-P<sub>xyIA</sub></a> mutagenesis (to eliminate the 1<sup>st</sup> illegal cutting site)</li> <li>Gel electrophoresis of secondary PCR product</li> <li>Ligation of <a href="#">R.G.T</a> and <a href="#">pSB1C3</a></li> <li>Transformation of ligation product</li> <li>Ligation of <a href="#">R.G.T</a> and <a href="#">Pveg-pSB1C3</a></li> <li>Transformation of ligation product</li> </ul>	<ul style="list-style-type: none"> <li>Check of plate with <a href="#">R.G.T-pSB1C3</a> transformed E.Coli</li> <li>Check of plate with <a href="#">Pveg-R.G.T-pSB1C3</a> transformed E.Coli</li> <li>Inoculation of E.Coli transformed with <a href="#">Pveg-R.G.T-pSB1C3</a></li> <li>Primary PCR for <a href="#">xyIR-P<sub>xyIA</sub></a> mutagenesis (to eliminate the 1<sup>st</sup> illegal cutting site)</li> <li>Gel electrophoresis of primary PCR product</li> <li>Gel purification of</li> </ul>	

		<ul style="list-style-type: none"> <li>Digestion of <a href="#">Pveg-pSB1C3</a> with XbaI and PstI</li> <li>Dephosphorylation of <a href="#">Pveg-pSB1C3</a> digestion product</li> <li>Gel electrophoresis of digestion product</li> <li>Gel Purification of <a href="#">Pveg-pSB1C3</a></li> </ul>		primary PCR product	
Result	<ul style="list-style-type: none"> <li>Got <a href="#">R.G.T</a> digested with XbaI and PstI</li> </ul>	<ul style="list-style-type: none"> <li>Got primary PCR product for <a href="#">xyIR-PxyIA</a> mutagenesis</li> <li>Got linear <a href="#">pSB1C3</a> digested with XbaI and PstI</li> <li>Got linear <a href="#">Pveg-pSB1C3</a> digested with XbaI and PstI</li> </ul>	<ul style="list-style-type: none"> <li>Secondary PCR yields no product</li> </ul>	<ul style="list-style-type: none"> <li>Got bacteria lawn on plate with <a href="#">R.G.T-pSB1C3</a> transformed E.Coli</li> <li>Got more primary PCR product for <a href="#">xyIR-PxyIA</a> mutagenesis</li> </ul>	
Discussion			<p>For secondary PCR</p> <ol style="list-style-type: none"> <li>the annealing PCR without primers did not work here</li> <li>The template for secondary PCR was not enough</li> </ol>	<p>For constructing <a href="#">R.G.T-pSB1C3</a></p> <ol style="list-style-type: none"> <li>the digestion of backbone is not complete</li> </ol>	

Remark	How do we confirm that the RBS has been linked with GFP?
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Week 3 (2012.6.25-2012.6.29)					
	Key Words: R.G.T-pSB1C3; <i>xyIR-P<sub>xyIA</sub></i> mutagenesis				
	Monday 2012.6.25	Tuesday 2012.6.26	Wednesday 2012.6.27	Thursday 2012.6.28	Friday 2012.6.29
Work done	<ul style="list-style-type: none"> <li>Secondary PCR for <i>xyIR-P<sub>xyIA</sub></i> mutagenesis (to eliminate the 1<sup>st</sup> illegal cutting site)</li> <li>Gel electrophoresis of secondary PCR product</li> <li>Gel Purification of secondary PCR product</li> </ul>	<ul style="list-style-type: none"> <li>Digestion of <i>BBa_J04450-pSB1A2</i> with EcoR I and Pst I</li> <li>Dephosphorylation of pSB1A2 digestion product</li> <li>Gel electrophoresis of digestion product</li> <li>Gel purification of pSB1A2</li> </ul>	<ul style="list-style-type: none"> <li>Digestion of <i>xyIR-P<sub>xyIA</sub></i> PCR mutagenesis product with XbaI I (to check the mutagenesis)</li> <li>Gel electrophoresis of digestion product</li> </ul>	<ul style="list-style-type: none"> <li>Digestion of <i>xyIR-P<sub>xyIA</sub></i> with EcoR I and Pst I</li> <li>Digestion clean up of <i>xyIR-P<sub>xyIA</sub></i></li> <li>Ligation of <i>xyIR-P<sub>xyIA</sub></i> and <i>pSB1A2</i></li> <li>Transformation of ligation product</li> <li>Digestion of <i>BBa_J04450-pSB1C3</i> with XbaI I and Pst I</li> <li>Dephosphorylation of <i>pSB1C3</i> digestion product</li> <li>Gel electrophoresis of</li> <li>Gel purification of</li> </ul>	<ul style="list-style-type: none"> <li>Check of plate with <i>xyRI-P<sub>xyIA</sub>-pSB1A2</i> transformed E.Coli</li> <li>Synthesis of <i>pTms</i> by direct annealing and extension of oligos</li> <li>Gel electrophoresis of PCR product</li> <li>PCR clean up of <i>pTms</i></li> </ul>

				<a href="#">pSB1C3</a>	
Result	. Got secondary PCR product for <a href="#">xyIR-P<sub>xyIA</sub></a> mutagenesis	. Got linear <a href="#">pSB1A2</a> digested with EcoR I and Pst I	. The 1 <sup>st</sup> mutagenesis of <a href="#">xyRI-P<sub>xyIA</sub></a> had been successfully done	. Got linear <a href="#">pSB1C3</a> digested with XbaI I and Pst I	. Got <a href="#">pTms</a> synthetic product
Discussion					
Remark					

## Week 4 (2012.7.2-2012.7.6)

**Key Words:** [R.G.T-pSB1C3](#); [xyIR-P<sub>xyIA</sub>-pSB1A2](#); [pTms-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>. Inoculation of E.Coli transformed with <a href="#">xyIR-P<sub>xyIA</sub> (1)-pSB1A2</a></li> <li>. Digestion of <a href="#">pTms</a> with XbaI I and Pst I</li> <li>. Ligation of <a href="#">pTms</a> and pSB1C3</li> <li>. Transformation of ligation product</li> </ul>	<ul style="list-style-type: none"> <li>. Check of plate with <a href="#">pTms-pSB1C3</a> transformed E.Coli</li> <li>. Plasmid extraction of <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a> for digestion confirmation</li> <li>. Digestion confirmation of <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a></li> <li>. Gel electrophoresis of</li> </ul>	<ul style="list-style-type: none"> <li>. Check of plate with <a href="#">R.G.T-pSB1C3</a> transformed E.Coli</li> <li>. Inoculation of E.Coli transformed with <a href="#">R.G.T-pSB1C3</a></li> <li>. Synthesis of <a href="#">pTms</a> by direct annealing and extension of oligos using modified protocol</li> <li>. Gel electrophoresis of</li> </ul>	<ul style="list-style-type: none"> <li>. Digestion of <a href="#">pTms</a> with XbaI I and Pst I</li> <li>. Digestion clean up of <a href="#">pTms</a></li> <li>. Ligation of <a href="#">pTms</a> and <a href="#">pSB1C3</a></li> <li>. Transformation of ligation product</li> <li>. Plasmid extraction of E.Coli transformed with</li> </ul>

			<p>digestion product</p> <p>Digestion of</p> <ul style="list-style-type: none"> <li><a href="#">xyIR-PxyIA-pSB1A2</a></li> </ul> <p>with Xba I</p> <ul style="list-style-type: none"> <li>Gel electrophoresis of digestion product</li> <li>Ligation of <a href="#">R.G.T</a> and <a href="#">pSB1C3</a></li> <li>Transformation of ligation product</li> </ul>	<p>synthetic product</p> <ul style="list-style-type: none"> <li>Gel purification of <a href="#">pTms</a></li> <li>Digestion of <a href="#">BBa_J04450-pSB1C3</a> with Xba I and Pst I</li> <li>Gel electrophoresis of digestion product</li> <li>Gel purification of <a href="#">pSB1C3</a></li> </ul>	<p><a href="#">R.G.T-pSB1C3</a></p> <ul style="list-style-type: none"> <li>Digestion confirmation of <a href="#">R.G.T-pSB1C3</a></li> <li>Gel electrophoresis of digestion product</li> </ul>
Result			<ul style="list-style-type: none"> <li>For constructing <a href="#">pTms-pSB1C3</a>, no colony showed on the plate</li> <li><a href="#">xyIR-PxyIA-pSB1A2</a> Was successfully constructed</li> </ul>	<ul style="list-style-type: none"> <li>Got new gel purified <a href="#">pTms</a> synthetic product</li> <li>Got <a href="#">pSB1C3</a> digested with Xba I and Pst I</li> </ul>	<ul style="list-style-type: none"> <li>For constructing <a href="#">R.G.T-pSB1C3</a> All the picked colonies showed negative confirmation result</li> </ul>
Discussion			<p>For constructing <a href="#">pTms-pSB1C3</a></p> <ol style="list-style-type: none"> <li>There may be some problem with the previous made linear <a href="#">pSB1C3</a></li> <li>non-specific product appeared in <a href="#">pTms</a></li> </ol>		

			synthesis product may interfere with the experiment		
Remark					

## Week 5 (2012.7.9-2012.7.13)

**Key Words:** [xyIR-P<sub>xyIA</sub>-R.G.T-pSB1A2](#); [pTms-pSB1C3](#); [pTG262](#)

	<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>Check of plate with <a href="#">pTms-pSB1C3</a> transformed E.Coli</li> <li>Digestion of <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a> with Spe I and pst I</li> <li>Gel electrophoresis check of digestion product</li> <li>Digestion clean up of <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a></li> <li>Digestion of R.G.T with Xba I and Pst I</li> <li>Digestion clean up of digestion product</li> </ul>	<ul style="list-style-type: none"> <li>Check of plate with <a href="#">R.G.T-xyIR-P<sub>xyIA</sub>-pSB1A2</a> transformed E.Coli</li> <li>Inoculation of E.Coli transformed with <a href="#">R.G.T-xyIR-P<sub>xyIA</sub>-pSB1A2</a></li> <li>Transformation of <a href="#">pTG262</a> into E.Coli</li> </ul>	<ul style="list-style-type: none"> <li>Check of plate with <a href="#">pTG262</a> transformed E.Coli</li> <li>Plasmid extraction of <a href="#">RBS(B)-BBa_E0840</a> - <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a> for digestion confirmation</li> <li>Digestion confirmation of <a href="#">RBS(B)-BBa_E0840</a> - <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a></li> <li>Gel electrophoresis of digestion product</li> </ul>	<ul style="list-style-type: none"> <li>Transformation of <a href="#">pTG262</a> into E.Coli</li> <li>Synthesis of <a href="#">pTms</a> using adjusted protocol</li> <li>Gel electrophoresis of <a href="#">pTms</a> synthetic product</li> <li>Gel purification of synthetic <a href="#">pTms</a> product</li> </ul>	<ul style="list-style-type: none"> <li>Check of plate with <a href="#">pTG262</a> transformed E.Coli</li> <li>Digestion of <a href="#">pTms</a> by Xba I and Pst I</li> <li>Digestion clean up of <a href="#">pTms</a></li> <li>Ligation of <a href="#">pTms</a> with <a href="#">pSB1C3</a></li> <li>Transformation of ligation product</li> </ul>

	<ul style="list-style-type: none"> <li>Ligation of R.G.T and <a href="#">xylR-P<sub>xylA</sub>-pSB1A2</a></li> <li>Transformation of ligation product</li> </ul>		<ul style="list-style-type: none"> <li>Annealing of the two oligos for synthesizing pTms</li> <li>Ethanol precipitation of annealing product</li> <li>Extension of annealing product</li> <li>Gel electrophoresis of extension product</li> </ul>		
Result	<ul style="list-style-type: none"> <li>For constructing <a href="#">pTms-pSB1C3</a>, no colony showed on the plate</li> </ul>		<ul style="list-style-type: none"> <li>For Transformation of pTG262, no colony showed on the plate</li> <li>Digestion confirmation of <a href="#">R.G.T-xylR-P<sub>xylA</sub>-pSB1A2</a> yields negative result</li> <li>Still got too many non-specific product for pTms extension</li> </ul>	<ul style="list-style-type: none"> <li>Got new gel purified pTms synthetic product</li> </ul>	<ul style="list-style-type: none"> <li>Got colonies on the plate with pTG262 transformed E.Coli</li> </ul>
Discussion	The purified pTms should be fine for ligation, better to do the experiment again with every step carefully checked to figure out the				

	problem				
Remark					

## Week 6 (2012.7.16-2012.7.20)

### Key Words: R.G.T-pSB1C3; pTms-pSB1C3; pTG262

	<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	<ul style="list-style-type: none"> <li>. Check of plate with pTms-pSB1C3 transformed E.Coli</li> <li>. Gel electrophoresis of pTms gel purification product</li> <li>. Digestion of R.G.T with Xba I and Pst I</li> <li>. Digestion clean up of R.G.T</li> <li>. Inoculation of E.Coli transformed with pTG262</li> </ul>	<ul style="list-style-type: none"> <li>. Plasmid extraction of pTG262</li> <li>. Digestion of pTG262 with</li> <li>. Gel electrophoresis of digestion product</li> <li>. Synthesis of pTms using adjusted protocol</li> <li>. Gel electrophoresis of pTms synthetic product</li> <li>. Gel purification of synthetic pTms product</li> <li>. Gel electrophoresis of pTms gel purification product</li> </ul>	<ul style="list-style-type: none"> <li>. Digestion of pTG262 with new enzyme</li> <li>. Gel electrophoresis of digestion product</li> <li>. Gel electrophoresis of pTms gel purification product using EB</li> <li>. Digestion of R.G.T by Xba I and Pst I</li> <li>. Digestion clean up of R.G.T</li> <li>. Ligation of R.G.T with pSB1C3</li> </ul>	<ul style="list-style-type: none"> <li>. Check of plate with R.G.T-pSB1C3 transformed E.Coli</li> <li>. Inoculation of E.Coli transformed with R.G.T-pSB1C3</li> <li>. Digestion confirmation of pTG262 with EcoR I, Xba I and Pst I</li> <li>. Synthesis of pTms using adjusted protocol</li> </ul>	<ul style="list-style-type: none"> <li>. Digestion of pTms by Xba I and Pst I</li> <li>. Phenol chloroform extraction of pTms</li> <li>. Ligation of pTms with pSB1C3</li> <li>. Transformation of ligation product</li> <li>. Plasmid extraction of R.G.T-pSB1C3 for digestion confirmation</li> <li>. Digestion confirmation of R.G.T-pSB1C3</li> <li>. Gel electrophoresis of</li> </ul>

			<ul style="list-style-type: none"> <li>Transformation of ligation product</li> </ul>	<ul style="list-style-type: none"> <li>Gel electrophoresis of <a href="#">pTms</a> synthetic product</li> <li>Phenol Chloroform extraction of synthetic <a href="#">pTms</a> product</li> <li>Gel electrophoresis of <a href="#">pTms</a> extraction product</li> </ul>	digestion product
Result	<ul style="list-style-type: none"> <li>For constructing <a href="#">pTms</a>-<a href="#">pSB1C3</a>, no colonies showed on the plate; No band showed on the gel after gel purification</li> </ul>	<ul style="list-style-type: none"> <li>For digestion of <a href="#">pTG262</a>, unexpected band pattern showed on the gel</li> <li>For synthesizing <a href="#">pTms</a>, no products showed on the gel after gel purification though suggested by nanodrop, there were some amount of DNA</li> </ul>	<ul style="list-style-type: none"> <li>For digestion of <a href="#">pTG262</a>, unexpected band pattern showed on the gel which was the same as the previous one</li> <li>For <a href="#">pTms</a>, very dark band showed at the expected size when the gel was stained with EB, which meant that gel purification had yielded poor recovery</li> </ul>	<ul style="list-style-type: none"> <li>A ^ illegal cutting site exists on <a href="#">pTG262</a></li> <li>Got large amount of <a href="#">pTms</a> synthetic product with little non-specific DNA</li> </ul>	<ul style="list-style-type: none"> <li><a href="#">R.G.T-pSB1C3</a> was successfully constructed</li> </ul>
Discussion		<p>The problem for <a href="#">pTG262</a> could be caused by poor enzyme activity, illegal cutting site or even poor technique, so better to do the same experiment again.</p>	<p>There could be some illegal cutting site on the shipped <a href="#">pTG262</a></p>	<p>Since it's hard to locate the illegal cutting site on the vector, we decided to use <a href="#">pDG1661</a> instead</p>	

		The problem for pTms could be that 0.01% SYBR Safe is not sensitive enough to detect small amount of DNA		
Remark	About the enzymes related to pTG262			

## Week 7 (2012.7.23-2012.7.27)

**Key Words:** pTms-pSB1C3; xyIR-P<sub>xyIA</sub> mutagenesis; ydcD; ydcE

	<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>. Check of plate with pTms-pSB1C3 transformed E.Coli</li> <li>. Inoculation of E.Coli transformed with pTms-pSB1C3</li> <li>. Primary PCR for xyIR-P<sub>xyIA</sub> mutagenesis (to eliminate the 2<sup>nd</sup> illegal cutting site)</li> </ul>	<ul style="list-style-type: none"> <li>. Plasmid extraction of pTms-pSB1C3 for digestion confirmation</li> <li>. Digestion confirmation of pTms-pSB1C3</li> <li>. Gel electrophoresis of digestion product</li> <li>. Inoculation of E.Coli transformed with pTms-pSB1C3</li> </ul>	<ul style="list-style-type: none"> <li>. Plasmid extraction of pTms-pSB1C3 for digestion confirmation</li> <li>. Digestion confirmation of pTms-pSB1C3</li> <li>. Gel electrophoresis of digestion product</li> <li>. Inoculation of E.Coli transformed with pTms-pSB1C3</li> </ul>	<ul style="list-style-type: none"> <li>. Check of plate with xyIR-P<sub>xyIA</sub>-pSB1A2 transformed E.Coli</li> <li>. Inoculation of E.Coli transformed with xyIR-P<sub>xyIA</sub>-pSB1A2</li> <li>. Plasmid extraction of pTms-pSB1C3 for digestion confirmation</li> <li>. Digestion</li> </ul>	<ul style="list-style-type: none"> <li>. PCR ydcD and ydcE from shipped plasmid</li> <li>. Digestion of ydcD and ydcE with XbaI and PstI</li> <li>. Ligation of ydcD and ydcE with pSB1C3 respectively</li> <li>. Transformation of ligation product</li> </ul>

	<ul style="list-style-type: none"> <li>. Gel electrophoresis of primary PCR product</li> <li>. Gel Purification of primary PCR product</li> </ul>	<ul style="list-style-type: none"> <li>. Secondary PCR for <i>xyIR-PxyIA</i> mutagenesis (to eliminate the 2<sup>nd</sup> illegal cutting site)</li> <li>. Gel electrophoresis of secondary PCR product</li> <li>. Gel Purification of secondary PCR product</li> </ul>	<ul style="list-style-type: none"> <li>. Digestion of <i>xyIR-PxyIA</i> with to confirm the mutagenesis</li> <li>. Digestion of <i>xyIR-PxyIA</i> with EcoR I and Pst I</li> <li>. Ligation of <i>xyIR-PxyIA</i> and <i>pSB1A2</i></li> <li>. Transformation of ligation product</li> </ul>	<p>confirmation of <i>pTms-pSB1C3</i></p> <ul style="list-style-type: none"> <li>. Gel electrophoresis of digestion product</li> </ul>	<ul style="list-style-type: none"> <li>. Plasmid extraction of <i>xyIR-PxyIA-pSB1A2</i> for digestion confirmation</li> <li>. Digestion confirmation of <i>xyIR-PxyIA-pSB1A2</i></li> <li>. Digestion of <i>xyIR-PxyIA-pSB1A2</i> with to confirm the mutagenesis</li> <li>. Digestion of <i>BBa_J04450-pSB1C3</i> with XbaI and Pst I</li> <li>. Dephosphorylation of <i>pSB1C3</i> digestion product</li> <li>. Gel electrophoresis of</li> <li>. Gel purification of <i>pSB1C3</i></li> </ul>
Result	<ul style="list-style-type: none"> <li>. Got primary PCR product for <i>xyIR-PxyIA</i> mutagenesis</li> </ul>	<ul style="list-style-type: none"> <li>. For constructing <i>pTms-pSB1C3</i> All the picked colonies showed negative confirmation result</li> <li>. Got secondary PCR</li> </ul>	<ul style="list-style-type: none"> <li>. For constructing <i>pTms-pSB1C3</i> All the picked colonies showed negative confirmation result</li> <li>. the 2<sup>nd</sup> point mutation</li> </ul>	<p>. <i>pTms-pSB1C3</i> was successfully constructed</p>	<ul style="list-style-type: none"> <li>. Got linear <i>pSB1C3</i> digested with XbaI and Pst I</li> <li>. <i>xyIR-PxyIA-pSB1A2</i> was successfully made</li> </ul>

		product for <a href="#">xyIR-P<sub>xyIA</sub></a> of <a href="#">xyIR-P<sub>xyIA</sub></a> was successfully done		
Discussion				
Remark	<a href="#">xyIR-P<sub>xyIA</sub></a> Point mutation enzyme			

## Week 8 (2012.7.30-2012.8.3)

### Key Words: [xyIR-P<sub>xyIA</sub>](#) mutagenesis; [ydcD-pSB1C3](#); [ydcE-pSB1C3](#)

	<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	<ul style="list-style-type: none"> <li>. Check of plate with <a href="#">ydcD-pSB1C3</a> transformed E.Coli</li> <li>. Inoculation of E.Coli transformed with <a href="#">ydcD-pSB1C3</a></li> <li>. Check of plate with <a href="#">ydcE-pSB1C3</a> transformed E.Coli</li> <li>. Inoculation of E.Coli transformed with <a href="#">ydcE-pSB1C3</a></li> </ul>	<ul style="list-style-type: none"> <li>. Plasmid extraction of <a href="#">ydcD-pSB1C3</a> for digestion confirmation</li> <li>. Digestion confirmation of <a href="#">ydcD-pSB1C3</a></li> <li>. Gel electrophoresis of digestion product</li> <li>. Plasmid extraction of <a href="#">ydcE-pSB1C3</a> for digestion confirmation</li> <li>. Digestion confirmation of <a href="#">ydcE-pSB1C3</a></li> </ul>	<ul style="list-style-type: none"> <li>. Digestion of <a href="#">BBa_J04450-pSB1A2</a> with EcoR I and Pst I</li> <li>. Dephosphorylation of pSB1A2 digestion product</li> <li>. Gel electrophoresis of digestion product</li> <li>. Gel purification of <a href="#">pSB1A2</a></li> <li>. Digestion of <a href="#">xyIR-P<sub>xyIA</sub></a> with to confirm the mutagenesis</li> </ul>	<ul style="list-style-type: none"> <li>. Check of plate with <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a> transformed E.Coli</li> <li>. Inoculation of E.Coli transformed with <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a></li> </ul>	<ul style="list-style-type: none"> <li>. Plasmid extraction of <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a> for digestion confirmation</li> <li>. Digestion confirmation of <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a></li> <li>. Digestion of <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a> with to confirm the mutagenesis</li> </ul>

	<ul style="list-style-type: none"> <li>Primary PCR for <i>xyIR-PxyIA</i> mutagenesis (to eliminate the 3<sup>rd</sup> illegal cutting site)</li> <li>Gel electrophoresis of primary PCR product</li> <li>Gel Purification of primary PCR product</li> </ul>	<ul style="list-style-type: none"> <li>Gel electrophoresis of digestion product</li> <li>Secondary PCR for <i>xyIR-PxyIA</i> mutagenesis (to eliminate the 3<sup>rd</sup> illegal cutting site)</li> <li>Gel electrophoresis of secondary PCR product</li> <li>Gel Purification of secondary PCR product</li> </ul>	<ul style="list-style-type: none"> <li>Digestion of <i>xyIR-PxyIA</i> with EcoR I and Pst I</li> <li>Ligation of <i>xyIR-PxyIA</i> and pSB1A2</li> </ul>		
Result	<ul style="list-style-type: none"> <li>Got primary PCR product for <i>xyIR-PxyIA</i> mutagenesis</li> </ul>	<ul style="list-style-type: none"> <li><i>ydcD-pSB1C3</i> was successfully constructed</li> <li><i>ydcE-pSB1C3</i> was successfully constructed</li> <li>Got secondary PCR product for <i>xyIR-PxyIA</i> mutagenesis</li> </ul>	<ul style="list-style-type: none"> <li>Got linear pSB1A2 digested with EcoR I and Pst I</li> <li>the 3<sup>rd</sup> point mutation of <i>xyIR-PxyIA</i> was successfully done</li> </ul>		<ul style="list-style-type: none"> <li><i>xyIR-PxyIA-pSB1A2</i> was successfully made</li> </ul>
Discussion					
Remark					

Week 9 (2012.8.6-2012.8.10)

Key Words: Sequencing

	<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					
Result					
Discussion					
Remark					

## Week 10 (2012.8.13-2012.8.17)

**Key Words:** [pTms-ydcD-pSB1C3](#); [xyIR-P<sub>xyIA</sub>-ydcE-pSB1A2](#); [pTms-E0240-pSB1C3](#)

	<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		<ul style="list-style-type: none"> <li>Digestion of <a href="#">pTms-pSB1C3</a> and <a href="#">xyIR-P<sub>xyIA</sub>-pSB1A2</a> with Spe I and Pst I</li> <li>Dephosphorylation of digestion product</li> <li>Digestion clean up of <a href="#">pTms-pSB1C3</a></li> <li>Digestion of <a href="#">ydcD-pSB1C3</a> and <a href="#">ydcE-pSB1C3</a> with Xba I</li> </ul>	<ul style="list-style-type: none"> <li>Digestion of <a href="#">ydcD-pSB1C3</a>, <a href="#">ydcE-pSB1C3</a> and <a href="#">E0240</a> with Xba I and Pst I</li> <li>Gel electrophoresis of digestion product</li> <li>Gel purification of <a href="#">ydcD</a>, <a href="#">ydcE</a> and <a href="#">E0240</a></li> <li>Ligation of <a href="#">E0240</a> and <a href="#">pTms-pSB1C3</a>, <a href="#">ydcD</a> and <a href="#">pTms-pSB1C3</a>, <a href="#">ydcE</a> and</li> </ul>	<ul style="list-style-type: none"> <li>Check of plate with <a href="#">pTms-E0240-pSB1C3</a>, <a href="#">pTms-ydcD-pSB1C3</a>, <a href="#">xyIR-P<sub>xyIA</sub>-ydcE-pSB1A2</a> transformed E.Coli</li> <li>Inoculation of E.Coli transformed with <a href="#">pTms-E0240-pSB1C3</a>, <a href="#">xyIR-P<sub>xyIA</sub>-ydcE-pSB1A2</a></li> </ul>	<ul style="list-style-type: none"> <li>Plasmid extraction of <a href="#">pTms-E0240-pSB1C3</a>, <a href="#">xyIR-P<sub>xyIA</sub>-ydcE-pSB1A2</a></li> <li>Digestion confirmation of <a href="#">pTms-E0240-pSB1C3</a>, <a href="#">xyIR-P<sub>xyIA</sub>-ydcE-pSB1A2</a></li> <li>Digestion of <a href="#">ydcD-pSB1C3</a> and <a href="#">ydcE-pSB1C3</a> with Xba I and Pst I</li> <li>Gel electrophoresis of</li> </ul>

		<p>and Pst I</p> <ul style="list-style-type: none"> <li>Gel electrophoresis of digestion product</li> <li>Gel purification of <i>ydcD</i> and <i>ydcE</i></li> </ul>	<p><i>xyIR-P<sub>xyIA</sub>-pSB1A2</i></p> <ul style="list-style-type: none"> <li>Transformation of ligation product</li> </ul>		<p>digestion product</p> <ul style="list-style-type: none"> <li>Gel purification of <i>ydcD</i> and <i>ydcE</i></li> </ul>
Result		<ul style="list-style-type: none"> <li>Got linear <i>pTms-pSB1C3</i> and <i>xyIR-P<sub>xyIA</sub>-pSB1A2</i> cut with Spe I and Pst I</li> <li>Got little recovery for gel purification</li> </ul>		<ul style="list-style-type: none"> <li>For constructing <i>pTms-ydcD-pSB1C3</i> no colonies showed on the plate</li> </ul>	<ul style="list-style-type: none"> <li><i>pTms-E0240-pSB1C3</i> was successfully constructed</li> <li>For constructing <i>xyIR-P<sub>xyIA</sub>-ydcE-pSB1A2</i>, all the picked colonies showed negative confirmation result</li> <li>Got <i>ydcD</i> and <i>ydcE</i> cut with Xba I and Pst I</li> </ul>
Discussion					
Remark					

Week 11 (2012.8.20-2012.8.24)

Key Words: *pTms-ydcD-pSB1C3*; *xyIR-P<sub>xyIA</sub>-pSB1A2*; *pTms-E0240-pSB3K3*; bacterial M9 medium

	Monday	Tuesday	Wednesday	Thursday	Friday
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	<b>2012.8.20</b>	<b>2012.8.21</b>	<b>2012.8.22</b>	<b>2012.8.23</b>	<b>2012.8.24</b>
Work done	<ul style="list-style-type: none"> <li>. Digestion of <a href="#">pTms-E0240-pSB1C3</a> with EcoR I and Pst I</li> <li>. Digestion clean up of <a href="#">pTms-E0240-pSB1C3</a></li> <li>. Digestion of <a href="#">BBa_J0445-pSB3K3</a> with EcoR I and Pst I</li> <li>. Dephosphorylation of digestion product</li> <li>. Gel purification of <a href="#">pSB3K3</a></li> <li>. Ligation of <a href="#">pTms-E0240</a> and <a href="#">pSB3K3</a>, <a href="#">ydcD</a> and <a href="#">pTms-PSB1C3</a>, <a href="#">ydcE</a> and <a href="#">xyIR-PxyIA-pSB1A2</a></li> <li>. Transformation of ligation product</li> </ul>	<ul style="list-style-type: none"> <li>. Check of plate with <a href="#">pTms-E0240-pSB3K3</a>, <a href="#">pTms-ydcD-pSB1C3</a>, <a href="#">xyIR-PxyIA-ydcE-pSB1A2</a> transformed E.Coli</li> <li>. Inoculation of E.Coli transformed with <a href="#">pTms-E0240-pSB3K3</a>, <a href="#">xyIR-PxyIA-ydcE-pSB1A2</a>, <a href="#">pTms-ydcD-pSB1C3</a></li> </ul>	<ul style="list-style-type: none"> <li>. Plasmid extraction of <a href="#">pTms-E0240-pSB3K3</a>, <a href="#">xyIR-PxyIA-ydcE-pSB1A2</a>, <a href="#">pTms-ydcD-pSB1C3</a></li> <li>. Digestion confirmation of <a href="#">pTms-E0240-pSB3K3</a>, <a href="#">xyIR-PxyIA-ydcE-pSB1A2</a>, <a href="#">pTms-ydcD-pSB1C3</a></li> <li>. Making of m9 salt and bacterial M9 medium for characterization</li> <li>. Inoculation of E.Coli carrying <a href="#">pTms-E0240-pSB3K3</a> and <a href="#">I20260-PSB3K3</a> with M9 medium</li> </ul>	<ul style="list-style-type: none"> <li>. Check of inoculation product</li> <li>. Making of new bacterial M9 medium with supplement using adjusted recipe</li> </ul>	
Result			<ul style="list-style-type: none"> <li>. <a href="#">pTms-E0240-pSB3K3</a>, <a href="#">xyIR-PxyIA-ydcE-pSB1A2</a>,</li> </ul>	<ul style="list-style-type: none"> <li>. Got no bacteria in all the inoculation product using M9 medium</li> </ul>	Got clear M9 medium without precipitation

			pTms-ydcD-pSB1C3 were successfully constructed	. Got precipitation in the newly made M9 medium after autoclave	
Discussion				The M9 salt should not be mixed with MgSO <sub>4</sub> and CaCl <sub>2</sub> before autoclave	
Remark: Before this week, separate the different construct					

## Week 12 (2012.8.27-2012.8.31)

**Key Words: [xyIR-P<sub>xyIA</sub>-pSB1C2](#); [xyIR-P<sub>xyIA</sub>-ydcE-pSB1C2](#); [pTms-E0240-pSB3K3](#) growth curve; [I20260-pSB3K3](#) growth curve; [pTms](#) characterization**

	<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	. Inoculation of E.Coli carrying pTms-E0240-pSB3K3, I20260-PSB3K3 and no construct with M9 medium	. Check of plate with <a href="#">xyIR-P<sub>xyIA</sub>-ydcE-pSB1C3</a> transformed E.Coli, <a href="#">xyIR-P<sub>xyIA</sub>-pSB1C3</a> transformed E.Coli . Inoculation of E.Coli transformed with <a href="#">xyIR-P<sub>xyIA</sub>-ydcE-pSB1C3</a> , <a href="#">xyIR-P<sub>xyIA</sub>-pSB1C3</a>	. Plasmid extraction of <a href="#">xyIR-P<sub>xyIA</sub>-ydcE-pSB1C3</a> , <a href="#">xyIR-P<sub>xyIA</sub>-pSB1C3</a> . Digestion confirmation of <a href="#">xyIR-P<sub>xyIA</sub>-ydcE-pSB1C3</a> , <a href="#">xyIR-P<sub>xyIA</sub>-pSB1C3</a>	. Growth curve measurement of E.Coli carrying pTms-E0240-pSB3K3, E.Coli carrying I20260-PSB3K3 in M9 medium	. Dilution of inoculation product to lag phase . Culture of the dilution product to mid-log phase . Characterization of pTms

	<ul style="list-style-type: none"> <li>Digestion of <i>xyIR-P<sub>xyIA</sub>-pSB1A2</i>, <i>xyIR-P<sub>xyIA</sub>-ydcE-pSB1A2</i> with EcoR I and Pst I</li> <li>Gel electrophoresis of digestion product</li> <li>Gel purification of <i>xyIR-P<sub>xyIA</sub></i> and <i>xyIR-P<sub>xyIA</sub>-ydcE</i></li> <li>Ligation of <i>xyIR-P<sub>xyIA</sub></i> and <i>pSB1C3</i>, <i>xyIR-P<sub>xyIA</sub>-ydcE</i> and <i>pSB1C3</i></li> <li>Transformation of ligation product</li> </ul>	<p>E.Coli transformed with <i>xyIR-P<sub>xyIA</sub>-pSB1C3</i></p> <ul style="list-style-type: none"> <li>Inoculation of E.Coli carrying <i>pTms-E0240-pSB3K3</i>, E.Coli carrying <i>I20260-PSB3K3</i> and E.Coli carrying <i>no construct</i> with M9 medium</li> </ul>	<ul style="list-style-type: none"> <li>Check of inoculation product</li> <li>Inoculation of E.Coli carrying <i>pTms-E0240-pSB3K3</i>, E.Coli carrying <i>I20260-PSB3K3</i> and E.Coli carrying <i>no construct</i> with M9 medium</li> </ul>	<ul style="list-style-type: none"> <li>Inoculation of E.Coli carrying <i>pTms-E0240-pSB3K3</i>, E.Coli carrying <i>I20260-PSB3K3</i> and E.Coli carrying <i>no construct</i> with M9 medium</li> </ul>	
Result	<ul style="list-style-type: none"> <li>the growth rate of E.Coli in the M9 medium is rather slow compared to that in LB</li> </ul>		<ul style="list-style-type: none"> <li><i>xyIR-P<sub>xyIA</sub>-ydcE-pSB1C3</i>, <i>xyIR-P<sub>xyIA</sub>-pSB1C3</i> were successfully constructed</li> <li>The growth rate of E.Coli in the new M9 medium is nearly the same as that in LB</li> </ul>	<ul style="list-style-type: none"> <li>The growth curve of <i>pTms-E0240-pSB3K3</i>, <i>I20260-PSB3K3</i> in E.Coli was plotted</li> </ul>	<ul style="list-style-type: none"> <li>Got the first set of data for characterization of <i>pTms</i></li> </ul>
Discussion	The supplement recipe should be changed further for the growth of E.Coli				

Remark				
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## Week 13 (2012.9.3-2012.9.7)

**Key Words: *xyIR-P<sub>xyIA</sub>-ydcE-pTms-ydcD-pSB1C3*; *pTms* characterization;  
*xyIR-P<sub>xyIA</sub>-E0240-pSB1A2* growth curve**

	<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	<ul style="list-style-type: none"> <li>. Inoculation of E.Coli carrying <i>pTms-E0240-pSB3K3</i>, E.Coli carrying <i>I20260-PSB3K3</i> and E.Coli carrying <i>construct</i> with <i>no</i> M9 medium</li> </ul>	<ul style="list-style-type: none"> <li>. Digestion of <i>pTms-ydcD-pSB1C3</i> with EcoR I and XbaI</li> <li>. Dephosphorylation of digestion product</li> <li>. Digestion clean up of <i>pTms-ydcD-pSB1C3</i></li> <li>. Digestion of <i>xyIR-P<sub>xyIA</sub>-ydcE-pSB1C3</i> with EcoR I and Spe I</li> <li>. Electrophoresis of digestion product</li> <li>. Gel purification of <i>xyIR-P<sub>xyIA</sub>-ydcE</i></li> </ul>	<ul style="list-style-type: none"> <li>. Dilution of inoculation product to lag phase</li> <li>. Culture of the dilution product to mid-log phase</li> <li>. Characterization of <i>pTms</i></li> <li>. Inoculation of E.Coli carrying <i>pTms-E0240-pSB3K3</i>, E.Coli carrying <i>I20260-PSB3K3</i> and E.Coli carrying <i>construct</i> with <i>no</i> M9 medium</li> </ul>	<ul style="list-style-type: none"> <li>. Dilution of inoculation product to lag phase</li> <li>. Culture of the dilution product to mid-log phase</li> <li>. Characterization of <i>pTms</i></li> <li>. Inoculation of E.Coli carrying <i>pTms-E0240-pSB3K3</i>, E.Coli carrying <i>I20260-PSB3K3</i> and E.Coli carrying <i>construct</i> with <i>no</i> M9 medium</li> </ul>	<ul style="list-style-type: none"> <li>. Dilution of inoculation product to lag phase</li> <li>. Culture of the dilution product to mid-log phase</li> <li>. Characterization of <i>pTms</i></li> <li>. Inoculation of E.Coli carrying <i>pTms-E0240-pSB3K3</i>, E.Coli carrying <i>I20260-PSB3K3</i> and E.Coli carrying <i>construct</i> with <i>no</i> M9 medium</li> </ul>

		<ul style="list-style-type: none"> <li>Dilution of inoculation product to lag phase</li> <li>Culture of the dilution product to mid-log phase</li> <li>Characterization of pTms</li> <li>Inoculation of E.Coli carrying <a href="#">pTms-E0240-pSB3K3</a>, E.Coli carrying <a href="#">I20260-PSB3K3</a> and E.Coli carrying <a href="#">no construct</a> with M9 medium</li> </ul>	<ul style="list-style-type: none"> <li>Ligation of <a href="#">xyIR-PxyIA-ydcE</a> and <a href="#">pTms-ydcD-pSB1C3</a></li> <li>Transformation of ligation product</li> </ul>	<ul style="list-style-type: none"> <li>Check of plate with <a href="#">xyIR-PxyIA-ydcE-pTms-yd</a> <a href="#">cD-pSB1C3</a> transformed E.Coli</li> </ul>	
Result				For constructing <a href="#">xyIR-PxyIA-ydcE-pTms-yd</a> <a href="#">cD-pSB1C3</a> , no colony shown on the plate	
Discussion					
Remark					

## Week 14 (2012.9.10-2012.9.14)

### Key Words: pTms characterization; Biobrick shipment

	<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	<ul style="list-style-type: none"> <li>. Dilution of inoculation product to lag phase</li> <li>. Culture of the dilution product to mid-log phase</li> <li>. Characterization of pTms</li> </ul>	<ul style="list-style-type: none"> <li>. Dilution of inoculation product to lag phase</li> <li>. Culture of the dilution product to mid-log phase</li> <li>. Characterization of pTms</li> </ul>	<ul style="list-style-type: none"> <li>. Dilution of inoculation product to lag phase</li> <li>. Culture of the dilution product to mid-log phase</li> <li>. Characterization of pTms</li> </ul>	<ul style="list-style-type: none"> <li>. Dilution of inoculation product to lag phase</li> <li>. Culture of the dilution product to mid-log phase</li> <li>. Characterization of pTms</li> </ul>	<ul style="list-style-type: none"> <li>. Dilution of inoculation product to lag phase</li> <li>. Culture of the dilution product to mid-log phase</li> <li>. Characterization of pTms</li> </ul>
Result					
Discussion					
Remark					

## Week 15 (2012.9.17-2012.9.21)

### Key Words: P<sub>xyIA</sub> characterization; Biobrick shipment

	<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					

