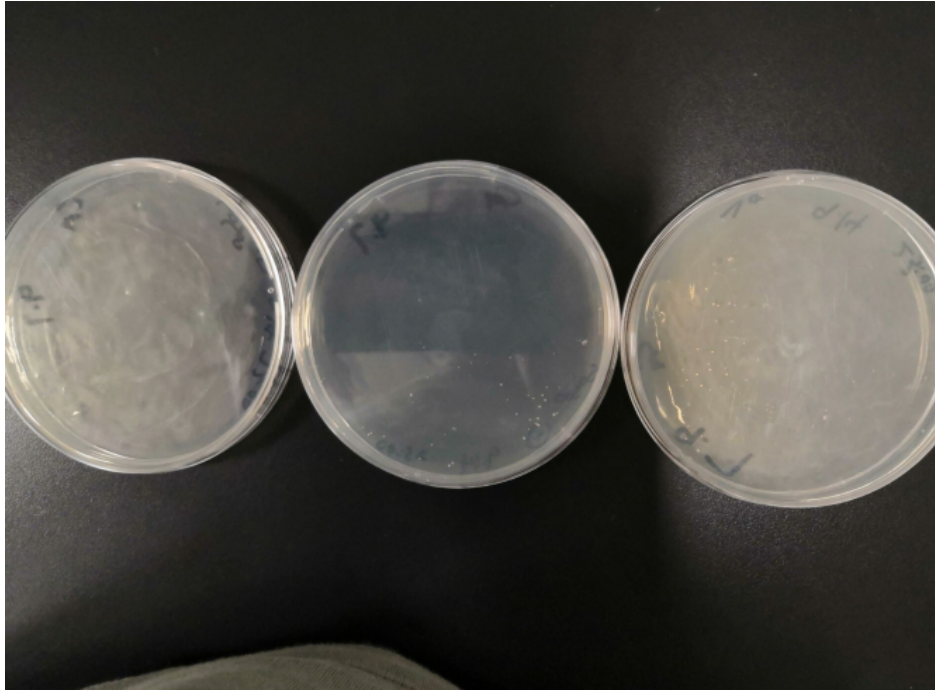# Team Name--XHD-Wuhan-China

2020.8.17

Reorganization and transformation validation

It's a good selection of six clones.

From left to right wereSOSOO ①, sosoo ② and vazyme.

Six clones were selected from sosoo and vazyme respectively.

Shaking at 37 ℃ at 220 rpm.

Clonal shaking bacteria.

2020.8.18

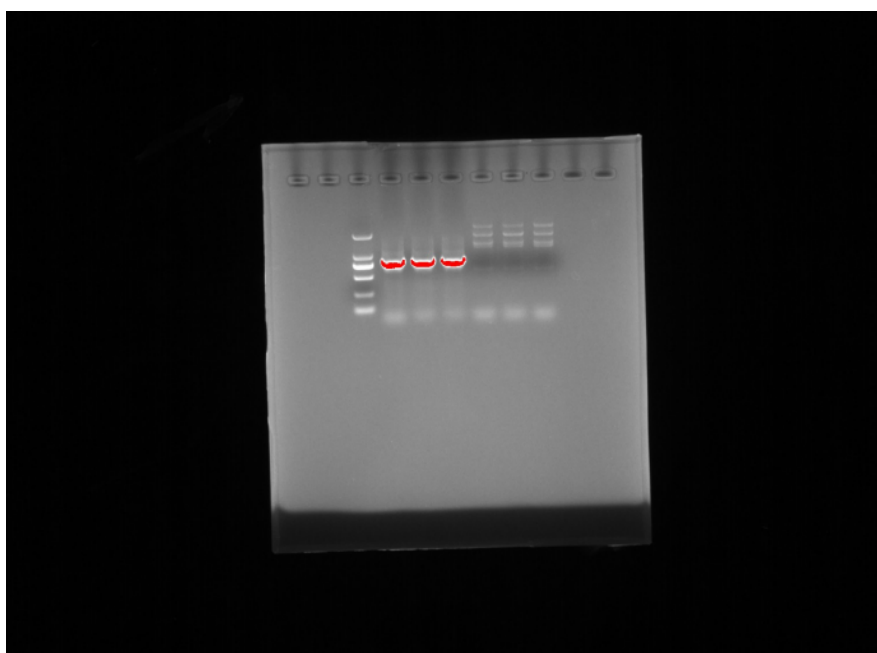1、Colony PCR: (860 BP) Qing family red enzyme

2 × T5 Super PCR Mix: 25μl

liner_ pSB1C3_ bottom: 2μl

Pasr-pSB1C3- bottom: 2μl

Template: 1 μl

dd H_2O: 20μl

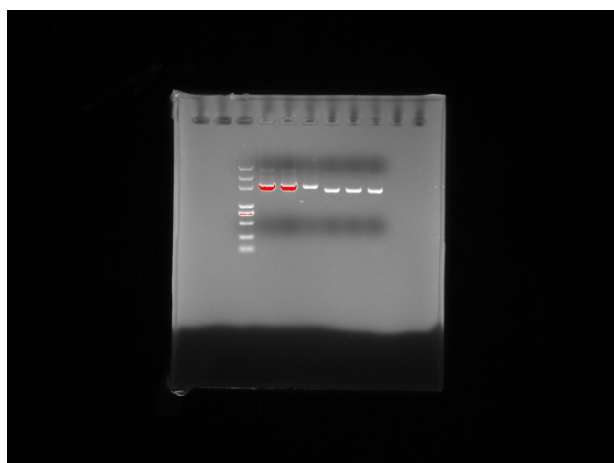No.1, 2 and 3 appeared normal band (2000 marker, 860bp target band)

2、The length of recombinant plasmid was verified by plasmid

Recombinant plasmid: 3275 BP

The concentration of recombinant plasmid was as follows：

No.	Sample ID	ID#	Sample Ty	SW (nm)	SW Abs	260 Abs (	280 Abs (	260 / 28(	260 / 23(	Conc. (ng	Dilution	Time
1	Pasr-pSB1C3质粒①	1	dsDNA	260	1.32	1.32	0.699	1.888	2.092	66	1	11:23:57
2	Pasr-pSB1C3质粒②	2	dsDNA	260	1.615	1.615	0.877	1.842	1.792	80.75	1	11:24:26
3	Pasr-pSB1C3质粒③	3	dsDNA	260	0.481	0.481	0.252	1.909	1.775	24.05	1	11:24:56
4	Pasr-pSB1C3质粒④	4	dsDNA	260	0.486	0.486	0.264	1.841	1.642	24.3	1	11:25:25
5	Pasr-pSB1C3质粒⑤	5	dsDNA	260	0.51	0.51	0.274	1.861	1.635	25.5	1	11:26:10
6	Pasr-pSB1C3质粒⑥	6	dsDNA	260	0.49	0.49	0.271	1.808	1.561	24.5	1	11:26:47

Electrophoresis verification:

the concentrations of No. 1, 2 and 3 were normal, and there was a normal band (5000marker, 3295bp target band)

3、Preservation of bacteria

2020.8.19

1、Transfer of Pasr-pSB1C3 plasmid strain by shaking last night

1: 100 transfer capacity
Three tubes of 5 mllb + 5ulcm + 50ul Pasr-pSB1C3 plasmid bacteria.
The plasmid was composed of 200 μl / L SBL and 20 μl / L PbSr 3.

2、Preparation of M9 medium

M9 mother liquor preparation: (980ml deionized water) high pressure sterilization
$Na_2HPO_4.12H_2O$: 17.1g (molecular weight 358.14)
KH_2PO_4: 3g (molecular weight 136.09)
NaCl:0.5g
NH_4Cl:1g
$1mMgSO_4.7H_2O$: 2.46g was added to 10ml dd H_2O and autoclaved
20% Glucose: 4g Glucose added to 20ml dd H_2O, filtered and sterilized
$1mCaCl_2.6H_2O$: 2.191g was added into 10ml dd H_2O, and autoclaved

2ml $MgSO_4$100μl $CaCl_2$ and 20ml filtered and sterilized Glucose were added into the above 980ml mother liquor.
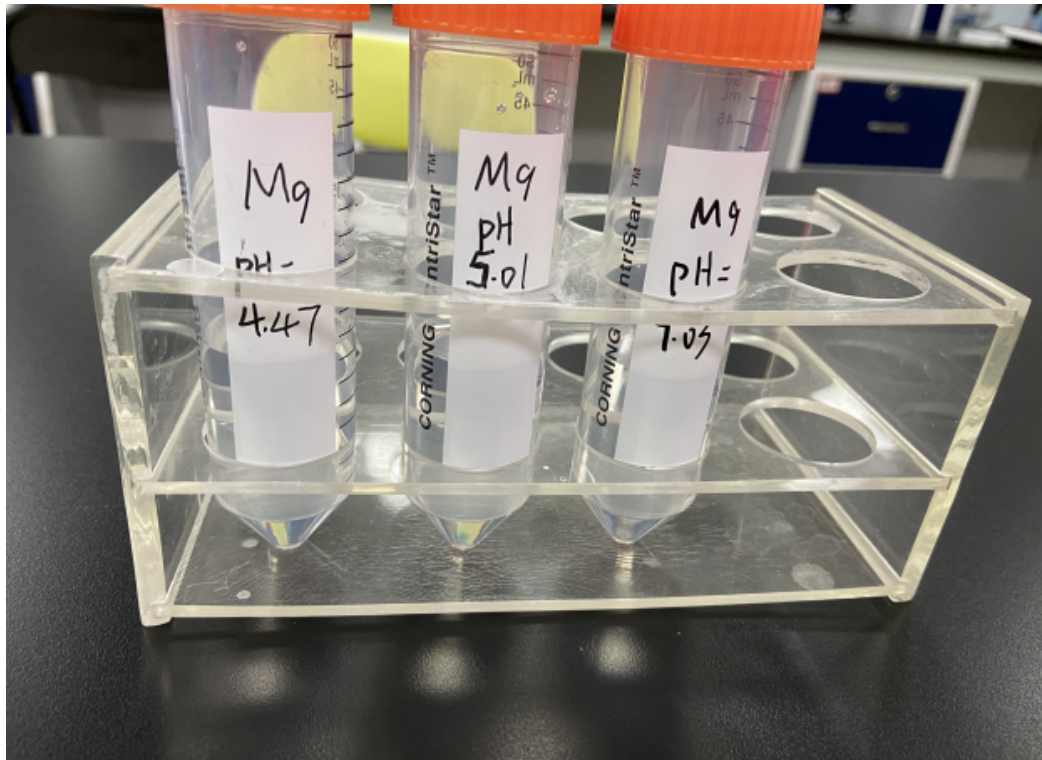M9 medium was prepared successfully.

3、The bacteria liquid was cultured to logarithmic growth stage and put into 4°C refrigerator to avoid the growth of bacteria as far as possible
Adjust the pH of M9 medium
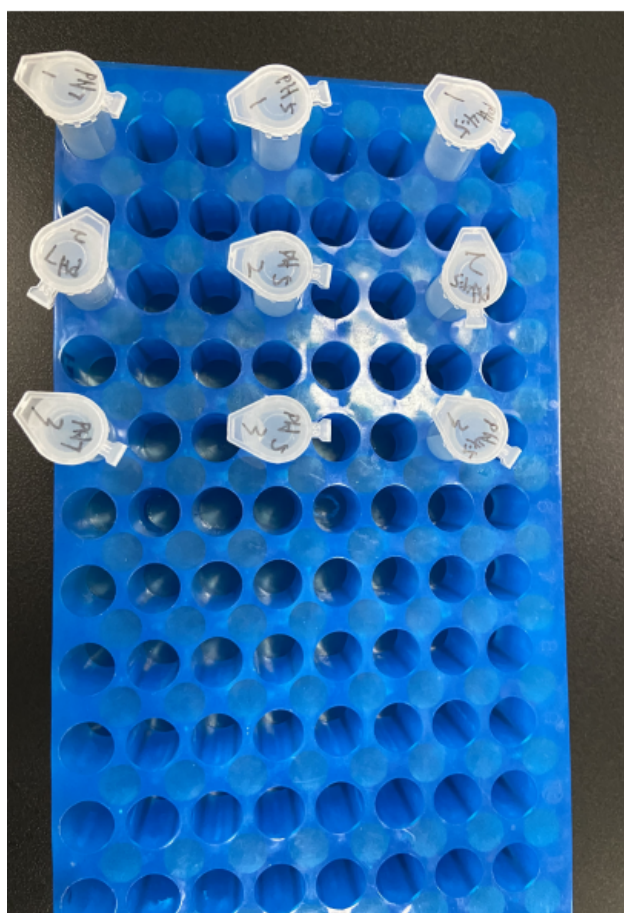The pH of the original medium was 7.03.
Firstly, 75 μl HCl was added, and the pH is 3.89. Secondly, 500 μl M9 medium was added, and the pH is 4.44. Thirdly, 20 μl M9 medium was added ,and the pH is 4.47.
Firstly , 80 μl HCl was added, and the pH is 4.43. Secondly, 500 μl M9 medium was added,and the pH is 4.93. Thirdly , 20 μll M9 medium was added , and the pH is 5.01.

1 ml of bacterial liquid (Pasr-pSB1C3 plasmid strain) in logarithmic growth phase was centrifuged at 3000 RMP for 5 min, and the supernatant was discarded
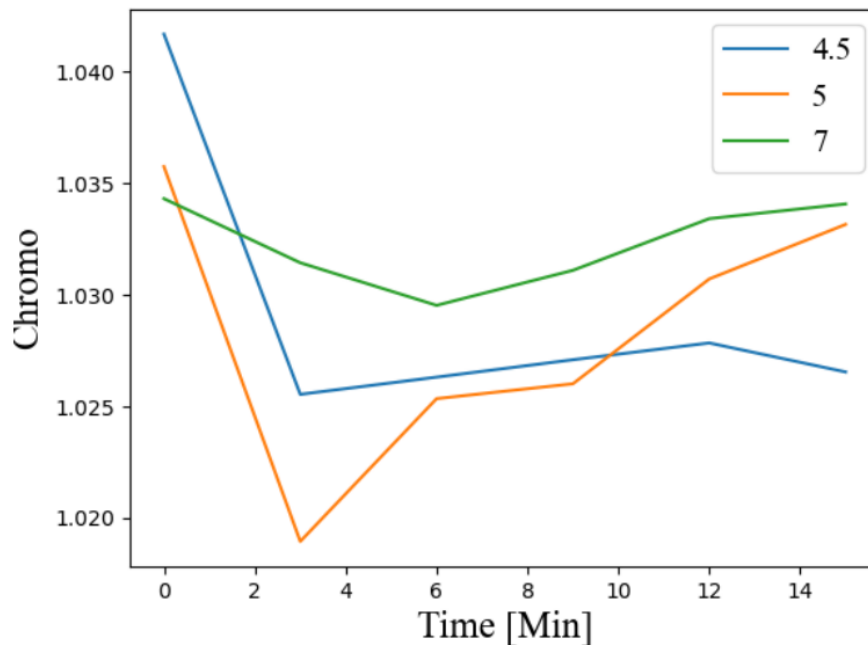
200 µl of the above medium was added, and three biological repeats were made for each gradient.

100 µl was added into 96 well plate and determined every 3 minutes for half an hour

The data of OD580 and OD600 were as follows:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		1	2	3	4	5	6			1	2	3	4	5	6	
2	D	0.695	0.529	0.693	0.039	0.04	0.039	Read 1:580	D	0.667	0.509	0.669	0.039	0.04	0.039	Read 1:600
3	E	0.683	0.702	0.708	0.041	0.041	0.041	Read 1:580	E	0.659	0.679	0.685	0.04	0.041	0.04	Read 1:600
4	F	0.761	0.759	0.739	0.041	0.039	0.039	Read 1:580	F	0.736	0.734	0.718	0.041	0.039	0.039	Read 1:600
5																
6		1	2	3	4	5	6			1	2	3	4	5	6	
7	D	0.636	0.495	0.633	0.039	0.04	0.039	Read 2:580	D	0.62	0.482	0.621	0.039	0.04	0.039	Read 2:600
8	E	0.632	0.657	0.661	0.041	0.041	0.04	Read 2:580	E	0.62	0.646	0.65	0.041	0.041	0.04	Read 2:600
9	F	0.715	0.709	0.696	0.041	0.039	0.039	Read 2:580	F	0.695	0.691	0.673	0.041	0.039	0.039	Read 2:600
10																
11		1	2	3	4	5	6			1	2	3	4	5	6	
12	D	0.633	0.491	0.631	0.038	0.04	0.039	Read 3:580	D	0.617	0.48	0.616	0.039	0.04	0.038	Read 3:600
13	E	0.63	0.655	0.657	0.041	0.041	0.04	Read 3:580	E	0.614	0.64	0.642	0.04	0.041	0.04	Read 3:600
14	F	0.711	0.702	0.693	0.041	0.039	0.039	Read 3:580	F	0.692	0.682	0.674	0.041	0.038	0.039	Read 3:600
15																
16		1	2	3	4	5	6			1	2	3	4	5	6	
17	D	0.629	0.491	0.628	0.039	0.04	0.039	Read 4:580	D	0.613	0.478	0.614	0.039	0.04	0.039	Read 4:600
18	E	0.628	0.655	0.654	0.041	0.041	0.041	Read 4:580	E	0.612	0.64	0.638	0.041	0.041	0.04	Read 4:600
19	F	0.709	0.7	0.699	0.041	0.039	0.039	Read 4:580	F	0.69	0.679	0.679	0.041	0.039	0.039	Read 4:600
20																
21		1	2	3	4	5	6			1	2	3	4	5	6	
22	D	0.627	0.49	0.625	0.039	0.04	0.039	Read 5:580	D	0.61	0.476	0.611	0.039	0.04	0.038	Read 5:600
23	E	0.629	0.653	0.653	0.041	0.041	0.041	Read 5:580	E	0.61	0.634	0.635	0.04	0.041	0.04	Read 5:600
24	F	0.715	0.706	0.708	0.041	0.039	0.039	Read 5:580	F	0.693	0.685	0.686	0.041	0.039	0.039	Read 5:600
25																
26		1	2	3	4	5	6			1	2	3	4	5	6	
27	D	0.627	0.49	0.625	0.039	0.04	0.039	Read 6:580	D	0.608	0.479	0.612	0.039	0.04	0.038	Read 6:600
28	E	0.626	0.651	0.652	0.041	0.041	0.04	Read 6:580	E	0.606	0.631	0.633	0.04	0.041	0.04	Read 6:600
29	F	0.725	0.713	0.713	0.041	0.038	0.039	Read 6:580	F	0.703	0.692	0.69	0.041	0.039	0.039	Read 6:600

2020.8.20

1、Transfer of Pasr-pSB1C3 plasmid strain shaken yesterday

1: 100 transfer capacity

The plasmid was composed of 200 μl LB, 20 μl Cm, and 200 μl Pasr-pSB1C3
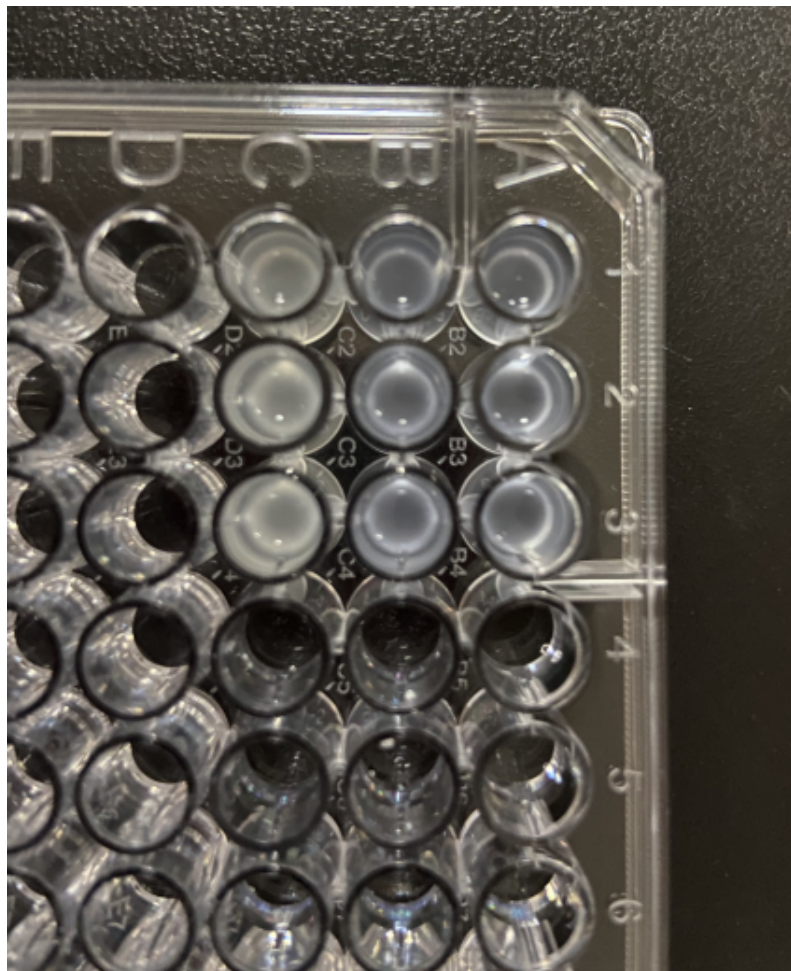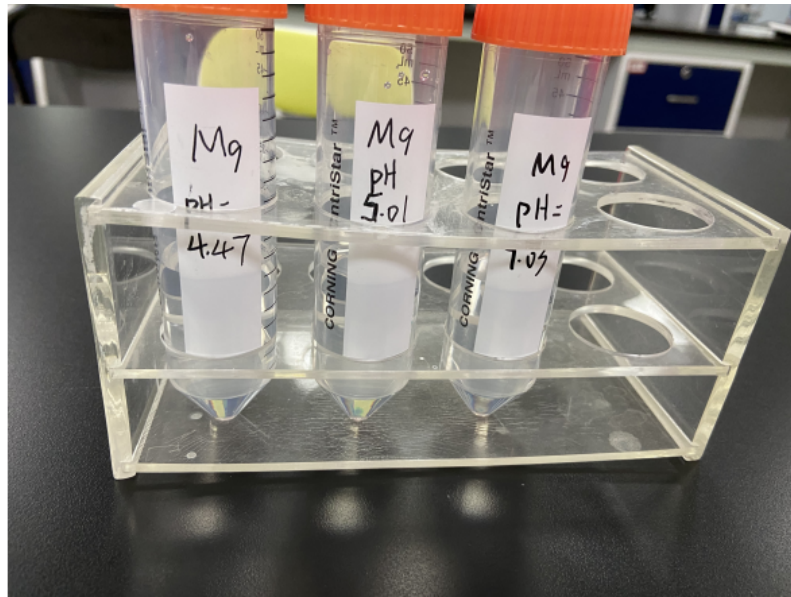
2 hours later, the OD was 0.454.

2、Transfer to M9 medium

Take 1000ml and centrifugate at 4000rpm for 10min.

Abandon supernatant solution.

200 μl M9 medium (pH = 4.5 , 5 , 7) was added.

Each pH was repeated in three groups.

Sampling:

When 200µl was added into each pore, the pH values were 4.5, 5 and 7 respectively.

The first three wells were samples, and the last three wells were M9 medium corresponding to pH.

The shaking time of the microplate :

In the first hour, measure every 3 minutes.

In the second hour, measure once every 10 minutes.

In the third hour, measure every 20 minutes.

At the fourth hour, the fifth hour and 30min.

The data are attached:

结果

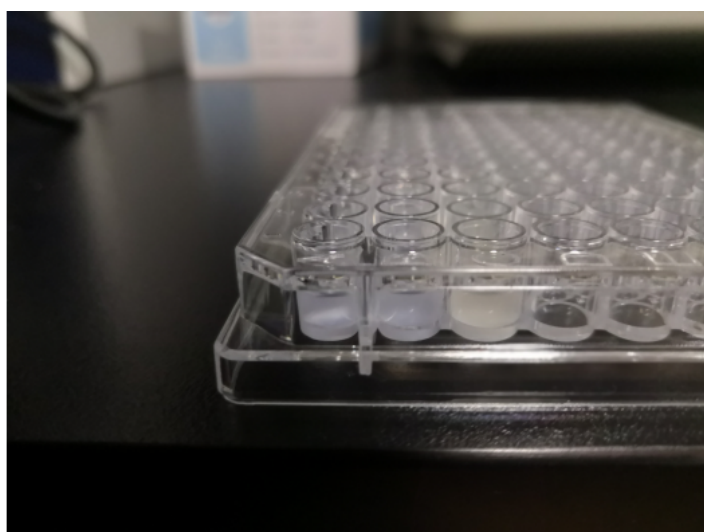|	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.515	0.573	0.527	0.039	0.039	0.038							Read 1:580
	0.497	0.539	0.507	0.040	0.039	0.038							Read 1:600
	0.517	0.571	0.530	0.039	0.039	0.038							Read 2:580
	0.500	0.546	0.512	0.040	0.039	0.038							Read 2:600
	0.515	0.567	0.530	0.039	0.039	0.038							Read 3:580
	0.497	0.541	0.511	0.040	0.039	0.038							Read 3:600
	0.515	0.564	0.529	0.039	0.039	0.038							Read 4:580
	0.496	0.539	0.511	0.040	0.040	0.039							Read 4:600
	0.516	0.564	0.530	0.039	0.039	0.038							Read 5:580
	0.497	0.539	0.512	0.040	0.039	0.038							Read 5:600
	0.516	0.565	0.530	0.040	0.039	0.039							Read 6:580
	0.498	0.540	0.512	0.040	0.039	0.038							Read 6:600
	0.517	0.567	0.531	0.039	0.039	0.039							Read 7:580
	0.498	0.543	0.513	0.040	0.039	0.039							Read 7:600
	0.518	0.570	0.532	0.039	0.039	0.038							Read 8:580
	0.498	0.545	0.514	0.039	0.039	0.038							Read 8:600
	0.521	0.574	0.533	0.040	0.039	0.038							Read 9:580
	0.500	0.550	0.514	0.040	0.039	0.038							Read 9:600
	0.524	0.577	0.536	0.039	0.039	0.038							Read 10:580
	0.502	0.552	0.516	0.040	0.039	0.038							Read 10:600
	0.528	0.581	0.539	0.039	0.039	0.038							Read 11:580
	0.506	0.556	0.518	0.040	0.039	0.038							Read 11:600
	0.533	0.587	0.543	0.039	0.039	0.038							Read 12:580
	0.510	0.561	0.522	0.040	0.039	0.038							Read 12:600
	0.536	0.588	0.547	0.039	0.039	0.038							Read 13:580
	0.513	0.563	0.525	0.040	0.039	0.038							Read 13:600
	0.541	0.592	0.550	0.039	0.039	0.038							Read 14:580
	0.517	0.567	0.527	0.040	0.039	0.038							Read 14:600
	0.543	0.597	0.552	0.039	0.039	0.038							Read 15:580
	0.520	0.571	0.529	0.040	0.040	0.039							Read 15:600
	0.549	0.600	0.554	0.039	0.039	0.038							Read 16:580
	0.524	0.574	0.531	0.039	0.039	0.038							Read 16:600
	0.551	0.602	0.557	0.040	0.040	0.038							Read 17:580
	0.526	0.575	0.533	0.040	0.039	0.038							Read 17:600
	0.553	0.605	0.560	0.039	0.039	0.038							Read 18:580
	0.529	0.578	0.537	0.040	0.040	0.039							Read 18:600
	0.556	0.608	0.562	0.039	0.039	0.038							Read 19:580
	0.531	0.582	0.539	0.040	0.039	0.038							Read 19:600
	0.559	0.611	0.564	0.040	0.040	0.038							Read 20:580
	0.534	0.584	0.541	0.040	0.039	0.038							Read 20:600
	0.563	0.616	0.568	0.040	0.040	0.038							Read 21:580
	0.538	0.589	0.544	0.040	0.040	0.038							Read 21:600
	0.561	0.615	0.568	0.040	0.039	0.038							Read 22:580
	0.540	0.593	0.549	0.040	0.039	0.038							Read 22:600
	0.567	0.614	0.574	0.039	0.039	0.038							Read 23:580
	0.547	0.593	0.555	0.040	0.039	0.038							Read 23:600
	0.574	0.619	0.582	0.039	0.039	0.038							Read 24:580
	0.554	0.599	0.563	0.040	0.039	0.039							Read 24:600
	0.580	0.624	0.588	0.040	0.039	0.038							Read 25:580
	0.561	0.604	0.570	0.039	0.039	0.038							Read 25:600
	0.586	0.628	0.597	0.039	0.039	0.038							Read 26:580
	0.567	0.609	0.579	0.040	0.039	0.038							Read 26:600
	0.590	0.634	0.604	0.040	0.039	0.038							Read 27:580
	0.572	0.615	0.585	0.040	0.039	0.039							Read 27:600
	0.599	0.639	0.607	0.039	0.039	0.038							Read 28:580
	0.582	0.621	0.589	0.040	0.039	0.038							Read 28:600
	0.603	0.639	0.607	0.040	0.039	0.038							Read 29:580
	0.587	0.622	0.592	0.040	0.039	0.038							Read 29:600
	0.606	0.639	0.606	0.039	0.039	0.038							Read 30:580
	0.592	0.623	0.591	0.040	0.039	0.038							Read 30:600
	0.602	0.636	0.602	0.039	0.039	0.038							Read 31:580
	0.589	0.620	0.588	0.040	0.039	0.038							Read 31:600
	0.601	0.629	0.598	0.040	0.039	0.038							Read 32:580
	0.588	0.615	0.584	0.040	0.040	0.038							Read 32:600
	0.602	0.618	0.589	0.039	0.039	0.038							Read 33:580
	0.590	0.604	0.576	0.040	0.039	0.038							Read 33:600
	0.608	0.609	0.583	0.039	0.039	0.038							Read 34:580
	0.597	0.596	0.570	0.040	0.039	0.038							Read 34:600
B	0.546	0.543	0.525	0.039	0.039	0.039							Read 1:580
	0.523	0.519	0.500	0.039	0.040	0.040							Read 1:600

B	0.546	0.543	0.525	0.039	0.039	0.039	Read 1:580
	0.523	0.519	0.500	0.039	0.040	0.040	Read 1:600
	0.526	0.517	0.512	0.039	0.039	0.039	Read 2:580
	0.512	0.504	0.496	0.039	0.039	0.040	Read 2:600
	0.529	0.517	0.511	0.040	0.039	0.039	Read 3:580
	0.513	0.504	0.496	0.039	0.040	0.039	Read 3:600
	0.532	0.519	0.510	0.039	0.039	0.039	Read 4:580
	0.514	0.505	0.495	0.039	0.040	0.040	Read 4:600
	0.533	0.520	0.513	0.040	0.039	0.039	Read 5:580
	0.515	0.506	0.498	0.039	0.039	0.039	Read 5:600
	0.536	0.524	0.517	0.039	0.039	0.039	Read 6:580
	0.519	0.510	0.502	0.039	0.040	0.040	Read 6:600
	0.542	0.527	0.517	0.039	0.039	0.039	Read 7:580
	0.524	0.513	0.501	0.039	0.040	0.040	Read 7:600
	0.547	0.529	0.521	0.039	0.039	0.039	Read 8:580
	0.530	0.515	0.505	0.039	0.039	0.040	Read 8:600
	0.549	0.533	0.526	0.039	0.039	0.039	Read 9:580
	0.531	0.519	0.510	0.039	0.040	0.039	Read 9:600
	0.555	0.539	0.531	0.039	0.039	0.039	Read 10:580
	0.536	0.523	0.515	0.039	0.039	0.039	Read 10:600
	0.560	0.546	0.538	0.039	0.039	0.039	Read 11:580
	0.542	0.530	0.521	0.039	0.039	0.040	Read 11:600
	0.567	0.555	0.544	0.039	0.039	0.039	Read 12:580
	0.548	0.539	0.527	0.040	0.040	0.039	Read 12:600
	0.573	0.563	0.552	0.039	0.039	0.039	Read 13:580
	0.554	0.546	0.535	0.040	0.040	0.040	Read 13:600
	0.579	0.570	0.556	0.039	0.039	0.039	Read 14:580
	0.559	0.552	0.539	0.039	0.039	0.039	Read 14:600
	0.583	0.579	0.560	0.039	0.039	0.039	Read 15:580
	0.565	0.561	0.543	0.040	0.040	0.040	Read 15:600
	0.590	0.588	0.566	0.039	0.039	0.039	Read 16:580
	0.570	0.568	0.547	0.039	0.039	0.040	Read 16:600
	0.596	0.592	0.572	0.040	0.039	0.040	Read 17:580
	0.576	0.572	0.553	0.039	0.039	0.039	Read 17:600
	0.600	0.597	0.576	0.039	0.039	0.039	Read 18:580
	0.581	0.578	0.558	0.039	0.040	0.040	Read 18:600
	0.603	0.603	0.580	0.039	0.039	0.039	Read 19:580
	0.583	0.583	0.562	0.039	0.040	0.039	Read 19:600
	0.609	0.611	0.585	0.039	0.039	0.039	Read 20:580
	0.588	0.591	0.567	0.039	0.040	0.039	Read 20:600
	0.612	0.618	0.590	0.039	0.039	0.040	Read 21:580
	0.591	0.597	0.571	0.039	0.039	0.039	Read 21:600
	0.621	0.621	0.602	0.039	0.039	0.039	Read 22:580
	0.601	0.602	0.584	0.039	0.039	0.039	Read 22:600
	0.630	0.626	0.612	0.039	0.039	0.039	Read 23:580
	0.610	0.609	0.593	0.039	0.040	0.039	Read 23:600
	0.635	0.633	0.620	0.039	0.039	0.039	Read 24:580
	0.615	0.615	0.602	0.039	0.040	0.040	Read 24:600
	0.640	0.637	0.623	0.039	0.040	0.039	Read 25:580
	0.619	0.619	0.604	0.039	0.040	0.039	Read 25:600
	0.640	0.642	0.628	0.039	0.039	0.039	Read 26:580
	0.621	0.624	0.610	0.039	0.039	0.040	Read 26:600
	0.643	0.650	0.633	0.039	0.039	0.039	Read 27:580
	0.624	0.632	0.615	0.039	0.040	0.040	Read 27:600
	0.647	0.655	0.641	0.039	0.039	0.039	Read 28:580
	0.628	0.637	0.624	0.039	0.039	0.039	Read 28:600
	0.650	0.659	0.649	0.039	0.039	0.039	Read 29:580
	0.633	0.642	0.632	0.039	0.040	0.040	Read 29:600
	0.657	0.658	0.650	0.039	0.039	0.039	Read 30:580
	0.641	0.642	0.633	0.039	0.040	0.039	Read 30:600
	0.661	0.657	0.651	0.039	0.039	0.039	Read 31:580
	0.645	0.642	0.637	0.040	0.040	0.040	Read 31:600
	0.666	0.657	0.650	0.040	0.039	0.039	Read 32:580
	0.651	0.643	0.636	0.039	0.040	0.040	Read 32:600
	0.671	0.655	0.643	0.039	0.039	0.039	Read 33:580
	0.656	0.641	0.629	0.039	0.039	0.039	Read 33:600
	0.667	0.645	0.639	0.039	0.039	0.039	Read 34:580
	0.652	0.631	0.625	0.039	0.039	0.039	Read 34:600
C	0.601	0.589	0.589	0.038	0.038	0.042	Read 1:580

Take photos after taking out the enzyme labeled instrument after running.

The bacteria with pH = 4.5 and pH = 5 turned blue and the acid induced promoter expression.

pH = 7 did not turn blue and the promoter expression was weak.

Drawing graphics: