LABNOTE-A

XMU-iGEM

Date: 7.17-7.30

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 ✓	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					

6M 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						

8 M 2014Y

NOTE :

2014-7-17

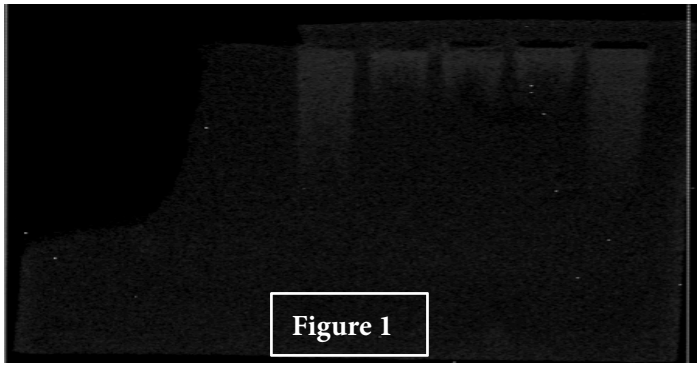
- Extraction of plasmids:

pLac	BBa_R0010	2014-P3-4G	200 bp
CheZ	BBa_K629003	2014-P1-18G	645 bp
T	BBa_B1005	2014-P3-5B	34 bp

- Preparation of competent bacteria:
- Measure the concentration of the plasmid we extracted this morning. We found:

BioBricks	Absorbance: 260/280	Measurement (μg/μL)
2014-P3-5B	0.98/1.77	464.1/54.0
2014-P3-4G	1.16/1/77	303.1/86.5
2014-P1-18G	1.27/2/16	218.0/70.4

- Enzyme Restriction:
  EcoR I, *Spe* I
- Verification: Agarose gel electrophoresis
- From left to right:
  Figure 1. 100Marker (2 μL)-5B-4G-18G-500Marker (5 μL)



- Tonight we took 10 μL Δ*CheZ* and DH5α strains re*Spec*tively on solid medium, placing them in a thermostatic incubator for 12 hours. Actually, we found that the diffusion of these two strains are the same. We su*Spec*ted that it is because we didn't use the semi-solid medium so that these bacteria cannot move.

2014-7-18

- Inoculation:
  BioBricks: 18G, 5B, 4G
- Strains: Δ*CheZ*, DH5α
- Enzyme Restriction:
  EcoR I, *Spe* I
- Verification： Agarose gel electrophoresis

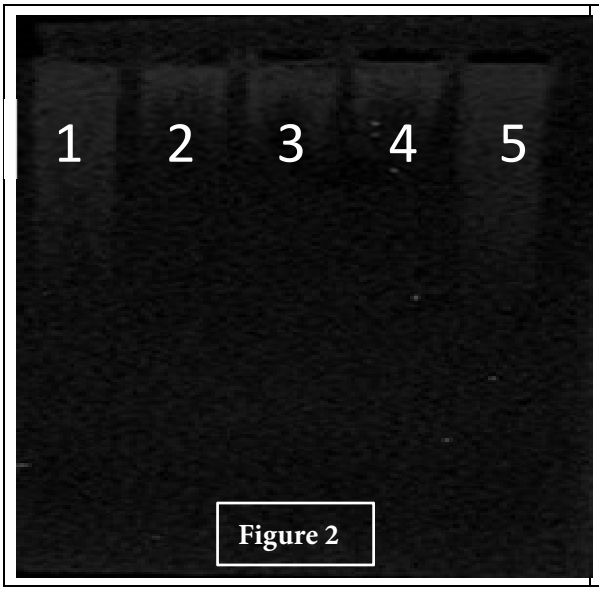


Figure 2

1: 100 bp marker;
2: BBa_B1005(terminator);
3: BBa_R0010(pLac);
4: BBa_K629003(*CheZ*);
5: 1kb marker.

Purpose: The verification of the BioBricks: BBa_B1005, BBa_R0010, BBa_K629003.
Results/discussion: We couldn't get any information from this figure. Finally we found the reason was that we used the wrong agar.

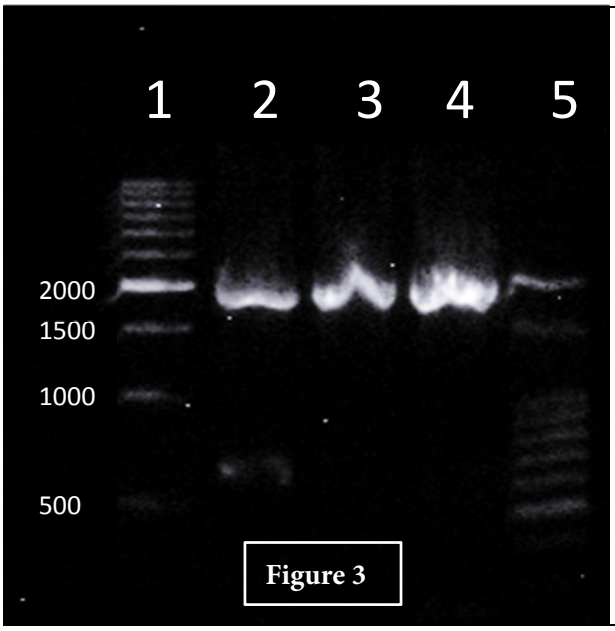


Figure 3

1: 500 bp marker;
2: BBa_K629003(*CheZ*);
3: BBa_B1005(terminator);
4: BBa_R0010(pLac);
5: 100 bp marker.

Purpose: The verification of the BioBricks: BBa_B1005, BBa_R0010, BBa_K629003.
Results/discussion: From the result, we could confirm that BBa_K629003 was true because of the band of 645 bp. But BBa_B1005 and BBa_R0010 were wrong, maybe our digestion was wrong or other influence factors.

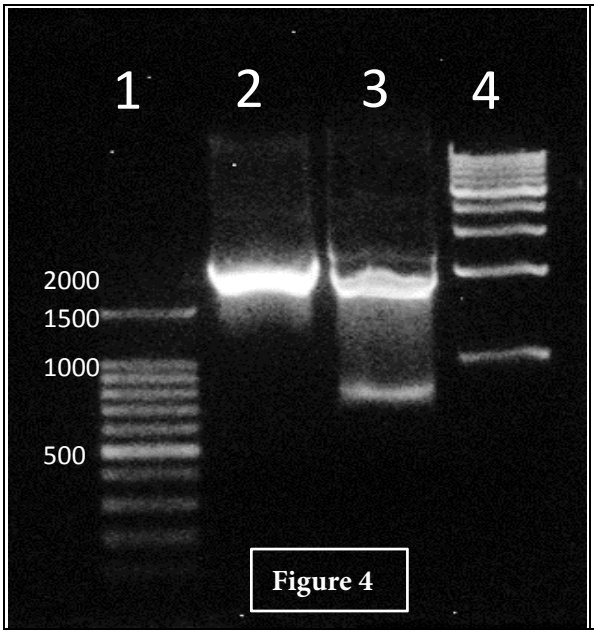


Figure 4

1: 100 bp marker;
2: BBa_B1005(terminator);
3: BBa_K629003(*CheZ*);
4: 1kb marker.

Purpose: The verification of the BioBricks: BBa_B1005, BBa_K629003, prepared for gel extraction and the ligation system.
Results/discussion: From the result, we could confirm that BBa_K629003 was true because of the length of band 3 is 645 bp. But BBa_K629003 was wrong, maybe the transformation was failed.

- Extraction of Plasmids:
  Tomorrow we would measure the concentration and purification of these plasmids.
- Inoculation
  BioBricks: 18G, 5B, 4G
- Strains: Δ*CheZ*, DH5α

2014-7-20

- Ligation
- Measure the concentration of the plasmid we connected. We connected:

BioBricks	Absorbance: 260/280	Measurement (μg/uL)
2014-P1-18G1	1.35	0.6
2014-P1-18G2	1.75	2.9
2014-P3-5B	1.42	44.3

- Conclusion: 18G2 is the purest gene.
- Ligation:

  1-*CheZ*, 2-T
- Conclusion:

  $V_1/V_2=3\times M_1\times C_2/1\times M_2\times C_1=3\times 645\times 44.3/1\times 2070\times 2.9=14.28$
- Total Volume: 20 μL
- Temperature: 16℃
- Preparation of the competent bacteria
- Extraction of the RBS

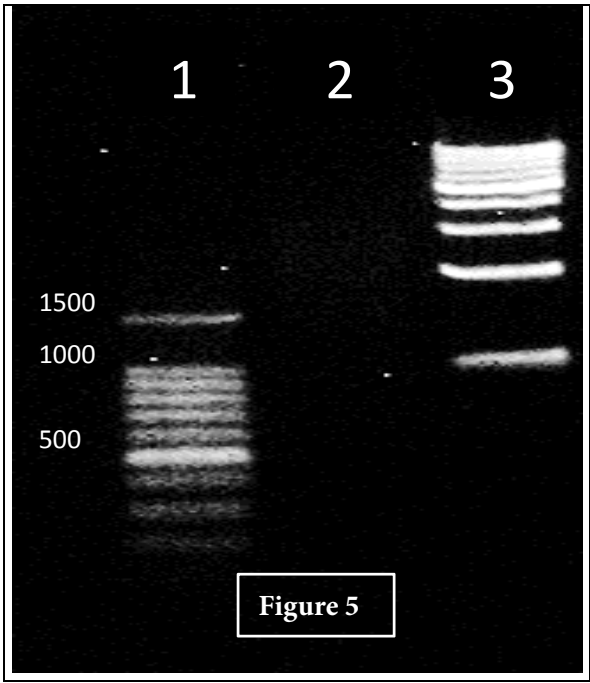


Figure 5

1: 100 bp marker;
2: BBa_B0034(RBS);
3: 1kb marker.

Purpose: The verification of the BioBrick: BBa_B0034.
Results/discussion: From the result, we couldn't get any information. Maybe our digestion was wrong or the transformation was failed.

- Measure the concentration of the RBS. We extracted:

BioBrick	Absorbance: 260/280	Measurement (μg/μL)
RBS	1.64	14.3

- Enzyme Restriction:
  EcoR I, *Spe* I
- Conclusion: Whether it is positive or negative, the Ligation system on the plates grew very dense colonies. RBS should be restricted and transferred again.

2014-7-21

- Conservation
- Enzyme Restriction

BioBrick	Single	Double
BBa_B0034(RBS)	EcoR I	EcoR I, *Spe* I

- Verification: Agarose gel electrophoresis

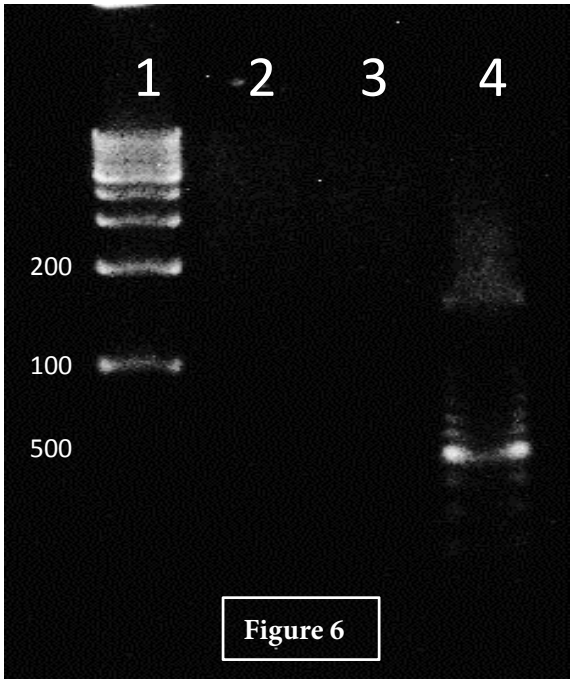


Figure 6

1: 1kb marker;
2: BBa_B0034(RBS) with double digestion;
3: BBa_B0034(RBS) with single digestion;
4: 100 bp marker.

Purpose: The verification of the BioBrick: BBa_B0034.
Results/discussion: From the result, we couldn't get any information. Maybe our digestion was wrong or other factors.

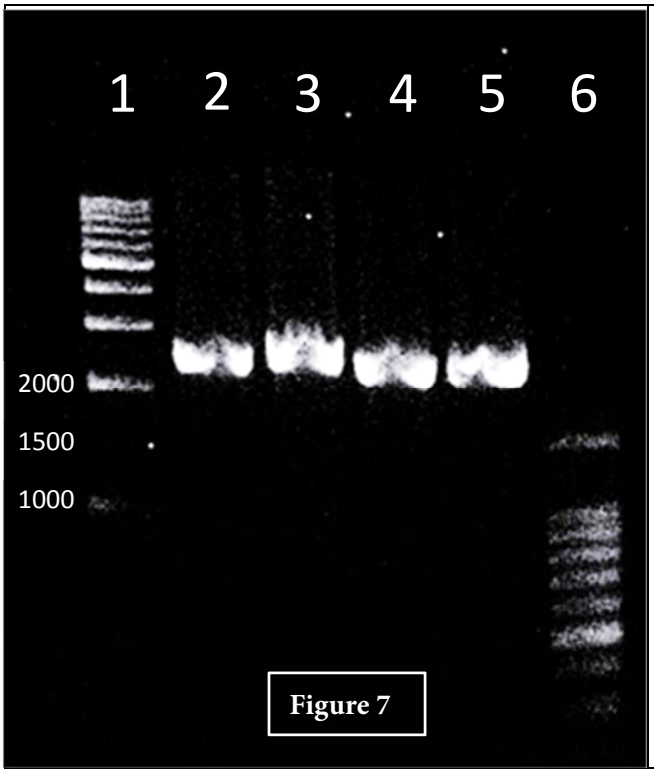
- Conclusion: We could see nothing but the markers.
- Configure Semi-solid medium

2014-7-22

- Preparation of the competent bacteria
- Transformation of RBS
- Enzyme Restriction

RBS	*CheZ*/*CheZ*+T
Spe I, *Pst* I	*Xba* I, *Pst* I

- Extraction of the Plasmids



1: 1kb marker;
2:BBa_K629003(*CheZ*)+BBa_B1005(terminator) with single digestion(1);
3:BBa_K629003(*CheZ*)+BBa_B1005(terminator) with double digestion(2);
4:BBa_K629003(*CheZ*)+BBa_B1005(terminator) with single digestion(1);
5:BBa_K629003(*CheZ*)+BBa_B1005(terminator) with double digestion(2);
6: 100 bp marker.
(1, 2 are different colonies on the same plate.)

Purpose: The verification of the BBa_K629003+ BBa_B1005 connection system.
Results/discussion: From the result, we found that we only got a skeleton about 2000 bp, the ligation between BBa_K629003 and BBa_B1005 failed. We need to try it again.

- Measure the Plasmids We Extracted:

	Absorbance: 260/280	Measurement (ng/ μL)
Positive	1.84	117.9
Negative	1.85	105.8

- Conclusion: Both are pure.
- Enzyme Restriction:

CheZ+T	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis

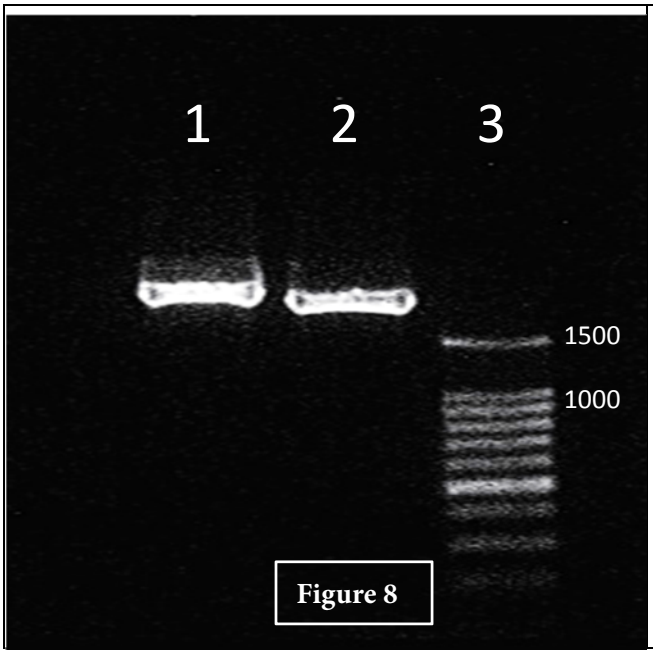


Figure 8

1: BBa_K629003(*CheZ*);
2: BBa_B1005(terminator);
3: 100 bp marker.

Purpose: The verification of BioBricks: BBa_K629003 and BBa_B1005.
Results/discussion: First, we forgot to add the 500 bp DNA marker. So we couldn't confirm the BioBricks what we wanted were correct or wrong.

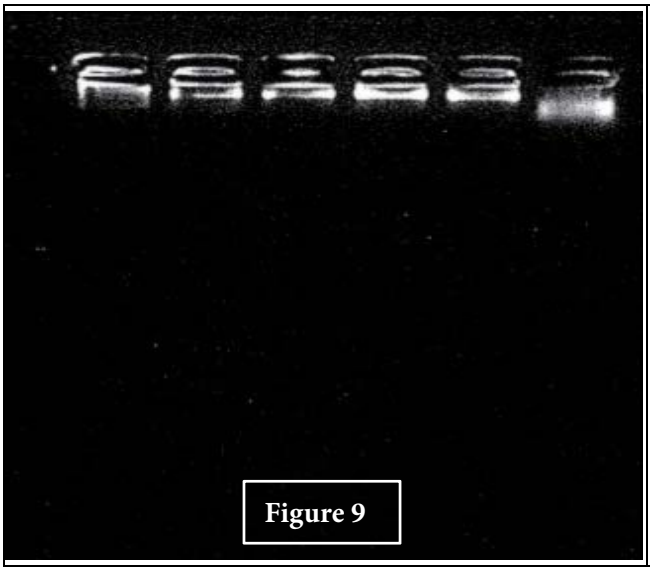


Figure 9

1: 100 bp marker;
2: BBa_K629003(*CheZ*);
3: BBa_B1005(terminator);
4: 100 bp marker.

Purpose: The verification of BioBricks: BBa_B1005 and BBa_K629003.
Results/discussion: We couldn't get any information from this figure. Finally, we found that the agar not fully immersed in the TAE buffer solution.

- Conclusion: We got everything but the phase of 500 bp Marker.
- Preparation of the Competent Bacteria

2014-7-23

- Extraction of the Plasmid

  2014-P1-18G
- Measure the Concentration of the Plasmid

	Absorbance: 260/280	Measurement (μg/μL)
2014-P1-18G	1.82	226.4

- Enzyme Restriction

	Single	Double
2014-P1-18G	EcoR I	EcoR I, *Spe* I

- Verification: Agarose gel electrophoresis

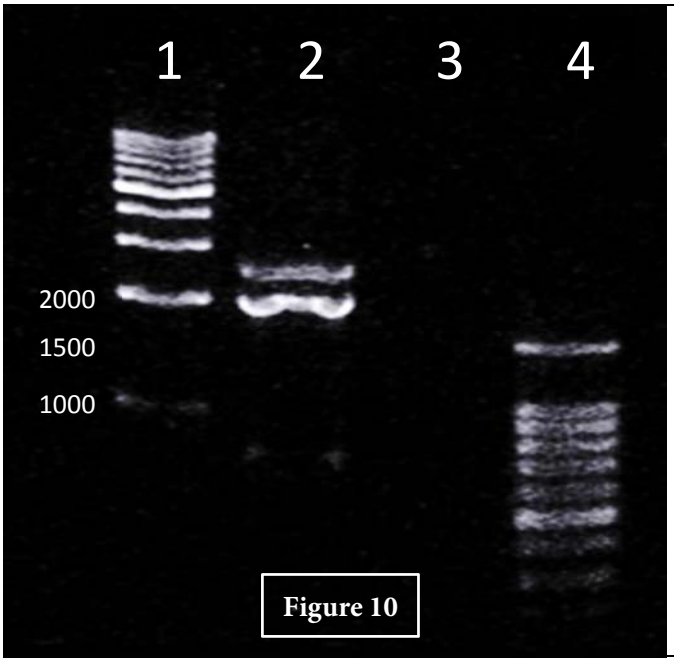


Figure 10

1: 1kb marker;
2:BBa_K629003(*CheZ*) with double digestion;
3:BBa_K629003(*CheZ*) with single digestion;
4: 100 bp marker.

Purpose: The verification of BioBrick: BBa_K629003.
Results/discussion: From the result of double digestion, we could know the bacteria contain the *CheZ* gene. Because there had a band about 645 bp, which was the theory length of *CheZ* gene.

CheZ	T
EcoR I/*Spe* I	EcoR I/*Xba* I

- Weighing

	Centrifuge Tube	All	Agarose gel	Volume
2014-P1-18G	0.912g	0.972g	0.060g	60 μL
2014-P3-5B	1.004g	1.103g	0.099g	99 μL

- Ligation
- Measure the Concentration of the Plasmid

	Absorbance: 260/280	Measurement (ng/μL)
2014-P1-18G1	1.29	45.1
2014-P1-18G2	1.86	6.4
2014-P3-5B	1.77	25.6

- *CheZ*-1, T-2
  V1/V2=3.74
- Verification: Agarose gel electrophoresis
- From left to the right: 100Marker-*CheZ*-T-1000Marker
- Ligation: *CheZ*+T
  Positive, Negative
- Measure the Concentration of RBS

RBS	Absorbance: 260/280	Measurement (ng/μL)
Plate1	1.80	458.3
Plate1’	1.88	261.3
Plate2	1.86	100.5
Plate2’	1.49	72.1

2014-7-24

- Preparation of the Competent Bacteria
- Extraction of the Plasmid: 2013-P3-4F

RBS-Plate1	EcoR I
RBS-Plate2	EcoR I
T	EcoR I

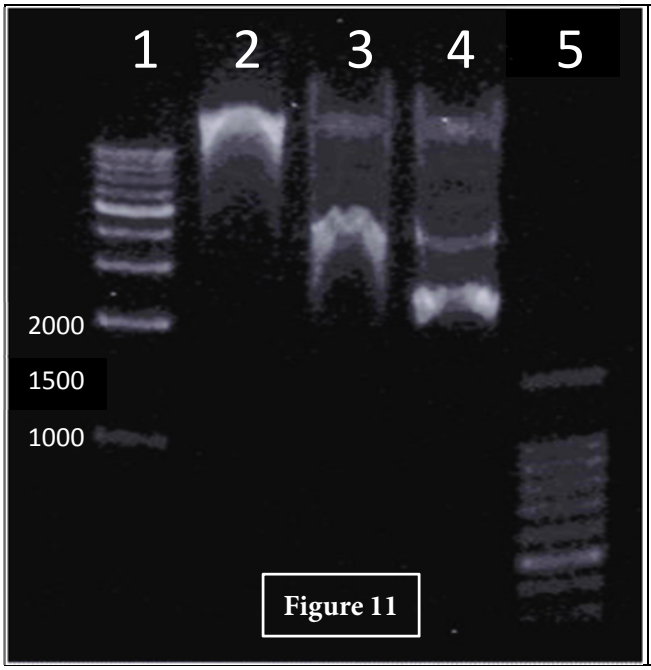


Figure 11

1: 1kb marker;
2: BBa_B0015(Double Terminator) ;
3: BBa_B0034(RBS(1.0))(3);
4: BBa_B0034(RBS(1.0)(4);
5: 100 bp marker.
(3 and 4 are different colonies on the same plate.)

Purpose: The verification of BioBricks: BBa_B0015 and BBa_B0034.
Results/discussion: The result was so bad because of the serious trailing phenomenon. We thought the reason lead to this phenomenon was that the dye time was not enough.

- Measure the Concentration of This Plasmid

	Absorbance: 260/280	Measurement (ng/μL)
RBS-Plate1 (twice)	1.85/1.85	525.7/495.6
RBS-Plate2	1.84	722.8
T	1.85	803.3

2014-7-25

- 100Marker-T-RBS (Plate1)-RBS (Plate2)-1000Marker
- Transformation

  RBS+TT *CheZ*+T (Positive) *CheZ*+T (Negative)
- Enzyme Restriction

RBS	EcoR I

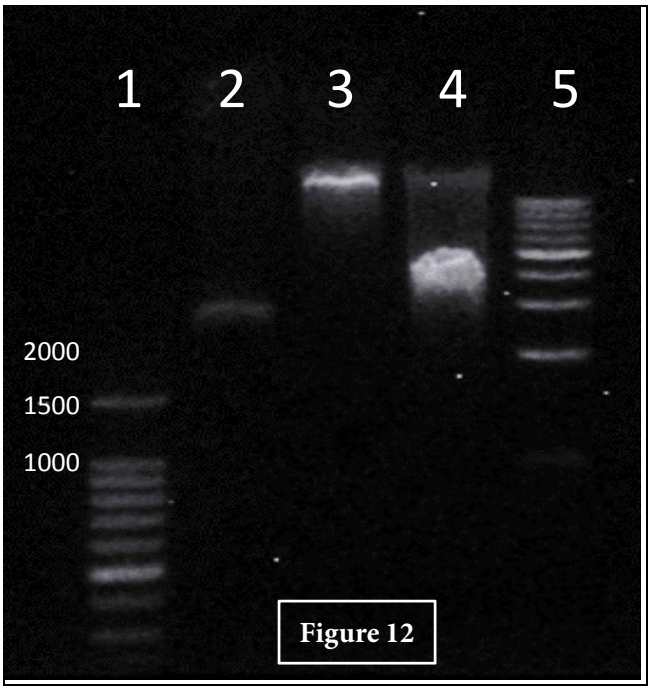


Figure 12

1: 100 bp marker;
2: BBa_B0015(Terminator);
3: BBa_B0034(RBS(1.0))(3);
4: BBa_B0034(RBS(1.0)(4);
5: 1kb marker.
(3 and 4 are different colonies on the same plate.)

Purpose: The verification of the BioBricks: BBa_B0015 and BBa_B0034.
Results/discussion: The result was so bad because of all the bands were far away from the theory length. We thought the reason leading to this phenomenon was that the digestion was wrong.

2014-7-26

- Enzyme Restriction
- Measure the Concentration of the 2013-P5-2M (There are two plates; the sample on each plate was divided into three samples).

	Absorbance: 260/280	Measurement (ng/μL)
1-1	1.83	492.4
1-2	1.80	367.2
1-3	1.84	854.4
2-1	1.83	549.4
2-2	1.80	498.7
2-3	1.89	503.6

- Enzyme Restriction

Single	RBS+T	*Xba* I
Double	*CheZ*+T	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis

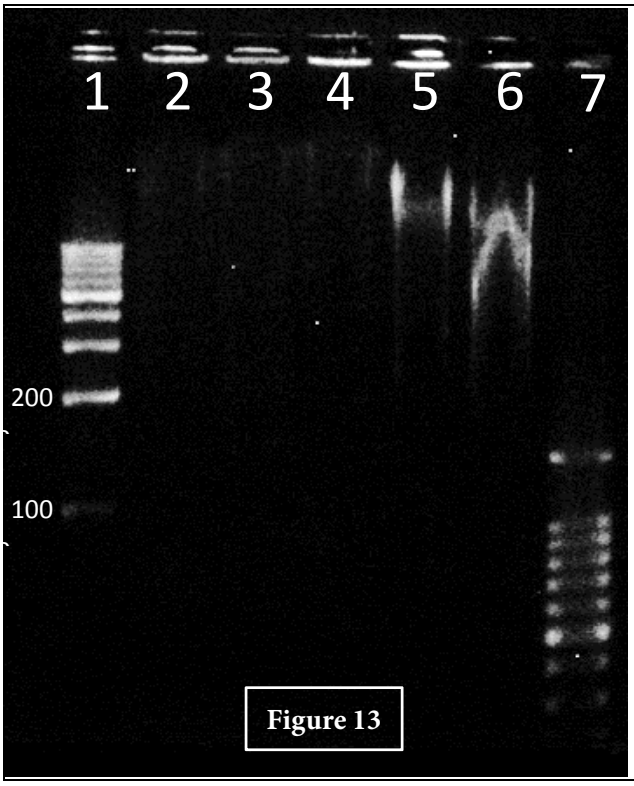


Figure 13

1: 100 bp marker;
2: BBa_B0034(RBS(1.0))(1);
3: BBa_B0034(RBS(1.0))(2);
4: BBa_B0015(terminator);
5: BBa_K629003(*CheZ*)+BBa_B1005(terminator) with single digestion;
6: BBa_K629003(*CheZ*)+BBa_B1005(terminator) with double digestion;
7: 1kb marker.
(1 and 2 are different colonies on the same plate.)

Purpose: The verification of the BioBricks: BBa_B0015, BBa_B0034 and the connection system BBa_K629003+BBa_B1005.
Results/discussion: We made a mistake as before that the agar not fully immersed in the TAE buffer solution. So we couldn't get any information from this image.

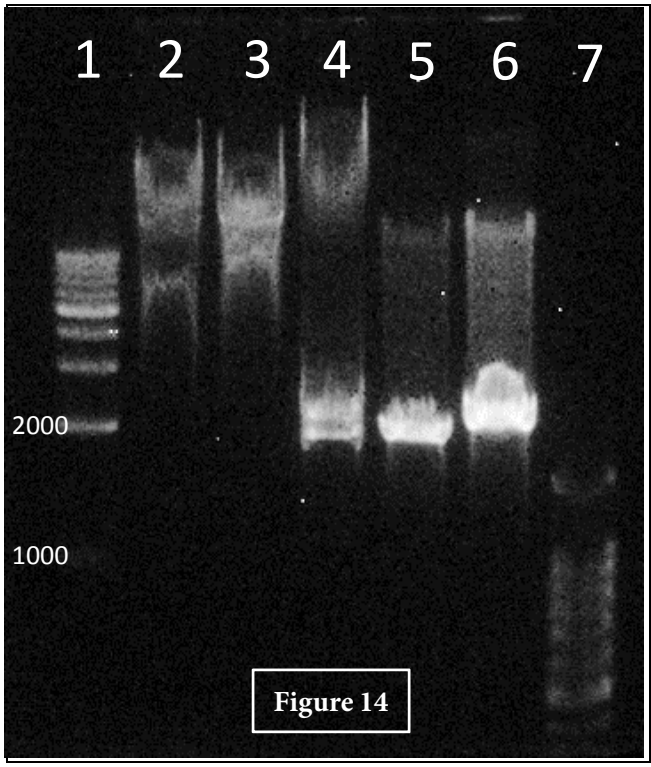


Figure 14

1: 1kb marker;
2: BBa_B0034(RBS(1.0))(2);
3: BBa_B0034(RBS(1.0))(1);
4: BBa_B0015(terminator);
5: BBa_K629003(*CheZ*)+BBa_B1005(terminator) with single digestion;
6: BBa_K629003(*CheZ*)+BBa_B1005(terminator) with double digestion;
7: 100 bp marker.
(1 and 2 are different colonies on the same plate.)

Purpose: Because we made a mistake that the agar not fully immersed in the TAE buffer solution. So we wanted to do that again.
Results/discussion: This phenomenon reminded us that we didn't have enough dyeing time. So we should pay more attention to some details.

- Preparation of the Competent Bacteria
- Enzyme Restriction

Single	T/*CheZ*+T *negative*	*Xba* I
Double	*CheZ*+T *negative*	*Xba* I, *Pst* I

- Verification: Agarose gel electrophoresis
  From left to the right: 100Marker-(*CheZ*+T *positive*)-(*CheZ*+T *negative*)-(2013-P3-4F)–(2013-P5-2M1)-(2013-P5-2M2)-1000Marker
- Verification: Agarose gel electrophoresis
  From left to the right: 100Marker-(2013-P3-4F *single*)-(*CheZ*+T *single*)-(*CheZ*+T *double)*-1000Marker

2014-7-27

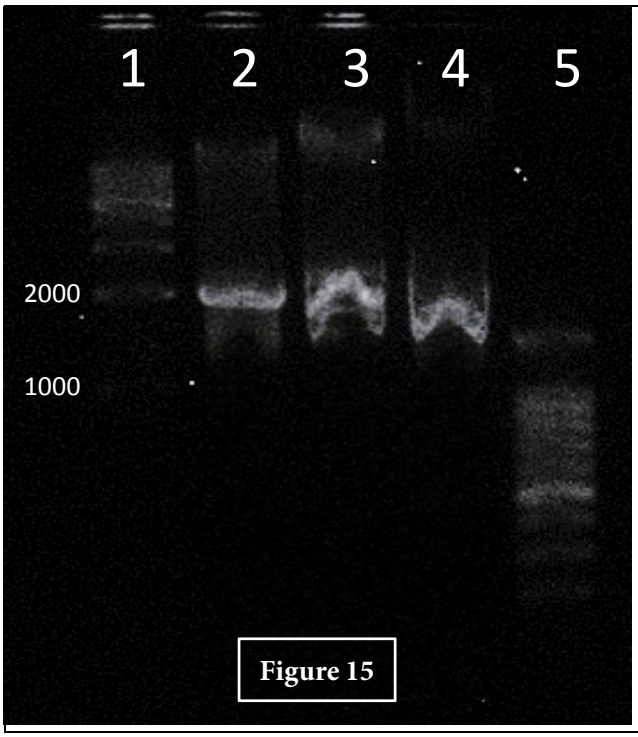


Figure 15

1: 100 bp marker;
2: BBa_B0015(terminator);
3:BBa_K629003(*CheZ*)+BBa_B1005(terminator) with single digestion;
4:BBa_K629003(*CheZ*)+BBa_B1005(terminator) with double digestion;
5: 1kb marker.

Purpose: The verification of BBa_B0015 and the connection system BBa_K629003+BBa_B1005.
Results/discussion: From the result, we couldn't determine whether BBa_B0015 was correct or not. At the same time, we got a bad result in the verification of the BBa_K629003+BBa_B1005 connection system, so the experiment failed.

2014-7-28

- Extraction of the Plasmid
- Measure the concentration of the Plasmid

	Absorbance: 260/280	Measurement(ng/μL)
2013-P5-2M	1.84	230.0
CheZ+T(+)1	1.83	123.1
CheZ+T(+)2	1.89	217.4
CheZ+T(+)3	1.84	363.7

- Enzyme Restriction

Single	2013-P5-2M	EcoR I
Double	*CheZ*+T(+)	EcoR I, *Spe* I

- Verification: Agarose gel electrophoresis

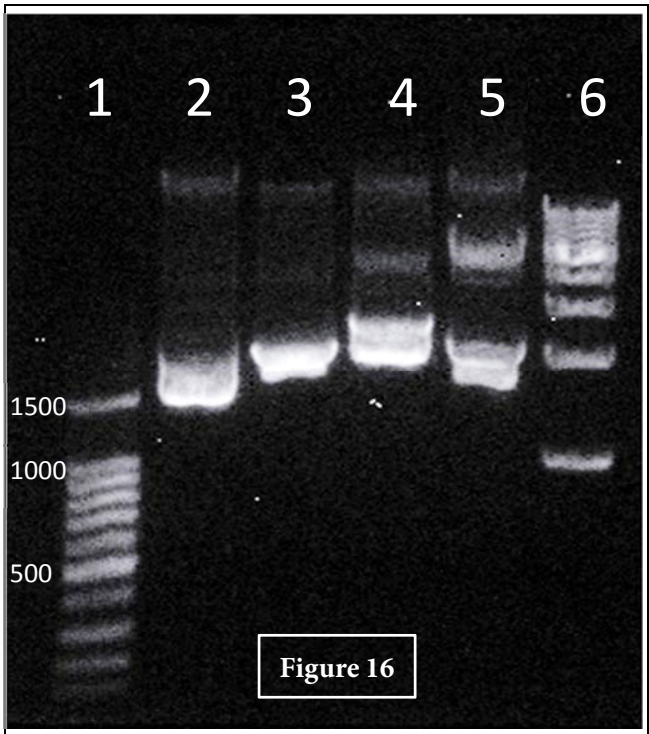


Figure 16

1: 100 bp marker;
2: BBa_B0034(RBS(1.0)) with single digestion;
3:BBa_K629003(*CheZ*)+BBa_B1005(terminator) with double digestion;
4:BBa_K629003(*CheZ*)+BBa_B1005(terminator) with double digestion;
5:BBa_K629003(*CheZ*)+BBa_B1005(terminator) with double digestion;
6: 1kb marker.
(2, 3 and 4 are different colonies on the same plate.)

Purpose: The verification of BBa_B0034 and the BBa_K629003+BBa_B1005 connection system.
Results/discussion: From the result, we could only get the length of plasmid skeleton, but we didn't know whether the bacteria contain the *CheZ*+T gene. What's more, we could found that the difference between bands was not obvious, maybe the digestion was not enough.

- Enzyme Restriction

Single	EcoR I	RBS
Double	EcoR I, *Spe* I	RBS

- Verification: Agarose gel electrophoresis
  From left to the right: 100Marker-(2013-P5-2M *single*)-(2013-P5-2M *double*)-1000Marker
- Measure the Concentration of the Plasmid(each sample was measured for 3 times)

	Absorbance: 260/280	Measurement (ng/μL)
2013-P5-2M	1.93/1.86/2.11	78.7/82.3/70.0
CheZ+T(+)1	2.07/1.84/1/86	105.9/82.7/82.3
CheZ+T(+)2	1.73/1.90/1/85	124.0/110.8/118.5
CheZ+T(+)3	1.98/1.83/1.81	92.3/100.9/104.1

- Enzyme Restriction

Single	*Xba* I	2013-P5-2M
Double	*Xba* I *Pst* I	*CheZ*+T

- Verification: Agarose gel electrophoresis
  From left to the right: 100Marker-(2013-P5-2M)-(2014-P1-18G)-1000Marker
- Ligation
- Measure the concentration of the plasmids

	Absorbance: 260/280	Measurement(ng/μL)
2014-P1-18G	1.59/1.58	20.7/20.2
2013-P3-2M	1.71	16.0

- 2014-P1-18G----1 2013-P3-2M------2
  V1/V2=1/2.5
- Preparation of the competent bacteria

2014-7-29

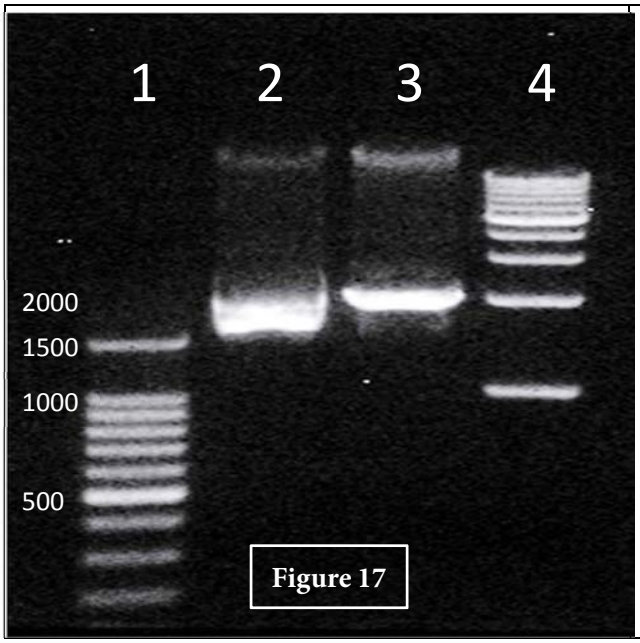


Figure 17

1: 100 bp marker;
2: BBa_B0034(RBS(1.0)) with single digestion;
3: BBa_B0034(RBS(1.0)) with double digestion;
4: 1kb marker.

Purpose: The verification of BBa_B0034.
Results/discussion: As we know, the plasmid contains BBa_B0034 with single digestion must longer than with double digestion. But the result was on the contrary, so we could get the conclusion that the plasmid we had was wrong.

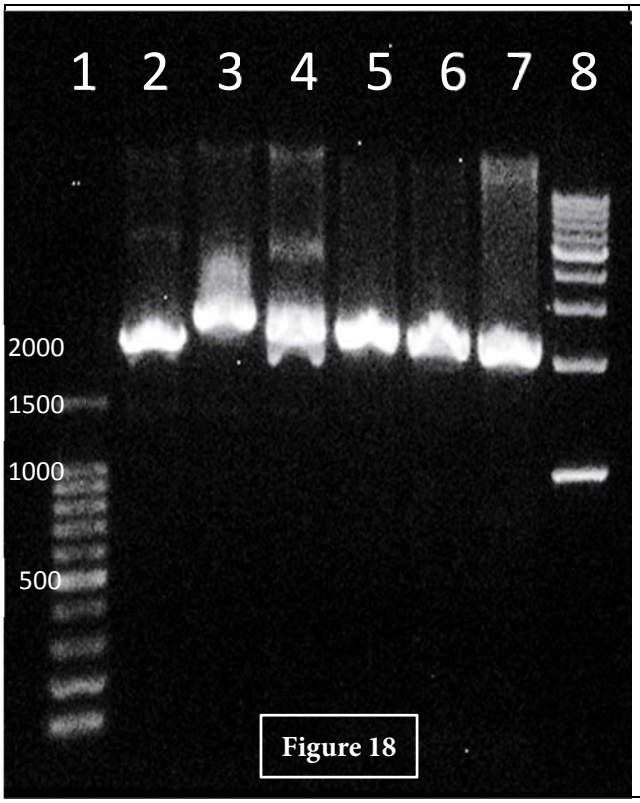


Figure 18

1: 100 bp marker;
2: BBa_B0034(RBS(1.0)) with double digestion(1);
3: BBa_B0034(RBS(1.0)) with double digestion(2);
4: BBa_B0034(RBS(1.0)) with double digestion(3);
5: BBa_B0034(RBS(1.0)) with double digestion(6);
6: BBa_B0034(RBS(1.0)) with double digestion(7);
7: BBa_B0034(RBS(1.0)) with double digestion(8);
4: 1kb marker.
(1, 2, 3, 6, 7, 8 were the different colonies on the same tablet)

Purpose: The verification of BBa_B0034.
Results/discussion: Because we didn't verified the bacteria contain BBa_B0034, we need to confirm again. From the result, we could only get the length of plasmid skeleton, but we didn't know whether the bacteria contained the *CheZ*+T gene. What's more, we could found that the difference between bands was not obvious, maybe the digestion was not compeletely.

2014-7-30

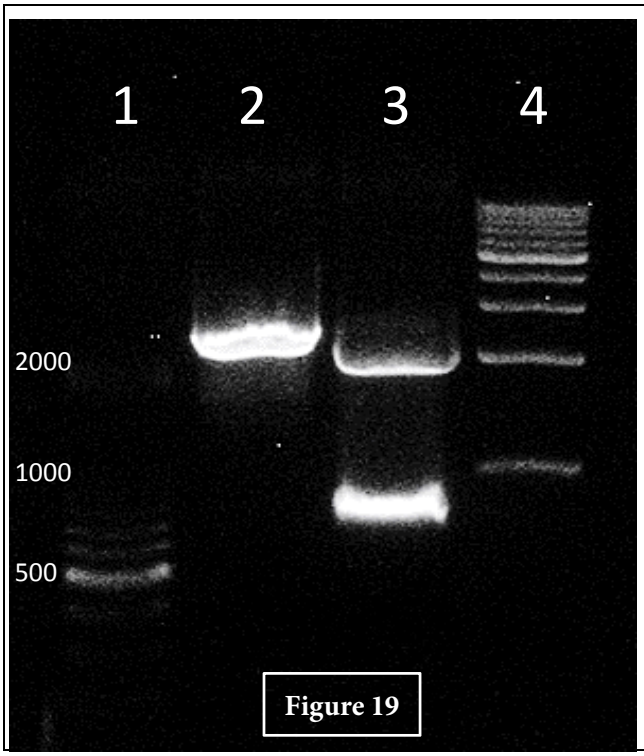


Figure 19

1: 100 bp marker;
2: BBa_B0034(RBS(1.0)) with double digestion;
3: BBa_K629003(*CheZ*) with double digestion;
4: 1kb marker.

Purpose: Preparation for the ligation between RBS and *CheZ*.
Results/discussion: The Biobrick BBa_B0034 after *Spe* I and *Pst* I double digestion, will remain 2070 bp skeleton. At the same time, we got *CheZ* gene with the *Xba* I and *Pst* I double digestion. At the end, we could use chemical transformation with gel extraction and the ligation between *CheZ* and RBS.