2009/8/21

2:00 Use the flow cytometery to test the tetR system.

13:00 There are enough colonies on each plate.

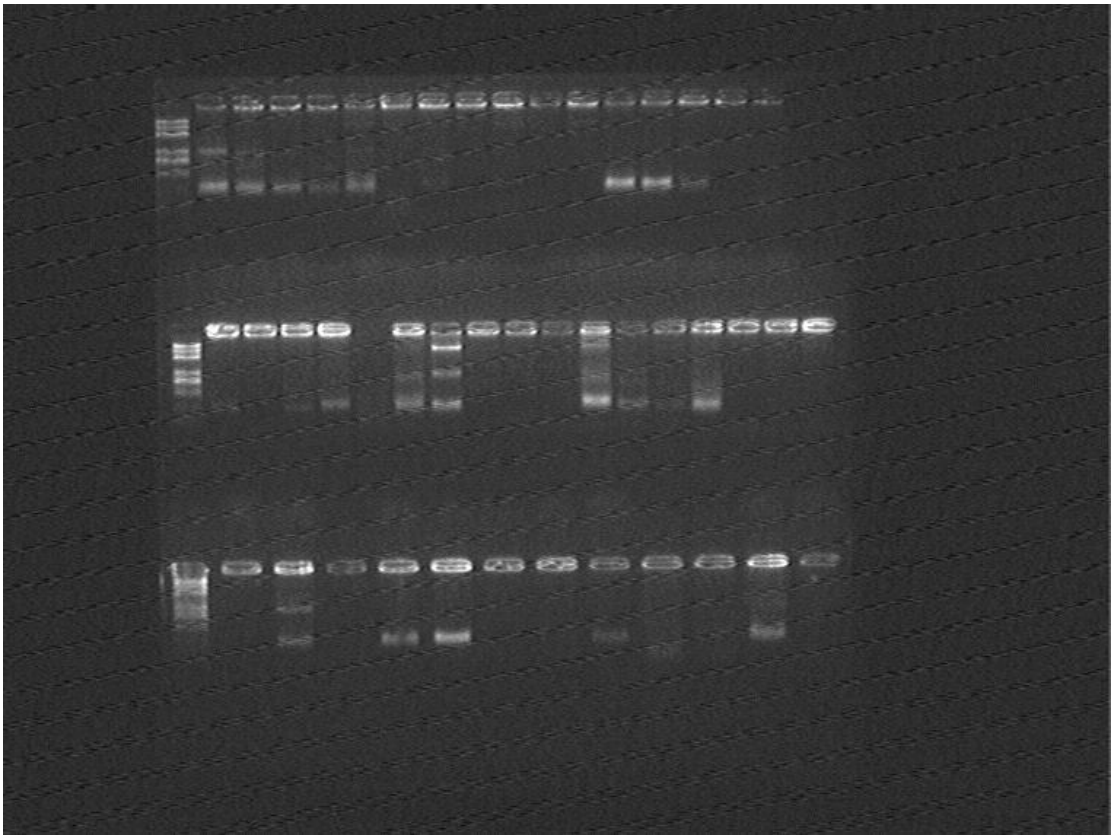
PCR the colonies to test if they are correct.

Total	10μL
Template	Colonies
For	0.5μL
Rev	0.5μL
Mix	5μL
ddH2O	3μL

20:40 Electrophoresis to test the PCR products.

The order of the samples: 1H, 2K, 1J, 2G, 5J, 2I, 2M, 5N, 1N.

Results:



5J2 and 2I1 is correct.

23:30 Pick the colonies and shake them in the incubator.

2009/8/22

12:00 Miniprep those plasmids.

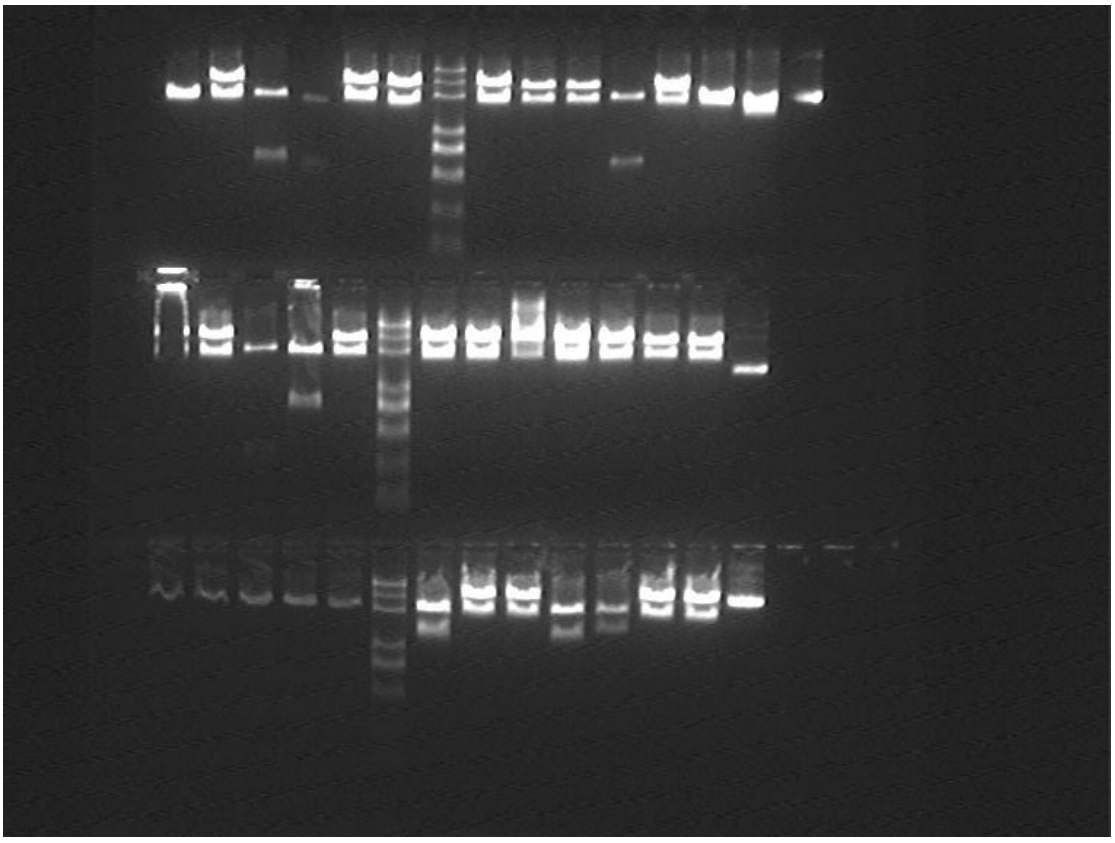
16:00 Digest those plasmids.

Total	20μL
Plasmids	4μL
EcoR1	1μL
Pst1	1μL
Buffer	2μL

ddH2O	12μL

23:00 Electrophoresis to the digestion products.

The order of the samples: plasmids control 1H1, 2K, 2G, 1H1, 1H2, plasmids control 1J4 1J, 1N, 1H3, 1H4, plasmids control 5J2, 5N, 2M, 2I1, 5J2.



All the clones have the right colonies except 5N.

2009/8/23

1:20 Cut the gel and recycle the right fragments.

13:30 Miniprep the plasmids of LacP+T7ptag 1-5N.

The concentrations of plasmids are about 350ng/μL.

14:30 Digest the plasmids into inserts.

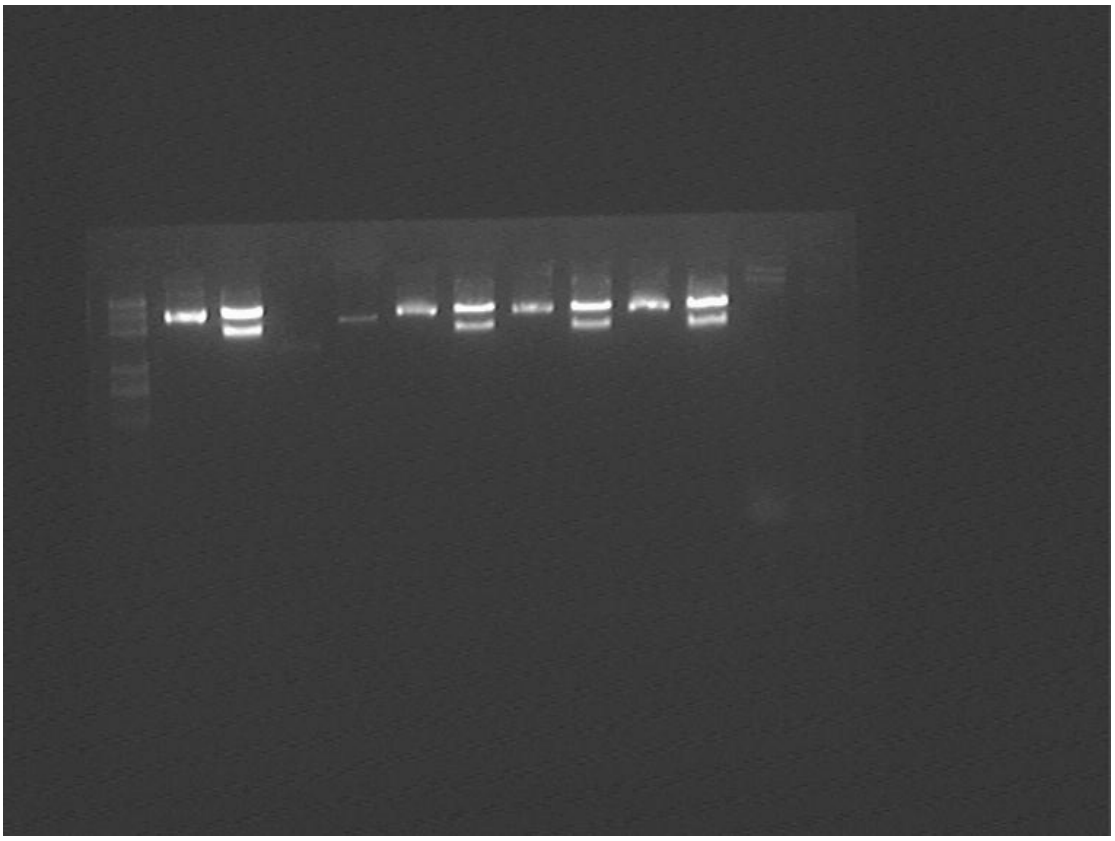
Total	**20μL**
Plasmids	3μL
Xba1	1μL
Pst1	1μL
Buffer	2μL
ddH2O	13μL

15:00 Start to digest.

19:30 Electrophoresis to test and recycle the digestion products.

The order of the samples: marker, plasmids control of 1-5N, digestion products of1-5N.

Results:



20:30 Shake the strain contains LuxR + SupD plasmids in the incubator.

21:00 Cut the gel and recycle those correct fragments.

2009/8/24

8:30 Miniprep the plasmids.

The concentration of the plasmids is about 130ng/μL.

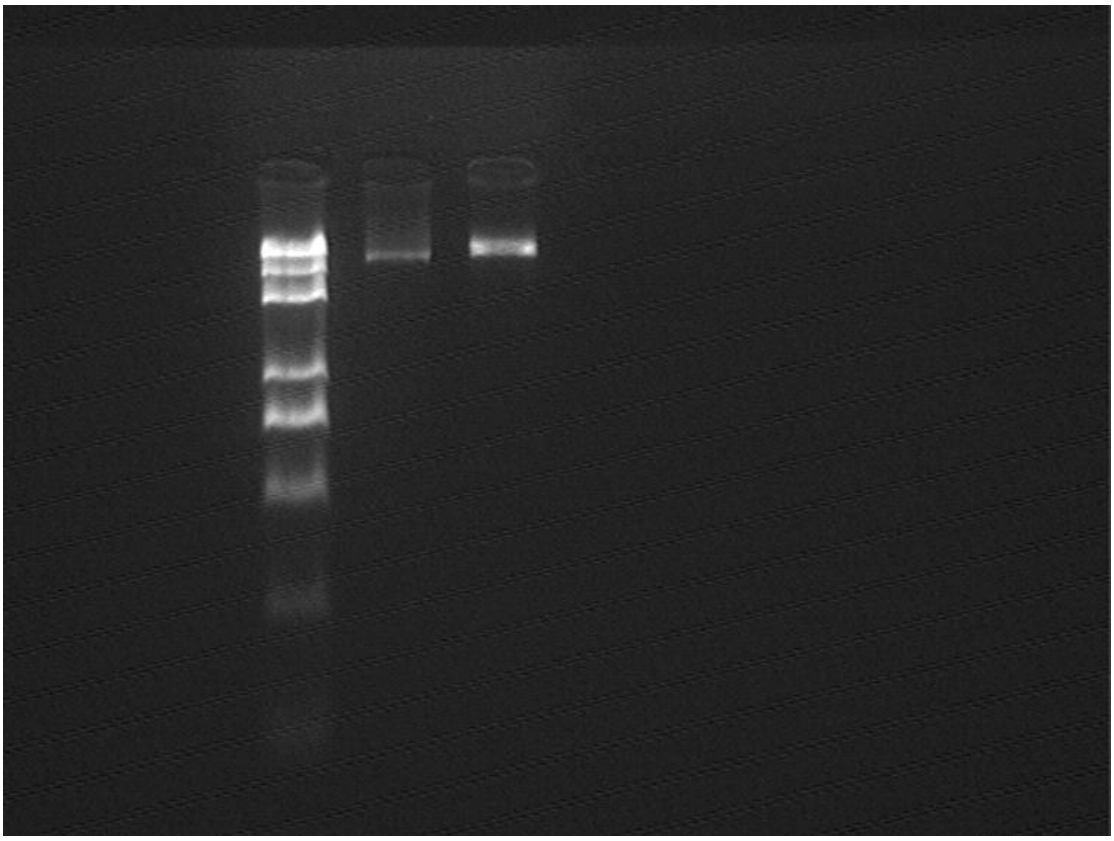
9:30 Digest the plasmids into vectors.

Total	**20μL**
Plasmids	10μL
Spe1	1μL
Pst1	1μL
Buffer	2μL
ddH2O	6μL

14:00 Electrophoresis to recycle the vectors.

The order of the samples: marker, plasmids control, digestion products.

Results:



The fragments are normal, so recycle them.

18:00 Link the inserts of LacP+T7ptag with the vectors of LuxP and SupD.

Ligation system:

Total	10μL
Vectors	1μL
Inserts	7μL
T4 ligase	1μL
Buffer	1μL

19:30 Transformation of the ligation products.

21:30 Start to incubate.

2009/8/25

10:30 There are colonies on all the plates.

Pick 4 colonies of each plate and shake them in the incubator.

22:30 Miniprep those plasmids.

The concentrations of the plasmids are about 150ng/μL.

2009/8/26

1:30 Digest the plasmids to test if they are correct.

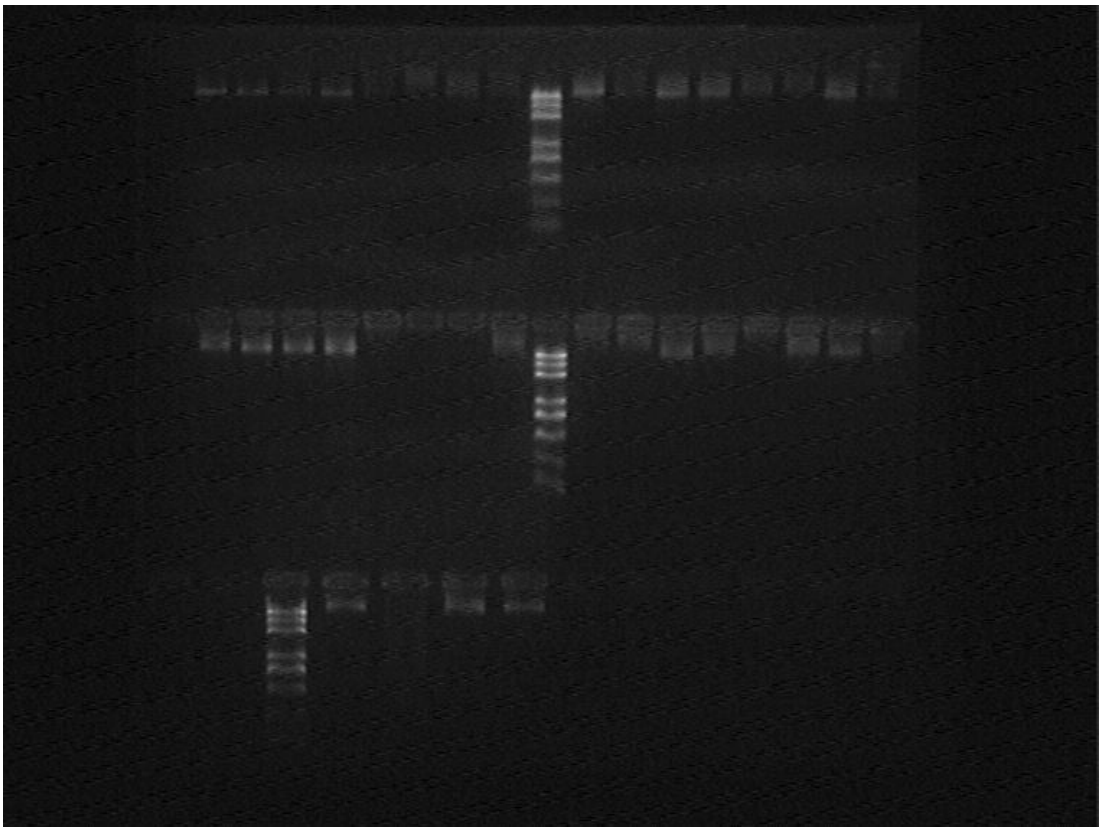
The digestion system:

Total	10μL
Plasmids	3μL
EcoR1	0.5μL
Pst1	0.5μL
Buffer	1μL
ddH2O	4.5μL

12:00 Electrophoresis to test the digestion products.

The order of the samples is alphabetical.

Results:



The results are not quite clear, may be because of the amount of the samples are not that enough.

23:00 Shake those strains again.

2009/8/27

12:30 Miniprep those plasmids.

18:00 Digest the plasmids to test if they are correct.

Total	**10μL**
Plasmids	8μL
EcoR1	0.5μL
Pst1	0.5μL
Buffer	1μL

23:00 Electrophoresis to test the fragments.

The order of the samples is also alphabetical.

The results are correct according to the electrophoresis.

2009/8/28

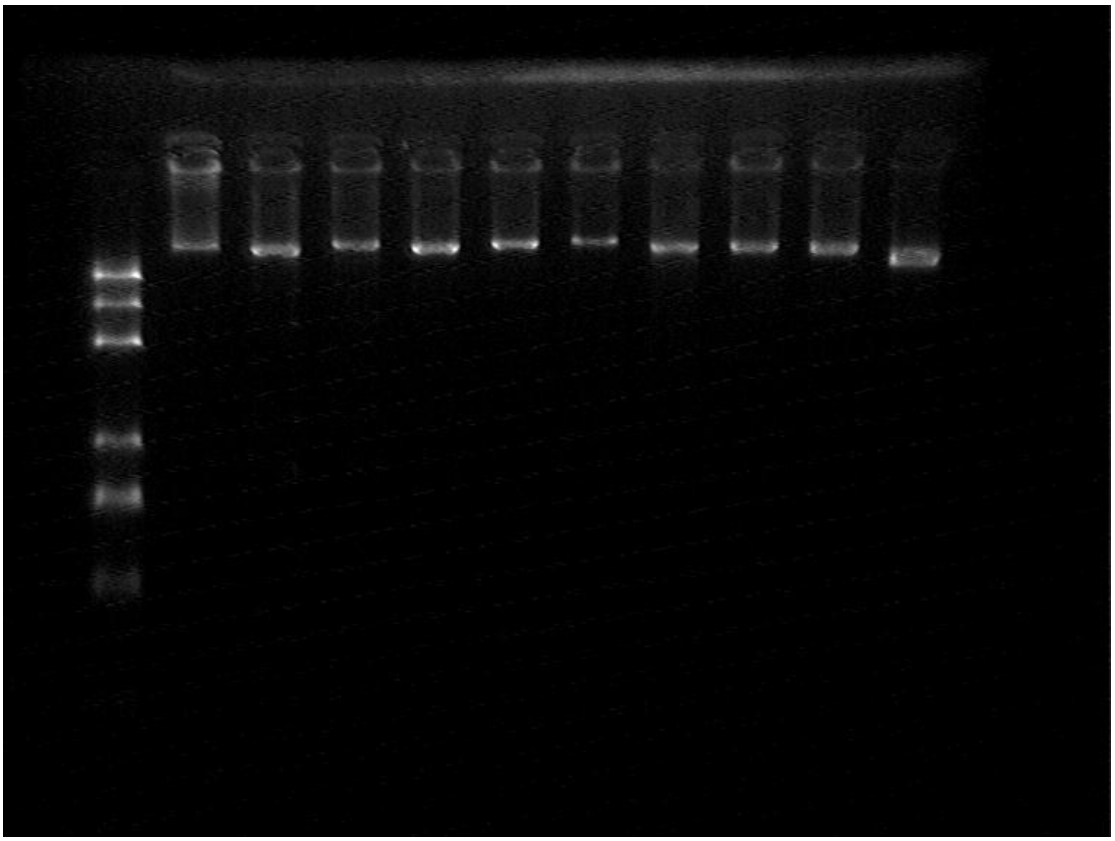
Digest AND GATE plasmids into vectors.

Total	**20μL**
Plasmids	10μL
Spe1	1μL
Pst1	1μL

Buffer	2μL
ddH2O	5μL

13:00 Electrophoresis to test and recycle the correct fragments.

Results:



The fragments are larger than expected.

2009/8/28

23:30 PCR those plasmids to test if they are correct.

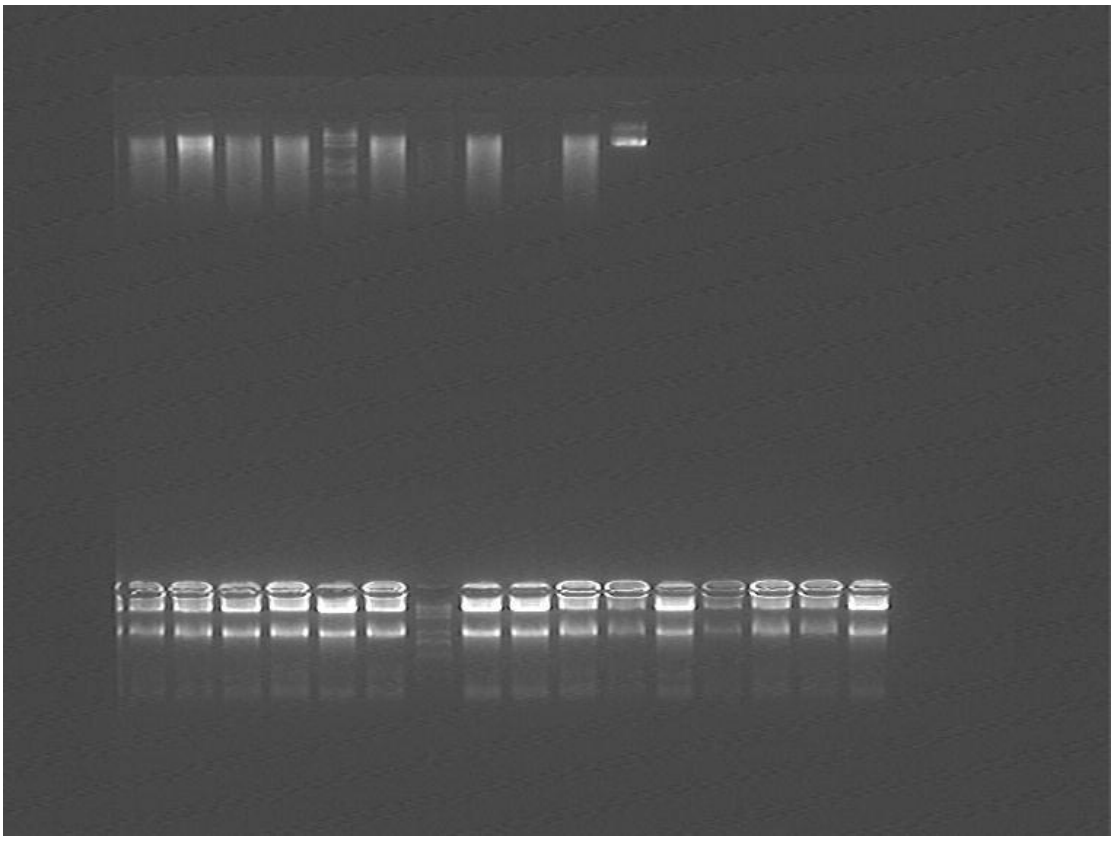
PCR system:

Total	**10μL**
Template	1μL
For	0.5μL
Rev	0.5μL
Mix	5μL
ddH2O	3μL

2009/8/29

12:00 Electrophoresis to test the PCR products.

Results:



All the plasmids are correct according to the PCR results.

2009/8/30

Start to test the function of the AND GATE.

The 2K one is better than the others.

2009/9/6

Start to construct the AND GATE: AraC+SupD+LacP+T7ptag.

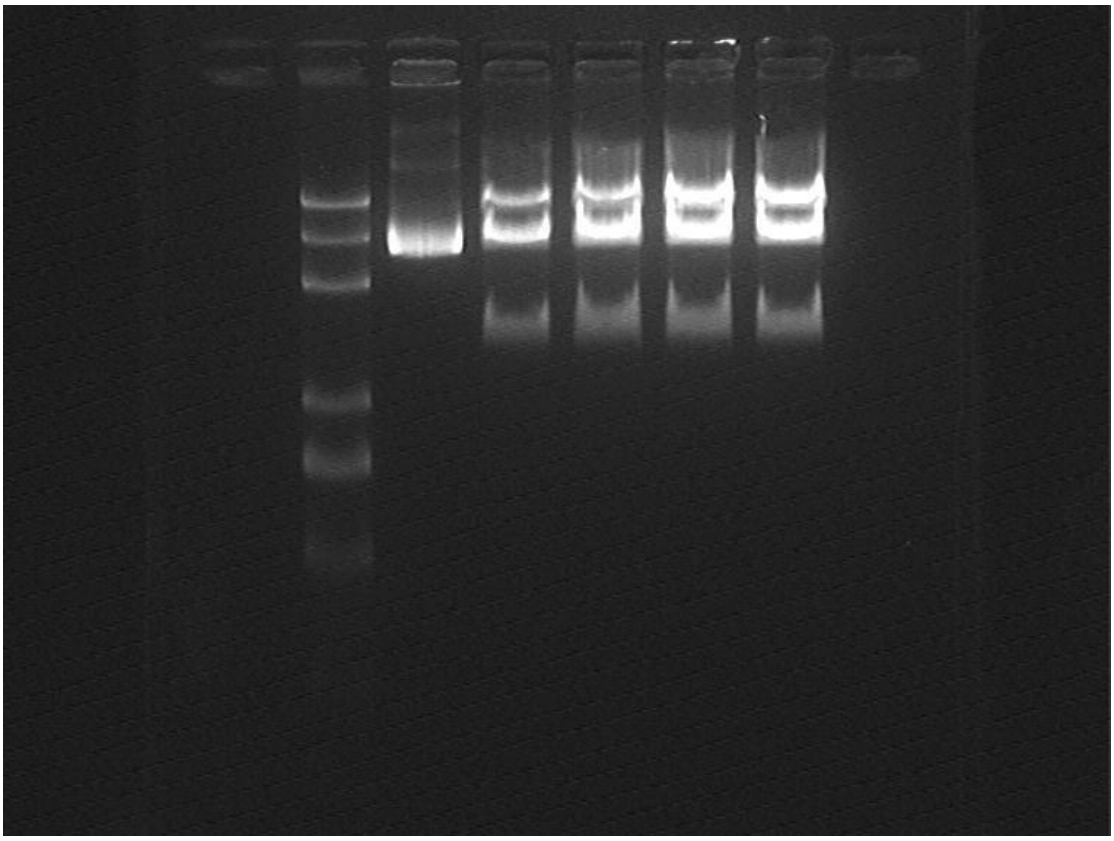
2009/9/8

14:00 Digest the plasmids AraC+SupD.

Total	**50μL**
Plasmids	10μL
EcoR1	1.5μL
Spe1	1.5μL
Buffer	5μL
ddH2O	32μL

20:00 Electrophoresis to recycle the correct fragments.

Results:



Recycle the fragments at about 1kb.

2009/9/9

21:00 Link the three fragments together.

Ligation system:

Total	10μL
Vectors	1μL
AraC+SupD	3μL
LacP+T7ptag	3μL
T4 ligase	1μL
Buffer	2μL

2009/9/10

Transformation

1:30 Start to incubate.

14:00 There are colonies on the plates of 1-5J, 1-2M, 1-2I, 1-2K.

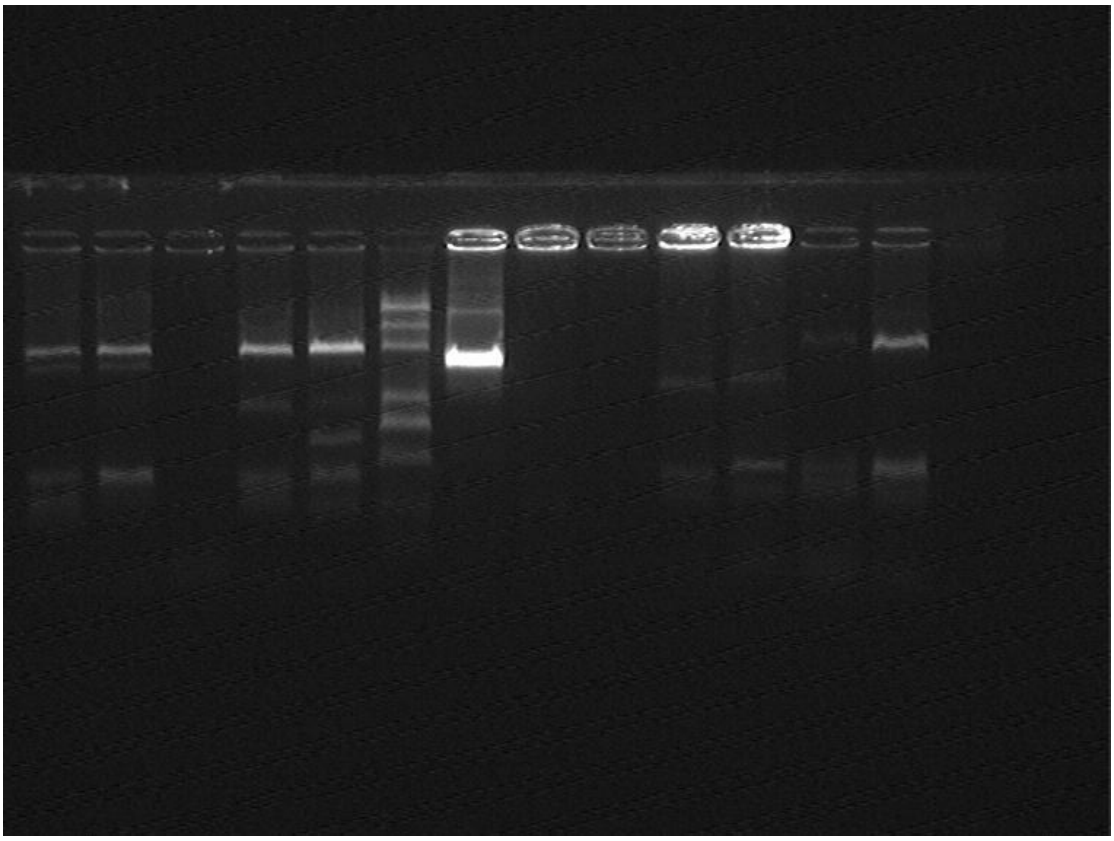
PCR the colonies to test if they are correct.

Total	10μL
Template	Colonies
For	0.5μL
Rev	0.5μL
Mix	5μL
ddH2O	4μL

2009/9/11

00:00 Electrophoresis to test the PCR products.

Results:



The fragments are not correct.

1:30 Pick those colonies and shake them in the incubator.

14:00 Miniprep those plasmids.

The concentrations of these plasmids are about 100ng/μL.

16:00 Digest the plasmids to test if they are correct.

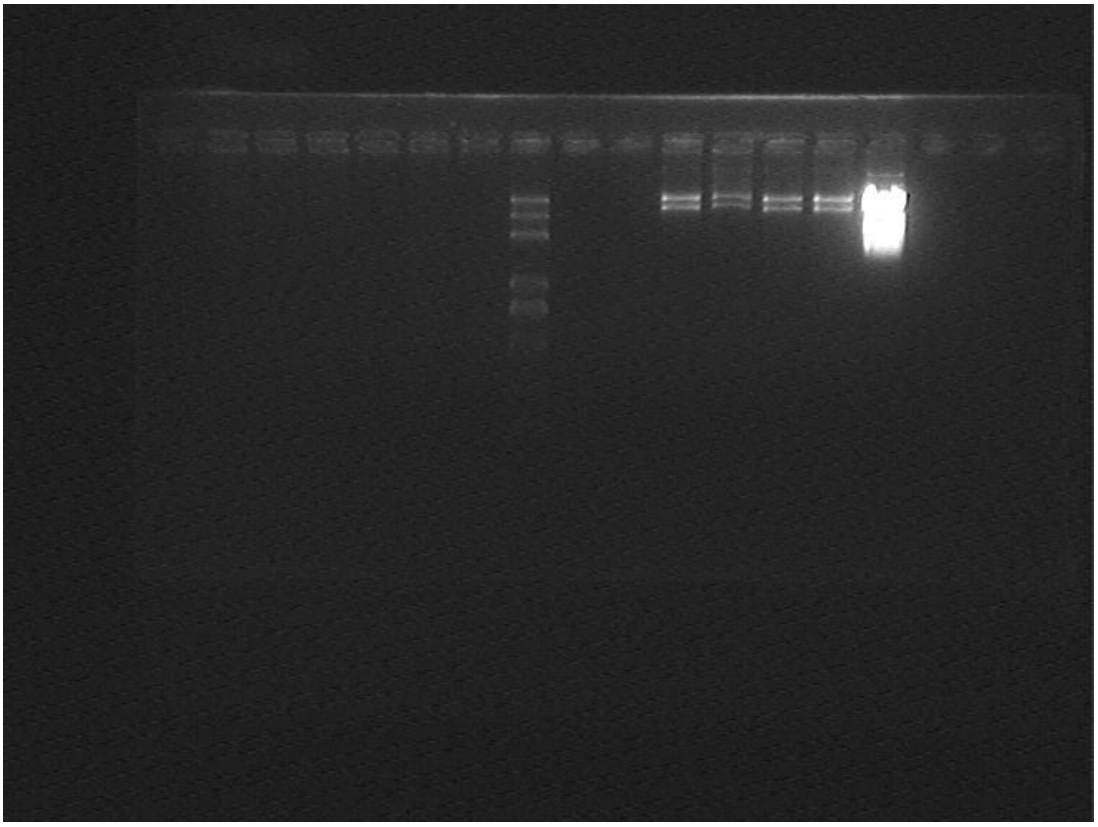
The digestion system:

Total	**10μL**
Plasmids	8μL
EcoR1	0.5μL
Pst1	0.5μL
Buffer	1μL

22:00 Electrophoresis to test the digestion fragments.

The order of the samples: 5J1-5J5, 2m, marker, plasmids control, 2I1, 2I2, 2I3, 2K1, 2K2, 2K3.

Results:



2I2, 2I3, 2K1, 2K2 are correct.

2009/9/12

11:00 Digest LacP+t7ptag plasmids, except 2I and 2K.

The digestion system:

Total	20μL
Plasmids	10μL
Xba1	1μL
Pst1	1μL
Buffer	2μL
ddH2O	5μL

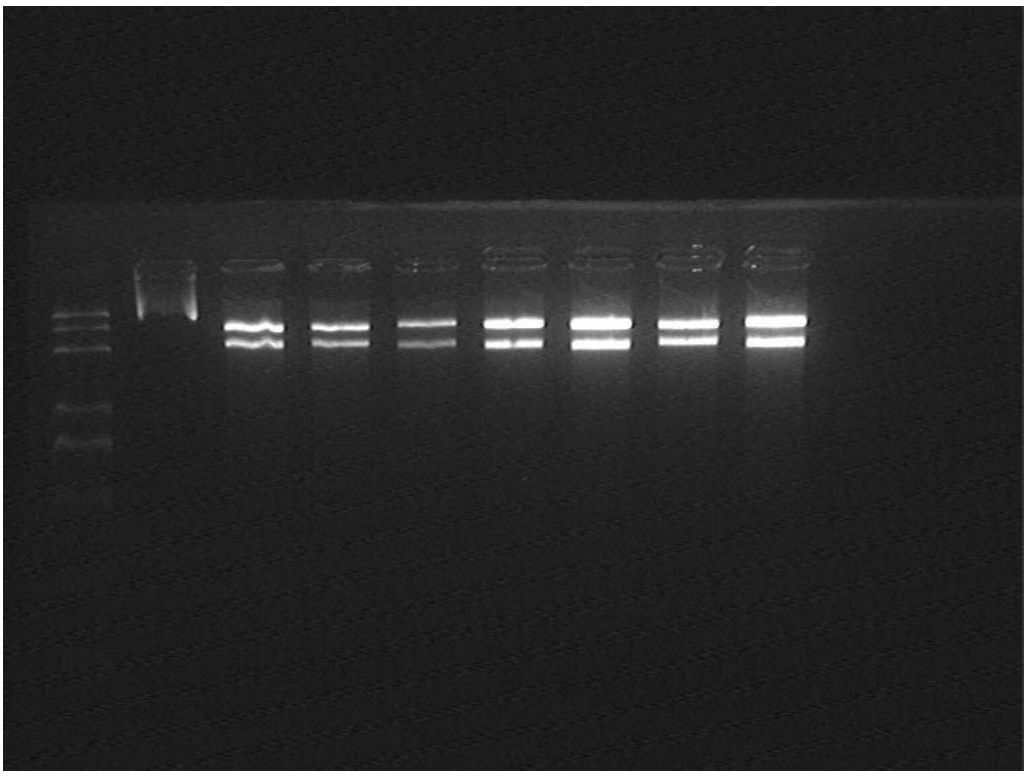
9:00 Shake T7 promoter+GFP JM109 stain in the incubator.

13:00 Prepare the competent cells.

15:30 Electrophoresis to test and recycle of the digestion products.

The order of samples: marker, plasmids control, 2G, 1J, 5N, 11N, 5J, 1H, 2M.

Results:



All the fragments are normal as expected, recycle the fragments of 3kb.

20:00 Shake the strain containing 1-7G plasmids.

2009/9/13

9:30 Miniprep the plasmids.

The concentrations of the plasmids are about 100ng/μL.

11:00 Digest the plasmids into vectors.

Digestion system:

Total	**20μL**
Plasmids	16μL
EcoR1	1μL
Pst1	1μL
Buffer	2μL

11:20 Start to digest.

22:00 Electrophoresis to test and recycle the digestion products.

Results:

The fragments are correct and recycle the fragments of about 3kb.

2009/9/14

00:30 Link the three fragments together.

Ligation system:

Total	**10μL**
Vectors	1μL
AraC+SupD	3μL
LacP+T7ptag	3μL
T4 ligase	1μL
Buffer	2μL

8:30 Transformation

10:10 Start to incubate.

23:30 Pick 3 colonies of each plate and shake them in the incubator.

2009/9/15

13:00 Miniprep those plasmids.

14:30 Digest the plasmids to test if they are correct.

The digestion system:

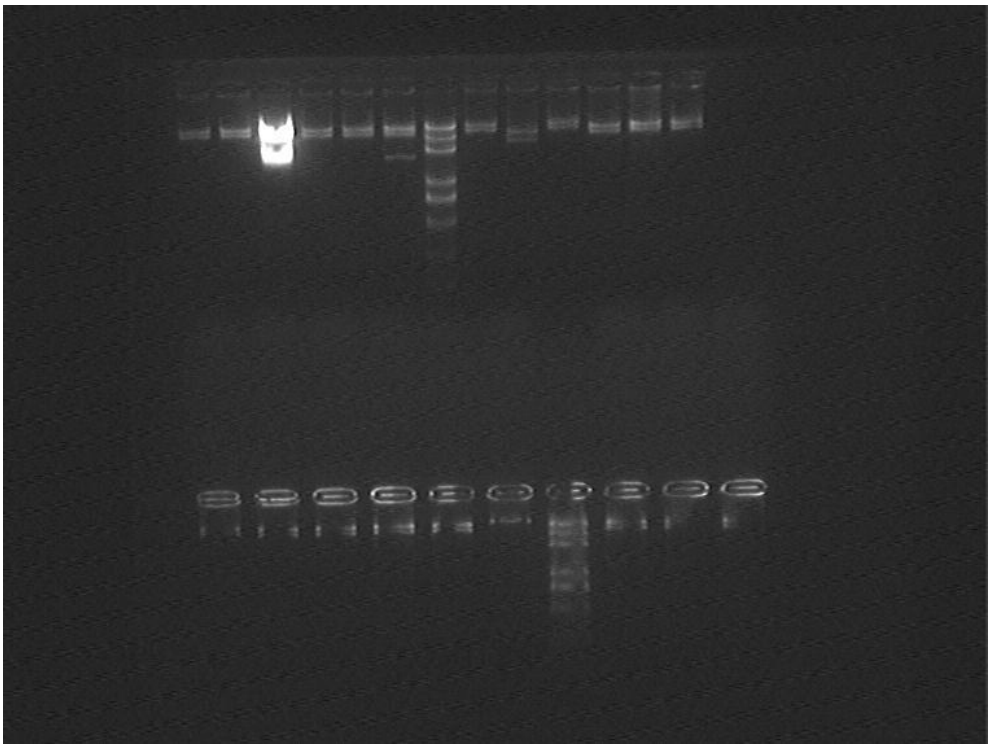
Total	10μL
Plasmids	8μL
EcoR1	0.5μL
Pst1	0.5μL
Buffer	1μL

15:30 Start to digest.

22:00 Electrophoresis to test the digestion products.

The order of the samples: 1H, 2G, marker, 5J, 1J; 5N, 11N, marker, 2M.

Results:



All the clones have correct colonies.

2009/9/17

Start to test the functions of the AND GATEs.