LABNOTE-D

XMU-iGEM

Date: 10.1-10.17

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	

10 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

						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						

11 M 2014 Y

NOTE :

2014-10-01

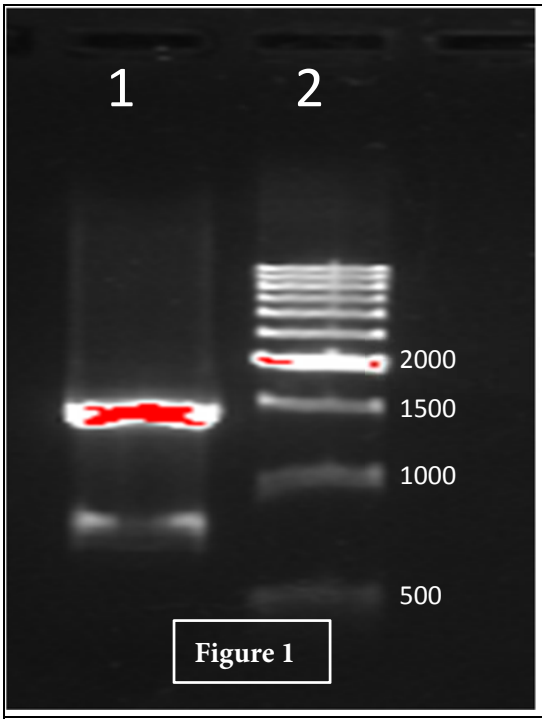


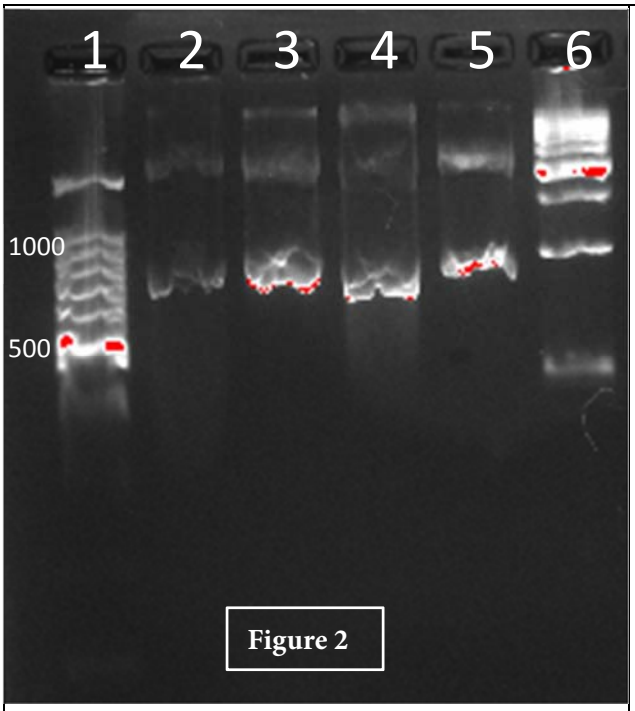
Figure 1

1: BBa_J61002;
2: 500 bp Marker.

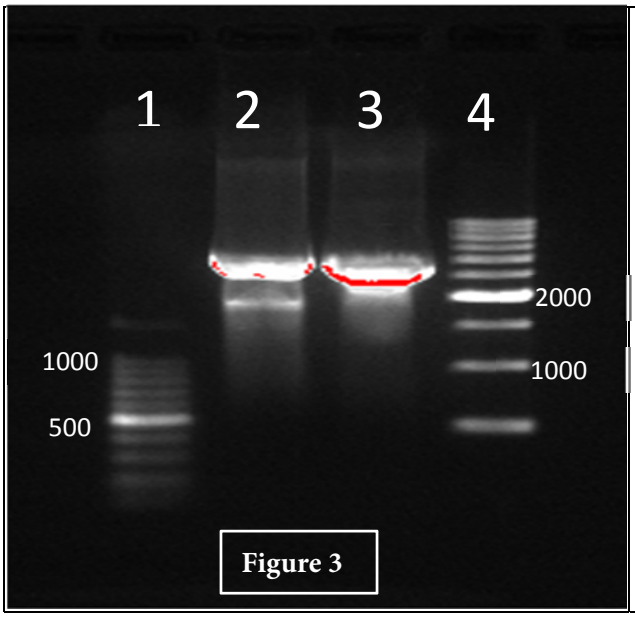
Purpose: The verification of BBa_K838000.
Results/discussion: The theoretical length of BBa_J61002 is 843 bp, so the length of the target gene on the image was right. But the band of the backbone was too short that the verification of the backbone was not correct.

- Verification: Agarose gel electrophoresis
  The backbone of 2014-P4-19L.
- Conclusion: The length of the backbone was shorter than it should be.

2014-10-02



1: 100 bp marker;
2: BBa_B0033;
3: BBa_B0033+BBa_K629003+BBa_B0015(1);
4: BBa_B0033+BBa_K629003+BBa_B0015(2);
5: BBa_B0032;
6: 500 bp marker.
((1), (2) are different colonies on the same plate.)
Purpose: Because after the sequence, we found the BBa_R0010+BBa_B0032+BBa_K629003+BBa_B0015 connection system lost RBS, so we wanted to know whether the plasmid BBa_B0032 containing the RBS or not.
Results/discussion: From the figure, we found the plasmid we signed BBa_B0032 didn't contain the RBS we wanted.



1: 100 bp Marker;
2: BBa_J04450 with single digestion(*EcoR* I);
3: BBa_J04450 with double digestion (*EcoR* I and *Pst* I);
4: 500 bp Marker.

Purpose: The verification of BBa_J04450.
Results/discussion: From the image, we could know that the lengths of the target genes which were restricted by single enzyme and double enzymes were the same. So the experiment failed.

- Verification: Agarose gel electrophoresis
  From left to the right: M(100)-(lock X P)-(new RBS X P)-M(500)
- Conclusion: Ptet_RBS, Ptet_crRNA_RBS are correct.

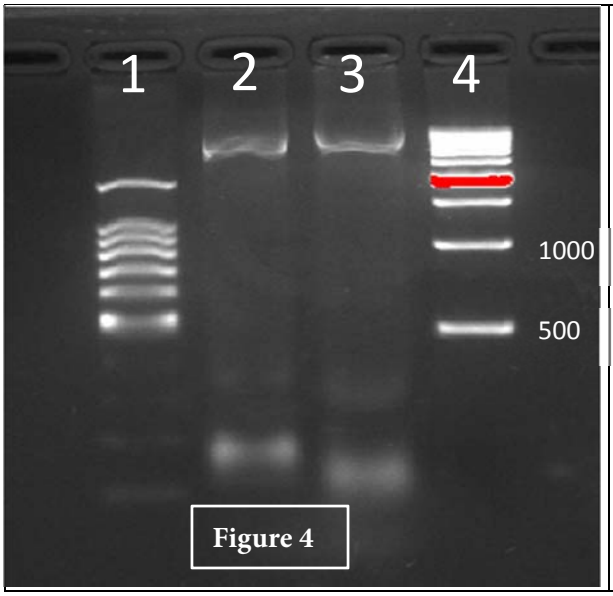


Figure 4

1: 100bp Marker;
2: Lock (restricted by double enzymes) ;
3: The new RBS;
4: 500bp Marker.

Purpose: The gel electrophoresis was prepared for the ligation of Lock and RBS.
Results/discussion: From the length we could know that the verification of PCR was correct.

The backbone of RFP and CheZ+TT.

- Conclusion: The length of the RFP backbone was longer than it should be, we couldn't see the bands of CheZ+TT.

2014-10-03

- Enzyme Restriction:
  Xba I, *Pst* I
- Verification: Agarose gel electrophoresis: (2014-P2-2L)-(2014-P2-2J)-(2L-18G-4F)-(2J-18G-4F)
- Verification: Agarose gel electrophoresis.
- Conclusion: The result of PCR was correct.

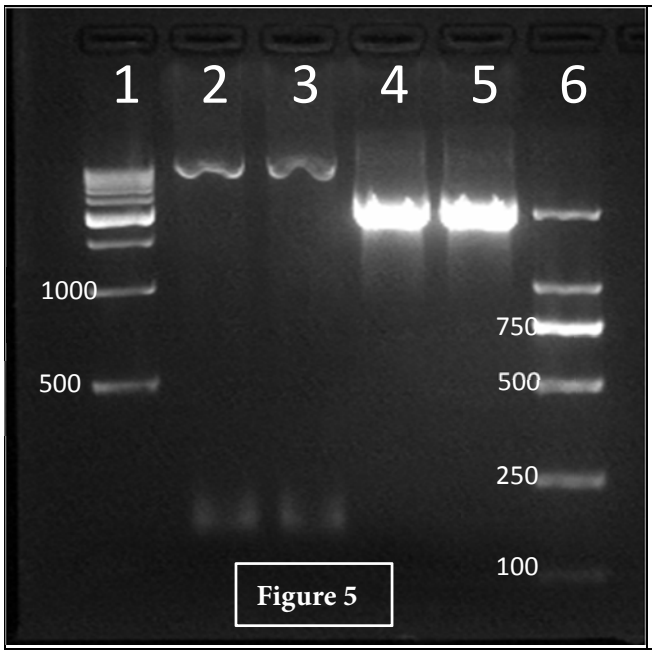


Figure 5

1: 500 bp Marker;
2: Ptet_aptamer_taRNA(E S);
3: Ptet_aptamer_taRNA(E S);
4: BBa_B0015;
5: BBa_B0014;
6: DL2000 Marker.

Purpose: The gel electrophoresis was prepared for the ligation of Ptet, aptamer, taRNA. It is called key.
Results/discussion: The length of the key is 187 bp theoretically and from line1 and line2 we could see that the ligation was successful.

2014-10-05

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

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

T/h	6F	3H	14A
0 h	0.20	0.30	0.15
2 h	0.20	0.30	0.15
24 h	1.45	0.80	0.50
30 h	1.75	1.05	0.70
45.5 h	2.70	1.60	1.50
52 h	3.00	1.90	2.00

2014-10-06

- Verification: Agarose gel electrophoresis
  The backbones of (2014-P1-18G)+(2013-P3-4F) and 2013-P3-4F
- Conclusion: We couldn't see the bands on the background clearly.

2014-10-13

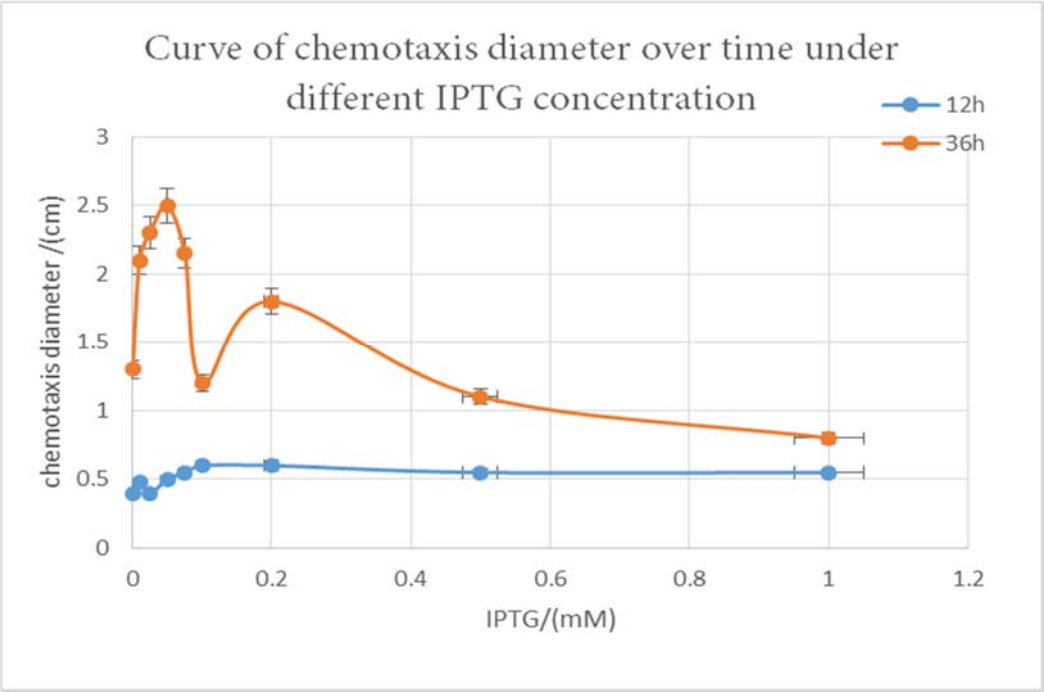
- The Experimental Plan:

  With 50 μg/ml Cm, the concentration gradients of IPTG is 0~1 mM

- Aim:

  We wanted to know the most appropriate concentration of IPTG:

The Concentration Gradient of IPTG/mM	The Chemotaxis Diameters in 12 h/cm	The Chemotaxis Diameters in 24 h/cm
0.00	0.40	1.30
0.01	0.48	2.10
0.03	0.40	2.30
0.05	0.50	2.50
0.08	0.55	2.15
0.10	0.60	1.20
0.20	0.60	1.80
0.50	0.55	1.10
1.00	0.55	0.80



- Conclusion:

  The most appropriate concentration of IPTG for *E. coli*'s chemotaxis is 0.25 mM~0.75 mM.

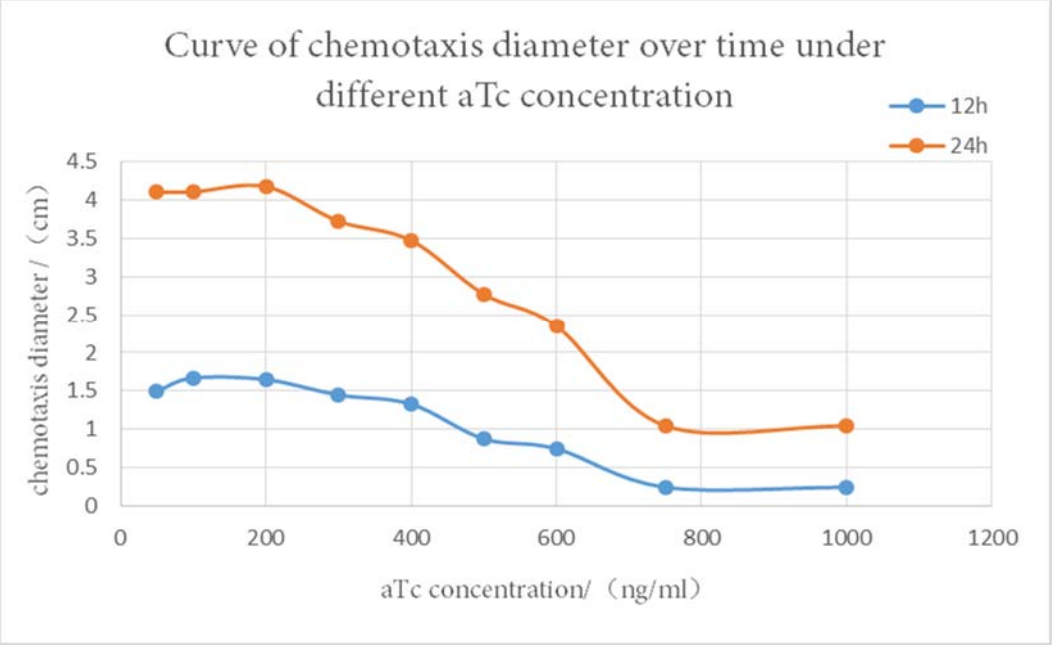
- The Experimental Plan:

  With 50 μg/ml, 0.01 mM IPTG, the concentration gradients of aTc is 50~1000 ng/ml.

- Aim:

  We wanted to know the most appropriate and the critical concentration for *E. coli*'s chemotaxis.

The Concentration Gradients of aTc/ng/ml	The Chemotaxis Diameters in 12h/cm	The Chemotaxis Diameters in 24h/cm
50	1.50	4.10
100	1.67	4.10
200	1.65	4.17
300	1.45	3.72
400	1.33	3.47
500	0.88	2.77
600	0.75	2.35
750	0.25	1.05
1000	0.25	1.05



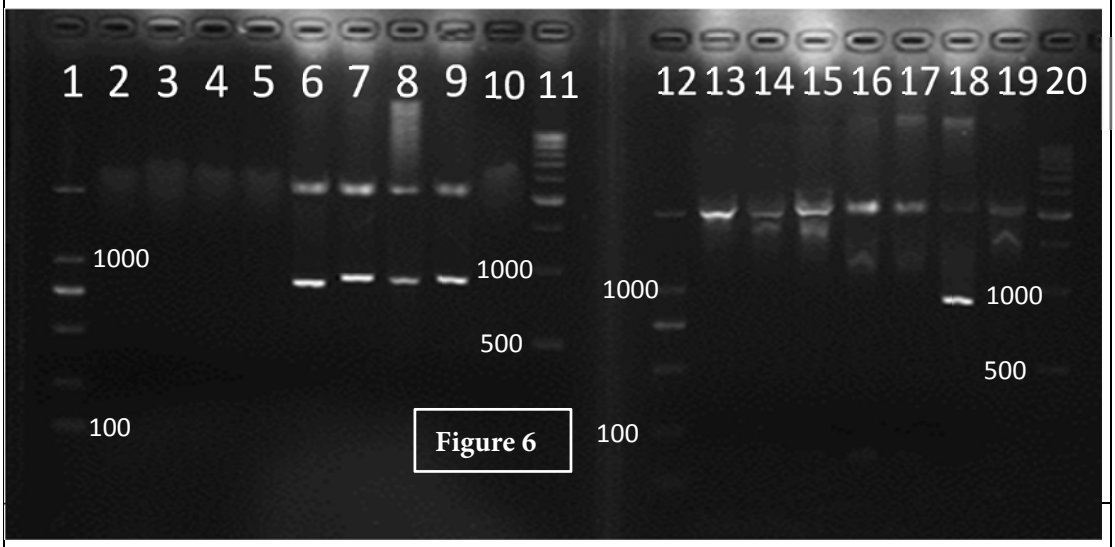


Figure 6

4: L2C(+1); 5: L2R(-1); 6: L2C(+1);
7: L2R(-1); 8: L2R-L2R(+1); 9: L2R(-1);
10: 500 bp Marker; 11: DL2000 Marker; 12: Pter_aptamer_taRNA(-1);
13: Pter_aptamer_taRNA(+1); 14:Pter_aptamer_taRNA+BBa_B0015;
15: Pter_aptamer_taRNA(-2); 16: Pter_aptamer_taRNA(-1);
17: BBa_K823000; 18: BBa_J04650;
19:BBa_K629003+BBa_B0015; 20: 500 bp Marker.

Purpose: The gel electrophoresis was prepared for the ligation of R2C, L2C and L2R.
Results/discussion: We found that the ligation system of L2R was successfully. What's more, the backbones of RFG and BBa_K629003+BBa_B0015 was also right. Unfortunately, we couldn't get any bands of R2Z and R2R in the image.

2014-10-15

- The Experimental Plan：
  With the 0.01 mM IPTG, 50 μg/ml Cm. And the single plot of IPTG is 0.25 mM and the single plot of the concentration gradients of Tet is 750~5000 ng/ml.
- Aim:
  We wanted to know the most appropriate concentration of the single plot aTc.

The concentration gradients of atC/(ng/ml)	12h The Chemotaxis Diameters towards aTc(d1)/cm	The Chemotaxis Diameters away from aTc(d2)/cm	Differences(d1-d2/cm	22h The Chemotaxis Diameters towards aTc(d1)/cm	The Chemotaxis Diameters away from aTc（d2）/cm	Differences(d1-d2)/cm	34h The Chemotaxis Diameters towards aTc(d1)/cm	The Chemotaxis Diameters away from aTc（d2）/cm	Differences（d1-d2）/cm
750	0.70	0.85	-0.15	1.40	1.55	-0.15	1.80	2.00	-0.20
1000	1.00	1.00	0.00	1.98	1.98	0.00	2.75	2.50	0.25
1500	0.65	0.80	-0.05	1.60	1.75	-0.15	1.75	1.85	-0.10
2000	0.75	0.80	-0.05	1.55	1.60	-0.05	1.90	1.90	0.00
2500	0.75	0.80	-0.05	1.40	1.40	0.00	1.80	1.90	-0.10
3000	0.80	0.85	-0.05	1.55	1.60	-0.05	2.40	2.25	0.15
3500	0.65	0.80	-0.15	1.36	1.45	-0.09	1.90	2.00	-0.10
4000	0.75	0.80	-0.05	1.45	1.50	-0.05	2.05	1.75	0.30
5000	0.90	0.90	0.15	1.50	1.60	-0.10	1.90	1.70	0.20

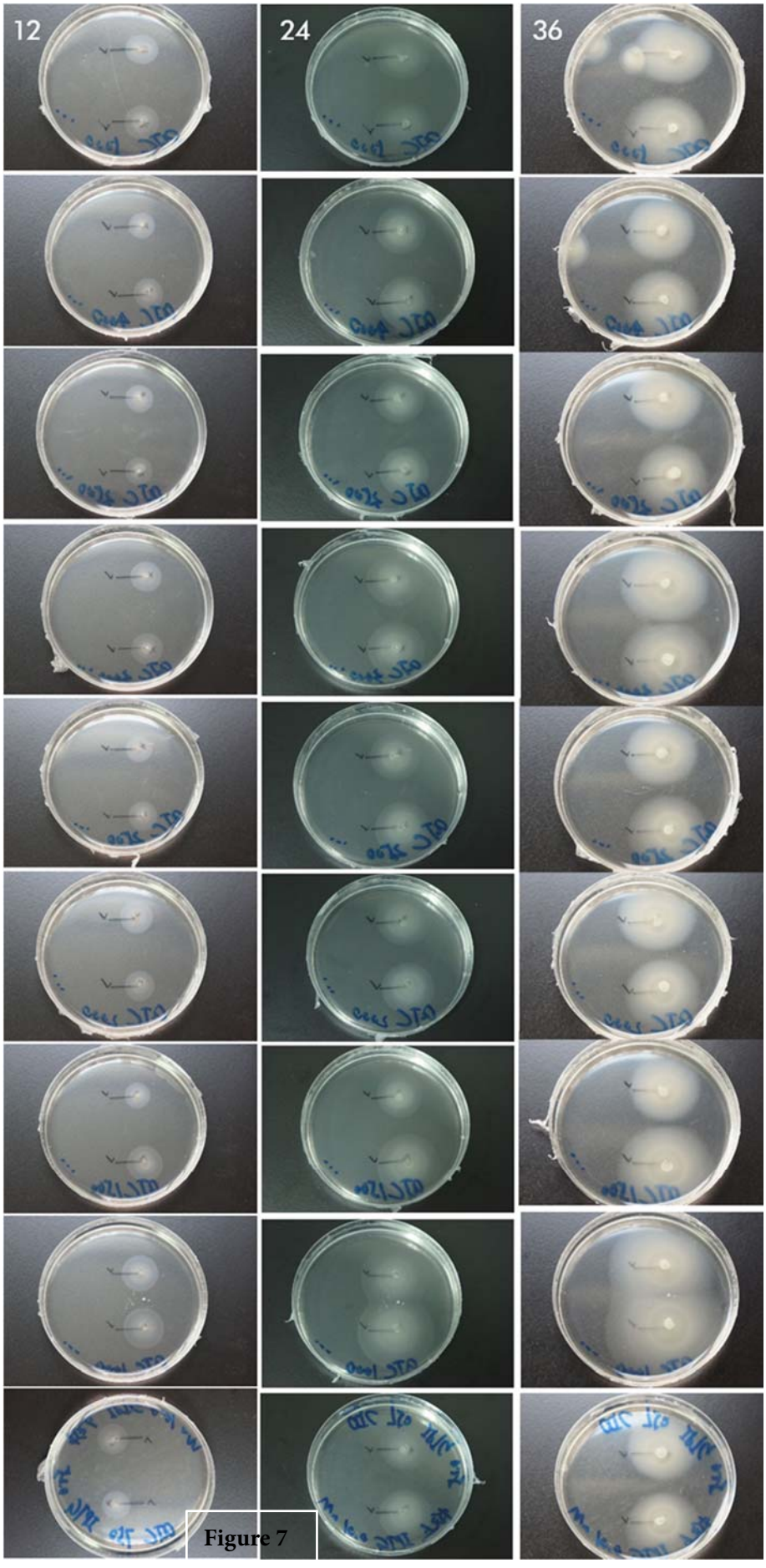


Figure 7

- Preparation of M63 semi-solid medium

M63 semi-solid medium/100mL	
Reagent	Quantities
KH_2PO_4	1.36 g
KOH	0.42 g
$(NH_4)_2SO_4$	0.2 g
$MgSO_4$	0.012 g
$FeSO_4$ [1]	$10.84*10^{-4}$ g
D-glucose	0.4 g
Glycerol	0.2 mL
Agar(gel strength>750 g/cm^2)	0.25 g
Asp [2]	$6.6*10^{-3}$ mg
Met, Leu, His, Thr (0.015g/mL)	1 mL

- Note：

1. $FeSO_4$ 1 :Add 10 μL 0.1 g/mL FeSO4 to the medium.
2. Asp 2 : Add 10 μL 66 mg/mL Asp to the medium.