Team Name--XHD-Wuhan-China

2020.8.10

1、 The plasmid Pasr-pUC19 was transformed into DH5 α

The plasmid lyophilized powder was centrifuged to ensure that 4ug powder was concentrated at the bottom of the bottle.

The lyophilized plasmid powder was dissolved in 40 UL of 1 × te buffer.

The mixture was centrifuged again and stored at −20 ℃.

Take 2.5μl and transfer to DH5 α receptive state.

Place on ice for 30 minute……

Heat shock 90s.

Place on ice for 2 min.

Add 900 μl LB liquid medium.

37 ℃ shaking table culture for 1 h.

It was coated on the plate containing amp and cultured at 37 ℃.

2、 The plasmid pUC19 was transformed into DH5 α

The pUC19 plasmid was transformed into DH5 α.

3、 Resuscitation of pSB1C3 plasmid bacteria

5 ml LB + 5 μl 34 mg / ml cm + 100 μl glycerol bacteria.

37 ℃ 220 RMP shaking table culture.

4、 PCR verification of electroporation mixed plasmid

Test top / test bottom and plasma top / plasma bottom were used as primers.

Preparation of 10 μl Max system.

5μl Max

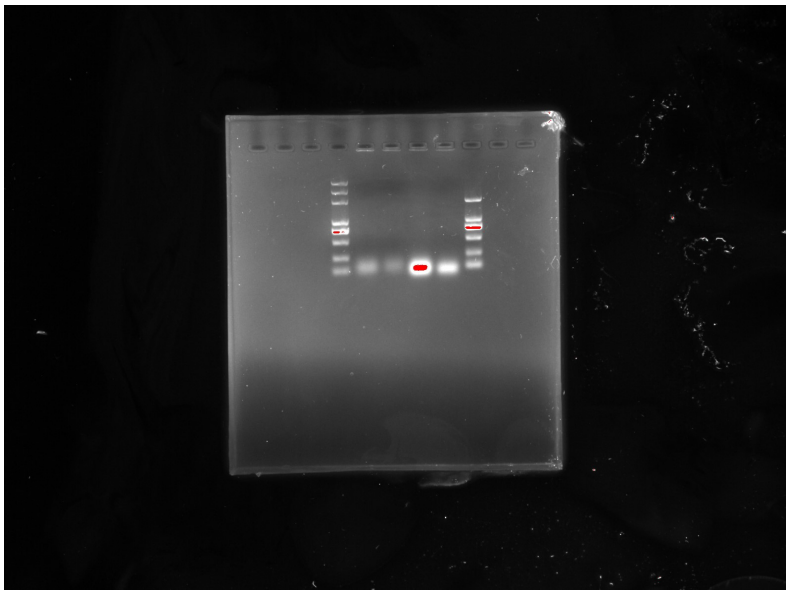
Primer on 1μl

1 μl primer

1 μl template bacteria

2μl dd H_2O

result:

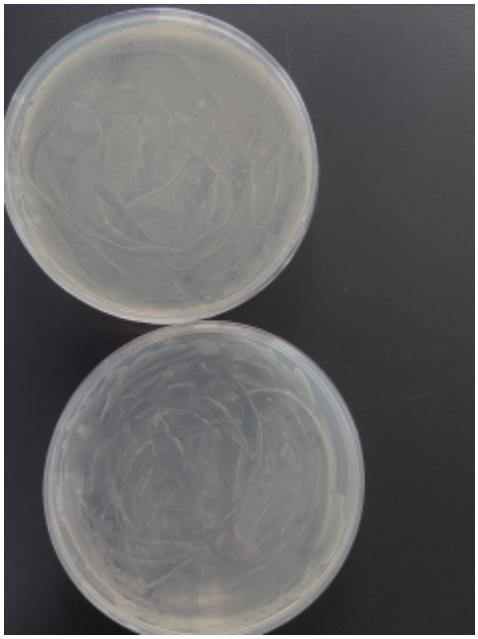


Verification failed

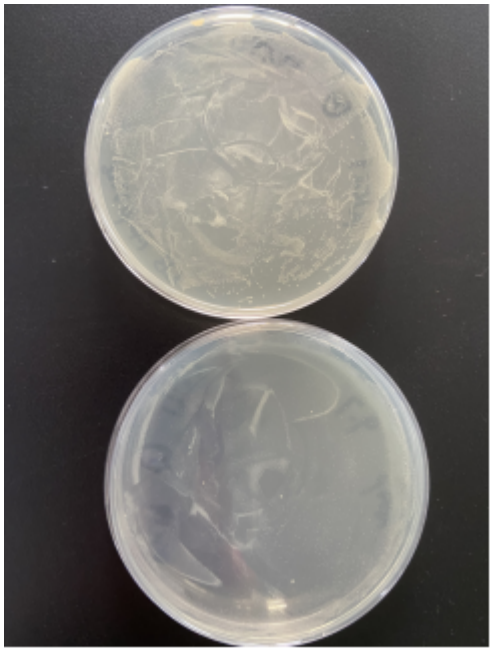
The literature shows that Dr strain needs special promoter to start expression, so it can not express
The plate of AMP may be infected with mixed bacteria, because the probability of bacterial growth is very low, not all the boards grow bacteria

2020.8.11

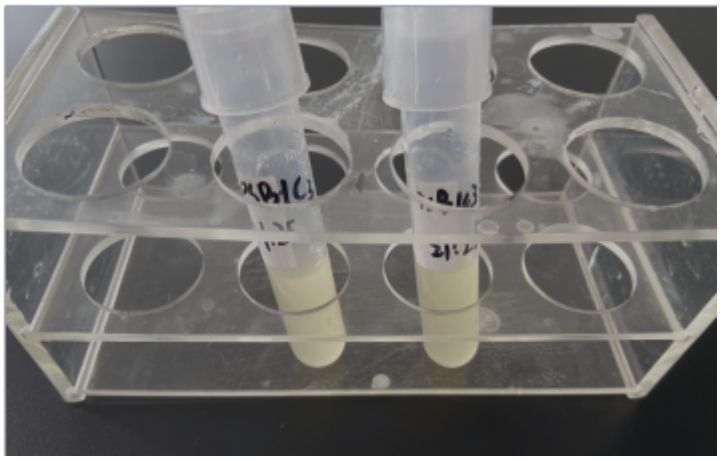
1、 Pasr-pUC19 coated yesterday was transformed into DH5 α flat panel Monoclonal (3 clones) and shaken in liquid LB medium containing amp



2、 The flat panel clones (three clones) transformed from pUC19 coated yesterday to DH5 α were shaken in liquid LB medium containing amp



3、 Transfer culture of pSB1C3 plasmid bacteria recovered yesterday (transferred to two tubes)



4、 Plasmid Extraction

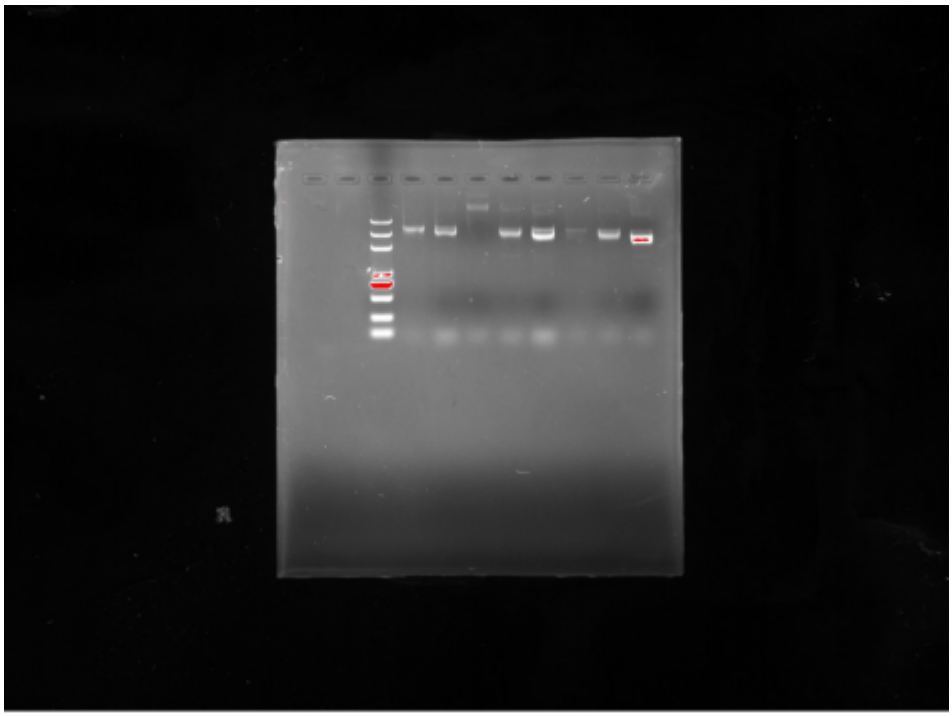
The plasmids were extracted from the above three kinds of bacteria

The plasmids Pasr-pUC19, pSB1C3 and pUC19 were extracted

Determination of concentration

	No.	Sample ID	ID#	Sample Ty	SW (nm)	SW Abs	260 Abs	280 Abs	260 / 28	260 / 23	Conc. (ng	Dilution	Time
2	1	PSB1C3	1	dsDNA	260	1.884	1.884	0.94	2.004	1.733	94.2	1	21:29:38
3	2	PSB1C3	2	dsDNA	260	2.414	2.414	1.176	2.053	2.114	120.7	1	21:30:03
4	3	Pasr-pUC19	3	dsDNA	260	1.108	1.108	0.574	1.93	1.314	55.4	1	21:30:40
5	4	Pasr-pUC19	4	dsDNA	260	2.188	2.188	1.093	2.002	1.798	109.4	1	21:31:06
6	5	Pasr-pUC19	5	dsDNA	260	2.435	2.435	1.227	1.985	1.744	121.75	1	21:31:33
7	6	pUC19	6	dsDNA	260	1.162	1.162	0.579	2.007	1.667	58.1	1	21:32:08
8	7	pUC19	7	dsDNA	260	1.852	1.852	0.925	2.002	1.848	92.6	1	21:32:32
9	8	pUC19	8	dsDNA	260	1.507	1.507	0.773	1.95	1.703	75.35	1	21:32:59

The length of plasmid was verified by electrophoresis



According to the concentration and electrophoretic bands, pSB1C3 was selected as the second tube and Pasr-pUC19 as the third tube for enzyme digestion and preservation

PUC19 select No. 2 tube preservation bacteria

2020.8.12

1、 Enzyme digestion

① Preparation of enzyme digestion system (20μl)

Pasr-pUC19 plasmid (120ng / μl): 7μl

EcoRI (10U/μl) :1μl

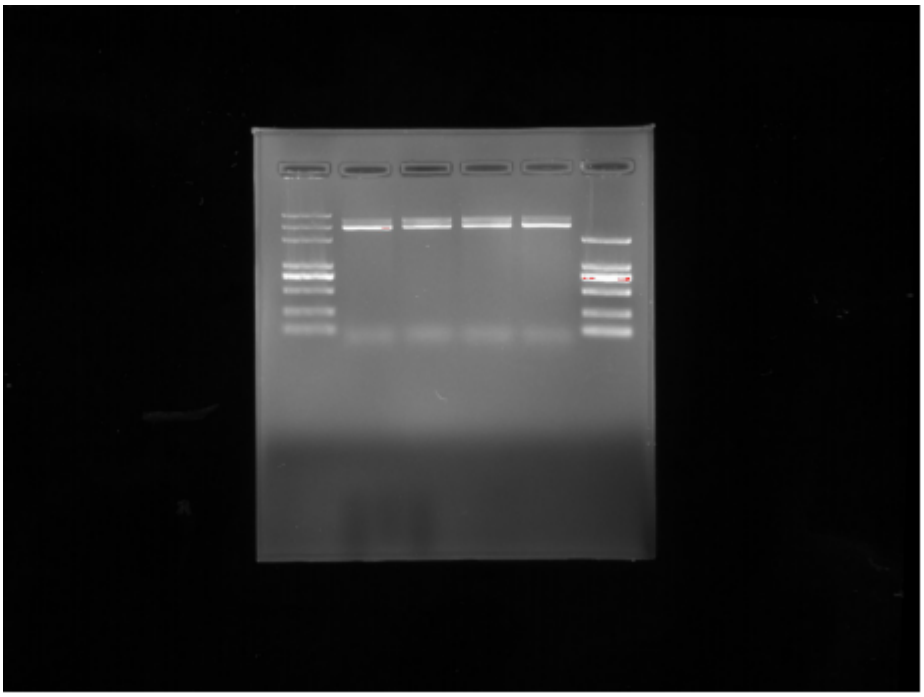
XbaI (10U/μl):1μl

10× buffer:2μl

dd H_2O:10μl

The enzyme was digested at 37 ℃ for 30 min and stored at 4 ℃

Results:



There was no target fragment in the 140 BP pasr, which might not have been cut

Note: later, it was found that my enzyme cutting time was set incorrectly. I set 30 minutes to 30 seconds, so there was no enzyme cutting

② Preparation of enzyme digestion system (20ul)

pSB1C3 plasmid (120ng / μl): 7μl

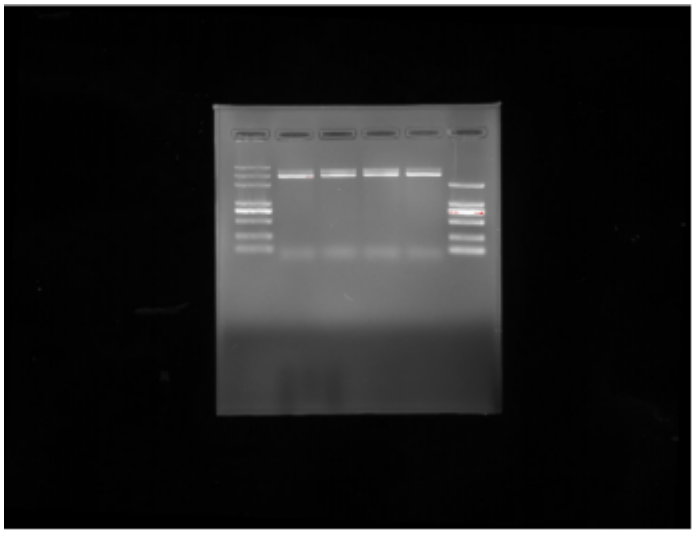
EcoRl (10U/μl) :0.5μl

Xbal (10U/μl):0.5μl

10× buffer:2μl

dd H_2O:10μl

The enzyme was digested at 37 ℃ for 30 min and stored at 4 ℃



Results:

The target fragment was most of pSB1C3 plasmid with normal band, but it was not found because it was only about 10 bp away from the complete plasmid

Method to determine whether the enzyme digestion was successful

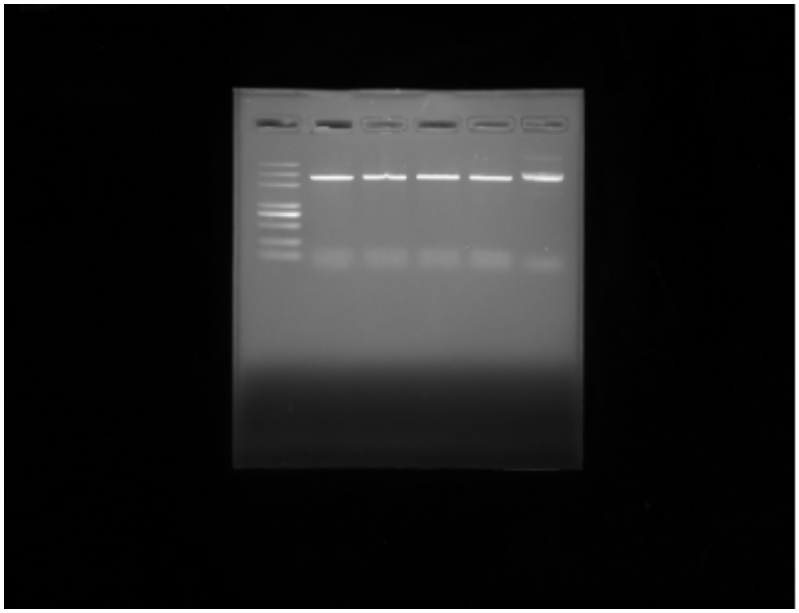
Improvement: a loop plasmid without enzyme digestion was selected as the control, and the linear plasmid was longer than the circular plasmid at the same length

For the time being, it is regarded as a successful enzyme digestion fragment for gel recovery

Note: later, it was found that my enzyme cutting time was set incorrectly. I set 30 minutes to 30 seconds, so there was no enzyme cutting

2、 The enzyme digestion of pasr was performed again

The system and conditions are the same as above.



The last pore is Pasr-pUC19 plasmid control

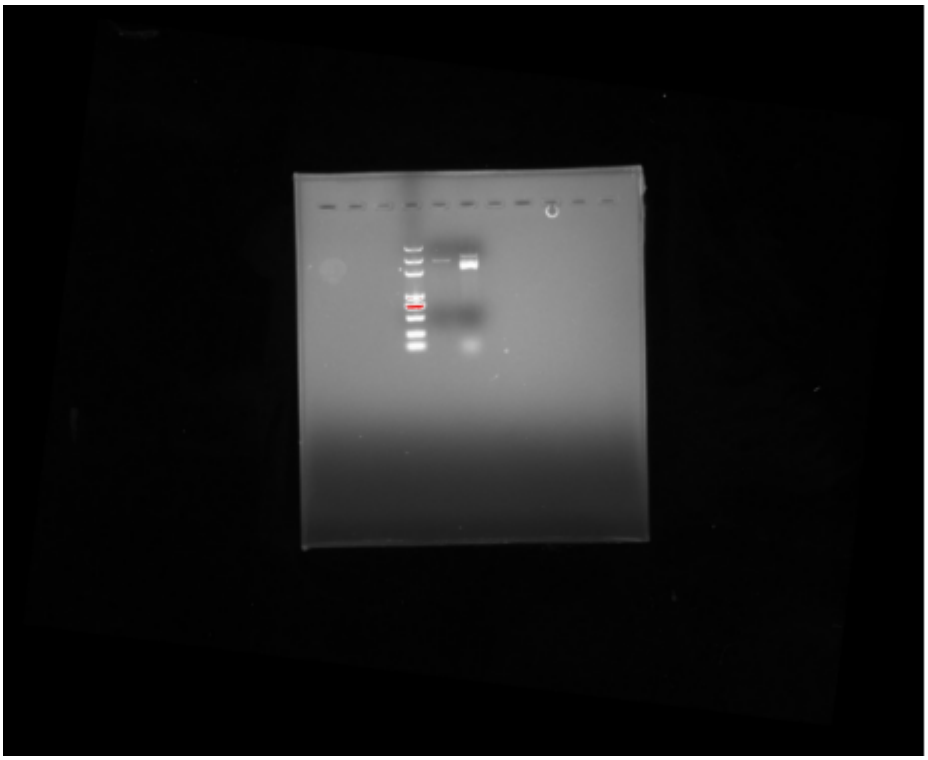
result:

There was no difference between the circular plasmid and the linearized fragment

Note: later, it was found that my enzyme cutting time was set incorrectly. I set 30 minutes to 30 seconds, so enzyme cutting is failed.

3、 pSB1C3 was digested by enzyme and recovered

1	No.	Sample ID	ID#	Sample Ty	SW (nm)	SW Abs	260 Abs	280 Abs	260 / 280	260 / 230	Conc. (ng	Dilution	Time
2	1	pSB1C3切回收	1	dsDNA	260	0.66	0.66	0.449	1.47	0.582	33	1	19:06:05



Results:

There is a problem with the concentration of the glue cutting recovery. It may be that W1 is wrongly added to W2 in the glue cutting recovery step (I don't remember).On the left side of the electrophoretic map is the tangent product of the enzyme recovered from gel cutting, and on the right side is the circular plasmid that has not been digested.However, due to the poor concentration of gel recovery, it may need to be renewed in the future conduct.

Note: Because enzyme digestion is failed, the recovered glue is also discarded.

2020.8.13

1、Preservation of bacteria

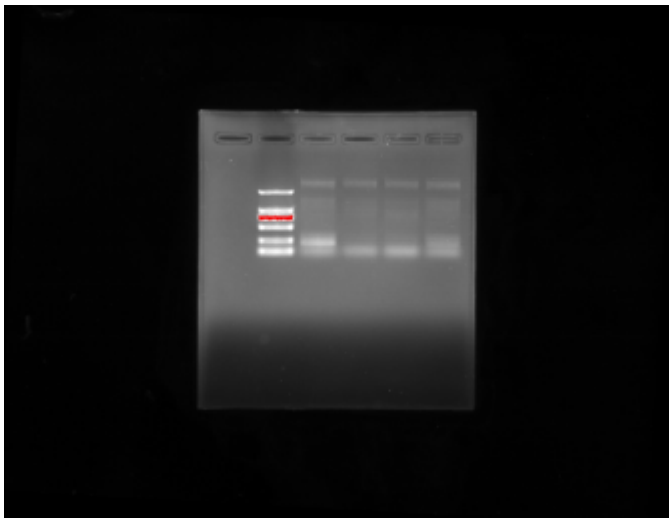
The DH5 α of Pasr-pUC19 plasmid was preserved

The DH5 α of pSB1C3 plasmid was preserved

The DH5 α of pUC19 plasmid was preserved

2、Pasr fragment was obtained by PCR

System 20μl



results:

140 BP is too short,

① Run electrophoresis with 2% gel may run out.

② Max enzyme PCR is not suitable for such a short fragment, you can try other enzymes.

3、Enzyme digestion

Recovery of pasr 140bp by cutting glue

	No.	Sample ID	ID#	Sample Ty	SW (nm)	SW Abs	260 Abs	280 Abs	260 / 280	260 / 230	Conc. (ng	Dilution	Time
1													
2	1	Pasr切胶回收片l	1	dsDNA	260	0.07	0.07	0.05	1.4	0.162	3.5	1	17:14:32

The concentration of glue recovery is too low

Cut the glue and recover the fragments below 500bp, and wash them with isopropanol

4、Re digestion of Pasr-pUC19

100bp length strips may not be suitable for 1% gels.

improvements:

① The enzyme digestion was repeated

② SDS was added to terminate the enzyme digestion reaction

③ After electrophoresis, 2% agarose gel was used for electrophoresis.

5、Enzyme digestion of pSB1C3 plasmid and Pasr-pUC19 plasmid

The system is the same as above, and each tube is made of 4 tubes of 20μl

The enzyme was digested at 37 ℃ for 30 min and inactivated at 65 ℃ for 5 min

Glue running verification

6、Glue cutting recovery

There is a problem with the gel cutting Recovery Kit. In recent days, the concentration of the kit is very low, so it has been discarded.

2020.8.14

1、Preparation of improved granules by shaking bacteria

Because the recovery of pSB1C3 plasmid gel cutting failed, it had to be redone

Pasr-pUC19 was not digested successfully, so we have to do it again

But the plasmid is gone, so shake the bacteria

Three tubes of pSB1C3 and Pasr-pUC19 were used

5 ml LB + 5μl Amp + 100μl pSB1C3 glycerol bacteria,

5 ml LB + 5μl Cm + 100μl Pasr-pUC19 glycerol bacteria,

37 ℃, 220 RMP overnight.

2020.8.15

1、The plasmid was extracted, the concentration was determined and the electrophoresis was detected

The concentration measured by a small spectrophotometer was as follows:

No.	Sample ID	ID#	Sample Ty	SW (nm)	SW Abs	260 Abs	280 Abs	260 / 280	260 / 230	Conc. (ng	Dilution	Time
1	PSB1C3 质粒①	1	dsDNA	260	2.158	2.158	1.062	2.032	2.026	107.9	1	12:41:54
2	PSB1C3 质粒②	2	dsDNA	260	2.828	2.828	1.42	1.992	1.999	141.4	1	12:42:28
3	PSB1C3 质粒③	3	dsDNA	260	2.654	2.654	1.299	2.043	2.065	132.7	1	12:42:59
4	PASR-PUC19 质粒①	4	dsDNA	260	2.732	2.732	1.361	2.007	2.057	136.6	1	12:43:32
5	PASR-PUC19 质粒②	5	dsDNA	260	2.054	2.054	1.038	1.979	2.014	102.7	1	12:44:05
6	PASR-PUC19 质粒③	6	dsDNA	260	2.619	2.619	1.31	1.999	2.019	130.95	1	12:44:33

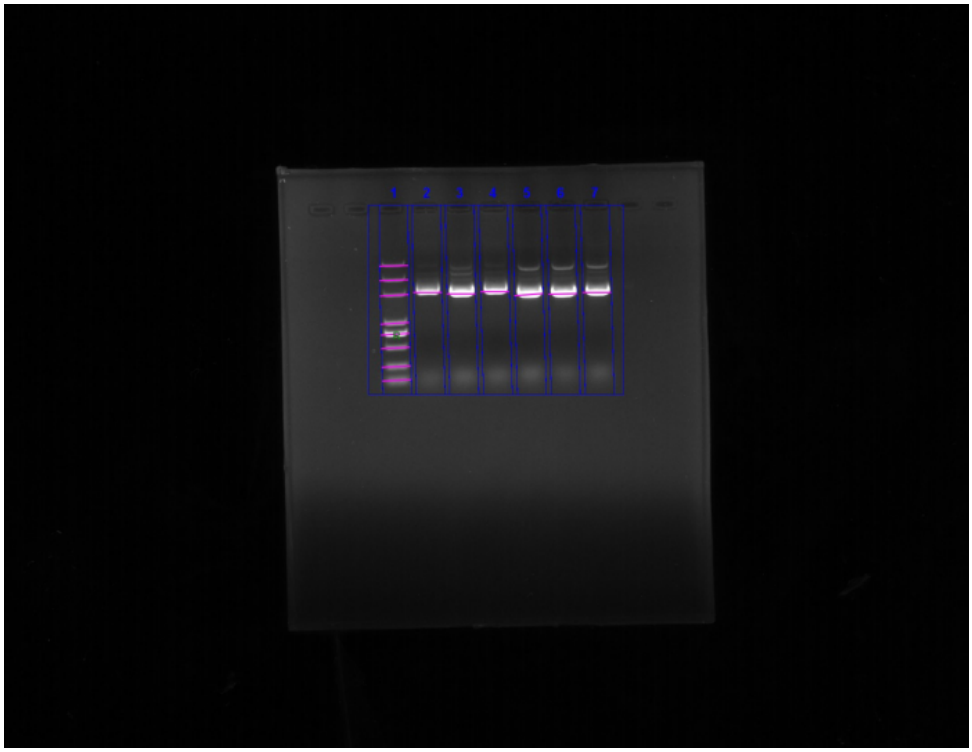
Secondary recovery:

Note: if you want to obtain a higher concentration of plasmids, you can add the centrifuged plasmid into the adsorption column for centrifugation

The lower plasmid concentration was higher than the first concentration

No.	Sample ID	ID#	Sample Ty	SW (nm)	SW Abs	260 Abs	280 Abs	260 / 280	260 / 230	Conc. (ng	Dilution	Time
1	PSB1C3 质粒①	1	dsDNA	260	4.35	4.35	2.399	1.813	1.043	217.5	1	12:51:25
3	PSB1C3 质粒②	3	dsDNA	260	3.341	3.341	1.683	1.985	1.944	167.05	1	12:59:30
4	PSB1C3 质粒②	4	dsDNA	260	2.88	2.88	1.429	2.015	1.993	144	1	13:00:02
5	Pasr-pUC19 质粒①	5	dsDNA	260	3.213	3.213	1.61	1.996	2.034	160.65	1	13:00:40
2	Pasr-pUC19 质粒②	2	dsDNA	260	3.289	3.289	1.795	1.832	1.197	164.45	1	12:52:28
6	Pasr-pUC19 质粒③	6	dsDNA	260	3.646	3.646	1.899	1.92	1.505	182.3	1	13:01:07

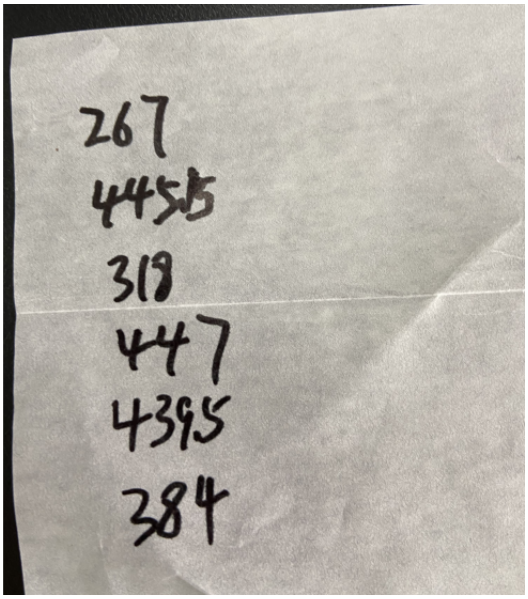
Electrophoresis verification：



The concentration was analyzed by image lab as follows:

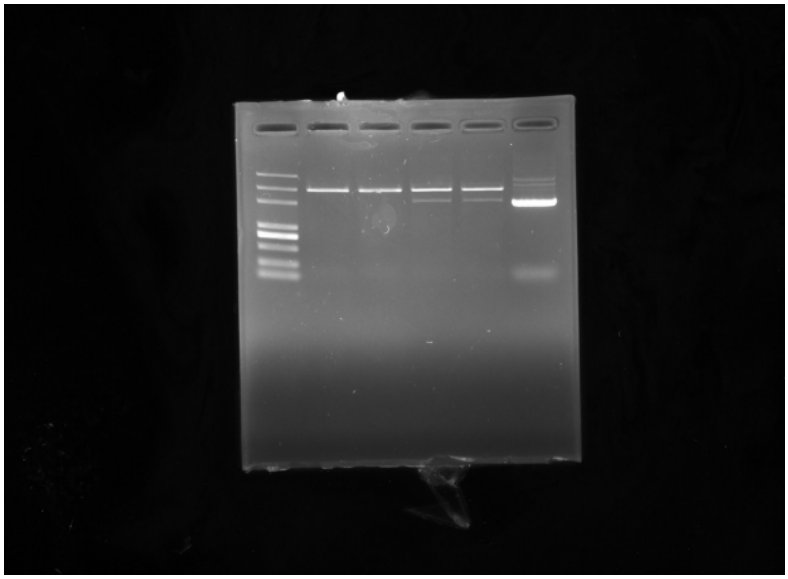
1	ZJY 2020-09-12 15 时 34 分									
2		Band No.	Band Labe	碱基对(bp	Relative	Volume(Ir	Abs. Quar	Rel. Quar	Band %	Lane %
3	泳道 1	1		不适用	0.323194	91840	不适用	0.173913	4.692034	4.449354
4		2		不适用	0.39924	123920	不适用	0.234661	6.330976	6.003527
5		3		不适用	0.479087	150480	不适用	0.284957	7.687906	7.290274
6		4		不适用	0.627376	270600	不适用	0.512422	13.82474	13.1097
7		5		不适用	0.684411	528080	不适用	1	26.9792	25.58378
8		6		不适用	0.756654	220840	不适用	0.418194	11.28254	10.69899
9		7		不适用	0.855513	284120	不适用	0.538025	14.51547	13.7647
10		8		不适用	0.927757	287480	不适用	0.544387	14.68713	13.92749
11	泳道 2	1		不适用	0.463878	942240	不适用	1.784275	100	60.13172
12	泳道 3	1		不适用	0.471483	1570760	不适用	2.974474	100	69.63823
13	泳道 4	1		不适用	0.460076	1118160	不适用	2.117406	100	59.96396
14	泳道 5	1		不适用	0.475285	1680680	不适用	3.182624	100	66.45841
15	泳道 6	1		不适用	0.471483	1547840	不适用	2.931071	100	66.91915
16	泳道 7	1		不适用	0.463878	1352240	不适用	2.560673	100	67.2288

The following concentrations were obtained: (in the order above)



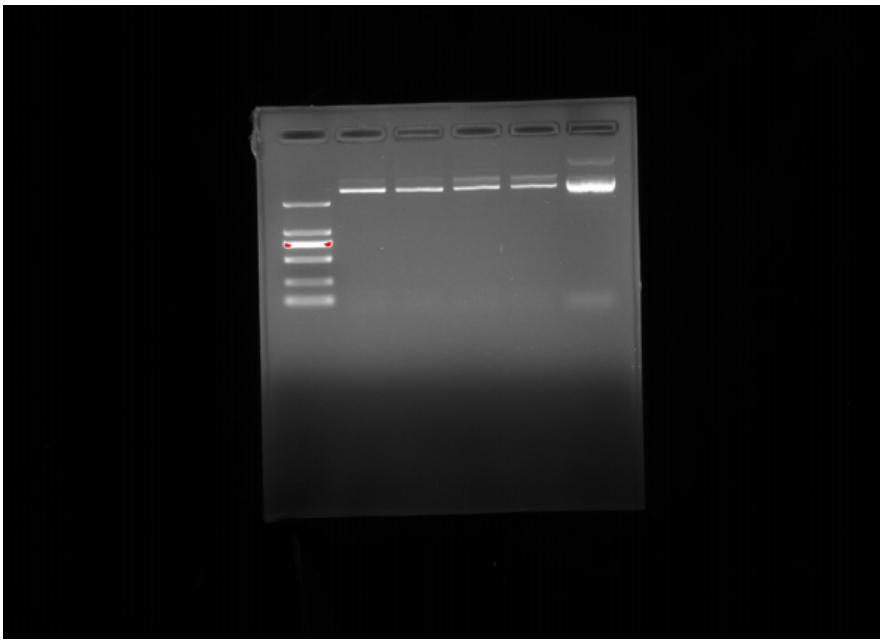
Enzyme digestion

The plasmid pSB1C3 was digested by enzyme：



Compared with the circular plasmid, the length of the control became longer.

Pasr-pUC19 plasmid was digested by enzyme:



results:

The length of 140 BP was not found, which was difficult to run. Therefore, we gave up the restriction enzyme to get the fragment.

2020.8.16

1、Pasr fragment was obtained by PCR

M13 FWD new and M13 rew new primers were selected to p down pasr gene (406bp)

System: (20ul) 4 tubes

Pasr-pUC19 plasmid: 1μl

M13-fwd-new：1μl

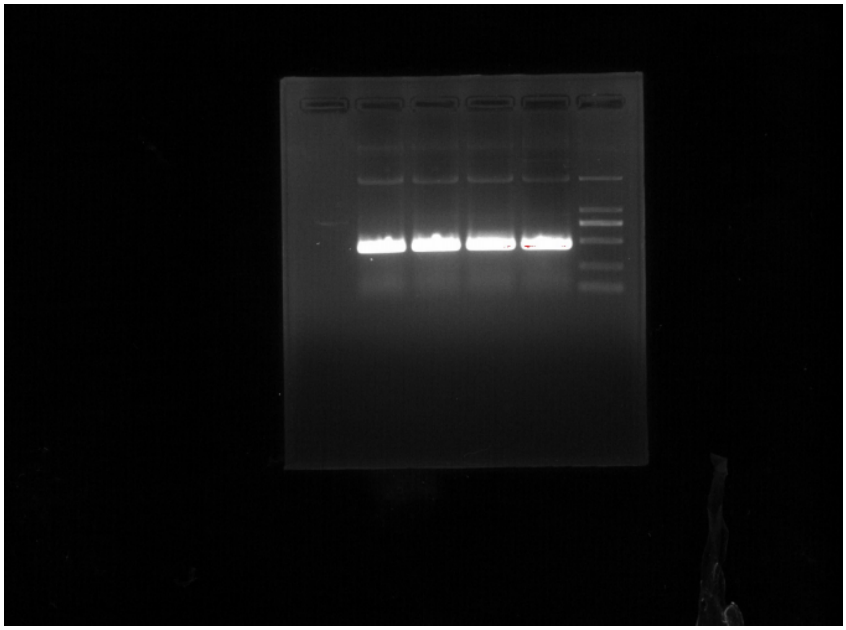
M13-rew-new：1μl

Max（2×）:10μl

dd H_2O：7μl

The program follows the saved Max program

Preparation of agarose gel 1.5% resin



2、The plasmid pSB1C3 was linearized by PCR (2894 BP)

System: (20μl) 4 tubes

pSB1C3 plasmid: 1μl

liner_ pSB1C3_ top: 1μl

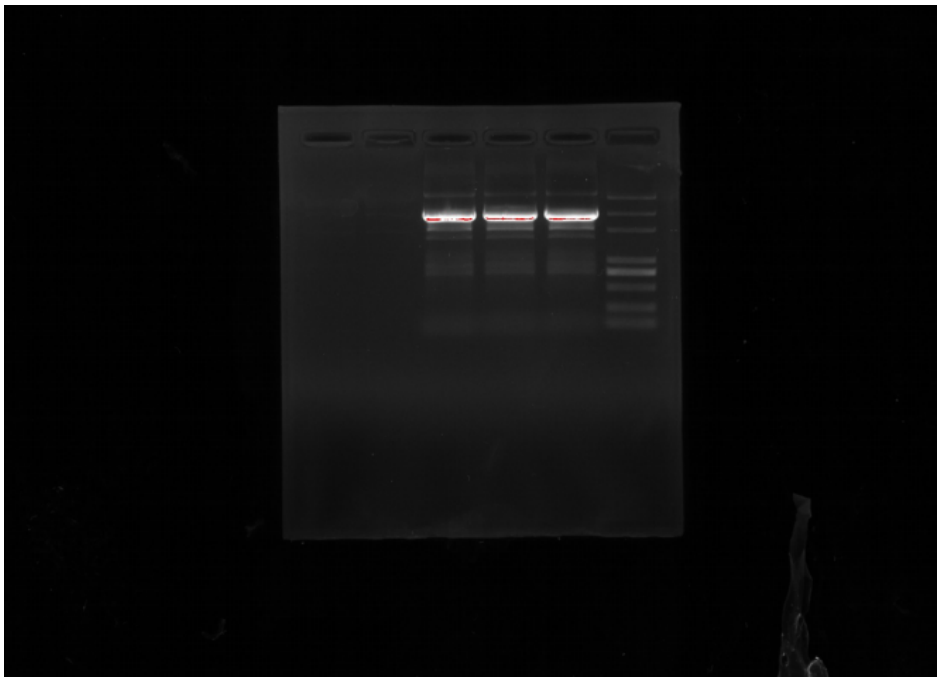
liner_ pSB1C3_ bottom：1μl

Max（2×）:10μl

dd H_2O：7μl

The program follows the saved Max program

Preparation of agarose gel 1% resin

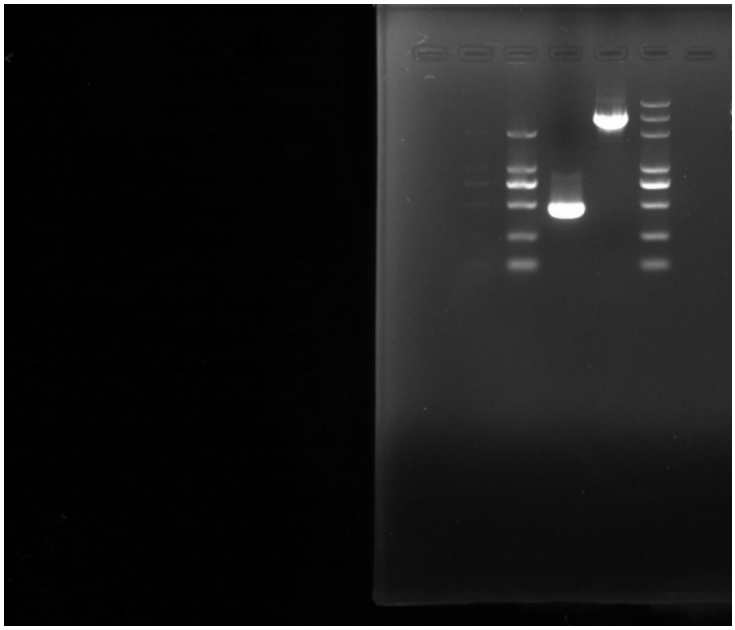


3、The above fragments were recovered by running glue and cutting glue (simgen for glue cutting recovery)

The concentration was determined after the glue was recovered

No.	Sample ID	ID#	Sample Ty	SW (nm)	SW Abs	260 Abs	(280 Abs	(260 / 280	(260 / 230	Conc. (ng	Dilution	Time
1	Pasr片段浓度	1	dsDNA	260	3.955	3.955	2.116	1.869	1.876	197.75	1	15:53:25
2	pSB1C3质粒线性化浓度	2	dsDNA	260	2.234	2.234	1.205	1.854	1.579	111.7	1	15:54:00

Run out to verify the cut and recovery strip and concentration



pSB1C3线性化片段（9.14）（									
	Band No.	Band Labe	碱基对(bp	Relative	Volume(Ir	Abs. Quar	Rel. Quar	Band %	Lane %
泳道 1	1		2,860.25	0.312757	1803556	不适用	3.380966	100	84.23313
泳道 2	1		5,000.00	0.242798	98534	不适用	0.184713	5.569158	5.117426
	2		3,000.00	0.304527	99788	不适用	0.187064	5.640034	5.182554
	3		2,000.00	0.374486	105260	不适用	0.197322	5.949313	5.466746
	4		1,000.00	0.526749	271852	不适用	0.509617	15.36512	14.11881
	5		750	0.592593	533444	不适用	1	30.15034	27.70476
	6		500	0.679012	254524	不适用	0.477133	14.38574	13.21887
	7		250	0.814815	195092	不适用	0.365722	11.02663	10.13223
	8		100	0.934156	210786	不适用	0.395142	11.91366	10.94731

1	Pasr片段（9.14）（								
2		Band No.	Band Labe	碱基对(bp	Relative	Volume(Ir	Abs. Quar	Rel. Quar	Band %	Lane %
3	泳道 2	1		2,000.00	0.310811	114076	不适用	0.241785	7.814045	7.020744
4		2		1,000.00	0.477477	216372	不适用	0.458602	14.82118	13.3165
5		3		750	0.554054	471808	不适用	1	32.31818	29.03716
6		4		500	0.648649	247798	不适用	0.525209	16.97381	15.25059
7		5		250	0.797297	168910	不适用	0.358006	11.5701	10.39547
8		6		100	0.927928	240920	不适用	0.510631	16.50268	14.82729
9	泳道 3	1		450.1521	0.671171	2317468	不适用	4.911888	100	80.61386

The concentrations were as follows:

The linear fragment concentration of pSB1C3 was 150 * 3.380966 = 507.1449

The concentration of pasr fragment was 150 * 4.911888 = 736.7832

4、Sosoo recombination and transformation (10ul system)

Sosoo: 5μl

Pasr fragment: diluted 10 times and added 1.5μl

Linearization pSB1C3: add 1.5μl after 10 times dilution

dd H_2O:2μl

The reaction time was 15 min at 50 ℃ and stored at 4 ℃.

5、Novozan recombination and transformation (20ul system)

Exnase II: 2μl

5 × CE II Buffer: 4μl

Pasr fragment: diluted 10 times and added 2μl

Linearization pSB1C3: add 1 μl after 100 times dilution

dd H_2O:9μl

The reaction time was 30 min at 37 ℃ and stored at 4 ℃ .