2009/7/23

11:30 Recycle all the inserts of SupD and terminator.

12:20 Digest high-copy bi-stable plasmids.

| Total | 20μL |
|---|---|
| Plasmids | 8μL |
| EcoR1 | 1μL |
| Pst1 | 1μL |
| Buffer | 2μL |
| ddH2O | 8μL |

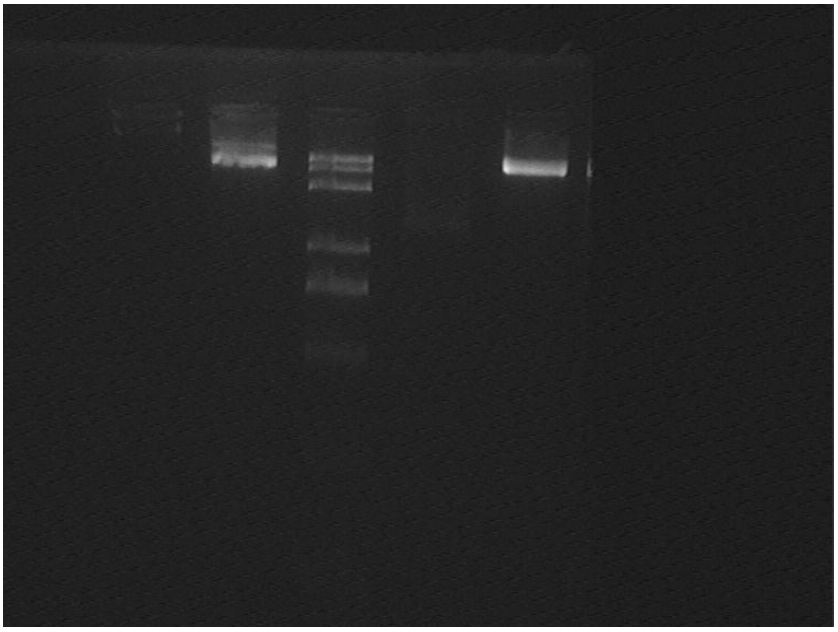
12:30 Start to digest.

12:40 Ligation of SupD+terminator and 1-18A (strongest promoter).

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

16:50 Electrophoresis to recycle the high-copy bi-stable digestion products.

The order and the amount of the samples: control plasmids 2μL, digestion products, 20μL marker 5μL.



2009/7/24

10:30 There are six clones on the plate. Shake those clones in the incubator.

PCR clones to test if they are correct.

| Total | 10μL |
|---|---|
| Template | Clones |

| | |
|---|---|
| **dNTP** | 2μL |
| **Buffer** | 1μL |
| **Taq** | 0.5μL |
| **For-primer** | 0.5μL |
| **Rev-primer** | 0.5μL |
| **ddH2O** | 5.5μL |

The results are not very good.

14:00 Transform the plasmids PKD46 from France in two tubes.

22:40 Miniprep 1-18A + SupD + terminator.

| Number of the plasmids | Concentration(ng/μL) |
|---|---|
| **Colony 1** | 223 |
| **Colony 2** | 355.05 |
| **Colony 3** | 148.15 |
| **Colony 4** | 124.33 |
| **Colony 5** | 113.34 |
| **Colony 6** | 146.85 |

2009/7/28

00:20 Digest 1-18A + SupD +terminator plasmids to test if they are correct. Digest them into vectors as well.

Digest into inserts:

| **Total** | **20μL** |
|---|---|
| **Plasmids** | 2μL |
| **EcoR1** | 1μL |
| **Pst1** | 1μL |
| **Buffer** | 2μL |
| **ddH2O** | 14μL |

Digest into vectors:

| **Total** | **20μL** |
|---|---|
| **Plasmids** | 3μL |
| **EcoR1** | 1μL |
| **Pst1** | 1μL |
| **Buffer** | 2μL |
| **ddH2O** | 13μL |

PCR at the same time:

| **Total** | **10μL** |
|---|---|
| **Template** | 0.5μL |
| **For** | 0.5μL |
| **Rev** | 0.5μL |

| | |
|---|---|
| **Mix** | 5μL |
| **ddH2O** | 3.5μL |

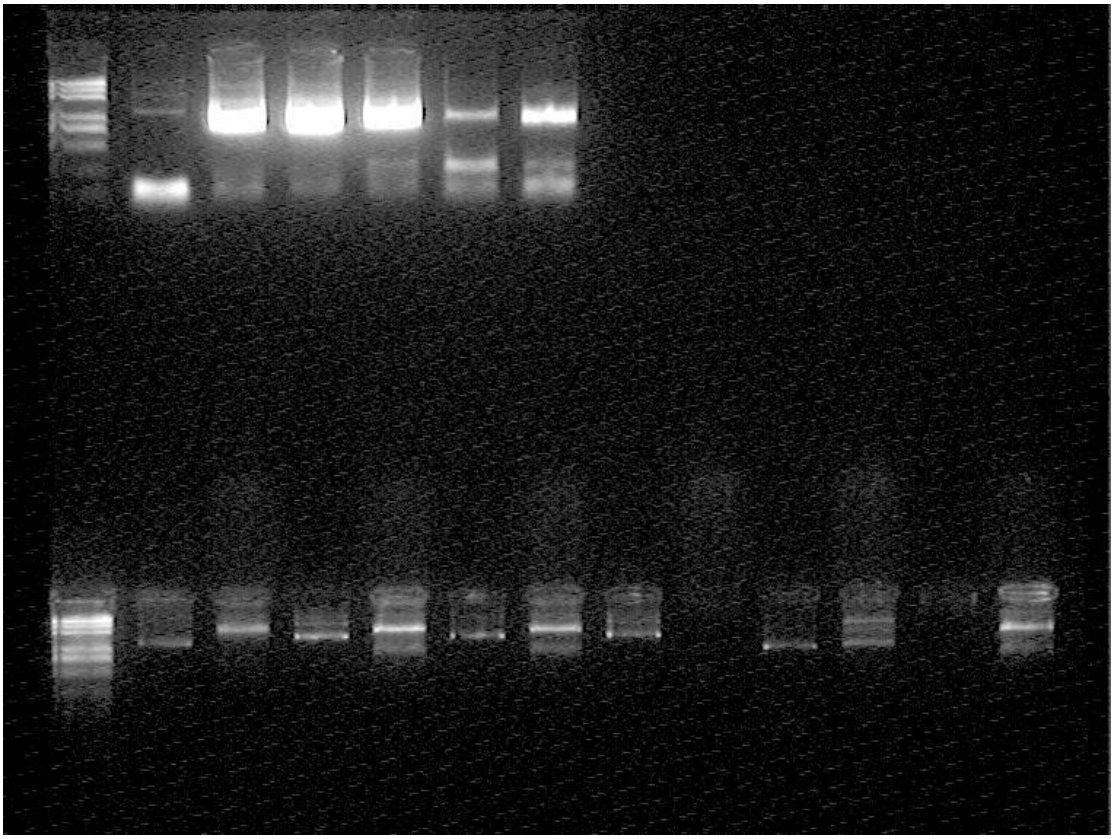
1:00 Start to digest.

1:30 Start to PCR.

12:00 Electrophoresis to test if the PCR products and digestion products are correct.

The order and the of the samples: marker, PCR products 1, PCR 2, PCR 3, PCR4, PCR5, PCR6, digestion control plasmids, digestion1, digestion2, digestion3, digestion4, digestion5, digestion 6.

Results:



16:00 Transfrom pKD3, pcp 20 to DH5a.

Digest 1-18C into vectors.

| **Total** | **20μL** |
|---|---|
| **Plasmids** | 3μL |
| **Spe1** | 1μL |
| **Pst1** | 1μL |
| **Buffer** | 2μL |
| **ddH2O** | 13μL |

20:00 Electrophoresis the digestion products:

The order and the amount of the samples: marker 10μL, plasmids control 2μL, digestion 1 15μL, digestion2 15μL.

Results:

The digestion products are correct.

2009/7/29

10:00 Recycle the digestion products.

Miniprep the plasmids of 1-18C and PYFP.

13:30 Digest plasmids PYFP from France.

| Total | 20μL |
|---|---|
| Plasmids | 4μL |
| EcoR1 | 1μL |
| Kpn1 | 1μL |
| Buffer | 2μL |
| ddH2O | 12μL |

13:48 Start to digest.

15:00 Transform psp20 into DH5a.

Ligation of 1-18C backbone and SupD+ terminator inserts.

15:30 PCR to standardize the PYFP plasmids.

Take a gradient from 54 centigrade to 58 centigrade.

| Total | 50μL |
|---|---|
| Template | 0.2μL |
| dNTP | 4μL |
| Buffer | 10μL |
| Phusion | 0.5μL |
| For-primer | 1.25μL |
| Rev-primer | 1.25μL |
| ddH2O | 32.8μL |

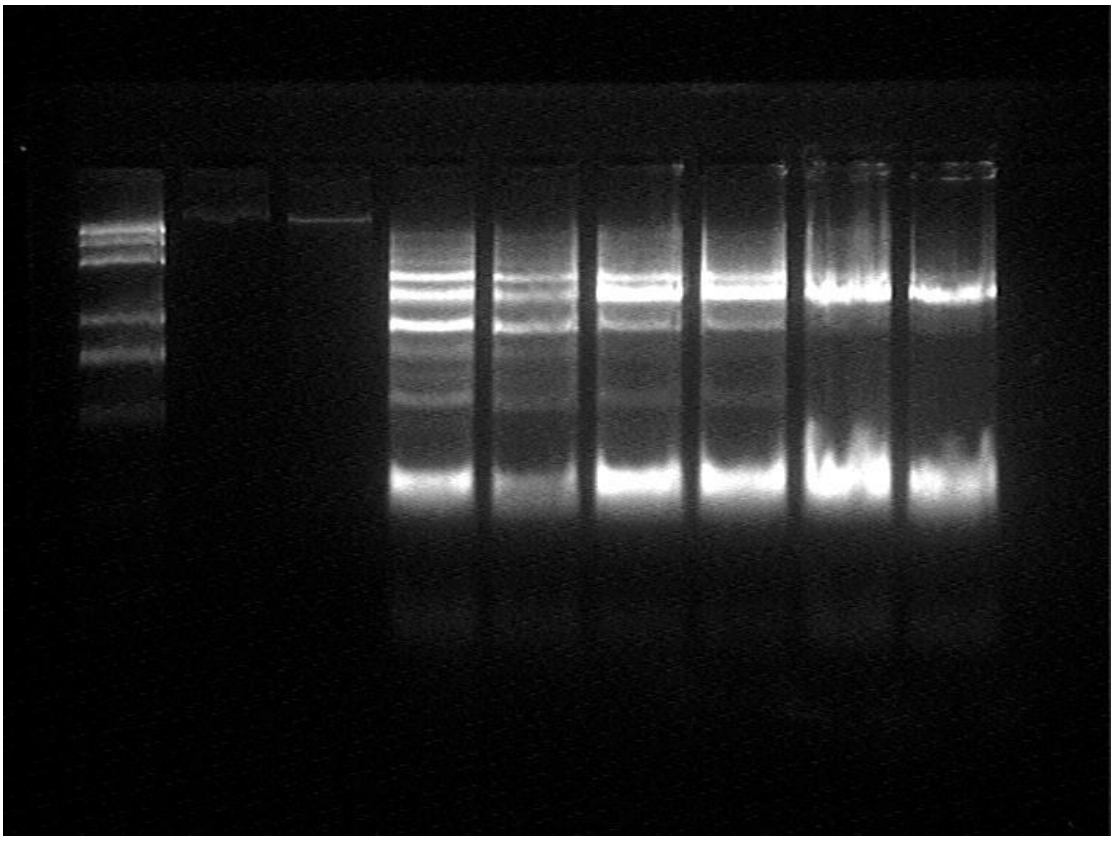
17:30 Digest the high-copy bi-stable PCR products.

| Total | 20μL |
|---|---|
| Fragments | 5μL |
| EcoR1 | 1μL |
| Kpn1 | 1μL |
| Buffer | 2μL |
| ddH2O | 10μL |

19:30 Electrophoresis the digestion products and PCR products.

The order of the samples: marker, plasmids control, digestion products, PCR products under 54 centigrade, PCR products under 56 centigrade, PCR products under 58 centigrade.

Results:



Digestion products are weird, while the PCR products under 58 centigrade are specialized. As a result, recycle the PCR products under 58 centigrade.

21:55 Digest PYFP plasmids.

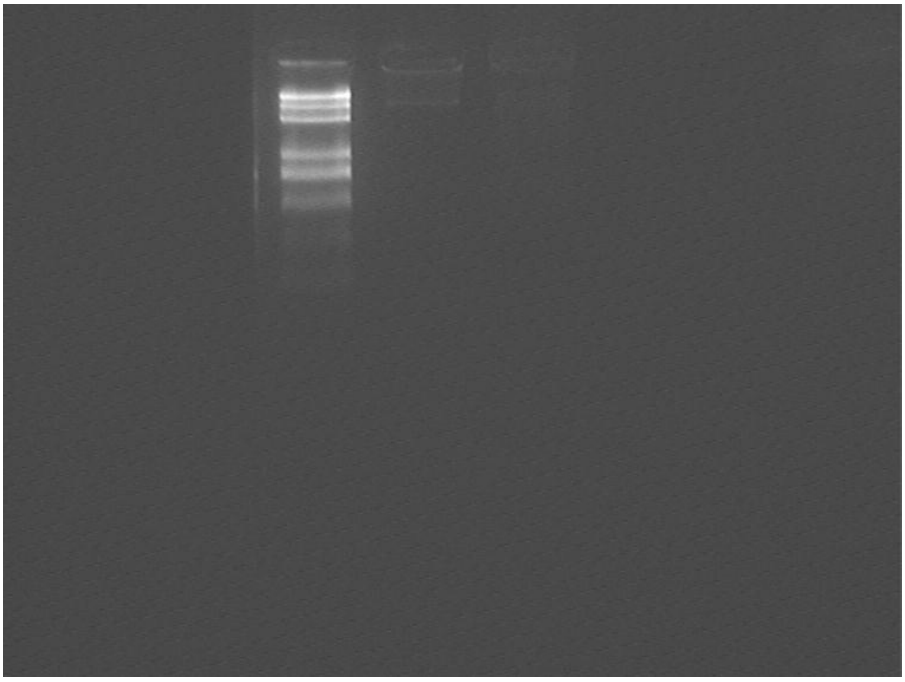
| Total | 20μL |
|---|---|
| Plasmids | 5μL |
| EcoR1 | 1μL |
| Kpn1 | 1μL |
| Buffer | 2μL |
| ddH2O | 10μL |

2009/7/30

9:00 Electrophoresis to test the products of the digestion.

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

Results:



It is difficult to tell the digestion fragments, maybe because of the poor concentration of the plasmids.

20:00 Digest the PYFP plasmids into vectors.

Digest to standardize:

| **Total** | **20μL** |
|---|---|
| **Plasmids** | 7μL |
| **Spe1** | 1μL |
| **Pst1** | 1μL |
| **Buffer** | 2μL |
| **ddH2O** | 9μL |

Digest to connect with high-copy bi-stable fragments.

| **Total** | **20μL** |
|---|---|
| **Plasmids** | 10μL |
| **EcoR1** | 1μL |
| **Kpn1** | 1μL |
| **Buffer** | 2μL |
| **ddH2O** | 6μL |

Digest the standardizing PCR fragments into inserts:

| **Total** | **20μL** |
|---|---|
| **Plasmids** | 17μL |
| **Xba1** | 1μL |
| **Buffer** | 2μL |

Digest T7P + 1-12M to get the high-copy backbone.

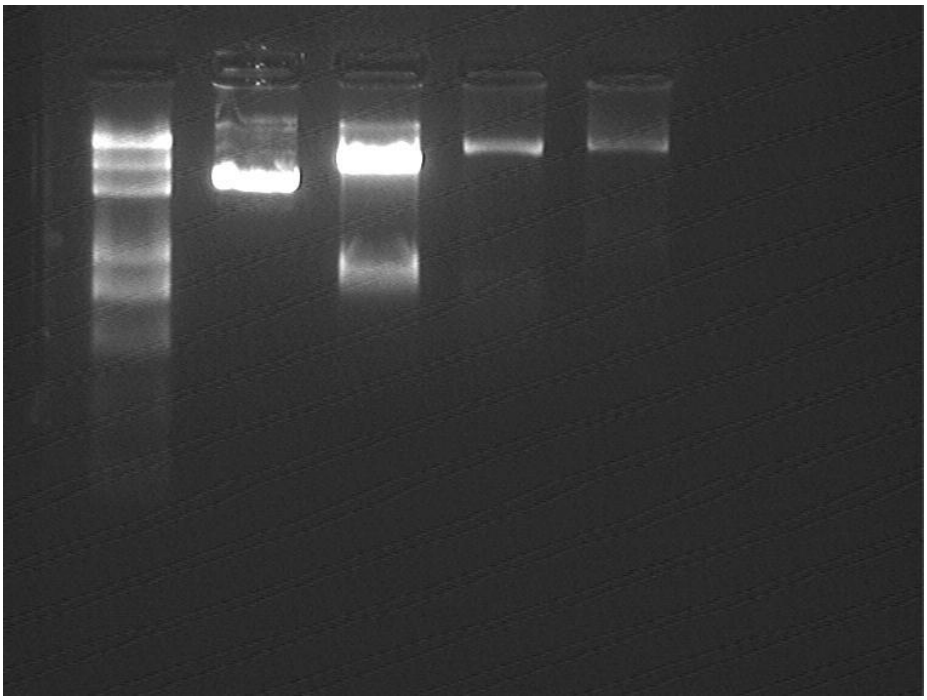
| Total | 20μL |
|---|---|
| Plasmids | 4μL |
| Xba1 | 1μL |
| Spe1 | 1μL |
| Buffer | 2μL |
| ddH2O | 12μL |

2009/7/31

10:30 Electrophoresis the digestion products.

The order of the samples: marker, digestion products for backbone, single digestion products of the standardized fragments, double digestion products of PYFP cut by Kpn1 and EcoR1, double digestion products of PYFP cut by Spe1 and Pst1.

Results:



Recycle the fragments.

12:00 Digest the single digestion products of PYFP by another enzyme.

| Total | 30μL |
|---|---|
| Fragments | 25.5μL |
| Apal1 | 1.5μL |
| Buffer | 3μL |

12:40 Start to digest.

14:00 Miniprep.

| Number of the plasmids | Concentration(ng/μL) |
|---|---|
| PKD46 | 53.68 |
| High-copy bi-stable | 102.61 |
| PKD3 | 139.91 |
| Psp20 | 72.955 |

| | |
|---|---|
| **PYFP** | 100.805 |

16:00 Digest high-copy bi-stable plasmids.

| **Total** | **20μL** |
|---|---|
| **Plasmids** | 15μL |
| **Xba1** | 1μL |
| **Nhe1** | 1μL |
| **Spe1** | 1μL |
| **Buffer** | 2μL |

16:30 Start to digest.

16:30 Start to recycle the fragments digested by ApaL1

17:30 Link the standard fragments with PYFP vectors, also high-copy bi-stable fragments with PYFP vectors.

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

22:00 Electrophoresis to test the digestion products.

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

Results:

Digestion is successful. Recycle the fragments of about 3kb.

22:30

Transform the ligation products into DH5a.

2009/8/1

00:30 Start to incubate.

13:30 There is no colony on the plates.

21:00 Digest the high-copy bi-stable plasmids and low-copy bi-table plasmids.

High-copy bi-stable plasmids:

| **Total** | **20μL** |
|---|---|
| **Plasmids** | 2μL |
| **EcoR1** | 1μL |
| **Spe1** | 1μL |
| **Buffer** | 2μL |
| **ddH2O** | 13μL |

Low-copy bi-stable plasmids:

| **Total** | **20μL** |
|---|---|
| **Plasmids** | 7μL |
| **EcoR1** | 1μL |
| **Nhe1** | 1μL |
| **Spe1** | 1μL |

| | |
|---|---|
| **Buffer** | 2μL |
| **ddH2O** | 8μL |

PYFP:

| | |
|---|---|
| **Total** | **20μL** |
| **Plasmids** | 10μL |
| **EcoR1** | 1μL |
| **Kpn1** | 1μL |
| **Buffer** | 2μL |
| **ddH2O** | 6μL |

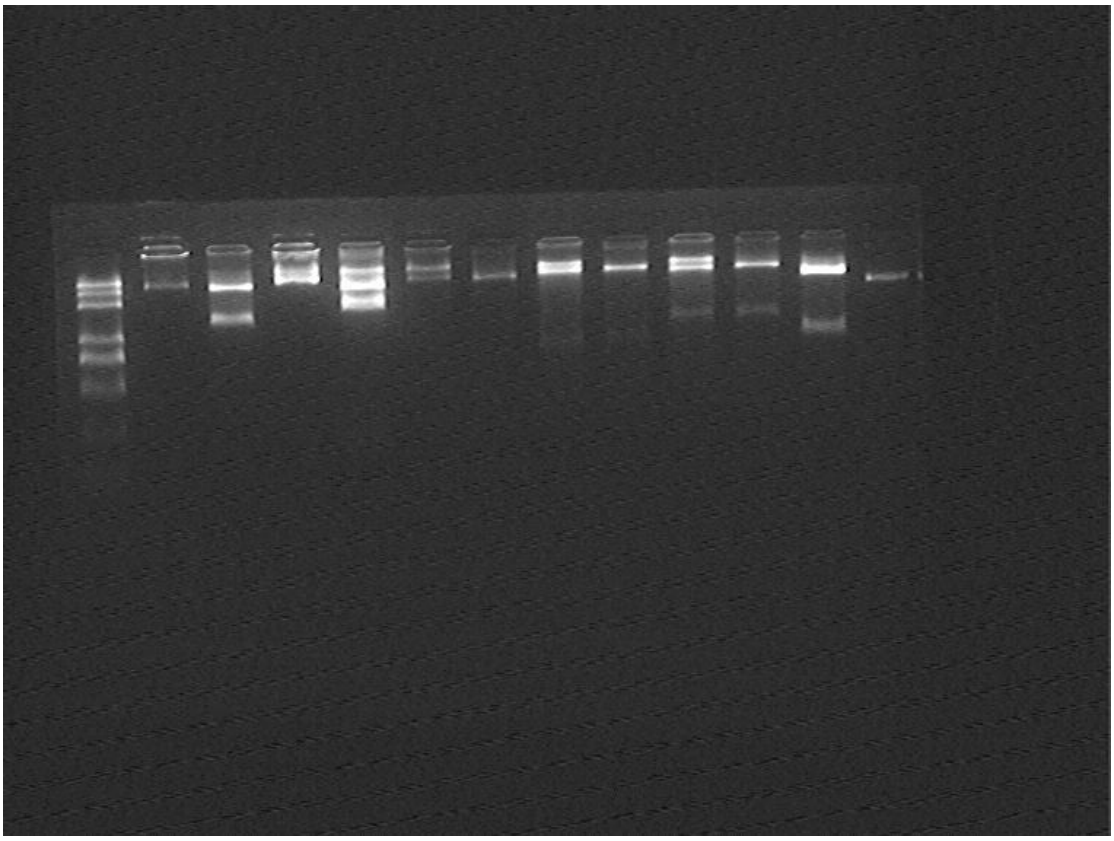
| | |
|---|---|
| **Total** | **20μL** |
| **Plasmids** | 10μL |
| **Pst1** | 1μL |
| **Spe1** | 1μL |
| **Buffer** | 2μL |
| **ddH2O** | 6μL |

Digest T7P + 1-12M for backbone:

| | |
|---|---|
| **Total** | **20μL** |
| **Plasmids** | 4μL |
| **EcoR1** | 1μL |
| **Spe1** | 1μL |
| **Buffer** | 2μL |
| **ddH2O** | 12μL |

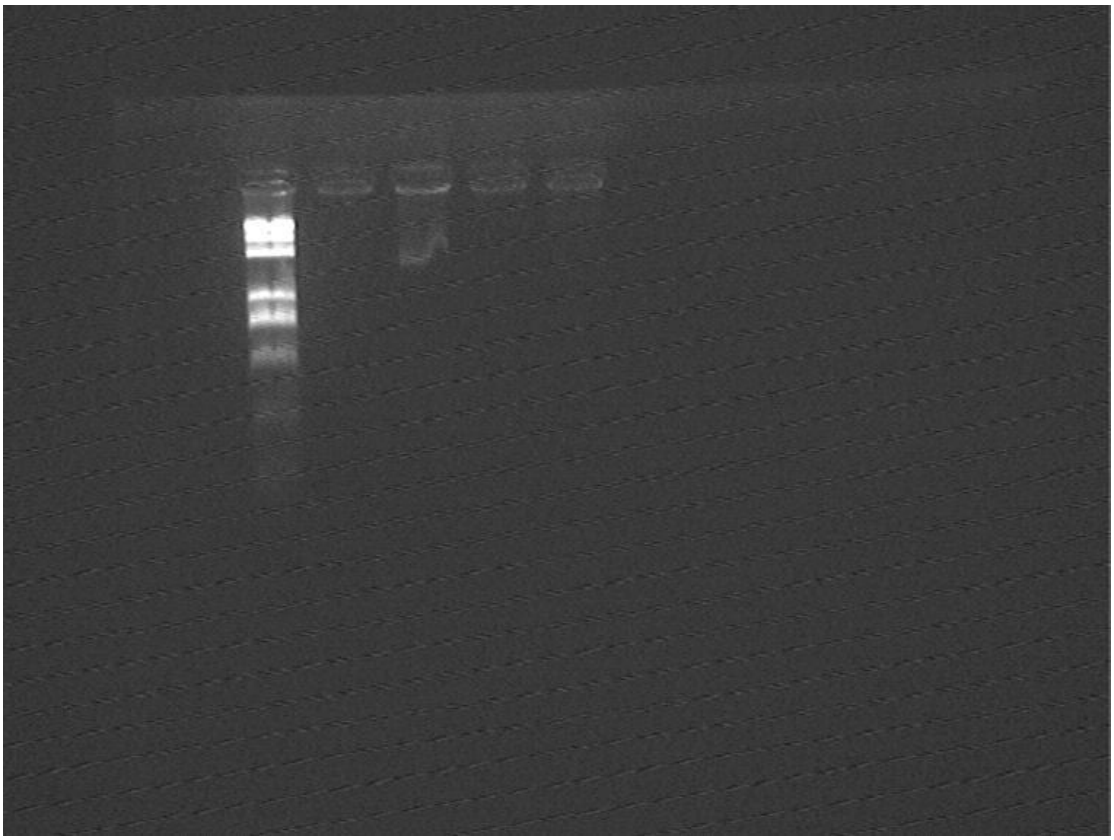
22:00 Start to digest.
2009/8/2
1:00 Electrophoresis to test the digestion products.

The order of the samples: marker, high-copy plasmids, high-copy digestion products, low-copy plasmids, low-copy digestion products, PYFP plasmids, PYFP digestion products by EK, PYFP digestion products by SP, T7P+1-12M plasmids, T7P+1-12M digestion products.
Results:



Electrophoresis the fragments used in the ligation the day before.

Results:



The concentrations of the fragments are too low, so that cause the ligation to fail.

2:00 Recycle the fragments.

4:00 Connect the bi-stable fragments, standard fragments with PYFP vectors.

Connect the high-copy bi-stable fragments and low-copy bi-stable fragments with the high-copy backbone.

| Total | 10μL |
|---|---|
| Vectors | 2μL |
| Inserts | 6μL |
| T4 ligase | 1μL |
| Buffer | 1μL |

13:00 Transform those ligation products into DH5a.

2009/8/3

8:30

There are many colonies on all the plates.

9:30 Start to shake the colonies in the incubator.

11:30 PCR to test the colonies at the same time.

| Total | 20μL |
|---|---|
| Template | Colonies |
| For | 1μL |
| Rev | 1μL |
| Mix | 10μL |
| ddH2O | 8μL |

17:00 Electrophoresis to test the PCR products.

The order of the samples: standardized PYFP, marker, PYFP with bi-stable parts, high-copy bi-stable parts, marker, low-copy bi-stable parts.

23:00 Miniprep.

| Number of the plasmids | Concentration(ng/μL) |
|---|---|
| PYFP with bi-stable parts 1 | 123.9 |
| PYFP with bi-stable parts 2 | 92.54 |
| PYFP with bi-stable parts 3 | 109.42 |
| PYFP with bi-stable parts 5 | 44.68 |
| Low-copy bi-stable parts 3 | 81.195 |
| Low-copy bi-stable parts 4 | 321.055 |
| High-copy bi-stable parts 3 | 160.98 |
| High-copy bi-stable parts 4 | 100.805 |
| PYFP with standardized fragments 5 | 55.82 |

2009/8/4

2:30 Digest and PCR those plasmids to test if they are correct.

PYFP with bi-stable parts:

| Total | 20μL |
|---|---|
| Plasmids | 3μL |
| EcoR1 | 1μL |

| | |
|---|---|
| **Kpn1** | 1μL |
| **Buffer** | 2μL |
| **ddH2O** | 13μL |

High-copy and low-copy bi-stable parts with high-copy backbone:

| **Total** | **20μL** |
|---|---|
| **Plasmids** | 3μL |
| **EcoR1** | 1μL |
| **Pst1** | 1μL |
| **Buffer** | 2μL |
| **ddH2O** | 13μL |

PYFP vectors with standardized fragments:

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

This is to test if the restriction enzyme sites are successfully been erased.

PCR to test standardization:

| **Total** | **20μL** |
|---|---|
| **Template** | 1μL |
| **For** | 1μL |
| **Rev** | 1μL |
| **Mix** | 10μL |
| **ddH2O** | 7μL |

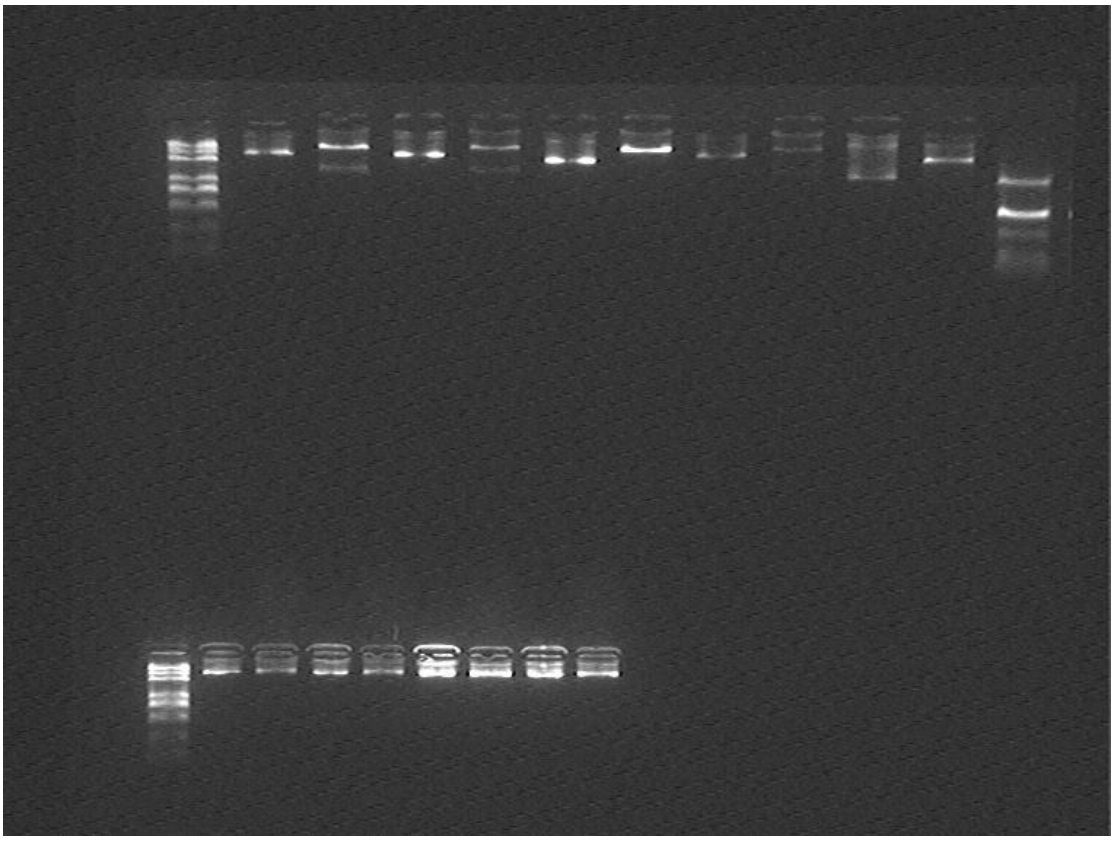
10:30 Electrophoresis to test if the PCR and digestion products are correct.

The order of the samples:

Marker, PYFP with bi-stable parts plasmids control, PYFP with bi-stable digestion products 1,2,3,5, PCR products of standardization, the digestion products of standardization.

Marker, low-copy bi-stable parts, high-copy bi-stable parts.

Results:



The PFYP with the bi-stable parts are correct.

16:00 Make up SOB.

100ML

2009/8/5

10:00 Prepare competent cells for electrical transformation.

20:00 Digest the PYFP with bi-stable parts to get the inserts.

| Total | 50μL |
|---|---|
| Plasmids | 10μL |
| Sap1 | 1.5μL |
| Sph1 | 1.5μL |
| Buffer | 5μL |
| ddH2O | 32μL |

2009/8/6

12:30 Recycle the digestion products.

13:30 Prepare SOC.

20:00 Electroporation:

Parameters: 50μLwith 400ng DNA fragments.

2.5kV, 25mF, 200ohms.

Incubate in SOC for 2h.

2009/8/7

There is one colony on the plate, but it is the result of reconnection of the plasmids.

2009/8/16

Test if the tetR promoter system works well in the low-copy backbone.

Get tetR+promoter+GRP from Wu Shuke.

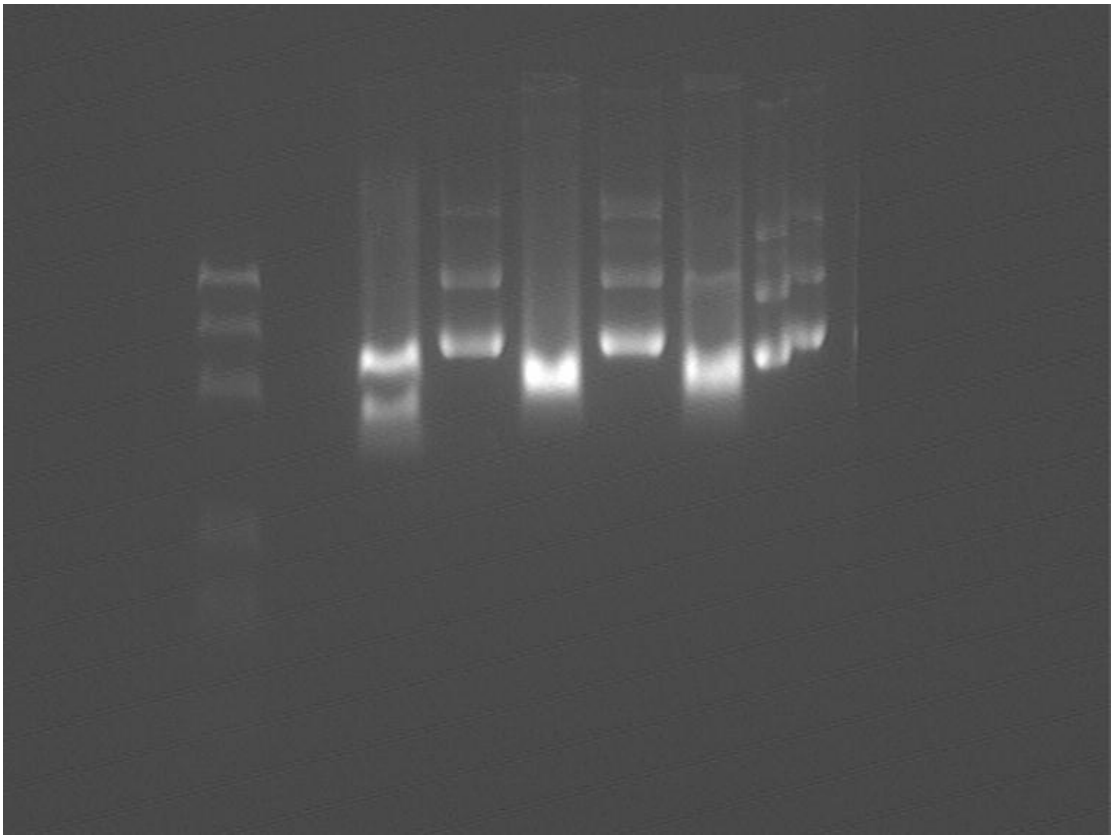
16:40 Digest the promoter and reporter system.

| Total | 50μL |
|---|---|
| Plasmids | 5μL |
| EcoR1 | 1μL |
| Pst1 | 1μL |
| Buffer | 2μL |
| ddH2O | 11μL |

20:00 Electrophoresis to recycle the inserts.

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

Results:



Only the first one is correctly digested, recycle them.

2009/8/17

10:00 Link the inserts with vectors with has Kanamycin resistance.

17:50 Transformation.

19:30 Start to incubate.

2009/8/18

10:00 There are many colonies on the plate.

PCR colonies to test if they are correct.

20:00 Pick the colonies to shake in the incubator.

2009/8/19

9:30 Mniprep the plasmids.

The general concentration of the plasmids are about 150ng/μL.

11:00 Digest and PCR those plasmids to test if they are correct.

The digestion system:

| Total | 20μL |
|---|---|
| Plasmids | 10μL |
| EcoR1 | 1μL |
| Pst1 | 1μL |
| Buffer | 2μL |
| ddH2O | 6μL |

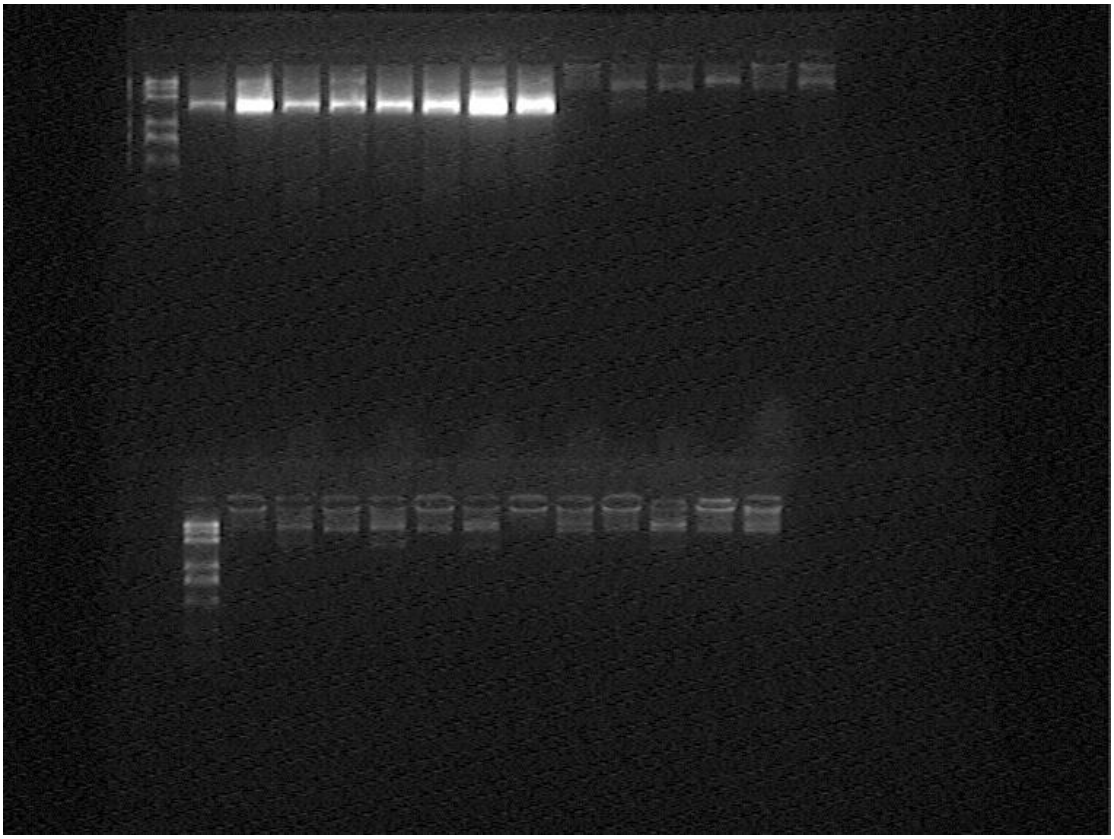
The PCR system:

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

12:00 Start to digest&PCR.

16:30 Electrophoresis to test the digestion and PCR products.

Results:



All the clones have correct colonies.

17:00 Induce the strain containing tetR and low-copy backbone by aTc.

22:00 Using flow cytometry to test the induction results.

There are about 5 folds between the induced sample and the uninduced one.

2009/8/20

Make a gradient to test the features of the tetR promoter system.

The gradient is from $10^{-2}$ to $10^{-6}$, control, negative control $10^{-2}$ , $10^{-3}$.

14:00 Recycle LacP vectors.

Miniprep Sal promoter + SupD + terminator.

18:40 Link the LacP vectors to T7ptag inserts.

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

19:00 Ligation starts.

20:30 Transformation

23:00 Start to incubate.