

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

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

Key Words: **Pveg-R.G.T-pSB1C3**; **xyIR-P_{xyIA}** mutagenesis

	Monday 2012.6.18	Tuesday 2012.6.19	Wednesday 2012.6.20	Thursday 2012.6.21	Friday 2012.6.22
Work done	<ul style="list-style-type: none"> PCR mutagenesis of BBa_E0840 (to add B.Subtilis RBS) Gel electrophoresis check of R.G.T PCR product PCR clean up of PCR product Digestion of R.G.T with XbaI and Pst I Digestion clean up of R.G.T Inoculation of shipped E.Coli carrying Pveg-pSB1C3 Spreading of shipped E.Coli carrying Pveg-pSB1C3 onto plate 	<ul style="list-style-type: none"> Primary PCR for xyIR-P_{xyIA} mutagenesis (to eliminate the 1st illegal cutting site) Gel electrophoresis of primary PCR product Gel purification of primary PCR product Digestion of BBa_J04450-pSB1C3 with XbaI and Pst I Dephosphorylation of pSB1C3 digestion product Gel electrophoresis of pSB1C3 Gel purification of pSB1C3 	<ul style="list-style-type: none"> Secondary PCR for xyIR-P_{xyIA} mutagenesis (to eliminate the 1st illegal cutting site) Gel electrophoresis of secondary PCR product Ligation of R.G.T and pSB1C3 Transformation of ligation product Ligation of R.G.T and Pveg-pSB1C3 Transformation of ligation product 	<ul style="list-style-type: none"> Check of plate with R.G.T-pSB1C3 transformed E.Coli Check of plate with Pveg-R.G.T-pSB1C3 transformed E.Coli Inoculation of E.Coli transformed with Pveg-R.G.T-pSB1C3 Primary PCR for xyIR-P_{xyIA} mutagenesis (to eliminate the 1st illegal cutting site) Gel electrophoresis of primary PCR product Gel purification of 	

		<ul style="list-style-type: none"> · Digestion of Pveg-pSB1C3 with Xbal I and Pst I · Dephosphorylation of Pveg-pSB1C3 digestion product · Gel electrophoresis of digestion product · Gel Purification of Pveg-pSB1C3 		primary PCR product	
Result	<ul style="list-style-type: none"> · Got R.G.T digested with Xbal I and Pst I 	<ul style="list-style-type: none"> · Got primary PCR product for xyIR-PxyIA mutagenesis · Got linear pSB1C3 digested with Xbal I and Pst I · Got linear Pveg-pSB1C3 digested with Xbal I and Pst I 	<ul style="list-style-type: none"> · Secondary PCR yields no product 	<ul style="list-style-type: none"> · Got bacteria lawn on plate with R.G.T-pSB1C3 transformed E.Coli · Got more primary PCR product for xyIR-PxyIA mutagenesis 	
Discussion			<p>For secondary PCR</p> <ol style="list-style-type: none"> 1. the annealing PCR without primers did not work here 2. The template for secondary PCR was not enough 	<p>For constructing R.G.T-pSB1C3</p> <ol style="list-style-type: none"> 1. the digestion of backbone is not complete 	

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_{xyIA}</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"> Secondary PCR for <i>xyIR-P_{xyIA}</i> mutagenesis (to eliminate the 1st illegal cutting site) Gel electrophoresis of secondary PCR product Gel Purification of secondary PCR product 	<ul style="list-style-type: none"> Digestion of <i>BBa_J04450-pSB1A2</i> with EcoR I and Pst I Dephosphorylation of pSB1A2 digestion product Gel electrophoresis of digestion product Gel purification of <i>pSB1A2</i> 	<ul style="list-style-type: none"> Digestion of <i>xyIR-P_{xyIA}</i> PCR mutagenesis product with XbaI I (to check the mutagenesis) Gel electrophoresis of digestion product 	<ul style="list-style-type: none"> Digestion of <i>xyIR-P_{xyIA}</i> with EcoR I and Pst I Digestion clean up of <i>xyIR-P_{xyIA}</i> Ligation of <i>xyIR-P_{xyIA}</i> and <i>pSB1A2</i> Transformation of ligation product Digestion of <i>BBa_J04450-pSB1C3</i> with XbaI I and Pst I Dephosphorylation of <i>pSB1C3</i> digestion product Gel electrophoresis of Gel purification of 	<ul style="list-style-type: none"> Check of plate with <i>xyRI-P_{xyIA}-pSB1A2</i> transformed E.Coli Synthesis of <i>pTms</i> by direct annealing and extension of oligos Gel electrophoresis of PCR product PCR clean up of <i>pTms</i>

				pSB1C3	
Result	. Got secondary PCR product for xyIR-P _{xyIA} mutagenesis	. Got linear pSB1A2 digested with EcoR I and Pst I	. The 1 st mutagenesis of xyRI-P _{xyIA} had been successfully done	. Got linear pSB1C3 digested with XbaI I and Pst I	. Got pTms synthetic product
Discussion					
Remark					

Week 4 (2012.7.2-2012.7.6)					
Key Words: R.G.T-pSB1C3; xyIR-P _{xyIA} -pSB1A2; pTms-pSB1C3					
	Monday 2012.7.2	Tuesday 2012.7.3	Wednesday 2012.7.4	Thursday 2012.7.5	Friday 2012.7.6
Work done		<ul style="list-style-type: none"> . Inoculation of E.Coli transformed with xyIR-P_{xyIA} (1)-pSB1A2 . Digestion of pTms with XbaI I and Pst I . Ligation of pTms and pSB1C3 . Transformation of ligation product 	<ul style="list-style-type: none"> . Check of plate with pTms-pSB1C3 transformed E.Coli . Plasmid extraction of xyIR-P_{xyIA}-pSB1A2 for digestion confirmation . Digestion confirmation of xyIR-P_{xyIA}-pSB1A2 . Gel electrophoresis of 	<ul style="list-style-type: none"> . Check of plate with R.G.T-pSB1C3 transformed E.Coli . Inoculation of E.Coli transformed with R.G.T-pSB1C3 . Synthesis of pTms by direct annealing and extension of oligos using modified protocol . Gel electrophoresis of 	<ul style="list-style-type: none"> . Digestion of pTms with XbaI I and Pst I . Digestion clean up of pTms . Ligation of pTms and pSB1C3 . Transformation of ligation product . Plasmid extraction of E.Coli transformed with

			<p>digestion product</p> <p>Digestion of</p> <ul style="list-style-type: none"> · xylR-P_{xylA}-pSB1A2 with XbaI I · Gel electrophoresis of digestion product · Ligation of R.G.T and pSB1C3 · Transformation of ligation product 	<p>synthetic product</p> <ul style="list-style-type: none"> · Gel purification of pTms · Digestion of BBa_J04450-pSB1C3 with XbaI I and Pst I · Gel electrophoresis of digestion product · Gel purification of pSB1C3 	<p>R.G.T-pSB1C3</p> <ul style="list-style-type: none"> · Digestion confirmation of R.G.T-pSB1C3 · Gel electrophoresis of digestion product
Result			<ul style="list-style-type: none"> · For constructing pTms-pSB1C3, no colony showed on the plate · xylR-P_{xylA}-pSB1A2 Was successfully constructed 	<ul style="list-style-type: none"> · Got new gel purified pTms synthetic product · Got pSB1C3 digested with XbaI I and Pst I 	<ul style="list-style-type: none"> · For constructing R.G.T-pSB1C3 All the picked colonies showed negative confirmation result
Discussion			<p>For constructing pTms-pSB1C3</p> <ol style="list-style-type: none"> 1. There may be some problem with the previous made linear pSB1C3 2. non-specific product appeared in pTms 		

			synthesis product may interfere with the experiment		
Remark					

Week 5 (2012.7.9-2012.7.13)					
Key Words: xylR-P_{xylA}-R.G.T-pSB1A2 ; pTms-pSB1C3 ; pTG262					
	Monday 2012.7.9	Tuesday 2012.7.10	Wednesday 2012.7.11	Thursday 2012.7.12	Friday 2012.7.13
Work done	<ul style="list-style-type: none"> Check of plate with pTms-pSB1C3 transformed E.Coli Digestion of xylR-P_{xylA}-pSB1A2 with Spe I and pst I Gel electrophoresis check of digestion product Digestion clean up of xylR-P_{xylA}-pSB1A2 Digestion of R.G.T with Xba I and Pst I Digestion clean up of digestion product 	<ul style="list-style-type: none"> Check of plate with R.G.T-xylR-P_{xylA}-pSB1A2 transformed E.Coli Inoculation of E.Coli transformed with R.G.T-xylR-P_{xylA}-pSB1A2 Transformation of pTG262 into E.Coli 	<ul style="list-style-type: none"> Check of plate with pTG262 transformed E.Coli Plasmid extraction of RBS(B)-BBa_E0840 - xylR-P_{xylA}-pSB1A2 for digestion confirmation Digestion confirmation of RBS(B)-BBa_E0840 - xylR-P_{xylA}-pSB1A2 Gel electrophoresis of digestion product 	<ul style="list-style-type: none"> Transformation of pTG262 into E.Coli Synthesis of pTms using adjusted protocol Gel electrophoresis of pTms synthetic product Gel purification of synthetic pTms product 	<ul style="list-style-type: none"> Check of plate with pTG262 transformed E.Coli Digestion of pTms by Xba I and Pst I Digestion clean up of pTms Ligation of pTms with pSB1C3 Transformation of ligation product

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

	problem				
Remark					

Week 6 (2012.7.16-2012.7.20)					
Key Words: R.G.T-pSB1C3; pTms-pSB1C3; pTG262					
	Monday 2012.7.16	Tuesday 2012.7.17	Wednesday 2012.7.18	Thursday 2012.7.19	Friday 2012.7.20
Work done	<ul style="list-style-type: none"> Check of plate with pTms-pSB1C3 transformed E.Coli Gel electrophoresis of pTms gel purification product Digestion of R.G.T with Xbal I and Pst I Digestion clean up of R.G.T Inoculation of E.Coli transformed with pTG262 	<ul style="list-style-type: none"> Plasmid extraction of pTG262 Digestion of pTG262 with Gel electrophoresis of digestion product Synthesis of pTms using adjusted protocol Gel electrophoresis of pTms synthetic product Gel purification of synthetic pTms product Gel electrophoresis of pTms gel purification product 	<ul style="list-style-type: none"> Digestion of pTG262 with new enzyme Gel electrophoresis of digestion product Gel electrophoresis of pTms gel purification product using EB Digestion of R.G.T by Xbal I and Pst I Digestion clean up of R.G.T Ligation of R.G.T with pSB1C3 	<ul style="list-style-type: none"> Check of plate with R.G.T-pSB1C3 transformed E.Coli Inoculation of E.Coli transformed with R.G.T-pSB1C3 Digestion confirmation of pTG262 with EcoR I, Xbal I and Pst I Synthesis of pTms using adjusted protocol 	<ul style="list-style-type: none"> Digestion of pTms by Xbal I and Pst I Phenol chloroform extraction of pTms Ligation of pTms with pSB1C3 Transformation of ligation product Plasmid extraction of R.G.T-pSB1C3 for digestion confirmation Digestion confirmation of R.G.T-pSB1C3 Gel electrophoresis of

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

		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; xylR-P _{xylA} mutagenesis; ydcD; ydcE					
	Monday 2012.7.23	Tuesday 2012.7.24	Wednesday 2012.7.25	Thursday 2012.7.26	Friday 2012.7.27
Work done	<ul style="list-style-type: none"> Check of plate with pTms-pSB1C3 transformed E.Coli Inoculation of E.Coli transformed with pTms-pSB1C3 Primary PCR for xylR-P_{xylA} mutagenesis (to eliminate the 2nd illegal cutting site) 	<ul style="list-style-type: none"> Plasmid extraction of pTms-pSB1C3 for digestion confirmation Digestion confirmation of pTms-pSB1C3 Gel electrophoresis of digestion product Inoculation of E.Coli transformed with pTms-pSB1C3 	<ul style="list-style-type: none"> Plasmid extraction of pTms-pSB1C3 for digestion confirmation Digestion confirmation of pTms-pSB1C3 Gel electrophoresis of digestion product Inoculation of E.Coli transformed with pTms-pSB1C3 	<ul style="list-style-type: none"> Check of plate with xylR-P_{xylA}-pSB1A2 transformed E.Coli Inoculation of E.Coli transformed with xylR-P_{xylA}-pSB1A2 Plasmid extraction of pTms-pSB1C3 for digestion confirmation Digestion 	<ul style="list-style-type: none"> PCR ydcD and ydcE from shipped plasmid Digestion of ydcD and ydcE with XbaI and Pst I Ligation of ydcD and ydcE with pSB1C3 respectively Transformation of ligation product

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

		product for xylR-P_{xylA} of xylR-P_{xylA} was		
Discussion		mutagenesis	successfully done	
Remark	xylR-P_{xylA} Point mutation enzyme			

Week 8 (2012.7.30-2012.8.3)					
Key Words: xylR-P_{xylA} mutagenesis; ydcD-pSB1C3 ; ydcE-pSB1C3					
	Monday 2012.7.30	Tuesday 2012.7.31	Wednesday 2012.8.1	Thursday 2012.8.2	Friday 2012.8.3
Work done	<ul style="list-style-type: none"> Check of plate with ydcD-pSB1C3 transformed E.Coli Inoculation of E.Coli transformed with ydcD-pSB1C3 Check of plate with ydcE-pSB1C3 transformed E.Coli Inoculation of E.Coli transformed with ydcE-pSB1C3 	<ul style="list-style-type: none"> Plasmid extraction of ydcD-pSB1C3 for digestion confirmation Digestion confirmation of ydcD-pSB1C3 Gel electrophoresis of digestion product Plasmid extraction of ydcE-pSB1C3 for digestion confirmation Digestion confirmation of ydcE-pSB1C3 	<ul style="list-style-type: none"> Digestion of BBa_J04450-pSB1A2 with EcoR I and Pst I Dephosphorylation of pSB1A2 digestion product Gel electrophoresis of digestion product Gel purification of pSB1A2 Digestion of xylR-P_{xylA} with to confirm the mutagenesis 	<ul style="list-style-type: none"> Check of plate with xylR-P_{xylA}-pSB1A2 transformed E.Coli Inoculation of E.Coli transformed with xylR-P_{xylA}-pSB1A2 	<ul style="list-style-type: none"> Plasmid extraction of xylR-P_{xylA}-pSB1A2 for digestion confirmation Digestion confirmation of xylR-P_{xylA}-pSB1A2 Digestion of xylR-P_{xylA}-pSB1A2 with to confirm the mutagenesis

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

Week 9 (2012.8.6-2012.8.10)
Key Words: Sequencing

	Monday 2012.8.6	Tuesday 2012.8.7	Wednesday 2012.8.8	Thursday 2012.8.9	Friday 2012.8.10
Work done					
Result					
Discussion					
Remark					

Week 10 (2012.8.13-2012.8.17)					
Key Words: pTms-ydcD-pSB1C3; xylR-P _{xylA} -ydcE-pSB1A2; pTms-E0240-pSB1C3					
	Monday 2012.8.13	Tuesday 2012.8.14	Wednesday 2012.8.15	Thursday 2012.8.16	Friday 2012.8.17
Work done		<ul style="list-style-type: none"> Digestion of pTms-pSB1C3 and xylR-P_{xylA}-pSB1A2 with Spe I and Pst I Dephosphorylation of digestion product Digestion clean up of pTms-pSB1C3 Digestion of ydcD-pSB1C3 and ydcE-pSB1C3 with Xba I 	<ul style="list-style-type: none"> Digestion of ydcD-pSB1C3, ydcE-pSB1C3 and E0240 with Xba I and Pst I Gel electrophoresis of digestion product Gel purification of ydcD, ydcE and E0240 Ligation of E0240 and pTms-pSB1C3, ydcD and pTms-pSB1C3, ydcE and 	<ul style="list-style-type: none"> Check of plate with pTms-E0240-pSB1C3, pTms-ydcD-pSB1C3, xylR-P_{xylA}-ydcE-pSB1A2 transformed E.Coli Inoculation of E.Coli transformed with pTms-E0240-pSB1C3, xylR-P_{xylA}-ydcE-pSB1A2 	<ul style="list-style-type: none"> Plasmid extraction of pTms-E0240-pSB1C3, xylR-P_{xylA}-ydcE-pSB1A2 Digestion confirmation of pTms-E0240-pSB1C3, xylR-P_{xylA}-ydcE-pSB1A2 Digestion of ydcD-pSB1C3 and ydcE-pSB1C3 with Xba I and Pst I Gel electrophoresis of

		and Pst I . Gel electrophoresis of digestion product . Gel purification of ydcD and ydcE	xylR-P _{xylA} -pSB1A2 . Transformation of ligation product		digestion product . Gel purification of ydcD and ydcE
Result		. Got linear pTms-pSB1C3 and xylR-P _{xyl} -pSB1A2 cut with Spe I and Pst I . Got little recovery for gel purification		. For constructing pTms-ydcD-pSB1C3 no colonies showed on the plate	. pTms-E0240-pSB1C3 was successfully constructed . For constructing xylR-P _{xylA} -ydcE-pSB1A2, all the picked colonies showed negative confirmation result . Got ydcD and ydcE cut with Xba I and Pst I
Discussion					
Remark					

Week 11 (2012.8.20-2012.8.24)					
Key Words: pTms-ydcD-pSB1C3; xylR-P _{xylA} -pSB1A2; pTms-E0240-pSB3K3; bacterial M9 medium					
	Monday	Tuesday	Wednesday	Thursday	Friday

	2012.8.20	2012.8.21	2012.8.22	2012.8.23	2012.8.24
Work done	<ul style="list-style-type: none"> Digestion of pTms-E0240-pSB1C3 with EcoR I and Pst I Digestion clean up of pTms-E0240-pSB1C3 Digestion of BBa_J0445-pSB3K3 with EcoR I and Pst I Dephosphorylation of digestion product Gel purification of pSB3K3 Ligation of pTms-E0240 and pSB3K3, ydcD and pTms-PSB1C3, ydcE and xylR-P_{xylA}-pSB1A2 Transformation of ligation product 	<ul style="list-style-type: none"> Check of plate with pTms-E0240-pSB3K3, pTms-ydcD-pSB1C3, xylR-P_{xylA}-ydcE-pSB1A2 transformed E.Coli Inoculation of E.Coli transformed with pTms-E0240-pSB3K3, xylR-P_{xylA}-ydcE-pSB1A2 pTms-ydcD-pSB1C3 	<ul style="list-style-type: none"> Plasmid extraction of pTms-E0240-pSB3K3, xylR-P_{xylA}-ydcE-pSB1A2, pTms-ydcD-pSB1C3 Digestion confirmation of pTms-E0240-pSB3K3, xylR-P_{xylA}-ydcE-pSB1A2, pTms-ydcD-pSB1C3 Making of m9 salt and bacterial M9 medium for characterization Inoculation of E.Coli carrying pTms-E0240-pSB3K3 and I20260-PSB3K3 with M9 medium 	<ul style="list-style-type: none"> Check of inoculation product Making of new bacterial M9 medium with supplement 	<ul style="list-style-type: none"> Making of new bacterial M9 medium with supplement using adjusted recipe
Result			<ul style="list-style-type: none"> pTms-E0240-pSB3K3, xylR-P_{xylA}-ydcE-pSB1A2, 	<ul style="list-style-type: none"> Got no bacteria in all the inoculation product using M9 medium 	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 ₄ and CaCl ₂ before autoclave	
Remark: Before this week, separate the different construct					

Week 12 (2012.8.27-2012.8.31)					
Key Words: xylR-P _{xylA} -pSB1C2; xylR-P _{xylA} -ydcE-pSB1C2; pTms-E0240-pSB3K3 growth curve; I20260-pSB3K3 growth curve; pTms characterization					
	Monday 2012.8.27	Tuesday 2012.8.28	Wednesday 2012.8.29	Thursday 2012.8.30	Friday 2012.8.31
Work done	. Inoculation of E.Coli carrying pTms-E0240-pSB3K3, I20260-PSB3K3 and no construct with M9 medium	. Check of plate with xylR-P _{xylA} -ydcE-pSB1C3 transformed E.Coli, xylR-P _{xylA} -pSB1C3 transformed E.Coli . Inoculation of E.Coli transformed with xylR-P _{xylA} -ydcE-pSB1C3,	. Plasmid extraction of xylR-P _{xylA} -ydcE-pSB1C3, xylR-P _{xylA} -pSB1C3 . Digestion confirmation of xylR-P _{xylA} -ydcE-pSB1C3, xylR-P _{xylA} -pSB1C3	. Growth curve measurement of E.Coli carrying pTms-E0240-pSB3K3, 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"> · Digestion of xylR-P_{xylA}-pSB1A2, xylR-P_{xylA}-ydcE-pSB1A2 with EcoR I and Pst I · Gel electrophoresis of digestion product · Gel purification of xylR-P_{xylA} and xylR-P_{xylA}-ydcE · Ligation of xylR-P_{xylA} and pSB1C3, xylR-P_{xylA}-ydcE and pSB1C3 · Transformation of ligation product 	<p>E.Coli transformed with xylR-P_{xylA}-pSB1C3</p> <ul style="list-style-type: none"> · Inoculation of E.Coli carrying pTms-E0240-pSB3K3, E.Coli carrying I20260-PSB3K3 and E.Coli carrying no construct with M9 medium 	<ul style="list-style-type: none"> · Check of inoculation product · Inoculation of E.Coli carrying pTms-E0240-pSB3K3, E.Coli carrying I20260-PSB3K3 and E.Coli carrying no construct with M9 medium 	<ul style="list-style-type: none"> · Inoculation of E.Coli carrying pTms-E0240-pSB3K3, E.Coli carrying I20260-PSB3K3 and E.Coli carrying no construct with M9 medium 	
Result	<ul style="list-style-type: none"> · the growth rate of E.Coli in the M9 medium is rather slow compared to that in LB 		<ul style="list-style-type: none"> · xylR-P_{xylA}-ydcE-pSB1C3, xylR-P_{xylA}-pSB1C3 were successfully constructed · The growth rate of E.Coli in the new M9 medium is nearly the same as that in LB 	<ul style="list-style-type: none"> · The growth curve of pTms-E0240-pSB3K3, I20260-PSB3K3 in E.Coli was plotted 	<ul style="list-style-type: none"> · Got the first set of data for characterization of pTms
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: xylR-P_{xylA}-ydcE-pTms-ydcD-pSB1C3 ; pTms characterization; xylR-P_{xylA}-E0240-pSB1A2 growth curve					
	Monday 2012.9.3	Tuesday 2012.9.4	Wednesday 2012.9.5	Thursday 2012.9.6	Friday 2012.9.7
Work done	<ul style="list-style-type: none"> Inoculation of E.Coli carrying pTms-E0240-pSB3K3, E.Coli carrying I20260-PSB3K3 and E.Coli carrying no construct with M9 medium 	<ul style="list-style-type: none"> Digestion of pTms-ydcD-pSB1C3 with EcoR I and XbaI I Dephosphorylation of digestion product Digestion clean up of pTms-ydcD-pSB1C3 Digestion of xylR-P_{xylA}-ydcE-pSB1C3 with EcoR I and Spe I Electrophoresis of digestion product Gel purification of xylR-P_{xylA}-ydcE 	<ul style="list-style-type: none"> Dilution of inoculation product to lag phase Culture of the dilution product to mid-log phase Characterization of pTms Inoculation of E.Coli carrying pTms-E0240-pSB3K3, E.Coli carrying I20260-PSB3K3 and E.Coli carrying no construct with M9 medium 	<ul style="list-style-type: none"> Dilution of inoculation product to lag phase Culture of the dilution product to mid-log phase Characterization of pTms Inoculation of E.Coli carrying pTms-E0240-pSB3K3, E.Coli carrying I20260-PSB3K3 and E.Coli carrying no construct with M9 medium 	<ul style="list-style-type: none"> Dilution of inoculation product to lag phase Culture of the dilution product to mid-log phase Characterization of pTms Inoculation of E.Coli carrying pTms-E0240-pSB3K3, E.Coli carrying I20260-PSB3K3 and E.Coli carrying no construct with M9 medium

		<ul style="list-style-type: none">· Dilution of inoculation product to lag phase· Culture of the dilution product to mid-log phase· Characterization of pTms· Inoculation of E.Coli carrying pTms-E0240-pSB3K3, E.Coli carrying I20260-PSB3K3 and E.Coli carrying no construct with M9 medium	<ul style="list-style-type: none">· Ligation of xylR-P_{xylA}-ydcE and pTms-ydcD-pSB1C3· Transformation of ligation product	<ul style="list-style-type: none">· Check of plate with xylR-P_{xylA}-ydcE-pTms-ydcD-pSB1C3 transformed E.Coli	
Result				For constructing xylR-P _{xylA} -ydcE-pTms-ydcD-pSB1C3, no colony shown on the plate	
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	<ul style="list-style-type: none"> · 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"> · 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"> · 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"> · 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"> · Dilution of inoculation product to lag phase · Culture of the dilution product to mid-log phase · Characterization of pTms
Result					
Discussion					
Remark					

Week 15 (2012.9.17-2012.9.21)					
Key Words: P _{xyIA} 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					

