0730

Measure the concentrations of Ligation products of reverse lacI + promoter

Sample	Concentration(×50μg/mL)
1	1.6536
2	2.4225
3	2.7404
4	2.8690
5	2.4858
6	2.5618

Enzyme digestion to test the ligation product

Plasmid	8μl
EcoRI	1.5 μl
PstI	1.5μl
10×H Buffer	2μl
ddH_2O	7μl
∑	20μl

The result is wrong, so MiniPrep plasmids containing reverse lacI or promoter respectively and measure its concentration

Sample	Concentration(×50μg/mL)
Promoter	2.5671
Reverse lacI	3.9938

Digest the above plasmids with EcoRI and XbaI or SpeI , 37 centigrade overnight

Plasmid(promoter)	10μl	Plasmid(reverse lacI)	10μl
EcoRI	1.5 μl	EcoRI	1.5 μl
XbaI	1.5μl	SpeI	1.5μl
10×M Buffer	2μl	10×H Buffer	2μl
ddH_2O	5μl	ddH_2O	5μl
∑	20μl	∑	20μl

0731

Using CIAP to recycle the vector

CIAP	0.5μl
Buffer	2.5 μl
Digestion product	20μl
ddH_2O	2μl
∑	25μl

37 centigrade, incubate for 30 min

Recycle the vector and fragment

Ligation of reverse lacI fragment into vector containing the promoter

Buffer	1μl
Ligase	1 μl

Vector(promoter)	2μl
Fragment(reverse lacI)	6μl
∑	10 μl

Transform ligation product into competent cells
Procedure:

Ice bath	30 min
Heat shock	90 s
Ice bath	2-3 min
Add 100 μl non-resistant LB	
Shaker, 37 centigrade	45 min
Plate on Amp plate	

0801
9:00 Pick up the colony and shake in the incubator

0802
11:00 Pick up 21 colonies and identify the results by PCR
It turns out that all the plasmids are self-ligation of the vector
21:00 ligate reverse lacI insert with lacI promoter again

0803
01:00 transformaiton
14:01 do the ligation again using the vector provided by WSK
20:00 transform the ligation product into DH5a

0804
Colony PCR
Lane1-39: my sample
Other lanes: WSK's sample
The number of the right-sized insert: 1,2,3,5,11,12,17,18,19,21,22,23,25,26,27,31,32,33,35,37
22:25 pick up the colonies 11,12,17,21,25,31,35 and shake in the 37 centigrade incubator
Since the vector used for ligation this time is enzyme digested overnight without addition of BSA by WSK, the PCR results finally has the right insert band, about 1.5 kb.

0805
Miniprep plasmid with No 11,12,17,21,25,31 and measure their concentration

Sample	Concentration(×50μg/mL)
11	3.7421
12	3.4852
17	1.9614
21	2.8062
25	5.3108
31	3.4985

Double digestion the plasmid (Fig 1)

Plasmid	5μl
EcoRI	1.5 μl
PstI	1.5μl
10×H Buffer	2μl
ddH_2O	10μl
∑	20μl

12:30 bath in 37 centigrade water

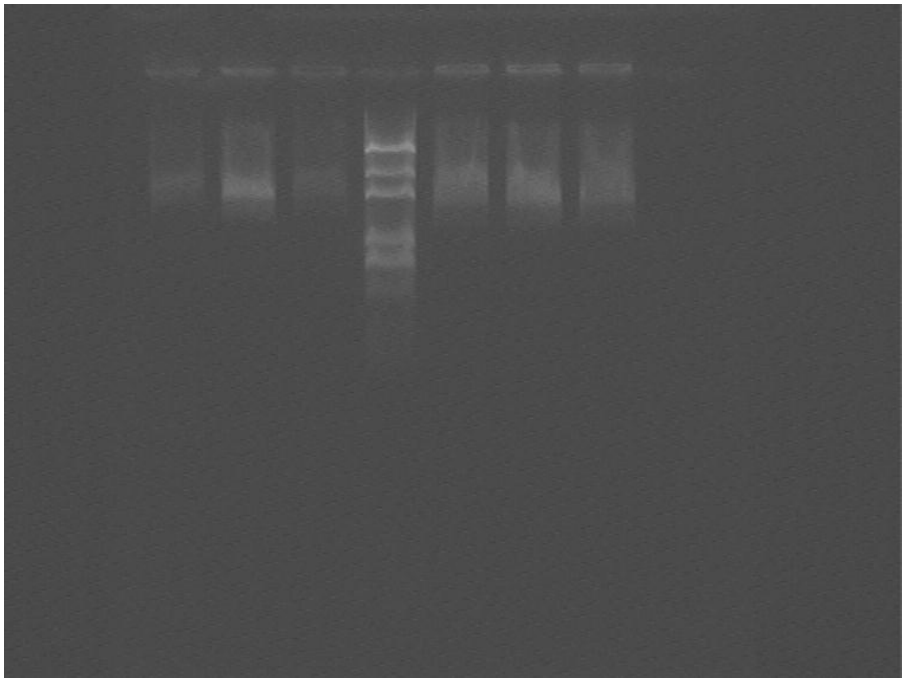

Figure 1

0806
Add SpeI and continue digestion (Fig 1)

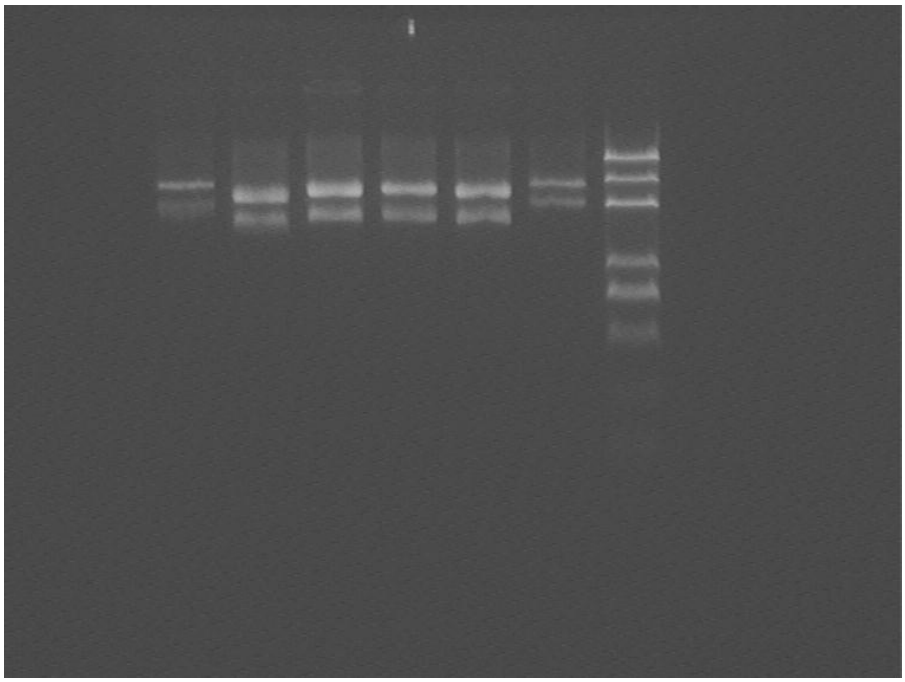

Figure 1

0807

Electrophoresis and cut the lacI insert band for recycle, measure the recycle product concentration

Sample	Concentration(×50μg/mL)
12	0.4524
17	0.5731

15:30 Ligation of lacI+promoter (insert) with GFP or supD

0808

18:00 colony PCR: 12lacI-supD, 17lacI-supD

2×superMix	**5μl**
Universal Primer (Formard)	0.25 μl
Universal Primer (Reverse)	0.25μl
ddH_2O	4.5μl
∑	10μl

21:30 Electrophoresis to check results

23:00 ligate the insert (reverse lacI+promoter) with vector (containing supD)

0809

The plate 12lacI-GFP has one colony, colony PCR to check the result

Miniprep 12-2supD and 12-5supD

Sample	Concentration(×50μg/mL)
2	1.3526
5	2.9598

Plasmid 12-2supD	**6µl**
EcoRI	1.5 µl
PstI	1.5µl
10×H Buffer	2µl
ddH_2O	9µl
∑	20µl

0810
Enzyme digest plasmid 5 to get the insert: reverse lacI+promoter (Fig 1)
Digestion confirmation (see Fig 2)
As shown in the figure, there are two bands, vector and insert respectively, it should be right
Recycle the insert in the gel and ligate it with GFP vector, then transform into DH5a

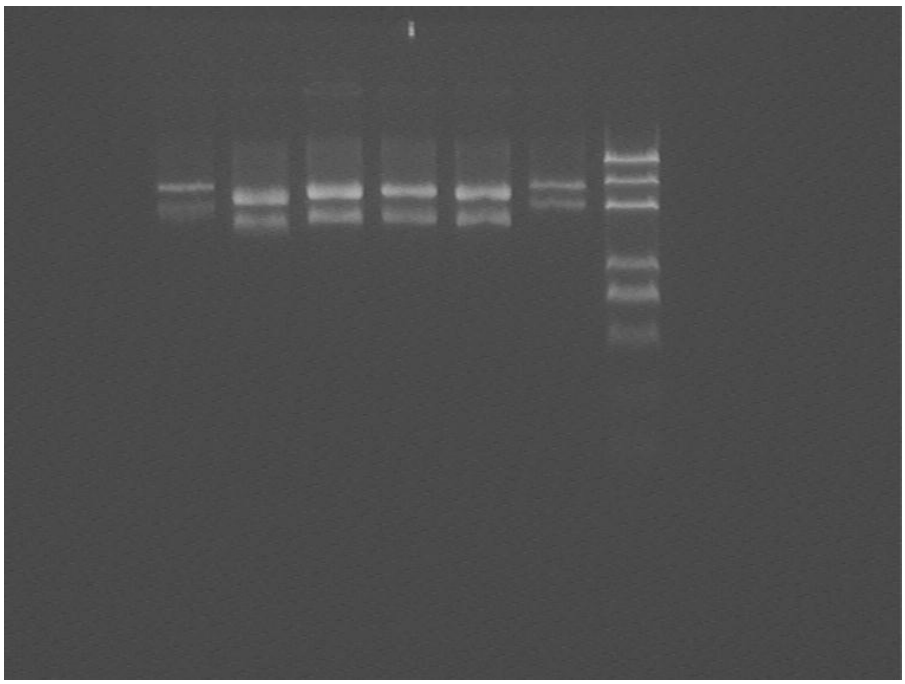

Figure 1

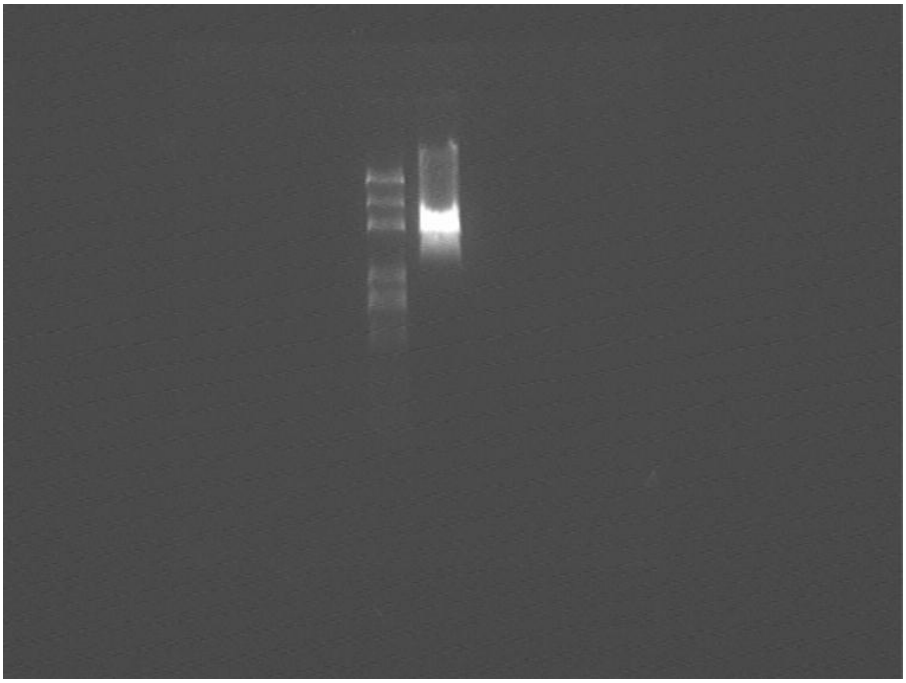

Figure 2

0811

Pick up nine colonies from the plate and PCR to check the results (Fig 1)

As shown in Fig 1, 5.1-5.6 are all achromatic, but electrophoresis results show that they are self-ligation. Shake colonies 5.7-5.9 which are green and shake them in the incubator, then MiniPrep plamid after 10 hours.

Sample	Concentration(×50μg/mL)
5.7	1.4882
5.8	1.9782
5.9	1.5369

The results indicate that lacI with degradation tag LVA may not inhibit the expression of GFP

Use synthesized primer and universal reverse primer to PCR tetR-GFP plasmid, electrophoresis and cut gel to recycle the insert.

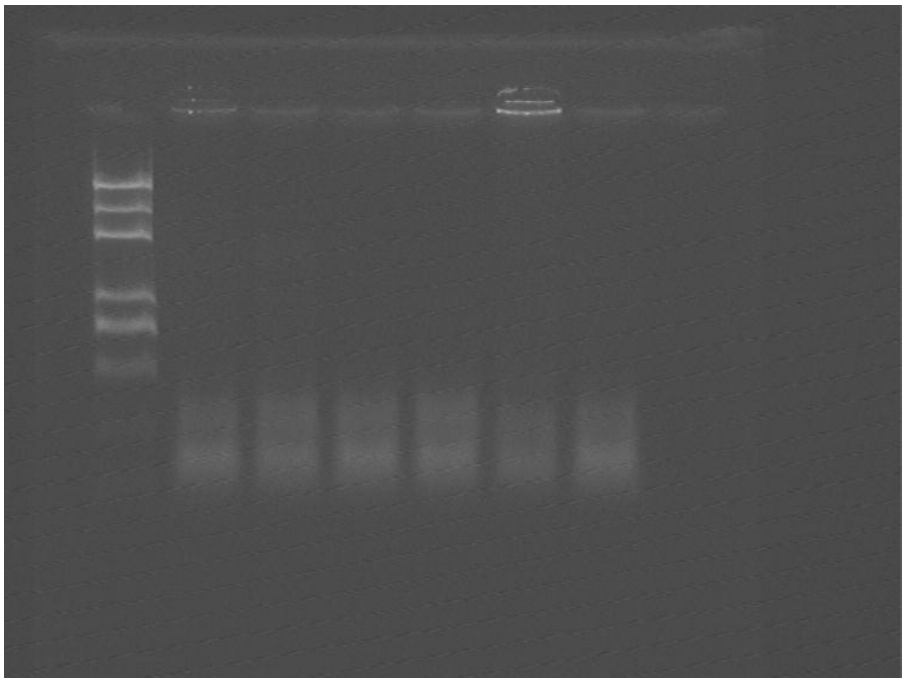

Figure 1

0812

Double enzyme digestion 5.7~5.9 plasmid, electrophoresis (Fig 1). It seems that only 5.8 insert is right Use delete LVA primer (forward primer as control) to PCR plasmid 5, 5.7, 5.8. As shown in Fig 2, to my surprise, 5F also shows positive result. Gel recycle of 5R. Since elongation time is short for 5.7 and 5.8, both results cannot be judged.

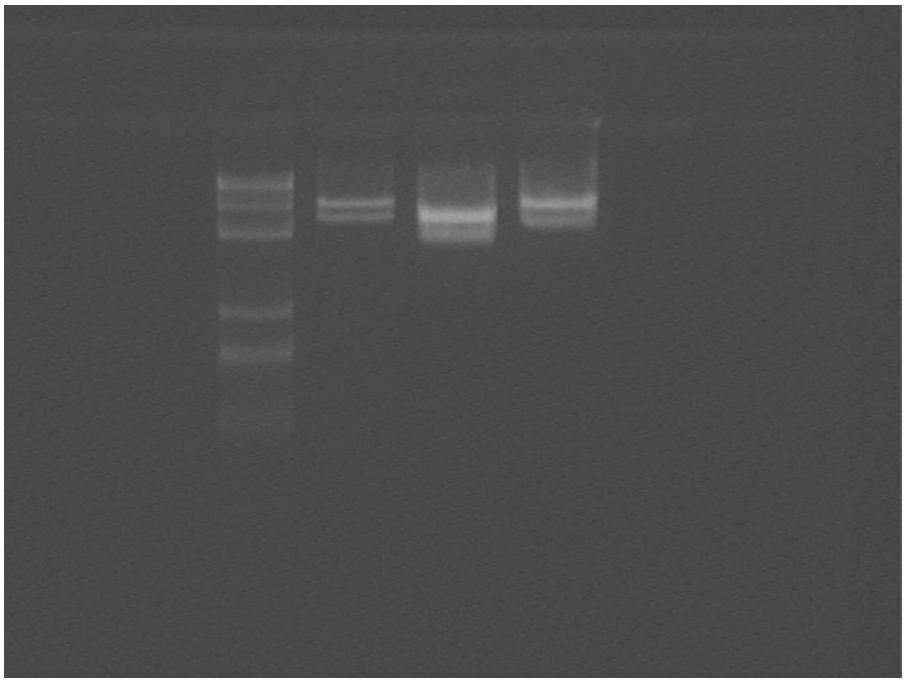

Figure 1

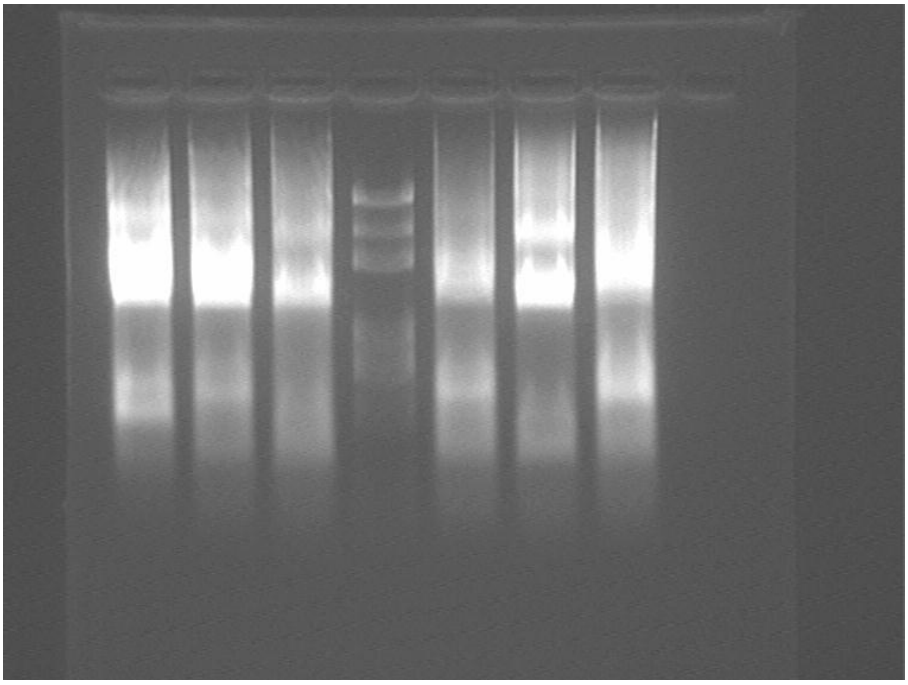

Figure 2

0813

Use dLVA primer to PCR plamid 5(no GFP), enzyme digestion and gel recycle the lacI insert (Fig 1), then ligate it with GFP vector.

Use dLVA primer to PCR plasmid 5.7 and 5.7(contain GFP), gel recycle to get the insert. (Fig 2)

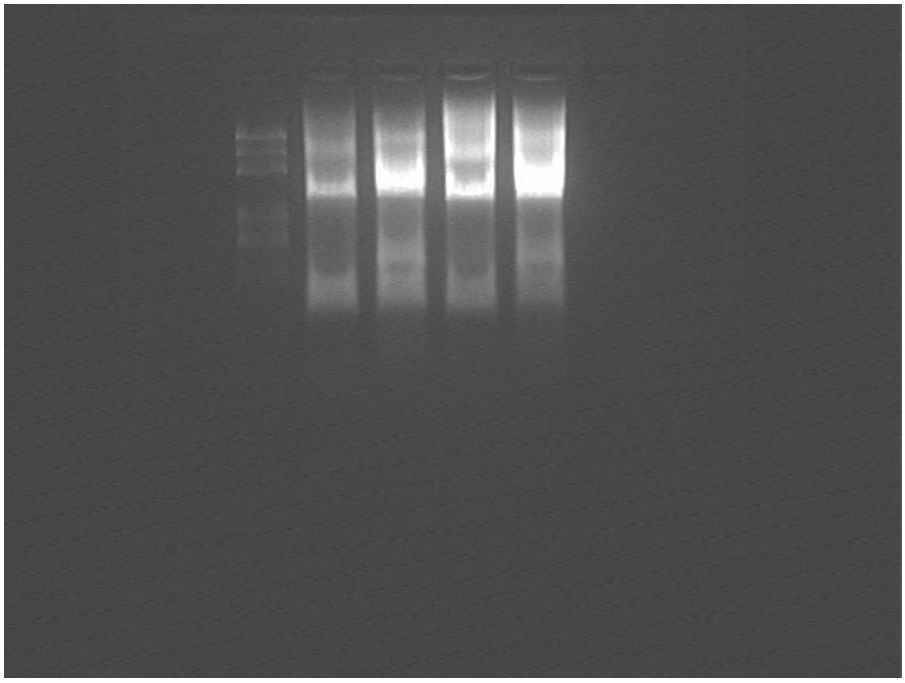

Figure 2

0816
Double enzyme digestion: lacI+promoter+GFP delete LVA plasmid (Fig 1)

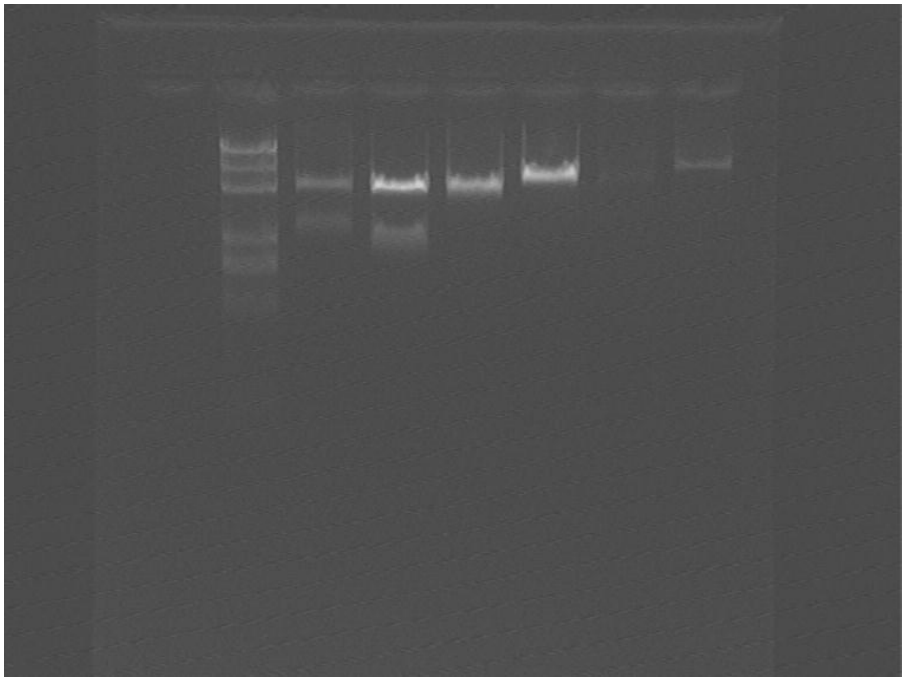

Figure 1