0904

Colony PCR results (Fig.1)

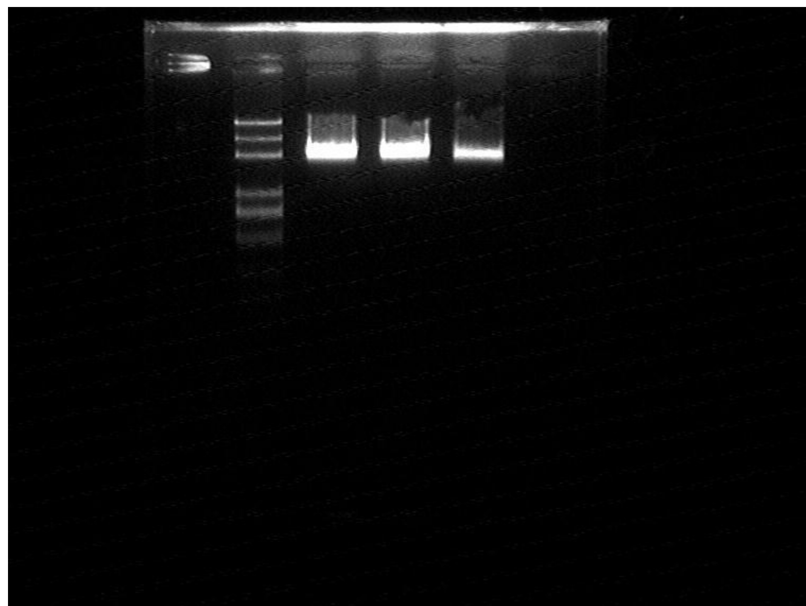

Figure 1

H1~5: sal-supD-lacI-T7ptag, Amp plate

S1~5: sal-supD, Amp plate (provided by WSK)

Miniprep S1, S2, S4

Double digestion (EcoRI+PstI): S1, S2, S4 (Fig.2)

40 uL S1(sal-supD, A^+K^+) is digested using EcoRI and PstI

S2(sal-supD, A^+K^+) is digested using EcoRI

Plasmid	**3μl**
EcoRI	1 μl
10×H Buffer	1μl
ddH$_2$O	5μl
∑	10μl

Prepare salicylate promoter insert and supD vctor

Sal-1M plasmid	**5μl**
XbaI	1 μl
PstI	1μl
10×M Buffer	2μl
ddH$_2$O	11μl
∑	20μl

supD+terminator plasmid	**5μl**
SpeI	1 μl
PstI	1μl
10×H Buffer	2μl
ddH$_2$O	11μl
∑	20μl

0905

Single digestion using EcoRI: supD+terminator plasmid

Double digestion using EcoRI and PstI: supD+terminator plasmid

Electrophoresis (Fig.1)

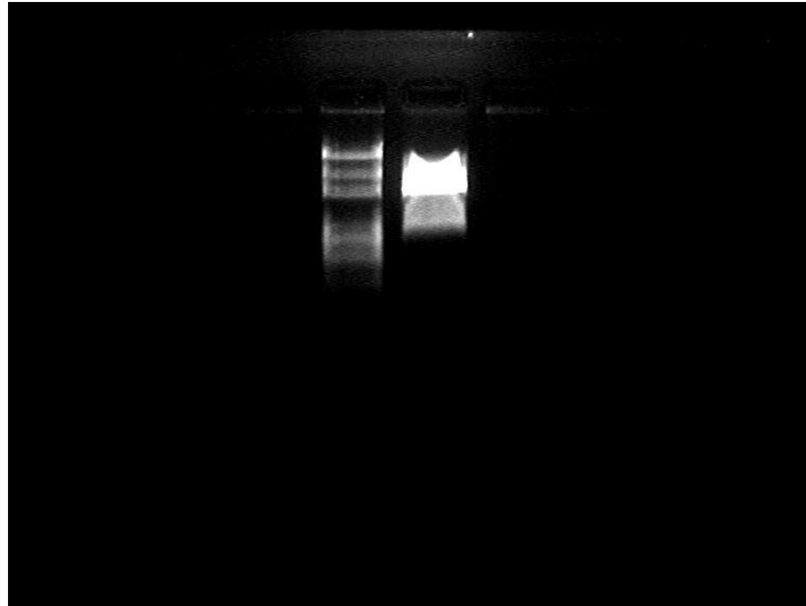

Figure 1

Lane1: plasmid 1	Lane2: plasmid 1 single digestion
Lane3: plasmid 1 double digestion	Lane4: plasmid 2
Lane5: plasmid 2 single digestion	Lane6: plasmid 2 double digestion

13:00 double digestion using EcoRI and XbaI: supD+terminator plamid

double digestion using EcoRI and SpeI: sal-1M plamid

16:00 double digestion using EcoRI and PstI (NEB enzyme): supD plasmid

21:00 electrophoresis to identify the results of digestion (Fig 2)

Lane 1: plasmid Lane 2: plasmid double digestion (EP)

There is a band whose length is about 300 bp and it demonstrates that the supD plasmid is right.

Recycle the Sal insert from gel (Fig 3). The insert is about 1.3 kb in length

DNA product purification: digested supD plasmid

22:30 ligation: sal (insert)+supD(vector), T4 DNA ligase (NEB), reaction under room temperature

Transformation

0906

01:00 plate (one is A^+K^+, the other is A^+)

Pick colonies from A^+K^+ plate and PCR (Fig 1)

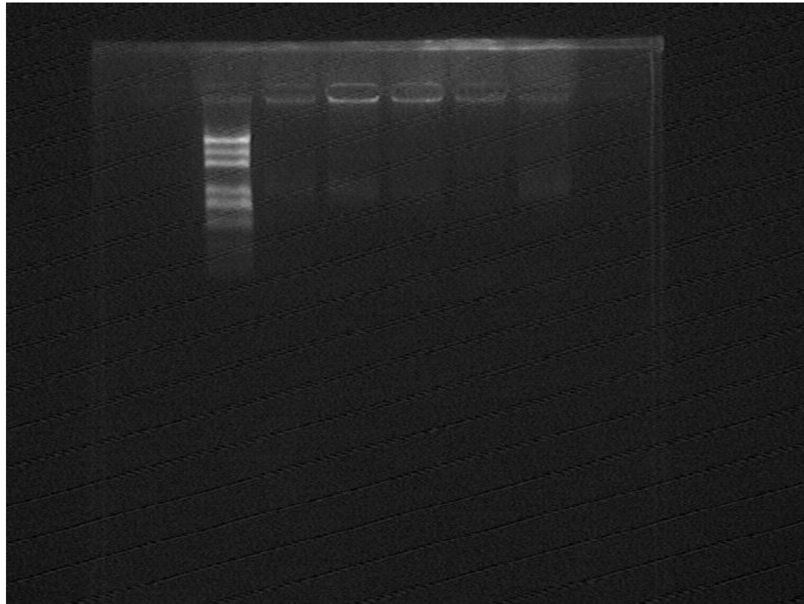

Figure 1

No.4 and No.5 are still transparent after 11 hours, MiniPrep plasmid from No.1~3 tube

0907

3:30 No.2 plasmid is double digested with EcoRI and SpeI, and the insert is given to ZHQ

No.2 plasmid is double digested with EcoRI and PstI, and the insert will be linked to another backbone (1-7G)

12:30 electrophoresis (Fig 1)

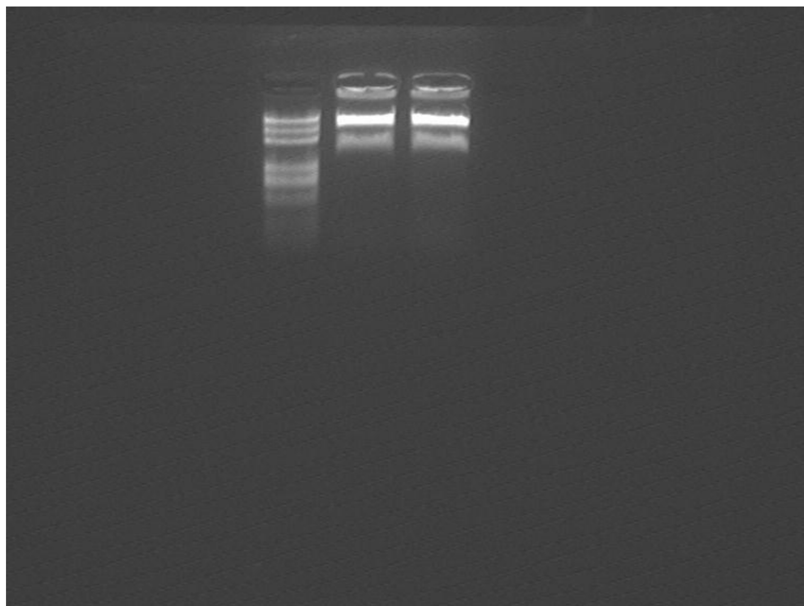

Figure 1

13:00 gel extraction: sal+supD insert (Fig 2)

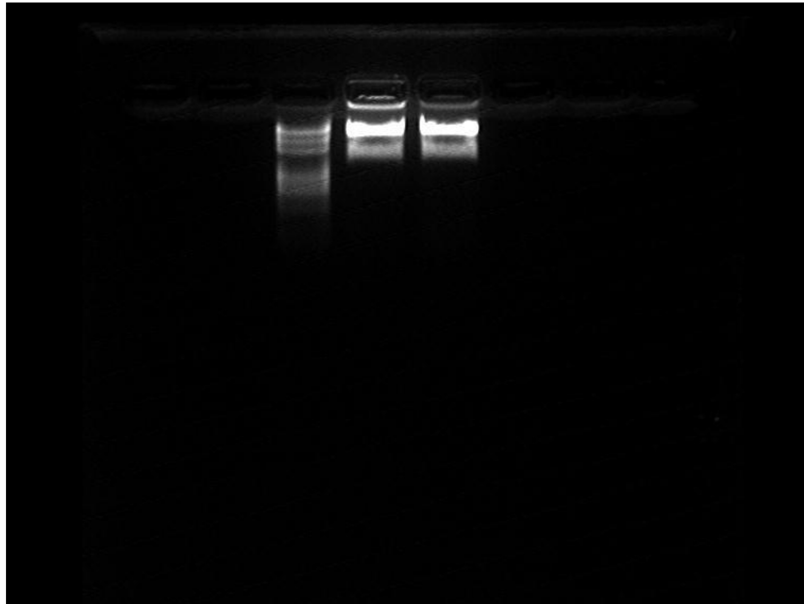

Figure 2

15:10 ligation: sal+supD (insert) and 1-7G (vector)

16:00 ligation

17:20 plate Amp plate and Kan plate. Amp plate is check if there is pollution of previous plasmid

0908

9:00 there is no colony on the Amp plate, shown that there is no pollution

Pick 5 colonies from Kan plate, PCR and shake in the incubator

20:30 double digestion to get 9 kinds of RBS+T7ptag+lacI: 1-1J, 1-5N, 1-1H, 1-2M, 1-2K, 1-5J, 1-2G, 1-1N, 1-2I

Plasmid	**3μl**
XbaI	1 μl
PstI	1 μl
Buffer	2μl
BSA	0.2 μl

22:15 MiniPrep the 5 tubes and numbered SS(1)~SS(5) 1-7G

3 μl plasmid is double digested with SpeI and PstI for recycle

3 μl plasmid is double digested with SpeI and PstI for identification

0909

00:30 gel extraction: 9×RBS+lacI+T7ptag, 9 insert in total (Fig 1)

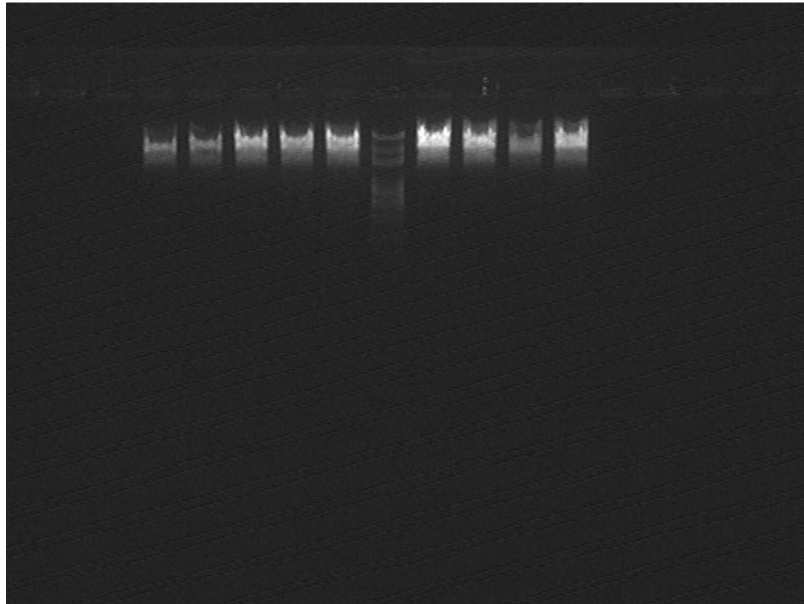

Figure 1

DNA product recycle: sal-supD 1-7G (digested with SpeI and PstI) as vector

Sal-supD 1-7G double digestion identification results (Fig2). The insert is about 1.4 kb in length and can hardly been seen on the gel. Only backbone can be seen in the figure.

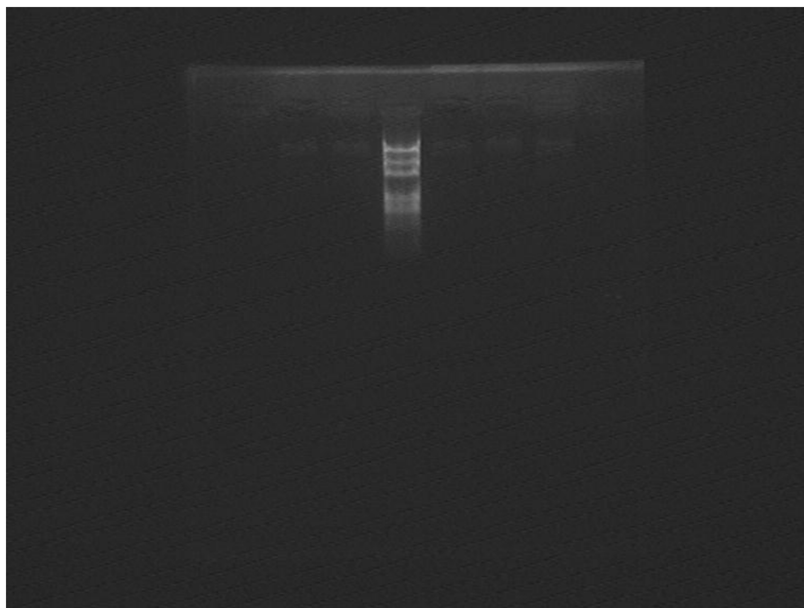

Figure 2

4:00 ligation: 9×RBS+lacI+T7ptag (insert) and sal-supD plasmid (vector)

11:30 transform ligation products into JM109

13:00 spread Kan plate

0910

00:00 only 1-5J, 1-2K, 1-5N, 1-1N, 1-1H plates have colonies. Colony PCR (Figure 1)

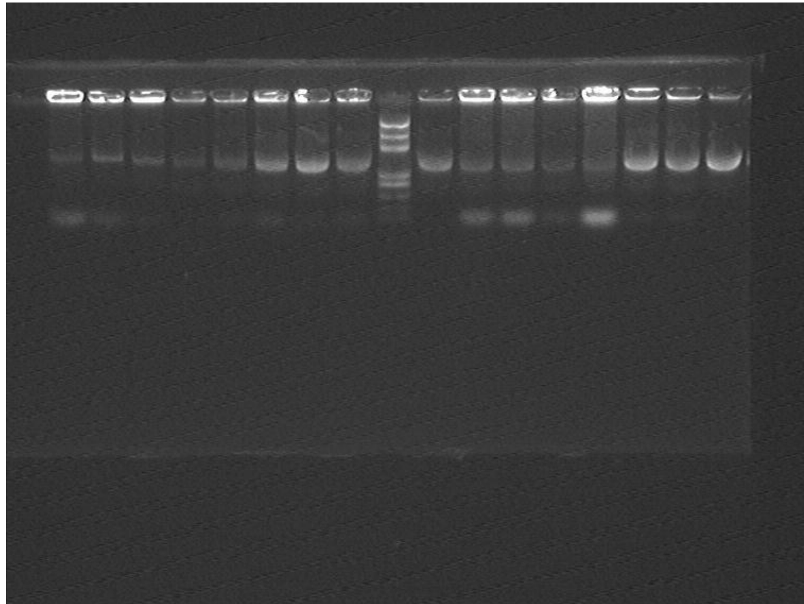

Figure 1

Double digestion with EcoRI and PstI: sal-supD plasmid, overnight

Double digestion with SpeI and PstI: sal-supD plasmid, overnight

10:00 electrophoresis: sal-supD plasmid (EP digestion) (Fig 2)

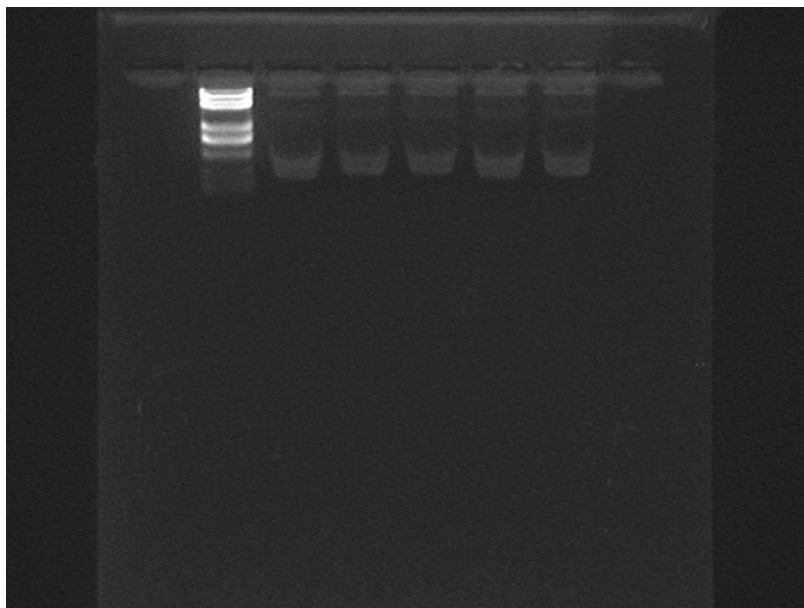

Figure 2

The band located at 1.3 kb is more clear than yesterday

16:00 double digestion: 9×RBS+lacI+T7ptag plasmid

21:30 electrophoresis, gel extract the insert (Figure 3)

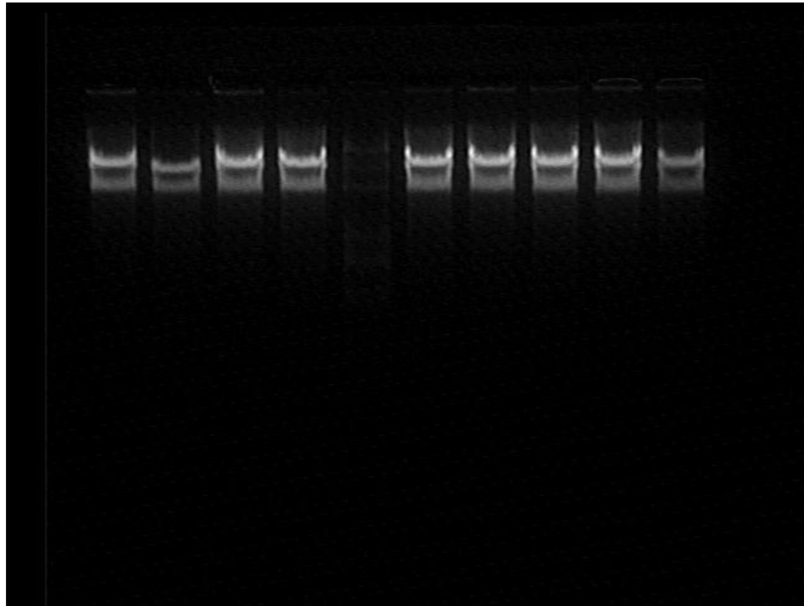

Figure 3

23:00 ligation: sal-supD + lacI-T7ptag

24:00 transformation

0911

01:35 plate

12:00 colony PCR

16:00 The PCR results are all negative

20:00 pick 5 colonies separately from 5J and 2G plate and grow in incubator

0912

13:00 MiniPrep 5J 1~5, 2G 1~5

14:00 double digestion with EcoRI and PstI to identify: 5J 1~5, 2G 1~5 plasmid (Fig 1)

0922

16:00 revive the 2G and 5J bacteria cell

20:00 induce 2G and 5J with IPTG and salicylate

22:00 use to measure the fluorescence intensity of induced cells

0923

12:00 1:50 revive the bacteria containing T7 promoter+GFP plasmid to $OD_{600nm} \approx 0.4$

18:00 prepare competent cell using the revived cell

18:30 transformation