LABNOTE-B

XMU-iGEM

Date: 8.1-8.31

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
31						

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	31		

7 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				

9 M 2014 Y

NOTE :

2014-8-1

- Polymerase Chain Reaction
- Measure the Concentration of the Plasmids

	Absorbance:260/280	Measurement(ng/μL)
2M+18G-1	1.87/1.85	112.8/146.8
2M+18G-2	1.86	87.5
2M+18G-3	1.87/1.82	150.4/148.5
2M+18G-4	1.87/1.83	121.6/135.0
2M+18G-5	1.85/1.90	135.9/134.7

- Conclusion: The 2nd sample's concentration was apparently lower than others. It was because the DNA supernatant was mostly kept on the wall of the centrifuge tube .
- Enzyme Restriction

CheZ+T	*EcoR* I, *Spe* I

- Verification: Agarose gel electrophoresis

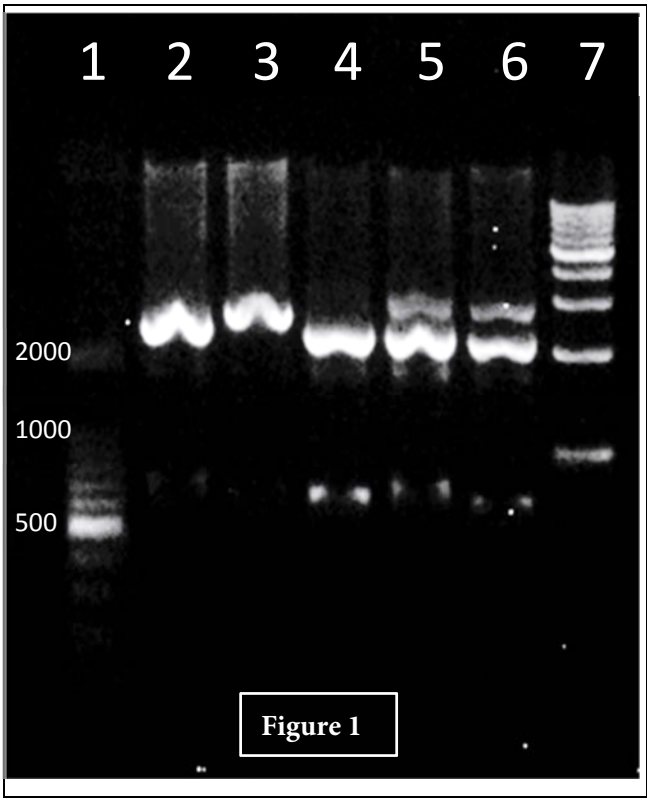


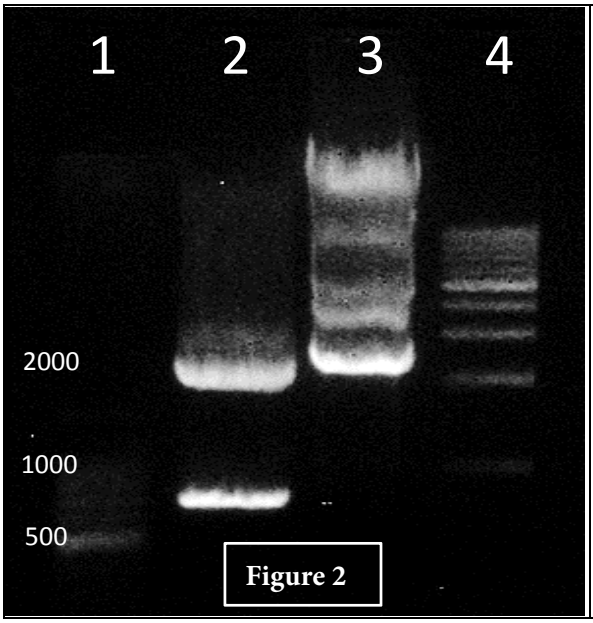
Figure 1

1: 100 bp marker;
2: BBa_B0034+BBa_K629003(1);
3: BBa_B0034+BBa_K629003(2);
4: BBa_B0034+BBa_K629003(3);
5: BBa_B0034+BBa_K629003(4);
6: BBa_B0034+BBa_K629003(5);
7: 1kb marker.

((1), (2), (3), (4), (5) are different colonies on the same plate)
Purpose: The verification of the connection system BBa_B0034+BBa_K629003.
Results/discussion: From the result, we could know that (3), (5) colonies containing the *CheZ* gene, (1), (4) colonies may have *CheZ* gene, but the result was not too ideal while the other colonies didn't have. In general, The effect of the gel electrophoresis was not very good.

2014-8-2

- Ligation
  RBS+*CheZ*+T
- Preparation for the Competent Bacteria
- Verification: Agarose gel electrophoresis



1: 100 bp marker;
2: BBa_B0034+BBa_K629003;
3: BBa_B0015(double terminator);
4: 1kb marker.

Purpose: Preparation for the ligation between RBS+*CheZ* and Double terminator.
Results/discussion: We wanted to use the plasmid containing double terminator as a backbone, then inserted RBS+*CheZ* gene to the backbone.

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
CheZ+RBS	1.68	20.9
TT	1.72	30.1

- *CheZ*+RBS-1, TT-2
  V_1/V_2=1.3

2014-8-3

- Select the Colonies and then transfer the plasmids into these colonies.

2014-8-4

- Measure the Concentration of the Plasmids (There were two samples from one plate, and each sample was measured three times)

	Absorbance: 260/280	Measurement (ng/μg)
2M-18G-4F1(+)	1.84/1.87/1.86	488.1/510.7/211.9
2M-18G-4F1(-)	1.81/1.87/1.85	505.7/603.5/546.5
2M-18G-4F2(+)	1.78/1.83/1.86	192.9/330.0/256.6
2M-18G-4F2(-)	1.85/1.84/1.86	256.6/189.1/261.1
2014-P3-14A	1.85/1.85/1.88	321.4/240.0/208.9
2014-P3-1N	1.74/1.84/1.85	467..9/327.1/377.7

- Enzyme Restriction

2M-18G-4F(1,2)(+,-)/2014-P3-1N/2014-P3-14A	*EcoR* I, *Spe* I

- Verification: Agarose gel electrophoresis

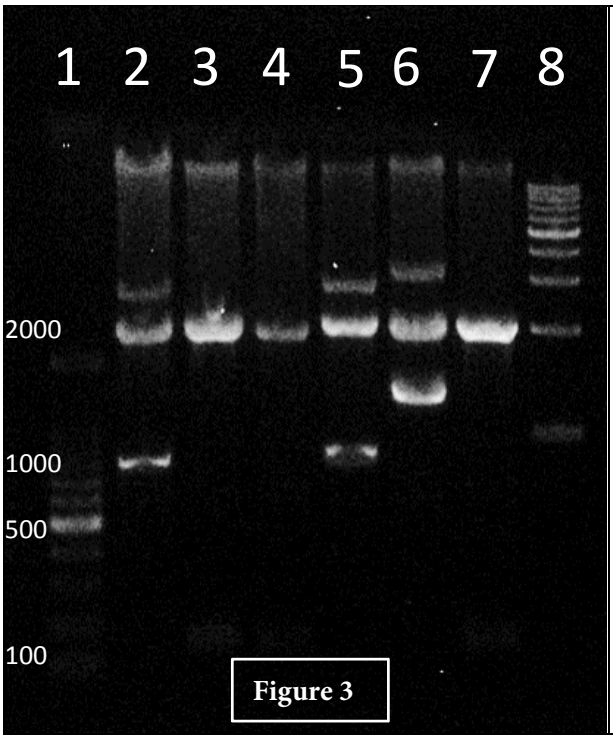


Figure 3

1: 100 bp marker;
2:BBa_B0034+BBa_K629003+BBa_B0015(+1)
3:BBa_B0034+BBa_K629003+BBa_B0015(+2)
4:BBa_B0034+BBa_K629003+BBa_B0015(-1);
5:BBa_B0034+BBa_K629003+BBa_B0015(-2);
6: BBa_I732820(LacI);
7: BBa_K206000.
8: 1kp marker.
(+1, +2, -1, -2 are the different colonies on the same plate.)

Purpose: The verification of the connection system RBS+*CheZ*+TT(BBa_B0015 as the backbone, BBa_B0034+BBa_K629003 as the insert gene), and BioBricks: BBa_I732820(LacI), BBa_K206000.
Results/discussion: BBa_B0034+BBa_K629003+BBa_B0015 was 786 bp. From the result, we could know that (+ 1) and (-2) with double enzyme digestion were right. But there still had bands near 3000 bp, which declared that double enzyme digestion not cut completely; (+ 2) and (-1) after double enzyme digestion, we could find the band near 2000 bp, thwas maybe the backbone, While the band between 100 bp to 200 bp may be 4F, so (+ 2) and (-1) must be self-join. The length of BBa_I732820 (LacI) was 1241 bp. From the result, we could know the bacteria had the LacI gene, but still had a band above 3000 bp, declared that the double enzyme digestion did not cut completely.1 BBa_K206000 gene after double enzyme digestion, we could got the 2070 bp backbone and the band of 130 bp, so the bacteria contain the BBa_K206000.

- Enzyme Restriction

pBAD/2014-P3-14A	LacI/2014-P3-1N
Spe I, *Pst* I	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis

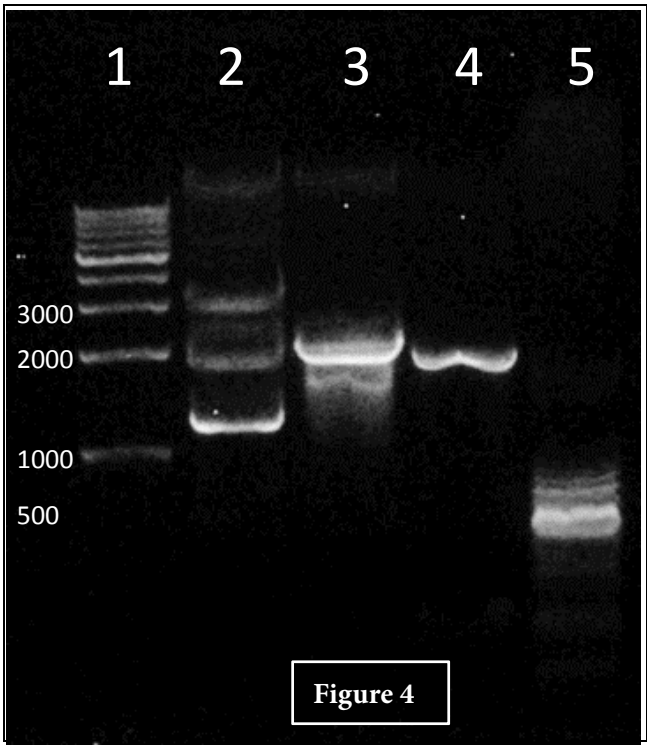


Figure 4

1: 1kb marker;
2: BBa_I732820(LacI);
3: BBa_k206000;
4: BBa_R0010;
5: 100 bp marker.

Purpose: The gel electrophoresis was prepared for the ligation between BBa_K206000(pBAD) and BBa_I732820(LacI).
Results/discussion: We used BBa_K206000(pBAD) as backbone, BBa_I732820(LacI) as insert gene. At the same time, we wanted to ligate BBa_R0010(pLac) with RBS+*CheZ*+TT. From the figure, we knew that all the length are correct, But the effect of the image was not very good. The possible reason was that the enzyme cutting time was too long or too short.

	Centrifuge Tube	All	Agarose gel
2014-P3-1N	0.910 g	0.974 g	0.064 g
2014-P3-14A	0.930 g	1.003 g	0.073 g

- Measure the Concentration of the Plasmids

	Absorbance: A260/A280	Measurement(ng/μL)
2014-P3-1N	1.92/1.77/1.87	3.3/4.5/3.3
2014-P3-14A	1.59	25.5

- 1N-1, 14A-2
  V1/V2=9
- Ligation

1N+14A	Positive	Negative
Enzyme	T4D	T4D

2014-8-5

- Transformation
  2014-P3-14G
- Ligation
  pLac+(RBS+*CheZ*+TT)

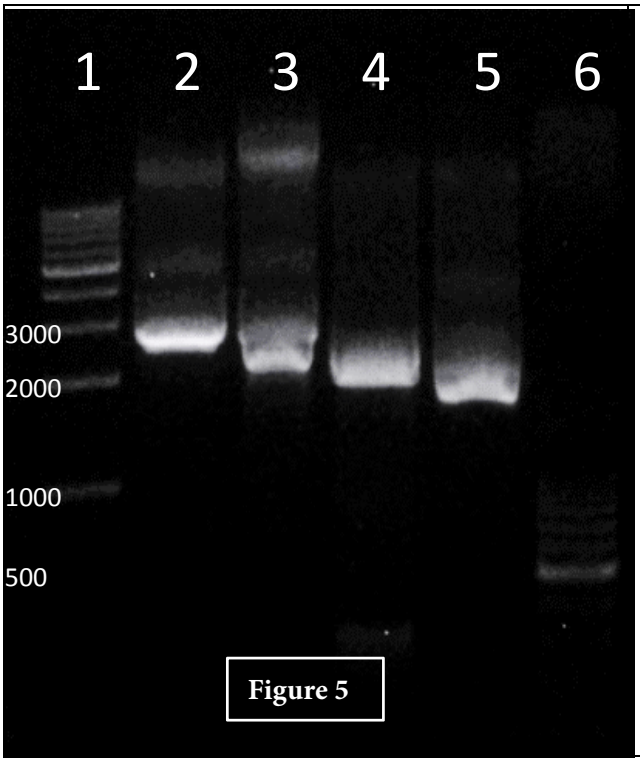


Figure 5

1: 1kb marker;
2: BBa_B0034+BBa_K629003+BBa_B0015(-2);
3: BBa_B0034+BBa_K629003+BBa_B0015(+1);
4: BBa_R0010(pLac) with double digestion;
5: BBa_R0010(pLac) with single digestion;
6: 100 bp marker.

Results/discussion: The result was so bad that we could got any conclution.

2014-8-6

- Measure the Concentration of the Plasmids:

	Absorbance: 260/280	Measurement(ng/μg)
2013-P3-4F	1.82/1.84	1025.3/761.2
2014-P2-2J1	1.84/1.81	842.6/809.9
2014-P2-2J2	1.82/1.83	601.1/692.9
2014-P2-1H1	1.71/1.70	111.6/141.6

- Enzyme Restriction

Single	2014-P2-2J/2014-P2-1H	*Xba* I
Double	2013-P3-4F	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis

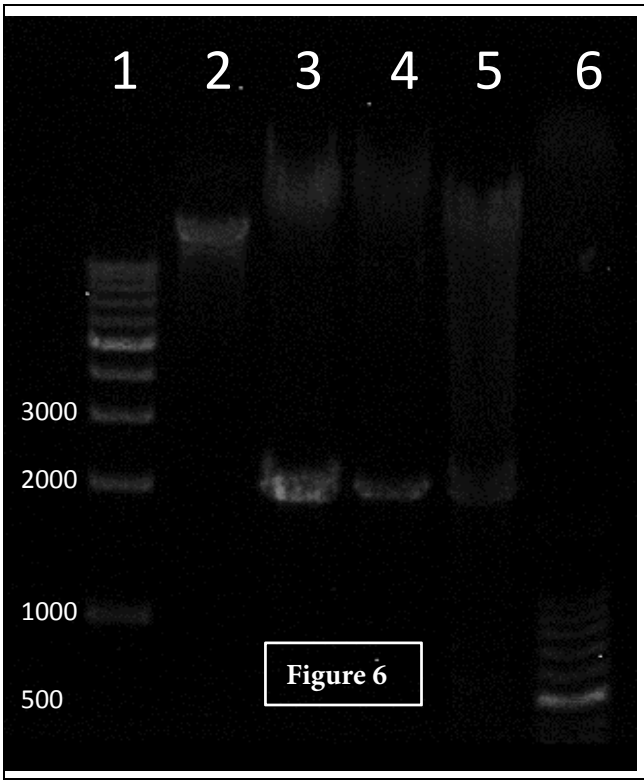


Figure 6

1: 1kb marker;
2: BBa_B0031(RBS(0.07));
3: BBa_B0032(RBS(0.3))(1);
4: BBa_B0032(RBS(0.3))(2);
5: BBa_B0015(double terminator);
6: 100 bp marker.
(1, 2 are different colonies on the same plate.)

Purpose: The verification of BioBricks: BBa_B0031, BBa_B0032, BBa_B0015.
Results/discussion: From the figure, we couldn't get a conclusion because of the tailing phenomenon.

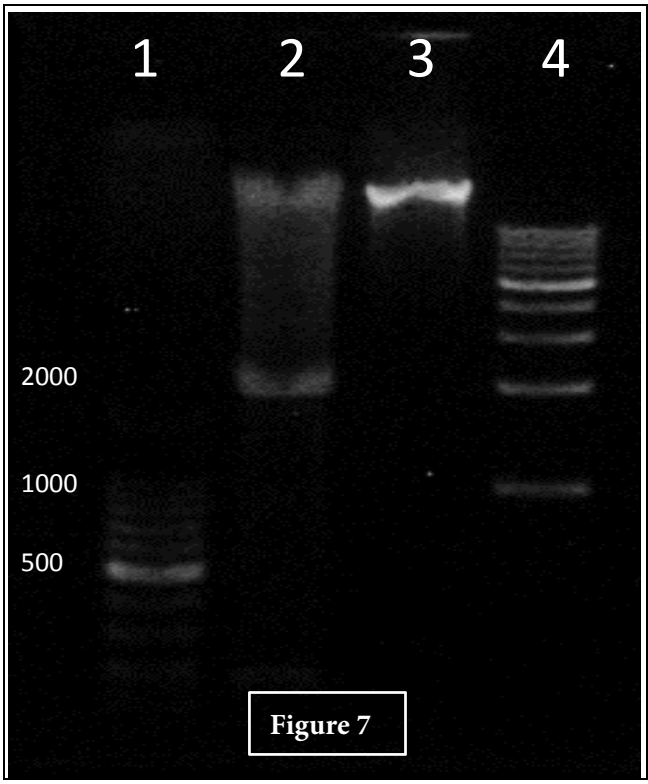


Figure 7

1: 100 bp marker;
2: BBa_B0015(double terminator);
3: BBa_B0031(RBS(0.07));
4: 1kb marker.

Purpose: The verification of BioBricks: BBa_B0015(double terminator) and BBa_B0031.
Results/discussion: From the length of gel electrophoresis bands, we could determine that the BBa_B0015(double terminator) gene we got was correct, but the BBa_B0031(RBS(0.07)) gene was wrong.

- Extraction of the Plasmids

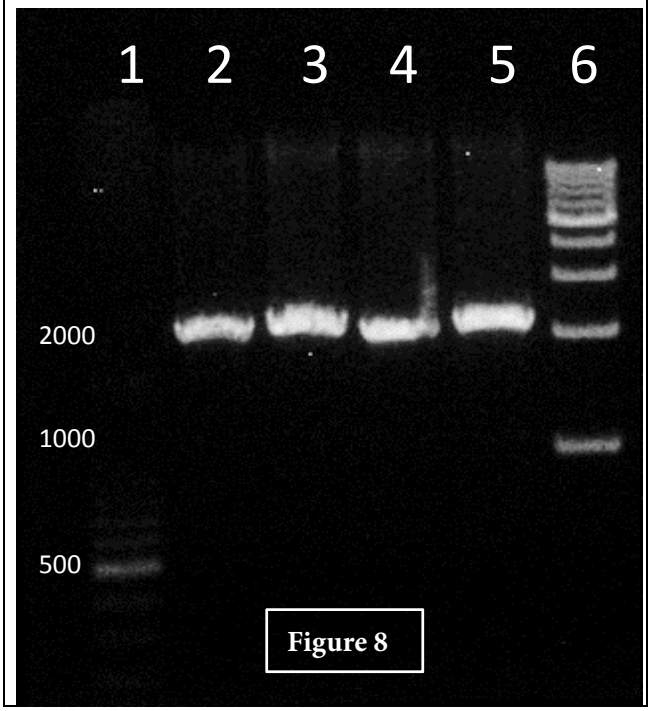


Figure 8

1: 100 bp marker;
2: BBa_K629003(1-1);
3: BBa_K629003(1-2);
4: BBa_K629003(2-1);
5: BBa_K629003(2-2);
6: 1kb marker.
((1-1), (1-2), (2-1), (2-2) are the different colonies.)

Purpose: We wanted to verify the plasmid we got contain the *CheZ* gene.
Results/discussion: From the figure, we just got backbone after the digestion, while we could determine they didn't have the *CheZ* gene.

- Measure the Concentration of the *CheZ*

	Absorbance: 260/280	Measurement(ng/ μL)
1-1	1.96	157.3
1-2	1.82	107.8
1-3	1.82	120.6
2-1	1.86	158.8
2-2	1.83	93.6

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement (ng/μL)
14A+1N(+)-1(1)	1.83/1.87/1.85	365.5/361.3/349.8
14A+1N(+)-1(2)	1.78	450.3
14A-1N(+)-1(3)	1.75/1.85/1.84	511.1/444.2/469.6
14A-1N(+)-2(1)	1.78/1.84/1.82	385.9/349.0/361.0
14A-1N(+)-2(2)	1.81	440.4
14A-1N(+)-2(3)	1.77/1.82	442.0/398.9
14A-1N(-)-1(1)	1.72/1.80	375.3/327.8
14A-1N(-)-1(2)	1.85/1.85	242.5/258.8
14A-1N(-)-1(3)	1.79/1.74/1.82	241.6/236.5/221.1

- Enzyme Restriction

14A+1N(+)/14A+1N(-)	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis

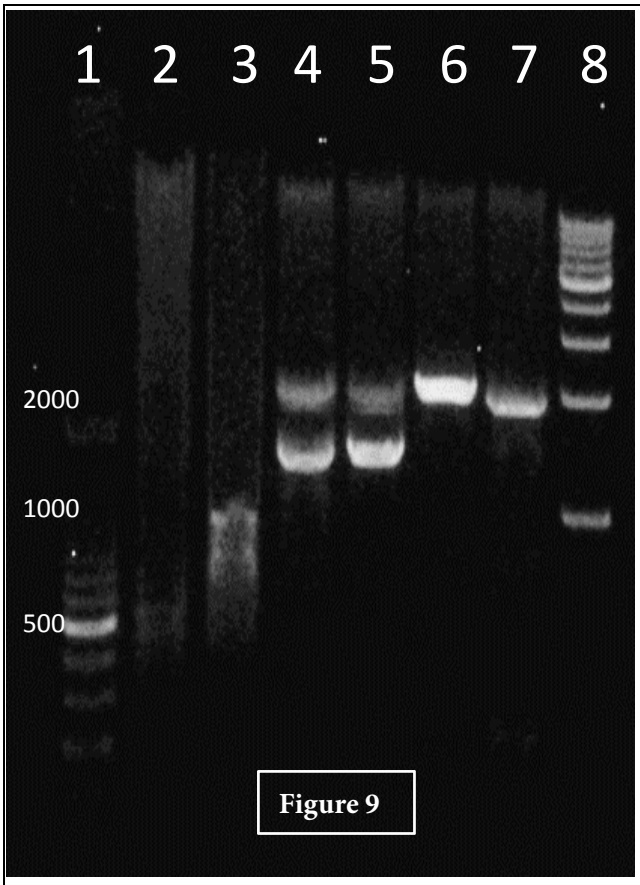


Figure 9

1: 100 bp marker;
2: BBa_J23101(pCONs)(1);
3: BBa_J23101(pCONs)(2);
4: BBa_k206000+BBa_I732820(+1);
5: BBa_k206000+BBa_I732820(+2);
6: BBa_k206000+BBa_I732820(-1);
7: BBa_k206000+BBa_I732820(-2);
8: 1kb marker.
((+1), (+2), (-1), (-2) are different colonies.)

Purpose: The verification of BioBrick BBa_J23101(pCONs) and the connection system BBa_k206000+BBa_I732820.

Results/discussion: First, the band of BBa_J23101(pCONs) was not clear, so we couldn't determine whether it was correct or wrong. Second, the connection system BBa_k206000+BBa_I732820 used BBa_k206000 as backbone, BBa_k206000 as insert gene. The length of both was1371 bp, from the figure, we could find (+1) and (+2) colonies was correct, while (-1) and (-2) are wrong, maybe self-join.

2014-08-07

- Verification: Agarose gel electrophoresis： *CheZ*

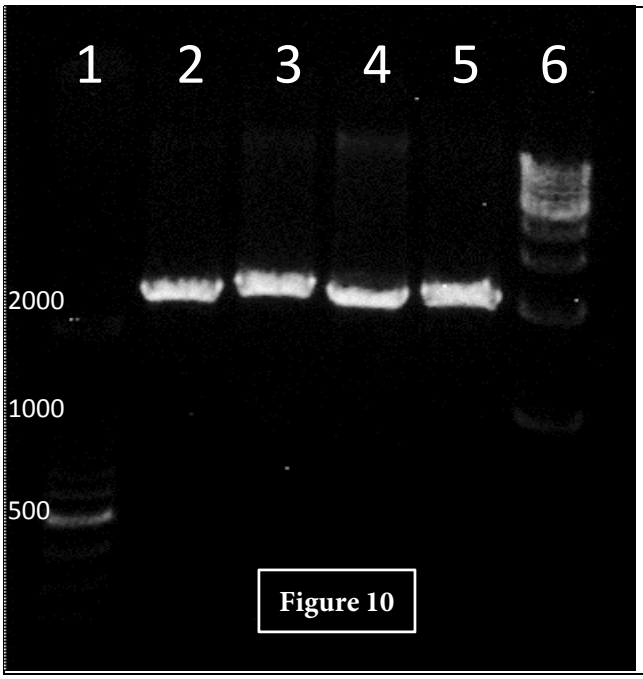


Figure 10

1: 100 bp marker;
2: BBa_K629003(1-1);
3: BBa_K629003(1-2);
4: BBa_K629003(2-1);
5: BBa_K629003(2-2);
6: 1kb marker.
((1-1), (1-2), (2-1), (2-2) are the different colonies.)

Purpose: We wanted to verify the plasmid we got containing the *CheZ* gene once more.
Results/discussion: From the figure, we just got backbone after the digestion, while we could determine they didn't have the *CheZ* gene.

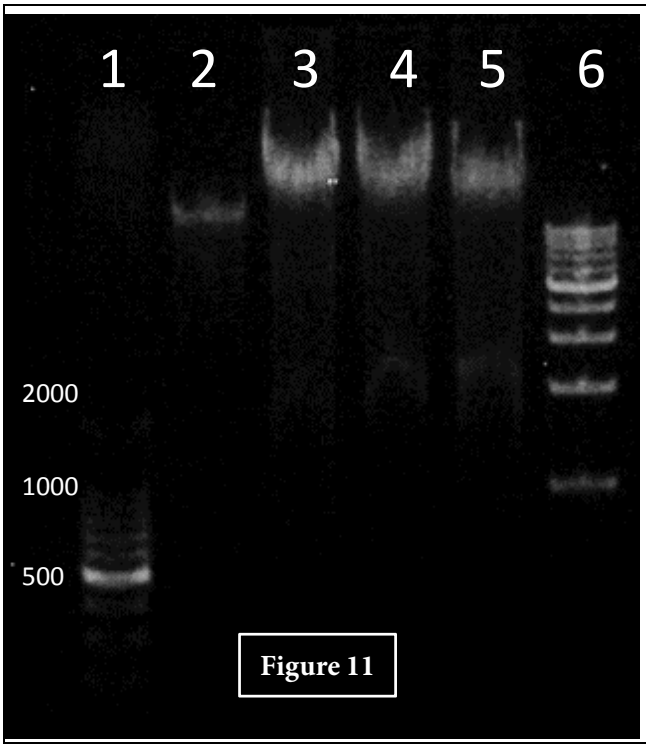


Figure 11

1: 100 bp marker;
2: BBa_J23101(pCONs)(1-4);
3: BBa_J23101(pCONs)(1-5);
4: BBa_J23101(pCONs)(2-3);
5: BBa_J23101(pCONs)(2-4);
6: 1kb marker.
((1-4), (1-5), (2-3), (2-4) are the different colonies.)

Purpose: We wanted to prove the validity of BBa_J23101(pCONs) gene once again.
Results/discussion: At the end, we found that the figure was still not clear, so we couldn't determine whether it was correct.

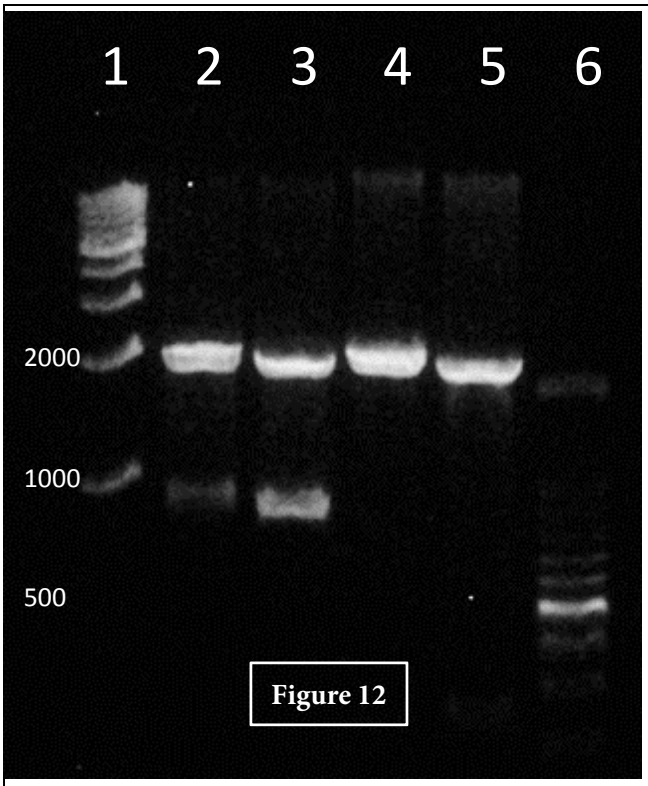


Figure 12

1: 1kb marker;
2:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(+1);
3:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(+2);
4: BBa_R0010(pLac)(1);
5: BBa_R0010(pLac)(2);
6: 100 bp marker.
((+1), (+2), (1), (2) are different colonies.)
Purpose: The verification of the connection system BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015.
Results/discussion: The length of the system was 986 bp, from the figure, we could declare that we connected BBa_R0010 and BBa_B0034+BBa_K629003+BBa_B0015 successfully.
Second, the BBa_R0010(2) was correct, while BBa_R0010(1) was wrong.

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
pLac+RBS+*CheZ*+TT(+)1	1.85	106.9
pLac+RBS+*CheZ*+TT(+)2	1.84	177.9
pLac_1	1.84	185.0
pLac_2	1.83	219.0

- Enzyme Restriction

  Preparation for the Competent Bacteria
- Measure the Concentration of the *CheZ*

	Absorbance: 260/280	Measurement(ng/μL)
1-1	1.85	127.8
1-2	1.84	148.4
1-3	1.85	109.5
1-4	1.84	159.1

- Verification: Agarose gel electrophoresis

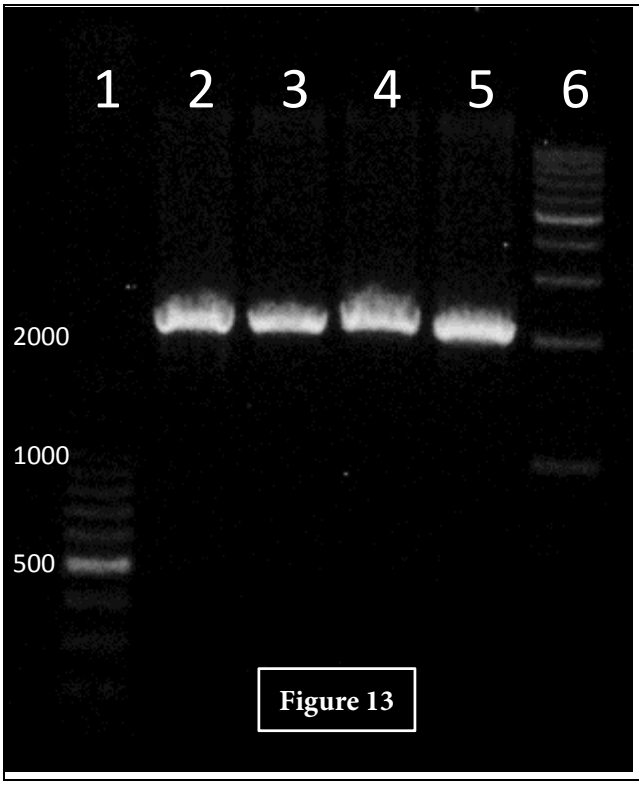


Figure 13

1: 100 bp marker;
2: BBa_K629003(1-1);
3: BBa_K629003(1-2);
4: BBa_K629003(2-1);
5: BBa_K629003(2-2);
6: 1kb marker.
((1-1), (1-2), (2-1), (2-2) are different colonies.)

Purpose: We wanted to verify the plasmids we got containing the *CheZ* gene once more.
Results/discussion: From the figure, we just got backbone after the digestion, while we could determine they didn't have the *CheZ* gene.

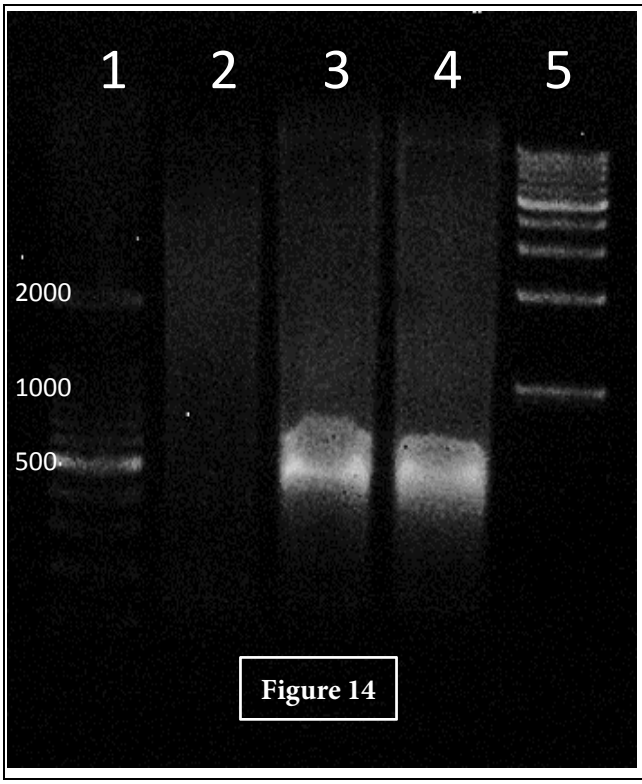


Figure 14

1: 100 bp marker;
2: BBa_J23101(pCONs)(1-5);
3: BBa_J23101(pCONs)(2-3);
4: BBa_J23101(pCONs)(2-4);
5: 1kb marker.
((1-5), (2-3), (2-4) are different colonies.)

Purpose: We wanted to prove the validity of BBa_J23101(pCONs) gene once again.
Results/discussion: At the end, we found that the figure still was not clear, so we couldn't determine whether it was correct.

- From left to the right: 100Marker-(2014-P3-18G_1)-(2014-P3-18G_2)-(1-1)-(1-2)-(2-

1)-(2-2)-1000Marker

- Conclusion: When we saw the photograph of thwas electrophoresis, we found that 2014-P3-18G_1 and 2014-P3-18G_2 hadn't been show on the graph, we guessed the reasons shown as the following: 2014-P3-18G_1 and 2014-P3-18G_2 weren't the correct plasmids of *CheZ*. The result of the electrophoresis of the 1-1 1-2 2-1 2-2 were not shown on the graph; there was only the graph of the vector. The problem was the BioBricks, we ought to begin with scratch.

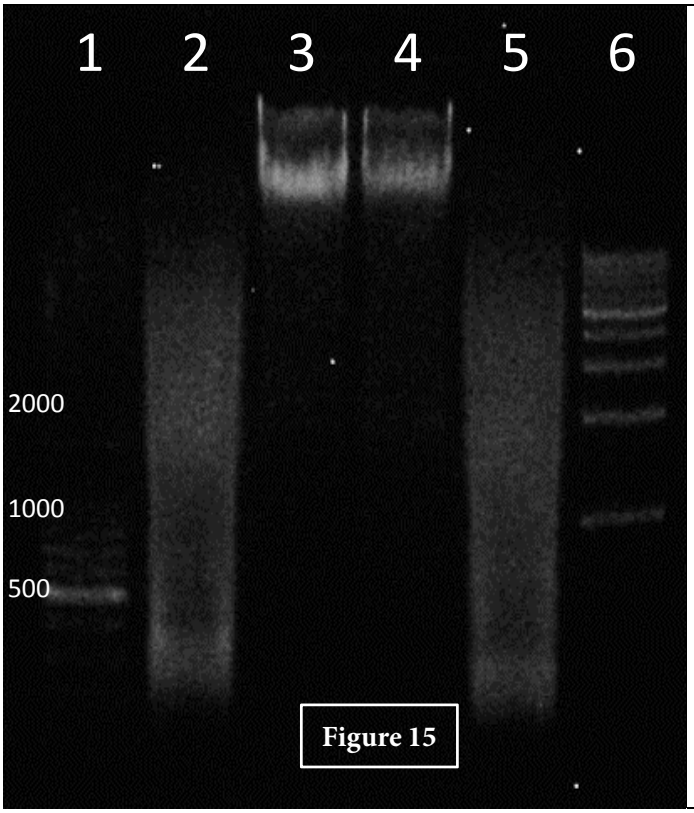


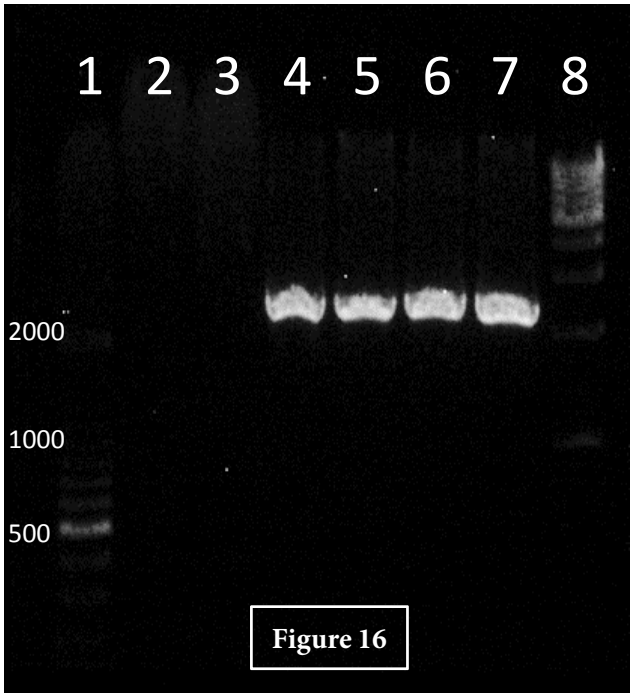
Figure 15

1: 100 bp marker;
2: BBa_J23101(pCONs)(2-3);
3: BBa_J23101(pCONs)(2-3)0;
4: BBa_J23101(pCONs)(2-4)2;
5: BBa_J23101(pCONs)(2-4)0;
6: 1kb marker.

Purpose: We wanted to prove the validity of BBa_J23101(pCONs) gene once again.
Results/discussion: We found that the figure still was not clear, so we couldn't determine whether it was correct.

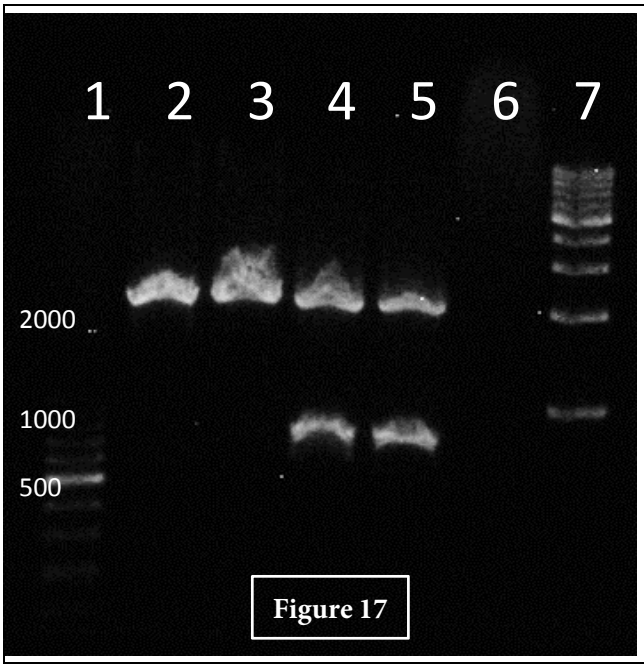
2014-08-08

- Verification: Agarose gel electrophoresis: *CheZ*



1: 100 bp marker;
2: BBa_K629003(1-1);
3: BBa_K629003(1-2);
4: BBa_K629003(2-1);
5: BBa_K629003(2-2);
6: 1kb marker.
((1-1) (1-2), (2-1), (2-2) are different colonies.)

Purpose: We wanted to verify the plasmid we got containing the *CheZ* gene once more. Results/discussion: From the figure, we just got backbone after the digestion, while we could determine they didn't have the *CheZ* gene.



1: 100 bp marker;
2: BBa_K629003(1-1);
3: BBa_K629003(1-2);
4: BBa_K629003(2-1);
5: BBa_K629003(2-2);
6: 1kb marker.
((1-1) (1-2), (2-1), (2-2) are different colonies.)

Purpose: We wanted to verify the plasmid we got containing the *CheZ* gene once more.
Results/discussion: From the figure, we just got backbone after the digestion, while we could determine they didn't have the *CheZ* gene.

2014-08-09

- Enzyme Restriction
- Verification: Agarose gel d

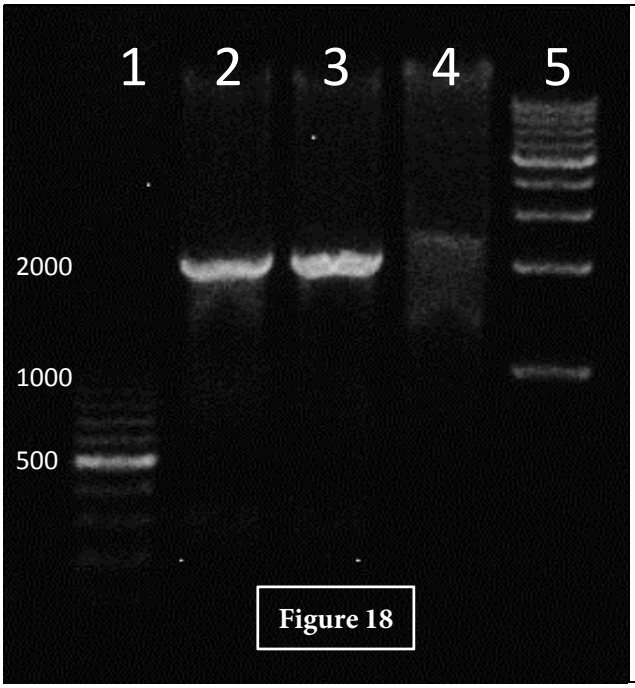


Figure 18

1: 100 bp marker;
2: BBa_R0010(1-1);
3: BBa_R0010(2-1);
4: BBa_J23101;
5: 1kb marker.
((1-1), (2-1) are different colonies.)

Purpose: We wanted to verify BBa_R0010 and BBa_J23101.
Results/discussion: From the figure, we could know that the BBa_R0010 was correct, while the BBa_J23101 was still not clear. So we couldn't determine whether the BBa_J23101 was correct or not.

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
2013-P5-18G	1.77/1.84	64.6/45.9

- Enzyme Restriction

2013-P5-18E	*EcoR* I

- Verification: Agarose gel electrophoresis
- From left to the right: 100Marker-(2013-P5-18E_2)-(2013-P5-18E_3)-1000Marker

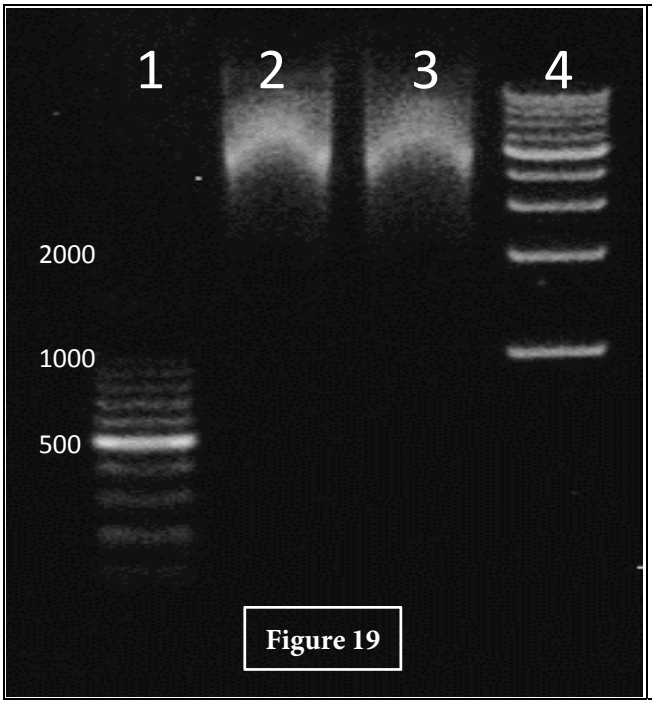


Figure 19

Results/discussion: We couldn't get a conclusion because of the tailing phenomenon.

- Conclusion: We could see the part of 200 bp， but every strip's color was lighter and dragged longer, we supposed that the enzyme restriction was not finished completely.

- Measure the Concentration of the Plasmids:

	Absorbance: 260/280	Measurement(ng/μL)
2014-P4-1H_1	1.86/1.89/1.85	566.6/570.6/404.5
2014-P1-18G_1	2.05/2.04/2.06	322.6/326.4/325.3
2013-P5-1H_2	1.83/1.83	217.9/322.2
2013-P1-18G_2	1.85/1.85/1.85	503.6/322.2/450.7

	Centrifuge Tube	All	Agarose Gel
2013-P3-3H_1-1	0.910 g	1.009 g	0.099 g
2013-P3-3H_2-1	0.887 g	0.991 g	0.104 g

	Absorbance: 260/280	Measurement(ng/μL)
2013-P3-3H_1-1	1.77	3.2
2013-P3-3H_2-1	1.72	3.1

2014-08-10

- Ligation

3H+(2M+18G+4F)	T4

- Enzyme Restriction

Single	2014-P4-1H_1	*EcoR* I
	2014-P4-1H_2	*EcoR* I
Double	2013-P1-18G_1	*EcoR* I, *Spe* I
	2013-P1-18G_2	*EcoR* I, *Spe* I

- Verification: Agarose gel electrophoresis

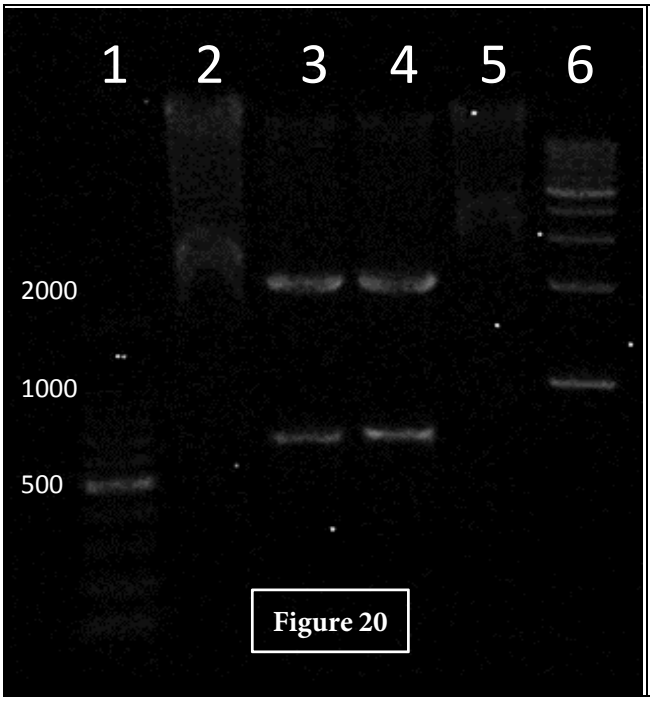


Figure 20

1: 100 bp marker;
2: BBa_B0031(RBS(0.07))(1);
3: BBa_K629003(*CheZ*)(1);
4: BBa_K629003(*CheZ*)(2);
5: BBa_B0031(RBS(0.07))(2);
6: 1kb marker.
((1), (2) are different colonies.)

Results/discussion: From the figure, we could determine that the BBa_K629003(*CheZ*) was correct, while the BBa_B0031(RBS(0.07)) was wrong.

- Measure the Concentration of the Plasmids

	Absorbance:260/280	Measurement(ng/μL)
2014-P2-6F	1.86/1.85/1.85	176.2/189.8/142.5

- Enzyme Restriction

2014-P2-6F	*EcoR* I

- Verification: Agarose gel electrophoresis
  From left to the right: 100Marker-(2014-P3-1N)-(2014-P2-6F)-1000Marker
- Enzyme Restriction

2014-P2-6F	*Spe* I, *Pst* I
2014-P3-1N	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis

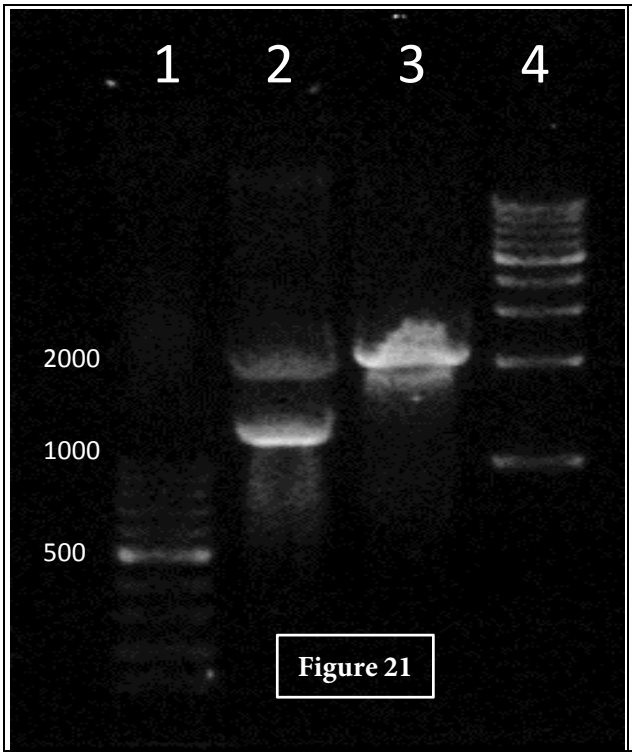


Figure 21

1: 100 bp marker;
2: BBa_I732820(LacI);
3: BBa_R0040(pTETR);
4: 1kb marker.

Results/discussion: From the figure, we could know that the BBa_I732820(LacI) was correct, at the same time, because the tripe of BBa_R0040(pTETR) above 2000 bp, so maybe it was correct too. And we could use it to connect with the connection system BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015.

	Centrifuge Tube	All	Agarose gel
2014-P3-1N	0.888g	0.946g	0.058g
2014-P2-6F	0.887g	0.941g	0.054g

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
2014-P3-1N	1.67/1.69	18.4/17.9
2014-P2-6F	1.95/1.79	8.5/11

(2014-P3-1N)----1, (2014-P2-6F)----2
V1/V2=1.000

- Conclusion: (2014-P3-4G)-(2013-P5-2M)-(2014-P1-18G)-(2013-P3-4F) was successfully ligated.

- Ligation

(2014-P3-1N)+(2014-P2-6F)　T4

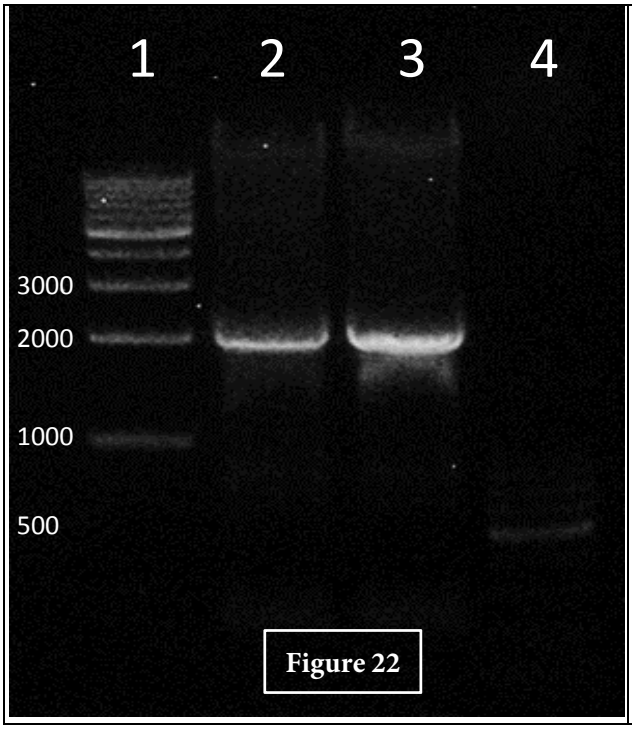


Figure 22

1: 1kb marker;
2: BBa_R0010(1-1);
3: BBa_R0010(2-1);
4: 100 bp marker.

Purpose: Prepare for the connection between BBa_R0010 and BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015.

Results/discussion: We used double enzyme to cut the plasmid which contained BBa_R0010 as the backbone, and the connection system BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 as the insert gene.

2014-08-11

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
3H-2M-18G-4F2.1(+)_2	1.78/1.77/1.80	385.8/409.3/306.3
3H-2M-18G-18G1.1(+)_1	1.81/1.84	260.1/553.0
3H-2M-15G-4F1.1(+)_2	1.78/1.83	448.7/292.5
3H-2M-18G-4F2.1(+)_1	1.82/1.85	134.0/221.8
4G-2M-18G-4F2.1(+)_2	1.86/1.86	905.2/258.1
4G-2M-18G-4F2.1(+)_1	1.84	271.5

2014-08-12

- Ligation

  CheZ+TT

- Enzyme Restriction

2014-P1-18G	*Xba* I, *Pst* I
2013-P3-4F	*Spe* I, *Pst* I

- Verification: Agarose gel electrophoresis

  From left to the right: 100Marker-(2014-P3-18G)-(2013-P3-4F)-1000Marker

- Enzyme Restriction

2014-P3-14A	*EcoR* I, *Spe* I
2014-P3-1N	*EcoR* I, *Xba* I

- Transformation of RBS

  The Plate of 2014-P4-1H was transformed successfully but the plate of 2013-P5-1H failed.

- Conclusion： There was no difference on the ampicillin resistance on these two plates.

	Centrifuge Tube	All	Agarose gel
2014-P1-18G	0.888 g	1.032 g	0.144 g
2013-P3-4F	0.886 g	0.960 g	0.074 g
14A+1N	0.892 g	0.984 g	0.092 g
3H+2M+18G+4F	0.985 g	0.953 g	0.058 g

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
2014-P1-18G	1.84	16.4
2013-P3-4F	1.77	20.3
14A+1N	1.96	149
3H+2M+18G+4F	1.67/1.71	13.8/14.0

- Ligation

  (2014-P1-18G) +(2013-P3-4F);

  (2014-P3-14A +2014-P3-1N) + (2013-P3-3H + 2013-P5-2M + 2014-P1-18G + 2013-P3-4F)

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
2014-P2-6F + 2014-P3-1N	1.87/1.87/1.85	610.9/848.1/539.2

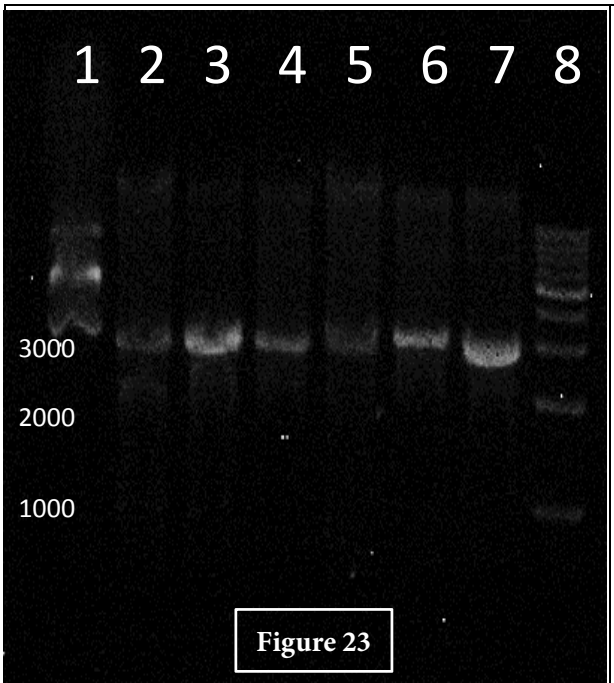


Figure 23

1: BBa_B0034+BBa_K629003+BBa_B0015;
2:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(1);
3:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(2);
4:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(3);
5:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(4);
6:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(5);
7:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(6);
8: 1kb marker.
((1), (2), (3), (4), (5), (6) are different colonies.)

Purpose: The verification of the connection systems: BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_B0034+BBa_K629003+BBa_B0015.

Results/discussion: BBa_B0034+BBa_K629003+BBa_B0015 connection system resulting from the single enzyme gene band length should be 3078 bp, and after the double enzyme gene band length should be 1008 bp, and the backbone length should be 2070 bp, according to the plastic figure, verify the correct connection system.

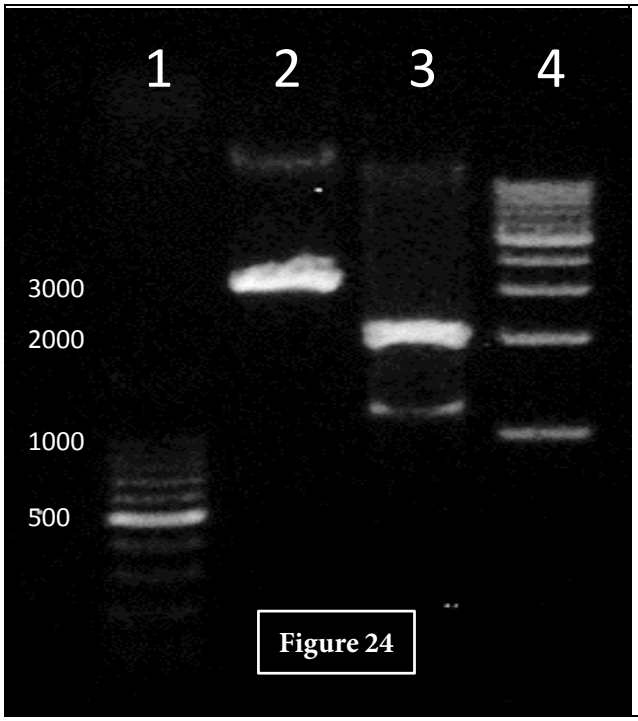


Figure 24

1: 100 bp marker;
2:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 with single digestion;
3:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 with double digestion;
4: 1kb marker.

Results/discussion: As we knew, the length of the BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 connection system was about 986 bp, while we got the strip above 1000 bp, so we couldn't determine that the connection system was correct.

2014-08-13

- Measure the Concentration of the Plasmids

  2014-P4-1H

	Absorbance: 260/280	Measurement(ng/μL)
1-1	1.85/1.86/1.85/1.85	300.1/228.3/239.1/391.6
2-1	1.83/1.87/1.87	149.9/107.0/154.2

- Enzyme Restriction

2014-P4-1H	*EcoR* I

- Verification: Agarose gel electrophoresis

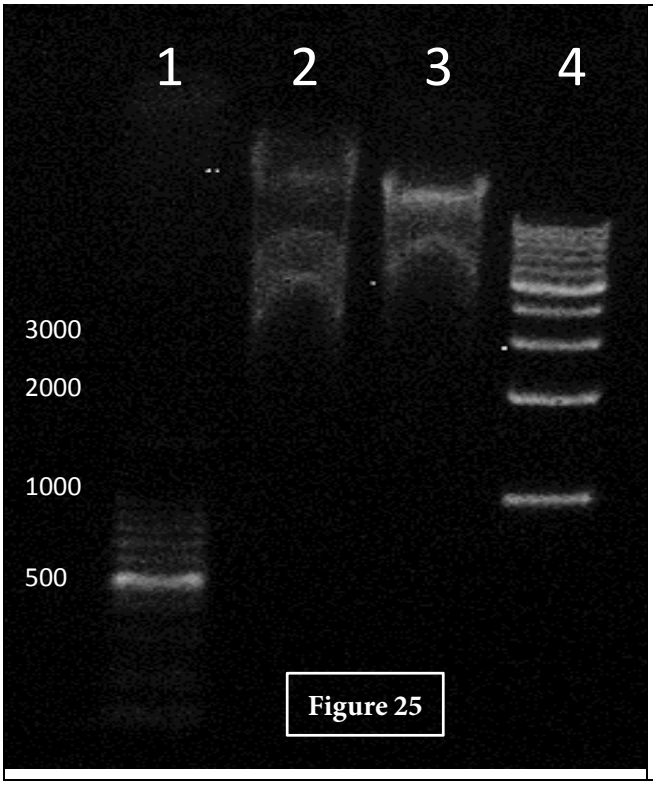


Figure 25

1: 100 bp marker;
2: BBa_B0031(RBS(0.07))(1.1);
3: BBa_B0031(RBS(0.07))(2.1);
4: 1kb marker.
((1.1), (2.1) are different colonies.)

Results/discussion: From the figure, we couldn't get any information about the gene we want, maybe the process of digestion and dye have some problems.

- Result: The middle two dragged with each other
- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement: (ng/μL)
13H-2M-18G-4F	1.86/1.84/1.81	438.9/412.9/576.6

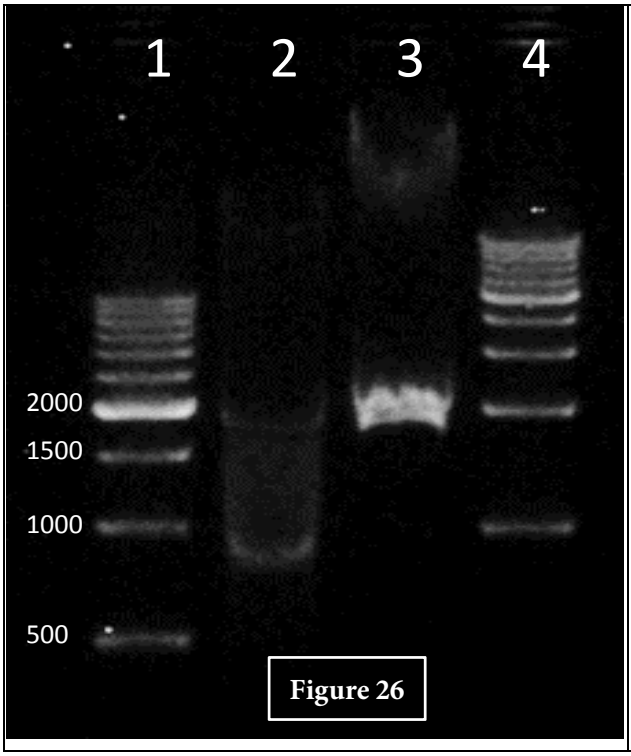


Figure 26

1: 500 bp marker;
2: BBa_R0040+ BBa_I732820 with double digestion;
3: BBa_R0040+ BBa_I732820 with single digestion;
4: 1kb marker.

Results/discussion: After gel electrophoresis, we couldn't get clear bands, so we don't know whether the connection system we got was correct or not.

2014-08-14

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement: ng/μL
6F-1N_1-2	1.75/1.66	63.7/66.8
6F-1N_2-2	1.68/1.90/1.59/1.67	78.3/61/87.3/74.3
1N_1	1.70/1.71/1.62/1.83	231.7/225.4/221/172.6
1N_2	1.81/1.84	175.7/207.5

- Enzyme Restriction

Single	1N	*EcoR* I
Double	6F-1N	*EcoR* I, *Spe* I

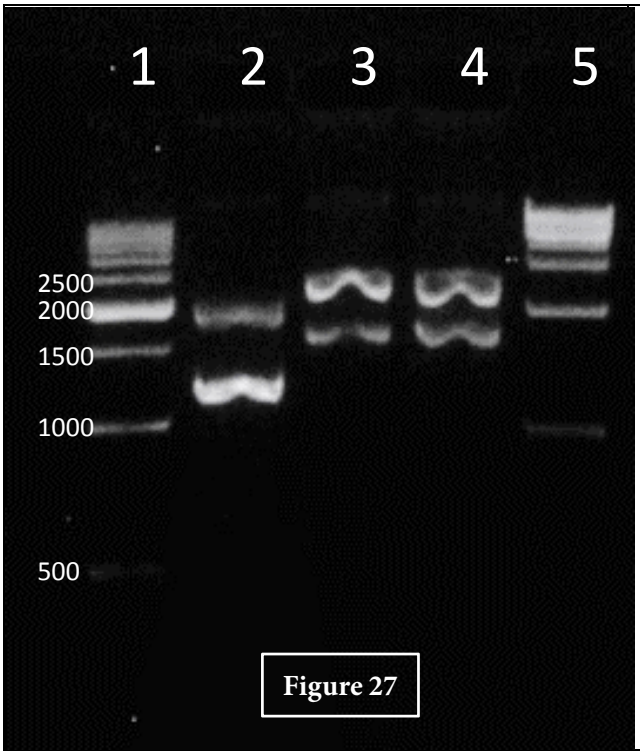


Figure 27

1: 500 bp marker;
2: BBa_I732820;
3: BBa_R0040+ BBa_I732820(1);
4: BBa_R0040+ BBa_I732820(2);
5: 1kb marker.
((1), (2) are different colonies on the same plate.)

Purpose: To verify the connection system BBa_R0040+ BBa_I732820.
Results/discussion: We usef the 2 times concentration of the original agar to reduce the *Spe*ed of the gene segment. After double enzyme digestion, we found that the length of the connection system BBa_R0040+BBa_I732820 longer than theoretical value, what's more, the frame length was longer than normal. So there might have some problems.

- Verification: Agarose gel electrophoresis

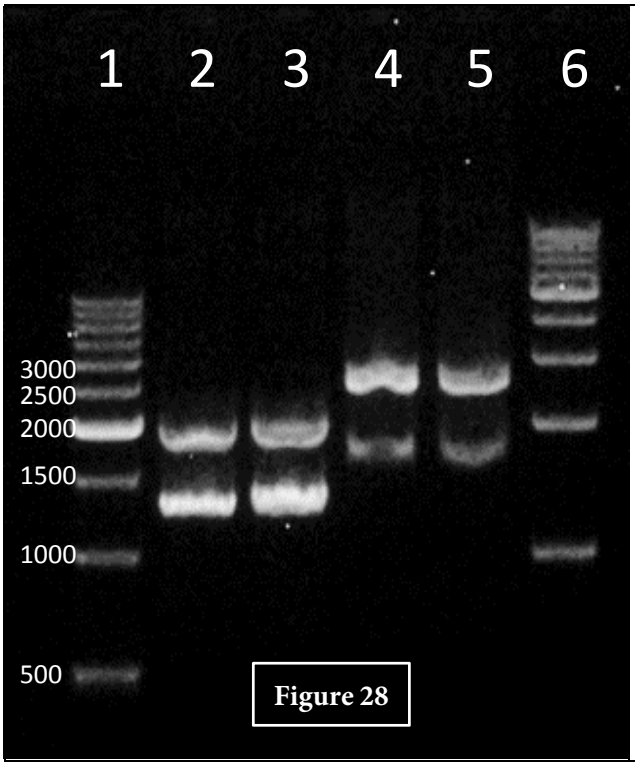


Figure 28

1: 500 bp marker;
2: BBa_I732820(LacI)(1);
3: BBa_I732820(LacI)(2);
4: BBa_R0040+ BBa_I732820(1);
5: BBa_R0040+ BBa_I732820(2);
6: 1kb marker.
((1), (2) are different colonies on the same plate.)

Purpose: We usef BBa_I732820(LacI) as reference to verify whether the connection system BBa_R0040+ BBa_I732820 was correct or not.
Results/discussion: The length of the BBa_R0040+ BBa_I732820 connection system was 1295 bp, only 54 bp longer than BBa_I732820(LacI), but by the figure, BBa_R0040+ BBa_I732820 connection system was more longer than BBa_I732820, what's more, the backbone was near 2500 bp, instead of 2070 bp, so the connection might be wrong.

- Measure the Concentration of the Plasmids:

	Absorbance: 260/280	Measurement (ng/μL)
(14A+1N)+(3H-2M-18G-4F)_2	1.85/1.85	327.9/335.4
(14A-1N)+(3H-2M-18G-4F)	1.83/1.87	167.4/154.9
(18G+4F)_2	1.86/1.83	157.4/133.2
(18G+4F)_1	1.87/1.84/1.86	170.8/176.1/173.9
(14A-1N)+(3H-2M-18G-4F)_1	1.85/1.86/1.86	370.5/401.5/341.3
2013-P5-1H	1.81/1.79/1.83	127.0/95.7/104.5

- Enzyme Restriction

3H-2M-18G-4F	
Single	*Pst* I
Double	*Xba* I, *Pst* I

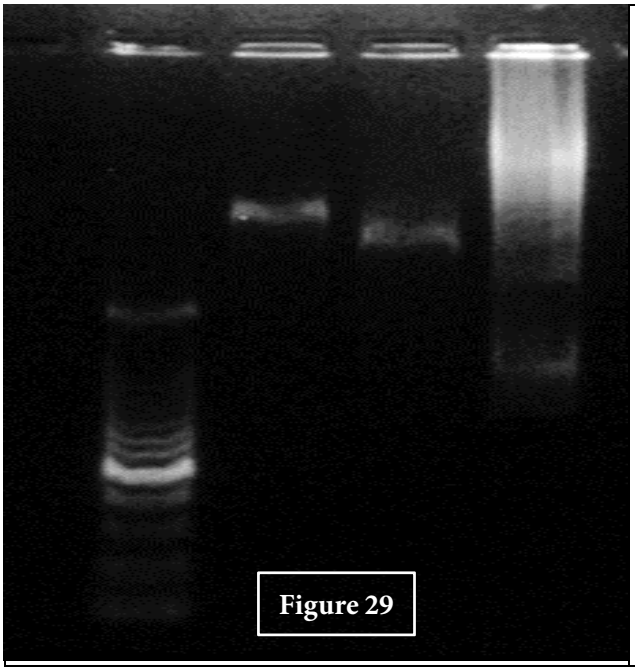


Figure 29

1: 100 bp marker;
2:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 with single digestion;
3:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 with double digestion;
4: 1kb marker.

Results/discussion: We have made the same mwastake as before that We don't have the AGAR gel completely immersed in the buffer. So we couldn't get the clear image we wanted.

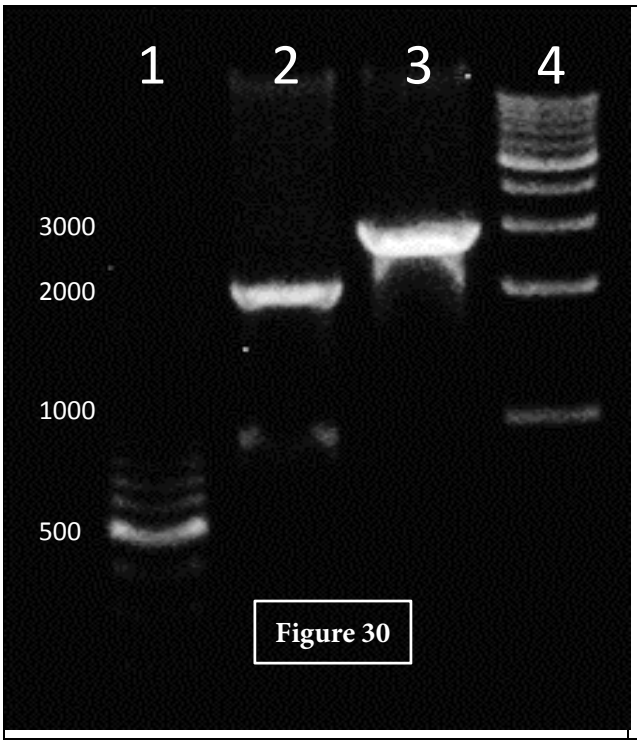


Figure 30

1: 100 bp marker;
2:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 with double digestion;
3:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 with Single digestion;
4: 1kb marker.

Results/discussion: The length of the connection system BBa_R0010+BBa_B0034+BBa_K629003+ BBa_B0015 was 986 bp. From the figure, we could determine that the bacteria contain the connection system was correct.

- Verification: Agarose gel electrophoresis

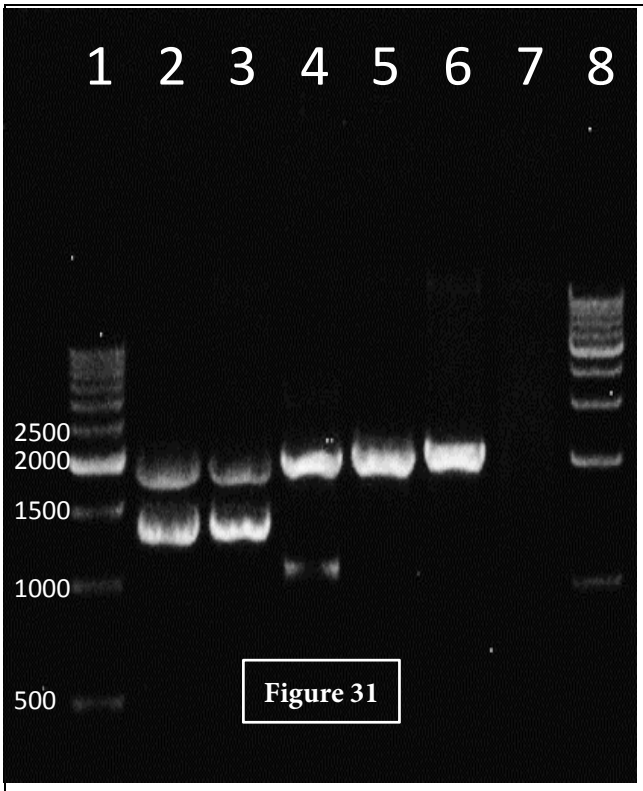


Figure 31

1: 500 bp marker;
2: BBa_K206000+ BBa_I732820+ BBa_R0010+ BBa_B0034+BBa_K629003+BBa_B0015(*CL-1*)(1);
3: BBa_K206000+ BBa_I732820+ BBa_R0010+ BBa_B0034+BBa_K629003+BBa_B0015(*CL-1*)(2);
4: BBa_K206000+ BBa_I732820+ BBa_R0010+ BBa_B0034+BBa_K629003+BBa_B0015 (*DH5α*);
5: BBa_K629003+ BBa_B0015(1);
6: BBa_K629003+ BBa_B0015(2);
7: BBa_B0030;
8: 1kb marker.
((1), (2) are different colonies.)

Purpose: The verification of the connection system BBa_K206000+BBa_I732820+ BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 transferred into *CL-1* and *DH5α*.
Results/discussion: From the result, the *CL-1* colonies got a 2000 bp band and a 1300 bp band, while *DH5α* colonies got a 2000 bp band and a 1kb band. So we could declare that the experiment failed.

2014-08-15

- Conservation

  14A-1N, 2013-P1-18G_1, 2014-P2-6F, 2014-P3-1N, 3H-2M-18G-4F

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
3F-2M-18G-4F_1	1.88	238.8
3F-2M-18G-4F_2	1.83/1.66/1.64	272.9/311.2/317.9
3F-2M-18G-4F_3	1.79/1.64/1.66	306.7/355.2/344.6
3F-2M-18G-4F_4	1.73/1.65	325.5/356.1
3F-2M-18G-4F_5	1.78	294.3
3F-2M-18G-4F_5	1.74	327.7
14A-1N_1	1.52/1.85/1.85	297.4/224.9/221.8
14A-1N_2	1.85	254.2
14A-1N_3	1.86	207.8
14A-1N_4	1.85	161.3
14A-1N_5	1.86	206.8
14A-1N_6	1.85	230.9
2014-P3-1N	1.86/1.87/1.85	228.2/267.6/227.7
2014-P2-6F	1.86/1.84/1.87	132.4/133.8/181.4
2013-P1-18G	1.85/1.88/1.86	218.5/238.6/257.3

- Enzyme Restriction

Single	2014-P2-6F	*Xba* I
Double	14A+1N/3H-2M-18G-4F/2014-P2-6F	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis

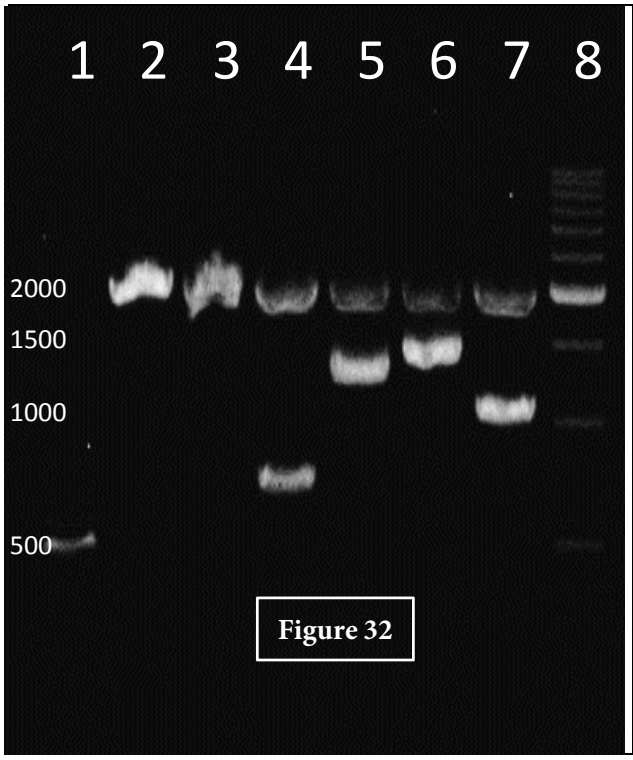


Figure 32

1: 100 bp marker;
2: BBa_R0040 with double digestion;
3: BBa_R0040 with single digestion;
4: BBa_K629003 with double digestion;
5: BBa_I732820 with double digestion;
6:BBa_K206000+BBa_I732820 with double digestion;
7:BBa_R0010+BBa_B0034+BBa_K629003+BBa_R0040 with double digestion;
8: 500 bp marker.
Purpose: The verification of BioBricks: BBa_R0040, BBa_K629003, BBa_I732820 and the connection system BBa_K206000+BBa_I732820 and BBa_R0010+BBa_B0034+BBa_K629003+BBa_R0040.
Results/discussion: We could determine that BBa_K629003 was correct, and the other are wrong.

- Enzyme Restriction

Single	2014-P2-6F	*Xba* I
Double	2014-P2-6F	*Xba* I, *Pst* I
	3H-2M-18G-4F	*EcoR* I, *Xba* I
	14A-1N	*EcoR* I, *Spe* I

- Enzyme Restriction

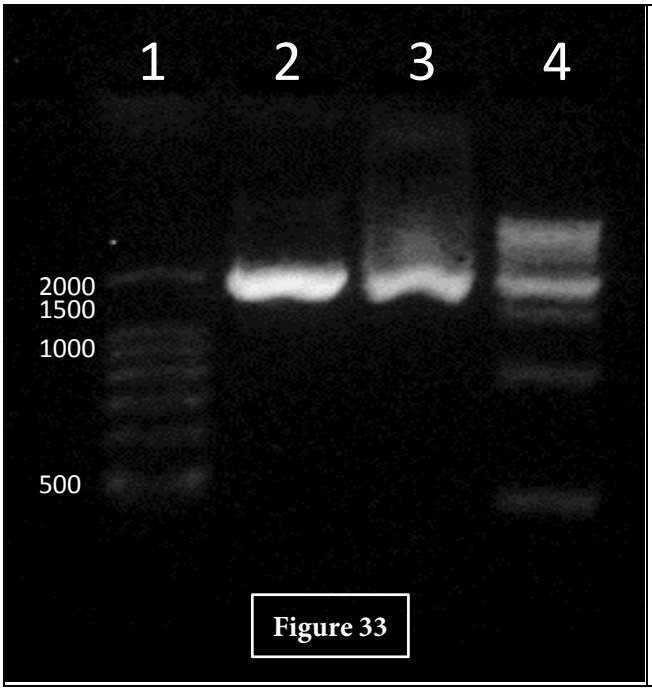


Figure 33

1: 100 bp marker;
2: BBa_R0040 with double digestion;
3: BBa_R0040 with single digestion;
4: 500 bp marker.

Results/discussion: From thwas figure, We found that the single enzyme digestion and double enzyme digestion bands of the same length. So we could determine that the plasmid we got was wrong.

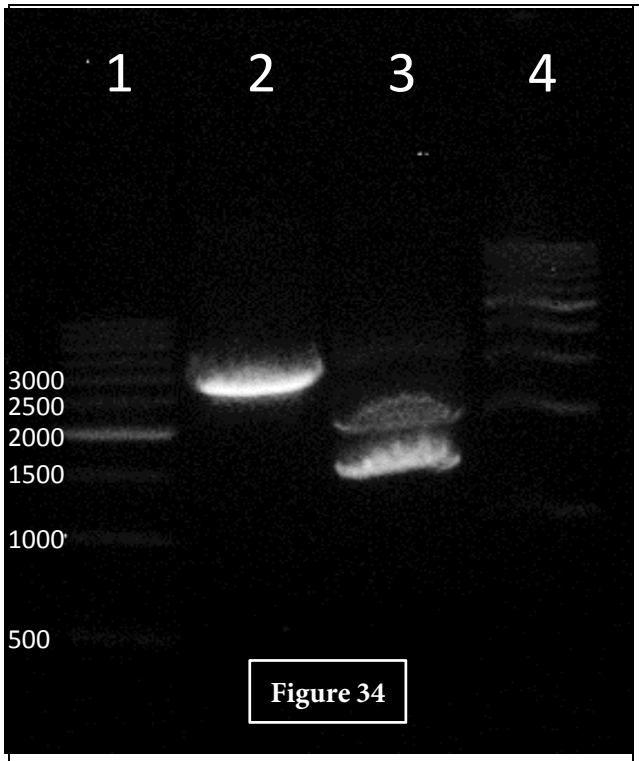


Figure 34

1: 500 bp marker;
2:BBa_R0010+BBa_B0034+BBa_K629003+BBa_R0040 with double digestion;
3:BBa_K206000+BBa_I732820 with double digestion;
4: 1kb marker.
Purpose: Use gel electrophoresis to got the BBa_R0010+BBa_B0034+BBa_K629003+BBa_R0040 connection system and BBa_K206000+ BBa_I732820 connection system, which could prepare for the next connection: BBa_K206000+ BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+ BBa_R0040.
Results/Discussion: In the connection system, we use the plasmid which contain the connection system BBa_R0010+BBa_B0034+BBa_K629003+BBa_R0040 as backbone, use BBa_K206000+ BBa_I732820 as insert gene. From the figure, we could know that the two connection system are correct. While in the figure, we found that near the 3000 bp, there had another band, it declared that the digestion was not complete.

- Ligation

	Centrifuge Tube	All	Agarose gel
14A-1N	0.902	1.028	0.126
3H-2M-18G-4F	0.929	1.069	0.140

- Enzyme Restriction

2014-P2-6F	*Spe* I, *Pst* I
2014-P3-1N	*Xba* I, *Pst* I

- Ligation: Positive and Negative
  (2014-P2-6F)+(2014-P3-1N)
  (14A-1N)—1, (3H-2M-18G-4F)—2
  V1/V2=8.87
- Verification: Agarose gel electrophoresis
- From left to the right:
  500Marker-(2014-P3-1N)-(2014-P1-6F)-1000Marker
  500Marker-(3H-2M-18G-4F)-(14A-1N)-1000Marker

	Centrifuge Tube	All	Agarose gel
2014-P3-1N	0.888	0.941	0.053
2014-P1-6F	0.913	1.013	0.100

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
2014-P3-1N	1.75/1.76/1.79	18.6/19.9/19.2
2014-P1-6F	1.79/1.85/1.91/1.88	19.4/20.9/20.0/21.1

- V1/V2=1.85

2014-08-16

- Ligation: Positive
  (2014-P3-1N)+(2014-P1-6F)
- Enzyme Restriction

2014-P1-18G	*Spe* I, *EcoR* I
2013-P3-4F	*EcoR* I, *Xba* I

- Verification: Agarose gel electrophoresis

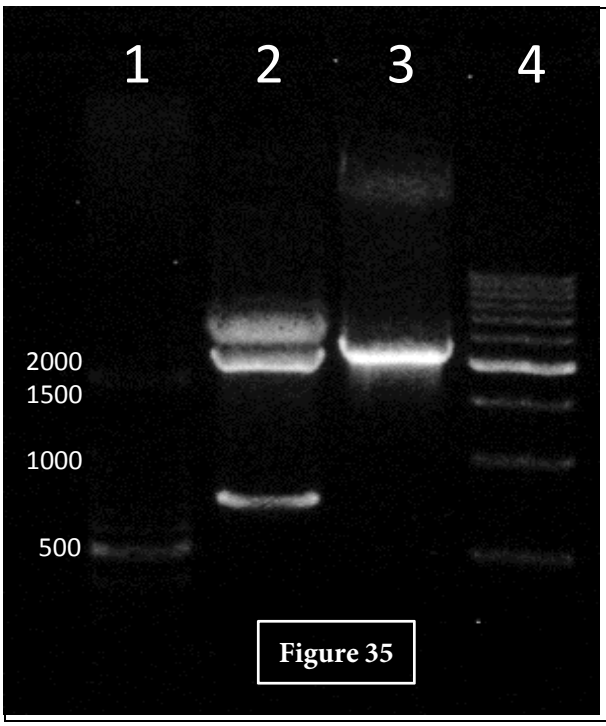


Figure 35

1: 100 bp marker;
2: BBa_K629003 with double digestion;
3: BBa_B0015 with double digestion;
4: 500 bp marker.

Results/discussion: From the figure, we could determine that the plasmid we got were all correct.

- Ligation
  3H-2M-18G-4F, 14A-1N

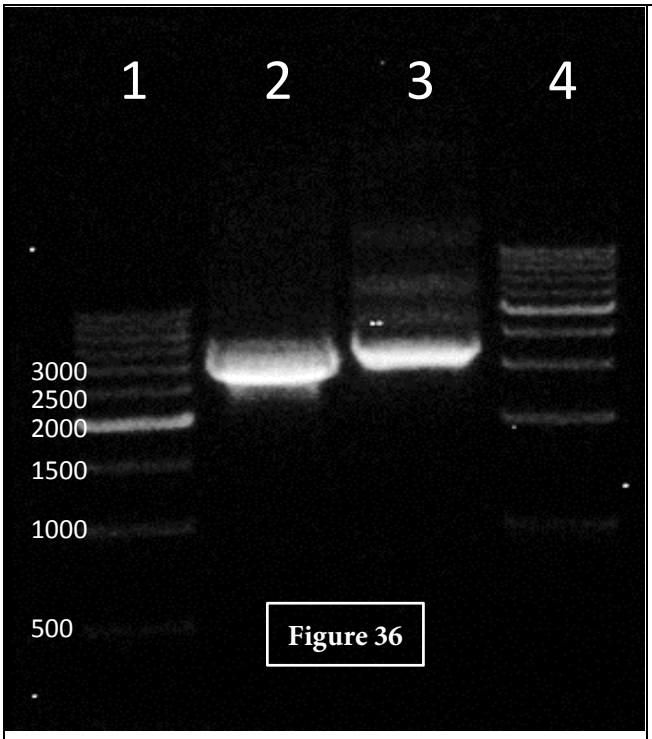


Figure 36

1: 500 bp marker;
2:BBa_R0010+BBa_B0034+BBa_K629003+BBa_R0040 with double digestion;
3:BBa_K206000+BBa_I732820 with double digestion;
4: 1kb marker.

Purpose: Use gel electrophoresis to got the connection system BBa_R0010+BBa_B0034+BBa_K629003+BBa_R0040 and BBa_K206000+BBa_I732820, prepare for the next connection: BBa_K206000+ BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+ BBa_R0040.

Results/discussion: In the connection system, we used the plasmid which contain the BBa_R0010+ BBa_B0034+BBa_K629003+BBa_R0040 connection system as backbone, use BBa_K206000+ BBa_I732820 as insert gene. From the figure, we could know that the connection system BBa_K206000+ BBa_I732820 was wrong, because we just got a band near 3000 bp, it declare that the digestion failed.

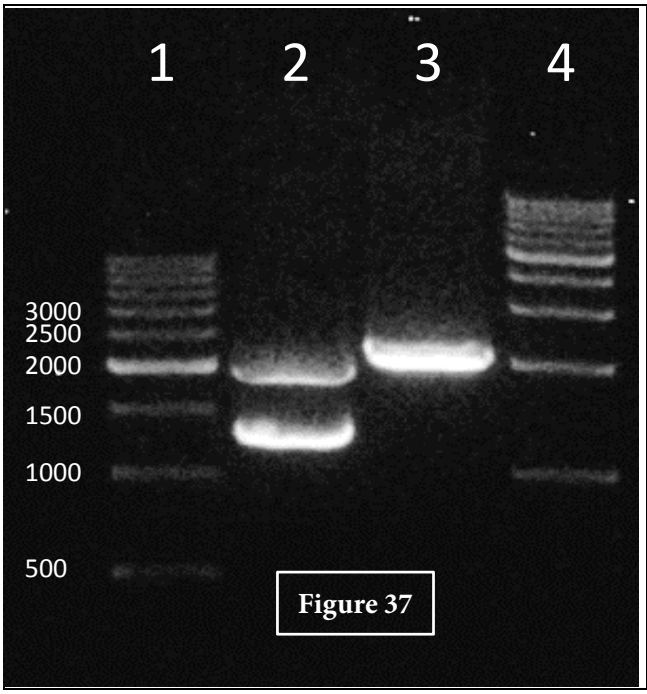


Figure 37

1: 500 bp marker;
2: BBa_I732820 with double digestion;
3: BBa_R0040 with double digestion;
4: 1kb marker.

Purpose: Use gel electrophoresis to get BBa_I732820 and BBa_R0040 gene bands, and then we could got the connection system BBa_R0040+BBa_I732820.

Results/discussion: In the connection system BBa_R0040+BBa_I732820, we used BBa_R0040 as backbone, BBa_I732820 as insert gene. So we cut the band of 1241 bp in BBa_I732820 and the band of 2124 bp in BBa_R0040, then gel extraction and ligate each other.

- Verification: Agarose gel electrophoresis

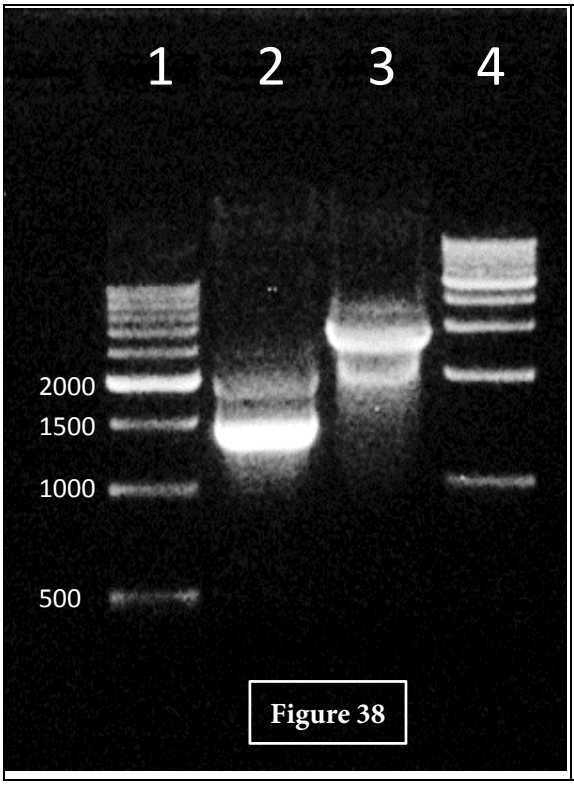


Figure 38

1: 500 bp Marker;
2: BBa_K206000+BBa_I732820;
3:BBa_BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015;
4: 1kb Marker.

Purpose: Prepare for the connection between BBa_K206000+BBa_I732820 and BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015. We use the BBa_K206000+BBA_I732820 as backbone and BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015 as the insert gene.
Results/discussion: From the figure, we know that the length of the BBa_K206000+BBa_I732820 was correct, but the length of BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015 was not correct. What's more, the band of thwas circuit was not very clear, may be the restriction time wasn't enough.

	Centrifuge Tube	All	Agarose gel
3H-2M-18G-4F	0.886 g	0.925 g	0.039 g
14A-1N	0.877 g	0.895 g	0.018 g

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
3H-2M-18G-4F	1.65/2.34/1.83	21.8/12/15.2
14A-1N	1.35/1.72	28.7/8.9

- 14A-1N—1 3H-2M-18G-4F—2
  V1/V2=2.3
- Ligation: Positive and Negative
- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement (ng/μL)
2014-P2-1L_1.1	1.74	51.6
2014-P4-1L_1.2	1.31	31.9
2014-P2-2L_2.1	1.84/1.83/1.86	230.4/197.4/117.4
2014-P2-2L_2.2	1.87	136.3

- Enzyme Restriction

2014-P2-2L	*EcoR* I

- Verification: Agarose gel electrophoresis
  From left to the right: 500Marker-(2014-P2-2L_2.1)-(2014-P2-2L_2.2)-(2014-P4-1L_1.1)

2014-08-17

- . Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement (ng/μL)
(6F+1N_1)+1	1.85/1.86/1.81/1.85	173.8/161.2/181.0/172.3
(6F+1N _1)-1	1.82/1.86/1.86//1.88/	233.1/221.0/221.8/220.5
	1.88/1.85/1.85	/219.5/228.6/220.6
(6F+1N_2)+1	1.74/1.76/1.79	159.3/119.5/99.2
(14A-1N+3H-2M-18G-4F_1)+1	1.86/1.80/1.87	205.9/791.0/627.6
(14A-1N+3H-2M-18G-4F_2)+1	1.78/1.80/1.79	288.6/320.6/384.3

- Enzyme Restriction: *Xba* I, *Pst* I
- Verification: Agarose gel electrophoresis
  From right to the left: 500Marker-(6F+1N *1+1*)-(6F+1N_1+1)-(6F+1N_2+1)-(14A-1N+3H-2M-18G-4F_1+1)-(14A-1N+3H-2M-18G-4F_1-1)-1000Marker
- Enzyme Restriction

6F-1N	*Spe* I, *Pst* I
3H-2M-18G-4F	*Xba* I, *Pst* I

- Part: 14A-1N+3H-2M-18G-4F

Single	*Xba* I
Double	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis
  From left to the right: 500Marker-(6F-1N)- (3H-2M-18G-4F)-(14A-1N+3H-2M-18G-4F Double)-(14A-1N+3H-2M-18G-4F Single)

	Centrifuge Tube	All	Agarose gel
(14A-1N+3H-2M-18G-4F)	0.901 g	0.928 g	0.050 g
6F-1N	0.895 g	0.937 g	0.042 g

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement (ng/μL)
6F-1N	1.88/1.82/1.67/1.82	18.3/19.5/22.8/20.2
3H-2M-18G-4F	1.83/1.79/1.77	17.7/18.1/18.4

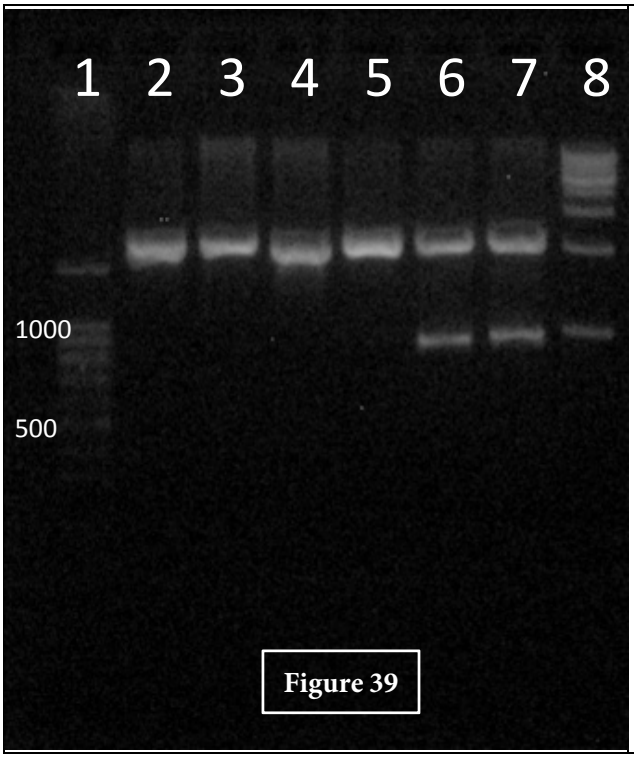
- Measure the Concentration of the Plasmids (Inter-Lab Study)

	Absorbance: 260/280	Measurement(ng/μL)
2014-P1-22I_1	1.75/1.84	104.2/122.1
2014-P1-22I_2	1.87/1.87	138.5/106.3
2014-P1-20K_1	1.85	180.0
2014-P1-20K_2	1.90/1.86	119.5/220.3
2014-P2-24B_1	1.87	134.2
2014-P2-24B_2	1.88/1.80/1.91	96.8/47.2/122.4

- Enzyme Restriction(Inter-Lab Study)

2014-P1-22I/2014-P1-20K/2014-P1/24B	pSB1C3	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis(Inter-Lab Study)



1: 100 bp marker;
2: BBa_K823012 with double digestion(1);
3: BBa_K823012 with double digestion(2);
4: BBa_K823005 with double digestion(1);
5: BBa_K823005 with double digestion(2);
6: BBa_E0240 with double digestion(1);
7: BBa_E0240 with double digestion(2);
8: 1kb marker.
(1, 2 are different colonies.)

Purpose: The verification of the BioBricks: BBa_K823012、BBa_K823005 and BBa_E0240.
Results/discussion: From thwas figure, we could confirm that the bacteria we got contain the BioBricks BBa_K823012, BBa_K823005 and BBa_E0240, because of the stripes about 1000bp and 2000 bp.

Conclusion: 2014-P2-24B was correct, and the fluorescent light showed 2014-P1-22I and 2014-P1-20K were correct.

- Ligation: Positive and Negative
  3H-2M-18G-4F—1 6F-1N—2
- Measure the Concentration of the Plasmids

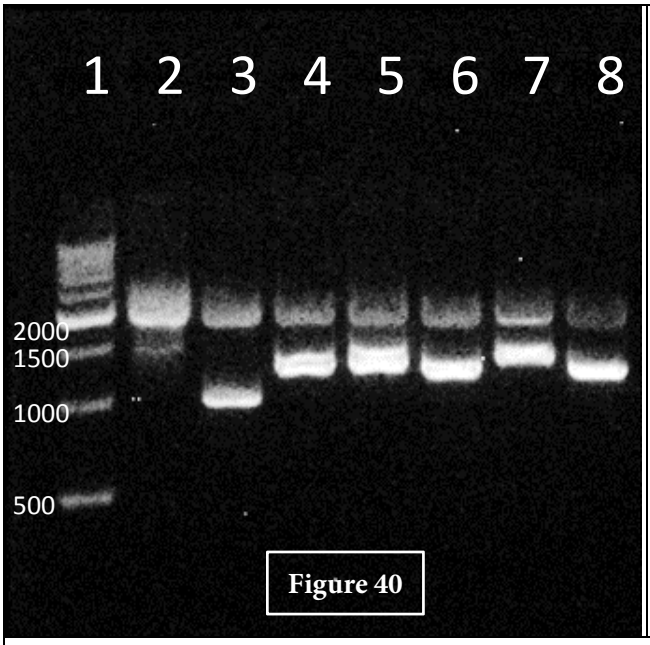


Figure 40

1: 500 bp Marker
2:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(pBAD+LacI+pLac+RBS+*CheZ*+TT)(1);
3:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(pBAD+LacI+pLac+RBS+*CheZ*+TT)(2);
4:BBa_R0040+BBa_I732820(pTETR+LacI)(1);
5:BBa_R0040+BBa_I732820(pTETR+LacI)(2);
6:BBa_R0040+BBa_I732820(pTETR+LacI)(3);
7:BBa_R0040+BBa_I732820(pTETR+LacI)(4);
8:BBa_R0040+BBa_I732820(pTETR+LacI)(5).
((1), (2), (3), (4), (5) are different colonies.)

Purpose: The verification of the BioBricks: BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_R0040+BBa_I732820. The circuit of BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 was detected from two different samples. One was positively ligated, the other was negatively ligated.

Results/discussion: The length of thwas circuit ought to be the same. But we surprwasingly found that they were not the same. What's more, the band of vector were not very clear.

As for the circuit of BBa_R0040+BBa_I732820, we chose 5 different samples from 4 different plates three of which was ligated positively. Fortunately the lengths of all the 5 sample were correct.

	Absorbance: 260/280	Measurement (ng/μL)
(14A-1N+3H-2M-18G-4F)_2+1	1.76/1.71/1.82/1.84	332.5/432.5/364.5/366.5
(14A-1N+3H-2M-18G-4F)_2-1	1.82/1.72/1.80	218.3/302.3/278.6
(6F-1N)_2-2	1.86/1.75	163.2/180.8
(6F-1N)_3+1	1.77/1.84	149.9/162.7
(6F-1N)_3-1	1.84/1.80	234.1/246.8
(6F-1N)_4-1	1.86/1.88	101.5/97.0
(6F-1N)_4-1	1.84/1.79	206.0/178.5

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
(18G+4F)_1+1	1.83/1.84	202.0/119.8
(18G+4F)_1+2	1.85/1.88	156.9/150.8
(18G+4F)_2+1	1.81/1.84	158.5/244.7
(18G+4F)_2+2	1.86/1.81	172.1/113.5
(18G+4F)_1-1	1.83/1.90/1.87	114.5/135.3/136.8
(18G+4F)_1-2	1.87/1.87	251.4/230.2

- Verification: Agarose gel electrophoresis

2014-08-18

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
2014-P4-18A_1.1	1.67/1.87	74.4/61.1
2014-P4-18A_1.2	1.87/1.87	45.1/45.9

- Enzyme Restriction

2014-P4-18A	*Xba* I, *Spe* I

- Verification: Agarose gel electrophoresis(Inter-Lab Study)

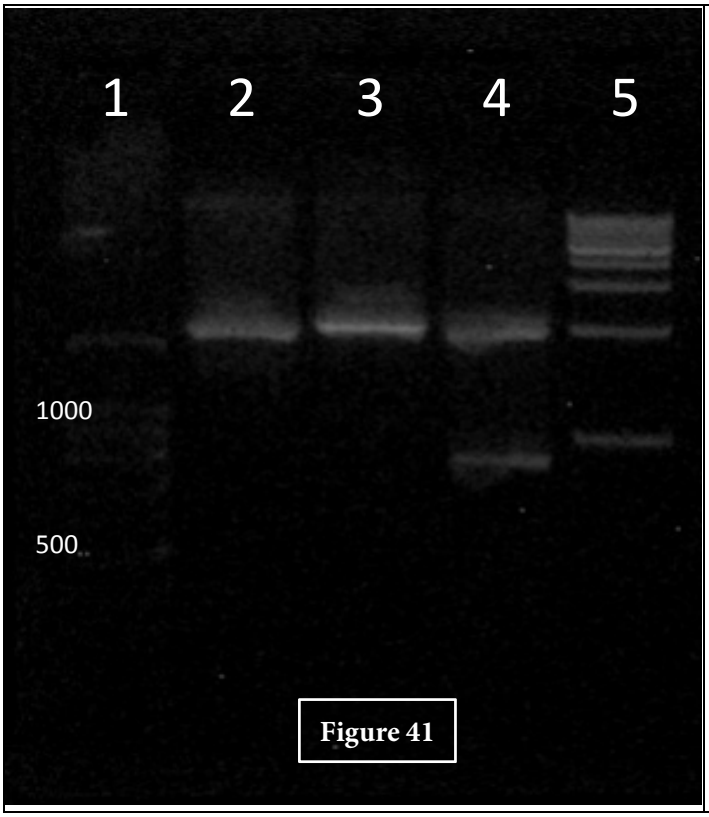


Figure 41

1: 100 bp marker;
2: BBa_K823005 with double digestion(*EcoR* I and *Pst* I);
3: BBa_K823012 with double digestion(*EcoR* I and *Pst* I);
4: BBa_E0240 with double digestion(*Xba* I and *Pst* I);
5: 1kb marker.

Purpose: Prepare for the BBa_K823005+ BBa_E0240 and BBa_K823012+BBa_E0240 connection system.
Results/discussion: We use BBa_K823005 、BBa_K823012 as backbone, BBa_E0240 as insert gene. From the figure, we could confirm that the gene bands we want are correct.

	Centrifuge Tube	All	Agarose gel
2014-P1-20K_1	0.883 g	0.919 g	0.885 g
2014-P1-22I_1	0.954 g	0.969 g	0.934 g
2014-P2-24B_1	0.885 g	0.934 g	0.049 g

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)

2014-P1-20K	1.08/0.97/0.85	109.7/418.6/32.1
2014-P1-22I	3.36/2.49	1.15/1.03
2014-P3-24B	1.15/1.03	30.5/153.3

- Ligation: 2014-P1-20K+2014-P1-24B
- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
14A-1N+3H-2M-18G-4F (+)_1	1.84/1.84/1.84/1.82	321.1/398.3/391.9/407.4
14A-1N+3H-2M-18G-4F(-)_1	1.84/1.79/1.80	387.8/391.2/414.0

- Enzyme Restriction: 14A-1N-3H-2M-18G-4F

Single	*Xba* I, *Pst* I
Double	*Xba* I

- Enzyme Restriction: 2014-P4-18A

Single	*Xba* I
Double	*Xba* I, *Pst* I

- Enzyme Restriction(Inter-Lab Study)

2014-P2-24B	*Xba* I, *Pst* I
2014-P1-20K	*Spe* I, *Pst* I
2014-P1-20I	*Spe* I, *Pst* I

- Verification: Agarose gel electrophoresis

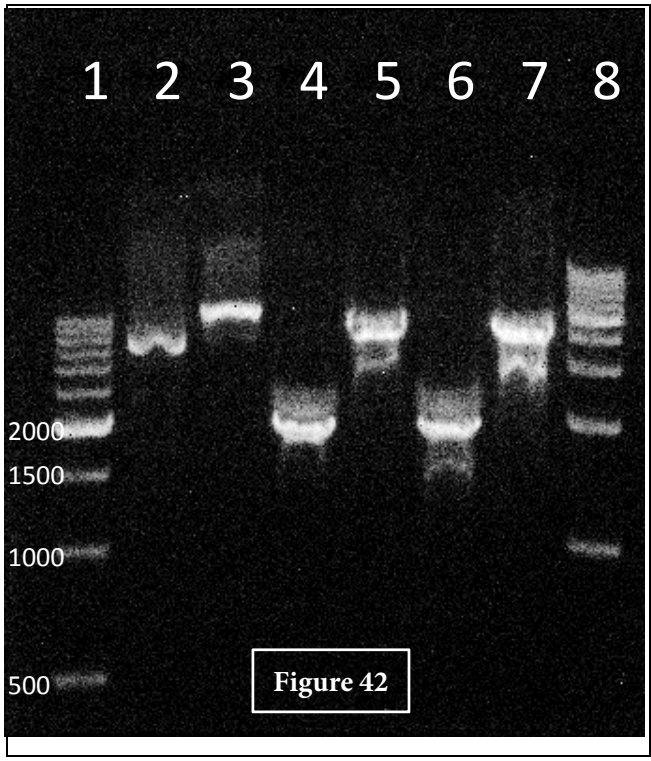


Figure 42

1: 500 bp Marker;
2: BBa_I20620(promoter+GFP)(1);
3: BBa_I20620(promoter+GFP) (2);
4:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(pBAD+LacI +pLac+RBS+*CheZ*+TT(1));
5:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(pBAD+LacI +pLac+RBS+*CheZ*+TT) (2);
6:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(pBAD+LacI +pLac+RBS+*CheZ*+TT) (3);
7:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(pBAD+LacI +pLac+RBS+*CheZ*+TT) (4);
8:1000 bp marker.
((1), (2), (3), (4) are different colonies)

Purpose: The verification of BioBrick: BBa_I20620 and BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015.
Results/discussion: The length of the BBa_R0010 which was restricted by double enzymes ought to be 919 bp and 2750 bp, clearly it was not correct. And the other which was restricted only by *Xba* I, whose theoretical length was 3669 bp, clearly shorter than what we saw on the image. As for the circuit BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015, although the image was not very clear, but the length of the 4 samples were correct.

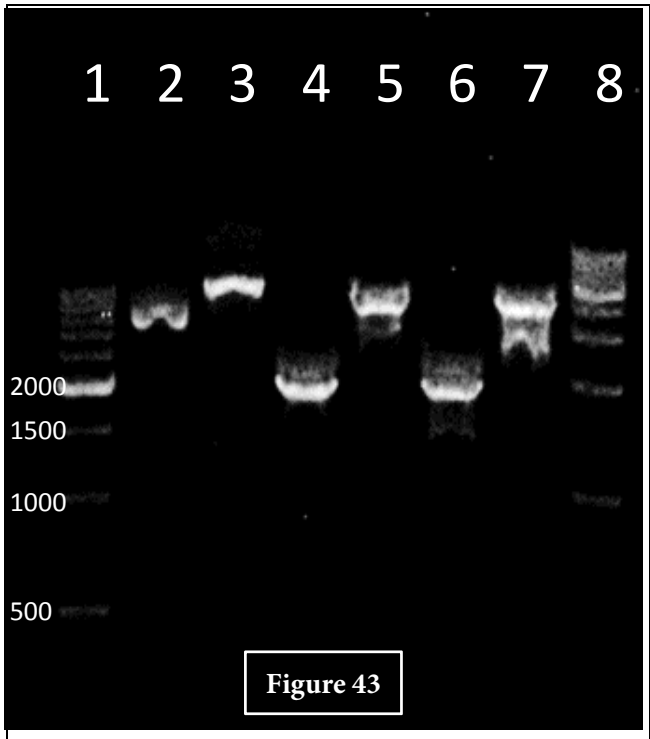


Figure 43

1: 500 bp Marker;
2: BBa_I20620(promoter+GFP)(1);
3: BBa_I20620(promoter+GFP) (2);
4:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(pBAD+LacI +pLac+RBS+*CheZ*+TT(1));
5:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(pBAD+LacI +pLac+RBS+*CheZ*+TT) (2);
6:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(pBAD+LacI +pLac+RBS+*CheZ*+TT) (3);
7:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(pBAD+LacI +pLac+RBS+*CheZ*+TT) (4);
8:1000 bp marker.
((1), (2), (3), (4) are different colonies)

Purpose: The verification of the BioBrick: BBa_I20620 and BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015.
Results/discussion: The length of the BBa_R0010 which was restricted by double enzymes ought to be 919 bp and 2750 bp, clearly it was not correct. And the other which was restricted only by *Xba* I, whose theoretical length was 3669 bp, clearly shorter than what we saw on the image. As for the circuit BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015, although the image was not very clear, but the length of the 4 samples were correct.

- Verification: Agarose gel electrophoresis(Inter-Lab Study)
- From left to the right: 100Marker-(2014-P1-20K)-(2014-P1-22I)-(2014-P2-24B)-1000Marker

	Centrifuge Tube	All	Electrophoresis
2014-P1-20K	0.914	0.970	0.056
2014-P1-22I	0.914	0.963	0.956
2014-P2-24B	0.897	0.956	0.059

- Measure the Concentration of the Plasmids(Inter-Lab Study)

	Absorbance: 260/280	Measurement(ng/μL)
2014-P1-20K	1.84	7.8
2014-P1-22I	1.76	6.8
2014-P2-24B	1.65	4.2

- Ligation

1. (2014-P1-20K)+(2014-P2-24B)

- 2014-P2-24B—1 2014-P1-20K—2

V1/V2=4:1

2. (2014-P1-22I)+(2014-P2-24B)

- 2014-P1-22I—1 2014-P2-24B—2

V1/V2=4.5:1

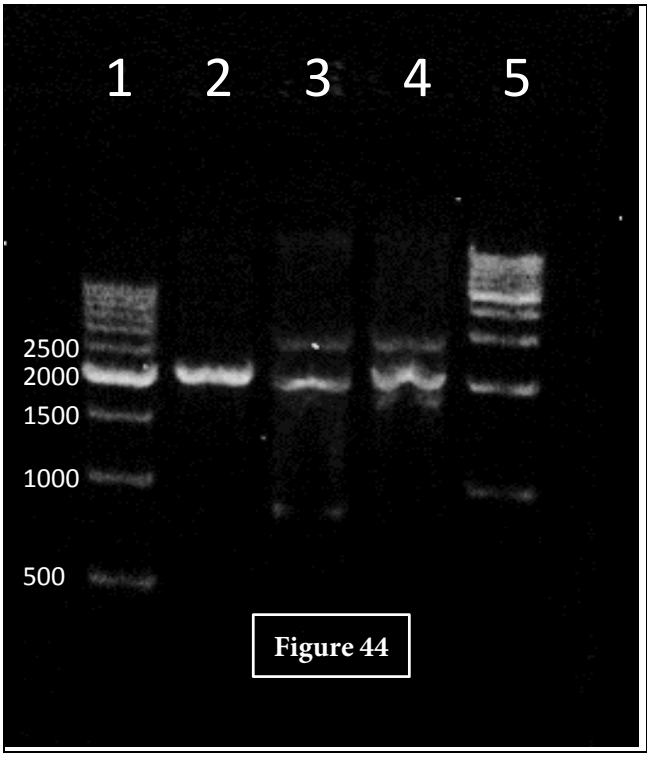


Figure 44

1: 500 bp Marker;
2: BBa_K629003+BBa_B0015(*CheZ*+TT)(1);
3: BBa_K629003+BBa_B0015(*CheZ*+TT) (2);
4: BBa_K629003+BBa_B0015(*CheZ*+TT) (3);
5: 1000 bp Marker.
((1), (2), (3) are different colonies.)

Purpose: The verification of the connection system BBa_K629003+BBa_B0015.
Results/discussion: The circuit were all ligated negatively, and we chose three samples two of which were from the same plate. We found that only the sample 2 was correct.

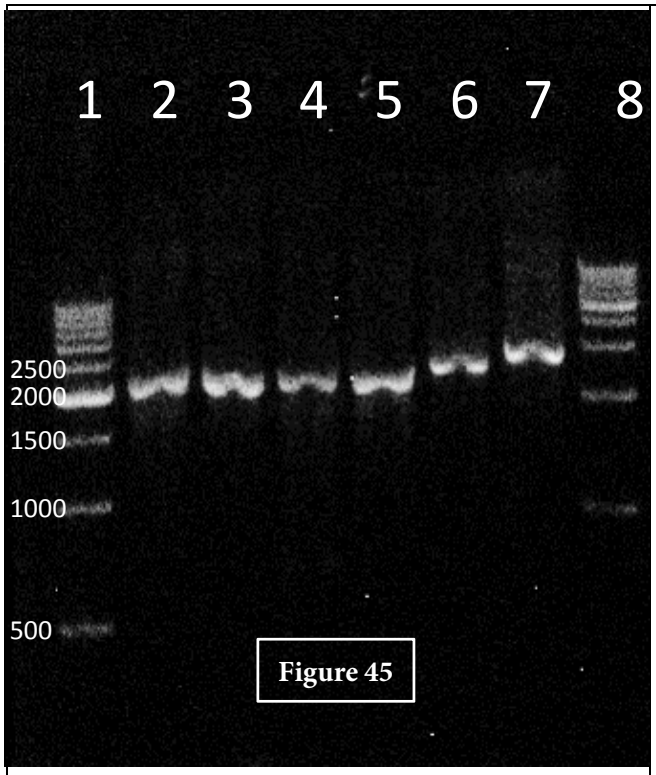


Figure 45

1: 500 bp Marker;
2: BBa_K629003+BBa_B0015(*CheZ*+TT)(1);
3: BBa_K629003+BBa_B0015(*CheZ*+TT)(2);
4: BBa_K629003+BBa_B0015(*CheZ*+TT)(3);
5: BBa_K629003+BBa_B0015(*CheZ*+TT)(4);
6: BBa_K629003+BBa_B0015(*CheZ*+TT)(5);
7: BBa_K629003+BBa_B0015(*CheZ*+TT)(6);
8: 1000 bp Marker.
((1), (2), (3), (4), (5), (6) are different colonies.)

Purpose: The verification of BioBrick: BBa_K629003+BBa_B0015(*CheZ*+TT). We chose four different samples and each pairwwase was from the same plate.

Results/discussion: The sample 1 was cut by two different enzymes. The first four were ligated positively. The exact length of the part ought to be 2814 bp and they were at the same length theoretically. None of them was correct.

2014-08-19

- Extraction of the Plasmids

  14A-1N+3H-2M-18G-4F(+)
  14A-1N+3H-2M-18G-4F(-)
  6F-1N+3H-2M-18G-4F(+)_1
  6F-1N+3H-2M-18G-4F(-)_2
  6F-1N+3H-2M-18G-4F(+)_2
  6F-1N+3H-2M-18G-4F(-)_2

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
14A-1N+3H-2M-18G-4F(+)	1.86/1.84/1.85	408.7/390.3/431.6
14A-1N+3H-2M-18G-4F(-)	1.76/1.84/1.82/1.84/1.85	496.6/442.7/447.0/480.8/494.7
6F-1N+3H-2M-18G-4F(+)_1	1.84/1.79/1.82	378.2/509.5/481.9
6F-1N+3H-2M-18G-4F(-)_1	1.85/1.85/1.84	389.1/440.3/380.7
6F-1N+3H-2M-18G-4F(+)_2	1.84/1.86/1.83	441.3/460.7/406.2
6F-1N+3H-2M-18G-4F(-)_2	1.86/1.84/1.84	465.4/424.6/398.9

- Enzyme Restriction

14A-1N+3H-2M-18G-4F(+)	*Xba* I, *Pst* I
14A-1N+3H-2M-18G-4F(-)	
6F-1N+3H-2M-18G-4F(+)_1	
6F-1N+3H-2M-18G-4F(-)_2	
6F-1N+3H-2M-18G-4F(+)_2	
6F-1N+3H-2M-18G-4F(-)_2	

- Verification: Agarose gel electrophoresis

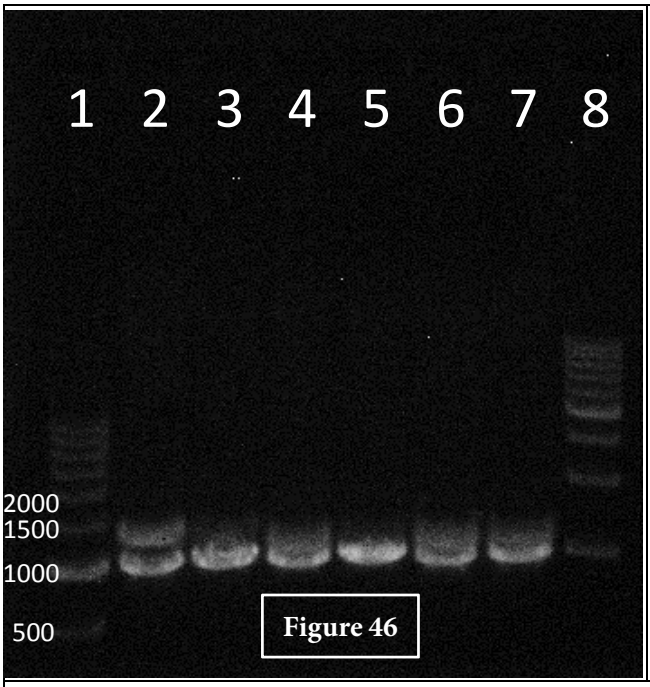


Figure 46

1: 500 bp Marker;
2:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(1);
3:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015(2);
4:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015(1);
5:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015(2);
6:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015(3);
7:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(4);
8: 1000 bp Marker.
((1), (2), (3), (4) are different colonies.)

Purpose: The verification of the connection systems: BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015.

Results/discussion: The length of BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 ought to be 2281 bp, and the length of BBa_R0040+BBa_I732820+ BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015 ought to be 2357 bp. Clearly all the image were wrong. Maybe it was because that the enzyme restriction time was too long so that the enzyme cut the spot which we didn't want them to do.

- Verification: Agarose gel electrophoresis

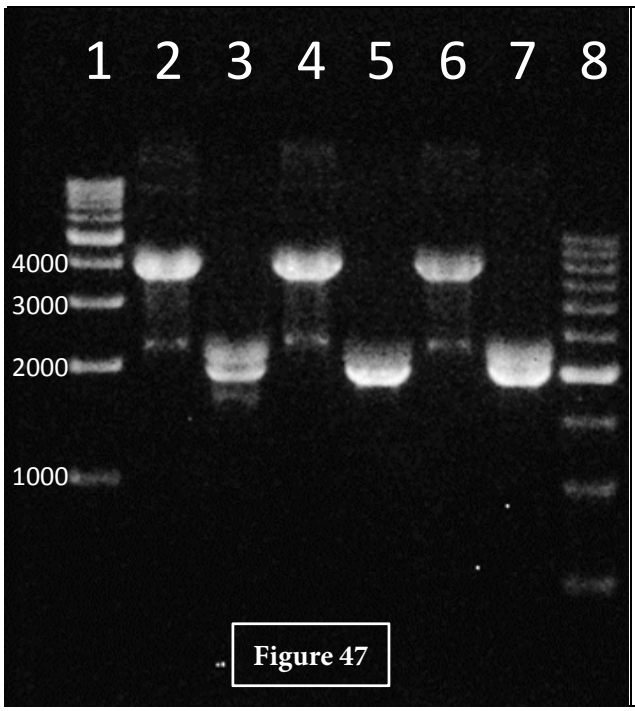


Figure 47

1: 1000 bp Marker;
2:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(1);
3:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015(2);
4:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(3);
5:BBa_R0040+BBa_I732820+BBa_R0010Z+BBa_B0034+BBa_K629003+BBa_B0015(4);
6: 500 bp Marker.
((1), (2), (3), (4) are different colonies.)
Purpose: The verification of the BioBricks was BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015.
Results/discussion: Regretfully all the images were wrong.

2014-08-20

- Verification: Agarose gel electrophoresis

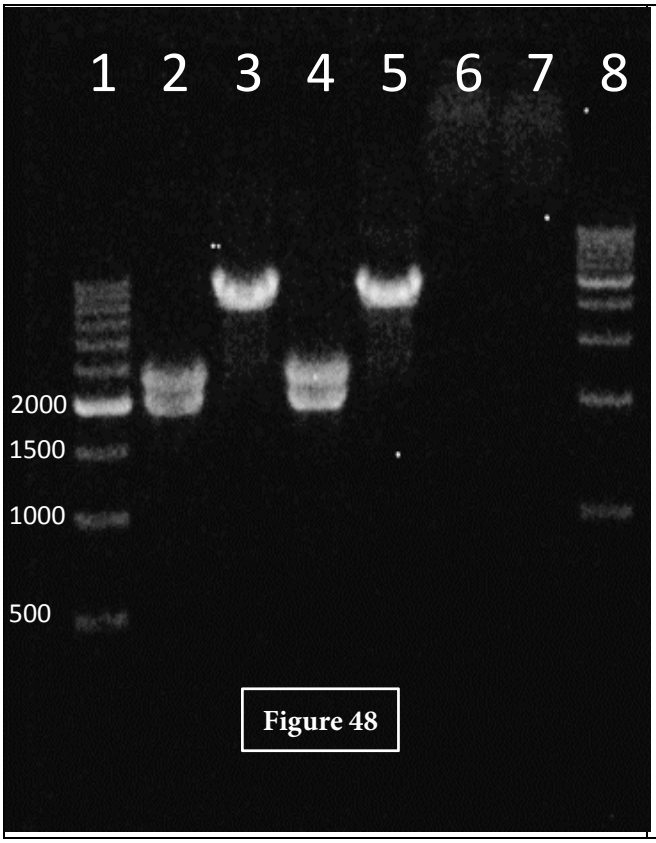


Figure 48

1:500 bp Marker;
2:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(1);
3:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015(2);
4:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015(3);
5:BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015(4);
8:1kb Marker.
((1), (2), (3), (4) are different colonies.)
Purpose: The verification of the connection system BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(pBAD+LacI+pLac+RBS+*CheZ*+TT).
Results/discussion: From the result we confirmed that the circuit of BBa_K206000+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 was successfully connected.

- Conservation:
  1.14A-1N+3H-2M-18G-4F(+)
  2.14A-1N+3H-2M-18G-4F(-)
- Extraction the Plasmids
- Measure the Concentration of the Plasmids

	Absorbance:260/280	Measurement(ng/μL)
14A-1N+3H-2M-18G-4F(+)	1.86/1.86	446.9/390.0
14A-1N+3H-2M-18G-4F(-)	1.87/1.88	345.2/399.0

- Measure the Concentration of the Plasmids(Inter-Lab Study)

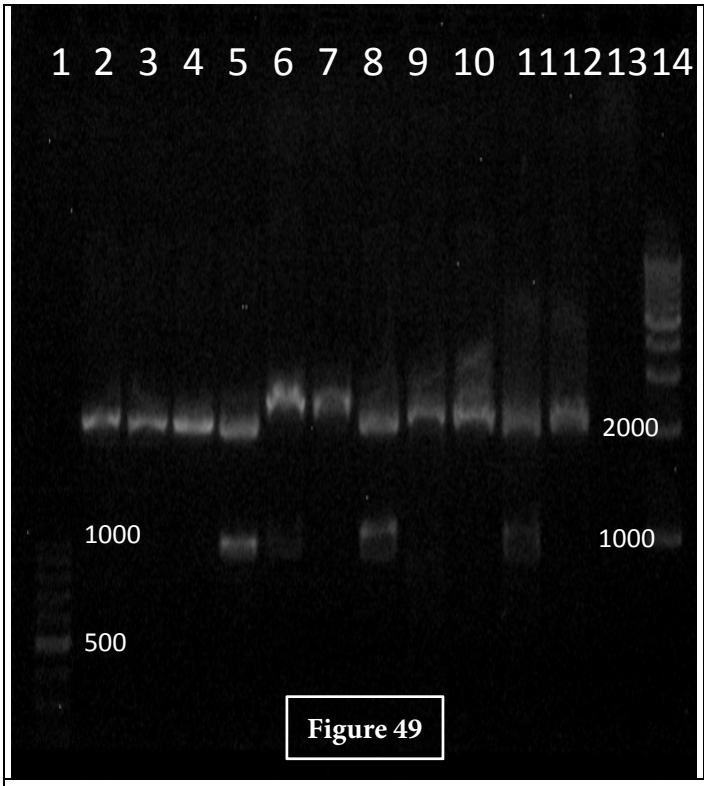


Figure 49

((1), (2), (3), (4), (5), (6), (7) are different colonies.)

Purpose: The verification of the BBa_K823005+ BBa_E0240 and BBa_K823012+ BBa_E0240 connection system.

Results/discussion: From thwas figure, we could confirm that the BBa_K823012+ BBa_E0240 (2) connection system was correct, which contain the stripes about 1000 bp and 2000bp. The stripe about 1000 bp was the length of BBa_K823012+ BBa_E0240, 2000 bp was the length of backbone pSB1C3. While the BBa_K823005+ BBa_E0240 connection system was wrong.

1: 100 bp marker;
2: BBa_K823005+ BBa_E0240 with double digestion(1);
3: BBa_K823005+ BBa_E0240 with double digestion(2);
4: BBa_K823012+ BBa_E0240 with double digestion(1);
5: BBa_K823012+ BBa_E0240 with double digestion(2);
6: BBa_K823012+ BBa_E0240 with double digestion(3);
7: BBa_K823005+ BBa_E0240 with double digestion(3);
8: BBa_K823012+ BBa_E0240 with double digestion(4);
9: BBa_K823012+ BBa_E0240 with double digestion(5);
10: BBa_K823012+ BBa_E0240 with double digestion(6);
11: BBa_K823012+ BBa_E0240 with double digestion(7);
12: BBa_K823005+ BBa_E0240 with double digestion(4);
13: BBa_K823005+ BBa_E0240 with double digestion(5);
14: 1kb marker.

- Brought up for 12 hours

	Absorbance: 260/280	Measurement(ng/μL)
22I+24B(-)	1.80/1.93	145.8/122.1
22I+24B(+)	1.72/1.90	129.8/103.5
20K+24B(+)	1.88/2.28/1.95	95.3/8.1/9.9

- Brought up for 2 hours

	Absorbance: 260/280	Measurement(ng/μL)
20K+24B	1.85/1.83	118.0/124.3
22I+24B	1.83	204.9

- Brought up for 7 hours

	Absorbance: 260/280	Measurement(ng/μL)
22I+24K	1.80	98.4
20K+24B	1.68	93.0

- Enzyme Restriction(Inter-Lab Study)

20K+24B/22I+24B	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis
  From left to the right: 100Marker-(20K+24B_1 2h)-(20K+24B_2 2h)-(22I+24B_2 2h)-(22I+24B 7h)-(20K+22B_1 negative 12h)-(22I+24B_1 12h)-(22I+24B_2 positive 12h)-(22I+24B_2 negative 12h)-(22I+24B_2 12h negative)-(24K+24B_1 positive 12h)-(20K+24B_2 positive 12h)- 1000Marker
- Measure the Concentration of the Plasmids
  6F-1N-3H-2M-18G-4F

	Absorbance: 260/280	Measurement (ng/μL)
+1	1.86/1.84	527/490.6
+2	1.86/1.79	415.9/449.1

2014-8-21

- Enzyme Restriction
- Verification: Agarose gel electrophoresis

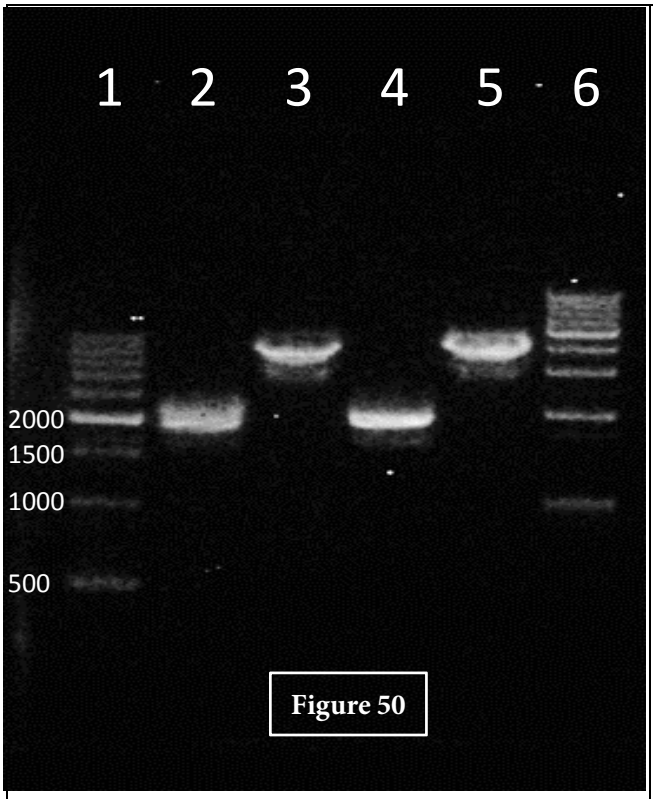


Figure 50

1: 500 bp Marker;
2:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(1);
3:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(2);
4:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(3);
5:BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015(4);
6: 1kb Marker.
((1), (2), (3), (4) are different colonies.)

Purpose: The verification of connection system BBa_R0040+BBa_I732820+BBa_R0010+BBa_B0034+BBa_K629003 +BBa_B0015.
Results/discussion: We chose 4 different samples from two different plates and all of them were ligated positively. The circuits which were restricted by two different enzymes couldn't been seen clearly. Fortunately, the circuits which were restricted by only one enzyme was proved to be correct.

- Enzyme Restriction

2014-P1-20K	*Spe* I, *Pst* I
2014-P2-24B	*Xba* I, *Pst* I
2013-P1-21F	*Xba* I, *Pst* I
2013-P1-12D	*Xba* I, *Pst* I

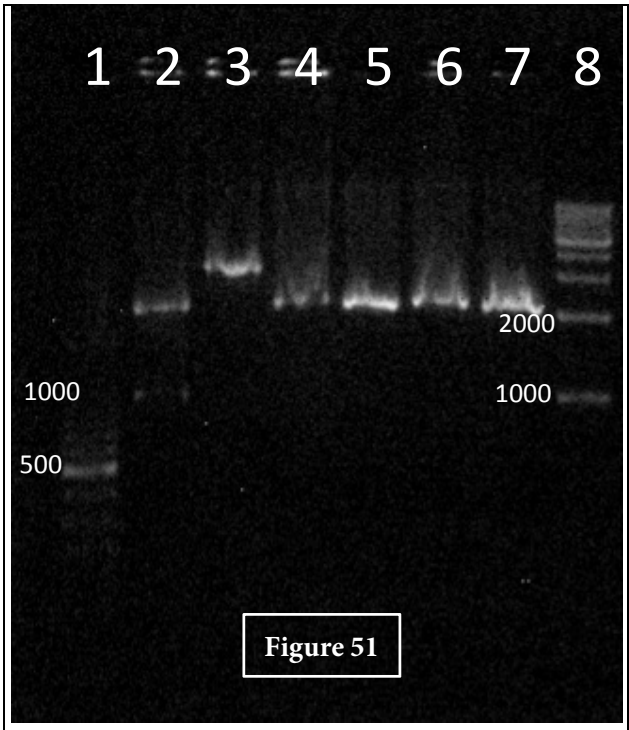


Figure 51

1: 100 bp marker;
2: BBa_K823005+ BBa_E0240 with double digestion(1);
3: BBa_K823005+ BBa_E0240 with single digestion(1);
4: BBa_K823005+ BBa_E0240 with double digestion(2);
5: BBa_K823005+ BBa_E0240 with double digestion(3);
6: BBa_K823005+ BBa_E0240 with double digestion(4);
7: BBa_K823005+ BBa_E0240 with double digestion(5);
8: 1kb marker.
(1, 2, 3, 4, 5 are different colonies.)

Purpose: The verification of the BBa_K823005+ BBa_E0240 connection system.
Results/discussion: From thwas figure, we could confirm that the BBa_K823005+ BBa_E0240 (1) connection system was correct, because of the stripes about 1000 bp and 2000 bp,
The stripe about 1000 bp was the band of BBa_K823005+ BBa_E0240, 2000 bp was the length of backbone pSB1C3.

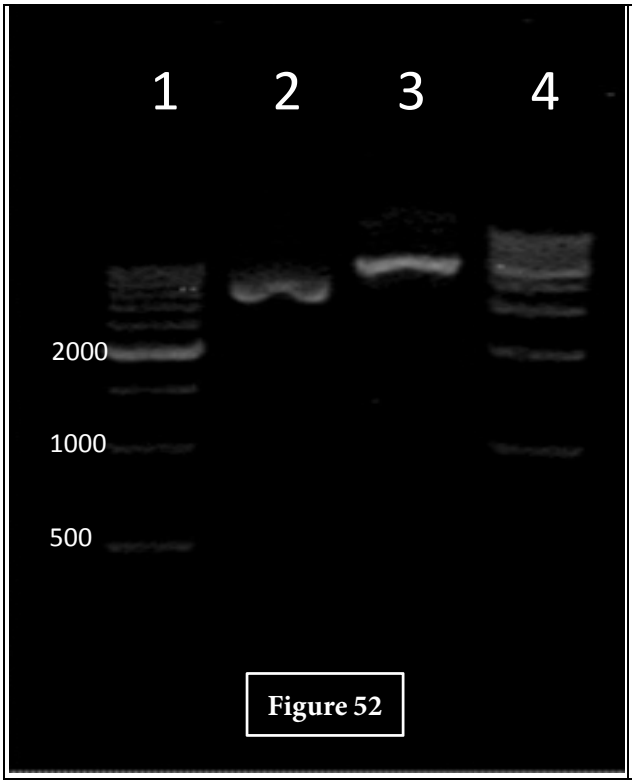


Figure 52

1: 500bp marker;
2: BBa_I20260 with double digestion;
3: BBa_I20260 with single digestion;
4: 1kb marker.

Purpose: The verification of the BBa_I20260 BioBrick.
Results/discussion: From this figure, we could found that the backbone from double digestion was correct because of the stripe near 3000 bp. At the same time, the band from single digestion was longer than backbone 1000 bp. while we couldn't confirm that the BioBrick was correct, because after double digestion, we just got a backbone, but couldn't get the band of BBa_I20260, which was 919 bp.

2014-08-22

- Enzyme Restriction

Double	2J(-)_1-2/2L(+)_1-1	*Xba* I, *Pst* I
Double	pLac	*Spe* I, *Pst* I

- Verification: Agarose gel electrophoresis
  From left to the right: 100Marker-[2L(+)_1-1]-[2J(-)_1-2]-500Marker

	Centrifuge Tube	All	Agarose gel
2L(+)_1-1	0.926 g	0.978 g	0.052 g
2J(-)_1-1	0.907 g	0.941 g	0.034 g
pLac	0.911 g	0.960 g	0.058 g

- Measure the Concentration of the Plasmids

	Absorbance:260/280	Measurement(ng/μL)
2L(+)_1-1	1.76/1.79	9.1/7.9
2J(-)_1-2	2.24/1.90	8.4/10.3
pLac	2.45/2.36	6.1/6.3

- 1—2014-P2-2L 2—2014-P2-2J 3—pLac
  V1/V3=3*M1*C3/1*M3*C3=0.757
  V2/V3=3*M2*C3/1*M3*C3=0.690
- Transformation

2013-P3-5G	Lux pR
2013-P3-5I	Lux pL
2013-P5-3K	Lux R
2013-P3-3I	Lux I
2014-P4-8O	RBS+Lux R+TT

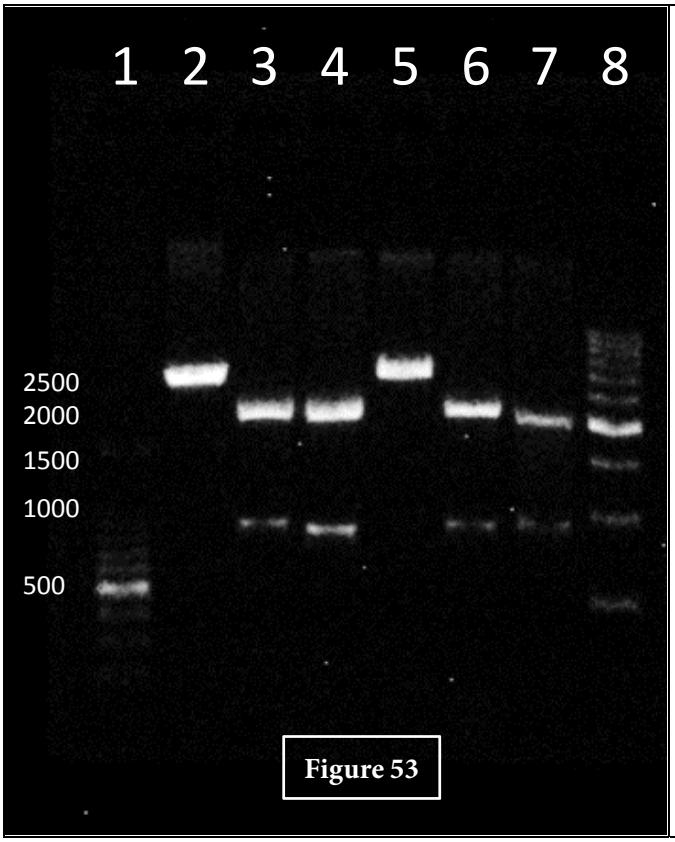


Figure 53

1: 100 bp Marker;
2: BBa_B0032(RBS0.3)(1);
3: BBa_B0032(RBS0.3)(2);
4: BBa_B0032(RBS0.3)(3);
5: BBa_B0033(RBS0.01)(1);
6: BBa_B0033(RBS0.01)(2);
7: BBa_B0033(RBS0.01)(3);
8: 500 bp Marker.
((1), (2), (3) are different colonies.)

Purpose: The verification of the BioBricks: BBa_B0032, which was negatively ligated from 3 different samples and BBa_B0033 which was positively ligated from 3 different samples.
Results/discussion: We could confirm that the samples which were restricted by 2 different enzymes was right. However, others were not true. Maybe the restriction time was not enough.

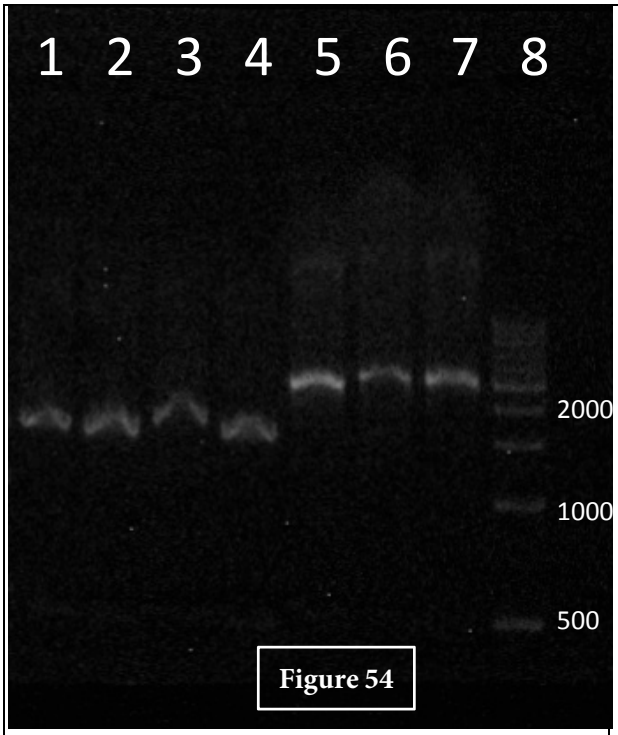


Figure 54

1: BBa_I20260 with double digestion(12-P2-17F)(1);
2: BBa_I20260 with double digestion(12-P2-17F)(2);
3: BBa_I20260 with double digestion(12-P2-17F)(3);
4: BBa_I20260 with double digestion(12-P2-17F)(4);
5: BBa_I20260 with double digestion(14-P4-18A)(1);
6: BBa_I20260 with double digestion(14-P4-18A)(2);
7: BBa_I20260 with double digestion(14-P4-18A)(3);
8: 500 bp marker.
Purpose: The verification of the BBa_I20260 BioBrick.
Results/discussion: Because we couldn't get a correction gene last time, so we used the plasmid from different year. While from the figure, we couldn't get a conclution after the digestion and gel electrophoresis, because the plasmid from 2012 year was about 3000 bp, the plasmid from 2014 year was about 5000 bp. What's worse, we couldn't get the band of BBa_I20260 BioBrick after double digestion.

2014-08-23

- Verification: Agarose gel electrophoresis

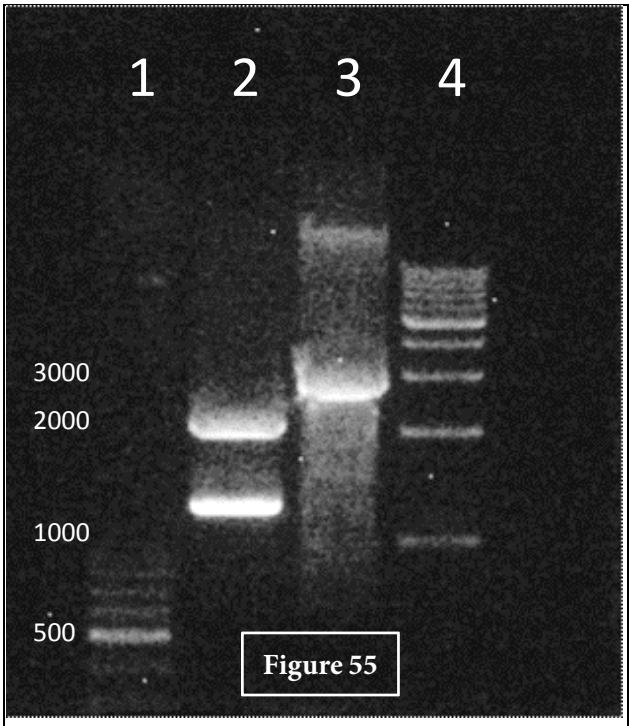


Figure 55

1: 100 bp Marker;
2:BBa_F2621(3OC6HSL Receiver Device);
3:BBa_B0034+BBa_K629003+BBa_B0015;
4:1kb Marker.

Purpose: The gel electrophoresis was prepared for the ligation between BBa_F2621 and BBa_B0034+BBa_K629003+BBa_B0015.
Results/discussion: We used the BBa_F2621 as backbone and BBa_B0034+BBa_K629003+BBa_B0015 as insert gene. From the figure, we knew that the BBa_F2621 was true. However the circuit BBa_B0034+BBa_K629003+BBa_B0015(RBS+*Che Z*+TT) was not correct. What's more, the image was not very clear.

	Centrifuge Tube	All	Agarose gel
2013-P1-21F	0.890 g	0.954 g	0.094 g
2M-18G-4F	0.876 g	0.958 g	0.082 g

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
2013-P1-21F	1.85	14.1
2M-18G-4F	1.70/1.78	27.1/23.9

- 2013-P1-21F—1 2M-18G-4F—2
  V1/V2=2.061
- Enzyme Restriction
  Xba I, *Pst* I
- Verification: Agarose gel electrophoresis
  From left to the right: 500Marker-(2014-P4-4B)-(2012-P -17F)-[2L(+)_1-2]-[2L(-)_1-1]-[2J(+)_1-1]
- Transformation
  2L(+)_1-1 2L(+)_1-2 2J(-)_1-1 2J(-)_1-2
- The Experiment Plan:

- Without Cm, Ara, the Concentration gradient of IPTG (0~10mM)
- Aim:

We couldnot find the maximum concentration of IPTG for the *E. coli*'s chemotaxis without arabinose.

We are going to find the range of the most effective concentration for IPTG regulation so that we could complete the orthogonal test.

The concentration of IPTG gradients/mM	The diameter of chemotaxis/cm
0	2.4
0.01	3.7
0.1	1.7
0.2	2.7
0.5	0
1	0
10	0

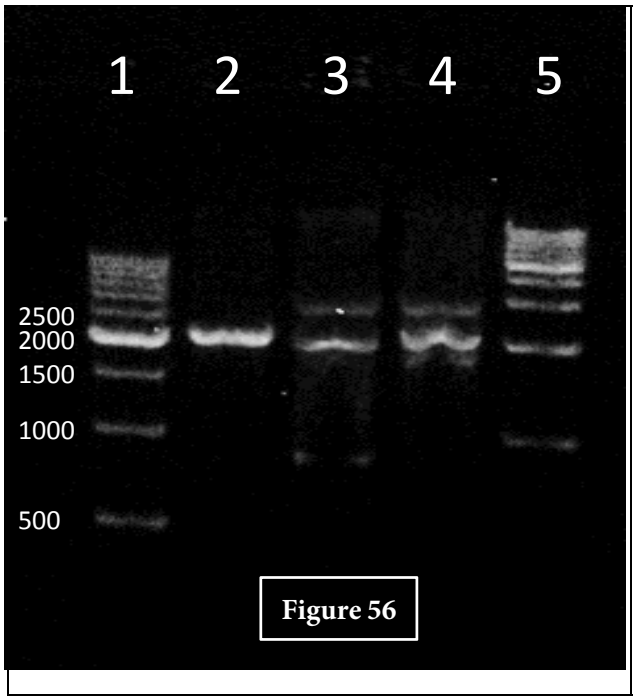


Figure 56

1: 500 bp Marker;
2: BBa_K629003+BBa_B0015(*CheZ*+TT)(1);
3: BBa_K629003+BBa_B0015(*CheZ*+TT)(2);
4: BBa_K629003+BBa_B0015(*CheZ*+TT)(3);
5: 1kb Marker
((1), (2), (3) are different colonies.)

Purpose: The verification of the connection system: BBa_K629003+BBa_B0015.

Results/discussion: The circuit were all ligated negatively, and we chose three samples two of which were from the same plate. We found that only the sample 2 was correct.

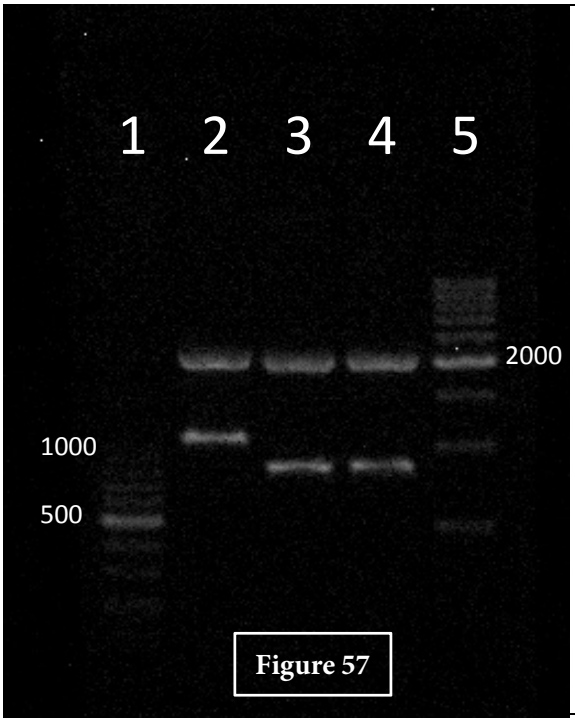


Figure 57

1: 100bp marker;
2:BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 in RP1616;
3: BBa_B0034+BBa_B0015(2);
4: BBa_B0034+BBa_B0015(1);
5: 500bp marker.
(1 and 2 are different colonies on the same plate.)

Purpose: The verification of the connection systems: BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_B0034+BBa_K629003+BBa_B0015.
Results/discussion: Because the length of BBa_R0010+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_B0034+BBa_K629003+BBa_B0015 are 1008 bp and 800 bp re*Spec*tive. From the figure, we could confirm that the connection system we got are all correct.

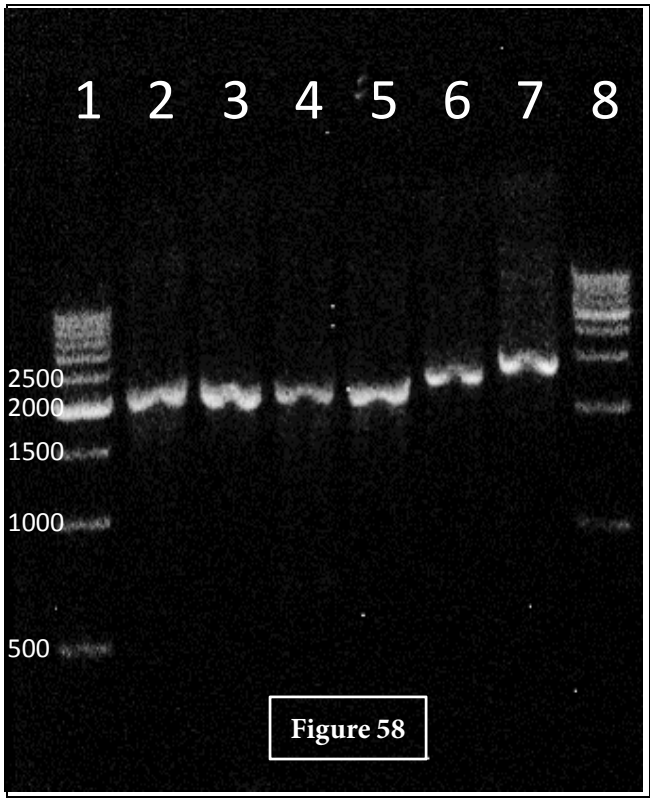


Figure 58

1: 500bp Marker;
2: BBa_K629003+BBa_B0015(*CheZ*+TT)(1);
3: BBa_K629003+BBa_B0015(*CheZ*+TT)(2);
4: BBa_K629003+BBa_B0015(*CheZ*+TT)(3);
5: BBa_K629003+BBa_B0015(*CheZ*+TT)(4);
6: BBa_K629003+BBa_B0015(*CheZ*+TT)(5);
7: BBa_K629003+BBa_B0015(*CheZ*+TT)(6);
8: 1kb Marker.
((1), (2), (3), (4), (5), (6) are different colonies.)

Purpose: The verification of the connection system:BBa_K629003+BBa_B0015.
Results/discussion: We chose four different samples and each pair was from the same plate. The sample 1-1 was cut by two different enzymes. The first four were ligated positively. The exact length of the part ought to be 2814bp and they were at the same length theoretically. None of them was correct.

2014-08-24

- Ligation: 2013-P3-5I+2012-P2-24I
- Enzyme Restriction: 2012-P2-17F

Single	*Pst* I
Double	*Pst* I, *Xba* I

- Verification: Agarose gel electrophoresis(Inter- Lab study)
  From left to the right: 500Marker-(2012-P2-22I)-(2013-P5-5I)-(2012-P2-17F Double)-(2012-P2-17F Single)-1000Marker
- Reback to dwassolve

Lux R	2014-P2-4L	Cm
RBS+Lux R+TT	2014-P4-7P	Amp
Lux R	2014-P2-4J	Cm

- Measure the concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/μL)
2014-P3-5I	1.37/1.33/1.88/1.86	46.1/36.0/15.9/15.6
2012-P2-24I	1.12/1.51/1.96/1.59/1.93	9.0/21.5/9.8/16.5/10.7

- 2012-P2-24I—1　　2013-P3-5I—2
  V1/V2=1.25
- Measure the Concentration of the Plasmids: 21F-2M-18G-4F

	Absorbance: 260/280	Measurement(ng/μL)
(-1)	1.84/1.80/1.85	211.3/217.9/240.1
(-2)	1.79/1.67/1.80/1.83	165.4/248.6/206.i8/176.1
(+1)	1.86/1.87/1.82	97.1/90.4/107

- Verification: Agarose gel electrophoresis: 12D-2M-18G-4F
  From left to the right: 1000Marker-(DH(2)-1 Double)-(DH(2)-1 Single)-(DH(2)+1 Double)-(DH(2)+1 Single)-(DH(1)-1 Double)-(DH(1)-1 Single)-(*CL-1*(2)-1 Double)-(*CL-1*(2)-1 Single)-(*CL-1*(1)+ Single)-(*CL-1*(1)-1 Double)-(*CL-1*(1)-1 Single)- (*CL-1*(1)-2 Double)-(*CL-1*(1)-2 Single)
- Enzyme Restriction

RFP	*Xba* I, *Pst* I
(2014-P1-20K)+(2014-P2-24B)	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis(Inter-Lab study)

From left to the right: 100Marker-(20K+24B)-(2013-P2-6F)-(2014-P4-6F)-1000Marker

	Centrifuge Tube	All	Agarose gel
20K+24B	0.926	0.966	0.030
2013-P2-6F	0.925	0.954	0.935
2014-P4-6F	0.890	0.935	0.045

- Transformation

2L(+)_1-1 2L(+)_1-2 2J(-)_1-1 2J(-)_1-2

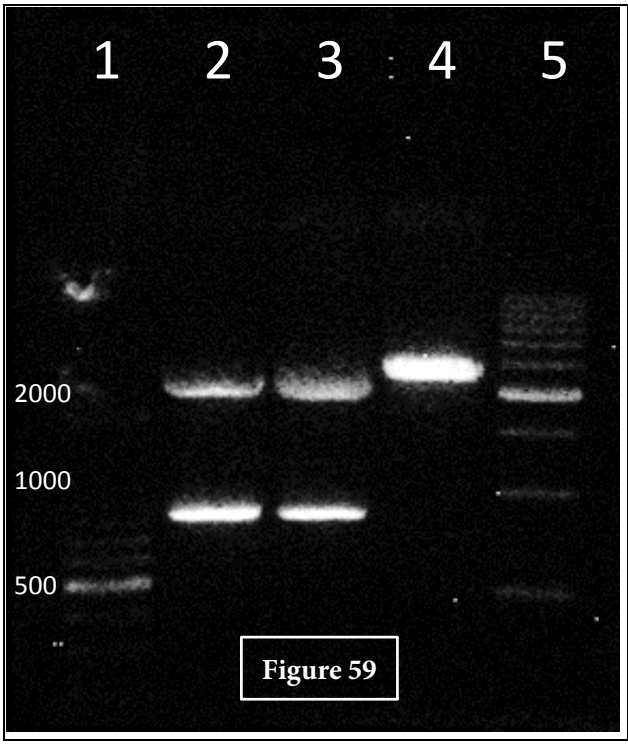


Figure 59

1: 100 bp Marker;
2:BBa_B0033+BBa_K629003+BBa_B0015;
3:BBa_B0032+BBa_K629003+BBa_B0015;
4: BBa_R0010(pLac);
5: 500 bp Marker.

Purpose: The gel electrophoresis was prepared for the ligation between BBa_R0010 and BBa_B0033+BBa_K629003+BBa_B0015 or BBa_B0032+BBa_K629003+BBa_B0015.
Results/discussion: We used the BBa_B0032/BBa_B0033+BBa_K629003+BBa_B0015 as the vector and BBa_R0010 as the insert gene. From the image we could see that the BBa_R0010 was not restricted entirely. But the length of BBa_B0032/BBa_B0033+BBa_K629003+BBa_B0015 was correct.

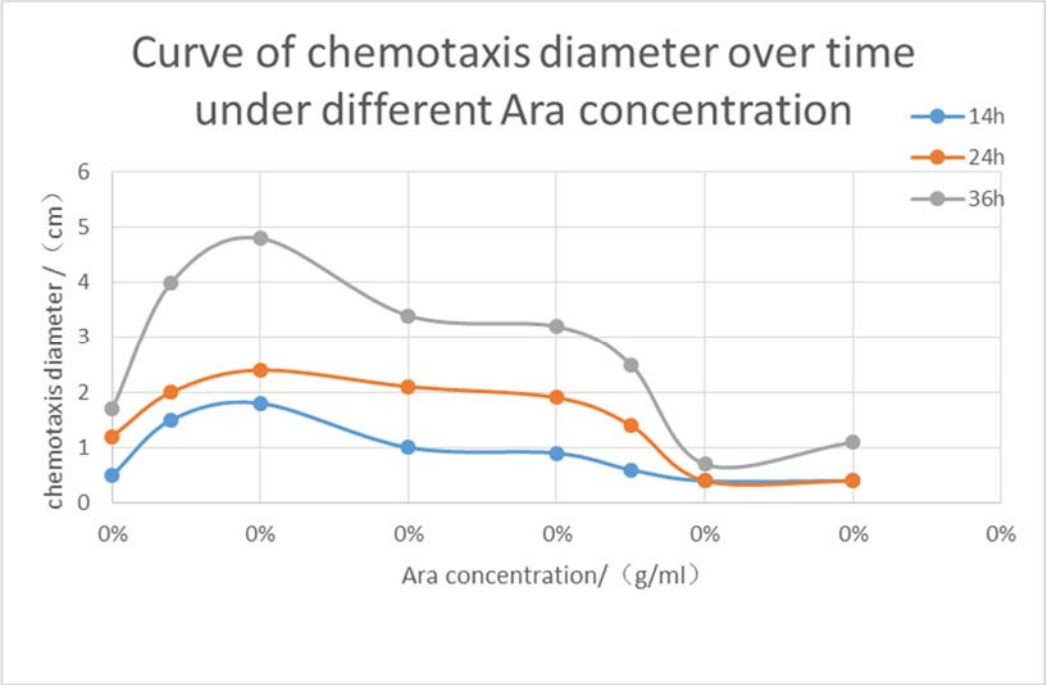
- The Exhibition Plan

With 0.01 mmol/mL IPTG and the concentration gradients of Cm.

- Aim:

Without resistance pressure, the bacteria lose its reswastant plasmid very easily at the same time they will lose *CheZ*, and as a result they lose the ability of chemotaxis. We need to find the appropriate concentration of antibiotics to make sure the multiplication of *E. coli* meanwhile we wanted to repress the growth of the bacteria that we didn't need.

The concentration of Cm/mM	The Chemotaxis Diameter/cm
0	0.5
20	0.7
30	1.6
40	0.6
50	3.5
100	1.6
200	2.5



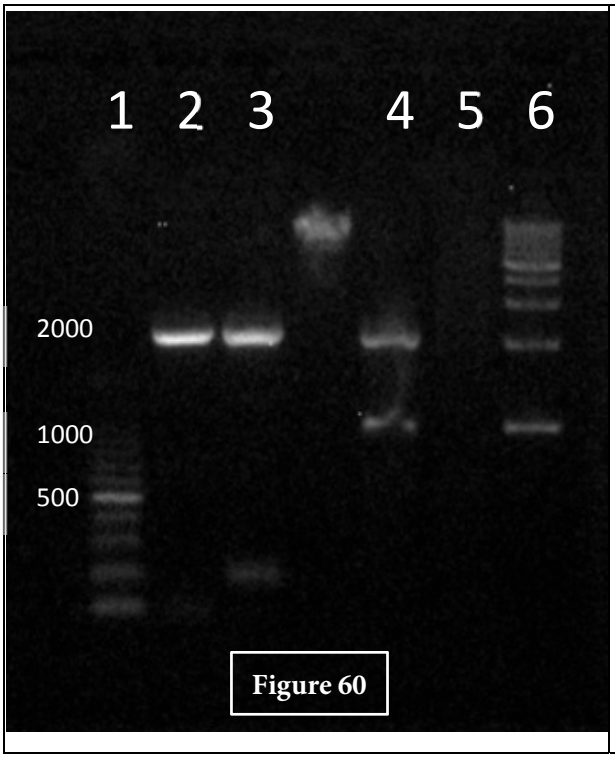


Figure 60

1: 100bp marker;
2: BBa_R0062(lux pR);
3: BBa_R0063(lux pL);
4: BBa_I0460(RBS +aiiA +TT)(1);
5: BBa_I0460(RBS +aiiA +TT)(2);
6: 1kb marker.
(1 and 2 are different colonies on the same plate)

Purpose: The verification of the BioBricks: BBa_R0062, BBa_R0063 and BBa_I0460.
Results/discussion: The length of lux pR, lux pL are 55 bp, the length of RBS +aiiA +TT was 969 bp. Therefore, from the length of gel electrophoresis bands, we could determine that the BioBricks BBa_R0062, BBa_R0063 and BBa_I0460 we got were all correct.

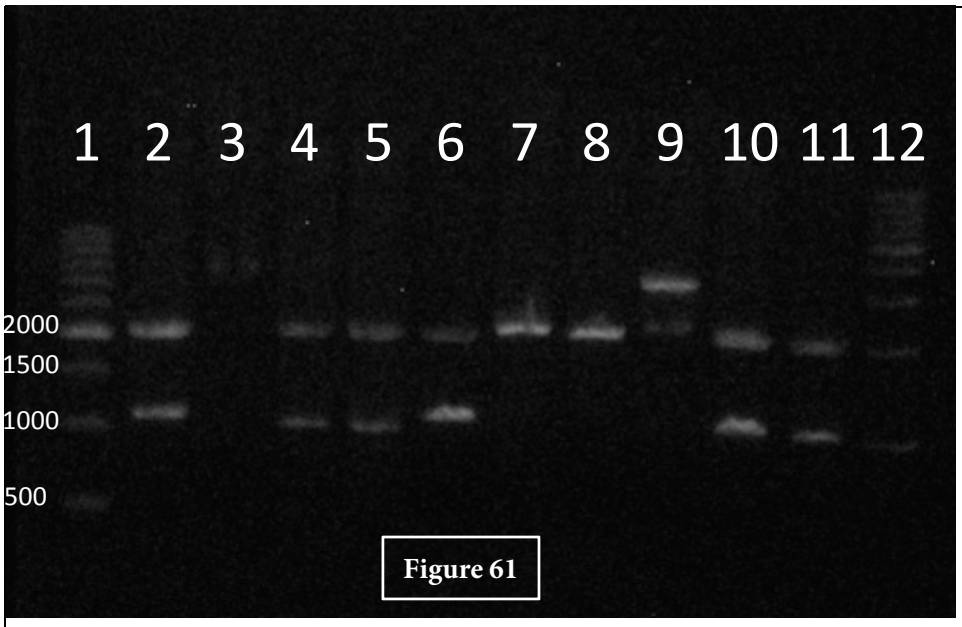


Figure 61

1: 500bp marker;
2: BBa_J04450 in *CL-1* with double digestion(XP);
3: BBa_I20260 with double digestion(XP);
4: BBa_B0033+BBa_K629003+BBa_B0015(1) with double digestion(XP);
5: BBa_B0033+BBa_K629003+BBa_B0015(2) with double digestion(XP);
6: BBa_B0033+BBa_K629003+BBa_B0015(3) with double digestion(XP);
7: BBa_B0033+BBa_K629003+BBa_B0015(4) with double digestion(XP);
8: BBa_B0032+BBa_K629003+BBa_B0015(1) with double digestion(XP);
9: BBa_B0032+BBa_K629003+BBa_B0015(2) with double digestion(XP);
10: BBa_B0032+BBa_K629003+BBa_B0015(3) with double digestion(XP);
11: BBa_B0032+BBa_K629003+BBa_B0015(4) with double digestion(XP);
12: 1kp marker.
(1, 2, 3, 4 are different colonies on the same plate)

Purpose: The verification of the connection systems: BBa_B0033+BBa_K629003+BBa_B0015 and BBa_B0032+BBa_K629003+BBa_B0015.
Results/discussion: The length of BBa_B0033(2) and BBa_B0032(4) are 799 bp and 801 bp re*Spec*tive. From the figure, we could find the corresponding bands, so we could confirm that BBa_B0033(1), (2) and BBa_B0032(3), (4) were all correct.

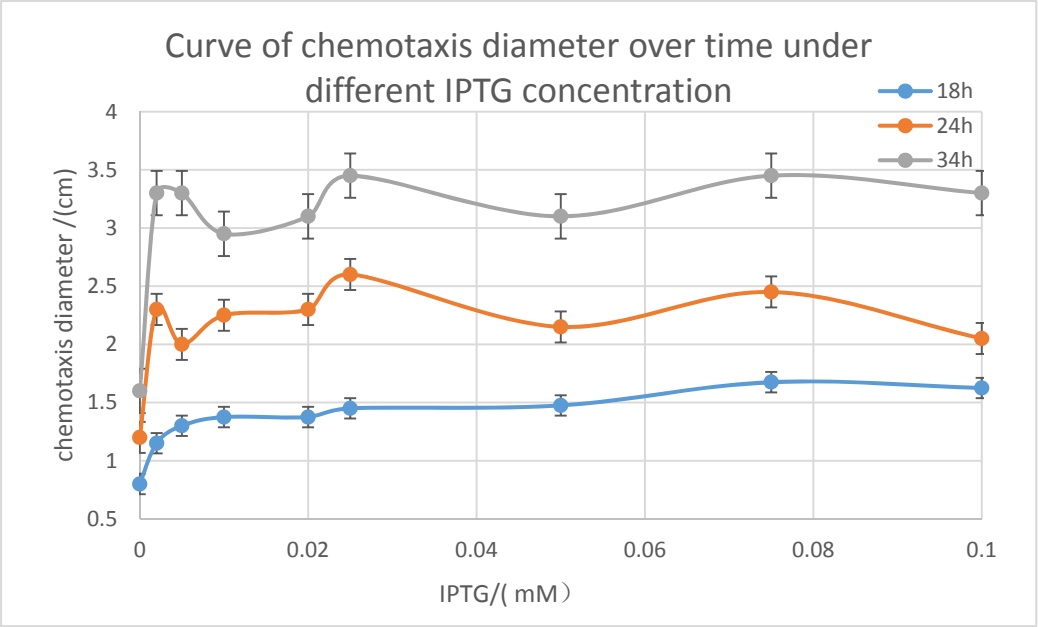
2014-08-25

- Extract the Plasmids
- The Experiment Plan:

  With concentration gradient of IPTG (improved, 0~0.2mM).
- Aim

  We wanted to gain the accurate critical concentration of IPTG for *E. coli*'s chemotaxis.

The concentration gradient of IPTG /mM	The chemotaxis diameters in 18h/cm	The chemotaxis diameters in 24h/cm	The chemotaxis diameters in 36h/cm
0	0.8	1.2	1.6
0.002	1.15	2.3	3.3
0.005	1.3	2	3.3
0.01	1.375	2.25	2.95
0.02	1.375	2.3	3.1
0.025	1.45	2.6	3.45
0.05	1.475	2.15	3.1
0.075	1.675	2.45	3.45
0.1	1.625	2.05	3.3

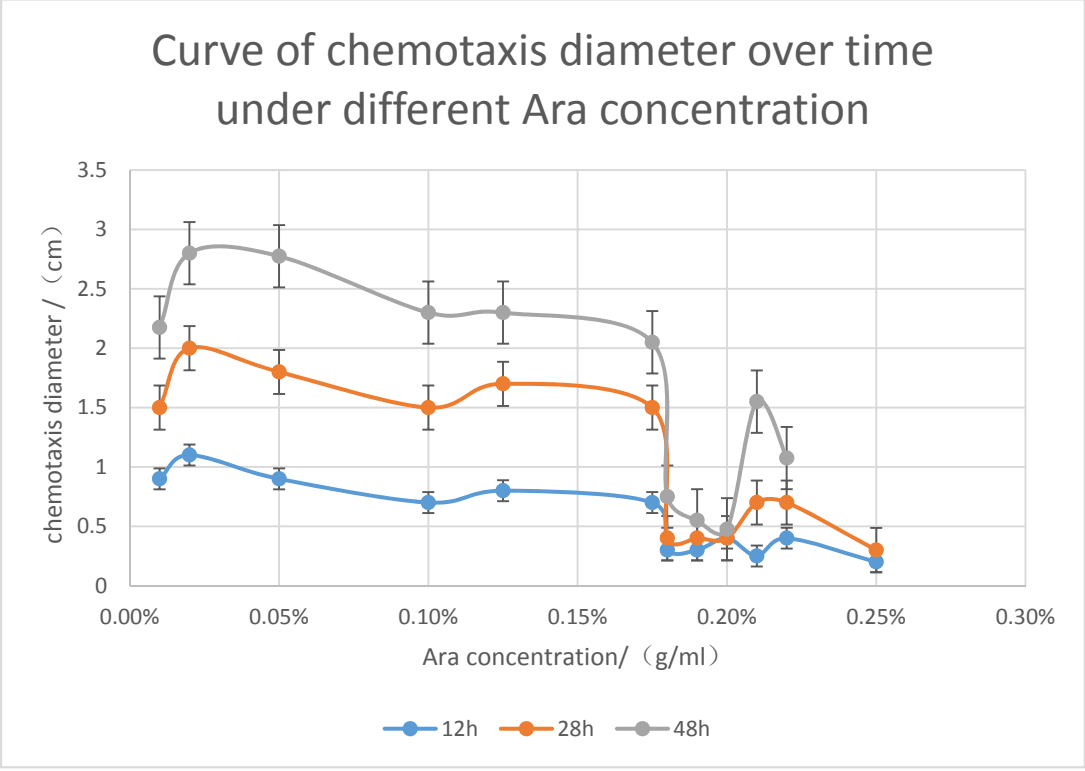


- The Experiment Plan:

  With concentration gradients of Ara (improved).
- Aim:

  We wanted to gain the accurate critical concentration of Ara.

The concentration gradients of Ara	The chemotaxis diameters in 12h/cm	The chemotaxis diameters in 28h/cm	The chemotaxis diameters in 42h/cm
0.01%	0.9	1.5	2.18
0.02%	1.1	2	2.8
0.05%	0.9	1.8	2.78
0.10%	0.7	1.5	2.3
0.125%	0.8	1.7	2.3
0.175%	0.7	1.5	2.05
0.18%	0.3	0.4	0.75
0.19%	0.3	0.4	0.55
0.20%	0.4	0.4	0.475
0.21%	0.25	0.7	1.55
0.22%	0.4	0.7	1.075
0.25%	0.2	0.3	



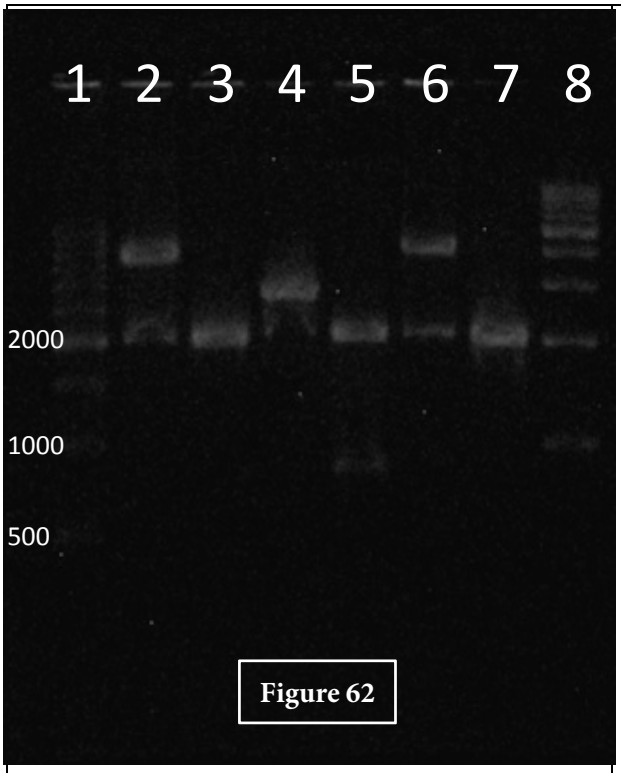


Figure 62

1: 500bp marker;
2: part(1) with single digestion(X);
3: part(1) with double digestion(XP);
2: part(2) with single digestion(X);
3: part(2) with double digestion(XP);
2: part(3) with single digestion(X);
3: part(3) with double digestion(XP);
4: 1kp marker.
(part=BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015. 1, 2, 3 are different colonies on the same plate)
Purpose: The verification of the connection system: BBa_F2621-BBa_B0034-BBa_K629003-BBa_B0015.
Results/discussion: The length of BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015 was 1994 bp, so we could get a 4014 bp band after single digestion. While we got 1994 bp band and 2070 bp band after double digestion. From the figure, we couldn't get a good result.

2014-08-26

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

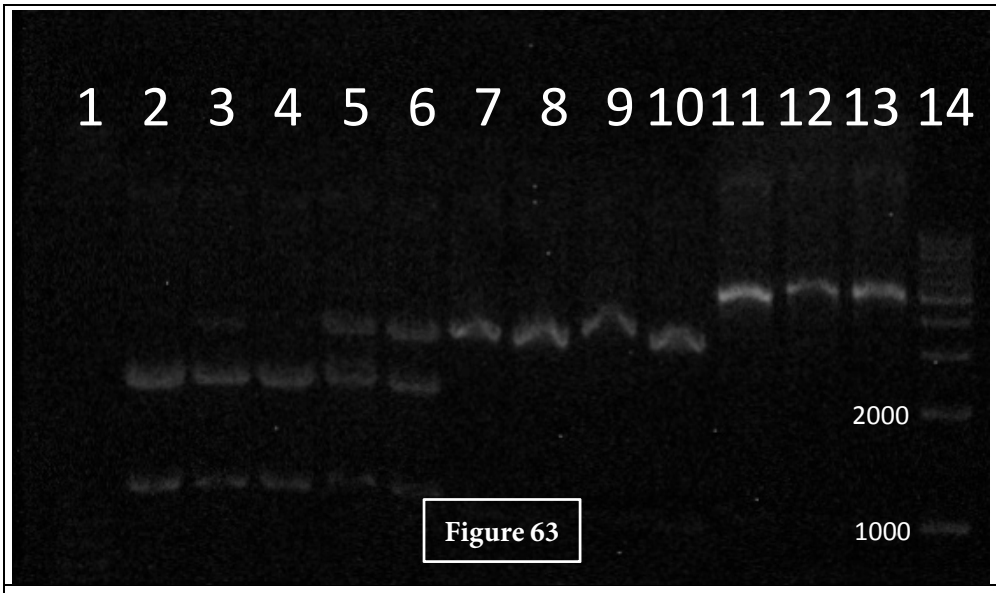


Figure 63

1: 100bp marker;
2: PART 1(1) with double digestion;
3: PART 1(2) with double digestion;
4: PART 2(1) with double digestion;
5: PART 2(2) with double digestion;
6: PART 2(3) with double digestion;
7: 1kb marker.
(PART1=BBa_B0033+BBa_B0034+BBa_K629003+BBa_B0015,
PART2= BBa_B0032+BBa_B0034+BBa_K629003+BBa_B0015)
(1, 2, 3 are different colonies on the same plate)

Purpose: The verification of the connection systems: BBa_B0032+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_B0033+BBa_B0034+BBa_K629003+BBa_B0015.
Results/discussion: The length of BBa_B0032+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_B0033+BBa_B0034+BBa_K629003+BBa_B0015 are 801 bp and 799 bp re*Spec*tive. From the figure, we found that the connection systems were all correct. While the BBa_B0032 still had some problems, because there were three bands in 4 and 5 runway.

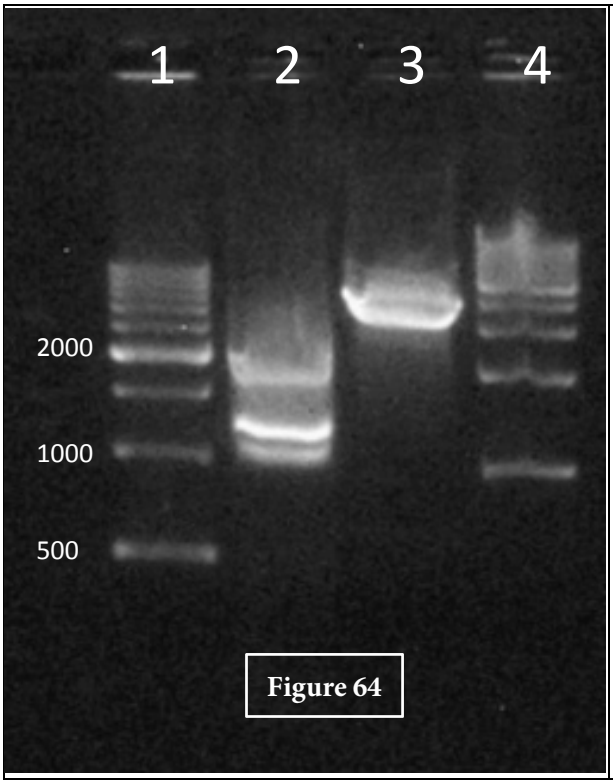


Figure 64

1: 500bp marker;
2:BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015 with triple digestion(XP Sac I);
3: BBa_K546000 with double digestion(SP);
4: 1kp marker.

Purpose: Prepare for the connection between BBa_K546000 and BBa_F2621-BBa_B0034-BBa_K629003-BBa_B0015.
Results/discussion: The length of BBa_F2621-BBa_B0034-BBa_K629003-BBa_B0015 was close to the backbone pSB1C3, so we couldn't separate them after common double digestion. At the end, we used the other enzyme Sac I to cut out the backbone. So we could find three bands from runway 2, and get the corresponding band about 1944 bp. While we got a dim result, maybe there were some factors during the whole process.

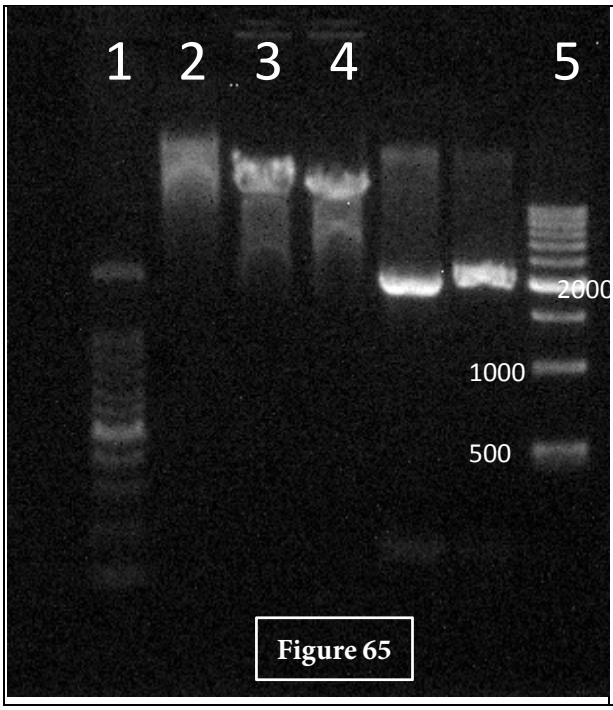


Figure 65

1: 100 bp marker;
2: BBa_I0462(RBS +lux R+TT);
3: BBa_C0062(lux R)(1);
4: BBa_C0062(lux R)(2);
5: 500 bp marker.
(1, 2 are different colonies on the same plate)

Purpose: The verification of the BioBricks: BBa_I0462 and BBa_C0062.
Results/discussion: The result was so bad because of the serious trailing phenomenon. We thought the reason was that the dye time was not enough.

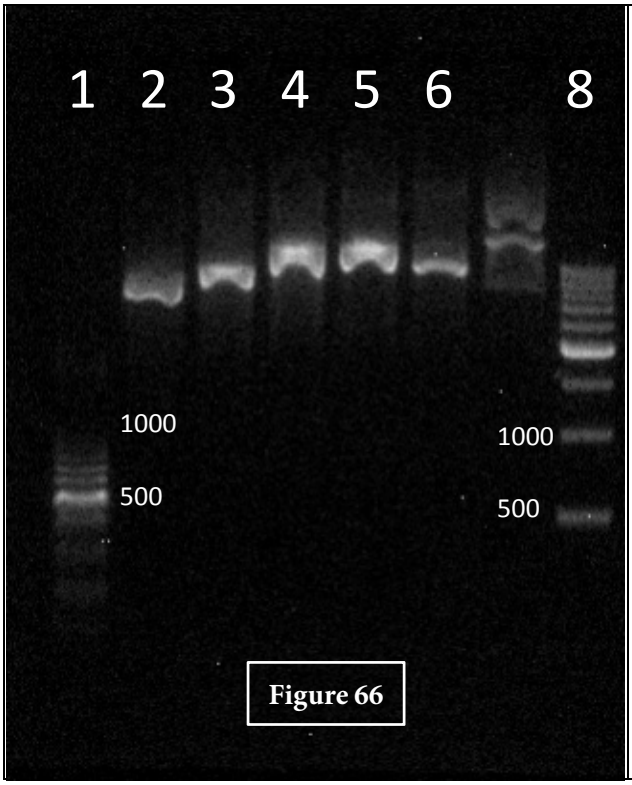


Figure 66

1: 100bp marker;
2: BBa_I20260 with double digestion (1);
3: BBa_I20260 with double digestion (2);
4: BBa_I20260 with double digestion (3);
5: BBa_I20260 with double digestion (4);
6: BBa_I20260 with double digestion (5);
7: BBa_I20260 with single digestion (5);
8: 500 bp marker.
(1, 2, 3, 4, 5, 6 are different colonies.)

Purpose: The verification of BioBrick BBa_I20260.
Results/discussion: from the figure, we couldn't get a conclusion after the digestion and gel electrophoresis, because the stripes were irregularity. What's worse, we couldn't get the band of BBa_I20260 BioBrick after double digestion.

2014-08-27

- Enzyme Restriction: Double

pBAD/pTETR	2M-18G-4F
Spe I *Pst* I	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis

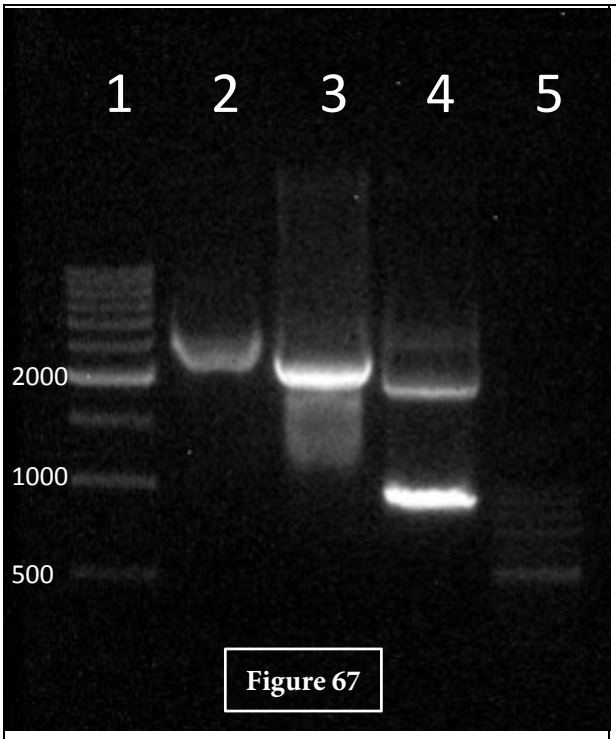


Figure 67

1: 500 bp marker;
2: BBa_R0040 with double digestion(SP);
3: BBa_K206000 with double digestion(SP);
4: BBa_B0034-BBa_K629003-BBa_B0015 with double digestion(XP);
5: 100 bp marker.

Purpose: Preparation for the connection between BBa_B0034+BBa_K629003+BBa_B0015 and promoter BBa_R0040, BBa_K206000 respective.
Results/discussion: We wanted to detect the strength of different promoter, so we used the plasmid containing promoter BBa_R0040 and BBa_K206000 as the backbone. Then we cleavaged the plasmid with *Spe* I and *Pst* I enzyme. And cleavage the plasmid containing BBa_B0034-BBa_K629003-BBa_B0015 with *Xba* I and *Pst* I enzyme to get the insert gene. From the figure, we found the corresponding bands what we wanted.

	Centrifuge Tube	All	Agarose gel
2014-P2-6F	0.909g	0.959g	0.050g
2014-P3-14A	0.919g	0.970g	0.051g
2M-18G-4F	0.911g	0.989g	0.078g

- Measure the Concentration of the Plasmids

	Absorbance: 260/280	Measurement(ng/ μL)
2014-P2-6F	1.65/2.33/2.04	5.1/3.0/3.4
2014-P3-14A	5.1/3.0/3.4	26.1/26.5
2M-18G-4F	1.80/1.62/1.60	13.7/16.7/17.4

- 1—2014-P2-4F, 2—2014-P3-14A, 3—2M-18G-4F

V1/V3=3*M1*C3/1*M3*C1=33.72(5:1)
V2/V3=3*M2*C3/1*M3*C2=5.1

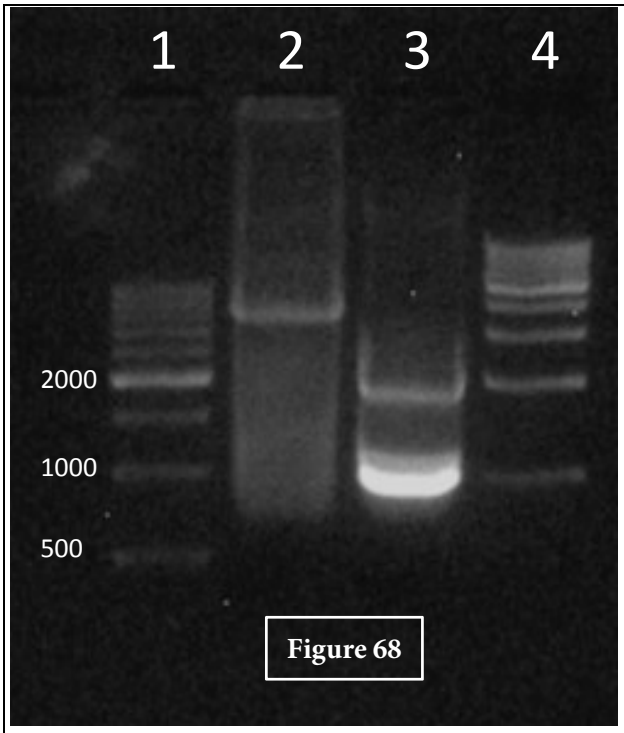


Figure 68

1: 500 bp marker;
2: BBa_K546000 with double digestion(SP);
3:BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015 with triple digestion(XP Sac I);
4: 1kb marker.

Purpose: Prepare for the connection between BBa_K546000 and BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015.

Results/discussion: Because the length of connection system BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015 was close to the backbone pSB1C3, so we used triple digestion to cut out the backbone. While from the figure, we couldn't get a good result, so we couldn't do the next experiment.

2014-08-28

- Extract the Plasmids: 2014-P2-6F
- Inoculation

2014-08-29

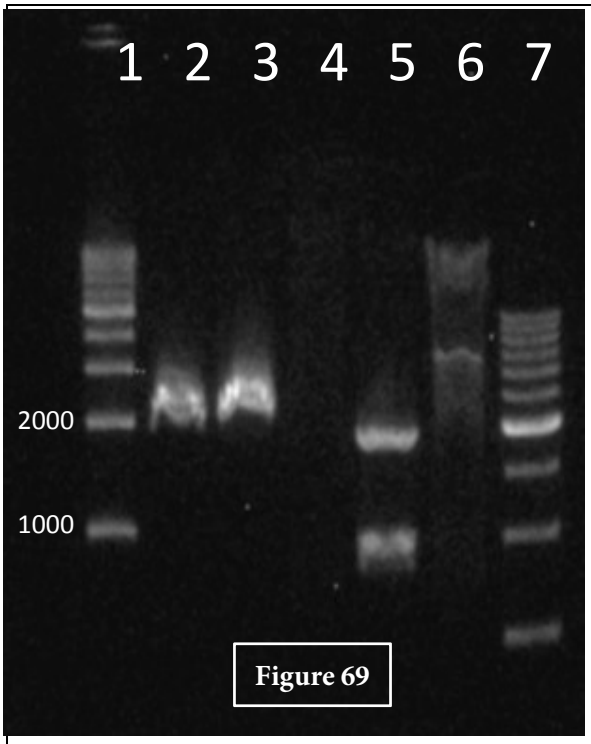


Figure 69

1: 1kp marker;
2:BBa_K546000+BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015(1) ;
3:BBa_K546000+BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015(2);
4: BBa_I13507(RBS+RFP+TT);
5: BBa_I13504(RBS+GFP+TT);
6: BBa_B0015;
7: 500 bp marker.
(1 and 2 are different colonies on the same plate)

Purpose: The verification of the connection system: BBa_K546000+BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015.

Results/discussion: The length of BBa_I13504 and BBa_K546000+BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015 are 875 bp and 3908 bp re*Spe*ctive. From the figure, we could determine that the connection system was wrong, because we couldn't get the corresponding bands after digestion, and the result was dim. While the BioBrick BBa_I13504 was correct. But the result of second lane was so bad because of the serious trailing phenomenon.

2014-08-30

- Enzyme Restriction: 2014-P2-6F 2014-P3-14A

Single	*Xba* I, *Pst* I
Double	*Xba* I

- Verification: Agarose gel electrophoresis

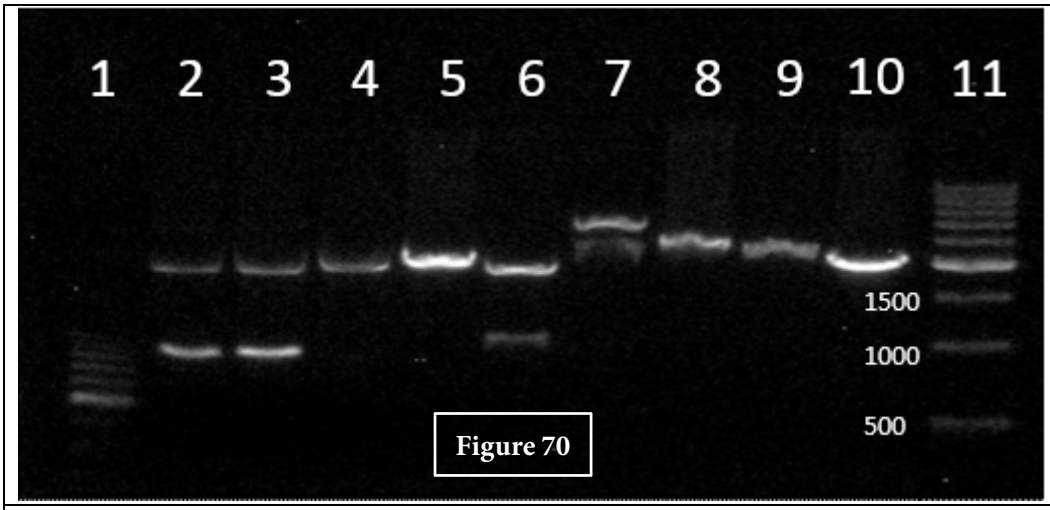


Figure 70

1: 100 bp marker;
2: BBa_K206000+BBa_B0034-BBa_K629003-BBa_B0015(1) with double digestion;
3: BBa_K206000+BBa_B0034-BBa_K629003-BBa_B0015(2) with double digestion;
4: BBa_K206000+BBa_B0034-BBa_K629003-BBa_B0015(3) with double digestion;
5: BBa_K206000+BBa_B0034-BBa_K629003-BBa_B0015(4) with double digestion;
6: BBa_R0040+BBa_B0034-BBa_K629003-BBa_B0015(1) with double digestion;
7: BBa_R0040+BBa_B0034-BBa_K629003-BBa_B0015(2) with double digestion;
8: BBa_R0040 with double digestion;
9: BBa_R0040+BBa_B0034-BBa_K629003-BBa_B0015(3) with double digestion;
10: BBa_R0040 with single digestion;
11: 500 bp marker.
(1, 2, 3, 4 are different colonies on the same plate)
Purpose: The verification of the connection systems: BBa_K206000+BBa_B0034-BBa_K629003+BBa_B0015 and 1BBa_R0040+BBa_B0034-BBa_K629003-BBa_B0015 .
Results/discussion: the length of the connection system BBa_K206000+BBa_B0034+BBa_K629003+BBa_B0015 and BBa_R0040+BBa_B0034+BBa_K629003+BBa_B0015 are 938 bp and 862 bp respective. After digestion, we found the corresponding band near 900bp from the figure, so we could confirm that the colonies BBa_K206000+BBa_B0034-BBa_K629003-BBa_B0015(1), BBa_K206000+BBa_B0034+BBa_K629003+BBa_B0015(2) and BBa_R0040+BBa_B0034-BBa_K629003-BBa_B0015(1) are correct.

- The Experiment Plan:

  With 50μg/ml, 0.02% Ara, 3 μL bacteria solution and different concentration IPTG (0.25 mM, 0.5 mM). The volume gradients of medium (3 ml, 4 ml, 5 ml).

  Aim: Verify the formation of the pattern of hyperbola.

The concentration of IPTG	3 ml medium	4 ml medium	5 ml medium
0.25 mM IPTG	1	2	3
0.5 mM IPTG	4	5	6

Conclusion: We could not get the pattern of hyperbola in the method of plate spread.

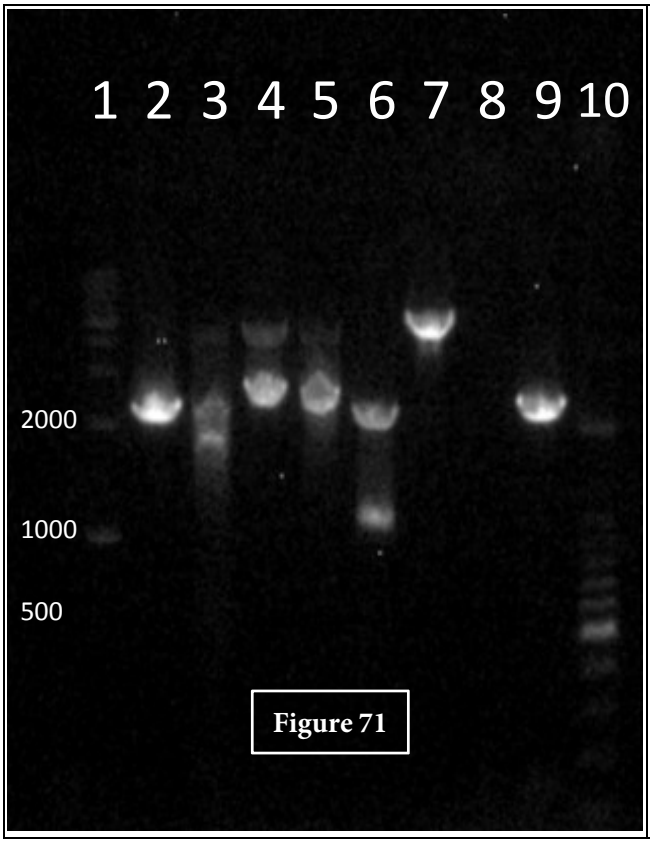


Figure 71

1: 1kb marker;
2: PARTB (1);
3: PARTB (1) with single digestion;
4: PARTB (2);
5: PARTB (3);
6: PARTB (4) with double digestion;
10: 100 bp marker.
(PARTB=BBa_K546000+BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015, 1, 2, 3, 4 are different colonies on the same plate)

Purpose: The verification of the connection system: BBa_K546000+BBa_F2621+BBa_B0034+BBa_K629003+BBa_B0015.

Results/discussion: From the result, we could only got the length of the backbone pSB1C3, but we didn't know whether the bacteria contain the CheZ+T gene. What's more, we found difference between the bands was not obvious, maybe the digestion time was not enough.

2014-08-31

- 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

  Separately into 5 ml LB liquid medium whose antibiotic concentration was 50 μg/ml. Culture for 12 h. Then transfer 50 μL bacterium solution into new LB liquid medium whose antibiotic concentration was 50 μg/ml to culture for 12 h.
- Characterization

  Then stab 3 μL bacterium medium into the M63 semi-solid medium at the dots. And culture the bacteria in constant temperature and humidity incubator at 37℃.
- Measure the radius of *E. coli*
- Extract the Plasmids
- The experiment Plan

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

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

The concentration gradients of IPTG/mM	12 h The chemotaxis diameter toward IPTG （d1） /cm	The chemotaxis diameters away from IPTG （d2） /cm	Difference（d1-d2）/cm	24 h The chemotaxis diameter toward IPTG （d1） /cm	The chemotaxis diameters away from IPTG （d2） /cm	Difference（d1-d2）/cm	36 h The chemotaxis diameter toward IPTG （d1） /cm	The chemotaxis diameters away from IPTG （d2） /cm	Difference（d1-d2）/cm
0.1	0.2	0.12	0.08	0.7	0.6	0.1	1.4	1.2	0.2
0.25	0.25	0.1	0.15	0.9	0.8	0.1	1.65	1.3	0.35
0.5	0.32	0.2	0.12	0.75	0.55	0.2	1.5	1.1	0.4
1	0.2	0.15	0.05	0.7	0.6	0.1	1.55	1.3	0.25

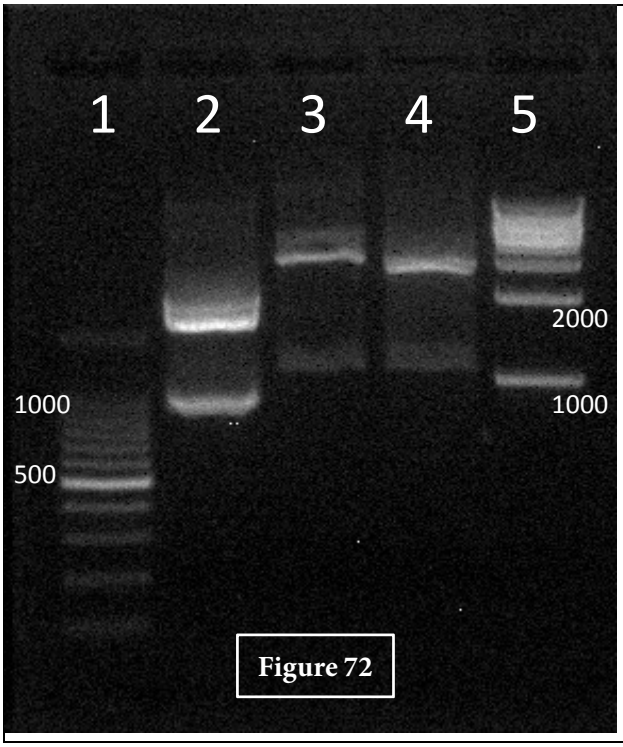


Figure 72

1: 100 bp marker;
2: BBa_K1412924 with double digestion;
4: pSB3K3(1) backbone with double digestion;
5: pSB3K3(2) backbone with double digestion;
3: 1kb marker.
((1), (2) are different colonies.)

Purpose: Because we couldn't get a correct plasmid from the transformation. So we wanted to connect the plasmid ourselves.
Results/discussion: We cut J23101+RBS0032+GFP+TT from BBa_K1412924, and use the pSB3K3 as backbone.