2009/7/3

Bi-stable plasmids from Chunbo Lou:

	I7100	PZSA1	PZSA2
Copy	High	Low	Low
Initial state	Green(C1)	Green(C1)	Red(C1434)
Stable state	Green(C1)	Green(C1)	Green(C1)
Resistant	Kan	Amp	Amp

20:00 Pick the single cloning and incubate.

2009/7/4

Prepare the samples of the bi-stable cells:

9:30 Prepare the first sample, the residual was diluted for a fold.

Pre pare one sample with the same method each hour.

17:30 Check those samples using the flow cytometer. There aren't strong red and green controls, so that the results are not convincing.

19:30 Check the parts:

Number	Size of backbone	Size of the insert	Location in the plate	Description	Plasmids
BBa_C0040			1-4A	Tet repressor + LVA	
BBa_C0012			1-2O	lacI + LVA	
BBa_J09250			2-9B	Constitutive GFP	
BBa_C0080			1-14L	araC + LVA	
BBa_K093012			3-13M	Constitutive GFP	
BBa-J3033			2-4O	B0034 + LuxR	
BBa_R0010			1-1D	LacI regulated promoter	
BBa_B0025			1-2E	Double terminators	
BBa_I14033			1-18P		

22:30 Transformation for the parts above.

1:45 The incubation was started.

2:00 Pick the pZSA GREEN and RED for incubation, 3-13M as a red control, 2-9B as a green control, 1-1D as a negative control.

2009/7/5

14:00 There isn't any colony on the plates, since using the Amp+ LB to incubate those cells.

Transform 1-18P, 2-9B, 3-13M, 1-2O, 1-4A again.

Check parts of rbs.

Choose the 6 strong ones for incubation.

23:40 Start to incubate.

2009/7/6

10:00 Those bacteria failed to grow, pick those colonies again and reincubate them.

11:30 Transformation: bi-stable high-copy plasmids.

20:30 Miniprep the plasmids below.

Concentration of those plasmids:

Number of the plasmids	Concentration(ng/μL)
1-1H	350
1-2L	212.5
1-11N	169.9
1-5J	471.1
1-5N	217.2
1-2M	154.1

2009/7/7

00:00 Pick the bi-stable colonies of high-copy and low-copy and incubate them.

8:00 Prepare the samples of the bi-stable cells, once an hour.

17:00 Controls are still not very good to get a good data.

18:00 Begin to make competent cells.

Incubate JM109 on a plate without resistance.

18:30 Transformation of 1-18A and 1-18C.

Information of 1-18A and 1-18C

2009/7/8

9:00 Pick JM109 into LB without any resistance for incubation (37 ° C)

10:00 Pick 1-18A, 1-18C into LB with Amp for incubation.

15:00 Prepare SOB.

17:00 Pick bi-stable cells of high-copy and low-copy to incubate.

22:10 Miniprep the plasmids of 1-18A and 1-18C

Number of the plasmids	Concentration(ng/μL)
1-18A	270
1-18C	167

23:10 Double digestion of 1-18A.

Total	20μL
Plasmids	4μL
Spe1	1μL
Pst1	1μL
Buffer	2μL
ddH2O	12μL

2009/7/12

Prepare competent cells.

Construction

8:00 The order of the samples: marker, plasmid control of 1-18A, digestion product of 1-18A

The digestion products run faster than the plasmids.

9:30 Shake 1-18A and 1-18C again.

15:30 Electrophoresis to test the digestion products

The order and the amount of the samples: marker 5μL, plasmids of high-copy bi-stable system 3μL, digestion products of hi-copy bi-stable system 20μL, plasmids of low-copy bi-stable system 5μL, digestion products of low-copy bi-stable system 20μL.

Results: Bands are smeared.

16:20 Digest high-copy bi-stable system and low-copy bi-stable system again.

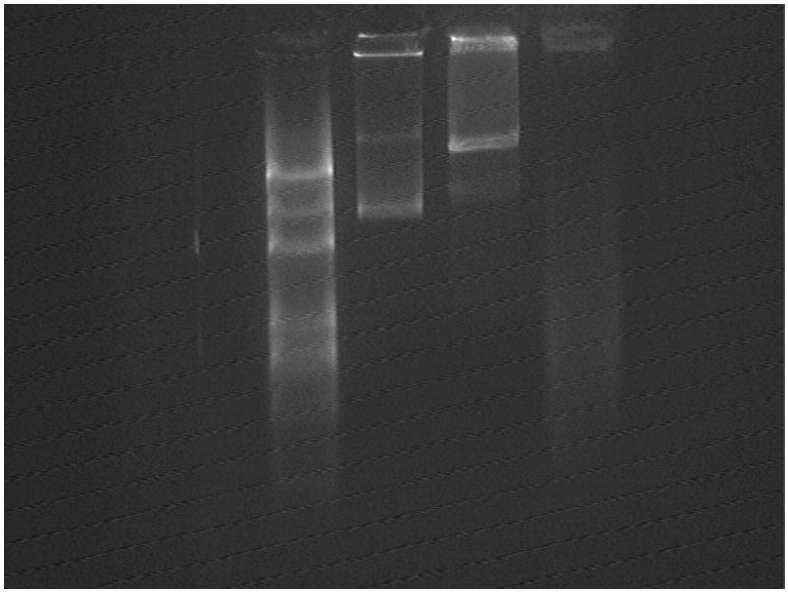
Total	**20μL**
Plasmids	4μL
EcoR1	1μL
Pst1	1μL
Buffer	2μL
ddH2O	12μL

22:00

Electrophoresis to test the digestion products

The order and the amount of the samples: marker 5μL, plasmids of high-copy bi-stable system 3μL, digestion products of hi-copy bi-stable system 20μL digestion products of low-copy bi-stable system 20μL.

Results:



High- copy plasmids have been successfully digested, while the low-copy plasmids are still digested to be smeared.

22:30 Miniprep of 1-18A and 1-18C

Number of the plasmids	**Concentration(ng/μL)**
1-18A	250
1-18C	350

23:00 Digest 1-18A.

Total	**20μL**

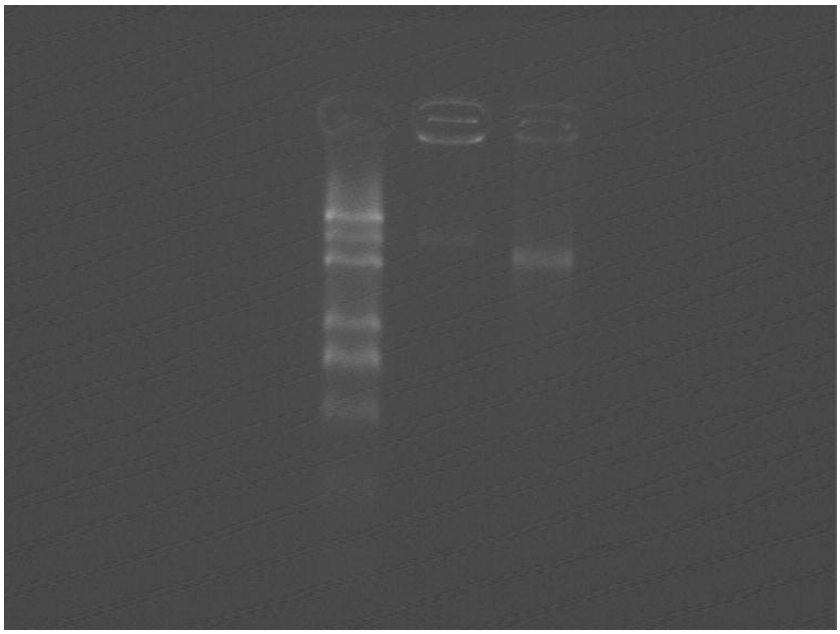
Plasmids	4μL
Spe1	1μL
Pst1	1μL
Buffer	2μL
ddH2O	12μL

2009/7/13

Electrophoresis to test the digestion products of 1-18A

The order and the amount of the samples: marker 5μL, plasmids of 1-18A 2μL, digestion products of 1-18A 20μL.

Results:



The digestion products still run slower than the uncut plasmids, but 1-18A is tested to be gel-bad according to the registry, so that the products are probably correct.

10:30

Continue to prepare competent cells.

13:50 Ligation of the 1-18A vector and 1-12O (GFP) inserts.

Total	**10μL**
Vectors	2μL
Inserts	6μL
T4 ligase	1μL
Buffer	1μL

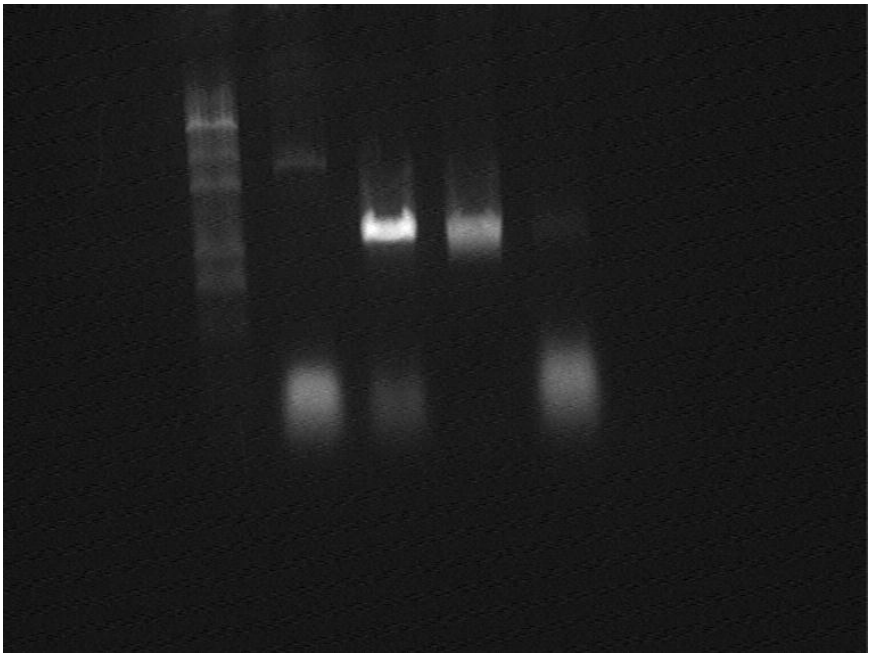
22:30 Transformation of the ligation products and 1-1D, 1-6I to test the efficiency of the competent cells.

2009/7/14

12:30 PCR to test GFP

15:30 Electrophoresis to test the PCR products.

The order and the amount of the samples: marker 5μL, clone 1 10μL, clone 2 10μL, clone 3 10μL, clone 4 10μL, clone 5 10μL.



The band appeared at about 1kb seems to be the correct products.

16:00 PCR bi-stable to insert into the chromosome.

Total	20μL
Template	1μL
dNTP	4μL
Buffer	2μL
Easypfu	1μL
For-primer	1μL
Rev-primer	1μL
ddH2O	10μL

20:30 Electrophoresis to test the PCR products.

The order and the amount of the samples: marker 5μL, bi—stable PCR products, plasmid control of low-copy bi-stable system, digestion products of low-copy bi-stable system.

Results:

PCR and digestion are both unsuccessful.

2009/7/15

21:30 PCR low-copy bi-stable system again (gradient).

Total	20μL
Template	0.5μL
dNTP	4μL
Buffer	2μL
Easypfu	1μL
For-primer	1μL
Rev-primer	1μL

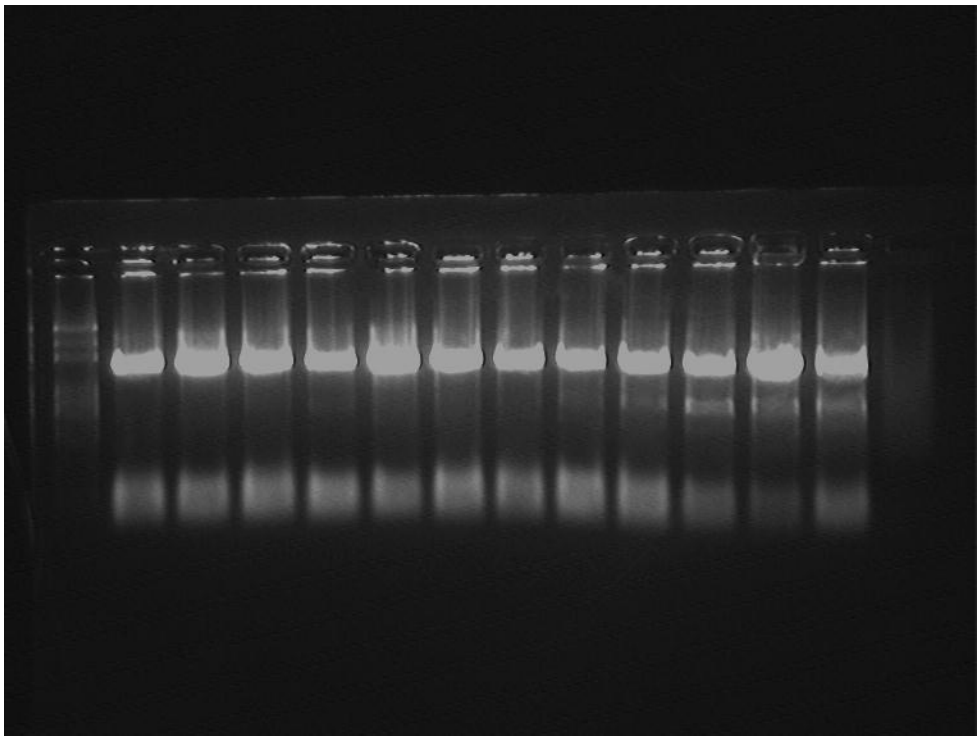
ddH2O	10.5μL

2009/7/16

11:30 Electrophoresis to test the PCR products.

The order and the amount of the samples: marker 5μL, bi—stable PCR products (from 50 to 61 centigrade)

Results:



All the products are correct according to the gel picture.

12:30 Recycle the correct bands.

13:10 PCR high-copy bi-stable system plasmids.

The system is the same with the high-copy plasmids.

16:30 Electrophoresis to test the PCR products.

The order and the amount of the samples: marker 5μL, bi—stable PCR products, control

Results:

PCR is unsuccessful.

2009/07/18

Prepare the high-copy bi-stable system samples for flow cytometer.

9:42 The first sample to be prepared.

2009/07/19

10:30 Miniprep 2-4O, 1-18P, low-copy bi-stable system

Concentration:

Number of the plasmids	Concentration(ng/μL)
2-4O	350

1-18P	250
Low-copy bi-stable system	125

12:00 Digest 1-18P, 2-4O, low-copy plasmids.

1-18P by Spe1 and Pst1;

2-4O by Xba1 and Pst1;

low-copy bi-stable system by EcoR1 and Pst1.

18:00 Recycle those digestion products.

22:40 Ligation of high-copy bi-stable system fragments and high-copy backbone.

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

2009/7/20

11:00 Transform 1-9H and 2-11P and the ligation products of high-copy bi-stable system.

2009/7/21

00:40 Digest SupD and terminator plasmids.

System of SupD:

Total	**20μL**
Plasmids	6μL
EcoR1	1μL
Spe1	1μL
Buffer	2μL
ddH2O	10μL

System of terminator 1-23L:

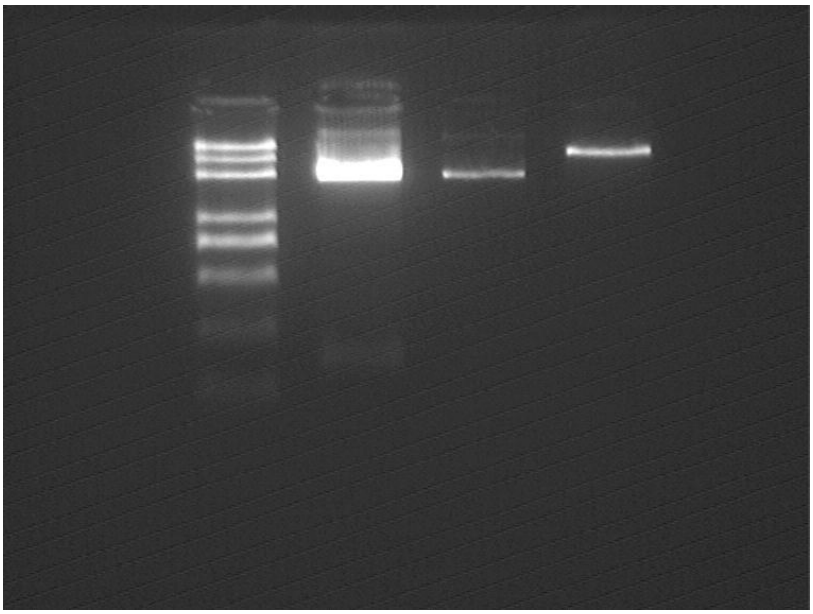
Total	**20μL**
Plasmids	6μL
EcoR1	1μL
Xba1	1μL
Buffer	2μL
ddH2O	10μL

1:00 Start to digest.

10:00 Electrophoresis to test the digestion products.

The order and the amount of the samples: marker 5μL, SupD digestion, 1-23L plasmids, 1-23L digestion.

Results:



The digestions are successful.

11:00 PCR the clones of high-copy bi-stable system to test if they are correct.

11:10 Recycle the fragments of SupD.

13:10 Ligation of the vectors of 1-23L and inserts of SupD.

System of ligation: 10μL

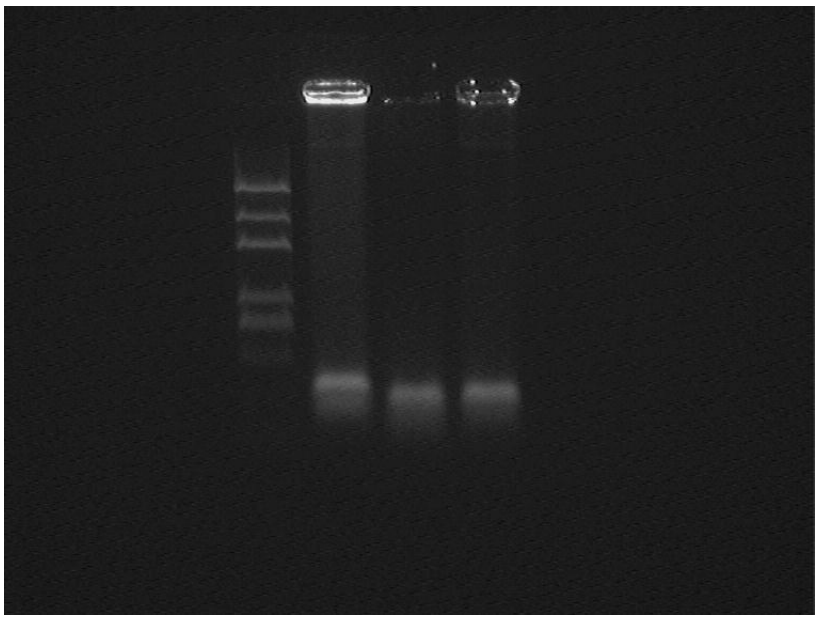
	Control system	1:7 system
Vectors	1μL	1μL
Inserts	0μL	7μL
Buffer	1μL	1μL
Ligase	1μL	1μL
ddH2O	7μL	0μL

13:10 Ligation starts.

13:30 Electrophoresis to test the PCR products.

The order and the amount of the samples: marker 5μL, bi-stable 1, bi-stable 2, bi-stable 3.

Results:



All of the three clones are incorrect according to the results of PCR.

14:21 Miniprep bi-stable 1,2,3,SupD1 and 1-9H.

Concentrations:

Number of the plasmids	Concentration(ng/μL)
Bi-stable 1	160
Bi-stable 2	200
Bi-stable 3	250
SupD1	250
1-9H	110

16:50 Digest the bi-stable system to test whether the ligations are correct

Total	10μL
Plasmids	1μL
EcoR1	0.5μL
Pst1	0.5μL
Buffer	1μL
ddH2O	7μL

17:15 Start to digest.

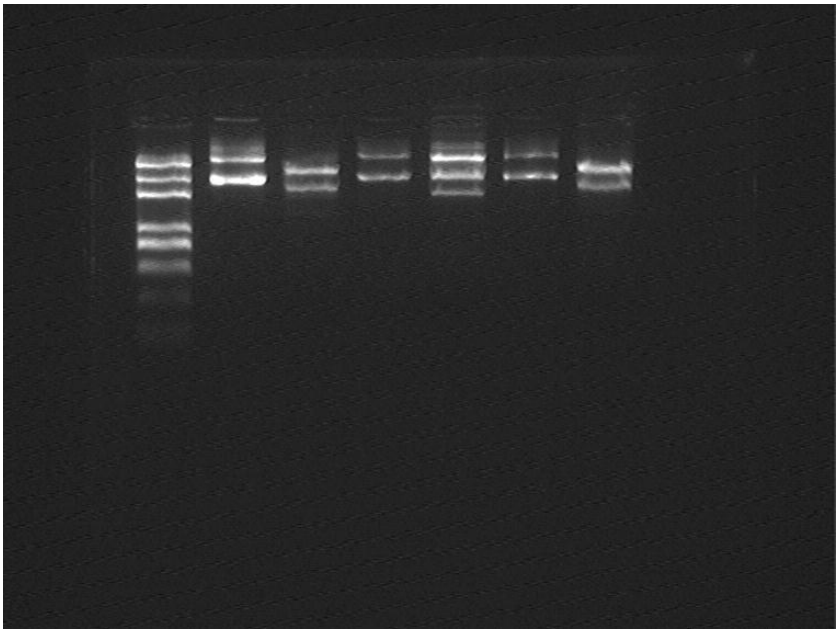
17:20 Transform the ligation products of SupD+1-23L backbone.

20:00

Electrophoresis to test the digestion products.

The order and the amount of the samples: marker 5μL, bi-stable plasmids1, bi-stable 1 digestion products, bi-stable 2 plasmids, bi-stable 2 digestion products, bi-stable plasmids 3, bi-stable 3 digestion products.

Results:



2009/7/22

10:00 There are 3 clones on the SupD + terminator plate.

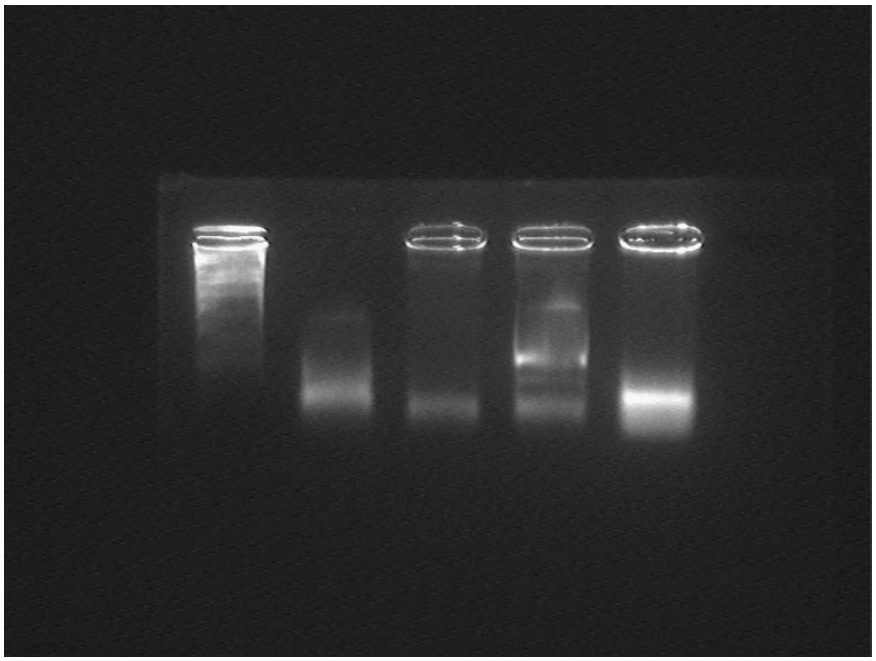
PCR to test whether the clones are correct.

Total	10μL
Template	Clones
dNTP	2μL
Buffer	1μL
Taq	0.5μL
For-primer	0.5μL
Rev-primer	0.5μL
ddH2O	5.5μL

14:00 Electrophoresis to test the PCR products.

The order and the amount of the samples: marker 5μL, negative control, colony 1, colony 2, colony 3.

Results:



It is difficult to say which colony is correct.

22:30 Miniprep SupD + terminator colonies 1,2,3.

Number of the plasmids	Concentration(ng/μL)
SupD1+1-23L	140
SupD2+1-23L	110
SupD3+1-23L	165

2009/7/23

0:30 Digest SupD+1-23L 1, 2, 3 to test whether the clones are correct.

Total	20μL
Plasmids	8μL
Xba1	1μL
Pst1	1μL
Buffer	2μL
ddH2O	8μL

PCR to test at the same time.

Total	10μL
Template	Clones
dNTP	2μL
Buffer	1μL
Taq	0.5μL
For-primer	0.5μL
Rev-primer	0.5μL
ddH2O	5.5μL

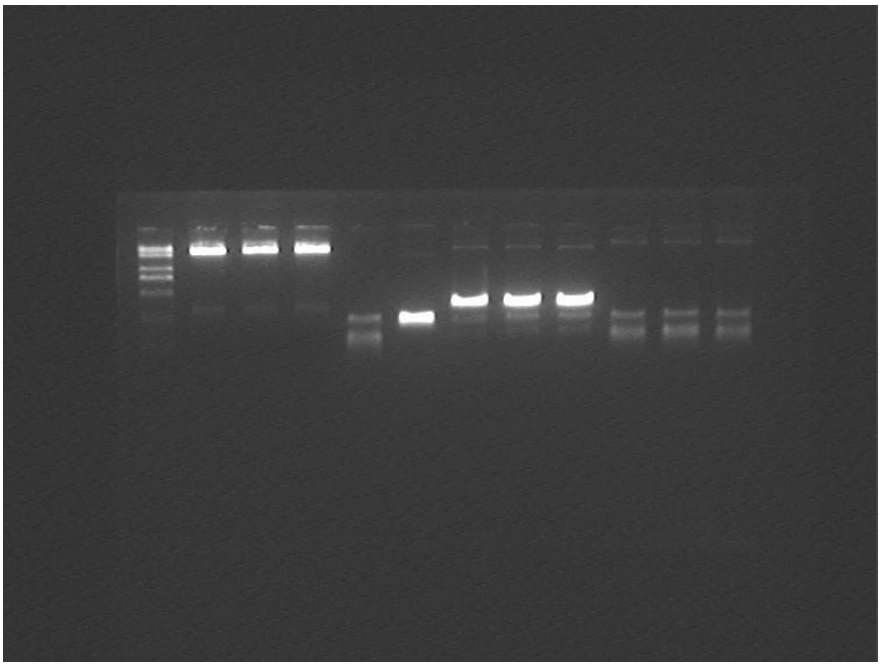
1:20 Start to digest.

1:45 Start to PCR.

10:40 Electrophoresis to test the PCR products.

The order and the amount of the samples: marker 5μL, colony 1 digestion products, colony 2 digestion products, colony 3 digestion products, positive control, PCR products of colony 1, PCR products of colony 2, PCR products of colony 3.

Results:



This result indicates that all the three colonies are correct.