LABNOTE-C

XMU-iGEM

Date: 9.1-9.30

Author: XMU-iGEM

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
	✓ 1	✓ 2	✓ 3	✓ 4	5	✓ 6
✓ 7	✓ 8	✓ 9	✓ 10	✓ 11	12	✓ 13
✓ 14	15	16	17	✓ 18	19	20
21	22	23	✓ 24	✓ 25	✓ 26	27
28	29	30				

9 M

2014 Y

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30					

8 M 2014 Y

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30					

10 M 2014 Y

NOTE :

2014-09-01

- Enzyme Restriction

Pomp1	3I		108 bp
Pomp2	3K		78 bp
Pomp3	3E		108 bp
Single		*Xba* I	
Double		*Xba* I, *Pst* I	

- Verification： Agarose gel electrophoresis.

From left to the right: 100Marker-(3I Double)-(3I Single)-(3K Double)-(3K Single)-(3E Double)-(3E Single)-500Marker

- Enzyme Restriction

P	*Spe* I, *Pst* I
2M+18G+4F	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis

From left to the right: 100Marker-500Marker-3I-3K-1000Marker

From left to the right: 100Marker-3E-(2M-18G-4F)-500Marker

	Centrifuge Tube	All	Agarose gel
3I(P1)	0.933 g	0979 g	0.046 g
3K(P2)	0.933 g	1.010 g	0.077 g
3E(P3)	0.905 g	0.992 g	0.087 g
2M-18G-4F	0.931 g	0.992 g	0.061 g

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
2014-P4-6F	3.91/7.03/2.32/1.47	2.9/3.3/1.7/7.4
2013-P2-6F	8.4/0.79	3.3/2.9
K+B	2.97	2.8
3I	1.86/1.41/1.71/1.68	24.5/87.0/17.6/17.9
3K	1.91/2.21	22.9/22.9
3E	2.19/1.77/1.98	24.5/31.8/25.9

- Ligation

3I+(2M-18G-4F)

3K+(2M-18G-4F)

The concentration gradients of Ara	12 h The chemotaxis diameter toward Ara（d1）/cm	The chemotaxis diameters away from Ara（d2）/cm	Difference（d1-d2）/cm	24 h The chemotaxis diameter toward Ara（d1）/cm	The chemotaxis diameters away from Ara（d2）/cm	Difference（d1-d2）/cm	36 h The chemotaxis diameter toward Ara（d1）/cm	The chemotaxis diameters away from Ara（d2）/cm	Difference（d1-d2）/cm
0.2%	0.12	0.2	-0.08	0.45	0.35	-0.1	1.25	0.9	0.35
0.5%	0.15	0.3	-0.05	0.8	0.6	-0.2	2.15	1.35	0.8
1%	0.15	0.15	0	0.5	0.5	0	1.25	1.25	0.25
2%	0.15	0.25	-0.1	0.75	0.65	-0.1	1.7	1.7	0.3

3E+(2M-18G-4F)
Lux pR+(RBS+GFP+TT)
Lux pR+(RBS+Lux)

- The Experiment Plan:

With 0.05% IPTG, 50 μg/ml Cm to to verify the concentration gradient of Ara in the method of single-point.

- Aim:

We want to know the most appropriate concentration of Ara for *E. coli* form the pattern of hyperbola.

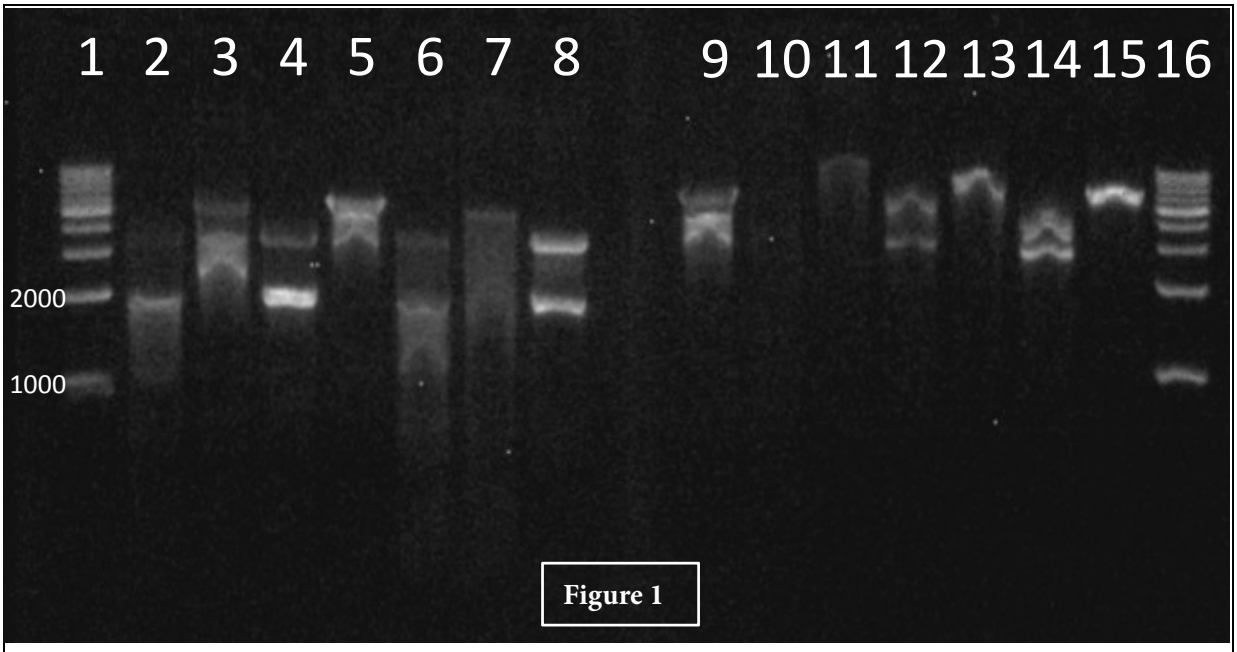

Figure 1

1: 1kb marker;
2: part in *DH5α* with double digestion(1);
3: part in *DH5α* with single digestion(2);
4: part in *DH5α* with double digestion(3);
5: part in *DH5α* with single digestion(4);
6: part in *DH5α* with double digestion(5);
7: part in *DH5α* with single digestion(6);
8: part in *CL-1* with double digestion(1);
9: part in *CL-1* with single digestion(2);
10: part in *CL-1* with double digestion(3);
11: part in *CL-1* with single digestion(4);
12: part in *CL-1* with double digestion(5);
13: part in *CL-1* with single digestion(6);
14: part in *CL-1* with double digestion(7);
15: part in *CL-1* with single digestion(8);
16: 1kb marker.
(part=BBa_K546000+BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015. 1, 2, 3, 4, 5, 6, 7, 8 were different colonies on the same tablet)

Purpose: The verification of the connection system: BBa_K546000+BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015.
Results/discussion: Because we didn't verify the bacteria containing BBa_B0034, so we needed to confirm again. From the result, we could only get the length of backbone, but we didn't know whether the bacteria contain the *CheZ*+T gene. What's more, we found the differences between bands were not obvious, maybe the digestion were not enough.

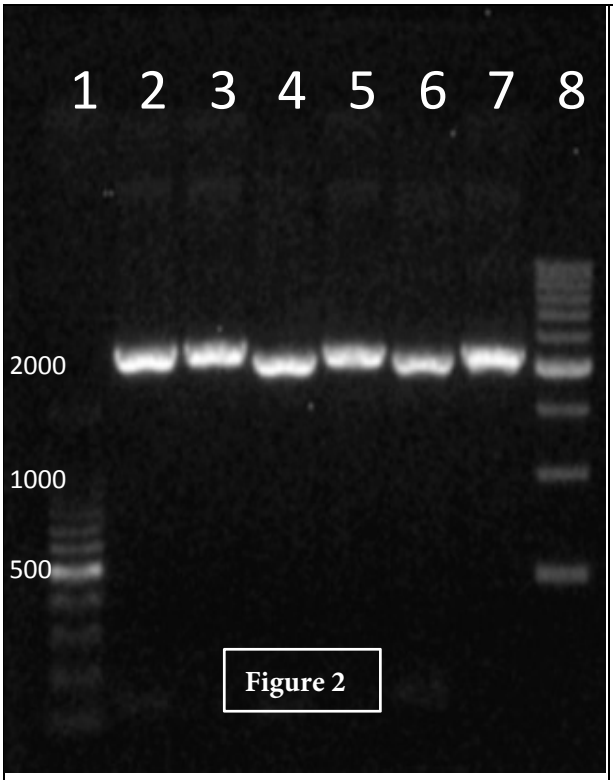

Figure 2

1: 100 bp marker;
2: BBa_R0083 with double digestion(XP);
3: BBa_R0083 with single digestion(X);
4: BBa_R0082 with double digestion(XP);
5: BBa_R0082 with single digestion(X);
6: BBa_R0084 with double digestion(XP);
7: BBa_R0084 with single digestion(X);
8: 500 bp marker.
(BBa_R0083 and BBa_R0082 are the promoter PomoR, which can regulate the expression of ompC. BBa_R0084 is the promoter PomoR, which can regulate the expression of ompF.)
Purpose: The verification of the BioBricks: BBa_R0082, BBa_R0083 and BBa_R0084.
Results/discussion: The respective lengths of Ba_R0083, BBa_R0082 and BBa_R0084 are 78 bp, 108 bp and 108 bp. From the figure, we could get a corresponding band near 100 bp after double digestion, while getting a corresponding band near 2000bp after single digestion, and the band with double digestion was lower 100bp than single digestion. So we could confirm that the plasmids we got were all correct.

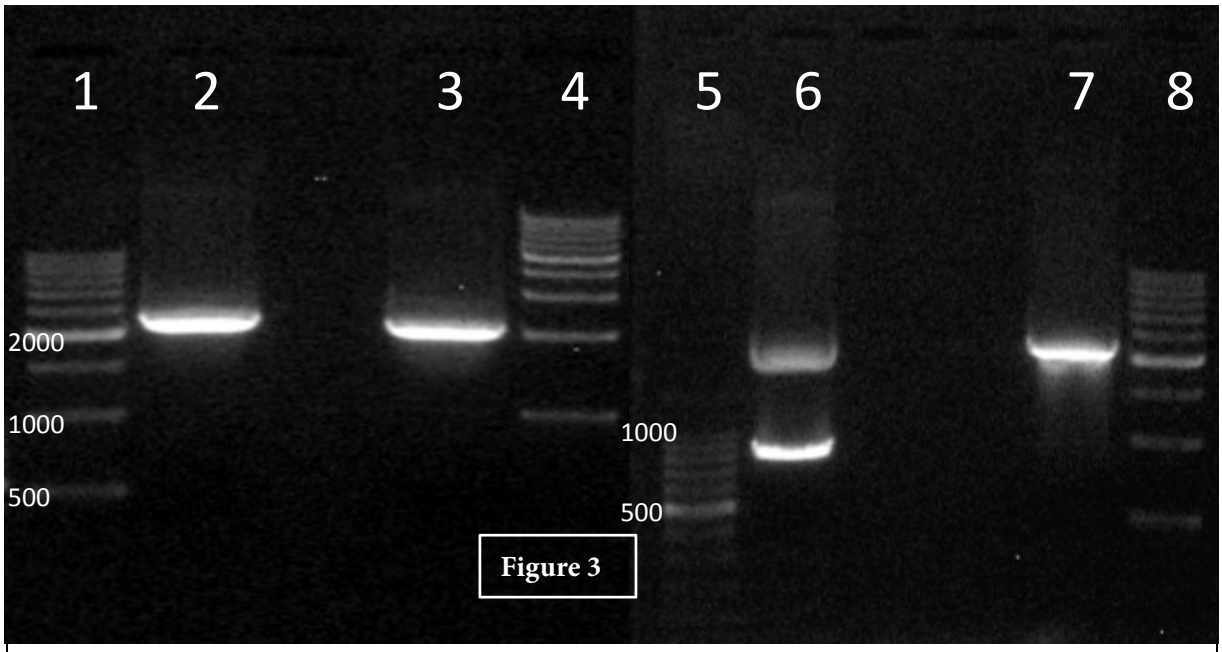

Figure 3

1: 500 bp marker;
2: BBa_R0082 with double digestion(SP);
3: BBa_R0083 with double digestion(SP);
4: 1kb marker;
Purpose: Respective preparation for the connection between BBa_R0082, BBa_R0083, BBa_R0084 and BBa_B0034 + BBa_K629003 + BBa_B0015.
Results/discussion: We used the plasmids which contain promoter as the backbone, so we digested the plasmid containing BBa_R0082, BBa_R0083 and BBa_R0084 with *Spe* I and *Pst* I enzyme. While we used the BBa_B0034+BBa_K629003+BBa_B0015 as the insert gene, whose length is 800 bp. so we digested the plasmids with *Xba* I and *Pst* I enzyme. Finally we could confirm that the plasmid were all we want to get.

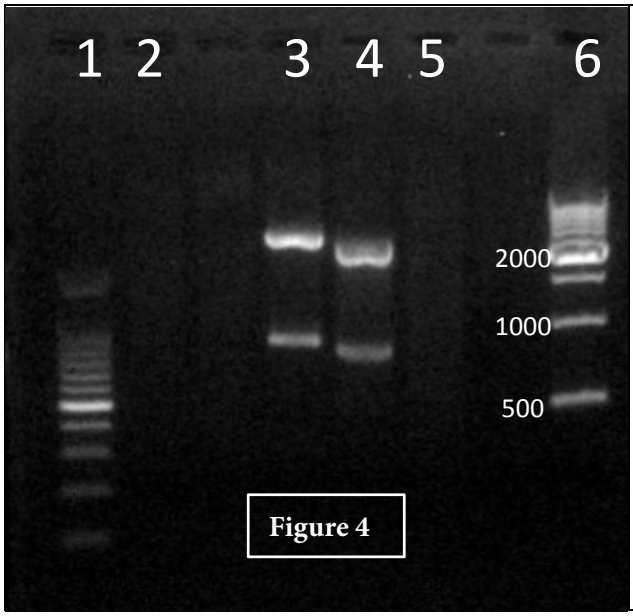

Figure 4

1: 100 bp marker;
2: BBa_I0462(RBS +Lux R+TT);
3: BBa_K805016(RBS +Lux I+TT)(1);
4: BBa_K805016(RBS +Lux I+TT)(2);
5: BBa_I13507(RBS+RFP+TT,);
6: 500 bp marker.
(1, 2 were different colonies on the same plate)

Purpose: The verification of the BioBricks: BBa_I0462, BBa_K805016, BBa_I13507.
Results/discussion: The length of BBa_K805016 was 798 bp, From the figure, we could determine that the BioBrick was correct. But others showed the serious trailing phenomenon.

2014-09-02

- The Experiment Plan
  With 50 μg/ml Cm, 1%Ara and the concentration gradients of IPTG are 0.1 mM, 0.25 mM, 0.5 mM, 1 mM.
- Aim
  We wanted to find the most appropriate concentration of IPTG.
- Conclusion

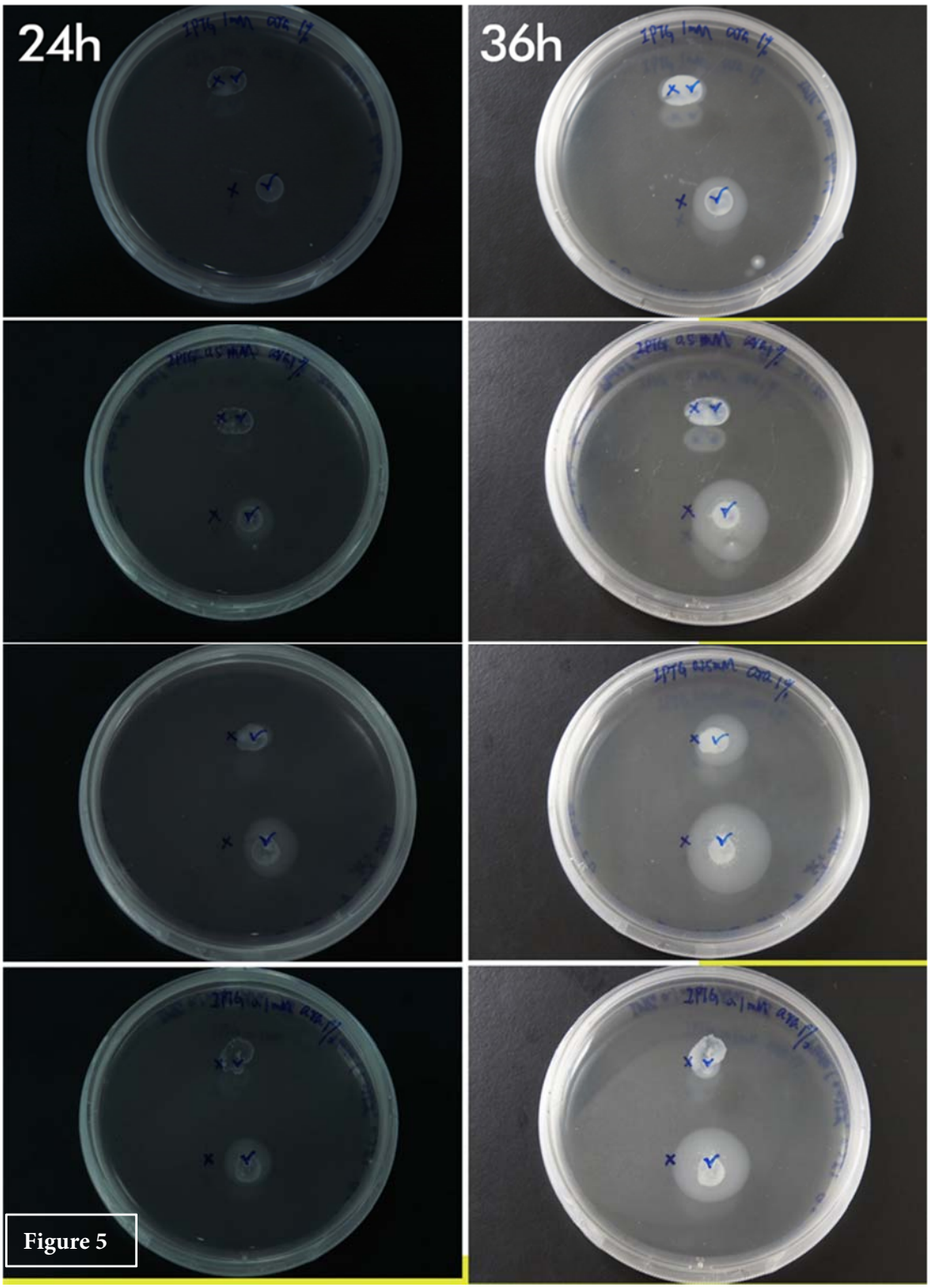

Figure 5

The most appropriate concentration is 0.25 mM.

- (Inter-Lab study)

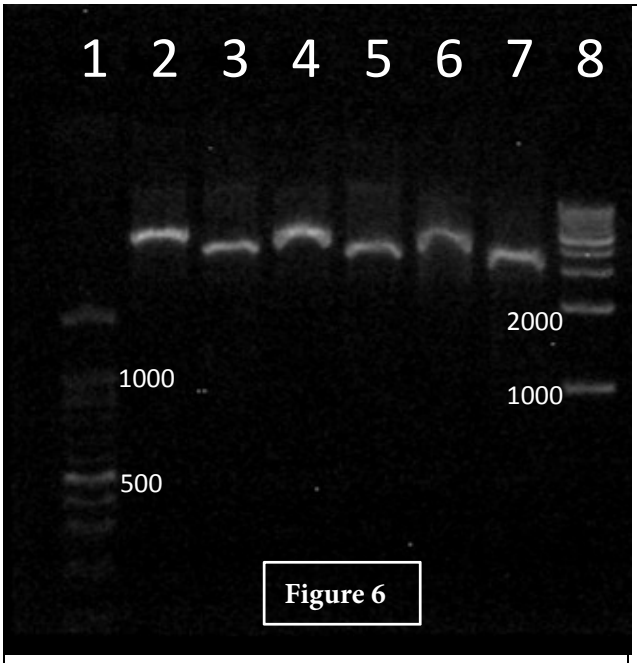

Figure 6

1: 100 bp marker;
2: BBa_I20260 with single digestion(12-P2-17F)(1);
3: BBa_I20260 with double digestion(12-P2-17F)(1);
4: BBa_I20260 with single digestion(12-P2-17F)(2);
5: BBa_I20260 with double digestion(12-P2-17F)(2);
6: BBa_I20260 with single digestion(12-P2-17F)(3);
7: BBa_I20260 with double digestion(12-P2-17F)(3);
8: 1kb marker.
(1, 2, 3 are the different colonies.)
Purpose: The verification of the BBa_I20260 BioBrick.
Results/discussion: From this figure, we could find that the fragments that were from single digestion was longer than double digestion. However we couldn't get a band near 1000 bp, which is the length of J23101+RBS0032+GFP+TT. So we couldn't confirm that the BBa_I20260 was correct.

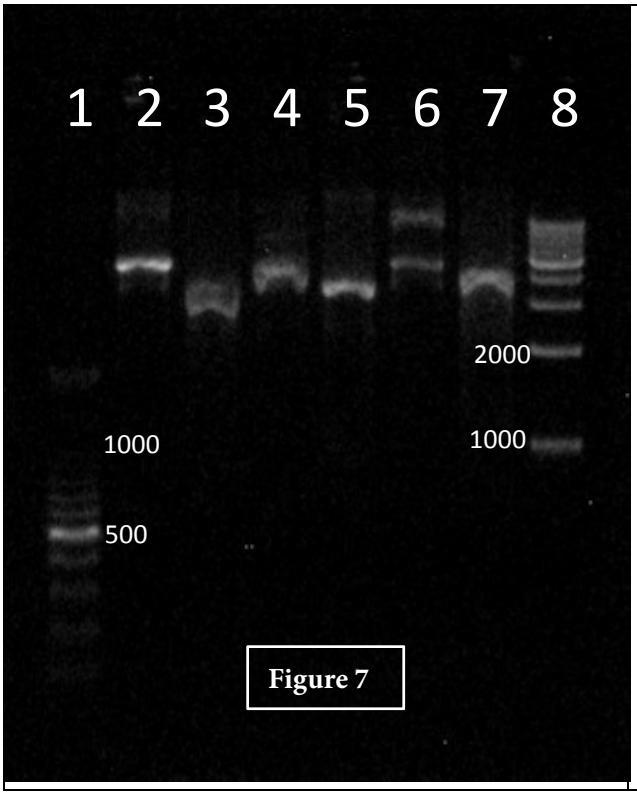

Figure 7

1: 100 bp marker;
2: BBa_I20260 with single digestion(12-P2-17F)(1);
3: BBa_I20260 with double digestion(12-P2-17F)(1);
4: BBa_I20260 with single digestion(12-P2-17F)(2);
5: BBa_I20260 with double digestion(12-P2-17F)(2);
6: BBa_I20260 with single digestion(12-P2-17F)(3);
7: BBa_I20260 with double digestion(12-P2-17F)(3);
8: 1kb marker.
(1, 2, 3 are the different colonies.)

Purpose: The verification of the BBa_I20260 BioBrick.
Results/discussion: From this figure, we could find that the fragments that were from single digestion was 1000 bp longer than double digestion. However, we couldn't get a band near 1000 bp, which was the length of J23101+RBS0032+GFP+TT. So we couldn't draw the conclusion that the BBa_I20260 was correct.

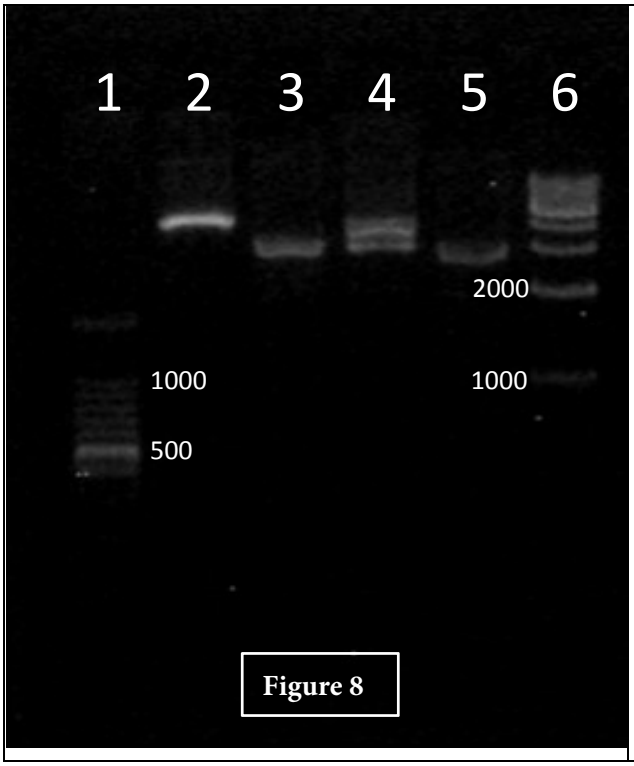

Figure 8

1: 100 bp marker;
2: BBa_K1412924+pSB3K3(1) with single digestion;
2: BBa_K1412924+pSB3K3(1) with double digestion;
2: BBa_K1412924+pSB3K3(2) with single digestion;
3: BBa_K1412924+pSB3K3(2) with double digestion;
6: 1kb marker.
(1, 2 are different colonies.)

Purpose: the verification of BBa_K1412924+pSB3K3 connection system.
Results/discussion: From this figure, we could find that the fragments were from single digestion was 1000 bp longer than double digestion. While we couldn't get a band near 1000 bp, which is the length of J23101+RBS0032+GFP+TT. So we couldn't confirm that the connection system was correct.

2014-09-03

- Enzyme Restriction
  Xba I, *Pst* I
- Verification: Agarose gel electrophoresis

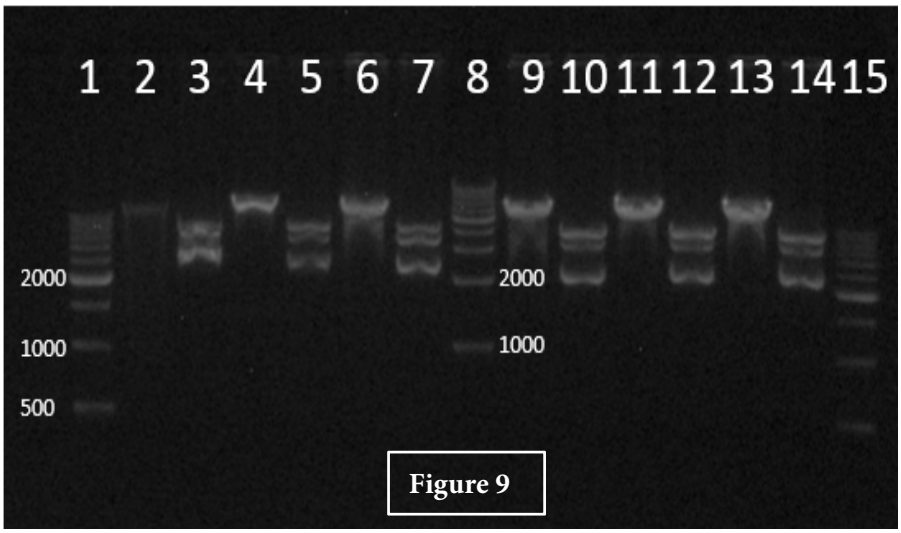

Figure 9

1: 500 bp marker;
2:partA1 with single digestion(P)(1);
3: partA1 with double digestion(XP)(1);
4: partA1 with single digestion(P)(2);
5: partA1 with double digestion(XP)(2);
6: partA2 with single digestion(P)(1);
7: partA2 with single digestion(P)(1);
8: 1kb marker;
9: partA2 with single digestion(P)(2);
10: partA2 with double digestion(XP)(2);
11: partA3 with single digestion(P)(1); 12: partA3 with double digestion(XP)(1);
13: partA3 with single digestion(P)(2); 14: partA3 with double digestion(XP)(2); 15: 500 bp marker.

(1 and 2 are different colonies on the same plate, they are all in the *CL-1* bacteria, partA = (*CL-1*)BBa_K546000+BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015+12F)
Purpose: We wanted to construct oscillation circuits, so we imported two plasmid into *CL-1* bacteria.
Results/discussion: The respective lengths of BBa_K546000+BBa_F2621-BBa_B0034+BBa_K629003+BBa_B0015 and 12F are 3908 bp and 2135 bp. In theory, we could get two bands near 5978 bp and 5376 bp after single digestion, while the two bands were too close to separate from the figure. In theory, we should get four bands 3908 bp, 2070 bp, 2135 bp and 3241 bp after double digestion, while 2070 bp and 2135 bp were too close to separate, so we found there were three bands only, but the third band was lighter than the other two. At the end, we chose the colony partA(2) for the next experiment.

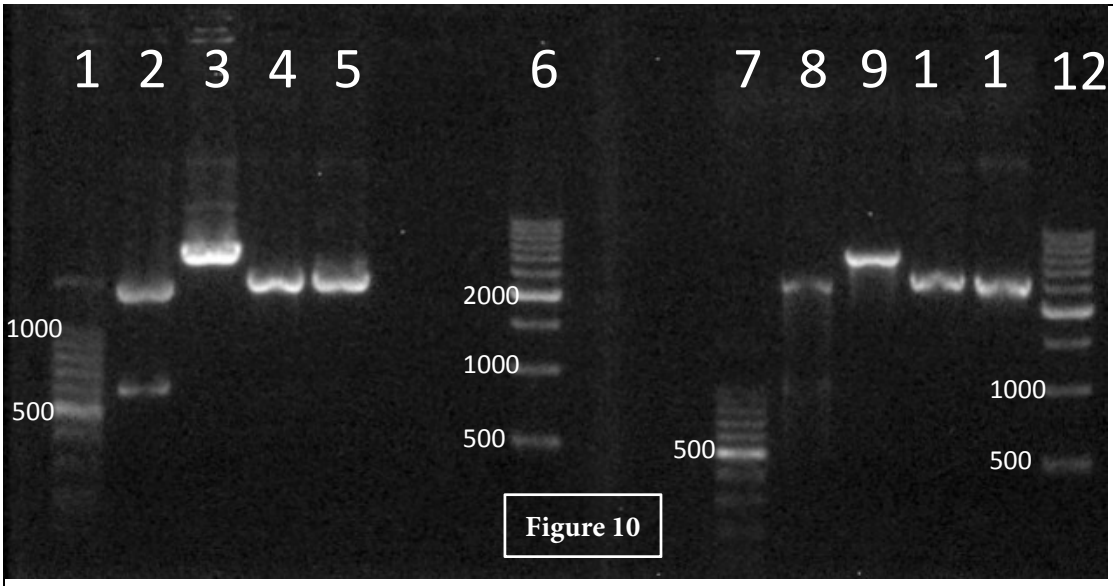

Figure 10

1: 100 bp marker; 2: PART1 with double digestion(1 XP);
3: PART1 with single digestion (1 X); 4: PART3 with double digestion (1 XP);
5: PART3 with single digestion (1 X);
6: 500 bp marker;
7: 100 bp marker;
8: PART2 with double digestion (1 XP);
9: PART2 with single digestion (1 X);
10: PART2 with double digestion (2 XP);
11: PART2 with single digestion (2 X);
12: 500bp marker.
(PART1=BBa_R0082+BBa_B0034+BBa_K629003+BBa_B0015
PART2=BBa_R0083+BBa_B0034+BBa_K629003+BBa_B0015
PART3=BBa_R0084+BBa_B0034+BBa_K629003-BBa_B0015)
(1, 2 are different colonies.)
Purpose: The verification of the connection systems: BBa_R0082+BBa_B0034+BBa_K629003+BBa_B0015, BBa_R0083+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_R0084+BBa_B0034+BBa_K629003+BBa_B0015.
Results/discussion: The lengths of BBa_R0083+BBa_B0034+BBa_K629003+BBa_B0015, BBa_R0082+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_R0084+BBa_B0034+BBa_K629003+BBa_B0015 were 886 bp, 916 bp and 916 bp. From the figure, we could get a band during 500 bp and 1000 bp when the connection system BBa_R0083+BBa_B0034+BBa_K629003+BBa_B0015 was digested with double enzyme. While the band with single digestion was longer than the length of backbone. So the connection system was correct. About connection system BBa_R0084+BBa_B0034+BBa_K629003+BBa_B0015, we found the band with double digestion and single digestion are the same, so our connection failed. As for the connection system BBa_R0082+BBa_B0034+BBa_K629003+BBa_B0015(1), we found the band near 1000 bp with double digestion. And the band with single digestion was longer than double digestion. So we could confirm that the connection system was correct.

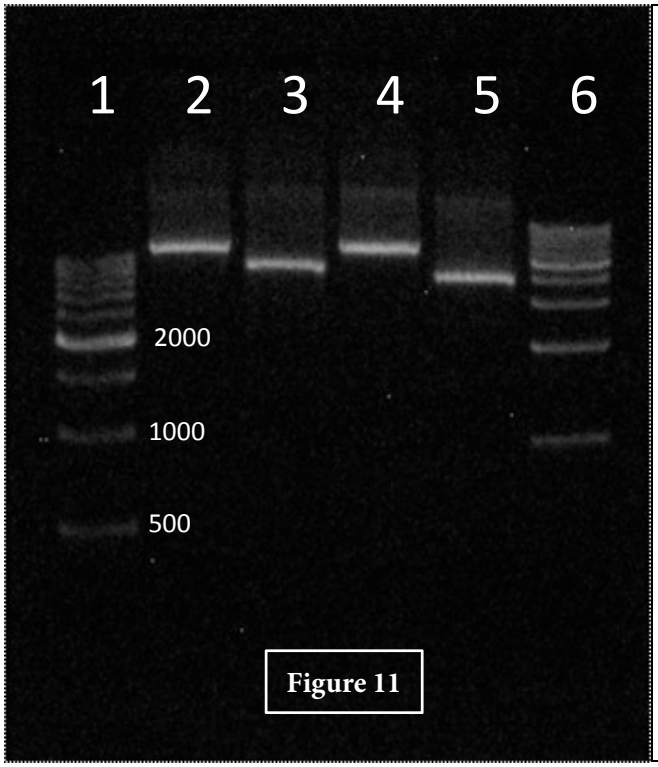

Figure 11

1: 500 bp marker;
2: BBa_I20260 with single digestion(12-P2-17F)(1);
3: BBa_I20260 with double digestion(12-P2-17F)(1);
4: BBa_I20260 with single digestion(12-P2-17F)(2);
5: BBa_I20260 with double digestion(12-P2-17F)(2);
6: 1kb marker.
(1, 2 are different colonies.)

Purpose: The verification of the BBa_I20260 BioBrick.

Results/discussion: From this figure, we could find that the fragments from single digestion was 1000 bp longer than double digestion. While we couldn't get a band near 1000 bp, which was the length of J23101+RBS0032+GFP+TT. So we couldn't confirm that the BBa_I20260 was correct.

2014-09-04

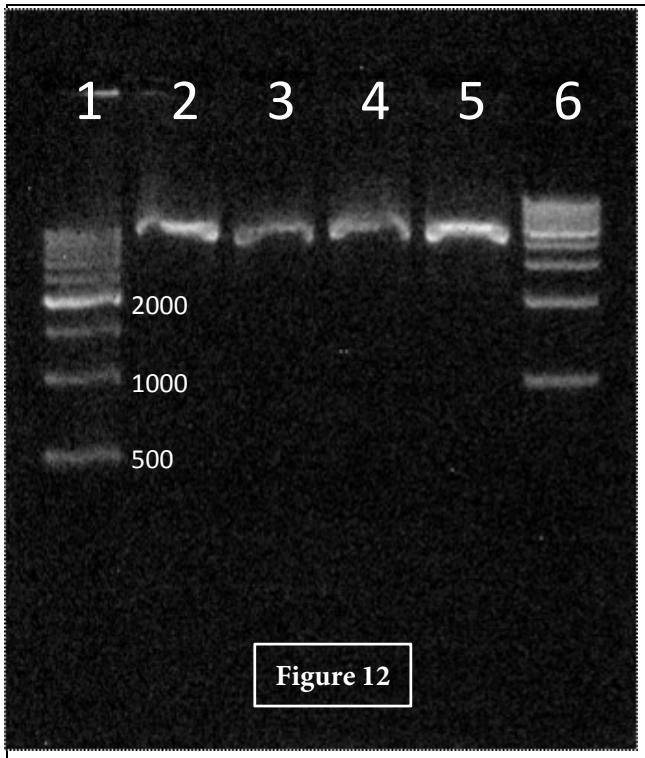

Figure 12

1: 500 bp marker;
2: BBa_I20260 with single digestion(12-P2-17F)(1);
3:BBa_I20260 with double digestion(12-P2-17F)(1);
4: BBa_I20260 with single digestion(12-P2-17F)(2);
5: BBa_I20260 with double digestion(12-P2-17F)(2);
6: 1kb marker.
(1, 2 are the different colonies.)
Purpose: The verification of the BBa_I20260 BioBrick.
Results/discussion: For we couldn't get good result from the digestion with *Xba* I and *Pst* I enzyme, so we tried the *EcoR* I and *Pst* I enzyme. From this figure, we could find that the fragment from single digestion and double digestion were near 5000 bp. While we couldn't get a band near 1000 bp from double digestion, which was the length of J23101+RBS0032+GFP+TT. So we couldn't confirm that the BBa_I20260 was correct.

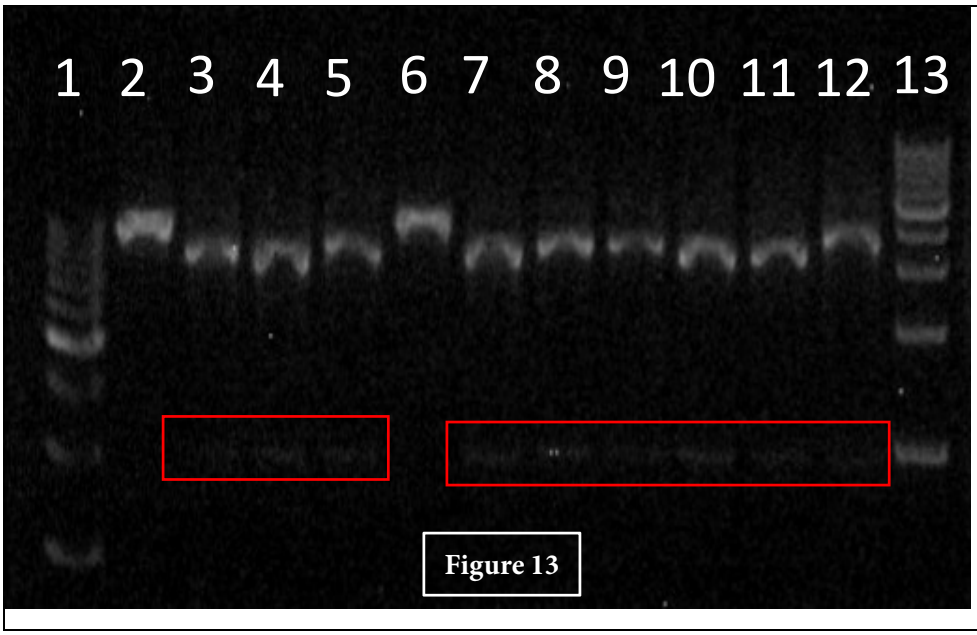

Figure 13

1. 500 bp Marker;
2. Single enzyme digestion (*Pst* I) with BBa_I20260 (reconstructed by XMU-China). Sample 1;
3. Double enzymes digestion (*EcoR* I and *Pst* I) with BBa_I20260 (reconstructed by XMU-China). Sample 1;
4. Double enzymes digestion (*EcoR* I and *Pst* I) with BBa_I20260 (reconstructed by XMU-China). Sample 2;
5. Double enzymes digestion (*EcoR* I and *Pst* I) with BBa_I20260 (reconstructed by XMU-China). Sample 3;
6. Single enzyme digestion (*Pst* I) with BBa_I20260(reconstructed by XMU-China). Sample 3;
7. Double enzymes digestion (*EcoR* I and *Pst* I) with BBa_I20260 (reconstructed by XMU-China). Sample 3;
8. Double enzymes digestion (*EcoR* I and *Pst* I) with BBa_I20260 (2012-Plate2-17F). Sample 1;
9. Double enzymes digestion (*EcoR* I and *Pst* I) with BBa_I20260 (2012-Plate2-17F). Sample 2;
10. Double enzymes digestion (*EcoR* I and *Pst* I) with BBa_I20260 (2012-Plate2-17F). Sample 3;
11. Double enzymes digestion (*EcoR* I and *Pst* I) with BBa_I20260 (2012-Plate2-17F). Sample 4;
12. Double enzymes digestion (*EcoR* I and *Pst* I) with BBa_I20260 (2014-Plate4-18A). Sample 1;
13. 1kb Marker.

Enzyme digestion verification of device BBa_I20260.

Results and discussion: It was very abnormal that we got puzzling results with double enzymes digestion by *Xba* I and *Pst* I. However, if we used *EcoR* I and *Pst* I instead, we found that the segments generated by single digestion was about 1000bp longer than double digestion whose backbone length isPSB3k3. We could also get segments slightly shorter than 1000bp which generated by double digestion, and that segments were highlighted by red frames. So we confirmed that device BBa_I20260 was correct. We took our actual measurement with the reconstructed device.

2014-09-06

- Activation of bacteria

  Use pipette to transfer 50 μL bacterium solution pLac-RBS(1.0)-*CheZ*-TT, pLac-RBS(0.01)-*CheZ*-TT, pLac-RBS(0.3)-*CheZ*-TT, pBAD-RBS(1.0)-*CheZ*-TT, pTet-RBS(1.0)-*CheZ*-TT) respectively into 5 ml LB liquid medium whose antibiotic concentration is 50 μg/ml. Culture for 12 h. Then transfer 50 μL bacterium solution into new LB liquid medium whose antibiotic concentration is 50 μg/ml to culture for 12 h.

- Characterization

  Then stab 3 μL bacterium medium into the M63 semisolid medium at the dots. And culture the bacteria in constant temperature and humidity incubator at 37℃.

- Measurement

  Measure the radius of *E. coli*.
  Cm=50 μg/ml, IPTG=0, ARA=0;

T/h	2M(A)	2M(B)	2L	2J
0 h	0.25	0.25	0.25	0.25
7 h	0.40	0.40	0.25	0.25
12 h	0.65	0.62	0.55	0.45

Cm=50 μg/ml, IPTG=0.025 mm, ARA=0;

T/h	2M(A)	2M(B)	6F(1-1)	6F(1-2)	14A
0 h	0.25	0.25	0.25	0.25	0.25
7 h	0.40	0.80	0.70	0.75	0.80
12 h	0.65	0.90	0.90	1.05	1.05

Cm=50 μg/ml, IPTG=0.025 mM, ARA=0.02%

T/h	2M(A)	2M(B)	2L	2J
0 h	0.25	0.25	0.25	0.25
7 h	0.3	0.65	0.25	0.25
12 h	0.55	0.75	0.6	0.45

- Activation of bacteria

  Use pipette to transfer 50 μL bacterium solution pLac-RBS(1.0)-*CheZ*-TT, pLac-RBS(0.01)-*CheZ*-TT, pLac-RBS(0.3)-*CheZ*-TT, pBAD-RBS(1.0)-*CheZ*-TT, pTet-RBS(1.0)-*CheZ*-TT respectively into 5 ml LB liquid medium whose antibiotic concentration is 50 μg/ml. Culture for 12 h. Then transfer 50 μL

bacterium solution into new LB liquid medium whose antibiotic concentration is 50 μg/ml to culture for 12 h.

- Characterization

  Then stab 3 μL bacterium medium into the M63 semisolid medium at the dots. And culture the bacteria in constant temperature and humidity incubator at 37℃.

- Measurement

  Measure the radius of *E. coli*.

  Cm=50 μg/ml, IPTG=0, ARA=0;

T/h	2M(A)	2M(B)	2L	2J
0 h	0.25	0.25	0.25	0.24
11 h15 min	0.5	0.55	0.25	0.24
24 h	0.6	0.65	0.25	0.26
30 h	0.95	1.10	0.26	0.25

Cm=50 μg/ml, IPTG=0.025 mM, ARA=0;

T/h	2M(A)	2M(B)	6F(1-1)	6F(1-2)	14A
0 h	0.25	0.25	0.25	0.25	0.25
11 h15min	0.49	0.88	0.68	0.80	0.83
24 h	1.00	1.70	0.90	1.15	1.20

Cm=50 μg/ml, IPTG=0.025 mm, ARA=0.02%

T/h	2M(A)	2M(B)	2L	2J
0 h	0.25	0.25	0.25	0.20
11 h15 min	0.30	0.75	0.30	0.20
24 h	0.35	0.90	0.30	0.25
30 h	0.60	1.40	0.30	0.25

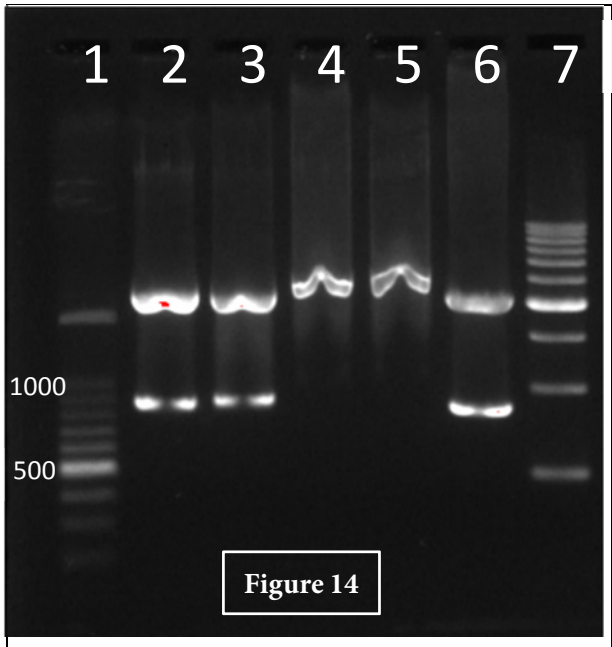

Figure 14

1: 100 bp marker;
2: PART1 with double digestion(1 XP);
3: PART1 with double digestion (2 XP);
4: PART3 with double digestion (1 XP);
5: PART3 with double digestion (2 XP);
6: PART2 with double digestion (1 XP);
7: 500 bp marker.

(PART1=BBa_R0083+BBa_B0034+BBa_K629003+BBa_B0015
PART2=BBa_R0082+BBa_B0034+BBa_K629003+BBa_B0015
PART3=BBa_R0084+BBa_B0034+BBa_K629003+BBa_B0015)

Purpose: The verification of the connection systems: BBa_R0083+BBa_B0034+BBa_K629003+BBa_B0015, BBa_R0082+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_R0084+BBa_B0034+BBa_K629003+BBa_B0015.

Results/discussion: The lengths of BBa_R0083+BBa_B0034+BBa_K629003+BBa_B0015, BBa_R0082+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_R0084+BBa_B0034+BBa_K629003+BBa_B0015 are 886 bp, 916 bp and 916 bp. From the figure, we could find the corresponding bands, so we confirmed the connection systems BBa_R0083+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_R0084+BBa_B0034+BBa_K629003+BBa_B0015 are correct, while BBa_R0082-BBa_B0034-BBa_K629003-BBa_B0015 was not correct.

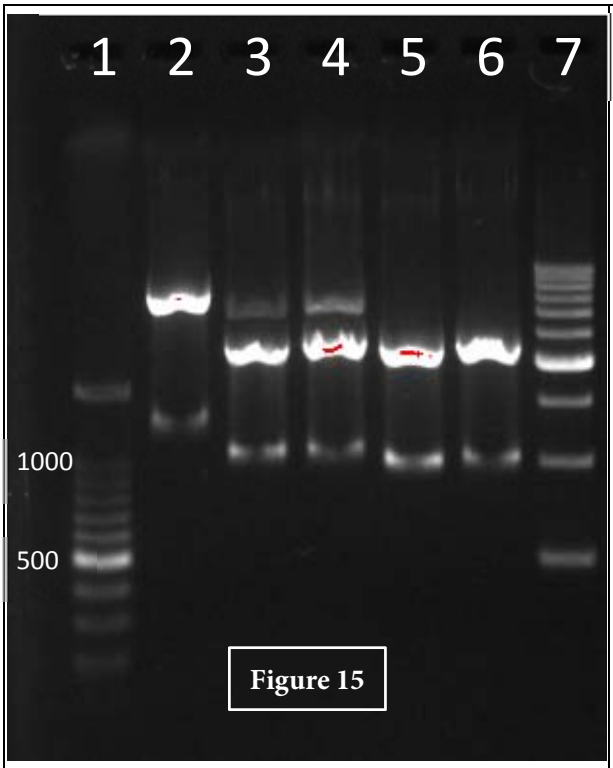

Figure 15

1: 100 bp marker;
2: 8D with double digestion(XP);
3: BBa_K145201 with double digestion (2.1 XP);
4: BBa_K145201 with double digestion (2.2 XP);
5: BBa_P0440 with double digestion (3.1 XP);
6: BBa_P0440 with double digestion (3.2 XP);
7: 500bp marker.
(2.1, 2.2, 3.1, 3.2 are different colonies.)
Purpose: The verification of the verification of BioBricks: 8D, BBa_K145201 and BBa_P0440.
Results/discussion: We wanted to use 8D as backbone which contained tetracycline resistance. BBa_K145201 contained a TetR generator, which could be used in the regulation of pTET. While BBa_P0440 only lacked a promoter relative to BBa_K145201. The lengths of BBa_K145201 and 8D are 883 bp and 840 bp. While the bands we got were all longer than 1000 bp, while the differences between BBa_K145201 and 8D was correct, so we considered the BioBrick was correct.

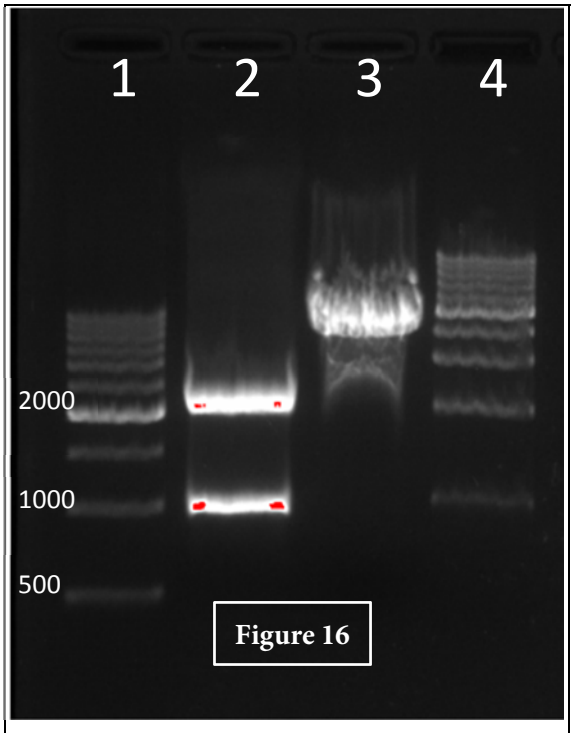

Figure 16

1: 500 bp marker;
2:BBa_K145201 with double digestion(ES);
3:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 with double digestion(EX);
4: 1kb marker.

Purpose: Preparation for the connection between BBa_K145201 and BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015.
Results/discussion: From the figure, we could find the corresponding bands were near 1000 bp and 2000 bp after double digestion. At the same time, we used the plasmids containing BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 as backbone. So after EX double enzyme digestion, we could get the band longer than 4300 bp.

2014-09-07

- Activation of bacteria

  Use pipette to transfer 50 μL bacterium solution pLac -RBS (1.0)-*CheZ*-TT, pLac-RBS(0.01)-*CheZ*-TT, pLac-RBS(0.3)-*CheZ*-TT, pBAD-RBS(1.0)-*CheZ*-TT, pTet-RBS(1.0)-*CheZ*-TT respectively into 5 ml LB liquid medium whose antibiotic concentration is 50 μg/ml. Culture for 12 h. Then transfer 50 μL bacterium solution into new LB liquid medium whose antibiotic concentration is 50 μg/ml to culture for 12 h.

- Characterization

  Then stab 3 μL bacterium medium into the M63 semisolid medium at the dots. And culture the bacteria in constant temperature and humidity incubator at 37℃.

- Measurement

  It's found that the Solid medium collapse, so the experiment fail.

2014-09-08

- Activation of bacteria

  Use pipette to transfer 50 μL bacterium solution pLac-RBS(1.0)-*CheZ*-TT, pLac-RBS(0.01)-*CheZ*-TT, pLac-RBS(0.3)-*CheZ*-TT, pBAD-RBS(1.0)-*CheZ*-TT, pTet-RBS(1.0)-*CheZ*-TT respectively into 5 ml LB liquid medium whose antibiotic concentration is 50 μg/ml. Culture for 12 h. Then transfer 50 μL bacteria solution into new LB liquid medium whose antibiotic concentration is 50 μg/ml to culture for 12 h.

- Characterization

  Then stab 3 μL bacterium medium into the M63 semisolid medium at the dots. And culture the bacteria in constant temperature and humidity incubator at 37℃.

- Measurement

  Measure the radius of *E. coli*.

  Cm=50 μg/ml, IPTG=0, ARA=0;

T/h	2M(A)	2M(B)	6F(1)	6F(2)	14A	2J
0 h	0.25	0.25	0.25	0.25	0.25	0.25
12 h	0.65	0.90	1.23	1.23	1.40	0.75
18 h	1.05	1.50	2.00	2.00	2.50	1.75

T/h	2M(A)	2M(B)	2L	2J
0 h	0.20	0.30	0.25	0.23
18 h	0.60	1.10	0.25	0.23
24 h	0.75	1.25	0.25	0.23

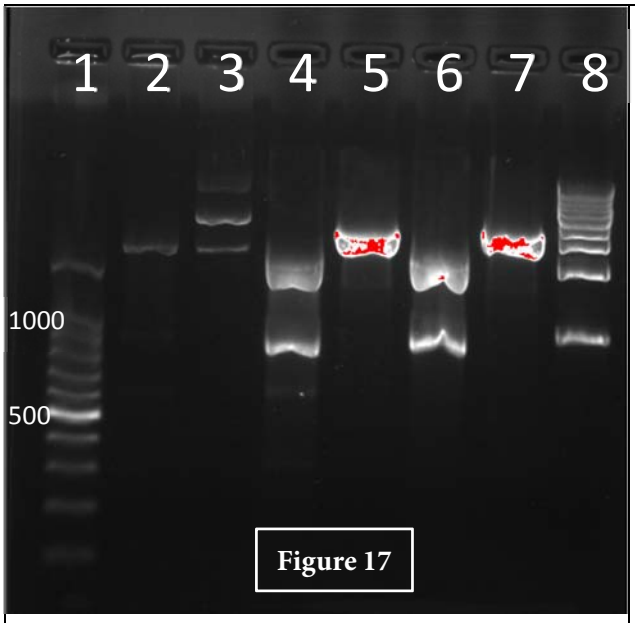

Figure 17

1: 100 bp marker;
2: PART1 in *CL-1* with double digestion(1 XP);
3: PART1 in *CL-1* with single digestion (1 X);
4: PART2 in *CL-1* with double digestion (1 XP);
5: PART2 in *CL-1* with single digestion (1 X);
6: PART2 in *CL-1* with double digestion (2 XP);
7: PART2 in *CL-1* with single digestion (2 X);
8: 500 bp marker.
(1 and 2 are the different colonies on the same plate.)

Purpose: The verification of the connection systems: BBa_R0083+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_R0082+BBa_B0034+BBa_K629003+BBa_B0015.

Results/discussion: The lengths of the connection systems BBa_R0083+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_R0082+BBa_B0034+BBa_K629003+BBa_B0015 are 886 bp and 916 bp, but we couldn't get a band near 886 bp with double digestion and also couldn't get a 2148bp band with single digestion. As for BBa_R0082+BBa_B0034+BBa_K629003+BBa_B0015 connection system, we could get a band near 1000 bp with double digestion and a 3000 bp band with single digestion. So we could confirm that the connection system BBa_R0082+BBa_B0034+BBa_K629003+BBa_B0015 was correct, while the length of BBa_R0083+BBa_B0034+BBa_K629003+BBa_B0015 was not correct.

Cm=50 μg/ml, IPTG=0.025 mM, ARA=0;

T/h	2M(A)	2M(B)	2J	2L
0 h	0.25	0.25	0.23	0.25
11 h15 min	0.575	0.60	0.23	0.25
24 h	0.75	1.10	0.23	0.25
30 h	0.85	1.30	0.23	0.25

Cm=0, IPTG=0, ARA=0

T/h	2M(A)	2M(B)	6F(1)	6F(2)	14A	2L
0 h	0.25	0.25	0.25	0.25	0.25	0.25
12 h	0.40	0.60	1.35	1.50	1.20	0.20
18 h	0.80	1.00	2.15	2.25	2.00	0.20

Cm=0, IPTG=0, ARA=0

2014-09-09

- Activation of bacteria

  Transfer 50 μL bacterium solution.

  pLac-RBS(1.0)-*CheZ*-TT, pLac-RBS(0.01)-*CheZ*-TT, pLac-RBS(0.3)-*CheZ*-TT, into new LB liquid medium whose antibiotic concentration is 50 μg/ml to culture for 12 h.

- Characterization

  Then stab 3 μL bacterium medium into the M63 semisolid medium at the dots. And culture the bacteria in constant temperature and humidity incubator at 37℃.

- Measurement

  Measure the radius of *E. coli*.

  6-Plate1: Cm=50 μg/ml, IPTG=0.025 mM, ARA=0;

T/h	2M(A)	2M(B)	2L	2J
0 h	0.25	0.25	0.25	0.25
12 h	0.6	0.8	0.25	0.25
24 h	1.325	2.4	0.225	0.25

  11-Plate2: Cm=50 μg/ml, IPTG=0.025 mM, ARA=0;

T/h	2M(A)	2M(B)	2J	2L
0 h	0.25	0.25	0.23	0.23
12 h	0.60	1.20	0.23	0.23
24 h	1.13	1.80	0.23	0.23
39 h	1.60	2.63	0.23	0.23

  4-Plate3: Cm=50 μg/ml, IPTG=0.025 mM, ARA=0;

T/h	2M(A)	2M(B)	2J	2L
0 h	0.25	0.25	0.23	0.25
12 h	0.75	1.10	0.23	0.25
24 h	1.33	2.40	0.23	0.25
39 h	Out of measure	Out of measure	0.23	0.25

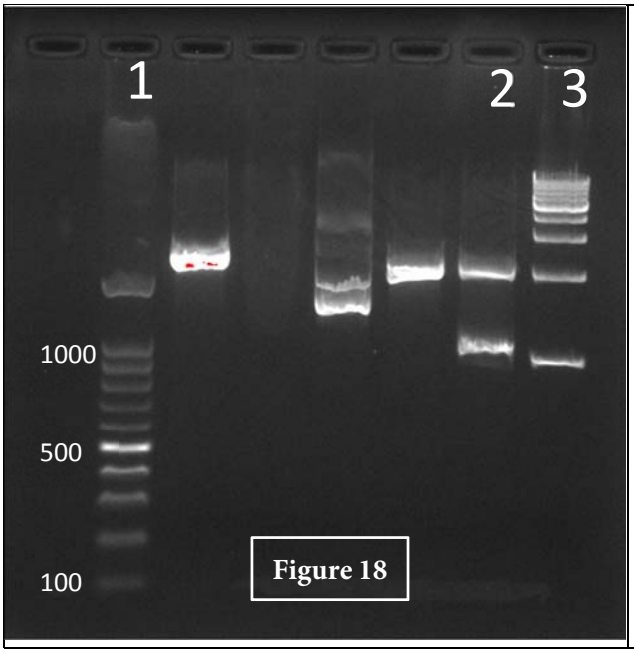

Figure 18

1: 100 bp marker;
2: BBa_I13507(RBS+RFP+TT);
3: 1kb marker.

Purpose: The verification of the BioBricks: BBa_I13507.
Results/discussion: The length of BBa_I13507 is 861 bp. From the figure, we could determine that the BioBrick was correct because of the corresponding bands were near 1000 bp and 2000 bp.

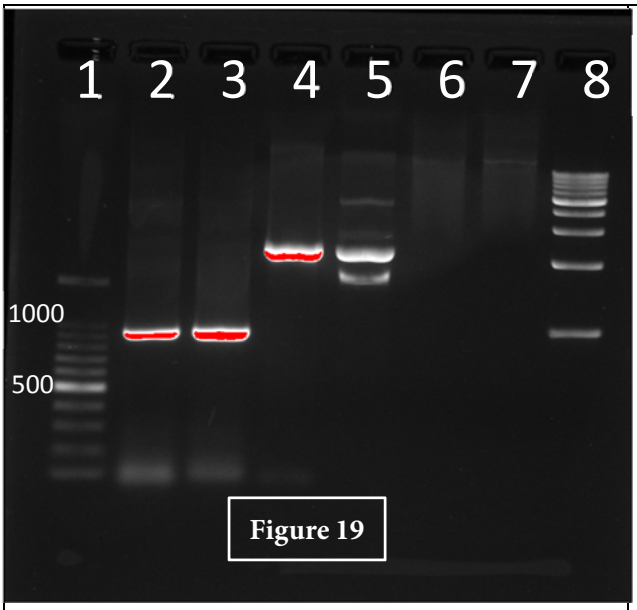

Figure 19

1: 100 bp marker;
2: rGFP(1) ,with double digestion;
3: rGFP(2),with double digestion;
4:.Fusion Promoter, with double digestion;
5: Fusion Promoter, with single digestion;
6:.Fusion Promoter, with double digestion;
7: Fusion Promoter, with single digestion;
8:1kbp marker.
(The rGFP is the GFE in reverse.)

Purpose: The verification of the products of PCR: rGFP, Fusion Promoter.
Results/discussion: The length of rGFP was 875 bp, and the length of Fusion Promoter was 130 bp. From the result, we could confirm that rGFP and Fusion Promoter were correct.

2014-09-10

- The Experiment Plan:
  With the 0.01 mM IPTG, 0.02%Ara or the 0.25 mM IPTG and its corresponding concentration gradients of Ara. We were going to use the concentration gradients of Ara in 1%, 5% and 10%.
- Aim:
  We wanted to get the most appropriate concentration of Ara to draw the parabola.
- Characterization:

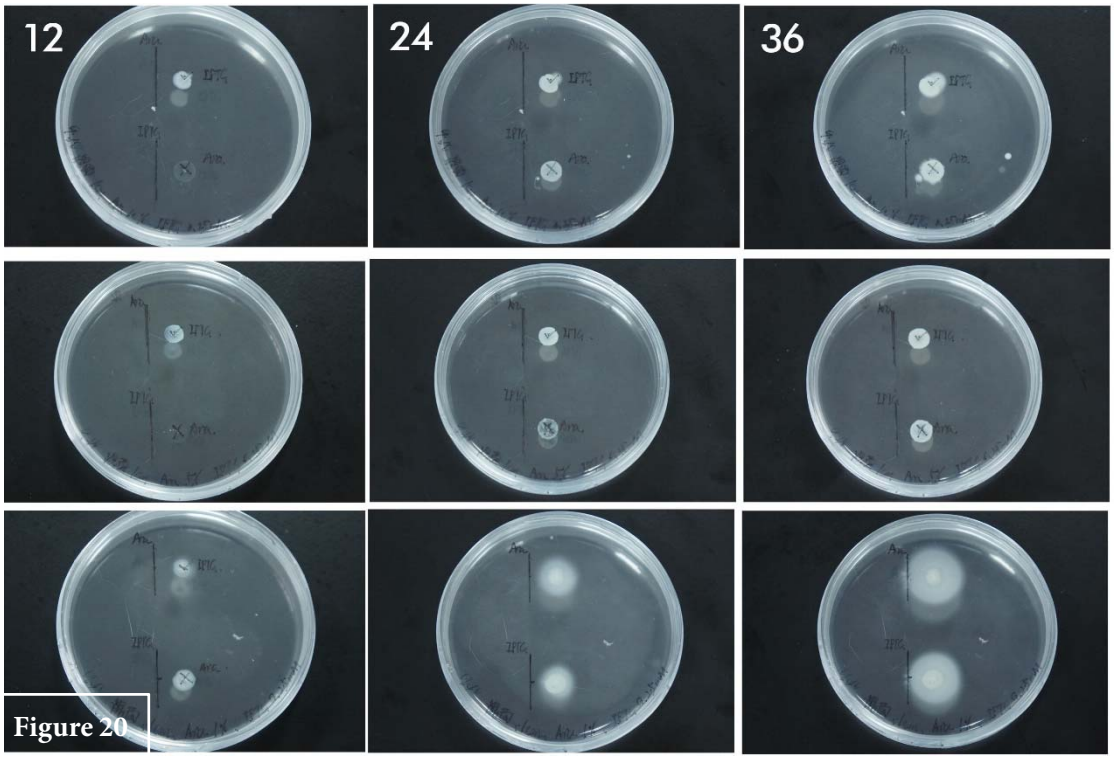

Figure 20

- Conclusion:

  With the 0.01 mM, 0.02% Ara in the medium, when plotting with 0.025 mM IPTG and drawing lines with 1% ARA, if the distance between the plot and the line was 1.5 cm, then the *E. coli* could form the parabola pattern.

2014-09-11

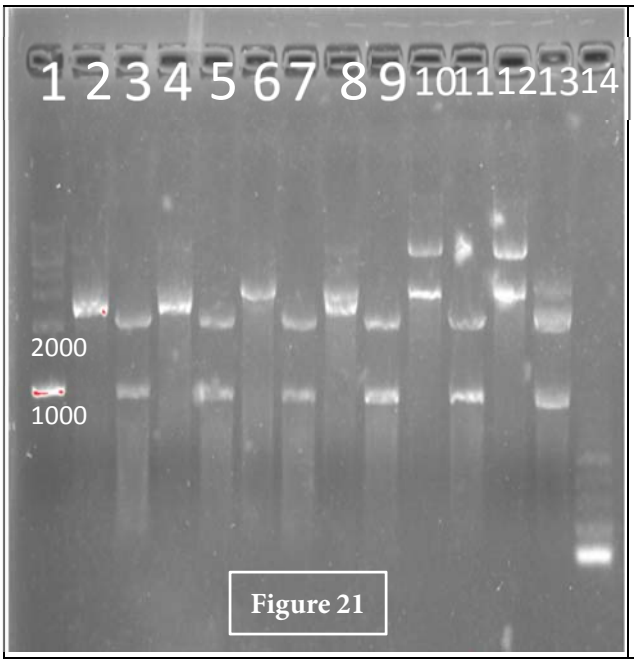

Figure 21

1: 1kb marker;
2, 4, 6, 8, 10, 12: Fusion:Promoter+pSB1C3, with single digestion;
3, 5, 7, 9, 11, 13: Fusion:Promoter+pSB1C3, with double digestion;
14: 100bp marker.

Purpose: To verify the PCR is correct or not.
Results/discussion: From the figure, we determined that PCR failed.

2014-09-13

- Activation of bacteria

  Use pipette to transfer 50 μL bacterium solution (pLac-RBS(1.0)-*CheZ*-TT, pLac-RBS(0.01)-*CheZ*-TT, pLac-RBS(0.3)-*CheZ*-TT) respectively into 5 ml LB liquid medium whose antibiotic concentration is 50 μg/ml. Culture for 12 h but no bacterium come back to life. Continue culture.

  Then transfer 50 μL bacterium solution into new LB liquid medium whose antibiotic concentration is 50 μg/ml to culture for 3 h.

- Characterization

  Then stab 3 μL bacterium medium into the M63 semisolid medium at the dots. And culture the bacteria in constant temperature and humidity incubator at 37℃.

2014-09-14

- Measurement

Measure the radius of *E. coli*.
Cm=50 μg/ml, IPTG=0.025 mM, ARA=0;

T/h	14A(1)	14A(2)	6F
0 h	0.25	0.25	0.25
12 h	0.40	0.40	0.40
24 h	0.60	0.65	0.90
36 h	0.75	0.83	1.18

Cm=50 μg/ml, IPTG=0, ARA=0;

T/h	2M (1)	2M(2)	6F	6F	14A
0 h	0.25	0.2	0.25	0.20	0.20
12 h	0.25	0.2	0.45	0.50	0.40
24 h	0.33	0.30	1.00	0.90	0.75
36 h	0.40	0.50	1.25	1.05	0.90

Cm=50 μg/ml, IPTG=0, ARA=0;

T/h	2M	6F	14A(1)	14A(2)
0 h	0.25	0.25	0.25	0.25
12 h	0.25	0.63	0.70	0.45
24 h	0.30	1.45	1.45	0.93
36 h	0.35	1.55	1.90	1.30

Use pipette to transfer 50 μL bacterium solution pLac-RBS(1.0)-*CheZ*-TT, pLac-RBS(0.01)-*CheZ*-TT, pLac-RBS(0.3)-*CheZ*-TT respectively into 5 ml LB liquid medium whose antibiotic concentration is 50 μg/ml. Culture for 12 h.

2014-09-18

Figure 22

1: BBa_J00450(backbone restricted by *EcoR* I);
2: BBa_J00450(backbone restricted by *EcoR* I and *Pst* I);
3:BBa_J00450(backbone restricted by *EcoR* I);
4: BBa_J00450(backbone restricted by *EcoR* I and *Pst* I);
5: 500 bp Marker;
6: BBa_J00450(backbone retricted by *Xba* I and *Pst* I).

Purpose: To verify the length of BBa_J00450.
Results/Discussion: The theoretical length of BBa_J00450 was 1069 bp. And we found that the length of the first and the third strip was right. However, when BBa_J00450 was restricted by double enzyme, the results of the verification was not correct. What's more, on the sixth strip, the length of the backbone(theoretical 2070 bp) was not correct.

2014-09-24

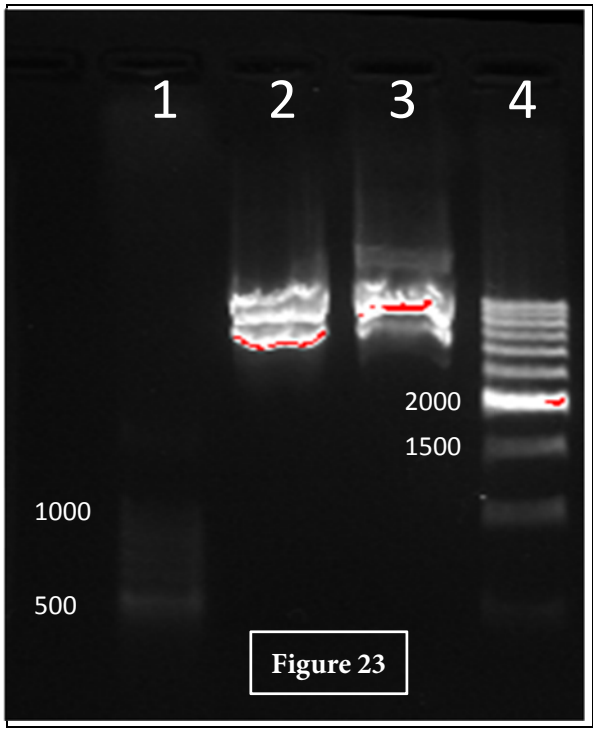

Figure 23

1: 100 bp marker;
2: BBa_J00450(backbone restricted by *EcoR* I);
3: BBa_J00450(backbone restricted by *EcoR* I and *Pst* I);
4: 500bp marker.

Purpose: To verify the length of BBa_J00450.
Results/Discussion: The strip of 100bp marker was dragged seriously and we couldn't see it clearly. And when BBa_J00450 was restricted by single enzyme, its theoretical length is 3139bp, which was just fitted what we showed. However, when it was restricted by double enzyme, its length was the same as when it was restricted by single enzyme, which was clearly not right. Unfortunately, all the strips was dragged seriously.

2014-09-25

- Extract the Plasmids: 2014-P2-6F
- Activation of bacteria

  Use pipette to transfer 50 μL bacterium solution pLac-RBS(1.0)-*CheZ*-TT, pLac-RBS (0.01)-*CheZ*-TT, pLac-RBS(0.3)-*CheZ*-TT respectively into 5 ml LB liquid medium whose antibiotic concentration is 50 μg/ml. Culture for 3 h.

2014-09-26

- Measurement: Measure the radius of *E. coli*.

Cm=50 μg/ml, IPTG=0.025 mM, ARA=0;

T/h	2M	6F	14A
0 h	0.25	0.20	0.25
12 h	0.30	0.20	0.35
36 h	0.60	0.70	0.70
48 h	0.70	0.80	0.80
59 h	1.20	1.50	1.20
72 h	1.60	1.70	1.60
83.5 h	1.70	1.80	1.80

Cm=50 μg/ml, IPTG=0.025 mM, ARA=0;

T/h	2M	2L-1	2L-1	2J-1	2J-1
0 h	0.25	0.25	0.25	0.25	0.25
12 h	0.40	0.30	0.35	0.30	0.30
36 h	0.60	0.35	0.40	0.65	0.70
48 h	0.75	0.70	0.70	0.85	0.90
59 h	1.10	0.90	1.00	1.30	1.30
72 h	1.50	1.30	1.35	1.70	1.40
83.5 h	1.80	1.55	1.60	2.20	1.70

Cm=50 μg/ml, IPTG=0.025 mM, ARA=0;

T/h	2M	6F	14A
0 h	0.25	0.25	0.30
12 h	0.55	0.60	0.80
36 h	0.75	0.90	0.90
48 h	1.05	1.50	1.50
59 h	1.25	1.50	1.75
72 h	1.25	1.50	1.75
83.5 h	1.70	1.80	1.80

Cm=50 μg/ml, IPTG=0.025 mM, ARA=0;

T/h	2M	6F	14A
0 h	0.25	0.25	0.25
12 h	0.25	0.25	0.3
36 h	0.50	0.70	0.40
48 h	0.80	0.73	0.60
59 h	1.20	1.15	1.00
72 h	1.40	1.30	1.40

Cm=50 μg/ml, IPTG=0.025 mM, ARA=0;

T/h	2M	2L-1	2L-2	2J
0 h	0.25	0.25	0.25	0.20
12 h	0.35	0.30	0.35	0.21
36 h	0.60	0.40	0.40	0.50
48 h	0.80	0.70	0.70	0.95
59 h	1.00	0.80	1.00	1.00
72 h	1.60	1.30	1.30	1.40
83.5 h	1.90	1.40	1.70	1.75

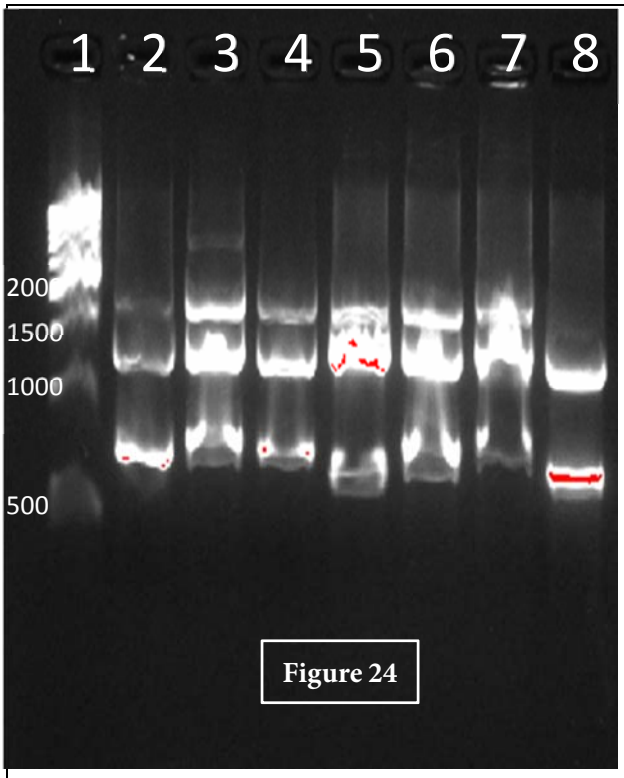

Figure 24

1: 500bp marker;
2:BBa_B0033+BBa_K629003+BBa_B0015(1);
3:BBa_B0033+BBa_K629003+BBa_B0015(2);
4:BBa_B0032(0.3RBS);
5:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015;
6:BBa_R0040+BBa_B0034+BBa_I1732100+BBa_B0015+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015;
7:BBa_K206000+BBa_B0034+BBa_I732100+BBa_B0015+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015;
8:BBa_J61002(RFP).
(1, 2 are different colonies.)

Purpose: The verification of the connection systems BBa_B0033+BBa_K629003+BBa_B0015, BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015, BBa_R0040+BBa_B0034+BBa_I1732100+BBa_B0015+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_K206000+BBa_B0034+BBa_I732100+BBa_B0015+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015, the length of BBa_B0032.

Results/Discussion: We could see from the image that all the strips were dragged seriously. All the circuits were different but they showed the same length, which meant that they were not all correct.