090613
13:00 pick one colony of Top10 containing T7 promoter plasmid. Incubate in 5 ml LB (Kan+).
14:00 adjust DNA imager with a mixture of 20ul Marker and 5ul genefinder. Image successfully captured.
13:00~14:00 transformation
Transform Plasmid pAra-T7ptag from Voigt lab to Top10 competent.
0.5ul plasmid diluted into 1ul. Then transform.

Add 500ul LB without antibiotic and shake in the incubator for 40 minutes.
Centrifuge to concentrate E.coli then Plate.

Keep the plate in 37 centigrade incubator.

15:00
Check up parts in partsregistry.
Results:

BBa_E0240	Rbs+GFP+terminator	Medium rbs	1-12M	Amp
BBa_E0840	Rbs+GFP+terminator	Strong rbs	1-12O	Amp
BBa_R0080	Ara-Promoter		1-12E	Amp
BBa_C0062	LuxR gene	No LVA	1-4O	Amp
BBa_C0051	CI repressor	+LVA	1-4E	Amp
BBa_C0179	LasR activator	No LVA	2-8M	Amp
BBa_C0079	LasR activator	LVA	1-14J	Kan
BBa_R0079	LasR/PAI promoter		1-12A	Amp

Dissolve parts in 15ul ddH2O store in PCR tube in -20

15:00
Sterile LB.
For plates(300ml+300ul Amp)
Liquid LB: 300ml with no antibiotics. 300ml with Amp. Dry in the hood.
21:00
Transformation (BBa_E0240&BBa_E0840 → Top10)
23:00
MiniPrep T7 promoter plasmid.
Digest with EcoRI and SpeI 37 centigrade over night

EcoRI	**0.5ul**
SpeI	**0.5ul**
10xH buffer	**2ul**
Plasmid	**10ul**
ddH2O	**7ul**

090614

10:00
Pour 1% agarose gel. Pre-mix the plasmid digestion product with 4ul geneFinder. Run the gel with DL2000 plus marker from Transgene. 60V to enter the gel, and then with 120V
The insert in lost.

10:50:
Pick 1-12M colony and 1-12O colony into 5ml Amp+ LB, shake in the incubator.
11:00
Sterilize LB.
14:30
Pour LB plates(Cm+)

090615

9:00
MiniPrep 1-12M and 1-12O.
Digest with EcoRI and Xbal

EcoRI	**1ul**
Xbal	**1ul**
10xM buffer	**5ul**
Plasmid	**43ul**

Transformation (pSalSer plasmid from Voigt Lab into Top10)
22:00
Digest 1-12M and 1-12O with EcoRI and SpeI overnight.

Xbal	**1ul**
PstI	**1ul**
10xM buffer	**5ul**
Plasmid	**43ul**

Digest T7 promoter with

SpeI	**1ul**
PstI	**1ul**
10xH buffer	**5ul**
Plasmid	**43ul**

090616
10:00: Gel (1-12M and 1-12O product)
Cut gel; Recycle the insert(about 1kb).
Wash with 30 ul Elution buffer, store in -20.

21:37
Gel to compare the concentration of insert (1-12M and 1-12O) and the vector(T7

promoter cut with SpeI and PstI)

Ligation 16 centigrade overnight

Ligase	**1ul**
Buffer	**1ul**
Vector	**1ul**
Insert	**7ul**

090617
Transformation(ligation product into Top10)
Miniprep SupD plasmid.
Use E.coli with T7ptag (From Voigt Lab) to make competent cell.
Pour LB plates with double antibiotics (Kan and Cm).
Transform the competent with SupD plasmid.

090618
The T7 promoter and 1-12O plate is filled with colonies, it is contaminated with uncutted vectors.
Pick 3 colonies and shake in 37 centigrade incubator.

5 Colonies grow on The Double antibiotic plate
Pick and shake in double antibiotic LB.

090619
Miniprep the Ligation (T7 promoter and 1-12O[rbs+GFP+terminator]) plasmid and GEL to see if the insert is successfully ligated into the vector.
Turn out a failure.

Miniprep the T7ptag and SupD double plasmid from the double antibiotic resistance E.coli.
Run plasmid.
It is proved that there are two plasmids in the Colony.

090620------090703 not in the lab

09-07-04
Back in the Lab.
Tranformation(Parts listed below)

BBa_I14033
BBa_C0040
BBa_C0012
BBa_J09250
BBa_C0080

BBa_B0034
BBa_K093012
BBa_J37033

090705
Pick colonies of the transformation and shake in the incubator.

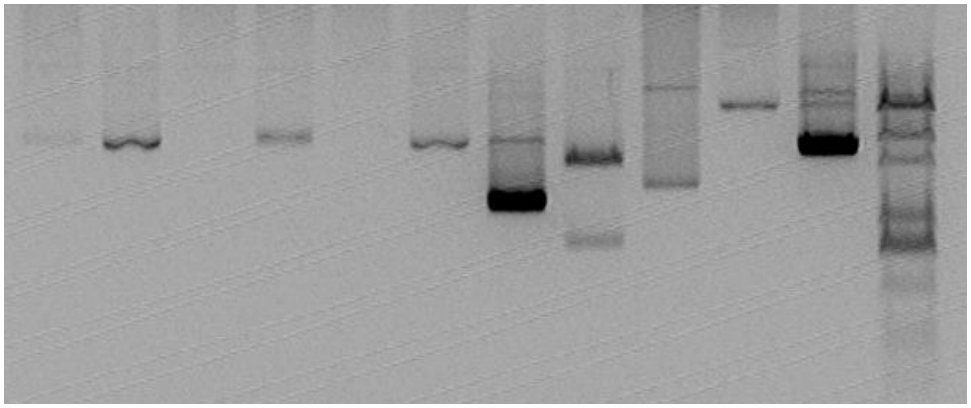
090706
MiniPrep tetR standard parts plasmid.(BBa_C0040)
Get 6 standard rbs plasmids From ShenShan.
21:40
Digest rbs

SpeI	**1.5ul**
PstI	**1.5ul**
10xH buffer	**5ul**
Plasmid	**5ul**
ddH2O	**37ul**

Digest tetR

XbaI	**1ul**
PstI	**1ul**
10xM buffer	**2ul**
Plasmid	**5ul**
ddH2O	**11ul**

090707
01:52
GEL to assess the digestion



CIP the 6 rbs vectors.
Add to the digestion product 5ul CIP Buffer and 1ul CIAP

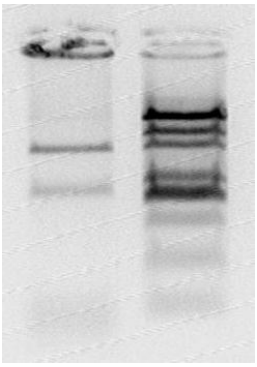
Run a GEL to recycle the insert of tetR.

The Hole is too large, the band is missing. (Never use the largest Cone for 20ul recycle any more)

03:24
Digest the tetR plasmid again.

090708
The Primer(Made of Standard Prefix and Suffix arrived).
Add ddH2O to each tube.

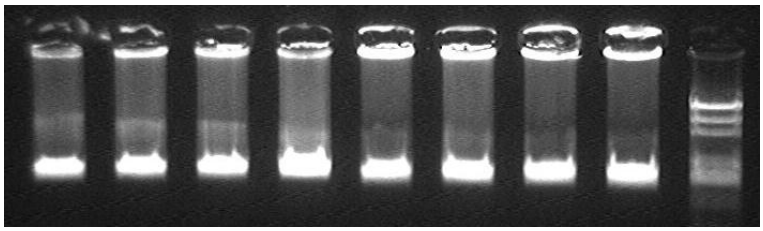
PCR tetR plasmid (MasterMix) to see whether the primers work.
PCR protocol.

 (the Band is too narrow which means that the PCR is not done very well)

Try Colony PCR
No Signal

Go to Lingli's Lab, do gradient PCR.
52, 53, 54, 55, 56, 57, 62, 64

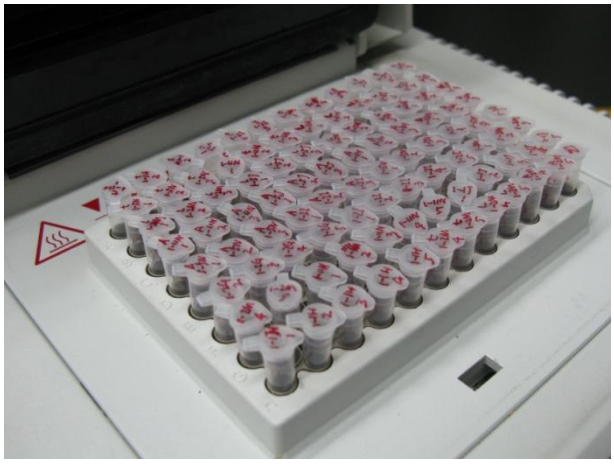
All of the temps works well.



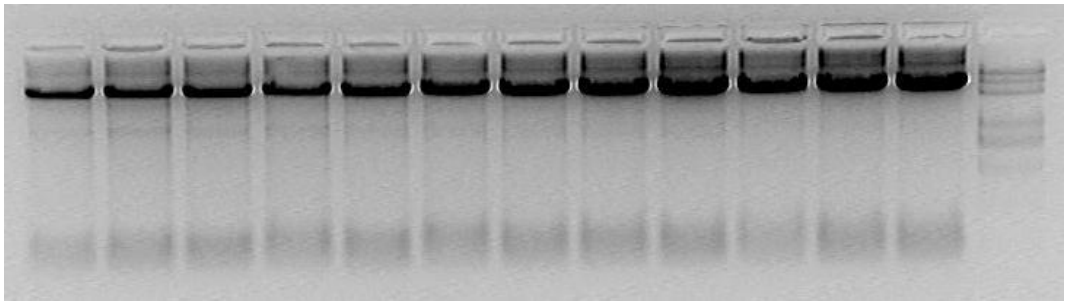
It turns out that the thermocycler in our lab has no hot cap. So the liquid is on the tube cap when exposed to 94 centigrade.

090709
10:00
Help ZhangHaoQian, ZhangGuoSheng, Wushuke and WangHao do assessment of

the colonies(Colony PCR). Make mixture for Colony PCR. There are 96 tubes in the PCR machine.



12:00
PCR T7ptag to standarlize the T7ptag gene.(the primer sequence here)
体系 here.
Condition here.
Use the machine in the New Life Science Building, ZebraFish Lab.
Do Gradient PCR, from 54 to 65 centigrade.
Use PFU
54,55,56,57,58,59,60,61,62,63,64,65.
Recycle by GEL.



All of the temps worked, and 65 is the best temp.

090710
The assessment PCR last night has stoped.
PCR the SupD plasmid to standarlize this SupD part.
TIxi
Condition Gradient PCR, 50, 55, 60, 65 centigrade. And use both pfu and mastermix. Since mastermix is supposed to be easier to use.
Results.
All the temps works. Recycle the Pfu ones.

Cut the PCR recycle product with EcoRI&SpeI and also EcoRI and PstI

Cut B0015 terminator with EcoRI and XbaI.
Cut tetR plasmid with EcoRI and PstI to get tetR backbone.

090710 16:26

Recycle SupD GEL, use 50ul EB to wash off.
The concentration of the recycle product is 35ng/ul
Use EcoRI and SpeI to cut SupD PCR product. Digestion starts from 18:40

19:40
And see whether the T7ptag and terminator vector has been digested.
CIP the terminator plasmid.
Recycle the backbone of tetR with GEL recycle Kit.

Recycle the T7ptag digestion product with DNA product recycle kit.

Ligation:

Ligase	**1ul**
Buffer	**1ul**
Terminator Vector	**1.5ul**
T7ptag Insert	**6.5ul**

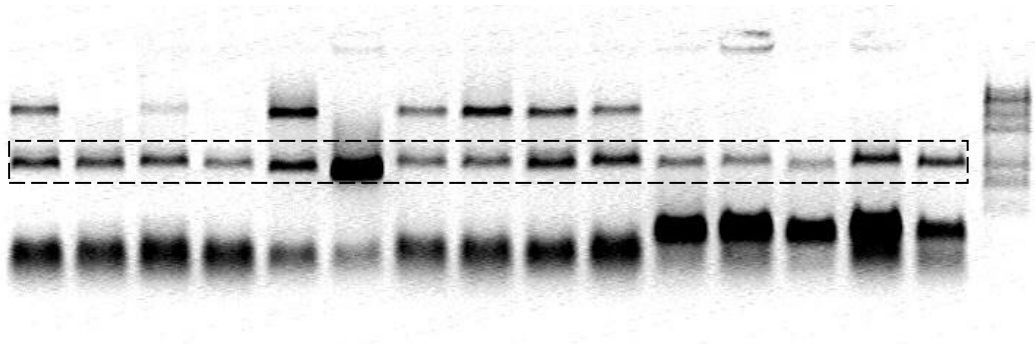
Ligase	**1ul**
Buffer	**1ul**
tetR Backbone	**1.5ul**
T7ptag Insert	**6.5ul**

Ligase	**1ul**
Buffer	**1ul**
WSK digestion	**1.2ul**
SupD Insert	**6.8ul**

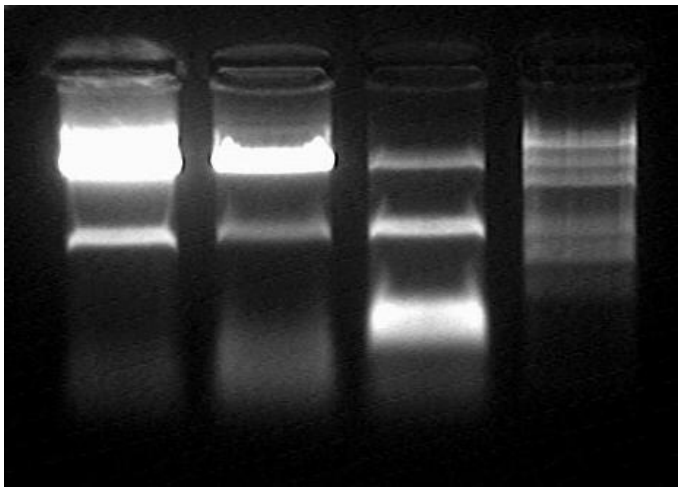
090711
9:00
Tranformation of the ligation products.
11:30
Plate the transformation product.
Into Incubator 37 centigrade.
16:15
Help wushuke do transformation. (5tubes t2, t3, I1, I2, control)

23:40
PCR to assess the T7ptag T7+terminator and SupD colonies. Pick 5 colonies from each plate PCR overnight.

090712
9:00
GEL to see the PCR product



There are contaminations.
Another PCR to see whether it is caused by the contamination of the ddH2O



There are still contaminations. Do not know the reason.

15:30
Wushuke's gel also has the contamination bands, even in those negative control without template.
It is supposed to be caused by primer contamination.

16:00
MiniPrep T7ptag(standardized) plasmid, T7ptag+terminator plasmid, and SupD(Standarlized) plasmid.
Send for sequencing

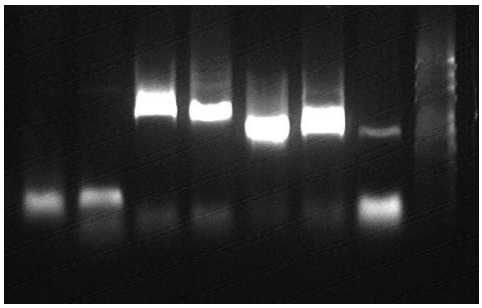
090714
16:00
MiniPrep low copy plasmid of the bistable plasmid.
Concentration = 130ng/ul
Enzyme Digestion with

EcoRI	**1ul**
PstI	**1ul**
10xH buffer	**2ul**
Plasmid	**7ul**
ddH2O	**9ul**

GEL:

Low copy bistable plasmid is lost.
Ask Louchunbo about it, the low copy plasmid is not standarlized.

090715
22:16
Help Wushuke with his PCR. The lacI1-1, lacI2-2, tetR2-2, tetR3-2, tetR3-1.

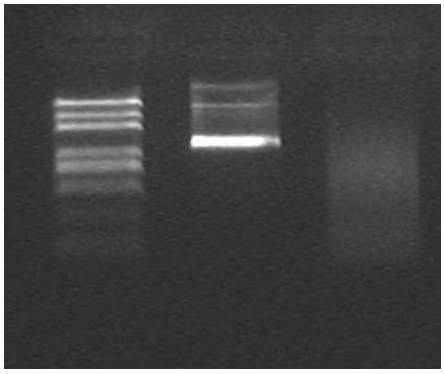


090716
MiniPrep 1-18P and 2-4O plasmid
Enzyme Digestion of 1-18P and 2-4O plasmid

SpeI	**1ul**
PstI	**1ul**
10xH buffer	**2ul**
1-18P Plasmid	**2ul**

ddH2O	**13ul**

XbaI	**1ul**
PstI	**1ul**
10xM buffer	**2ul**
2-4O Plasmid	**10ul**
ddH2O	**6ul**



Several Trials and failures, 2-4O can not be digested normally, at least be TaKaRa Enzymes.
Check up the parts and found
BBa_J09855 Constitutive LuxR with pLuxR 1-9H pSB1A2
Transformation of the parts by shenshan
Shake the constructed 1-2M+T7ptag2+terminator in the incubator.
090720
23:16
MiniPrep the 1-2M+T7ptag2+terminator plasmid. Cut with XbaI and PstI over night.
090721
12:00
GEL purification of rbs+T7ptag+terminator.

Ligation
1ul ligase
7ul rbs-T7ptag-terminator(XP) insert
1ul 1-18A vector
1ul ligation buffer

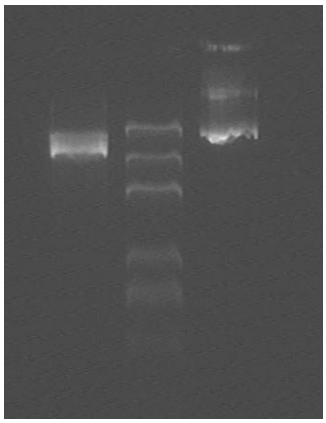
20:30
Transformation

090722

Reverse Mutation of T7ptag
12:00
PyroBEST PCR T7ptag(mutation)

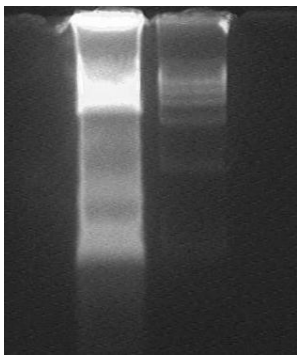
PyroBEST polymerase 0.25ul
2.5uM dNTP 4ul
Buffer 5ul
Primer For: 1ul
Primer Rev: 1ul
Template: 0.6ul
ddH2O: 37.8ul

14:00
Double Digest of J09855
SpeI 1ul
PstI 1ul
10xH 2ul
Plasmid 3ul
ddH2O 13ul



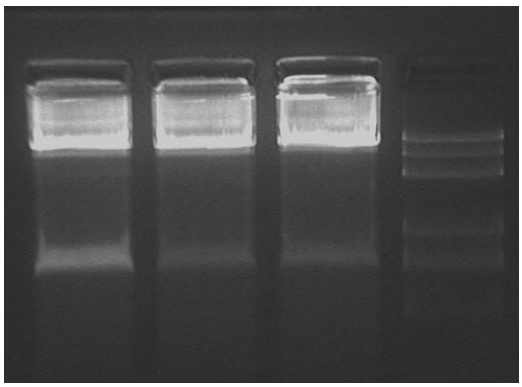
The digestion is weird, maybe GEL BAD

18:00
1% agarose DL15000 Marker, GEL

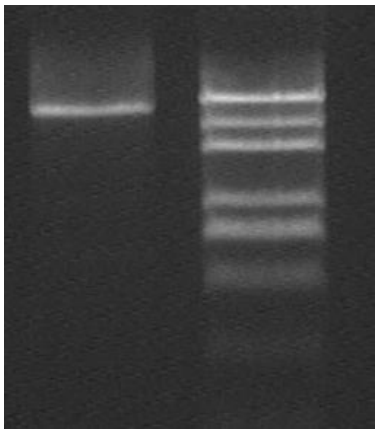


Assume the result of the reverse mutation PCR is not correct. I redo PCR with Phusion.

Use a gradient
Set the annealing step to 55 60 63 centigrades.
1. 98(30s)
2. 98(10s)
3. annealing(30s)
4. Extension (2min30s)
5. GO TO 2. 35 cycles
6.72(10 min)
7. 4(hold).
GEL



GEL to assess whether the recycled DNA from PYROBEST PCR is correct:



It is correct! It is 2600+2000=about 4600bp

BLUNT KINATION reaction:
Measure the concentration of the purified DNA, it is about 1.5038ng/ul
C=1.5038ng/ul*50/300/4.7*10^3g/mol=0.05333pmol/ul.
17ul*0.05333pmol/ul=0.907pmol

According to the protocol, 1pmol is needed for BLUNT Kination reaction.

So:
DNA fragment 17ul
10xBlunting Kination Buffer 2ul
Blunting Kination Enzyme Mix 1ul
ddH2O 0ul

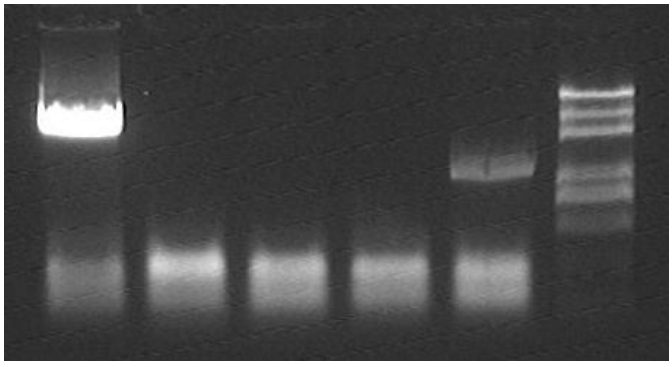
37centigrade for 10 min, and then 70 centigrade for 10 min.

Take 5 ul of the blunting solution, mix it with 5ul ligation buffer which is designed for Blunt end ligation. For 1h it ligates.

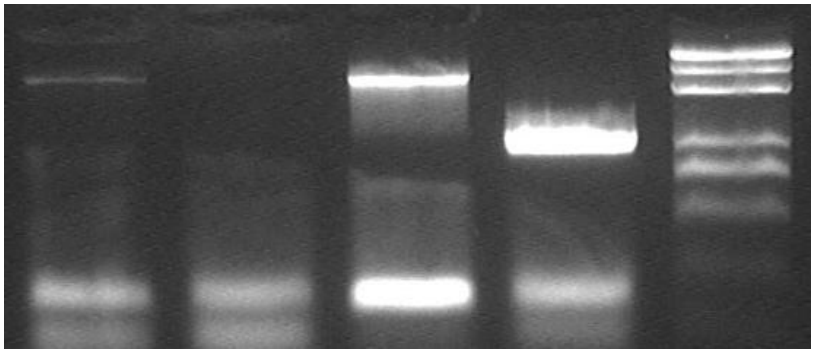
Transformation.

090723:
PCR assessment of the T7ptag Reverse Mutation Strain.

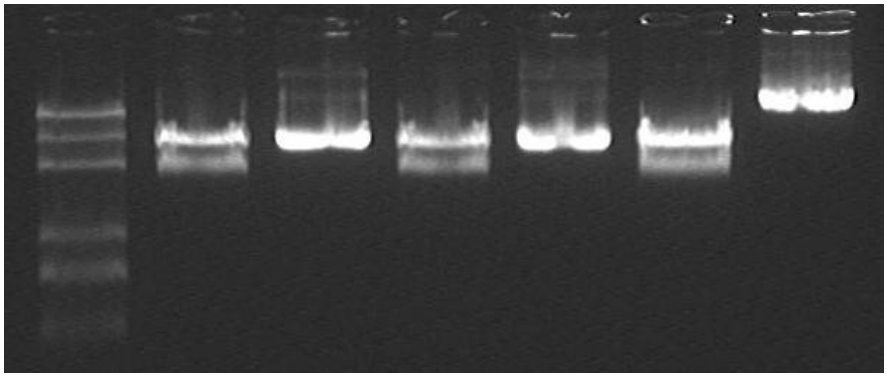
FastTaq PCR protocol.



As seen in the gel picture, number one is correct, but in fact PCR assessment sometimes is not so accurate, so I PCR again to get more correct colonies.



Miniprep number 1, 7, 8.
Enzyme digestion assessment:
EcoRI and PstI



Lane1 is marker
Lane 2 is number 1 plasmid(EP digest)
Lane 3 is number 1 plasmid.
Lane 4 is number 7 plasmid(EP digest)
Lane 5 is number 7 plasmid.
Lane 6 is number 8 plasmid(EP digest)
Lane 7 is number 8 plasmid.

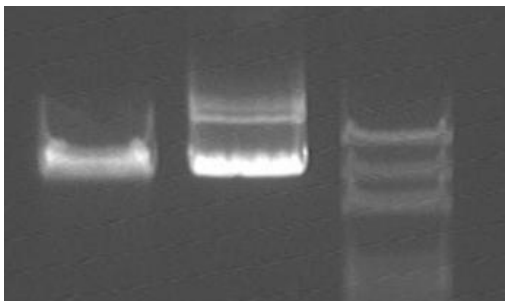
So that Number1 and Number7 is correct, but Number 8 may be the result of two linearlized plasmids ligated together.

So far the Reverse mutation work is done, and number 1 is sent for sequence. (Correct).

Several days in shanghai

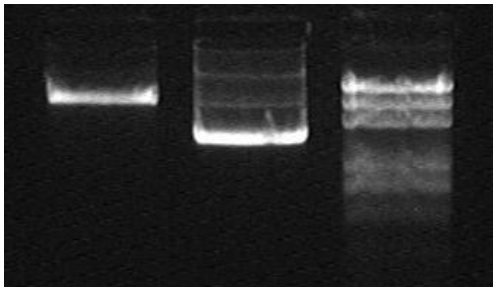
090727

Enzyme Digestion of J09855 plasmid.
SpeI 1ul
PstI 1ul
10xH Buffer 2ul
Plasmid 3ul
ddH2O 13ul



It is strange that the linearlized plasmid is as large as the super coiled plasmid, maybe GEL BAD or something.

Digest Wushuke's constitutive tetR + tetP with SpeI and PstI



Cip for 20 min and Purify.

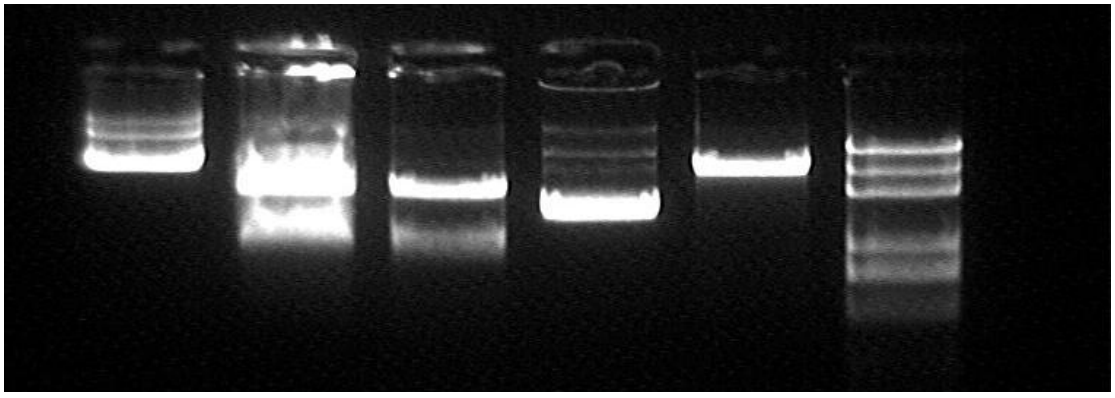
Digest E0840 with EcoRI and XbaI overnight.
Digest J09855 with EcoRI and SpeI overnight.

Ligation overnight:

Wushuke's constitutive tetR + tetP (SP).
E0840(XP) insert from shenshan.
1ul ligase
1ul ligation Buffer.

Wushuke's constitutive tetR + tetP (SP).
SupD+terminator(XP) digested earlier.
1ul ligase
1ul ligation buffer.

2009 07 28
11:00
Transformation.
GEL:
Digest E0840 with EcoRI and XbaI overnight.
Digest J09855 with EcoRI and SpeI overnight.



Lane 1 is the J09855 plasmid
Lane 2 and lane 3 is the digestion product.
Lane 4 is the E0840 plasmid
Lane 5 is the digestion product of E0840.

GEL purification.
Ligation:
J09855 LuxR-luxP(ES) 6.5ul
E0840 rbs-GFP-terminator(EX) 1.5ul
Ligase 1ul
Ligation buffer 1ul

Pick colonies of Wushuke's tetR-tetP-GFP and tetR-tetP-supD.
PCR overnight for assessment, and at the same time shake them in the incubator.

2009 07 29
Miniprep of the tetR-GFP number2 and number 5 and tetr-supD number 1.
Assessment by enzyme digestion.

In fact the insert is about the same length with the backbone, so the assessment failed.

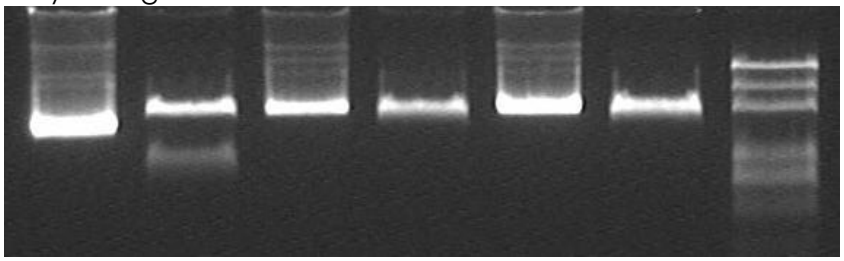
Then I find that the colonies are green, I think it is due to the basal expression of the tet promoter. So the tetR is not enough, I plan to express tetR under a stronger promoter. It is J23100.

Ligation:
J23100(XP) with both rbs-lacI-terminator and rbs-tetR-terminator.

Assessment of the LuxR-luxP-GFP construct.

The colonies seem to be white, but there are 2 green colonies.
I shake both of the colonies in the incubator for assessment.

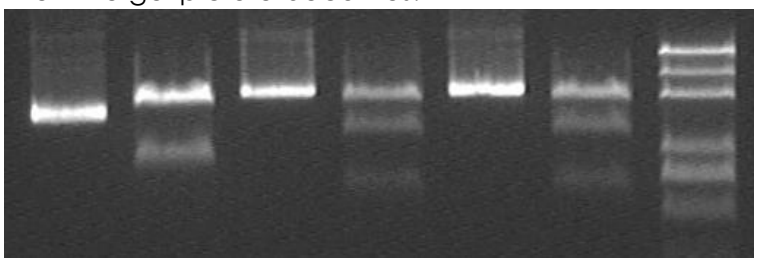
2009 07 30
Miniprep that LuxR-luxP-GFP plasmid(both green and white)
Enzyme digest with EcoRI and PstI



The first lane is the green plasmid, and the second is the digested green plasmid.

The 3 and 5 lane are the white colonies, and 4 and 6 are those digested.

Add SphI to the Digestion, since SphI has cut site inside of LuxR.
Then the gel picture becomes:



So the white colonies are the correct ones.

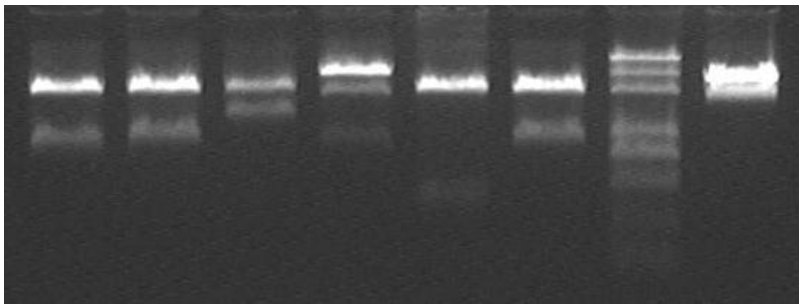
12:30
PCR assessment of the J23100-tetR and the J23100-lacI
Since the false colonies is red, due to the RFP in the J23100, I pick both red and white colonies.

Shake in the incubator.

2009 07 31

Miniprep J23100-tetR & J23100-lacI. Both Red and white ones.

Enzyme digest with EcoRI and PstI

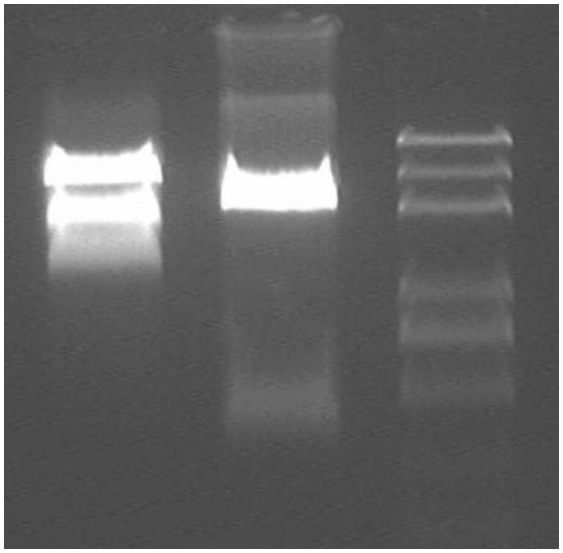


Lane1 is the correct J23100-tetR
Lane2 is the contaminated J23100-RFP
Lane3 is the correct J23100-LacI
Lane4 is the Contaminated J23100-RFP

Enzyme Digestion of SupD-terminator(EX)

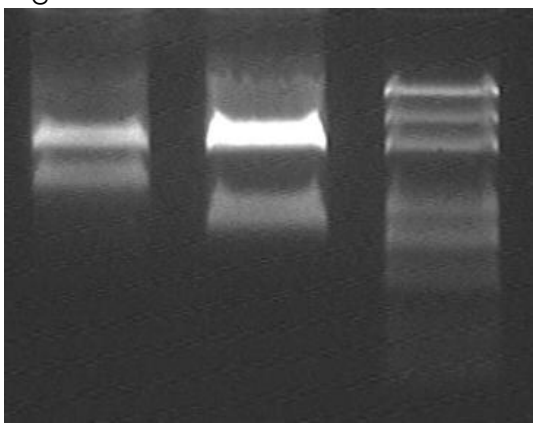
Ligation:
LuxR-LuxP insert(ES)
SupD-terminator(EX)

Enzyme Digestion of T7ptag-terminator with HindIII, XbaI and PstI
Since T7ptag-terminator is almost as large as the backbone, which is 3kb, HindIII has its cutting site inside of the backbone.



GEL purification of the T7ptag-terminator fragment.

Digest J23100-tetR and J23100-LacI with EcoRI and SpeI.



GEL purification of the insert.

Ligation
Rbs 1-2M(SP)vector
T7ptag-terminator insert(XP)

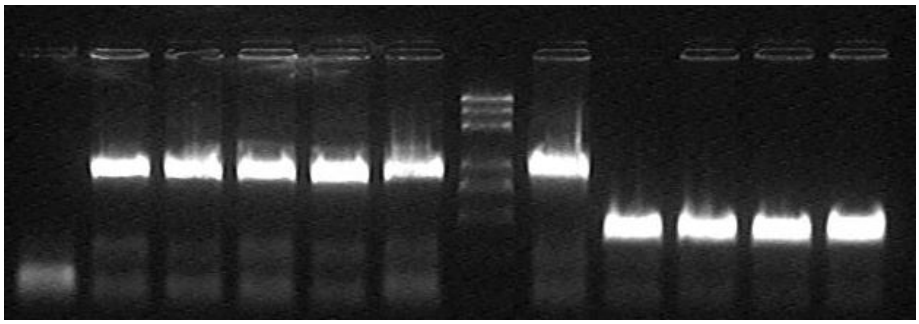
Ligation
J23100-tetR insert
tetP(EX) vector

Transformation of Ligation
Rbs 1-2M(SP)vector
T7ptag-terminator insert(XP)

2009 08 01
Transformation:
J23100-tetR-tetP and LuxR-LuxP-SupD

PCR assessment for the J23100-tetR- tetP and LuxR-LuxP-SupD
Overnight

2009 08 02
PCR product → GEL

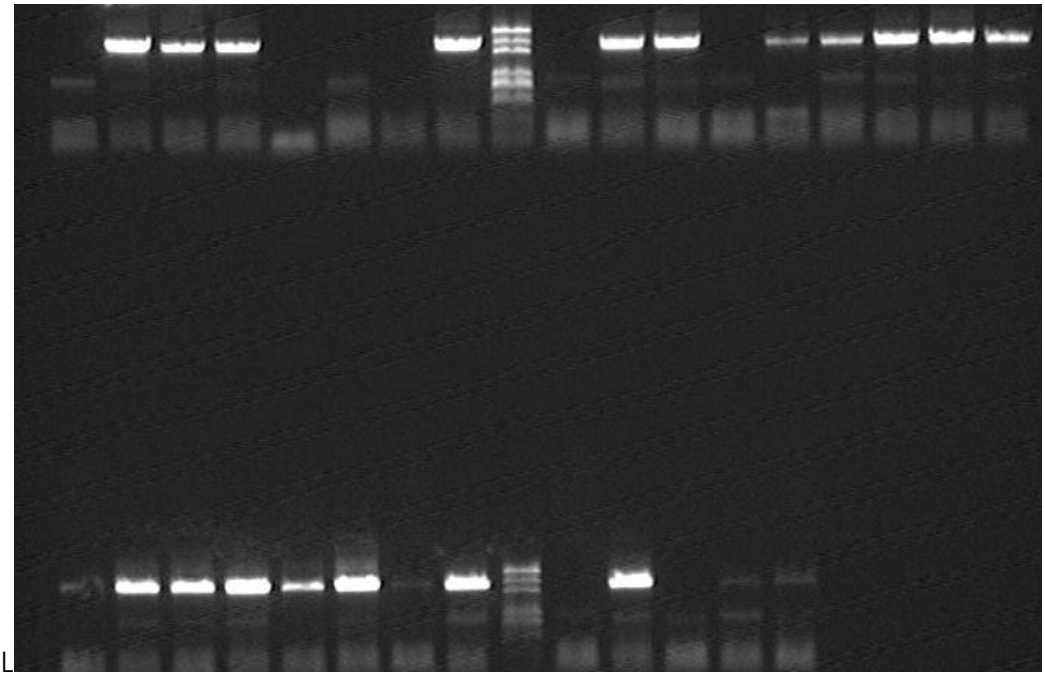


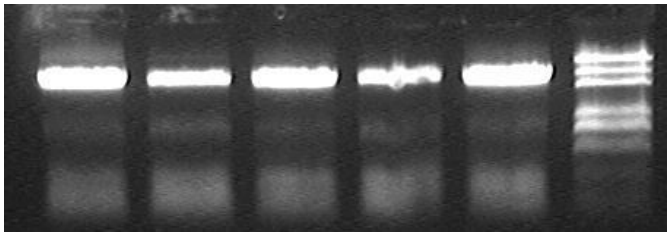
Number2-6 J23100-tetR-tetP is correct.
Number1 of LuxR-LuxP-SupD is correct.

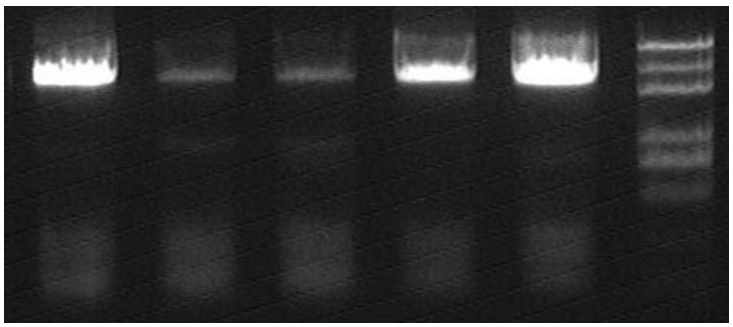
Digestion of the J23100-tetR-tetP
With EcoRI and SpeI overnight

2009 08 03
01:30
Help Zhangguosheng pick colonies and PCR
10:00

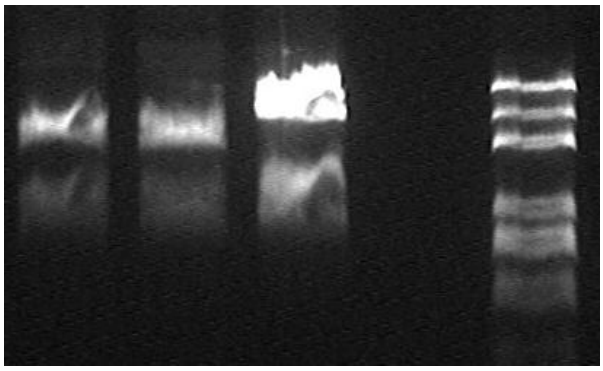
GEL







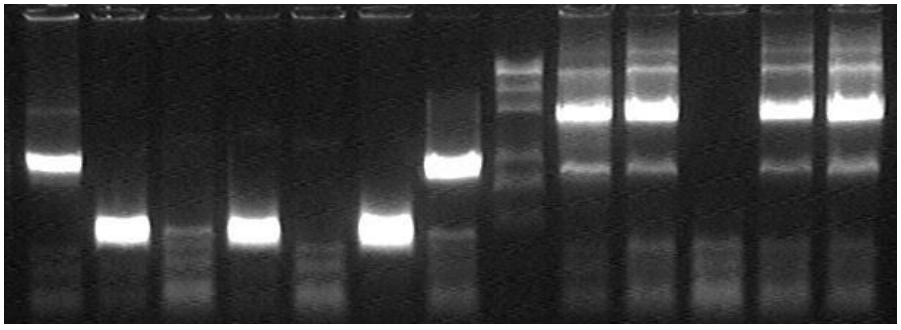
Enzyme Digest of the J23100-tetR-tetP with EcoRI and SpeI



GEL Purification.
Ligation:
J23100-tetR-tetP ES insert
E0840 EX vector and SupD-terminator EX vector

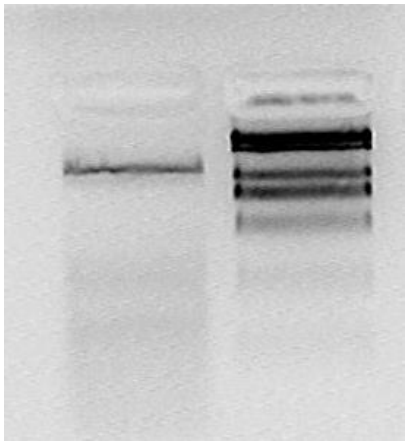
18:30
Transformation.

2009 08 04
PCR assessment



Shake the BL21 strain transformed with T7promoter-GFP by WangHao
Induce with IPTG.
The induced one has fluorescence

2009 08 05
Pick standard part I0500
Phusion PCR



GEL purification and then cut with EcoRI and SpeI.
Purify from digestion.

2009 08 06

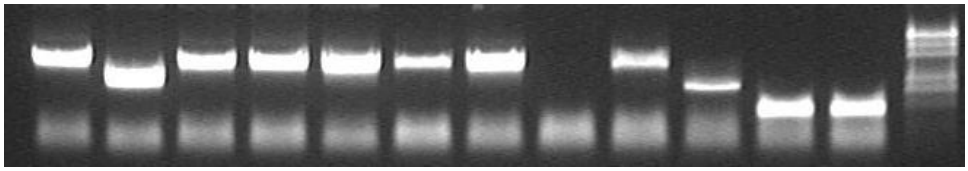
Ligation of I0500(ES) to SupD-terminator and E0840 vector.

Transformation

In order to get a Kan resistant back bone, I transformed the pSB1K3 part from plate1-7A.

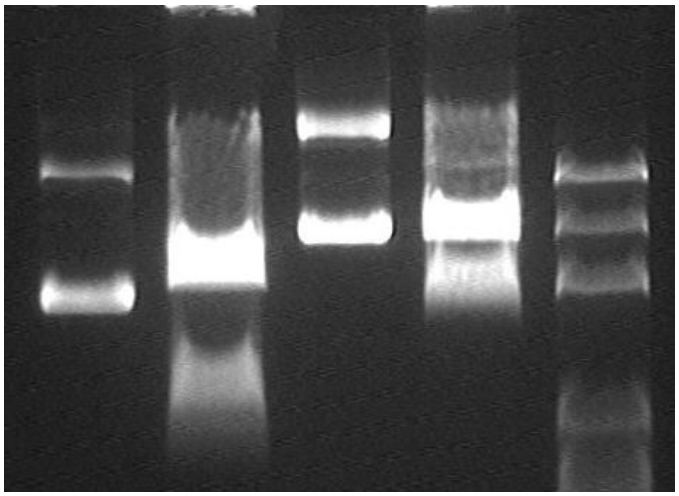
2009 08 07

Shake the 1-7A in the incubator.
PCR assessment of the I0500-GFP clone.



But it is no need to care about which is right, cause Gaorencheng has worked out a AraC and PBad promoter that works well.

2009 08 08
Miniprep the 1-7A plasmid.
Digest with EcoRI and PstI

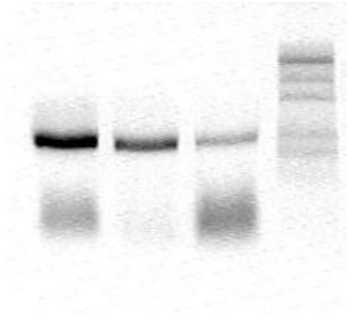


GEL purification.
Store the backbone.

Ligation
T7promoter-GFP EP insert
pSB1K3 EP backbone

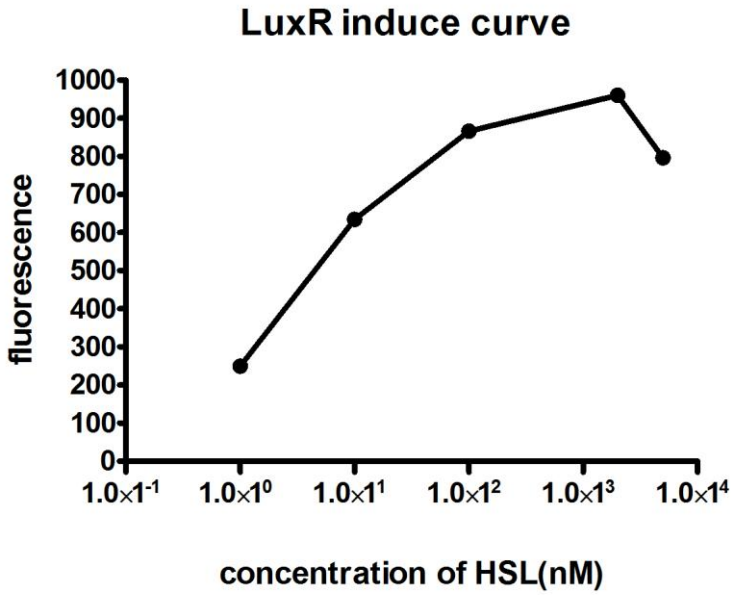
Transformation

2009 08 09
Pick colonies to do PCR assessment of the T7promoter→pSB1K3



The 3 colonies are correct ones.

Induction of the LuxR-LuxP-GFP
A gradient of concentration:
5uM, 2uM, 100nM, 10nM, 1nM, 0
Use flocytometry to detect the fluorescence.



It shows a ten fold induction.
However the basal is very high.

Miniprep the LuxR-LuxP-SupD plasmid and the T7promoter- GFP-pSB1K3 plasmid.

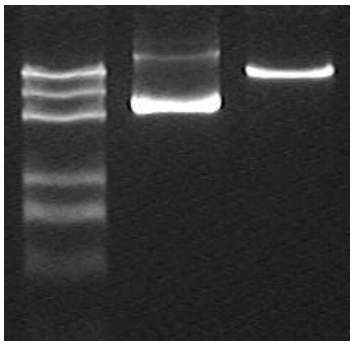
090811

10:00
Help Zhangguosheng with his GEL
15:00
Enzyme Digestion of the LuxR-LuxP-T7ptag by XbaI and PstI

2009 08 12

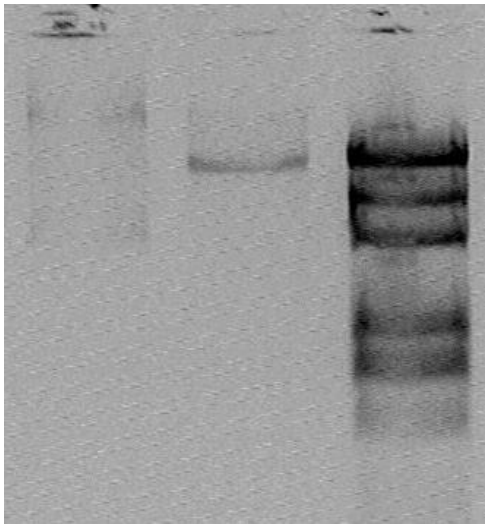
2009 08 13
Miniprep the Low copy plasmids, and digest with EcoRI and PstI.

Digest the LuxR-LuxP-SupD plasmid with SpeI and PstI, CIAP and then purify.



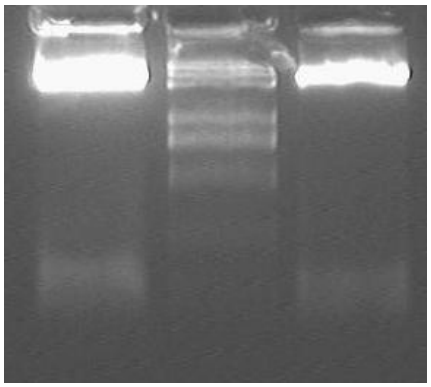
Ligation:
Insert: XP digestion of AraC-T7ptag insert (9x rbs)
Vector: LuxR-LuxP-SupD(SP)

2009 08 14
Shake the 3 counter plasmid in the incubator.
Miniprep the SupD-terminator plasmid. Digest with EcoRI and XbaI to make vectors.



Help Wushuke induce the pLac-RFP-JM109.
Miniprep the 3 counter plasmid.

2009 08 15
11:00
Phusion PCR of the 3 counter plasmid.



GEL purification.
Digest with EcoRI and PstI

Ligation:
pSB1K3 backbone 1 ul
T3 pol insert 7ul