Category

The category seems strange. But it can work as normal one when you download the pdf and want to go to one part by clicking the category.

Cns1 and Cns2

6.25th，Tuesday
participator： Zhang Chenming Qiu Qinling
purpose： Use XbaI , EcoRI(For Cns2 and its pYES2 backbone) and HindIII(cns3 and its pYES2 backbone) to digest fragement and vector. Processing purification for future ligation.
process：
1. digest
The digestion dose cohort is as followed:

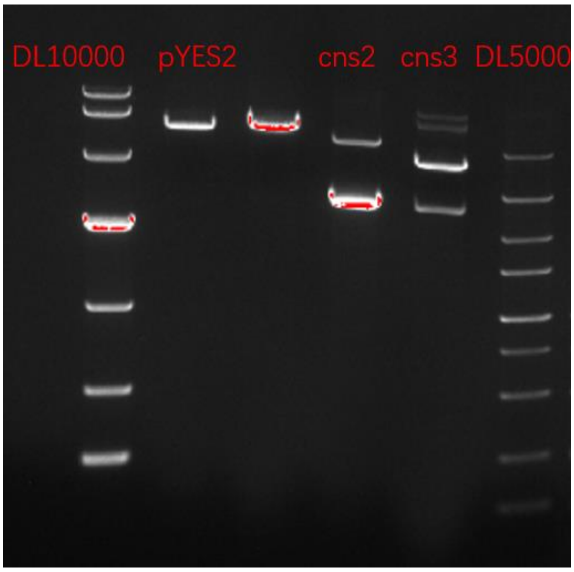
10X green buffer	2.5ul
EcoRI (for cns2) HindIII(for cns3)	1ul
Fragment/ Vector	4ul
ddH2O	16.5ul
Total	25ul

Incubate at 37 ℃ for 45min
Inactive at 80℃ for 15min
Then processing gel extraction for purification.
2. ligase

Ligastion Buffer (10x)	2uL	
CNS2bb	8.5uL	9.82ng/uL
CNS2	8.5uL	5.01ng/uL
T4 ligase	1uL	
ddH2O	5uL	
Total	20uL	

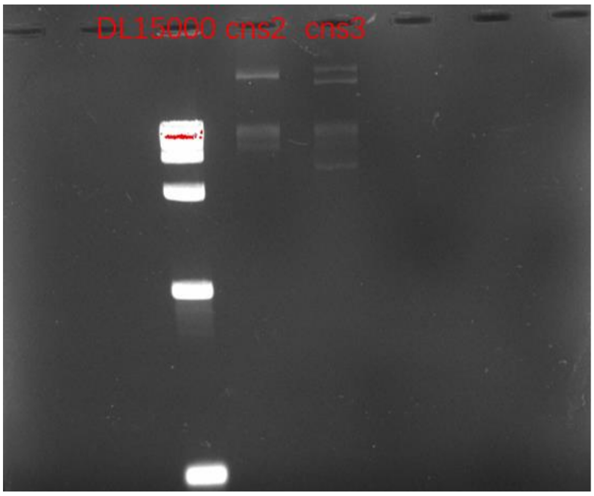
results:

1.digest



digest and gel extraction of cns2+cns3+BB

2.ligase



cns2(Left1)cns3(Left2)liagetoYES2 success!

conclusions: digestion is successful.

next step: transform the ligase product into trans5a

6.26th, Wednesday

participator: Zhang Chenming Qiu Qinling

purpose: transform the ligase product into trans5a and spread plate to get the single colony

1.transform

15uL the ligase product with 100uL Trans5a

42° C from 90s, 37° C 100 rmp from 1h

2.ligase (re)

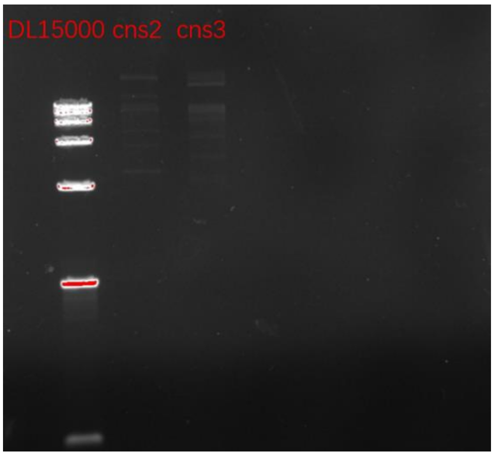
Ligastion Buffer (10x)	2uL	
CNS2bb	5uL	9.82ng/uL
CNS2	12uL	5.01ng/uL
T4 ligase	1uL	

ddH2O	5uL	
Total	20uL	

3. spraed plate

three plates for cns2, every plate adds 150ul bacterial fluid

results:



cns2(Left1)cns3(Left2)liagetoYES2 success!

conclusions: we got cns2 gene fragment

next step: transform the ligase product (re) into trans5a

6.27th, Thursday

participator: Zhang Chenming Qiu Qinling

purpose: transform the ligase product into trans5a and spread plate to get the single colony; verify transfomation;

process:

1. transform (re)

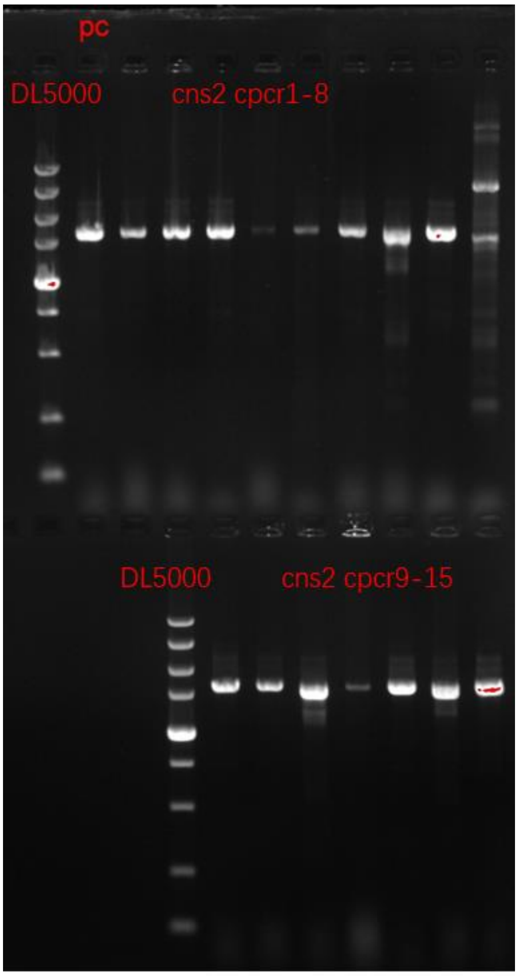
15uL the ligase product with 100uL Trans5a

42° C from 90s, 37° C 100 rmp from 1h

2. CPCR

2X T5Mix	5uL
Colony	1uL
F	0.5uL
R	0.5uL
DdH2O	3uL
Total	10uL

results:



cns2 CPCR, 15 samples

conclusions: we get the E.coli with cns2-pYES2

next step: Liquid culture to get more right plasmids; stock the right E.coli.

6.28th Friday

participator: Zhang Chenming Qiu Qinling

purpose: get more right plasmids ;stock the right E.coli

process:

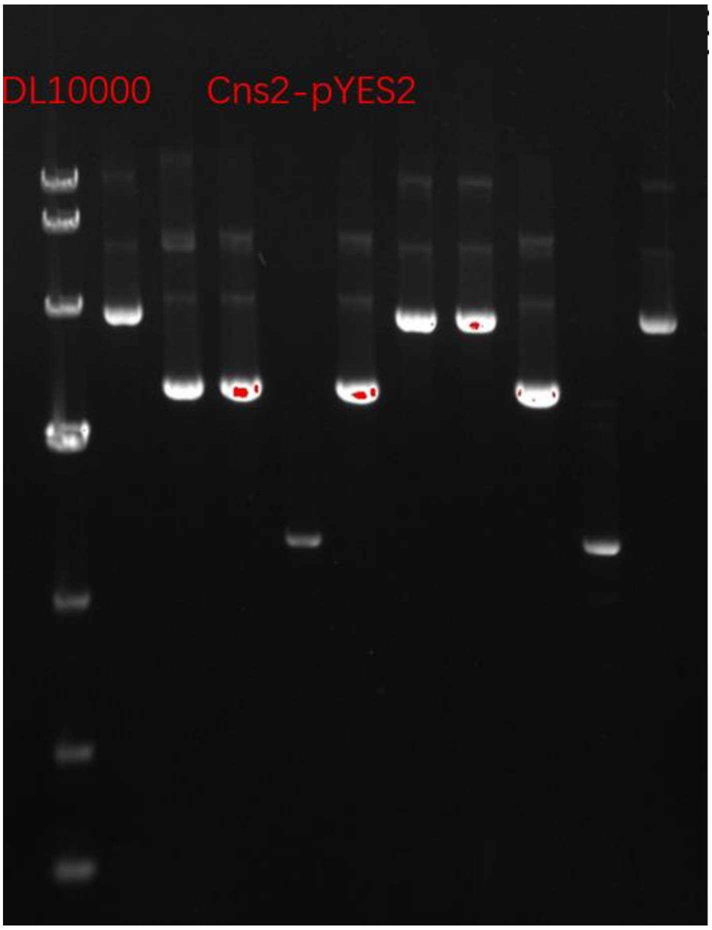
1.plasmids extraction

2.stock the bacterial glycerol

1V bacteria liquid into 1V 50% glycerin of each and mix up

results:

1.plasmids extraction



cns2-pYES2 plasmids

conclusions: some of the plasmids are not correct, the correct plasmids are 2、3、5、8

next step: Liquid culture to get more right plasmids

7.2nd Tuesday

participator: Zhang Chenming Qiu Qinling

purpose: get the colony which has cns2-pYES2

process:

1.resuscitation culture glycerol bacteria (cns2)

results: -

conclusions: -

next step: digest to get cns2 gene

7.3rd Wednesday

participator: Zhang Chenming Qiu Qinling

purpose: get cns2 gene, and insert it into pYES2

process:

1.digest

Quick buffer (10x)	2.5uL
Plasmid	4uL

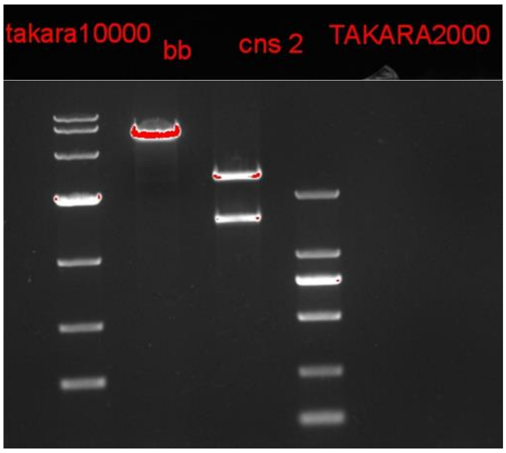
EcoR I	1uL
Xho I	1uL
ddH2O	16.5uL
Total	25uL

2.gel extraction

3.ligase

T4 ligase	1uL	
10 X buffer	2uL	
ddH2O	6.7uL	
cns2	3.0uL	13.08ng/uL
pYES2	7.3uL	6.9ng/uL
total	20uL	

results:



gel extraction of cns2 & BB

conclusions: we get cns2 fragment, and we can do next step

next step: transform ligase product into E.coli DH5α.

7.4th Thursday

participator: Zhang Chenming Qiu Qinling

purpose: Transform the ligase product into DH5a and cultivate it on the LB solid medium with A+ to select monoclonal colony. After this we will choose some monoclonal colony and hydroponics to attain more plasmids for next stage.

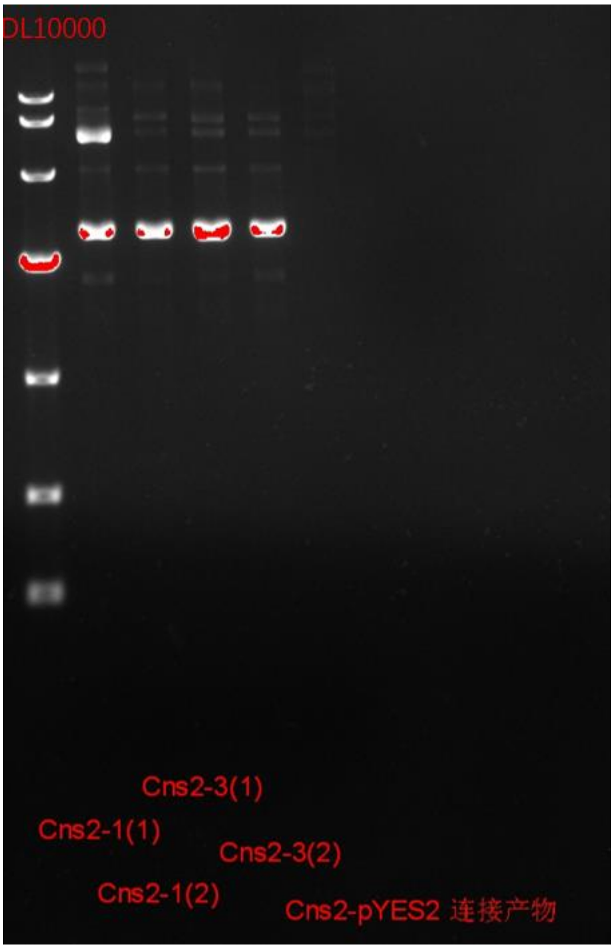
process:

1.transform

15uL the ligase product with 100uL Trans5a

42° C from 90s, 37° C 180 rmp from 1h

2. hydroponics E.coli which have cns1-PUC
3. plasmid extraction to get cns2-pYES2
results:



cns2 ligase products
conclusions: we might get wrong plasmids
next step: transform cns2-pYES2;digest cns1-PUC to get cns1 fragment

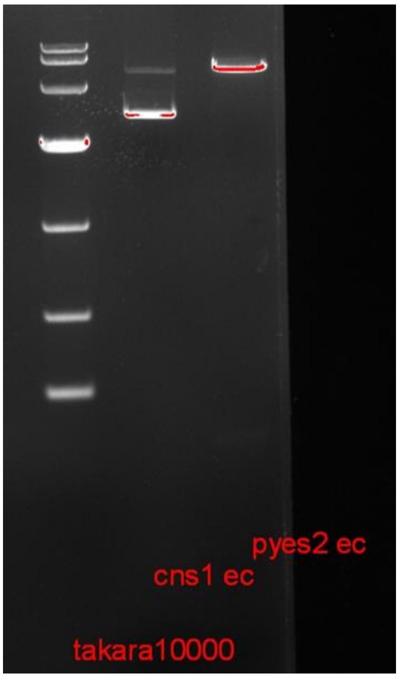
7.8th Monday
participator: Zhang Chenming　Qiu Qinling
purpose: get the colony which has cns2-pYES2;get cns1 fragment
process:
1. transform
2. digest

Quick buffer (10x)	2.5uL
cns1-PUC	4uL
Hind III	1uL
EcoR I	1uL
ddH2O	16.5uL

Total	25uL

Quick buffer （10x）	2.5uL
pYES2-NTA	4uL
Hind III	1uL
EcoR I	1uL
ddH2O	16.5uL
Total	25uL

results:



cns1-puc、pyes2 gel extraction

conclusions: failed, because cns1 can not be separated

next step: verify pYES2-cns2; attain more plasmids

7.9th Tuesday

participator: Zhang Chenming Qiu Qinling

purpose: verify pYES2-cns2 by CPCR and digest; attain more pYES2-NTA、cns1-PUC

process:

1.CPCR

	Volume
T5 Super Mix	5uL

F	0.5uL
R	0.5uL
colony	2ul
ddH2O	2uL

Procedure	T	time
Initial denaturation	98℃	3min
Denaturation	98℃	10s
Annealing	50℃	10s
Extension	72℃	20s
Final extension	72℃	5min
	4℃	Hold
2-4 repeat 35times		

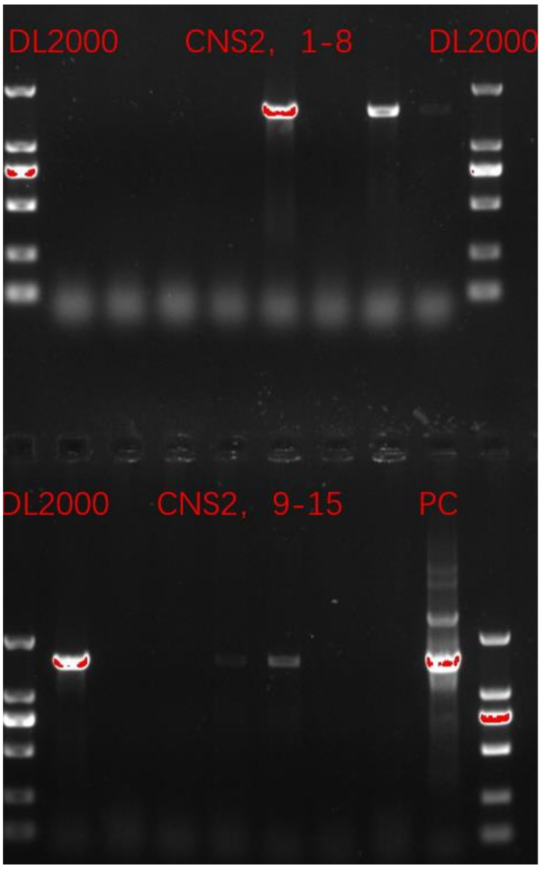
2.digest

Quick buffer（10x）	1.5uL
Plasmid	2uL
EcoR I	0.7uL
Xho I	0.7uL
ddH2O	10.5uL
Total	15uL

3.hydroponics E.coli which have cns1-PUC or pYES2-NTA

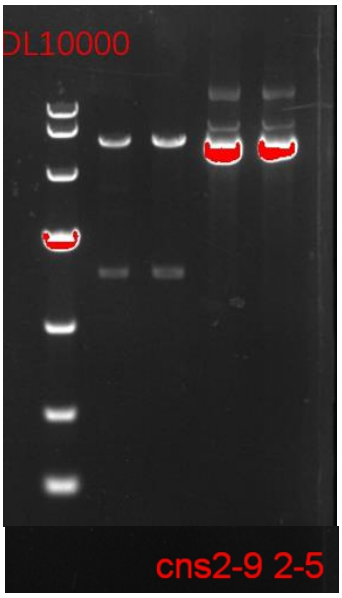
results:

1.CPCR



cns2-pYES2 CPCR

2. digest



cns2-9, 2-5 digest

conclusions: cns2-9, cns2-5 are correct

next step: Liquid culture and stock the E. coli

7.10th Wednesday

participator: Zhang Chenming Qiu Qinling

purpose: extract plasmid and stock the E. coli with the right plasmid

process:

1. plasmid extraction
we attain pYES2-cns2 & cns1-PUC & pYES2-NTA
2.stock the bacterial glycerol
we stock the E.coli with cns1-PUC
results: -
conclusions: -
next step: plasmid extraction

7.12th Friday
participator: Zhang Chenming Qiu Qinling
purpose: attain more cns1 & cns2 fragment for next step
process:
1.PCR

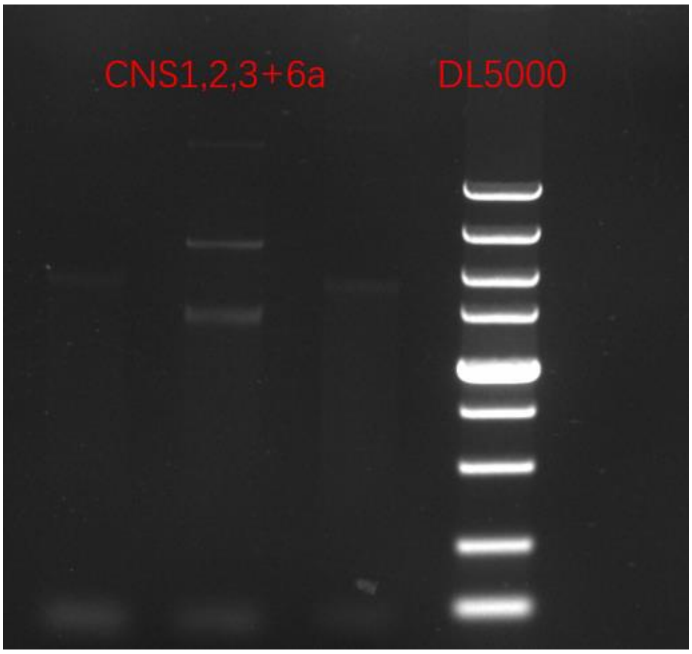
	Volume
T5 Super Mix	25uL
cns1+aF	1uL
puc-SP 通用 R	1uL
Template	1uL
ddH2O	22uL

	Volume
T5 Super Mix	25uL
cns2+aF	1uL
puc-SP 通用 R	1uL
Template	1uL
ddH2O	22uL

Procedure	T	time
Initial denaturation	98℃	3min
Denaturation	98℃	10s
Annealing	62℃	15s

Extension	68℃	3min
Final extension	68℃	5min
	4℃	Hold
2-4 repeat 30times		

results:



cns1，2，3+6a PCR

conclusions: failed

next step: re PCR

7.16th Tuesday

participator: Zhang Chenming Qiu Qinling

purpose:

process:

1.PCR

	Volume
T5 Super Mix	25uL
cns1+aF	1uL
puc-SP 通用 R	1uL
Template	1uL
ddH2O	22uL

	Volume
T5 Super Mix	25uL
cns2+aF	1uL
puc-SP 通用R	1uL
Template	1uL
ddH2O	22uL

Procedure	T	time
Initial denaturation	98℃	3min
Denaturation	98℃	10s
Annealing	57℃	15s
Extension	68℃	2min
Final extension	68℃	5min
	4℃	Hold
2-4 repeat 35times		

cns1 try two step PCR

2. cycle pure

Cns1-1	98.13ng/uL
Cns1-2	98.50ng/uL

3. digest

Quick buffer（10x）	2.5uL
Cns1	2uL
EcoR I	1uL
Hind III	1uL
ddH2O	18.5uL
Total	25uL

Quick buffer （10x）	2.5uL
pYES2-NTA	4uL
EcoR I	1uL
Hind III	1uL
ddH2O	16.5uL
Total	25uL

4.gel extraction
5.ligase

Buffer	4L	
Cns1	17uL	4ng/uL
BB	14uL	
T4 Ligase	2uL	
ddH2O	3uL	
Total	40uL	

16° C for 12h+
results: -
conclusions: -
next step: cns2-pYES2 verity by digest ;transform cns1-pYES2 into DH5a

7.17th Wednesday
participator: Zhang Chenming Qiu Qinling
purpose: verity cns2-pYES2;get single colony of cns1-pYES2
process:
1.transform
2.digest

Quick buffer （10x）	5uL
fragment	7.8uL
Mfe I	1uL
ddH2O	36.2uL
Total	50uL

3.ligase
results: -

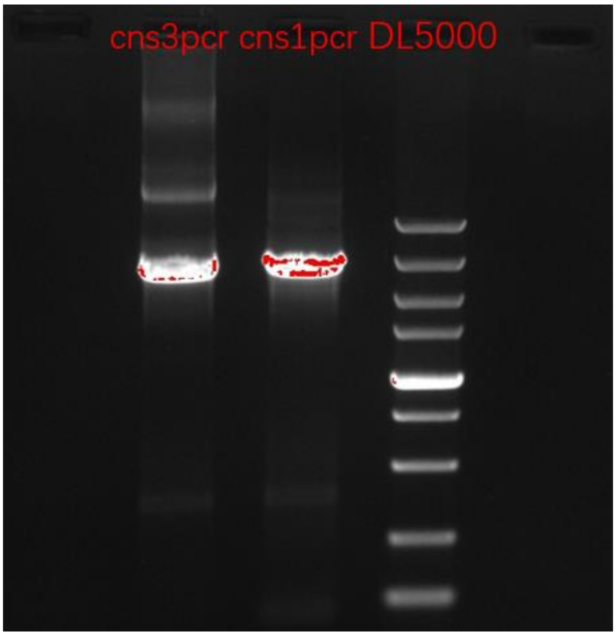
conclusions: -
next step: transform

7.18th Thursday
participator: Zhang Chenming Qiu Qinling
purpose:
process:
1. PCR

	Volume
T5 Super Mix	5uL
F	0.5uL
R	0.5uL
Template	0.5uL
ddH2O	3.5uL

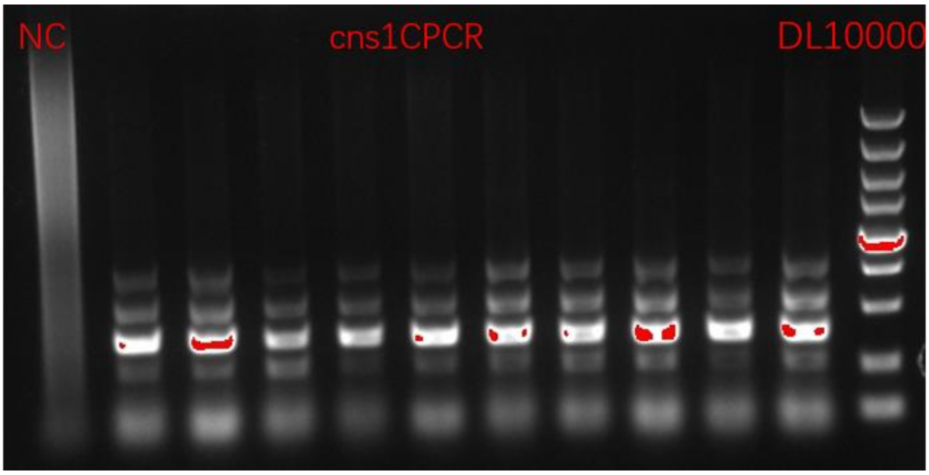
Procedure	T	time
Initial denaturation	98℃	3min
Denaturation	98℃	10s
Annealing	57℃	10s
Extension	72℃	6s
Final extension	72℃	3min
	4℃	Hold
2-4 repeat 39times		

results:



cns1&cns3 PCR
conclusions: -
next step: digest

7.19th Friday
participator: Zhang Chenming Qiu Qinling
purpose: verity the transform
process:
1.CPCR
results:
1.CPCR



cns1 colony PCR
conclusions: -
next step: Liquid culture

7.21st Sunday
participator: Zhang Chenming Qiu Qinling、罗雅欣
purpose: verity cns1-pYES2 & cns2-pYES2
process:

1. digest

Quick buffer (10x)	5uL
Fragment	10uL
Mfe I	1uL
ddH2O	34uL
Total	50uL

37° C for 1h

Quick buffer (10x)	5uL
fragment	15uL
Xho I	1uL
ddH2O	29uL
Total	50uL

results: -
conclusions: -
next step: transform

7.24th Wednesday
purpose: construct the pYES2-CNS1 plasmid
process:
1. perform enzyme digestion on pYES2 plasmid and the PCR production of CNS1 gene

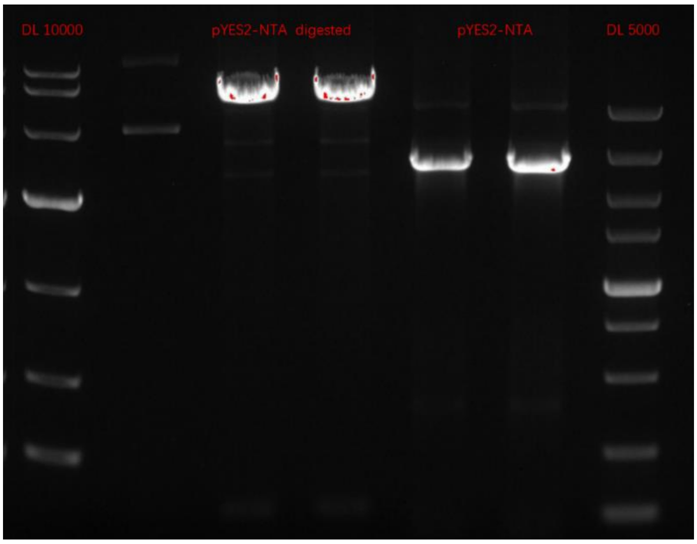
Quick buffer (10x)	5uL
pYES2/ CNS1	20uL
EcoR I	1uL
Hind III	1 uL
DdH2O	23uL
Total	50uL

2. perform agarose electrophoresis on digestion products to check the production and purify it
3. using E.Z.N.A.® Gel Extraction Kit to rescue and purify the production
4. perform ligation reaction on the rescued products

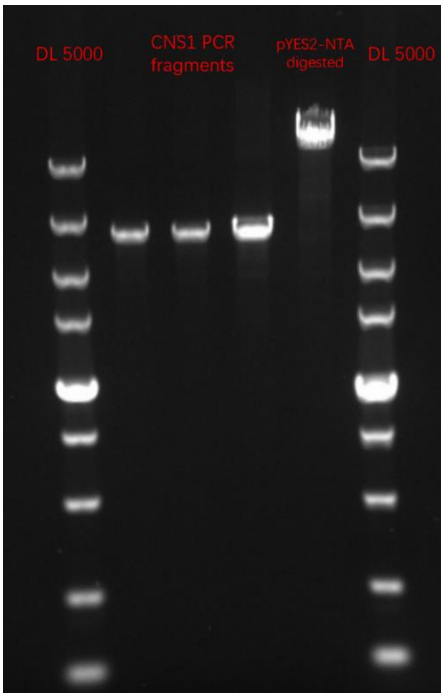
Ligation Buffer (10x)	2uL	
pYES2	3uL	23ng/uL
CNS1	9uL	10ng/uL
T4 ligase	1uL	
ddH2O	5uL	
Total	20uL	

results:

pYES2-NTA digested



CNS1 PCR fragments



conclusions: finished constructing of plasmids pYES2-CNS1

next step: Transform the plasmids pYES2-CNS1 into DH5-α

7.25th， Thursday

purpose: Transform the plasmids pYES2-CNS1 into DH5α

process:

1. transform

results: -

conclusions: successfully transformed the plasmids pYES2-CNS1 into DH5α

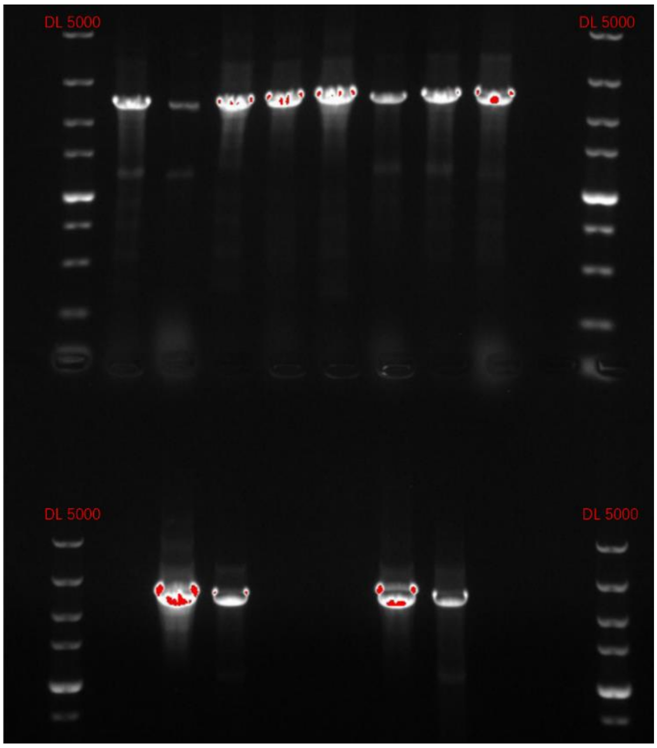
next step: Transform the plasmids pYES2-CNS1 into DH5α.

7.26th，Friday

Purpose: using PCR(colony) to identify the positive colony.
Process: 10uL ddH20 for dissolve the colony ,and 2uL of the solution for PCR reaction.
Colony PCR system:

T5mix	5uL	Process:	98℃	3min
Primer F	0.5uL		98℃	10s
Primer R	0.5uL		50℃	10s
Template	2uL		72℃	1min
ddH20	2uL		72℃	5min
Total	10uL		4℃	hold

Results:

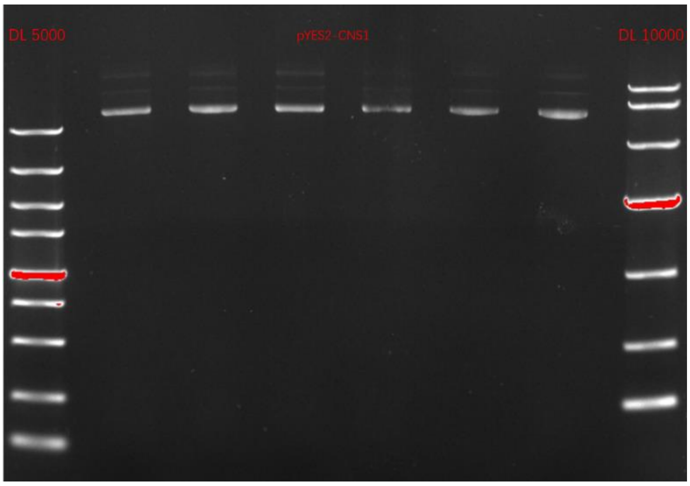


PCRcolony pYES2-CNS1
Conclusions: successfully identified the positive colonies.
Next step: extract plasmids pYES2-CNS1 from the positive colonies

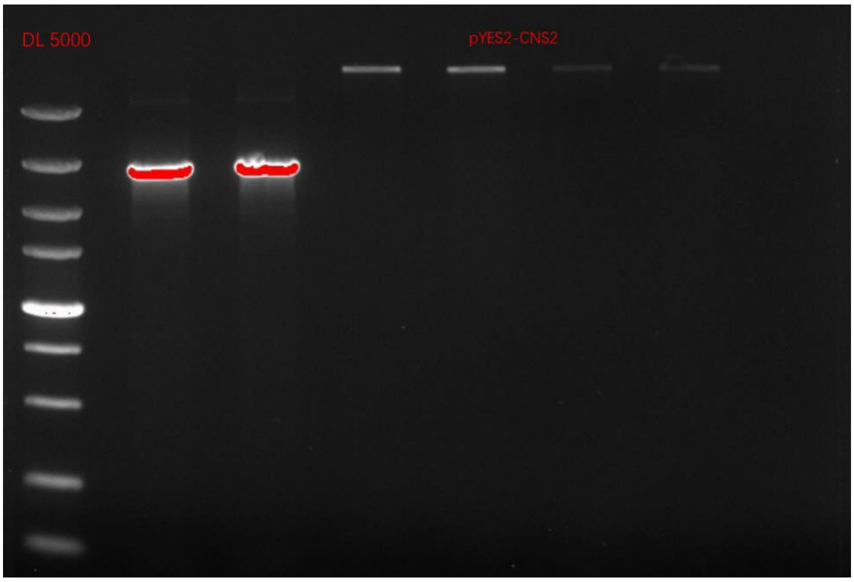
7.27th，Saturday
Purpose: extract plasmids pYES2-CNS1 from the positive colonies.
Process:
liquid culture the positive colony identified yesterday for ~8 hours
using E.Z.N.A.® Plasmid Mini Kit I to extract and purify plasmids from the liquid culture.
using agarose electrophoresis(purification) to identify and purification the plasmid.
using E.Z.N.A.® Gel Extraction Kit to rescue and purification the plasmid.

Results:

①pYES2-CNS1



pYES2-CNS2



Conclusions: successfully extracted and purified the right-connected plasmids.

Next step: extract plasmid pYES2-CNS2 from the positive colony

7.28th, Sunday

Purpose:

1.extract plasmid pYES2-CNS2 from the positive colony using the preserved culture in the 4 ℃ fridge(positive colony No.6 & 9)

2.send the CNS1-pYES2 sample to Tsingke to sequencing it (CNS1-1: 3&4&5; CNS1-2: 2&3&6)

3.double enzyme digest plasmid CNS2-pYES2& CNS1-pYES2 to prepare for the construction of plasmid CNS1-CNS2-pYES2

Process:

send the CNS1-pYES2 sample to Tsingke to sequencing it (CNS1-1: 3&4&5; CNS1-2: 2&3&6)

using E.Z.N.A.® Plasmid Mini Kit I to extract and purify plasmid from the 2X2X2.5 ml liquid culture (cns2-pYES2-6/9-1/2)

double enzyme digestion of plasmid CNS2-pYES2

Quick buffer （10x）	2uL
pYES2/ CNS1	8uL
EcoR I	1uL
Hind III	1 uL
DdH2O	8uL
Total	20uL
process	37℃× 40min

PYES2-CNS2-6-2&9-2

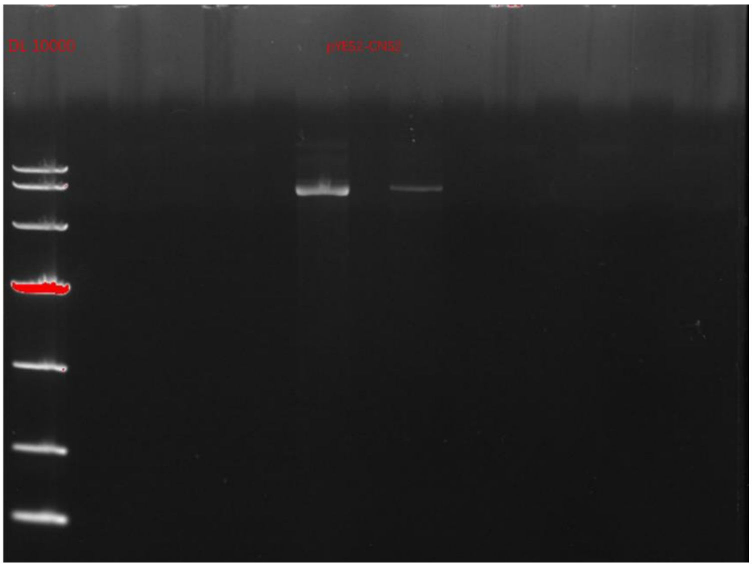
Quick buffer （10x）	2uL
pYES2/ CNS1	4uL
EcoR I	1uL
Hind III	1 uL
DdH2O	12uL
Total	20uL
process	37℃× 40min

using agarose electrophoresis(purification) to identify and purification the plasmid
using E.Z.N.A.® Gel Extraction Kit to rescue and purification the plasmid
using agarose electrophoresis (verification) to measure the concentration
ligation reaction（insert CNS1 fragment into CNS2-pYES2）,
stay overnight stockd in 16℃ .

10×Buffer	2uL
Backbone 1(CNS2-pYES2)	3.3uL
CNS1-1	6.4uL
T4 ligase	1uL
ddH2O	7.3uL
Total	20uL

10×Buffer	2uL
Backbone 2(CNS2-pYES2)	7.1uL
CNS1-1	6.8uL
T4 ligase	1uL
ddH2O	3.1uL
Total	20uL

Results:
pYES2-CNS2-69



Conclusions: the concentration of the digestion products is too low to carry out the ligation reaction
the sequencing result suggested that we have constructed CNS1-pYES2 successfully.
Next step: redigesting the plasmid CNS2-pYES2 to construct the plasmid CNS1-CNS2-pYES2.

7.29th，Monday

Purpose: redigesting the plasmid CNS2-pYES2 and constructing the plasmid CNS1-CNS2-pYES2
Process:
using E.Z.N.A.® Plasmid Mini Kit I to extract and purify plasmid from the 2X2X2.5 ml liquid culture （cns2-pYES2-6/9-1/2）
double enzyme digest the plasmid CNS2-pYES2
L6-1&9-1

Quick buffer （10x）	2uL

pYES2/ CNS1	8uL
EcoR I	1uL
Hind III	1 uL
DdH2O	8uL
Total	20uL
Procedure	37℃× 40min

L6-2&9-2

Quick buffer （10x）	2uL
pYES2/ CNS1	4uL
EcoR I	1uL
Hind III	1 uL
DdH2O	12uL
Total	20uL
Procedure	37℃× 40min

using agarose electrophoresis(purification) to identify and purification the plasmid
using E.Z.N.A.® Gel Extraction Kit to rescue and purification the plasmid
using agarose electrophoresis(verification) to measure the concentration
3.ligation reaction（insert CNS1 fragment into CNS2-pYES2）, stay overnight stockd in 16℃ .
L6-1&9-1

Buffer	2uL
Backbone 1	3.3uL
CNS1-1	6.4uL
T4 ligase	1uL
ddH2O	7.3uL
Total	20uL

L6-2&9-2

Buffer	2uL

Backbone 2	7.1uL
CNS1-1	6.8uL
T4 ligase	1uL
ddH2O	3.1uL
Total	20uL

Next steps: Transfer plasmid CNS1-CNS2-Pyes2 into DH5α and liquid culture.

7.30th，Tuesday

Process:

1.Transition: CNS1-2-pYES2 （1-① 1-2；1-②；NC）

joining plasmids (20uL) → 50uL competent cells

YPD liquid culture for 1 hour

280uL ddH2O attenuate

spread plate with 100uL.

results: plate lost

conclusions：successfully transformed the plasmid into DH-5a

7.31，Wednesday

Purpose：transformation verification, insurance for failed connection/transformation reaction

Process：

Colony PCR

	1	2
2X T5 Super Mix	12.5	12.5
cns1-aF	1	1
pUC-SPR 通用	1	1
Template	1（1+colony）	1（1+colony）
ddH2O	9.5	9.6
total	25	25
sample	CNS1-2-PYES2-1-2 ①	CNS1-2-PYES2-1-2 ②

process	Temperature	Time

Initial Denaturation	98℃	2min
Denaturation	98℃	10s
Annealing	58℃	10s
Extension	72℃	15s
Final Extension	72℃	5min
Hold	4℃	Forever

Transformation

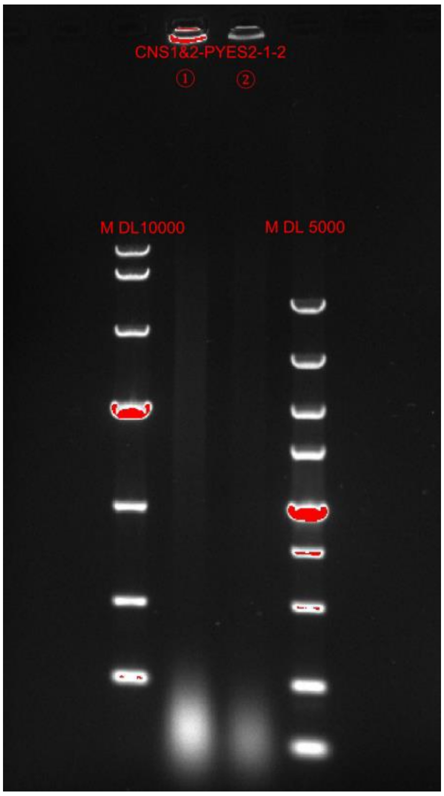
PYES2-CNS1-CNS2-1-1-1	PYES2-CNS1-CNS2-1-1-2	PYES2-CNS1-CNS2-1-2	NC

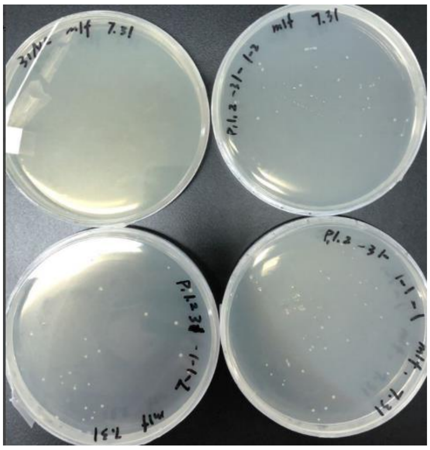
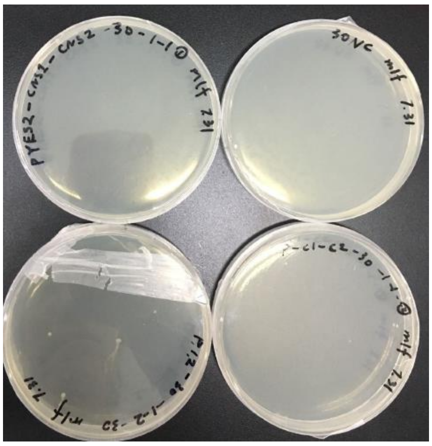
Spread plate

sample 0730	PYES2-CNS1-CNS2-1-1-1	PYES2-CNS1-CNS2-1-1-2	PYES2-CNS1-CNS2-1-2	NC
sample 0731	PYES2-CNS1-CNS2-1-1-1	PYES2-CNS1-CNS2-1-1-2	PYES2-CNS1-CNS2-1-2	NC

Results:

PCR(colony)-false positive



Conclusions: probably caused by transformation mistakes.
Next step: PCR (colony) to select positive colonies

8.1st, Thursday
Purpose: Colony-PCR o select positive colonies
Process:
1.PCR(colony) process

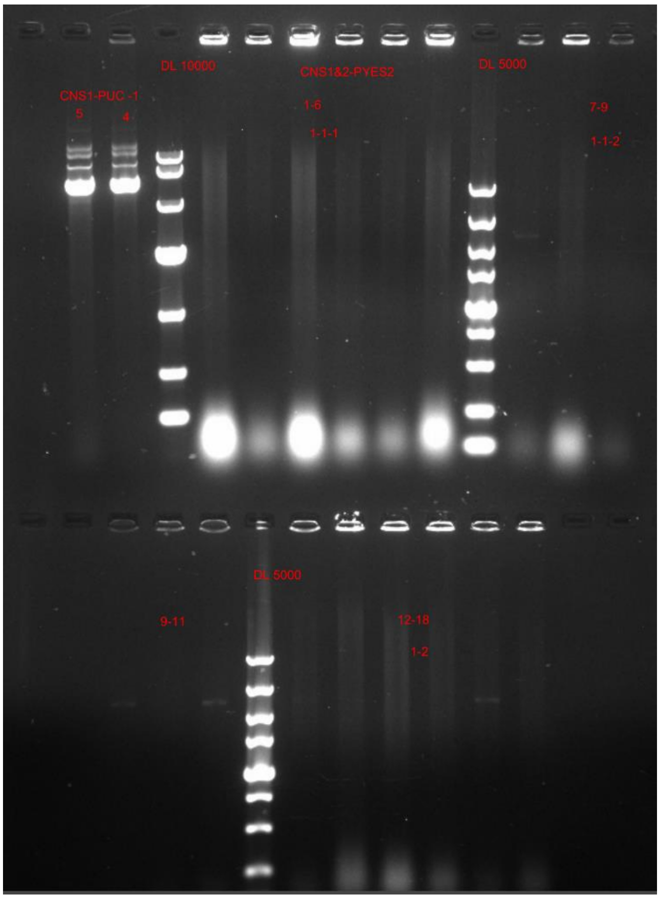
process	Temperature	Time
Initial Denaturation	98	2min
Denaturation	98	10s
Annealing	58	10s
Extension	72	15s
Final Extension	72	5min
Hold	4	Forever

	1
2X T5 Super Mix	10
cns1-aF	1
CYCter-R	1
Template	1 (1+colony)
ddH2O	7
total	20

liquid culture

1-1-2 ①	1-1-2 ②	1-1-2 ③	1-1-2 ④
1-2 ①	1-2 ②	1-2 ③	1-2 ④

Results:
4 positive colonies （2-1、2-4、2-6、3-5）



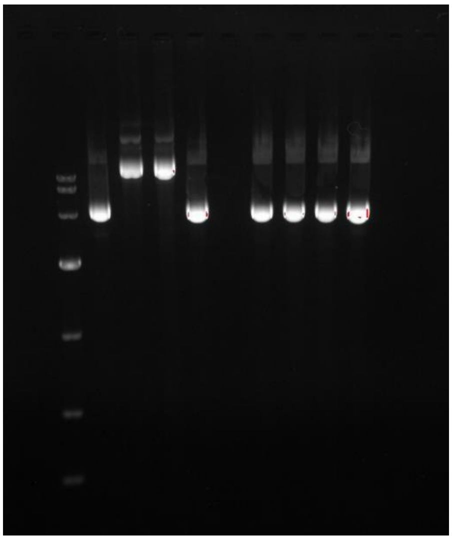
Conclusions: successfully selected the positive colonies
Next step: Plasmid extraction、Sequencing

8.2nd，Friday
Purpose: Plasmid extraction, then digest the plasmid by Hind III and Xho I
Process:
Plasmid extraction 8 samples
Digestion system

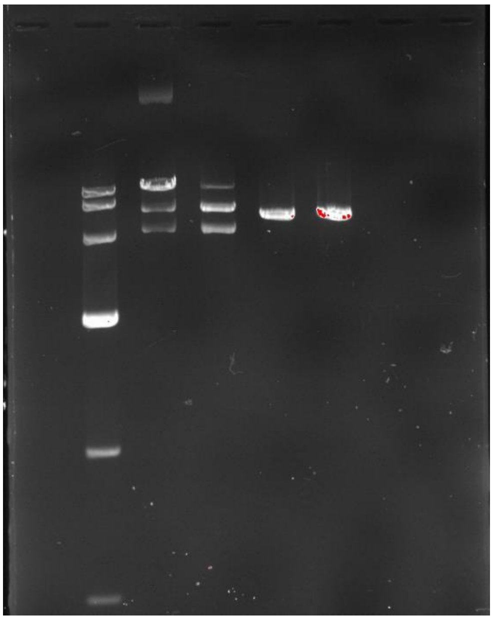
	1-1-2 ④	1-1-2 ⑤
Hind III	0.5	0.5
Xho I	0.5	0.5
ddH2O	4	4

Template	4	4
10X Green Buffer	1	1
Total	10	10

Results:
plasmid extraction check



DL1000,1-2④，1-2⑤，1-1-2①，1-1-2⑥



positive sample sent to sequencing
Conclusions: 1-2④，1-2⑤ positive
Next step: transformation

8.3rd，Saturday
Purpose: Digest to verify pYES2-cns2
Process:
1st Digestion

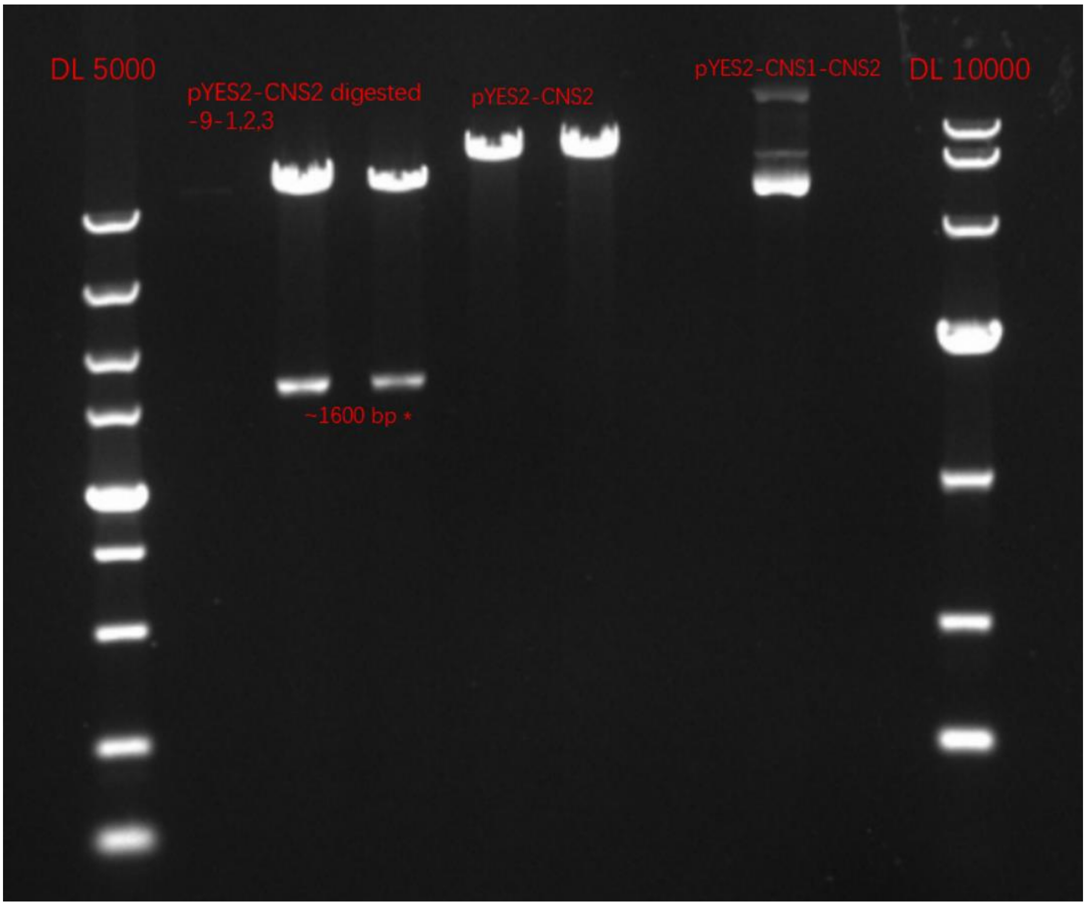
	2-3	2-6	2-9
Hind III	1	1	
Xho I	1	1	
ddH2O	14	14	
Template	2	2	
10X Green buffer	2	2	
Total	20	20	

2. 2nd Digestion

	2-9-1	2-9-2	2-9-3	NC1	NC2	NC3	NC4	NC5
Hind III	1	1	1	1	0	0	0	0
Xho I	1	1	1	0	1	0	0	0
ddH2O	14	14	14	15	15	18	16	20
Template	2	2	2	2	2	0	2	0
10X Green buffer	2	2	2	2	2	2	2	0
Total	20	20	20	20	20	20	20	20

Results：

2nd digestion



Conclusions: pYES2-Cns2 is correct contenting GAL1 and T7 promoter.
Next step: sequencing the pYES2-Cns1-Cns2 plasmid and start the construction of pYES2-Cns1ACns2

8.5th, Monday

Purpose: cut off the terminator of cns1 and promoter of cns2 then link the terminal together for the fusion protein gene of cns1Acns2.

Process:

for the reason that Mef I need longer time to be digested, we choose to digest EcoR I first, then Mef I.

Digest pYES2-CNS1-CNS2 by EcoR I

Quick buffer （10x）	2uL
pYES2-CNS1-CNS2	4uL
EcoR I	1uL
ddH2O	13uL
Total	20uL

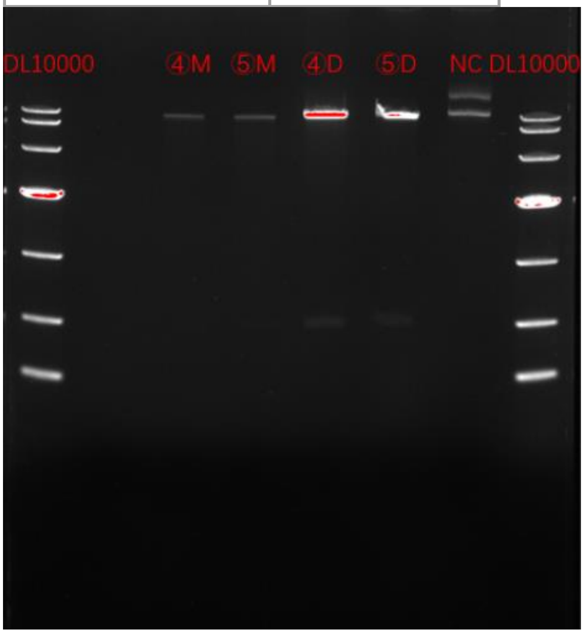
2. Cycle pure

Mistakes:

First add digest productions into columns then add in CP buffer and vortex to mix them up. (The correct step should be mix them up in EP columns then add the mix into the colunms for filter.)
Finally get 25uL solution/column.
3. Double enzyme digestion
Digest pYES2-CNS1-CNS2 by EcoRI and MfeI (classical digestion system)
we find the enzymes produced by NEB that can digest both sites together.

8.6th，Tuesday
Purpose：digest pYES2-CNS1-CNS2 by EcoRI and MfeI, then ligase the product by T4 DNA ligase（NEB）.
Process：
1. Gel Extraction，then one drop measured concentration

name	concentration
P12MED（D1）	11.31ng/ul
P12MED（D2）	9.61ng/ul
P12MEM3（M3）	6.46ng/ul
P12MEM	3.07ng/ul



M=only digested by EcoR I; D=digested by EcoR I&Mfe I
CNS12-PYES2-EcoRI MfeI Digest
2. ligase

	D1-①	D1-②	D2-①	D2-②	M3
T4 ligase	1ul	1ul	1ul	1ul	1ul
10 X buffer	2ul	2ul	2ul	2ul	2ul

ddH2O	12ul	12ul	15ul	12ul	12ul
template	5ul	5ul	2ul	5ul	5ul
total	20ul	20ul	20ul	20ul	20ul

Next step:
transform the ligase product into E.coli DH5α.
transform pYES2-CNS1 and pYES2-CNS2 into E.coli DH5α.
liquid culture to attain more plasmids for next stage.

8.7th, Wednesday

Purpose: Transform the ligase product、pYES2-CNS1 and pYES2-CNS2 into DH5a and cultivate it on the LB solid medium with A+ to select monoclonal colony. After this we will choose some monoclonal colony and hydroponics to attain more plasmids for next stage.

Process:

1.Transform linked plasmids product、pYES2-CNS1 and pYES2-CNS2

	pYES2-cns1A2①	pYES2-cns1A2②	pYES2-cns1A2③	pYES2-cns1A2④	pYES2-cns1A2⑤	PC（pYES2-NTA）	NC（ddH2O）	pYES2-CNS1	pYES2-CNS2
DH5a	50ul	50ul	50ul	50ul	33ul	33ul	33ul	50ul	50ul
plasmid	20ul	20ul	20ul	20ul	20ul	10ul	10ul	20ul	20ul

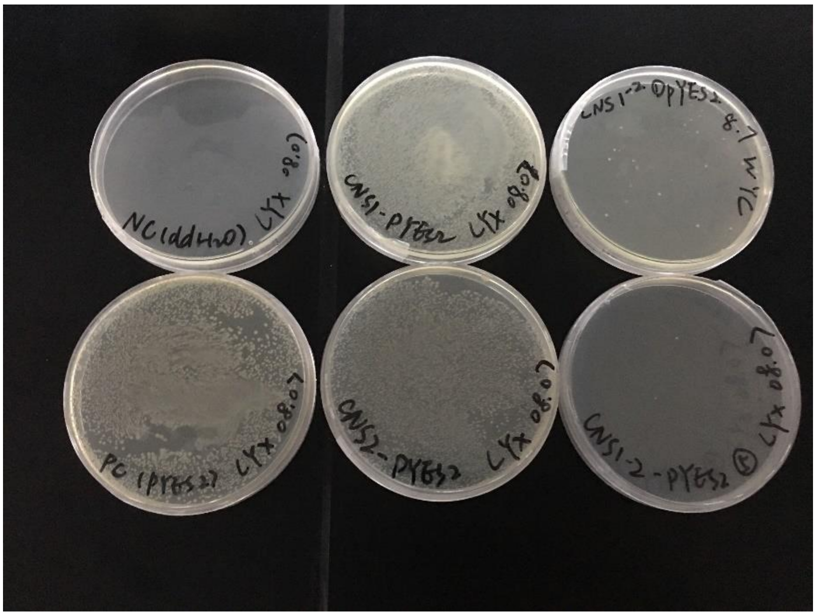
Mistake: We should just use 1ul-2ul pYES2-CNS1、pYES2-CNS2 and pYES2-NAT in transformation, because they are plasmid not the ligase product.

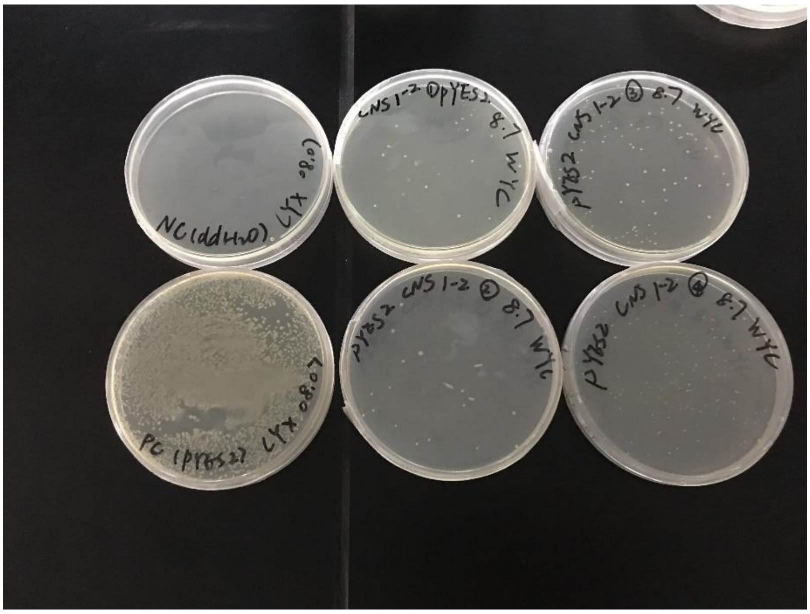
2.Spread plate

We used nine LB solid medium with A+, then 37° C, 20.5h.

Results:

from top to bottom, from left to right: NC、PC、pYES2-CNS1、pYES2-CNS2、pYES2-CNS1ACNS2①、pYES2-CNS1ACNS2⑤





As we can see , PC、pYES2-CNS1、pYES2-CNS2 have too many colonies.NC doesn't have colony, which is a nice result. All pYES2-CNS1-2 have colonies, not too much, but this result is in our expectation.

Conclusions: We don’t know if the transformation was successful, so we need do colony PCR at next step.

Next step:

1、Colony PCR to verify the positive transformation.

2、Hydroponics Extract plasmid pYES2-CNS1-2、pYES2-CNS1 and pYES2-CNS2 .

3、Digest pYES2-CNS1-2 by BamH I, after this ligase the digested product.

8.8th，Thursday

Purpose:

① attain sufficient **cns1*cns2 plasmids(digested by EcoR I & Mef I)**, carry out another digestion by BamH I and ligation to get the final pYES2-cns1Acns2 plasmid.

② enlarge the quantities of pYES2-CNS1 、pYES2-CNS2 plasmids by hydroponics the colonies.

process:

1.Choose monoclonal colony for colony PCR and liquid culture

pYES2-CNS1*2 (①-⑤)×2; pYES2-CNS1 & pYES2-CNS2 ×3/per; PC×2;

	Volume
T5 Super Mix	10uL
T7F	1uL
CYCterR	1uL
colony	touch
ddH2O	8uL
Total	20uL

Procedure	T	time
Initial denaturation	98℃	3min
Denaturation	98℃	10s
Annealing	50℃	10s
Extension	72℃	1min30s
Final extension	72℃	5min
	4℃	Hold
2-4 repeat 35times		

2. gel electrophoresis

20 samples each 5ul and we used DL2000 (for cns2 and PC), DL5000 (for cns1 and cns1a2)

3. plasmid extraction (from 8h hydroponics)

4. mono-enzyme digestion CNS1A2 by BamH I, then gel extraction

	cns1a2, 1-①	cns1a2, 1-②	cns1a2, 2-①	cns1a2, 2-②	cns1a2, 5-②
BamH I	1uL	1uL	1uL	1uL	1uL
ddH2O	13uL	13uL	13uL	13uL	13uL
Template	4uL	4uL	4uL	4uL	4uL

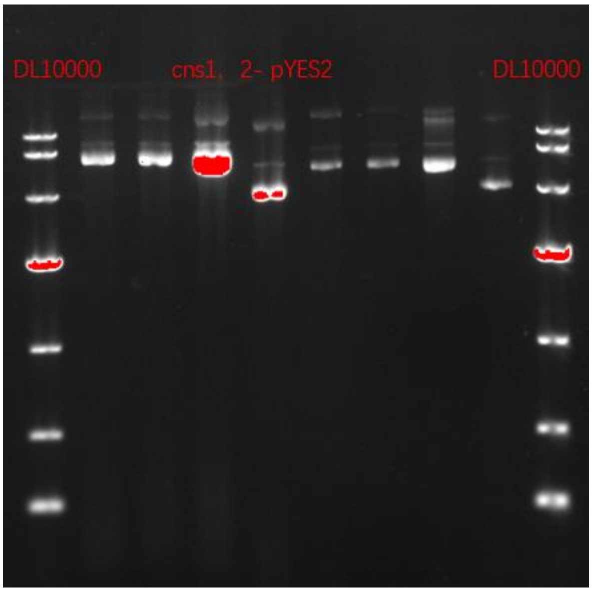
10X Green buffer	2uL	2uL	2uL	2uL	2uL
Total	20uL	20uL	20uL	20uL	20uL

5. joining（CNS1*2，self）

	CNS1A2①	CNS1A2②	CNS1A2③	CNS1A2④	CNS1A2⑤
Buffer	2ul	2ul	2ul	2ul	2ul
DNA	4ul	3.8ul	3ul	2.2ul	4.1ul
T4 Ligase	1ul	1ul	1ul	1ul	1ul
ddH2O	13ul	13.2ul	14ul	14.8ul	12.9ul
Total	20ul	20ul	20ul	20ul	20ul

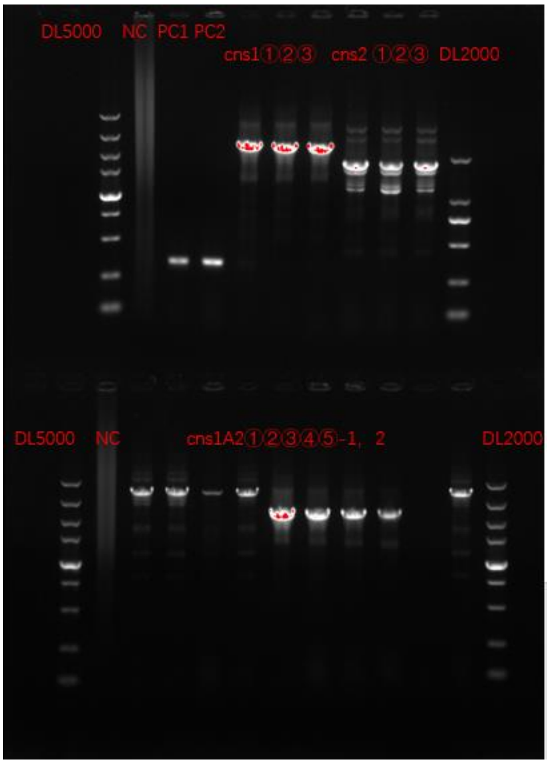
Results:

1.plasmids extraction



cns1,2-pYES2

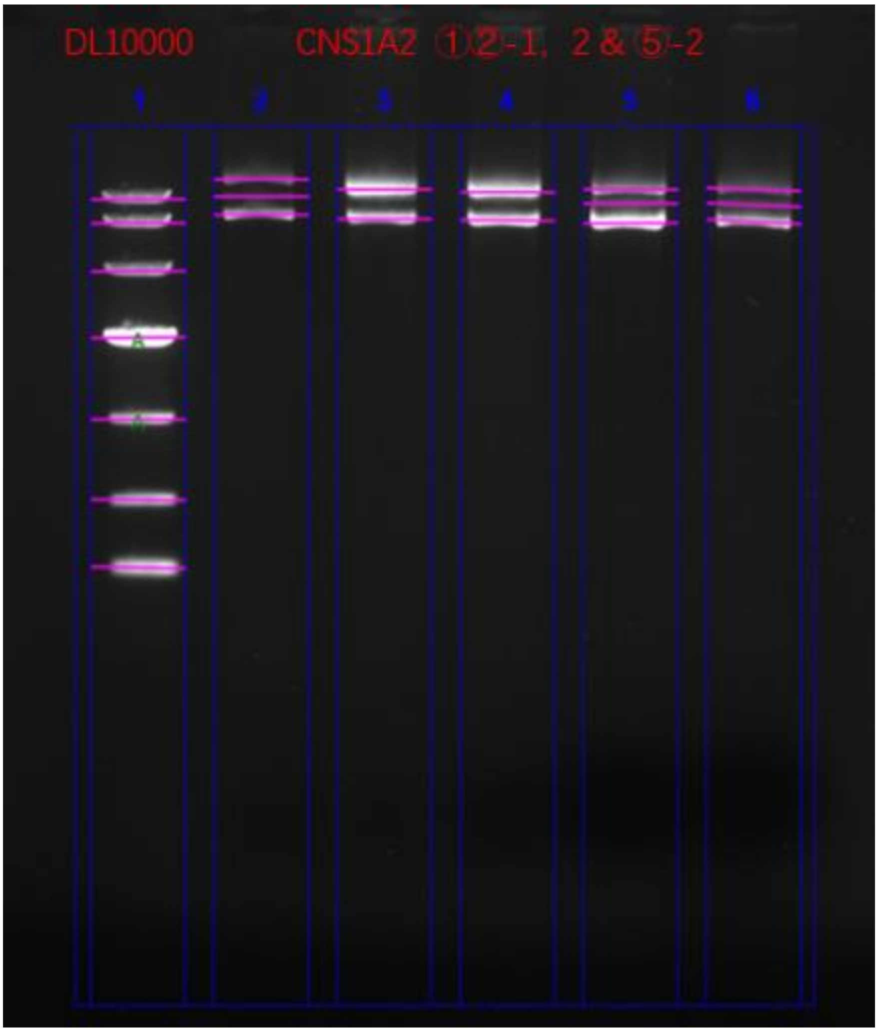
2. gel electrophoresis



CPCR cns1&cns2&cns1A2

only CNS1A2 ①②-1，2 & ⑤-2；and CNS1&CNS2-①，②，③ are correct

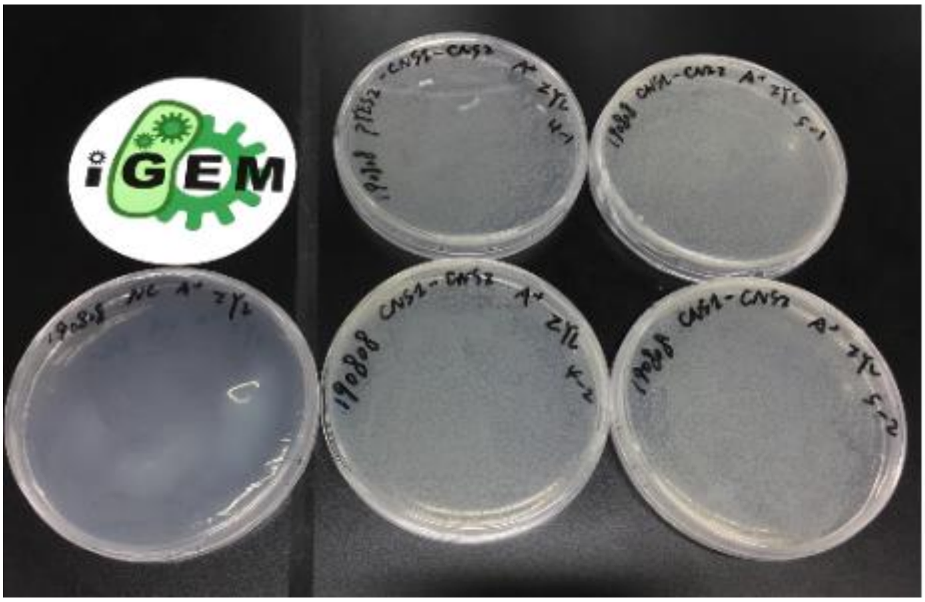
4. monoenzym digestion & gel extraction



PURI of cns1acns2

All sample had some failure digestion.

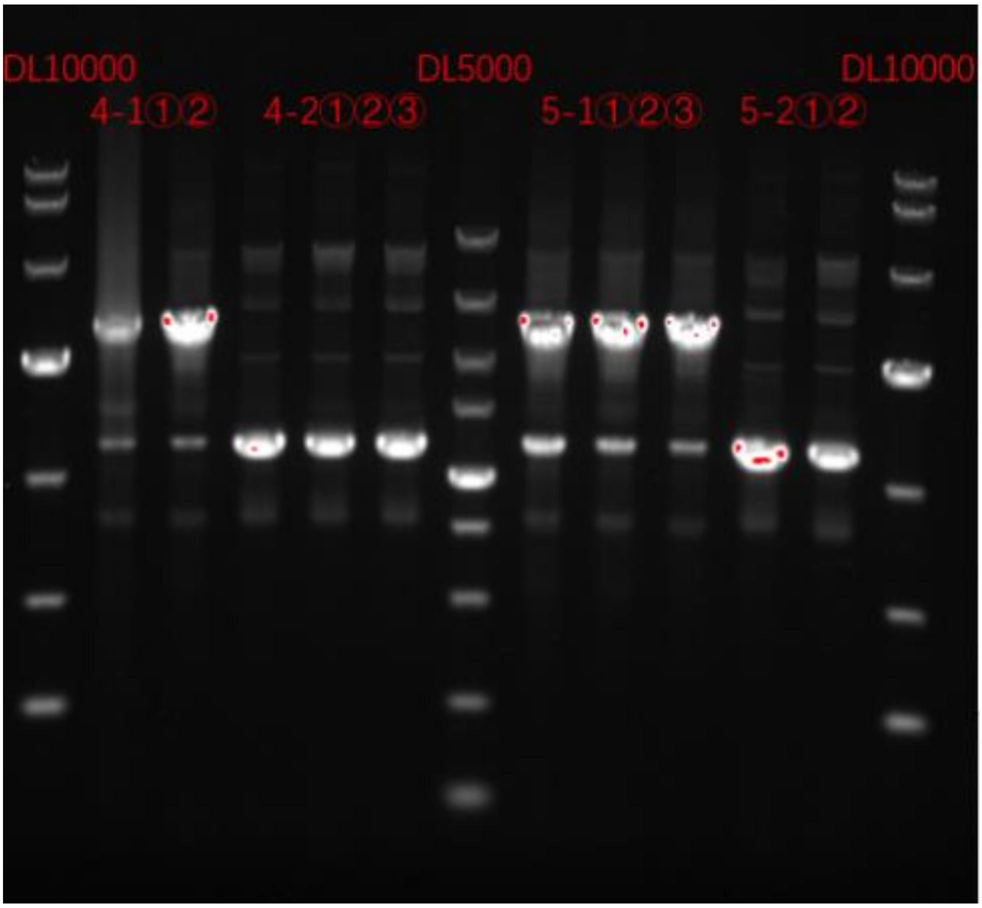
6. another transformation (CNS1-2), 37℃, 8-9h



conclusions:

pYES2-CNS1-2's transformation is successful, but next time if we want transform plasmid we can only use 50ul bacterial fluid without centrifugation.
Next step:
Transform plasmids CNS1*2; pick and enlarge CNS1-2 transformed colonies; extract pure plasmids CNS1-2 and CNS1, CNS2.

8.9th，Friday
Purpose:
transform plasmids CNS1*2 to get amplified constructed plasmid CNS1A2-pYES2; ②purify plasmid CNS1-2-pYES2.
Process:
1.transform plasmids CNS1*2-pYES2 into Trans5α, culture in A+ plates in 37℃.
(CNS1*2-pYES2 ①-⑤+NC)
2.pick single colonies from CNS1-2-pYES2 (4-1; 4-2; 5-1; 5-2) for colony PCR as well as hydroponics.
（4-1-①&②；4-2-①&②&③；5-1-①&②&③；5-2-①&② +NC）
3. gel electrophoresis
We used DL5000 and DL10000，10 samples and 1 NC.
4. liquid culture, first we chose 10 samples，but after 5h we didn't see the LB become turbid，so we chose 8 samples again.
results:
1.CPCR cns1-2



4=from plate ④; 5=from plate ⑤

cpcr cns1-2

conclusions: we might get the right pYES2-CNS1-2 plasmids

Next step:

attain sufficient pYES2-CNS1-CNS2 plasmids

verify pYES2-CNS1*CNS2 plasmid is correct by colony PCR

8.10th, Saturday

Purpose:

1. attain sufficient pYES2-CNS1-CNS2 plasmids
2. verify pYES2-CNS1*CNS2 plasmid is correct by colony PCR
3. hydroponics pYES2-CNS*CNS2 colony to attain sufficient pYES2-CNS1*CNS2 plasmids.

Process:

1. Plasmid extraction

after 20h hydroponics, we attain sufficient pYES2-CNS1-CNS2 plasmids. And chose three of them to stock under -20℃.

2. colony PCR

2samples (pYES2-CNS1*2 colonies)

1st

	Volume

T5 Super Mix	10uL
T7F	1uL
CYCter R	1uL
colony	touch
ddH2O	8uL
Total	20uL

Procedure	T	time
Initial denaturation	98℃	3min
Denaturation	98℃	10s
Annealing	50℃	10s
Extension	72℃	1min30s
Final extension	72℃	5min
	4℃	Hold
2-4 repeat 35times		

2nd

	V
T5 Super Mix	10uL
T7F	1uL
CYCterR	1uL
colony	touch
ddH2O	8uL
Total	20uL

	T	time
Initial denaturation	98℃	2min

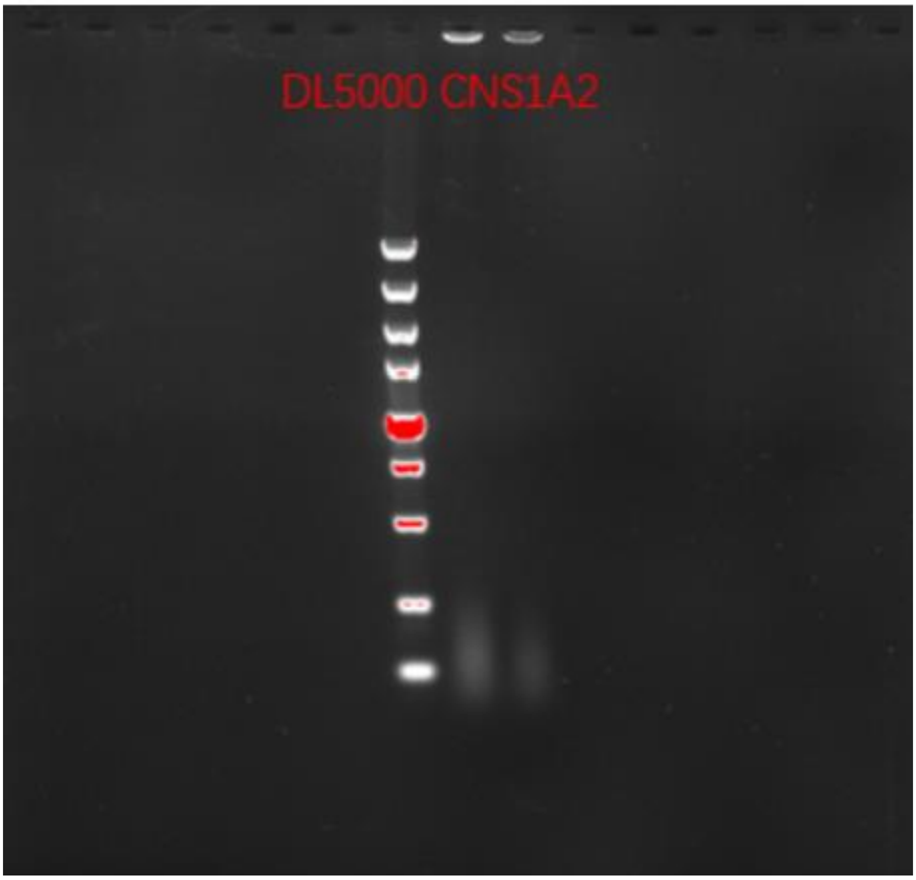
Denaturation	98℃	10s
Annealing	58℃	10s
Extension	72℃	45s
Final extension	72℃	5min
	4℃	Hold
2-4 repeat 29times		

3.gel electrophoresis
1st colony PCR’s result is strange, so we took the 2nd CPCR and the result showed our transformation is a failure.
Negative results for CNS1*2 colonies×2
4.transformation
（the former linked CNS1*2 plasmid×5）
Negative results for all 5 transformed samples.
5.ligation
（the former digested CNS1*2②&③）

②	13ng/uL	50ng	3.8uL
③	16.6ng/ul	60ng	3.6uL

	2	3
Buffer	2ul	
DNA	3.8ul	3.6ul
Ligase	1ul	
ddH2O	13.2ul	13.4ul
Total	20ul	

results:
1.CPCR of CNS1A2



CNS1A2 clonyPCR.

Conclusions: ligation and transformation are failed.

Next step: verify pYES2-CNS1*CNS2 plasmid is correctly linked by colony PCR transform ligase product into trans5α.

8.11th，Sunday

Purpose: ①attain correct pYES2-CNS1*CNS2 plasmid, ②transform CNS2-pYES2 for glycerin bacteria

Process:

1.transform

50ul trans5a + 20ul linked product (CNS1a2) X2

50ul trans5a + 20ul linked product (NC)

50ul trans5a + 2ul pYES2-NJA , (NC for the plate)

2. transformation 2

50uL trans5α +2uL CN2-pYES2

【concentration check】:

Sample\ data	260/280	260/230	Ng/uL
Elution buffer			0
CNS1-2 4-1 ①	1.848	2.480	203.62
CNS1-2 4-1 ②	1.817	2.624	97.73

CNS1A2 linear ①	2.026	-0.030	-2.15
CNS1A2 linear ②	- 178.379	-0.007	-0.66
CNS1A2 linear ③	4.297	-0.005	-0.60
CNS1A2 linear ④	2.454	-0.039	-1.86
CNS1A2 linear ⑤	1.916	0.280	-1.98

we found that the concentration of our linear plasmids CNS1*2 are all minus value,

The problem can be caused by:

① we had put the digested plasmid in fridge for a long time (from 8/8 to 8/11), they have been digested by DNase.

the kit we used for gel extraction was damaged, for that participators in other groups suffered similar problems (they found their samples are in a particular low concentration after gel extraction).

Results：

The reasons that we always get negative results when transform plasmids CNS1*2 into Trans5α are the damaged linear plasmid samples and it was caused by the above mentioned points.

Next step：Construct plasmid CNS1a2 from the very begin & stock CNS2-pYES2 glycerin bacteria.

8.12th，Monday

Purpose：get pYES2-CNS1*CNS2 positive colony，hydroponics colonies to attain enough pYES2-CNS1*CNS2 plasmids

Process：

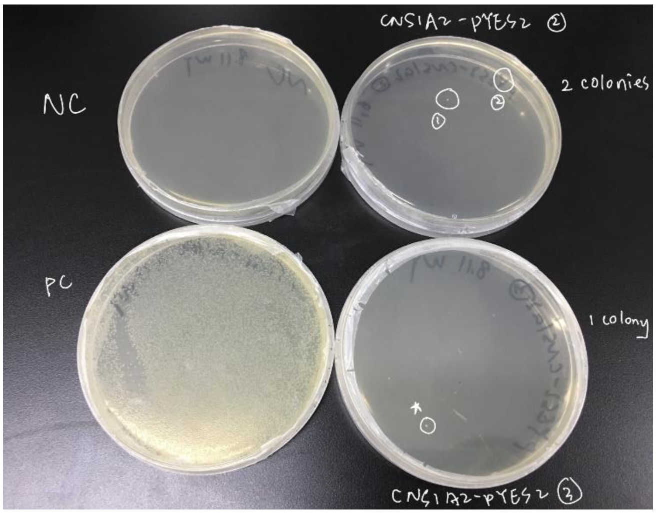
1.colony PCR and liquid culture

（the transformation products CNS1*2-pYES2-Trans5α done yesterday）

Only 3 colonies formed on the 2 plates.

CNS1*2-pYES2-①：2 colonies

CNS1*2-pYES2-②：1 colony

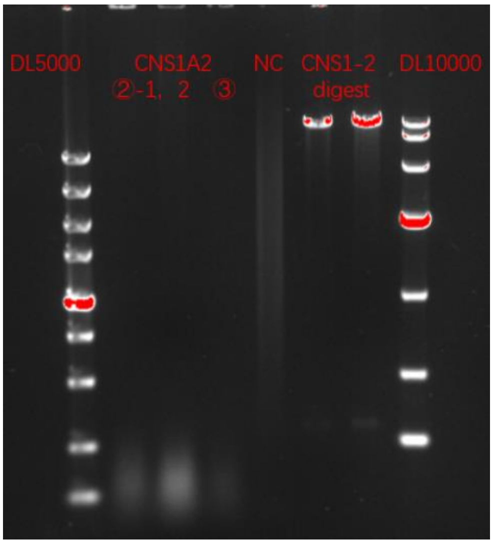


2. Digestion （CNS1-2-BamHI）

10×buffer	3uL
BamH I	1uL
CNS1-2	①4uL (800ng) / ② 10uL(970ng)
ddH2O	22uL / 16uL
Total	30uL
30℃ for 30min	

3. gel electrophoresis

For both colony PCR products and digested plasmids.

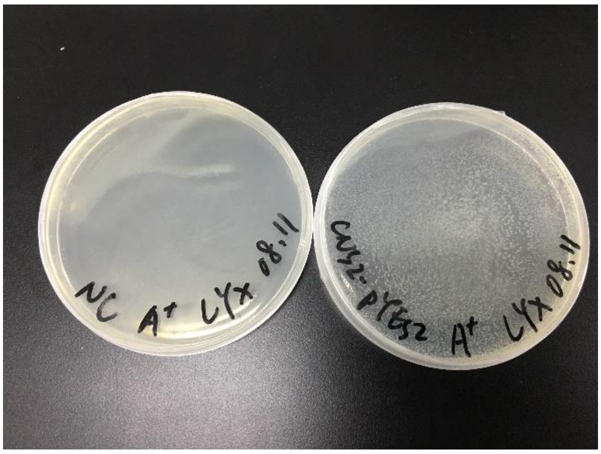


cpcr cns1a2 & digest cns1-2.

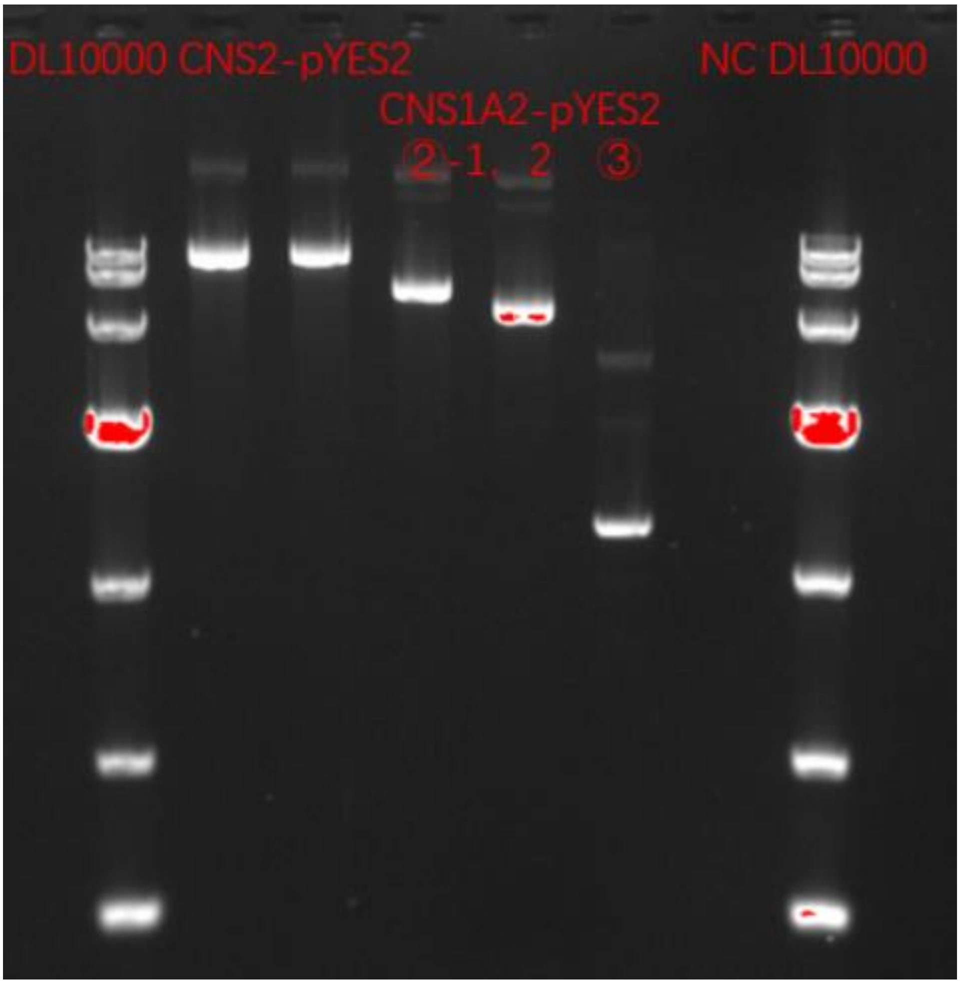
4. ligation

we use cycle pure kit for purifying our digested plasmids.

5. glycerin bacteria storage:



pick 2 colonies from the transformation plate
liquid culture for 10 hours
transfer 500uL bacteria solution into 500uL glycerin of each and mix up
stock the glycerin and bacteria mixture under -80℃.
6. plasmid extraction and electrophoresis
(for CNS2-pYES2 ×2 & CNS1*2-pYES2 (from colonies) ×3)



plasmid CNS2 & CNS1A2.

Results:

we successfully stockd glycerin bacteria containing plasmid CNS2-pYES2.

the transformation products of plasmid CNS1*2-pYES2 we did yesterday are negative results.

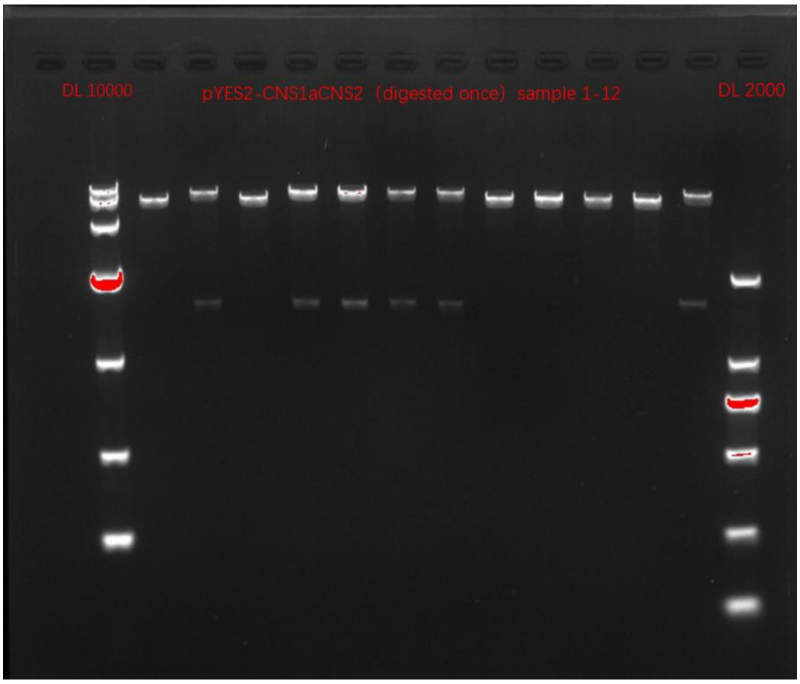
Next step:
① stock our linked plasmid CNS1-2-pYES2 (digested by BamH I) under -20℃ temporarily.
(for that we are not able to buy restriction enzyme Mef I in time)
② transform the linked products to get the bacteria during middle processes for later need.

8.13th，Tuesday
Purpose：attain the colony which have pYES2-CNS1*CNS2（only digest by Bam HI）
Process：
1.transformation
4 samples , ①-2 and ②-2 add 10ul linked product，①-1 and ②-1 add 20ul linked product.
2.spare plate
4 plates，37°C for > 18h
Results：
Next step：chose some colonies and extract plasmids, then digest them to verify if they are positive.

8.14th，Wednesday
Purpose：verify the colonies which have pYES2-CNS1*2
Process：
liquid culture：12 colonies from pYES2-CNS1*2 plates,37°C 200rmp for 12h
Plasmid extraction：12 samples

	V
Xho I	0.5ul
BamH I	0.5ul
ddH2O	6ul
Green Buffer	1ul
Template	2ul
Total	10ul

transform pYES2-CNS1 and pYES2-CNS1-CNS2 into BY4741
Results：
digestion:
sample 1,3,8,9,10,11 is approximately 8000bp
sample 2,4,5,6,7,12 is approximately 9000bp and 1600bp
the ideal fragments should be 8629bp and 1605bp.



Conclusion: sample 2, 4, 5, 6, 7, 12 should be pYES2-CNS1*CNS2, sample 1, 3, 8, 9, 10, 11 could be pYES2-CNS2(7544bp).

We need sequencing to verify the positive results and use one negative result to compared.

Next step: sequencing the pYES2-Cns1*2 plasmid and start to transform plasmids to yeast.

8.17th Saturday

participator: Wu Yi、Shen Xinran

purpose: to make sure that transform is successful

process:

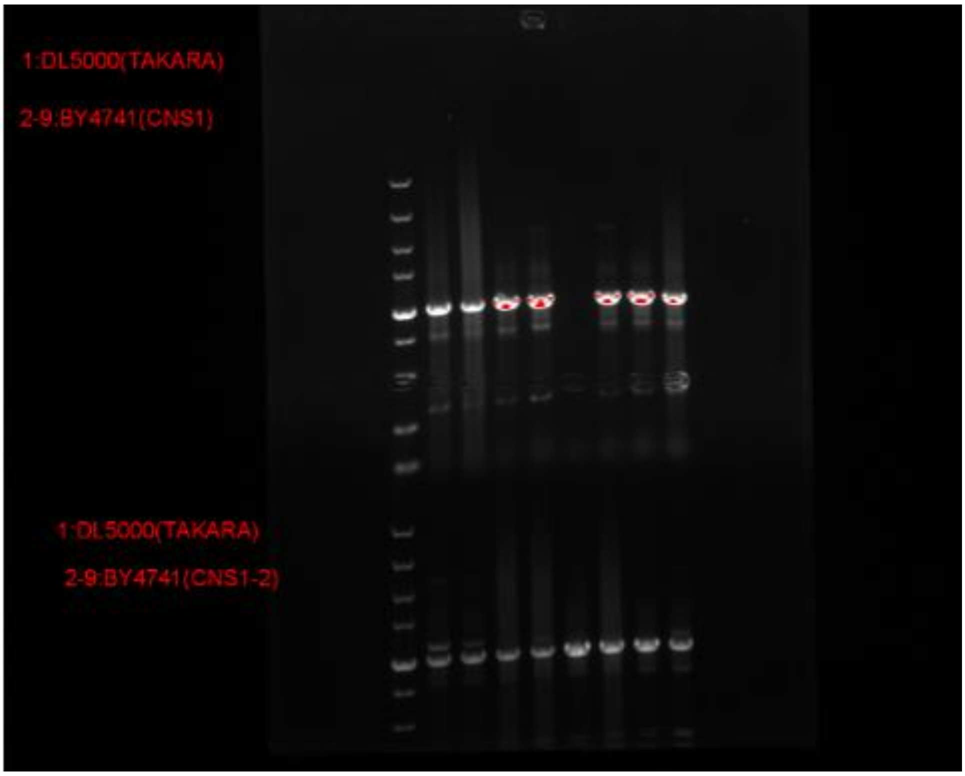
1. CPCR

	V
T5 Super Mix	5uL
T7F	0.5uL
CYCterR	0.5uL
clony	touch
ddH2O	4uL
Total	10uL

2. gel electrophoresis

use DL5000 from TAKARA

results:



cpcr by4741(cns1,cns1-2)

conclusions: the primers don 't specific.we can not judge if the transform is successful.

next step: design new primers

8.18th, Sunday

Purpose: design new primers that are more specific, order enzyme Mfe I.

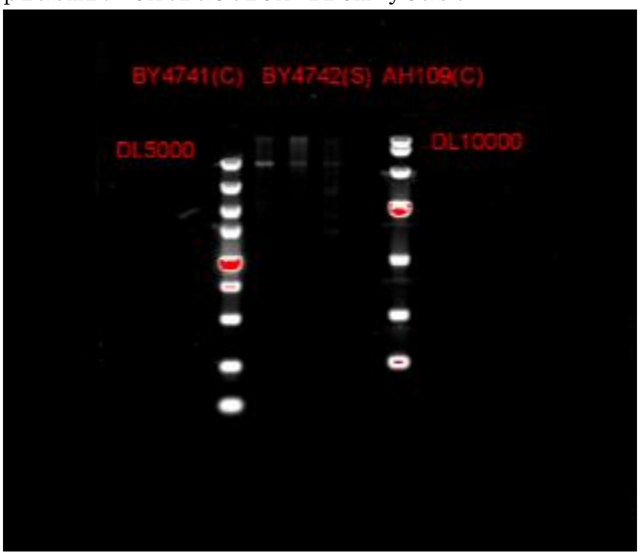
Process: design two primers

CNS1-2-F: GGGAATATTAAGCTTAAAAAAATGGC

CNS1-2-R: TTCGAAGGGCCCTCTAGACT

results:

2.plasmid extraction from yeast



plasmid extraction from yeast.

conclusions: -
Next step: colony PCR (E.coli pYES2-CNS1a2 to test the new primers, exert digestion , joining reaction to pYES2-CNS1*CNS2 to construct pYES2-CNS1aCNS2.)

8.19th, Monday
participator: Wu Yi、Shen Xinran、Liu Yuxi、Lingfeng Mao
purpose: colony PCR(E.coli pYES2-CNS1a2 to tset the new primers, exert digesition , joining reaction to pYES2-CNS1aCNS2 to construct pYES2-CNS1*CNS2
process:
1. PCR(colony)pYES2-CNS1a2 only digest by BamH I

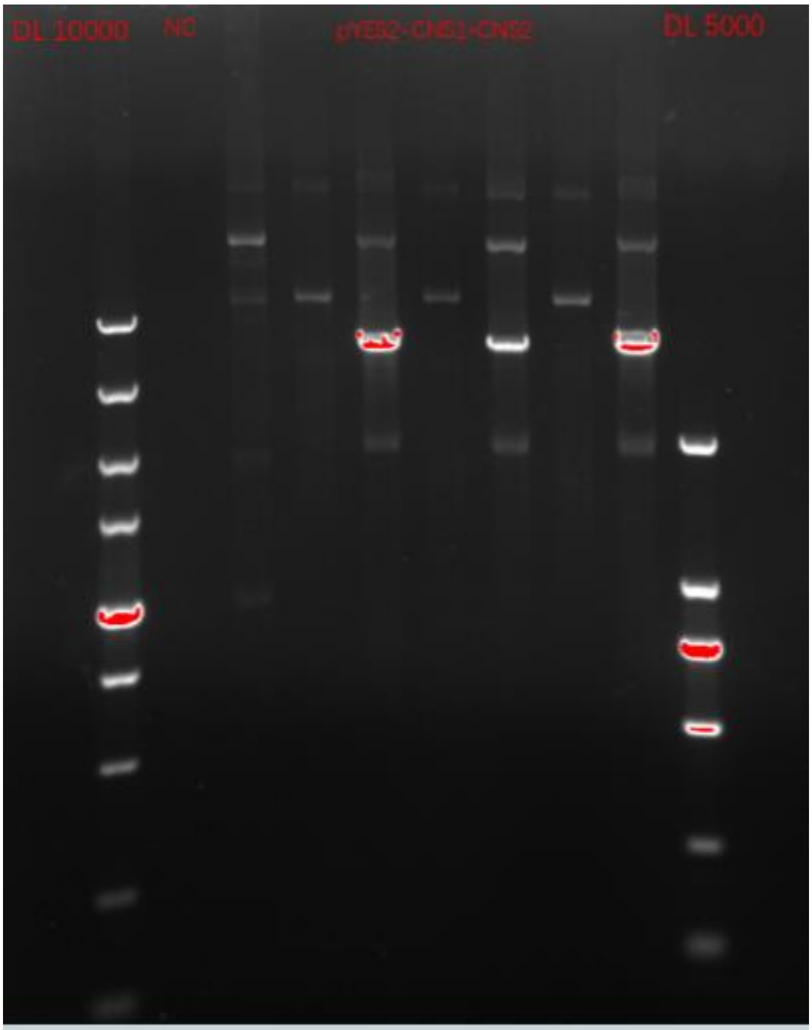
T5 super Mix	5uL
T7F	0.5uL
CYCterR	0.5uL
colony	1uL
ddH2O	3uL
total	10uL

2.colony pcr (chemical conversion of BY4741-cns1;BY4741-cns1-2)recorded somewhere else

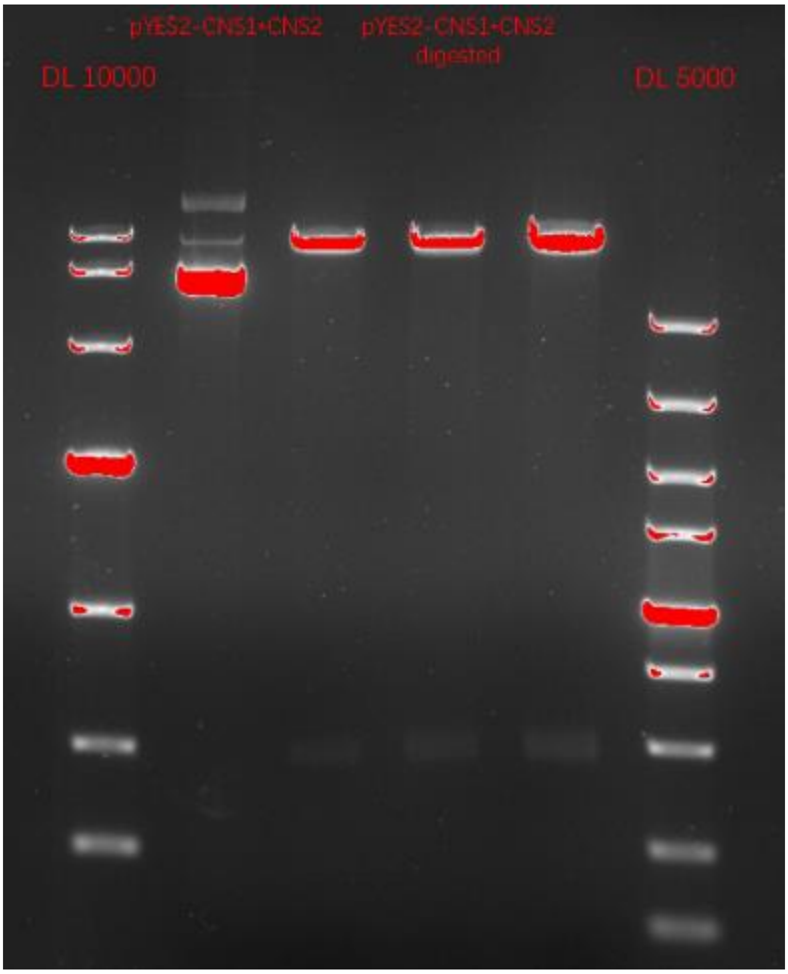
digestion 0919	ul
Mfe I	1
EcoR I	1
2x smart cut mix	2
digested plsmid(pYES2-CNS1aCNS2)	4
ddH2O	12
total	20

joining reaction 0919	ul
T4 Ligase	1
10X T4 Ligase buffer	2
joined plasmid(pYES2-CNS1aCNS2)	10
ddH2O	7
total	20

results:

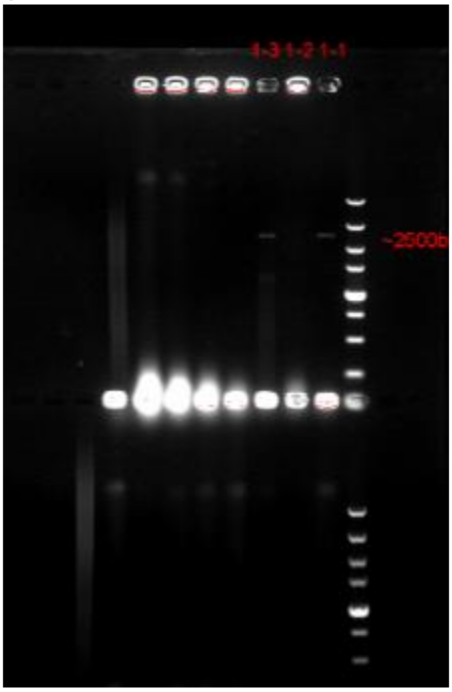


PCR(colony)pYES2-CNS1a2 only digest by BamH I: the result is confusing

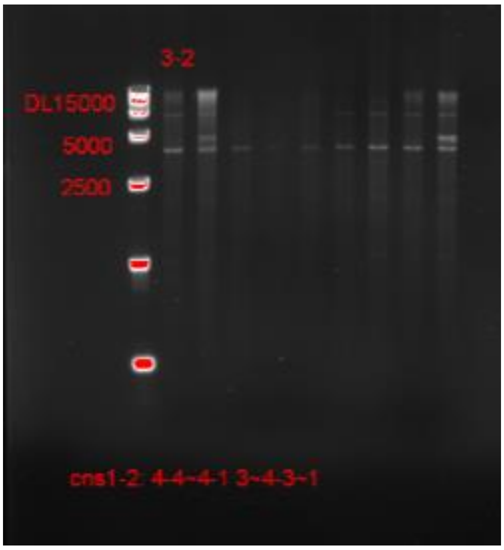


the ideal fragment are 564bp and 9310bp

digestion:



colony PCR of yeast plasmid CNS1&1-2



yeast extract plasmid CNS1-2

conclusions: the diegstion succeeded

next step: transform the joined plasmid pYES2-CNS1*CNS2 into DH-5α to amplify it

8.20th，Tuesday

participator：Shen Xinran、Lingfeng Mao

Purpose：transform the joined plasmid pYES2-CNS1aCNS2 into DH-5α to amplify it，check the new designed primer

Process：

1、transformation

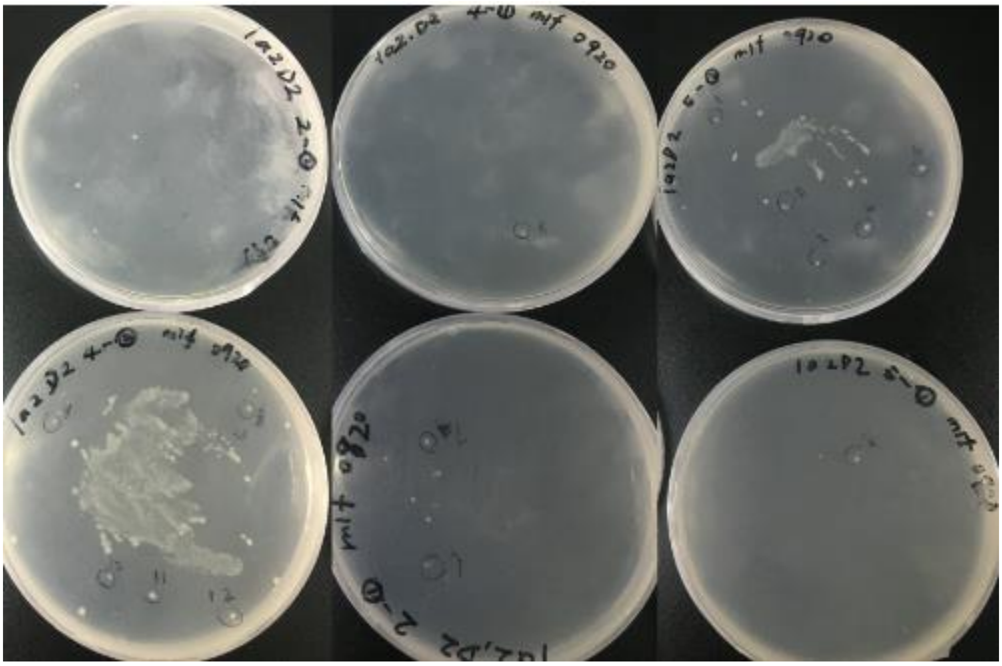
sample 4,2,5X2, 5 and 10 ul joined plasmids added

Spread plate

LB, A+, sample 4,2,5X2, 5,4000RMP, 3min, 50ul remained

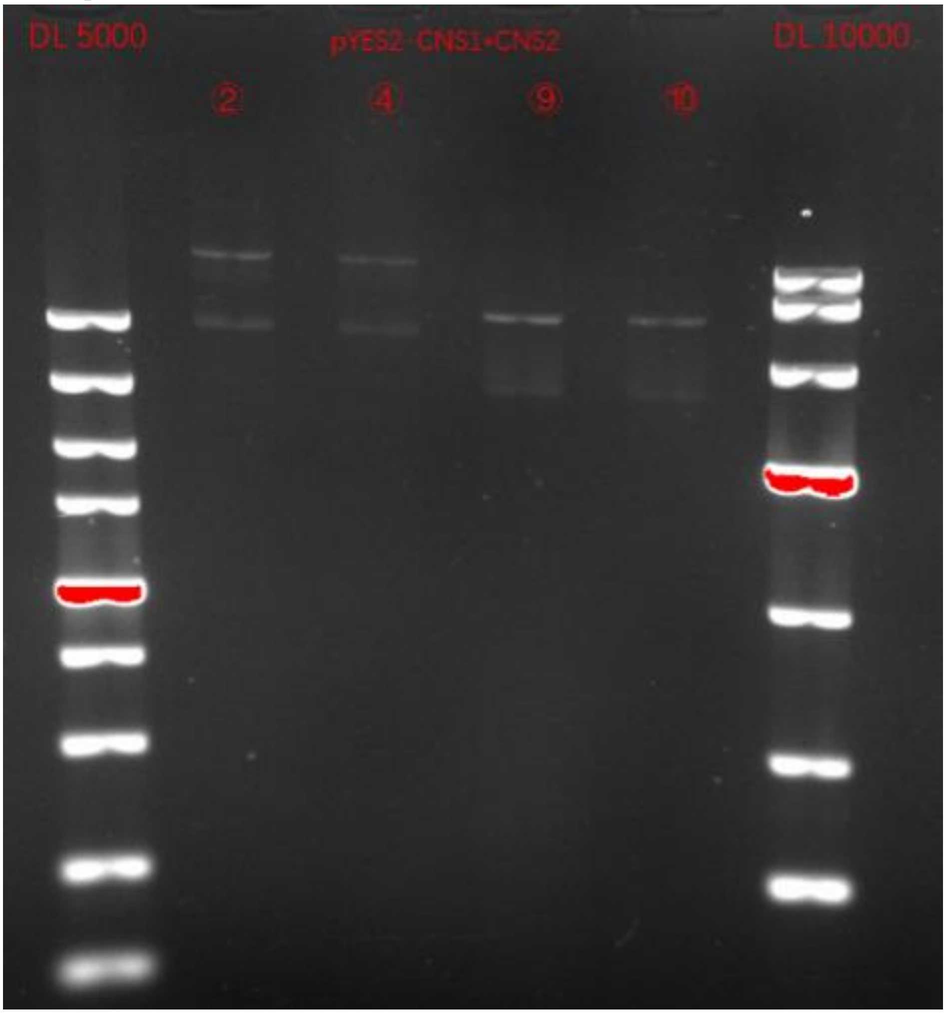
	T	time
Initial denaturation	98℃	1min
Denaturation	98℃	10s
Annealing	58℃	10s
Extension	72℃	1min
Final extension	72℃	5min
	4℃	Hold
2-4 repeat 30times		

Results：



Different growth condition could be caused by the improper steps in the transformation, and, to speak of, the unspreaded colony in the middle of the dish should be the result of partly resuspend culture before spread plate.

spread plate



the result is confusing

PCR(pYES2-CNS1*CNS2)

Conclusions: the plate could be used in the next step, the new designed primer is specific

next step: PCR(colony) to select possible positive colony, enzyme digesting to verify the plasmid.

8.21st, Wednesday

Purpose: exert PCR colony to verify the positive colony, amplifying the positive colony with liquid culture and extract plasmids from it

exert PCR (gradient) and oligo 7 to check the primers

Process:

liquid culture : sample 2, 4, 6, 8, 10, 12, 14 from the plate

PCR colony sample①(1-15) from the plate

	T	time
Initial denaturation	98℃	3min
Denaturation	98℃	10s
Annealing	58℃	10s
Extension	72℃	1min
Final extension	72℃	2min
	8℃	Hold
2-4 repeat 25times		
CNS1-2-F	CNS1-2-R	

gradient PCR （plasmid pYES2-CNS1aCNS

	T	time
Initial denaturation	98℃	3min
Denaturation	98℃	10s
Annealing	60±5℃	10s
Extension	72℃	1min
Final extension	72℃	2min
	8℃	Hold

2-4 repeat 25times		
CNS1-2-F	CNS1-2-R	

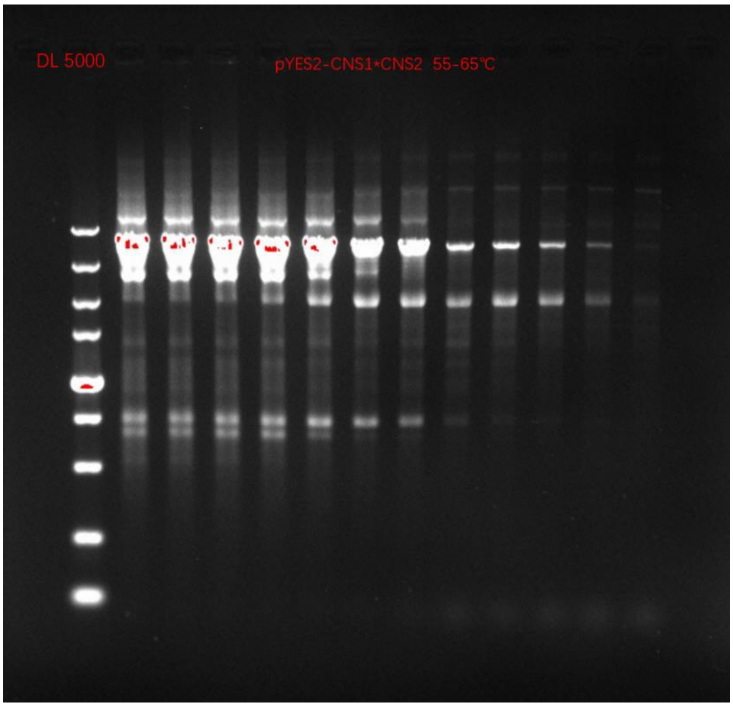
PCR colony sample②(1-13) from the plate 8 rows

	T	time
Initial denaturation	98℃	3min
Denaturation	98℃	10s
Annealing	58℃	10s
Extension	72℃	1min
Final extension	72℃	2min
	8℃	Hold
2-4 repeat 25times		
T7 F	CYCter R	

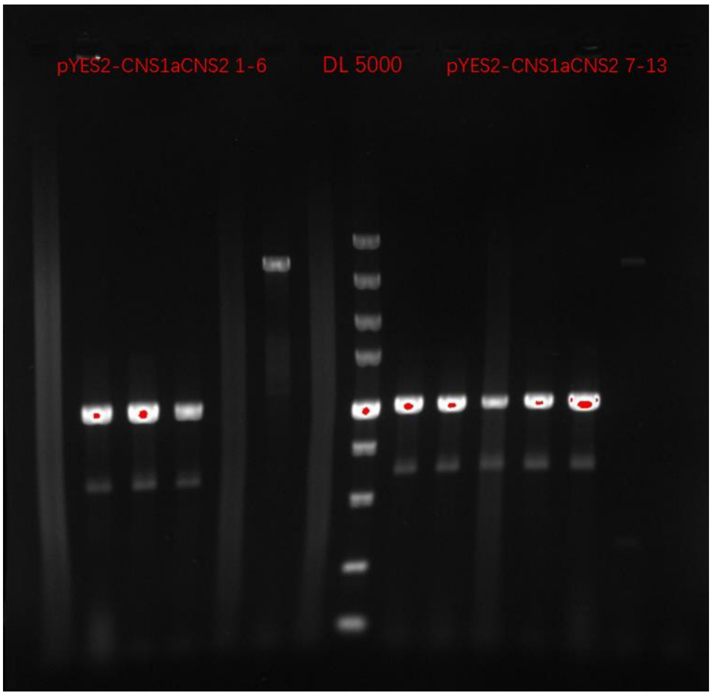
extract plasmid from the liquid culture
Results:
all samples are negative

the optimal temperature is 60.5 ℃, with ideal fragment 4050bp and 2000bp, 750 bp noise fragment.

oligo 7 shows there are fault binding site of the primers on the plasmid, in line with the PCR results.



Sample 6, 13,~4000bp, are positive, the negative samples with 2000bp fragments are confusing because we should not have plasmid at this size.



Conclusions: Our plate could be contaminated by unknown plasmids, positive colony sample 6, 13 could be our ideal plasmid, which needs next steps to verify. The new primers could be used in PCR(colony), but is not specific enough to be used in sequencing.

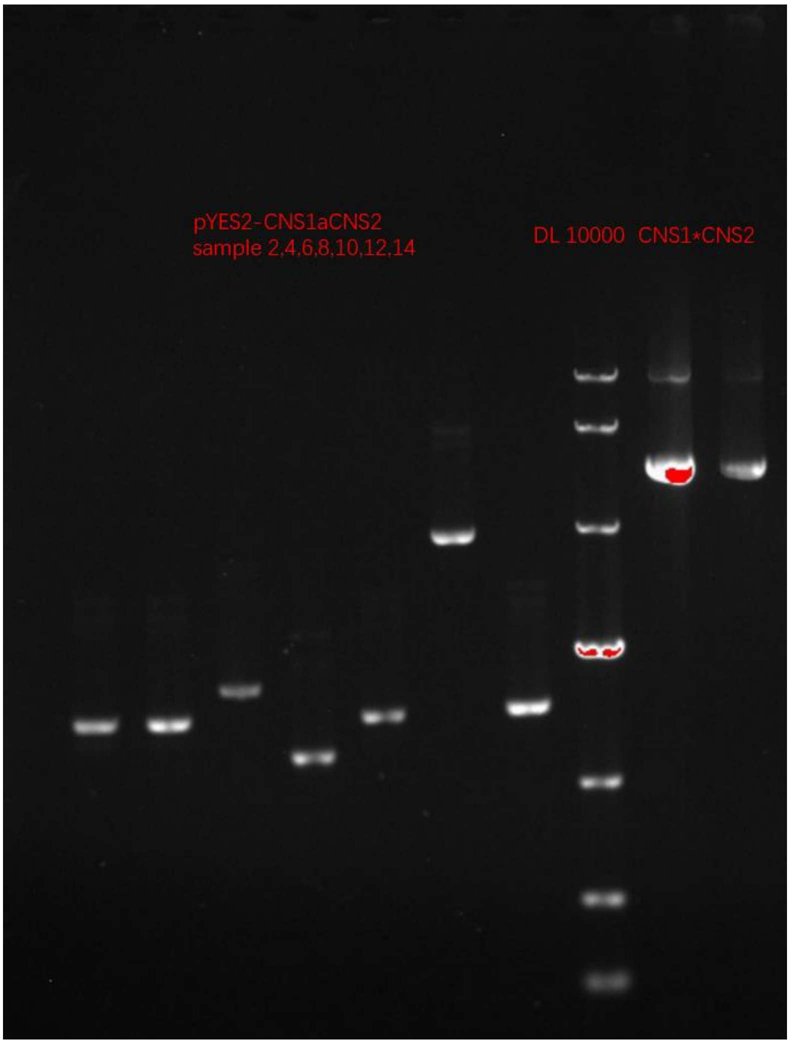
Next step: enlargement culture sample 6,13 and extract plasmids to verify it.

8.22nd, Thursday

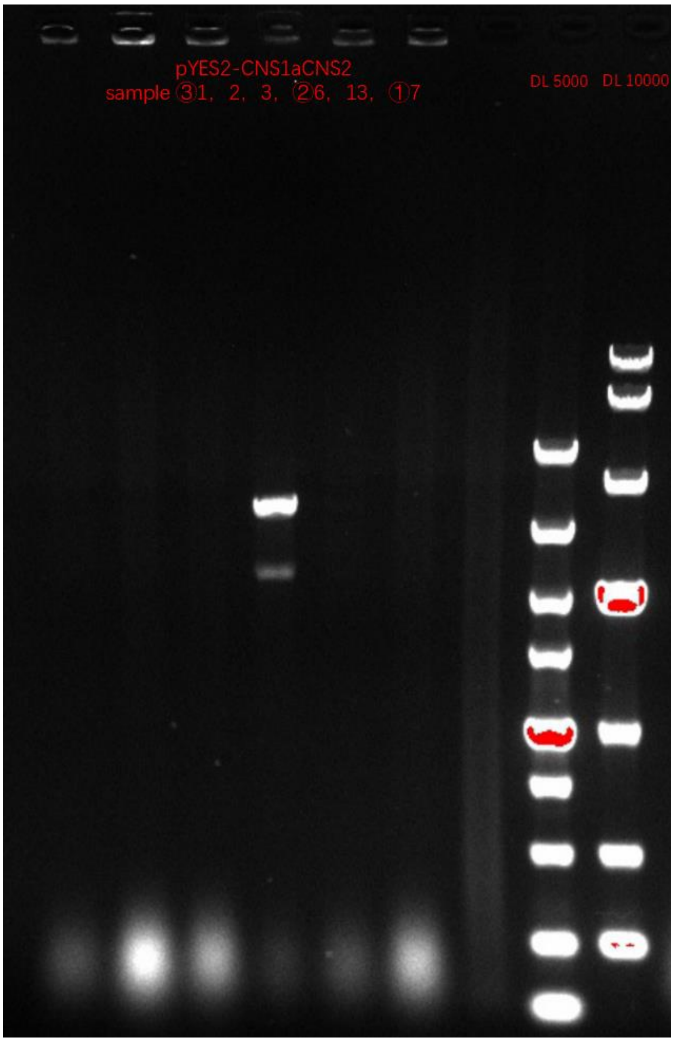
Purpose: verification of the plasmids CNS1a2.
Process: enlargement culture sample 6,13 and extract plasmids to verify it
liquid culture of sample ② 6,13

	T	time
Initial denaturation	98℃	3min
Denaturation	98℃	10s
Annealing	60.5℃	10s
Extension	72℃	1min
Final extension	72℃	2min
	8℃	Hold
2-4 repeat 25times		
CNS1-2-F	CNS1-2-R	

Results:
plasmid CNS1aCNS2 sample ①2,4,6,8,10,12,14: our plate could be contaminated by unknown plasmids.



PCR colony sample③1, 2, 3, ②6, 13, ①7 from the plate: sample 6, 13 are~3500bp, they are positive.



conclusion: sample 6, 13 could be our ideal plasmids.
Next step: extract plasmids from liquid culture and verify sample 6, 13.

8.23th Friday

purpose:
1. extract plasmids from liquid culture and verify sample 6, 13
2. PCR verify sample 6 and choose the best primers.
3. digestion of plasmid sample 6.
process:
1. extract plasmids （sample3, 6, 7, 13） from liquid culture
2. PCR

T5 super mix	5uL
Former Primer*	0.5uL
Posterior Primer*	0.5uL
plasmid	0.5uL
ddH2O	3.5uL
total	10uL

Initial denaturation	98℃	3min
Denaturation	98℃	10s

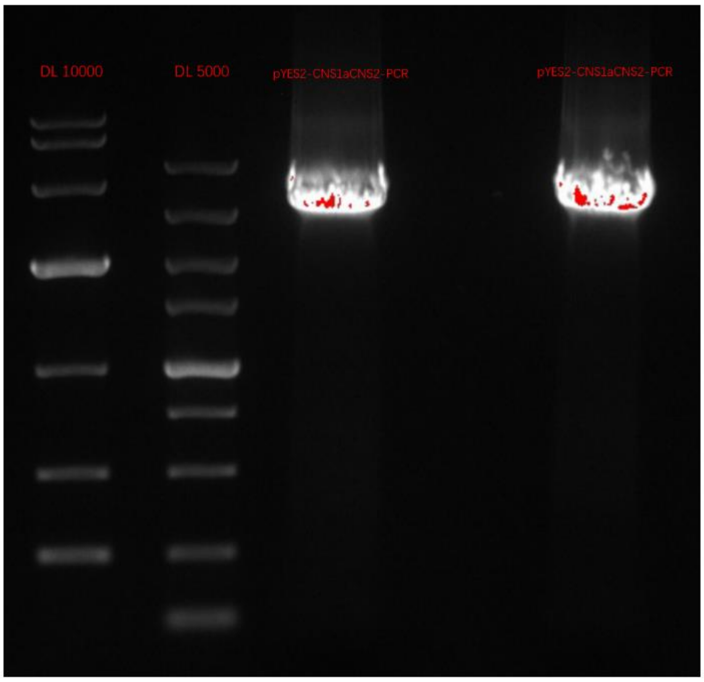
Annealing	58.5℃	10s
Extension	72℃	1min
Final extension	72℃	2min
	12℃	Hold
2-4 repeat 20times		

3.digestion
results:
plasmids: sample 6 is positive.
2. the results of PCR is negative.
digestion
3. the results of digestion is negative，only one sample has a positive result.
conclusion:
1.we have successfully get positive pYES2-CNS1a2 plasmid from liquid culture.
next step:
PCR again and gel extraction.

8.24th Saturday
purpose：PCR verify sample 6 and gel extraction.
process：
1.PCR（total 50uL）

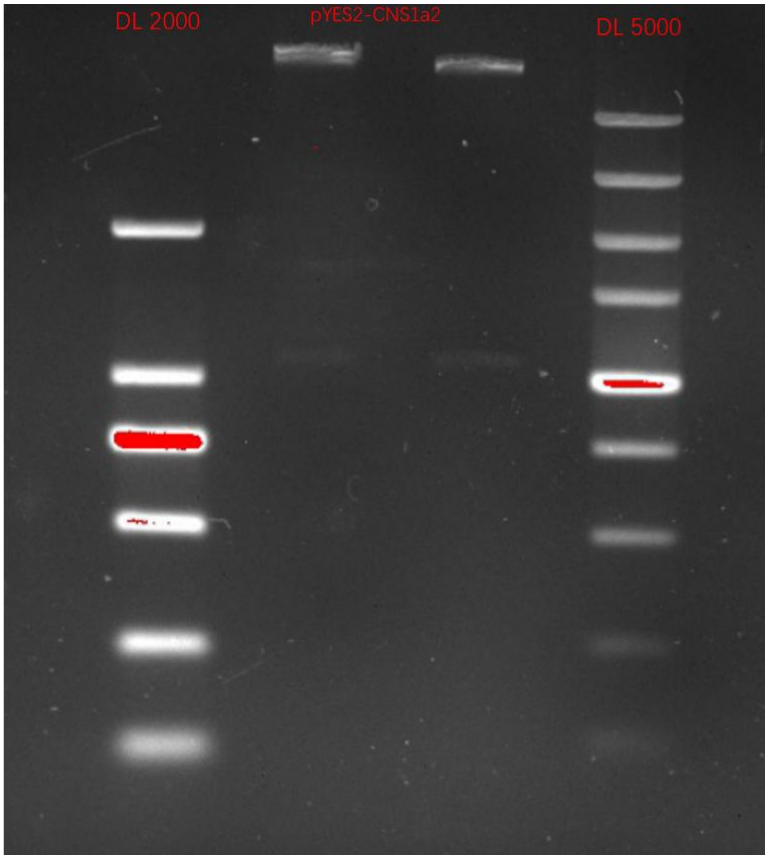
Initial denaturation	98℃	3min
Denaturation	98℃	10s
Annealing	60.5℃	10s
Extension	72℃	1min
Final extension	72℃	2min
	8℃	Hold
2-4 repeat 25times		
CNS1-2-F	CNS1-2-R	

2.gel extraction
results：
1.PCR



sample 6 gets a positive result.
2. gel extraction
we get gel extraction and send the sample for sequencing.
conclusion:
1. our pYES2-CNS1a2 sample is positive.
next step: exert double digestion to verify the plasmid pYES2-CNS1a2

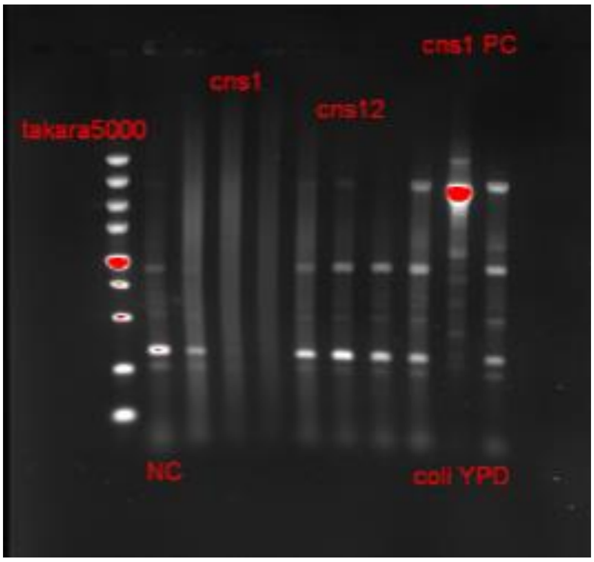
8.25th Sunday
purpose: exert double digestion to verify the plasmid pYES2-CNS1a2
process:
classical double digestion system(Bam HI Xba I)
amplifying culture of the positive sample
results:
1.digest



The thin ~1100kb band is the ideal band

double digestion

2. CPCR



CPCR cns1、cns1-2 coli

conclusion：Although possibly impure, the plasmid sample could be the wanted plasmid. We successfully constructed the plasmid pYES2-CNS1a2

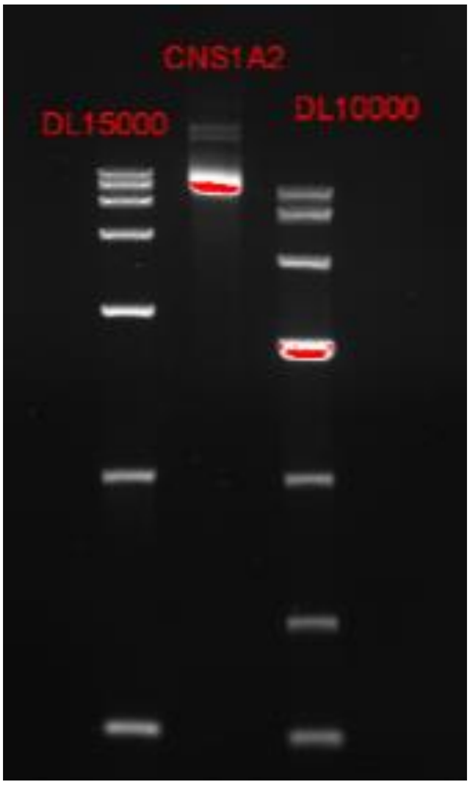
next step： transform the plasmid into yeast strain BY4741

8.26th Monday

participator： -

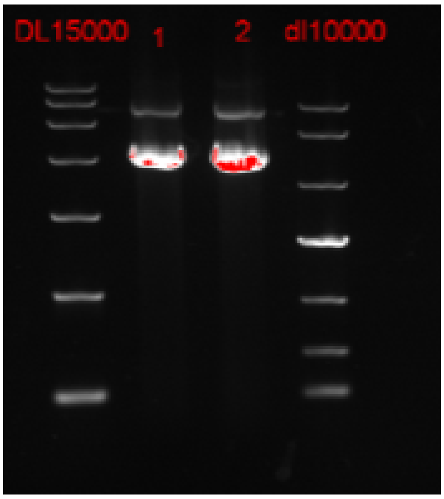
purpose： insure the plasmid we’ ve extracted is what we want.

process:
1. plasmids extraction
results:



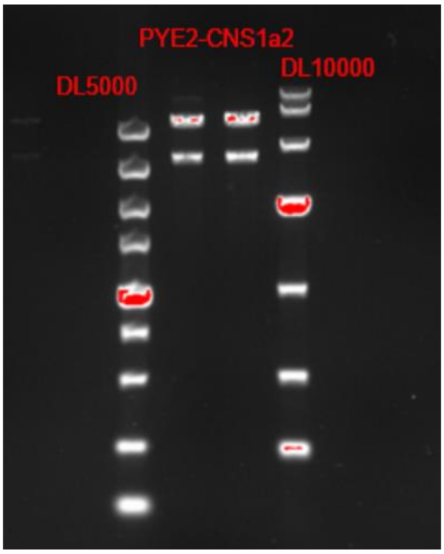
plasmid CNS1A2 sample 6
conclusion: succeed
next step: verify that cns1a2’ s transformation in yeast is succeed

8.27th Tuesday
participator: -
purpose: verify that cns1a2’ s transformation in yeast is succeed
process:
1. plasmids extraction
2. digestion
results:
1. plasmids extraction



plasmids cns1A2-pYES2

2.digestion



digestion CNS1A2

conclusion: succeed

next step: use SDS-PAGE to verify the molecular weight tof protein product.

chemical transformation of Saccharomyces Cerevisiae

8.14th, Wednesday

Purpose:

the 1st try to confirm that chemical transformation is practical and find a proper condition for yeast transformation.

Process:

transform plasmids CNS1-pYES2 & CNS1-2-pYES2 into competent cells of strain BY4741.

Process of transformation① (BY4741) :

① Take 100 μl of melted BY4741 competent cells on ice, add 1-3 μg of pre-cooled target plasmid, Carrier DNA (96 ° C water bath for 3 min, fast ice bath for 3 min,

repeat) 10 μl, PEG/LiAc Mix 500 μl and mix several times with a 30 ° C water bath for 30 min (flip 6-8 times at 15 min).
② Move the competent cells to a 42 ° C water bath for 15 min (flip 6-8 times at 7.5 min).
③ Centrifuge at 5000 rpm for 40 s. Discard the supernatant, resuspend ddH2O 400 μl, centrifuge for 30 s and discard the supernatant.
④ ddH2O 50 μl resuspend, plate, incubate at 29 ° C for 48-96 h.
transform plasmids yeGFP into competent cells of strain AH109.
Process of transformation (AH109) :
① Take 100 μl of AH109 competent cells on ice, add 2-5 μg of pre-cooled target plasmid, Carrier DNA (95-100 ° C, 5 min, fast ice bath, repeat) 10 μl, PEG/LiAc Mix 500 μl and mix several times with a 30 ° C water bath for 30 min (flip 6-8 times at 15 min).
② Place the tube in a 42 ° C water bath for 15 min (flip 6-8 times at 7.5 min).
③ Centrifuge at 5000 rpm for 40 s Discard the supernatant, resuspend ddH2O 400 μl, centrifuge for 30 s and discard the supernatant.
④ ddH2O 50 μl resuspend, plate, incubate at 29 ° C for 48-96 h.
【totally 8 plates: 2 AH109 with ye-GFP-pYES2; 2 BY4741 with CNS1-pYES2 and CNS1-2-pYES2 of each; 2 NC (one for ddH2O, one for none)】
Results:
①After around 24 hours, plate containing transformed AH109 (with plasmids yeGFP-pYES2) appear dense colonies.
NC and BY4741 do not appear colony.
②After around 40 hours, plates containing transformed BY4741 (with plasmids CNS1-pYES2 and CNS1-2-pYES2) appear several colonies clearly.
Next step:
Try another transformation protocol; or one more chemical transformation using thses competent cells.

8.15th, Thursday
Process:
1.Microscopic examination
colonies on 2 plates(of transformed AH109 with yeGFP-pYES2)
2. plate streaking
(⑤→YPD×1, SD×1; ⑥→YPD×1, SD×1; Seed fungi liquid of BY4742→SD×1)
we streak some yeasts from transformed plates into new YPD and SD plates to see which kind of plate makes transformed AH109 strain grow faster.
Results:
1.The transformed colonies are all yeasts, without other microorganisms.
2.After 24-48 hours

The plate streaking results of transformed AH109 strain (with yeGFP-pYES2 plasmids); this strain growt faster on SD plates than YPD plates.

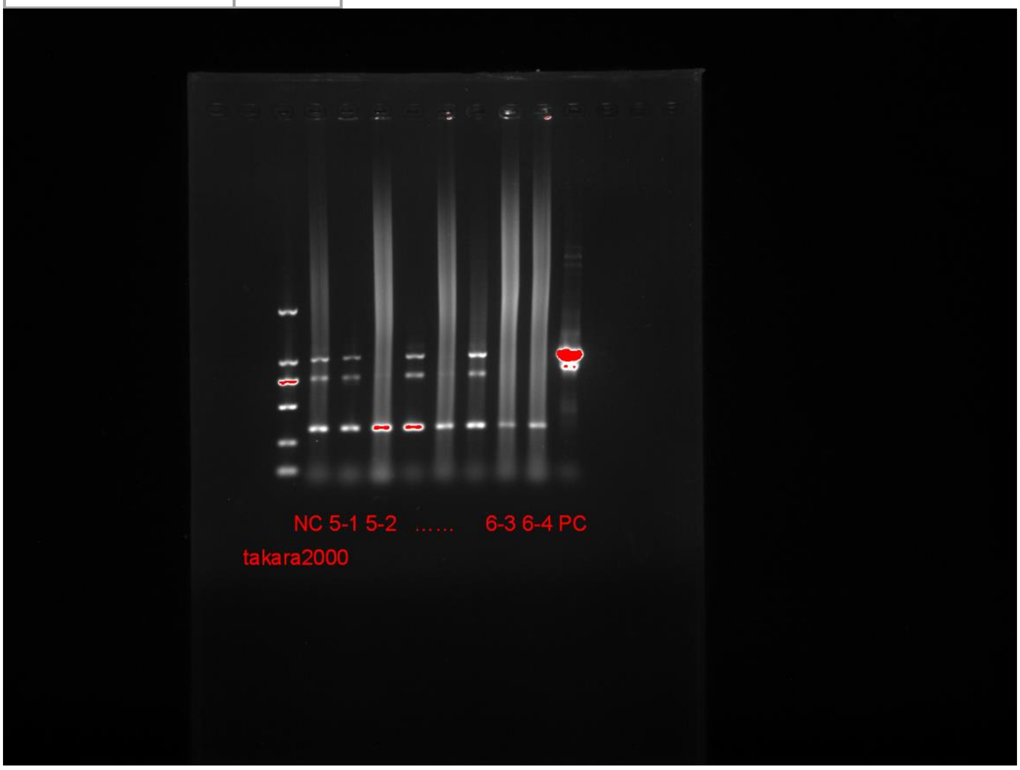
8.16th，Friday

Purpose： Verify that the colonies are yeasts with our interested plasmids.

Process：

colony PCR & gel electrophoresis

2×mix	5uL
Forward primer	0.5uL
Reverse primer	0.5uL
Colonies	Touch
ddH2O	4uL
Total	10uL



We chose 5-1, 5-2 and 6-3 as positive results.

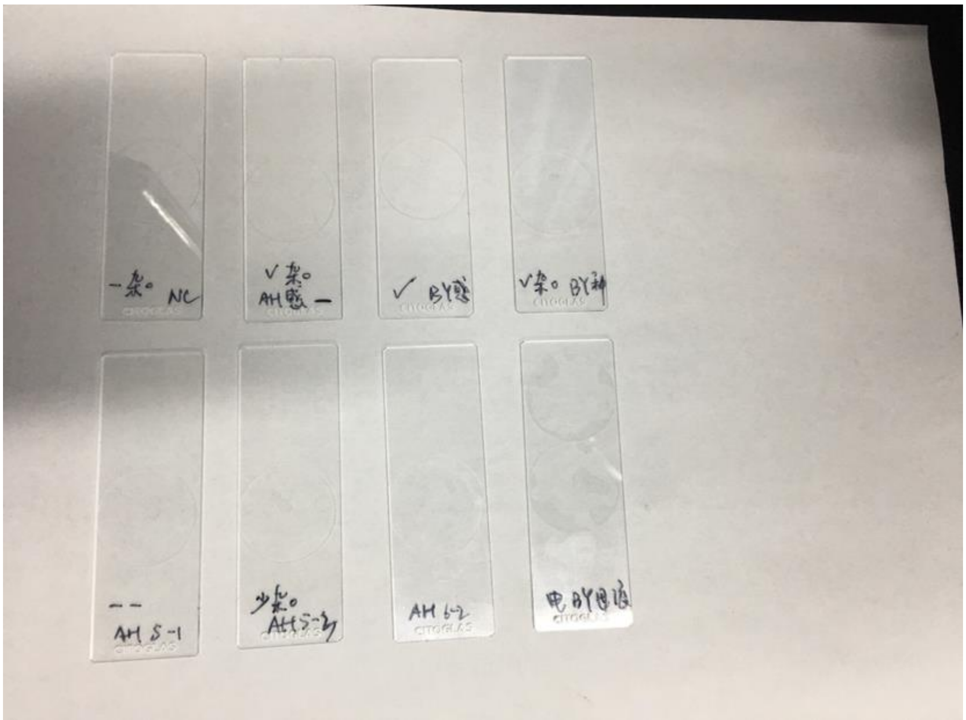
Yeast plasmid extraction & gel electrophoresis (plasmids are extracted from colonies that electronically transformed the 2nd time (colonies are liquid cultured for 24 hours))

Result:

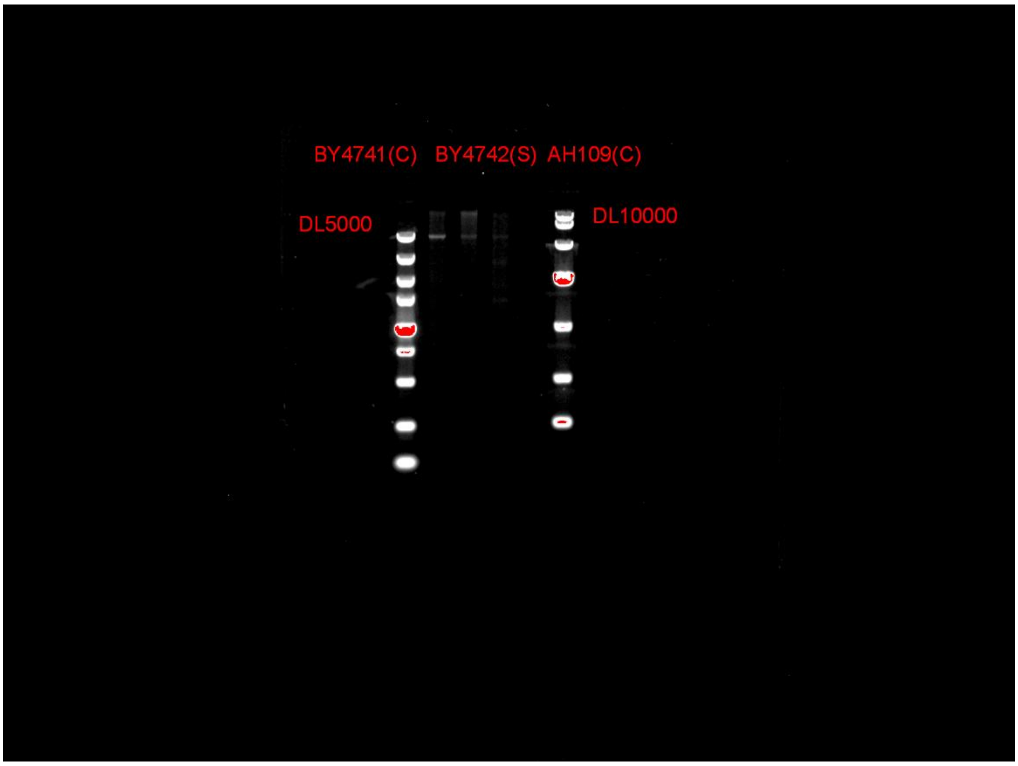
Got negative results of no band is showed. (picture is deleted)
Reasons:
Liquid culture period should be longer for more yeast to extract plasmids.
Enlarge cultivation
(positive colonies mentioned above ×3（5-1, 5-2 and 6-3）; competent cells ×2（of AH109&BY4741 bought from shanghaiweidi company）; seed yeast liquid of BY4742 ×1) each 10uL added into 5mL YPD liquid culture.
Next step：extract plasmid from enlarged yeast culture and gel electrophoresis; colony PCR of transformed BY4741 strain.

8.17th, Saturday
Process：
colony PCR of transformed BY4741 strain（3×4）
liquid culture the colonies of transformed BY4741

8.18th，Sunday
Process：
1.Microscopic examination(of 7 liquid cultured yeasts: AH109 with yeGFP-pYES2×3(⑤-1，⑤-3，⑥-2); BY4741 competent cells; AH109 competent cells; BY4742 seed solution; NC)
2.plasmid extraction & electrophoresis
(of BY4741 (C) & BY4742 (S) & AH109 (C); C means competent cells, and S means seed solution.)
3.liquid culture some colonies from streaked plates of the first chemically transformed AH109 with yeGFP.(2×4： ⑤-YPD；⑤-SD；⑥-YPD；⑥-SD)
Results:
1.The liquid cultured yeasts(mentioned above) appear epinephelos in a unusual way: after around 24 hours liquid culture, the 3 (BY4741 competent cells; AH109 competent cells; BY4742 seed solution) appear epinephelos slightly.
While after another period around 17hours, the lasted 4 have 3 got epinephelos, and NC is one of the 3.



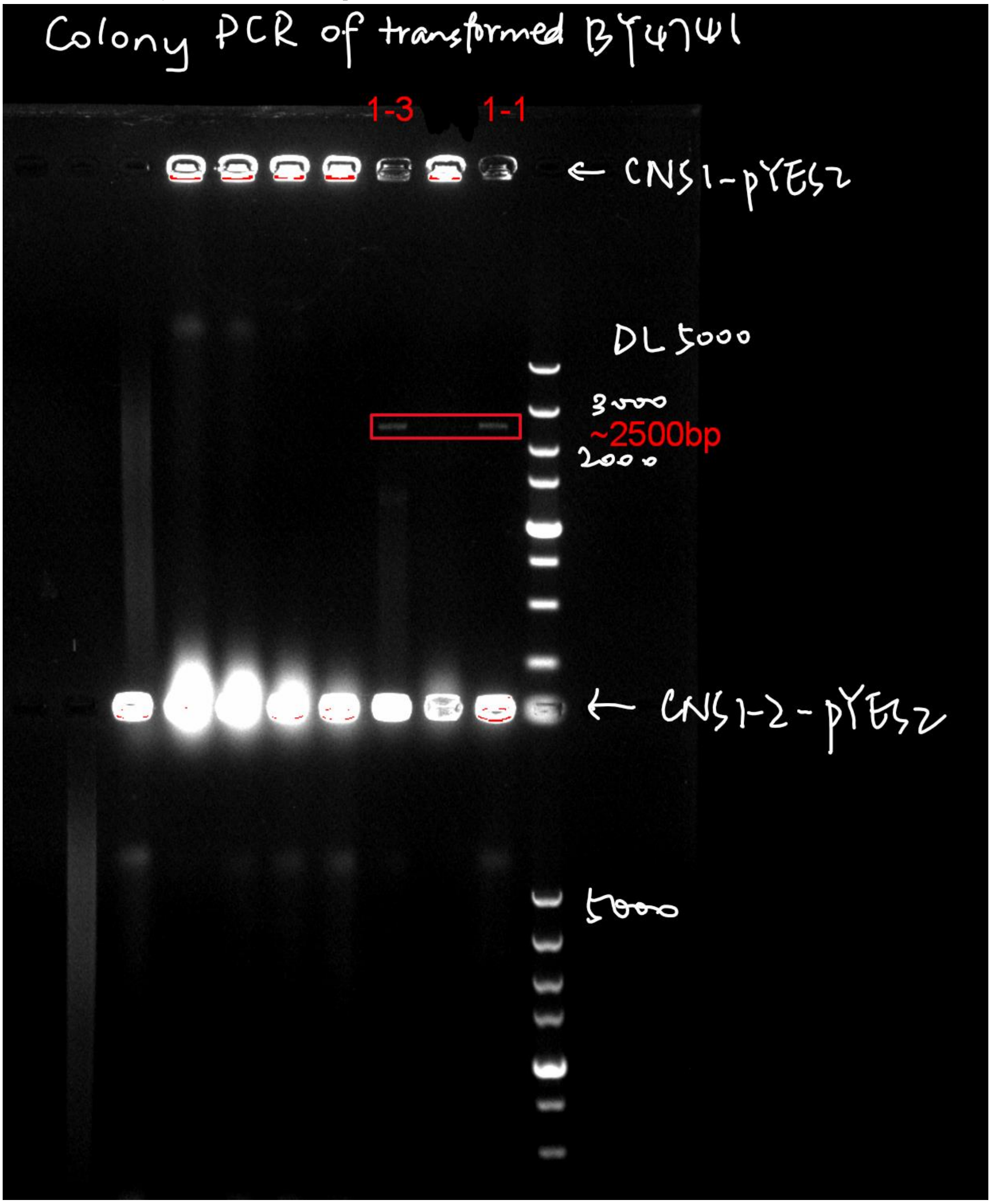
2. The extraction of the 3 kinds of strains all have bands around 7000-10000bp, and bands of BY4741 (C) & BY4742 (S) appear around 5000bp as well. we also speculate that unexpected bands appear on AH109 (C) are result from the infected bacteria.
liquid culture some colonies from streaked plates of the first chemically transformed AH109 with yeGFP.
(2×4： ⑤-YPD；⑤-SD；⑥-YPD；⑥-SD)



Conclusions:
① our seed solution of BY4742 have no plasmid originally.
Next step:
① extraction plasmids from liquid cultured BY4741-CNS1/1-2-pYES2.

② extraction plasmids from liquid cultured AH109-yeGFP-pYES2.

8.19th, Monday
Purpose:
Extract plasmids from transformed BY4741 with CNS1/1-2-pYSE2; compare the growth rate of AH109 in different liquid mediums.
Process:
1.colony PCR of transformed BY4741 & electrophoresis
(16×transformed BY4741: CNS1-pYES2 ①-1, 2, 3, 4 & ②-1, 2, 3, 4; CNS1-2-pYES2 ③-1, 2, 3, 4 & ④-1, 2, 3, 4)
positive result: ①-1 & ①-3 seem have expected bands. other results cannot be determined by this electrophoresis.

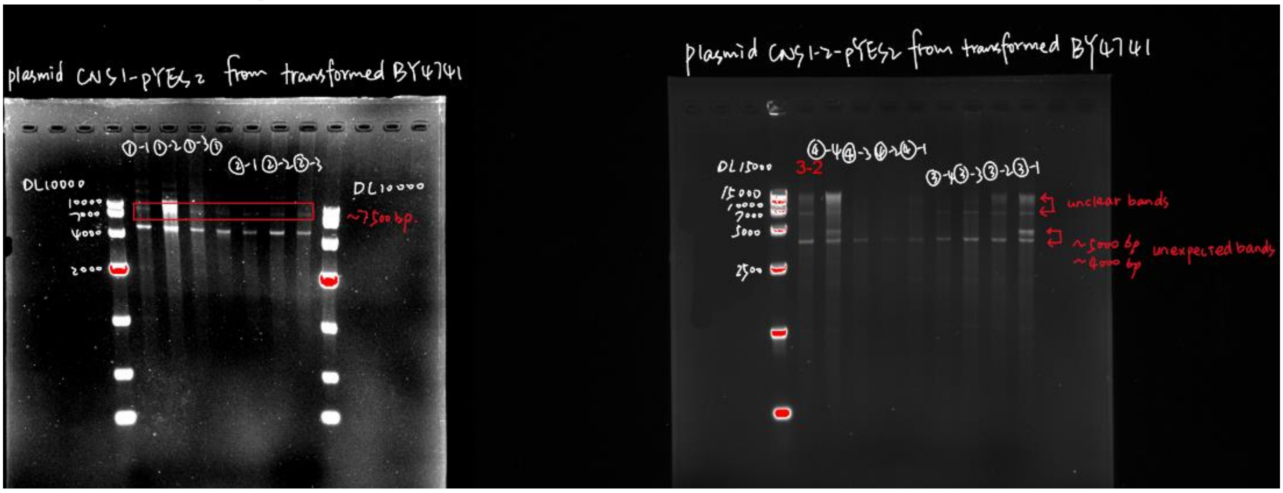


2.Plasmid extraction & gel electrophoresis

(16×transformed BY4741: CNS1-pYES2 ①-1, 2, 3, 4 & ②-1, 2, 3, 4; CNS1-2-pYES2 ③-1, 2, 3, 4 & ④-1, 2, 3, 4)

① CNS1-pYES2 should be around 8700bp, however if the plasmids are in supercoil structure, the bands around 7500 bp should be what we expect.

② ② CNS1-2-pYES2 should be around 10200bp, while there are several bands near 10000bp and all the sample appear bands around 4000/5000bp. the former bands may contain expected bands, the later ones can be mysterious.



3. Liquid culture some strains for different purpose. (from 8:35 p.m.)

① AH109(competent cells) in YPD / YPDA medium + AH109(seed cells from streaked plates) in YPD / YPDA medium

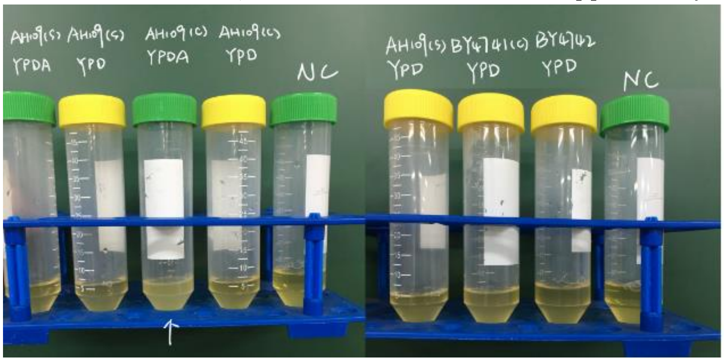
—— to test the growth rate of AH109 in liquid culture with or without Adenine.

——also to attain seed solution of strain AH109 for further experiments.

② BY4741 (competent cells) in YPD +BY4742(seed solution cells) in YPD medium

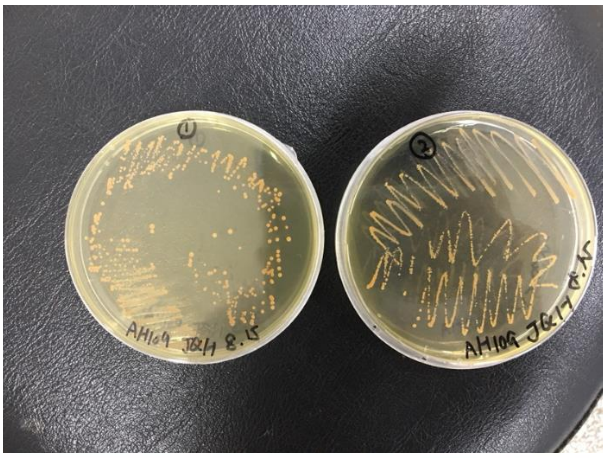
—— to attain yeasts in logarithmic stage for competent cell preparation.

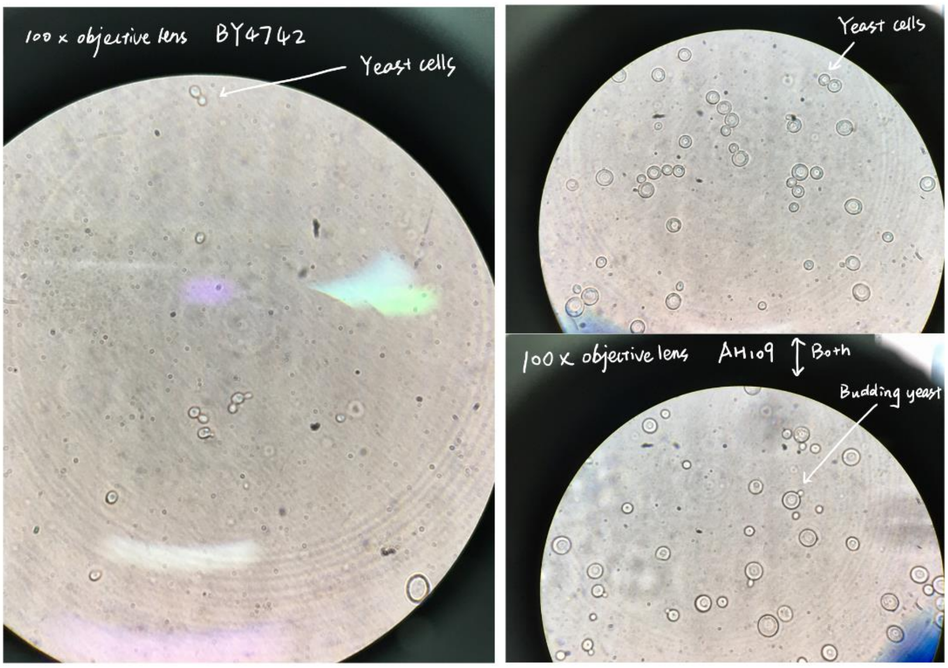
After 13 hours, AH109(C) in YPDA is apparently epinephelos.



4. Microscopic examination

(for BY47412 seed solution; AH109 colonies on streaked plates)





Conclusions:

① after colony PCR and plasmid extraction and electrophoresis we still cannot confirm that we have transformed our two plasmids into the yeasts, so we need further confirmation.

Next step:

double enzyme digestion and sequencing the plasmids of positive results; liquid culture the other strains, prepare for competent cell preparation.

8.20th, Tuesday

Purpose: ①compare the growth rate of AH109 in YPD with it in YPDA medium. ② double enzyme digestion to confirm that the transformed yeasts are with our plasmids of CNS1-pYES2 & CNS1-2-pYES2.

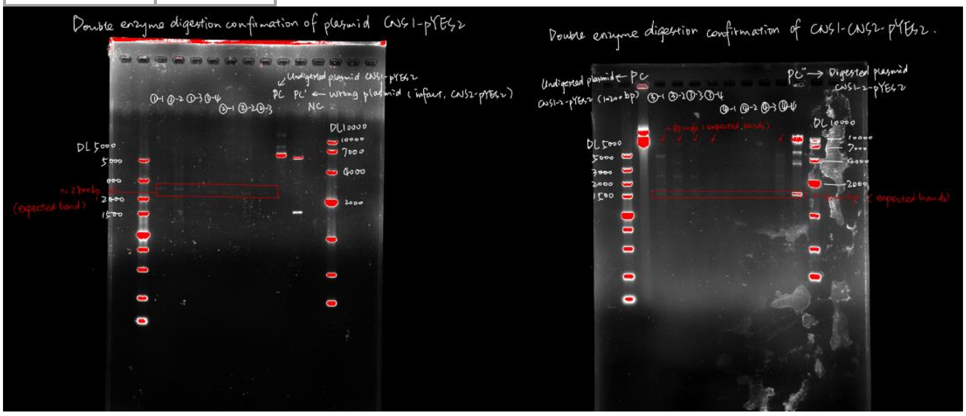
Process:

1. double enzyme digestion & electrophoresis.

10×quick cut mix	2uL
Hind III	1uL
Xba I	1uL

CNS1-pYES2	5uL
ddH2O	11uL
Total	20uL
10×quick cut mix	2uL
EcoR I	1uL
Xba I	1uL
CNS1-2-pYES2	5uL
ddH2O	11uL
Total	20uL

Process	
37℃	45min
60℃	15min
4℃	hold



2.Chemically transformation of CNS2-pYES2 into BY4741.

3.Glycerin bacteria storage (of the positive results CNS1-pYES2: ①-1, ①-2, ②-2; CNS1-2-pYES2: ③-1, ③-2, ③-3)

Conclusions:

① our transformation of CNS1-pYES2 & CNS1-2-pYES2 into BY4741 seems successful.

Next step:

① Try to prepare chemical transformation cells according to another protocol(B).

② linearize the plasmids of positive results, and sequencing the products of gel cycling.

8.21st, Wednesday

Purpose:

② confirm the correct plasmids from transformed BY4741 by sequencing.

② test another chemical transformation protocol(B).

Process：

1.Carry out the protocol of chemical transformation competent cells preparation and transform. （20/ 30/ 50 times attenuated solution of AH109 with CNS3-pYES2 and BY4741 with CNS1-pYES2 each.）

【A little bit different from the original one.】

① cells preparation:

pick out colonies and liquid culture overnight.

Activation: 20/ 30/ 50 times attenuate the solution of yeasts in 5mL YPD liquid medium. 30 ℃ for 4-5 hours culture.

2000 rpm × 2 min, discard the supernate.

1mL 1×TE buffer resuspend, transfer the solution into 1.5mL tube.

2000 rpm × 2 min, discard the supernate ; resuspend with 1mL 1×TE buffer.

2000 rpm × 2 min, discard the supernate; ≤ 300uL 1×TE resuspend.

② transformation

prepare the carrier DNA (96℃ water bath for 3min, ice bath for 3min,then repeat one time.)

Add 500uL mixture of PEG&LiAc, 5uL carrier DNA and around 2ug plasmids into a tube with 100uL prepared competent cells, then mix all them up.

42℃ water bath for 30min, rotate for mix up after 15min.

③ spread the solution on SD medium plates.

2000 rpm × 3-5 min， discard the supernate, 1mL YPDA resuspend.

2000 rpm × 2 min, discard the supernate, 50uL YPDA(A+) resuspend.

2.revive the glacerine yeasts with plasmids CNS1-pYES2 & CNS1-2-pYES2.

through streaking the solution on SD medium plates.

(prepare for next stage of protein expression.)

Next step：

② another transformation of plasmid CNS3-pYES2 into BY4741.

③ PCR a fragment from the plasmid the former days got to confirm them. (replace former planed monoenzyme digestion.)

8.22nd，Thursday

Process：

1. Another chemical transformation of CNS3-pYES2 into BY4741 competent cells made by ourselves according to protocol B.
2. Microscopic examination(of two AH109 seed solution)

results：

conclusions：

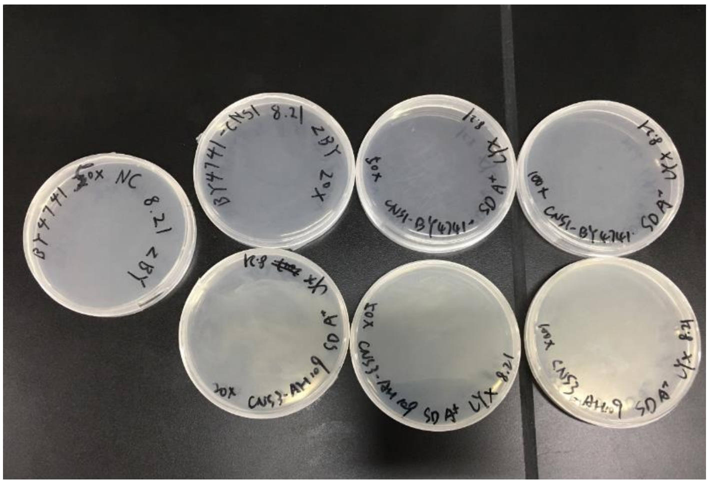
next step：

8.24th, Saturday

【results of the other day】：

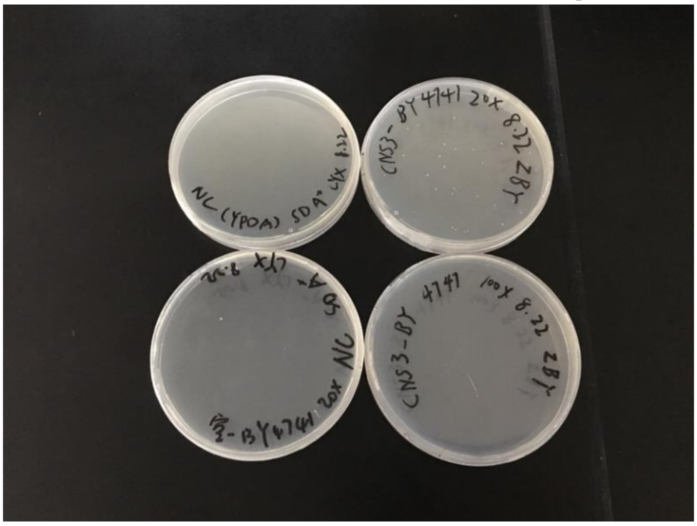
① the results of the first chemical transformation according to protocol B.

(of 20/ 50/ 100 times attenuated competent cells transformed with plasmids CNS1-pYES2 into BY4741 & CNS3-pYES2 into AH109)



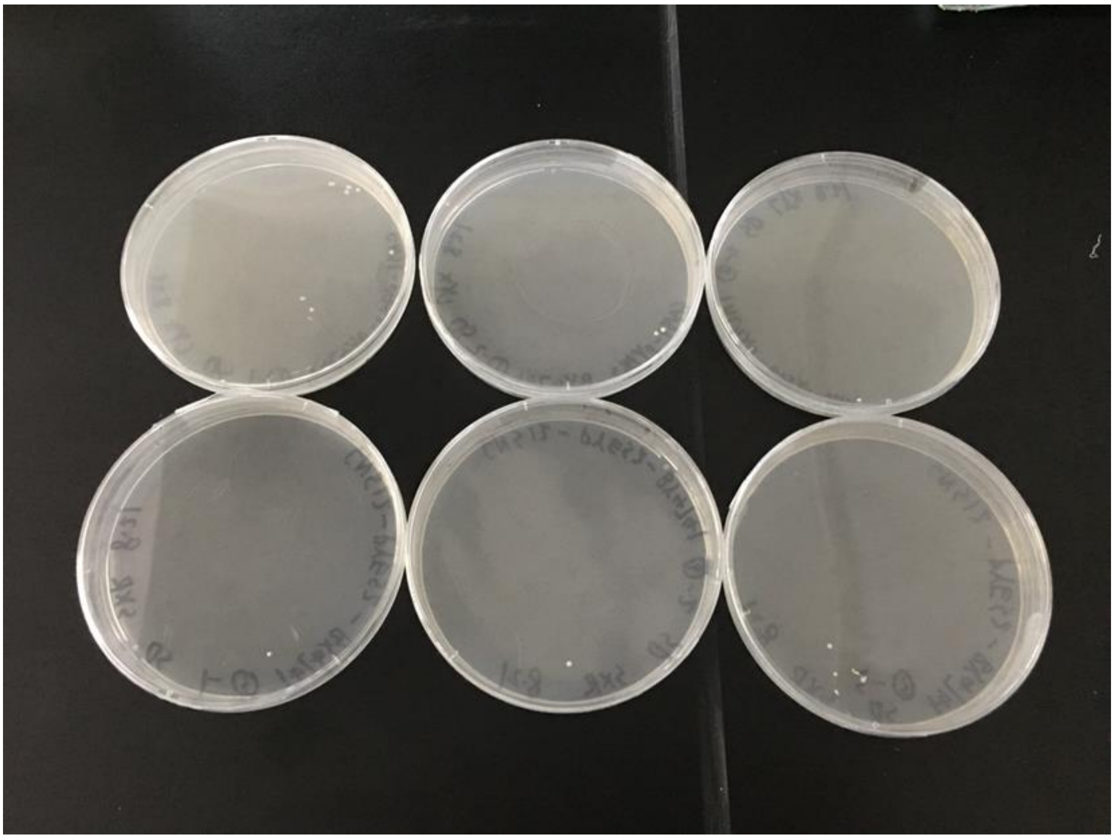
plates spreaded with AH109 show different degrees of biofilm, while plates spreaded with BY4741 hasn't show any colony.

② the results of the second chemical transformation according to protocol B. (of 20/ 100 times attenuated BY4741 competent cells transformed with plasmids)



while the plate using 20 times attenuated competent cell solution appears lots of separated colonies, which seem a perfect result; the plate using 100 times attenuated competent cell solution appears only 1 colony, which means it is over attenuated.

③ the results of revived glycerin yeasts with CNS1-pYES2 & CNS1-2-pYES2.



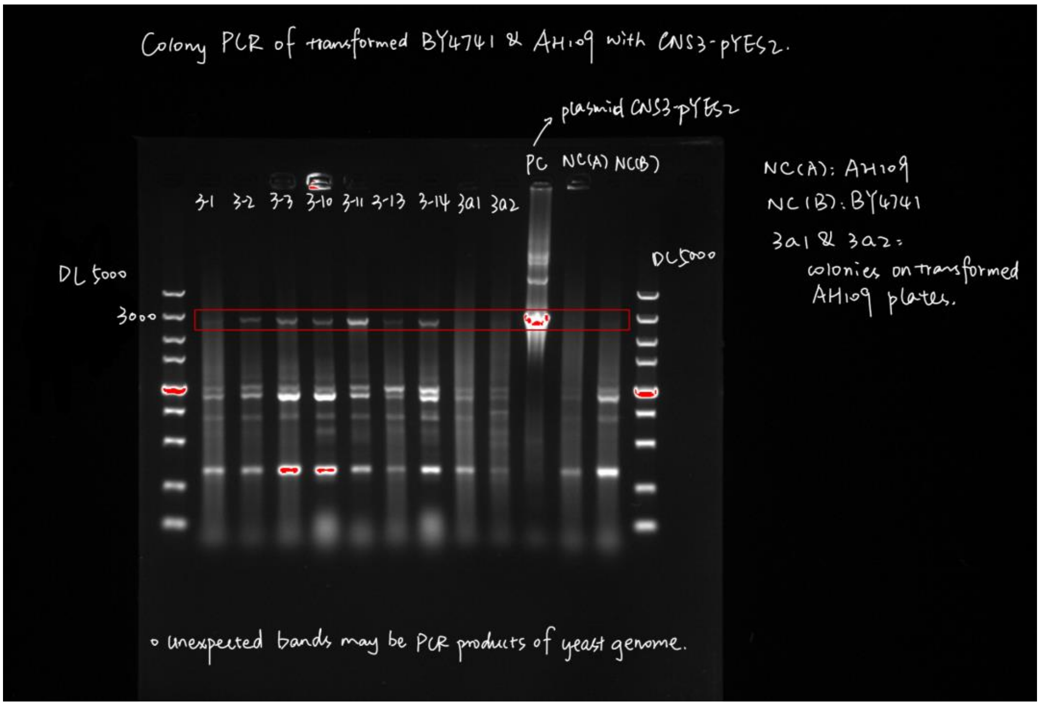
all of the plates appear several colonies. all of the plates appear several colonies.

【Today's records:】
purpose:
① compare the growth rate of BY4741 in YPD liquid medium with in SD liquid medium.
② liquid culture the colonies of transformed BY4741 with CNS3-pYES2.
③ liquid culture the colonies of revived BY4741 with CNS1-pYES2 and CNS1-2-pYES2.
④ confirm that AH109 without transform any plasmids is also able to growth in SD plates.
process:
liquid culture the colonies of transformed BY4741 with CNS3-pYES2 (5: 20 p.m.)
(in YPD liquid culture and SD liquid culture, each for 3 tubes.)
liquid culture the colonies of revived BY4741 with CNS1-pYES2 (6: 50 p.m.) and CNS1-2-pYES2 (7: 30 p.m.).
spread AH109 on SD plates.
(competent cells & seed solution)
conclusions:
next step:

8.25th, Sunday
1. microscopic examination
(of liquid cultured BY4741 with CNS3-pYES2.)
【we found that all the YPD medium are infected with bacteria. the YPD liquid medium is contaminated.】
2. colony PCR & electrophoresis
(of transformed BY4741 and AH109 with CNS3-pYES2.)

2×Mix	5uL
F primer	0.5uL
R primer	0.5uL
Yeasts	Touch
ddH2O	4uL
Total	10uL

Temperature	Time
98℃	10min
98℃	10s
50℃	10s
72℃	45S
	2-4 steps repeat 34 times
72℃	5min
4℃	Hold



Positive results: 3-1, 3-2, 3-3, 3-11, 3-13, 3-14 (all from transformed BY4741.) Negative results: 3a1, 3a2 (both from transformed AH109.)

3. liquid culture transformed BY4741 (with CNS3-pYES2) in SD liquid medium. (of 3-1→3-3, 3-11→3-14.)

8.26th, Monday

1. colony PCR of transformed BY4741 with CNS1 & CNS1-2 -pYES2
negative results.
2. induce the expression of CNS1, CNS1-2 and CNS3 with galactose.
【while, they all got infected with bacteria.】

8.27th, Tuesday

1. plasmid extraction of CNS1, CNS1-2 and CNS3
【we got very low concentration products, failed.】
2. induce the expression of CNS1 & CNS1-2 again.

Cns3

Gradient PCR for constitutive promoters

2019年8月20日星期二

Date: 08/20/2019

Purpose: determine the optimal annealing temperature for constitutive promoters **pTDH3, pTEF1, pTEF2, pTPS1 and pPDA1** of *Saccharomyces cerevisiae* BY4742

Process:

- Prepare the PCR reaction system，each promoter has 6 PCR systems and a negative control:

Gradient PCR - 1st

	Reagent	Volume/μL	Negative Control/μL
1	Forward Primer	0.4	0.4
2	Reverse Primer	0.4	0.4
3	Template: genome of BY4742 (1:10)	4.2	/
4	I-5™ 2×High-Fidelity Master Mix	5	5
5	Sterilized Deionized Water	/	4.2
6	Total	10	10

- Set PCR program:

PCR program - 1st

	Process	Temperature/ ℃	Duration	Remark
1	Initial Denaturation	98	2 min	
2	Denaturation	98	10 s	
3	Annealing	60±5	15 s	pTDH3
4				pTEF2
5				pPDA1
6		62±5		pTPS1
7				pTEF1
8	Extension	72	21 s	To step 2 for 29 cycles
9	Final Extension	72	3 min	
10	Hold	4	∞	

- Perform nucleic acid electrophoresis using 1% agarose gel dyed by Ethidium bromide.

- In the first electrophoresis, both pTDH3 and pTEF2 groups showed no positive results, thus a second PCR reaction was performed.
  PCR systems are as follows, both groups have 6 systems and a negative control:

Gradient PCR - 2nd

	Reagent	Volume/μL	Negative Control/μL
1	Forward Primer	0.4	0.4
2	Reverse Primer	0.4	0.4
3	Template: genome of BY4742 (1:5)	3	/
4	I-5™ 2×High-Fidelity Master Mix	5	5
5	Sterilized Deionized Water	1.2	4.2
6	Total	10	10

PCR programs are set as follows:

PCR program - 2nd

	Process	Temperature/ ℃	Duration	Remark
1	Initial Denaturation	98	2 min	
2	Denaturation	98	10 s	
3	Annealing	62±10	15 s	
4	Extension	72	21 s	To step 2 for 29 cycles
5	Final Extension	72	3 min	
6	Hold	4	∞	

- Perform nucleic acid electrophoresis using 1% agarose gel dyed by Ethidium bromide.

Results:

Gradient PCR for pTPS1, pTEF1, pTDH3 and pPDA1 - 1st Round

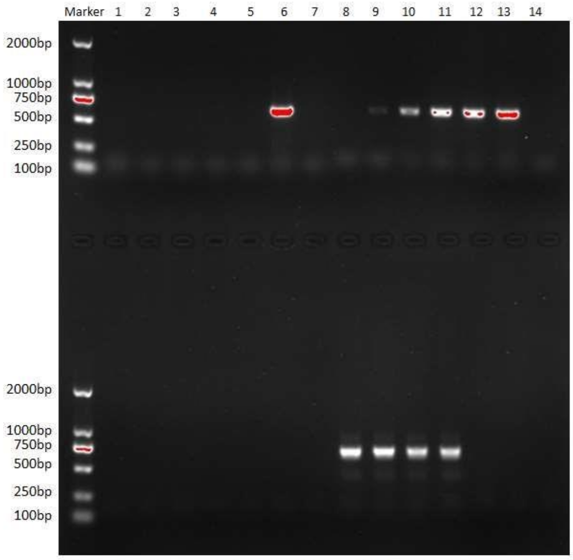


Figure 1 First round of gradient PCR for pTPS1, pTEF1, pTDH3 and pPDA1
Line 1 Lane 1-7: pTPS1 1-6, NC; Lane 8-14: pTEF1 1-6, NC
Line 2 Lane 1-7: pTDH3 1-6, NC; Lane 8-14: pPDA1 1-6, NC
Marker: Takara DL2000

【】“

Gradient PCR for pTEF2 - 1st Round

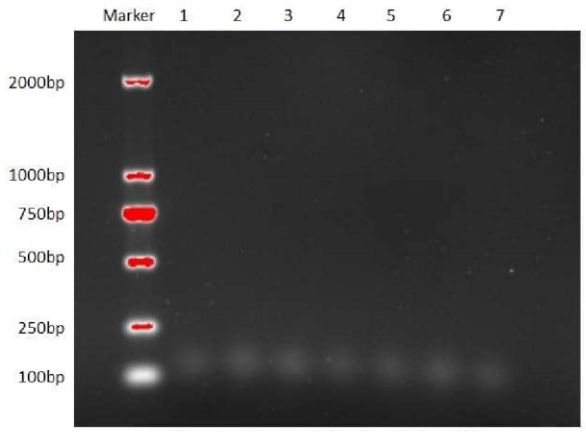


Figure 2 **First round of gradient PCR for pTEF2**
Lane 1-6: pTEF2 1-6; Lane 7: NC
Marker: Takara DL2000

It can be seen that both pTDH3 and pTEF2 had no positive results, so we added more templates into the system and changed the annealing temperature to 62±10℃ in the subsequent reaction.

Gradient PCR for pTEF2 and pTDH3 - 2nd Round

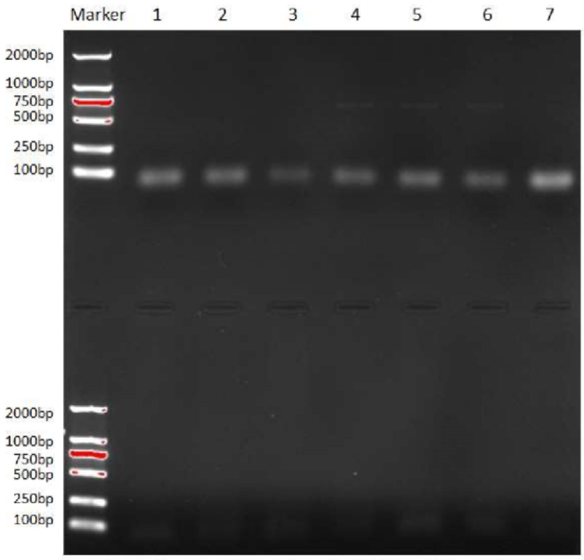


Figure 3 **Second round of gradient PCR for pTEF2 and pTDH3**
Line 1 Lane 1-6: pTEF2 1-6; Lane 7: NC
Line 2 Lane 1-6: pTDH3 1-6; Lane7: NC
Marker: Takara DL2000

Conclusions:

1. From the positive results we chose 56℃ as the optimal annealing temperature for pTPS1, pTEF1 and pPDA1;
2. In the second round of reaction, pTEF2 showed faint results, thus a median annealing temperature of 60℃ was selected for subsequent reactions;
3. We that differences between genomes of *Saccharomyces Cerevisiae* BY4742 and standard strain may attribute to the negatitive results of gradient PCR on pTDH3, so a re-design of primers is needed.

Next step:

- Amplification PCR

Amplification PCR for constitutive promoters

2019年8月22日星期四

Date: 08/21/2019

Purpose: Amplicate copies of constitutive promoters for later digestion and ligation

Process:

- Prepare the PCR reaction system，each promoter（pTPS1, pTEF1, pPDA1, pTEF2)has 2 systems and a negative control:

Amplification PCR

	Reagent	Volume/μL	Negative Control/μL
1	Forward Primer	2	1
2	Reverse Primer	2	1
3	Template	3	/
4	I-5™ 2×High-Fidelity Master Mix	25	12.5
5	Sterilized Deionized Water	18	10.5
6	Total	50	25

- Perform nucleic acid electrophoresis using 1% agarose gel dyed by Ethidium bromide.

- Set PCR program:

Amplification PCR Program

	Process	Temperature/ °C	Duration	Remark
1	Initial Denaturation	98	2 min	
2	Denaturation	98	10 s	
3	Annealing	60	15	pTEF2
4		56		pTPS1
5				pTEF1
6				pPDA1
7	Extension	72	21 s	To step 2 for 34 cycles
8	Final Extension	72	3 min	
9	Hold	4	∞	

Result:

Amplification PCR for constitutive promoters

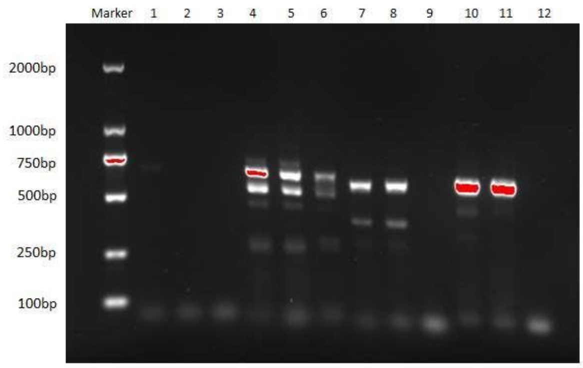


Figure 1 **Amplification PCR for constitutive promoters**
Lane 1-3: pTEF2 1, 2, NC; Lane 4-6: pPDA1 1, 2, NC
Lane 7-9: pTPS1 1, 2, NC; Lane 10-12: pTEF1 1, 2, NC
Marker: Takara DL2000

Conclusion:

- Attempts to amplicate pTEF2 had failed, but amplication on pPDA1, pTEF1 and pTPS1 showed favorable results. The bands in the NC group of pPDA1 may be attributed to a fault when adding samples;

Next step:

- Enzymatic digestion and ligation

Enzyme digestion for and ligation between constitutive promoters and plasmid yeGFP-pYES2

2019年8月22日星期四

Date: 08/21/2019

Purpose: Digest constitutive promoters pTPS1, pPDA1 and pTEF1 ,as well as plasmid yeGFP-pYES2 using restricted endonuclease Spe Ⅰ and Hind Ⅲ; then link fragments of constitutive promoters and plasmid yielded from digestion using T4 DNA ligase to construct plasmid pTPS1/pPDA1/pTEF1-yeGFP-pYES2;

Process:

- Purify products collected from amplication PCR using Cycle Pure Kits;
- A subsequent nucletic acid concentration determination was performed on NanoDrop micro spectrophotometer;
- Run double enzyme digestion:

Double Enzyme Digestion System

		pPDA1	pTEF1	pTPS1	yeGFP-pYES2 plasmid
1	Spe Ⅰ*/μL	1	1	1	1
2	Hind Ⅲ*/μL	1	1	1	1
3	10*Buffer/μL	2	2	2	2
4	Fragments/μL	3.7	3.7	6	6
5	Sterilized Deionized Water/μL	12.3	12.3	10	10
6	Total/μL	20			
7	*From Takara Biomedical Technology (Beijing) Co., Ltd.				

Double Enzyme Digestion Program

		Temperature/ ℃	Duration
1	Digestion	37	90 min
2	Hold	4	∞

- Run nucleic acid electrophoresis using 1% agarose gel dyed by Ethidium bromide to determine the validation of digestion, then purify products collected from double enzyme digestion. Fragments of constitutive promoters are collected using Cycle Pure Kits, and plasmids are yielded via gel extraction;

- A subsequent nucletic acid concentration determination was performed on NanoDrop micro spectrophotometer;

- Aftering determining nucletic acid concentration, enzymic ligation system conducted at the temperature of 15℃ and incubated overnight:

Enzymatic Ligation System

	A	B	C	D
1		pTEF1	pTPS1	pPDA1
2	10*Buffer/μL	2	2	2
3	T4 Ligation Enzyme/μL	1	1	1
4	Digested yeGFP-pYES2 plasmid/μL	8	8	8
5	Template/μL	3.5	3.5	4
6	Sterilized Deionized Water/μL	5.5	5.5	5
7	Total/μL	20		

- Perform nucleic acid electrophoresis using 1% agarose gel dyed by Ethidium bromide.

Results:

Spe I and Hind III enzyme digestion for pTPS1, pTEF1, pPDA1 and yeGFP

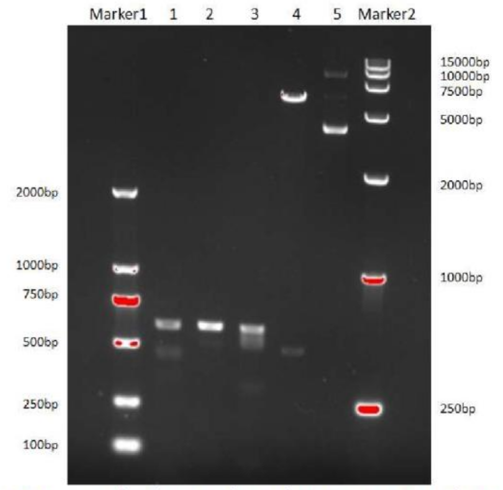


Figure 1 Spe I and Hind III enzyme digestion for pTPS1, pTEF1, pPDA1 and yeGFP
Lane 1: pTPS1; Lane 2: pTEF1; Lane 3: pPDA1
Lane 4: Digested yeGFP-pYES2; Lane 5: Original yeGFP-pYES2
Marker 1: Takara DL2000; Marker 2: Takara DL15000

Conclusions:

- The lengths of pTPS1, pTEF1 and pPDA1 is 620bp, 640bp and 674bp repectively. And digested plasmid fragments should have lengths around 6000bp and 500bp, so it can be concluded that enzyme digestion was a success;

Next step:

- Plasmid transformation and colony PCR verification.

Plasmid transformation and colony PCR on E.Coli DH5α

2019年8月23日星期五

Date: 08/22/2019 - 08/23/2019

Purpose: Transform products of enzymatic ligation into E.Coli DH5α and select positive colonies and amplify copies of desired pTEF1/pTPS1/pPDA1 - yeGFP - pYES2 plasmid; colony PCR is conducted to verify that constitutive promoters are properly linked upstream of yeGFP;

Process:

- Plasmid transformation:

1. Each tube contains 50μL competent E.Coli DH5α and 10 μL plasmid, the negative control group contains 50μL competent E.Coli DH5α and 10 μL ddH_2O;
2. Incubate on ice for 30 minutes;
3. Heat shock at 42℃ for 78 seconds;
4. Instantly transfer the tubes on ice and let sit for 10 minutes;
5. Add 400μL liquid LB medium into each tube and vibrate on a shaker for 60 minutes at 37℃;
6. Add 200μL cultured medium onto solid LB plate with 0.1% Ampicilin and incubate at 37℃ overnight;

- Conlony PCR:

On the next day single colonies was selected and colony PCR was conducted, reaction systems were mixed as follows, each group had 10 systems and a negative control:

Colony PCR System

	Reagent	Volume/μL	Negative Control/μL
1	Forward Primer	0.4	0.4
2	Reverse Primer	0.4	0.4
3	Template	2	/
4	2×T5 Super PCR Mix (Colony)	5	5
5	Sterilized Deionized Water	2.2	4.2
6	Total	10	10

Amplification PCR Program

	Process	Temperature/ ℃	Duration	Remark
1	Initial Denaturation	98	2 min	
2	Denaturation	98	10 s	
3	Annealing	56	15 s	pTPS1
4				pTEF1
5				pPDA1
6	Extension	72	21 s	To step 2 for 29 cycles
7	Final Extension	72	3 min	
8	Hold	4	∞	

- Perform nucleic acid electrophoresis using 1% agarose gel dyed by Ethidium bromide.
- True positive colonies were then selected and put into 3 mL liquid LB medium with 0.1% Ampicilin for overnight culture.

Results:

Overnight culture of E.Coli DH5α transformed with pPDA1/pTEF1/pTPS-yeGFP-pYES2

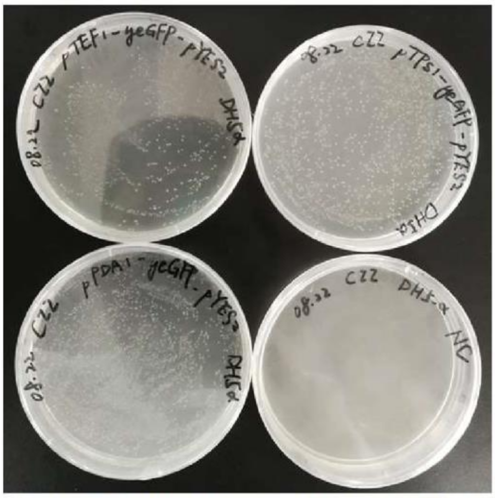


Figure 1 Overnight culture of E.Coli DH5α transformed with pPDA1/pTEF1/pTPS-yeGFP-pYES2

Colony PCR after transformation of pTEF1-yeGFP-pYES2

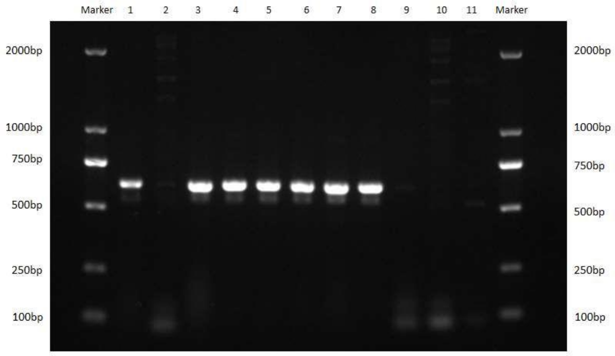


Figure 2 **Colony PCR after transformation of pTEF1-yeGFP-pYES2**
Lane 1-10: E.Coli DH5α pTEF1-yeGFP-pYES2 single colonies 1-10; Lane 11:NC
Marker: Takara DL2000

Colony PCR after transformation of pTPS1-yeGFP-pYES2

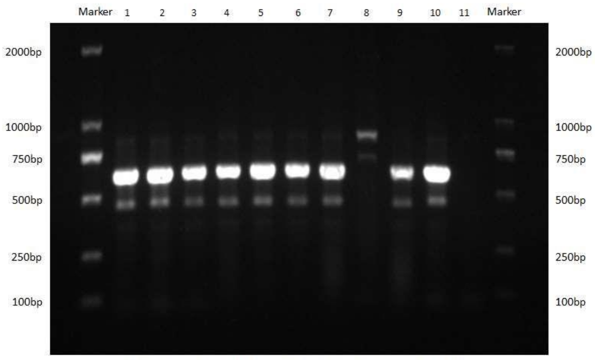


Figure 3 **Colony PCR after transformation of pTPS1-yeGFP-pYES2**
Lane 1-10: E.Coli DH5α pTPS1-yeGFP-pYES2 single colonies 1-10; Lane 11:NC
Marker: Takara DL2000

Plasmid extraction and verification.

Colony PCR after transformation of pPDA1-yeGFP-pYES2

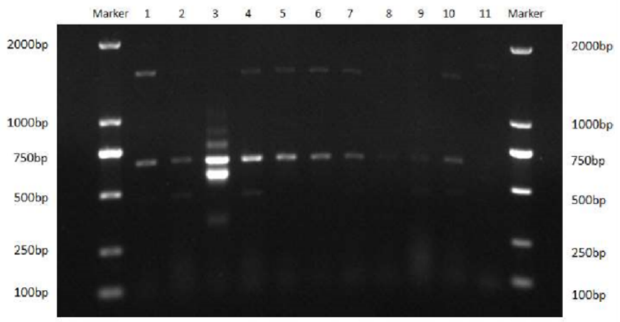


Figure 4 Colony PCR after transformation of pPDA1-yeGFP-pYES2
Lane 1-10: E.Coli DH5α pPDA1-yeGFP-pYES2 single colonies 1-10; Lane 11:NC
Marker: Takara DL2000

Conclusions:

1. Plasmid transformation was a success;
2. True positive single colonies include pTEF1 1,3,4,5,6,7,8; pTPS1 1,2,3,4,5,6,7,9,10; pPDA1 1,2,3,4,5,6,7,10;

Next step:

p

Plasmid extraction and enzyme digest validation

2019年8月24日星期六

Date: 08/25/2019

Purpose: extract plasmids amplified in DH5α and verify their positivity via enzyme digestion;

Process:

- Before extraction glycerol bacteria was mixed by adding 500μL LB culture into 500μL 50% glycerol and then stored at -80℃;
- Plasmid extraction was conducted according to the protocols provided in Plasmid Mini Kit I by Omega Biotechs;
- A subsequent nucleic acid concentration determination was performed on NanoDrop micro spectrophotometer;
- Carry out enzymatic digestion:

Enzyme Digestion System

		Plasmid
1	Spe Ⅰ*/μL	0.5
2	Hind Ⅲ*/μL	0.5
3	10*Buffer/μL	1
4	Template/μL	3
5	Sterilized Deionized Water/μL	5
6	Total/μL	10
7	*From Takara Biomedical Technology (Beijing) Co., Ltd.	

Plasmid yeGFP - pYES2 was used as a contrast.

Enzyme Digestion Program

		Temperature/ ℃	Duration
1	Digestion	37	120 min
2	Hold	4	∞

- Perform nucleic acid electrophoresis using 1% agarose gel dyed by Ethidium bromide.
- Ture positive plasmids were then sent to Tsingke Chengdu for gene sequencing;

Results:

Spe I and Hind III digestion for plasmid pTEF1-yeGFP-pYES2 extracted from single colonies

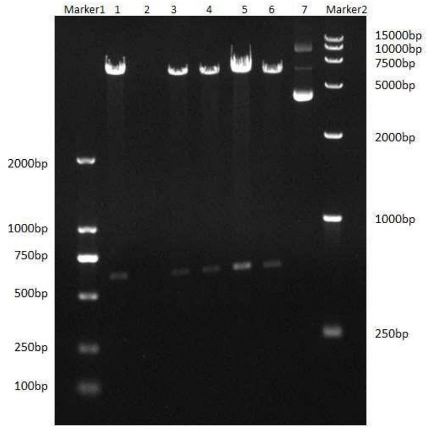


Figure 1 Spe I and Hind III digestion for plasmid pTEF1-yeGFP-pYES2 extracted from single colonies
Lane 1-6: pTEF1-yeGFP-pYES2 extracted from single colony 1,3,4,5,6,7
Lane 7: Plasmid yeGFP-pYES2
Marker 1: Takara DL2000; Marker 2: Takara DL15000

Spe I and Hind III digestion for plasmid pTPS1-yeGFP-pYES2 extracted from single colonies

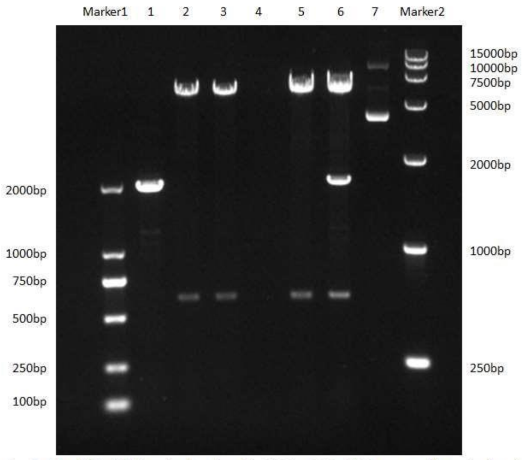


Figure 2 **Spe I and Hind III digestion for plasmid pTPS1-yeGFP-pYES2 extracted from single colonies**
Lane 1-6: pTPS1-yeGFP-pYES2 extracted from single colony 2,3,5,6,9,10
Lane 7: Plasmid yeGFP-pYES2
Marker 1: Takara DL2000; Marker 2: Takara DL15000

Spe I and Hind III digestion for plasmid pPDA1-yeGFP-pYES2 extracted from single colonies

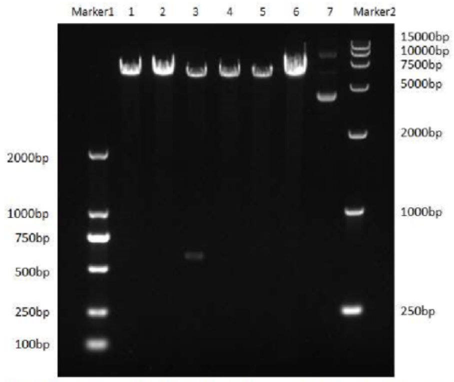


Figure 3 **Spe I and Hind III digestion for plasmid pPDA1-yeGFP-pYES2 extracted from single colonies**
Lane 1-6: pPDA1-yeGFP-pYES2 extracted from single colony 1,2,3,5,6,10
Lane 7: Plasmid yeGFP-pYES2
Marker 1: Takara DL2000; Marker 2: Takara DL15000

Conclusion:

Among the single colonies selected, pTEF1 1,4,5,6,7 pTPS1 3,5,9,10 and pPDA1 3 were ture positive but still needs gene sequencing for further verification.

Construction of the pYES2-HisG plasmid-round 1

2019年8月4日星期日

- [x] **Gradient PCR and amplification for HisG domain**

- date：20190803
- purpose：

find the appropriate annealing temperature(primer:mf-hisg/yh-hisg/hisg-only) by gradient PCR, and obtain a large number of HisG fragments by PCR

- process：

1. Prepare for the following system:
   Among them, MF and YH each has 8 tubes, and HisG only has 12 tubes.

gradient PCR system

	A	B
1		10ul for each tube
2	template DNA	0.5ul
3	primer F	0.4ul
4	primer R	0.4ul
5	Mix	5ul
6	ddH20	3.7ul

2. Run gradient PCR as followed and perform Agarose gel electrophoresis on the product:

gradient PCR

	A	B	C	D
1	predegeneration	98℃	2min	
2	degeneration	98℃	10s	
3	annealing	54-66℃	15s	
4	extend	72℃	mf/yh:15s;HisG: 8s	35 cycle
5	final extend	72℃	5min	
6	final hold	4℃	∞	

3. According to the results obtained in the previous step, prepare for system as follows:
- Among them, MF/YH/HisG each has 4 tubes.

amplification PCR system

	A	B
1		50ul for each tube
2	template DNA	2ul
3	primer F	2ul
4	primer R	2ul
5	Mix	25ul
6	ddH20	19ul

4. Run amplification PCR reaction as follows and perform Agarose gel electrophoresis on the product:

amplification PCR reaction

	A	B	C	D
1	predegeneration	98℃	2min	
2	degeneration	98℃	10s	
3	annealing	mf/yh:61.3℃;HisG:60.5℃	15s	
4	extend	72℃	mf/yh:15s;HisG:8s	35 cycle
5	final extend	72℃	5min	
6	final hold	4℃	∞	

5. Implement gel extraction to obtain the target segment.

- results:

1. The gel phote after gradient PCR as followed:

figure1

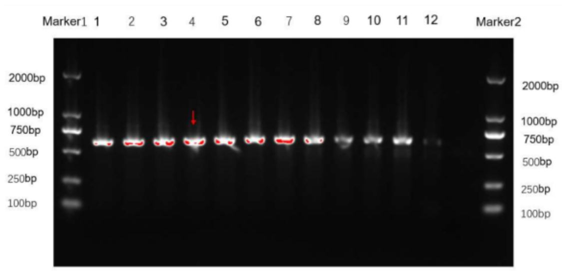


Figure1. 1~12 lane: HisG-only PCR product with a gradient of 54-66 ℃。Red arrow: Target product。Marker1: Takara DL2000 DNA Marker。Marker2: Takara DL2000 DNA Marker 。

figure2

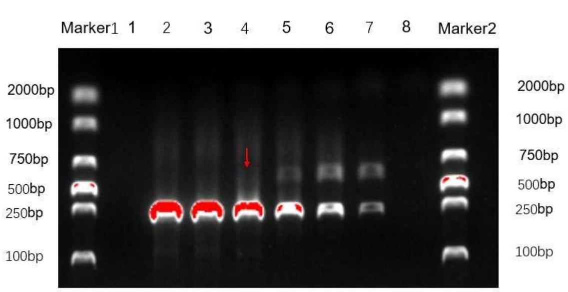


Figure2. 1~8 lane: MF PCR product with a gradient of 54-66 ℃。Red arrow: Target product。Marker1: Takara DL2000 DNA Marker。Marker2: Takara DL2000 DNA Marker 。

figure3

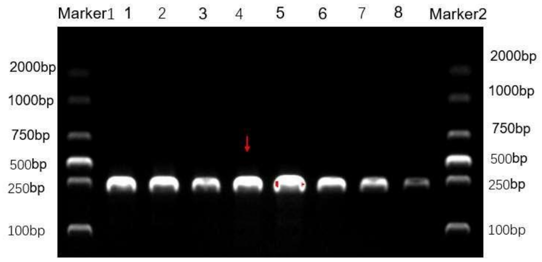


Figure3. 1~8 lane: YH PCR product with a gradient of 54-66 ℃。Red arrow: Target product。Marker1: Takara DL2000 DNA Marker。Marker2: Takara DL2000 DNA Marker。

2. The gel photo after amplification PCR as followed:

figure4

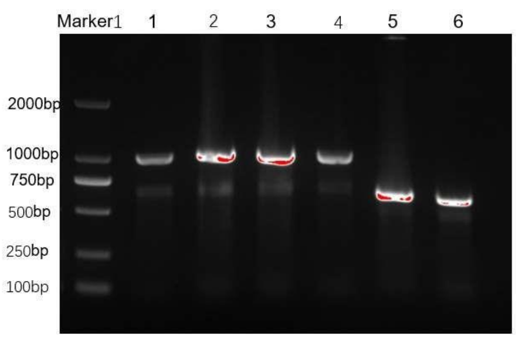


Figure4. 1~4 lane: MF PCR product。 5~6 lane: HisG-only PCR product。Marker1: Takara DL2000 DNA Marker。

figure5

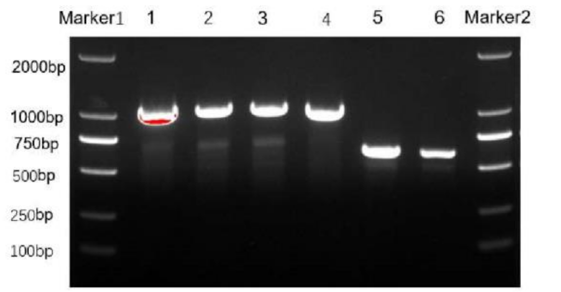


Figure5. 1~4 lane: YH PCR product。 5~6 lane: HisG-only PCR product 。Marker1: Takara DL2000 DNA Marker。Marker2: Takara DL2000 DNA Marker。

- conclusions:

1. The suitable annealing temperature of mf/yh is 61.3℃.
2. The suitable annealing temperature of HisG-only is 60.5℃.

- next step:

Perform enzyme digestion reaction on the product of glue recycling and pYES2 plasmid.
ligation of the two fragments obtained from the emzyme digestion.

Enzyme digestion and ligation reaction

- date: 20190805
- purpose:

Perform enzyme digestion reaction on HisG fragment(the product of glue recycling yesterday) and pYES2 plasmid.
Explore the optimal time of the enzyme digestion reaction.
Ligation of the two fragments obtained from the emzyme digestion.

- process:

1. Prepare for the following enzyme digestion system:

Among them, HisG only/MF /YH each has 1 tubes, and pYES2 only has 3 tubes.

enzyme digestion system

	A	B	C
1	all: 25ul	HisG	pYES2
2	DNA	18ul	12ul
3	Xba Ⅰ	1ul	1ul
4	Hind Ⅲ	1ul	1ul
5	ddH2O	2.5ul	8.5ul
6	green buffer	2.5ul	2.5ul

2. Run the enzyme digestion reaction with 37℃ for 3h.
3. Take 5ul from pYES2 tubes for agarose gel electrophoresis verification.
4. Measure the concentration of the products.
5. Prepare for the following ligation system with 3 tubes:

ligation system

	A	B	C
1	all:20ul	MF/YH	ONLY
2	10X T4 buffer	2ul	2ul
3	pYES2	5ul	5ul
4	HisG	4ul	1.5ul
5	T4 ligase	1ul	1ul
6	ddH20	8ul	10.5ul

6. Run the ligation reaction with 16℃ for 8h overnight,then

put the product n the refr gerator -20℃ storage

- results:

1. The gel phote after enzyme digestion reaction as followed:

figure1

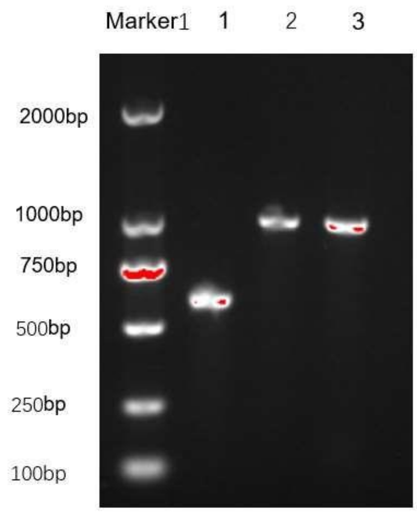


Figure1. 1 lane: HisG-only enzyme-digested product。2 lane: MF enzyme-digested product。3 lane: YH enzyme-digested product。 Marker1: Takara DL2000 DNA Marker。

figure2

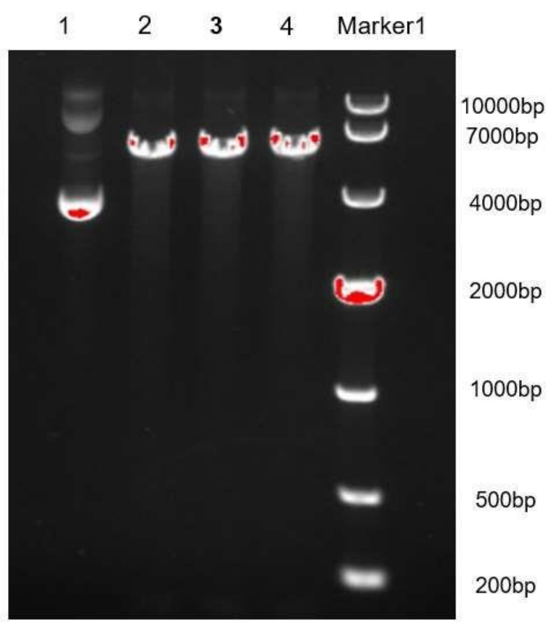


Figure2. 1 lane: pYES2 plasmid。2~4 lane: pYES2 enzyme-digested product。Marker1: Takara DL10000 DNA Marker。

- next step:

ransform the p ds pY S2 sG nto D 5α

asm

Expression and validation of pYES2-HisG

- date: 20190805
- purpose:

Transform pYES2-HisG plasmid into DH5α.
Individual bacterial colonies PCR to obtain positive clone.
Extract and sequence.

- process:

1. Transform pYES2-HisG into DH5α, and culture for 16 hours at 37℃.
2. Prepare for the colonies PCR system.

Among them, MF/YH/HisG each has 5 tubes.

colonies PCR system

	A	B
1		10ul for each tube
2	Individual bacterial colony	/
3	primer F	0.4ul
4	primer R	0.4ul
5	T5 super Mix	5ul
6	ddH20	4,2ul

3.Run the colonies PCR reaction as follows and and perform Agarose gel electrophoresis on the product:

colonies PCR

	A	B	C	D
1	predegeneration	98℃	2min	
2	degeneration	98℃	10s	
3	annealing	mf/yh:61.3℃;HisG:60.5℃	15s	
4	extend	72℃	15s	30 cycle
5	final extend	72℃	3min	
6	final hold	4℃	∞	

4. Liquid culture: select the positive clones in the previous step for liquid culture.

- results:

Though colonies PCR has positive results, no e-coli growth was observed after 24h liquid culture.

- conclusions:

We speculated that the results of colony PCR were all false positive results, that is, plasmids adhered to the surface of bacteria but did not transform into bacteria.

- next step:

Do another round.

Construction of the pYES2-HisG plasmid-round 2

2019年8月4日星期日

- date：20190809~20190814
- purpose：

Construction of the pYES2-HisG plasmid for the second time.

- process：

The steps are exactly the same as those of the first experiment.

- results：

1. The gel phote after enzyme digestion reaction as followed:

figure1

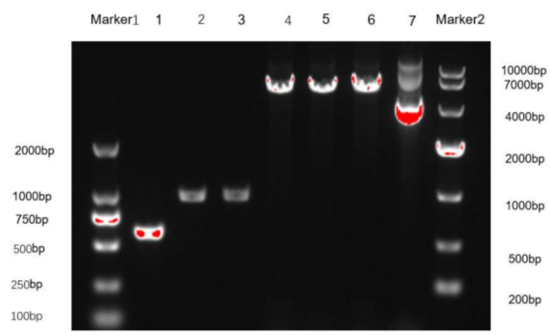


Figure1. 1 lane： HisG-only enzyme-digested product。2 lane： MF enzyme-digested product。3 lane： YH enzyme-digested product。 Marker1： Takara DL2000 DNA Marker。 4~6 lane： pYES2 enzyme-digested product。 7 lane： pYES2 plasmid。Marker2： Takara DL2000 DNA Marker。Marker2： Takara DL10000 DNA Marker。

2. The conlony PCR results as followed:

figure2

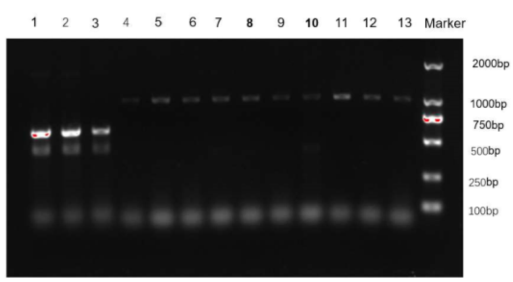


Figure2. 1-3 lane： HisG-only 。4-13 lane： MF。Marker： Takara DL10000 DNA Marker。

figure3

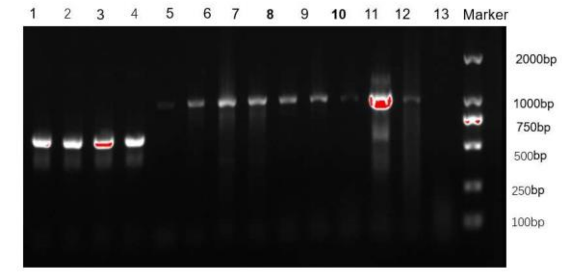


Figure3. 1-4 lane： HisG-only 。4-13 lane：YH。Marker：Takara DL10000 DNA Marker。

3. The results of plasmid extraction after liquid culture as followed:

figure4

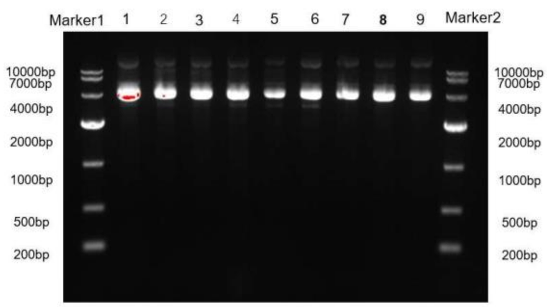


Figure4. 1-6 lane： HisG-only-pYES2 。7-9 lane：MF-pYES2。Marker1 and2：Takara DL10000 DNA Marker。

figure5

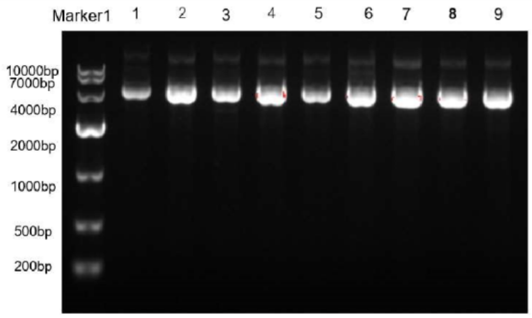


Figure5. 1-6 lane： YH-only-pYES2 。7-9 lane：MF-pYES2。Marker1：Takara DL10000 DNA Marker。

- conclusions:

1. With the experience of the first round and the problem of glue recycling kit excluded, our results of this round are obviously better than that of the first round.
2. We cannot determine whether all these plasmids are empty vectors or the desired results, which requires further PCR verification.

- next step：

PCR verification

Construction of the pYES2-HisG plasmid-round 3

2019年8月4日星期日

- date：20190811~
- purpose：

Construction of the pYES2-HisG plasmid for the third time.

- process：

The steps are exactly the same as those of the previous experiment.

- results：

1. The gel phote after enzyme digestion reaction as followed:

figure1

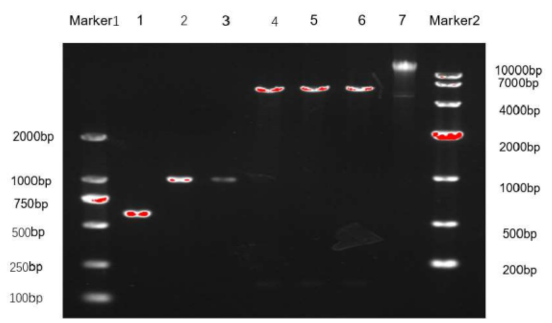


Figure1. 1 lane：HisG-only enzyme-digested product。2 lane：MF enzyme-digested product。3 lane：YH enzyme-digested product。Marker1：Takara DL2000 DNA Marker。4~6 lane： pYES2 enzyme-digested product。7 lane：pYES2 plasmid。Marker2：Takara DL2000 DNA Marker。Marker2：Takara DL10000 DNA Marker。

2. The conlony PCR results as followed:

figure2

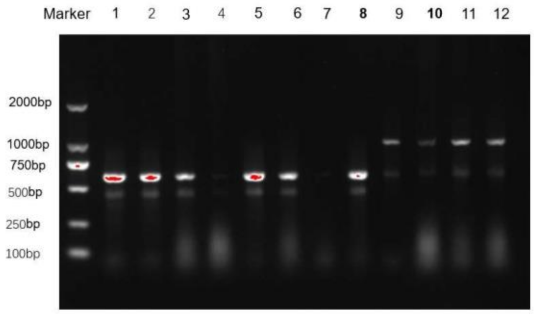


Figure2. 1-8 lane： HisG-only 。8-12 lane：MF。Marker：Takara DL2000 DNA Marker。

figure3

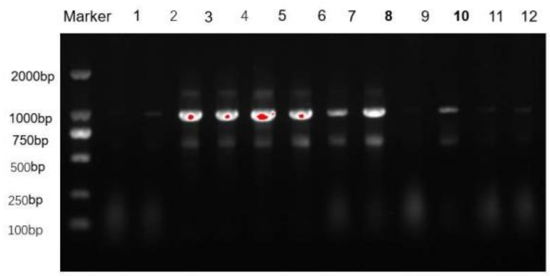


Figure3. 1-4 lane： YH 。5-12 lane：MF。Marker：Takara DL2000 DNA Marker。

figure3

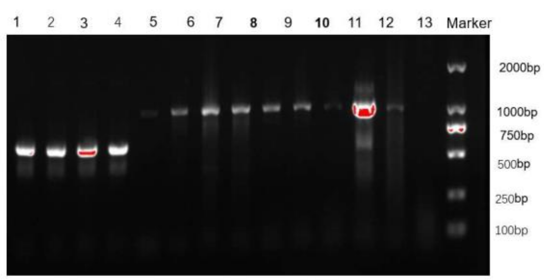


Figure3. 1-4 lane： HisG-only 。4-13 lane：YH。Marker：Takara DL10000 DNA Marker。

3. The results of plasmid extraction after liquid culture as followed:

- conclusions:

- next step:

plasmid extraction

2019年8月19日星期一

- date：20190817
- purpose：to test the plasmids extracted
- process:

perform double endonuclease restriction for 45min.

Table1

	A	B
1	XbaI	1ul
2	HindIII	1ul
3	ddH2O	13ul
4	buffer	2ul
5	plasmids	3ul

- results:

no fragments which are about 1000bp or 600bp. (sad

- conclusion:

the plasmids got last time are no-load plasmids.

- next step:

do the next round of Cns3-hisG experiment.

Construction of the pYES2-HisG round4

2019年8月19日星期一

- purpose: **the amplification of hisG domain**
- process: 1) PCR of the hisG domain in three aspects, only-hisG\mf-hisG\yh-hisG as before.

but we change the 'final elogation' time to 2min from 5min.

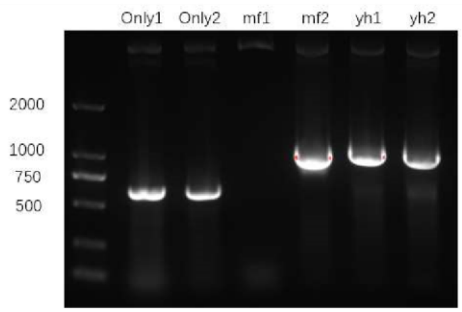
2) gel extraction (1.1% agarose; 35min)

3) concentration test

- results: GEL GRAPH

1. PCR.

image.png



Amplification PCR to retain enough fragments. There are no lines in the mf1 lane, it is probably because only one primer was added. Other lanes are quiet well.

2. concentrations:

Table1

	A	B
1	name	concentration(ng/ul)
2	mf(230)	131.59
3	yh1	88.40
4	yh2(230)	50.94
5	only1	64.46
6	only2	60.24

- conclusion:
  successfully get three fragments. but the 230 value is abnormal in two of them.
- next step:
  do enzymatic digestion and ligation the next day.

2019年8月20日星期二

- purpose: to perform enzymatic digestion and liagation for the next day's transformation.
- process:
  - enzymatic digestion
    -

Table2

	A	B	C
1	all：20ul	HisG	pYES2
2	DNA	18ul	12ul
3	Xba Ⅰ	1ul	1ul
4	Hind Ⅲ	1ul	1ul
5	ddH2O	2.5ul	8.5ul
6	green buffer	2.5ul	2.5ul

- ○ gel extraction and column extraction
- ○ ligation

2019年8月22日星期四

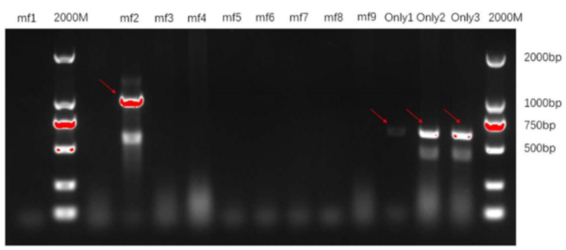
- Purpose : to get the real positive results.
- Process:

1. transfrmation. Transfer ligations to the transa E.coli.
2. Colony PCR for the positive results. The system and conditions used are as before.

- Results:

1. only-hisG: 1\2\3\4\\7\9
2. mf: 1\2\8
3. Yh:1\2\8

image.png



Notice: gel shows that maybe mf is 2 only, but I just forgot the correct order.

- Conclusion: there are positive results in the colony step.
- Next steps: extract plasmids from the colonies selected, then perform enzyme digestion to test and verify.

2019年8月23日星期五

- purpose: to get the cloned plasmids for sequencing.
- process: 1. plasmid extraction; 2. send to sequence.
- results: successful groups,
  a. only-hisG: 1\2\3\5\7\9
  b. mf: 1\2
  c. yh: 2\8
- next step: to do the protein experiments.

Electroporation

2019年7月31日星期三

Purpose:
Culturing the S.cerevisiae BY4742 to logarithmic phase.

Procedure:
Transfer 20μl YPD liquid medium with overnight cultured S.cerevisiae BY4742 to 500ml fresh YPD liquid medium in 2L Erlenmeyer flask, culture at Constant Temperature Incubator Shaker with 30℃ & 220rpm for 10h. Measure its OD600, concentrate the S.c if OD600 is greater than 0.8 and less than 1.2.

Result:
OD600=0.293 (in the evening before we store it in 4℃) Temporarily store the liquid medium in 4℃.

2019年8月1日星期四

Purpose:
Collect the concentrated S.cerevisiae BY4742 and prepare for electroporation.

Procedure:
Take the liquid medium out of 4℃ refrigerator and measure its OD600.
Transfer the liquid medium into several 50ml centrifugal tubes.
Centrifuge at 4℃ 4000g for 20mins.
Discard the supernate and resuspend all the S.c with 80ml 4℃ sterile ddH2O totally.
Centrifuge at 4℃ 4000g for 20mins.
Discard the supernate and resuspend all the S.c with 40ml 4℃ 1mol/L sterile sorbitol solution.
Collect all the liquid into one 50ml centrifugal tube.
Centrifuge at 4℃ 4000g for 20mins.
Discard the supernate and resuspend all the S.c with 0.8ml 4℃ 1mol/L sterile sorbitol solution.
Pack each 100μl S.c medium in one 1.5ml EP tube and quickly freeze them by liquid nitrogen.
Store the S.c in -80℃ refrigerator.

Result:

OD600=1.424 (in the morning before we wash it)

Get 16X100μl competent S.cerevisiae BY4742, stored in -80℃ refrigerator.

2019年8月2日星期五

Purpose:
Transfer the pYES2 plasmid into competent S.cerevisiae BY4742 by setting voltage and time gradients.

Procedure:
Take the competent S.cerevisiae BY4742 out of -80℃ refrigerator and recover it in ice.
Measure the concentration of pYES2.
Set voltage and time gradients as follows.

Table1

	time	1500V	1625V	1750V	E	F
1	2ms	1	4	7		
2	6ms	2	5	8		
3	10ms	3	6	9		

Add 50μl competent S.cerevisiae BY4742 and 1μl pYES2 into electroporation cup.
Electroporate each group.
Add 1ml 4℃ 1mol/L sterile sorbitol solution in each electroporation cup and wait for 1min.
Test the result by spread plate for 3-6 days (50μl mixed liquids on SD-Ura solid culture medium and culture at 30℃).

Result:
The concentration of pYES2 is 87.88 ng/μl.
1500V & 2ms has 11 colonies as showed.

IMG_20190804_195132.jpg

thumbnail

2019年8月6日星期二

Purpose:
Transfer four of the individual colonies into SD-Ura culture medium to amplify the BY4742 S.c.
Try other electroporation conditions.

Procedure:
Take the competent S.cerevisiae BY4742 out of -80℃ refrigerator and recover it in ice.
Measure the concentration of pYES2.

Set voltage and time gradients as follows.

Table2

	time	1200V	1350V	1500V	E	F
1	1ms	1	4	7		
2	2ms	2	5	8		
3	4ms	3	6	9		

Add 50μl competent S.cerevisiae BY4742 and 1μl pYES2 into electroporation cup.
Electroporate each group.
Add 1ml 4℃ 1mol/L sterile sorbitol solution in each electroporation cup and wait for 1min.
Test the result by spread plate for 3-6 days (50μl mixed liquids on SD-Ura solid culture medium and culture at 30℃).

Result:
The concentration of pYES2 is 123.57 ng/μl.
There are many colonies except 1350V & 2ms.

2019年8月7日星期三

Purpose:
Collect the concentrated S.cerevisiae AH109 and prepare for electroporation.

Procedure:
Take the liquid medium out of 4℃ refrigerator and measure its OD600.
Transfer the liquid medium into several 50ml centrifugal tubes.
Centrifuge at 4℃ 4000g for 20mins.
Discard the supernate and resuspend all the S.c with 80ml 4℃ sterile ddH2O totally.
Centrifuge at 4℃ 4000g for 20mins.
Discard the supernate and resuspend all the S.c with 40ml 4℃ 1mol/L sterile sorbitol solution.
Collect all the liquid into one 50ml centrifugal tube.
Centrifuge at 4℃ 4000g for 20mins.
Discard the supernate and resuspend all the S.c with 0.5ml 4℃ 1mol/L sterile sorbitol solution.
Pack each 100μl S.c medium in one 1.5ml EP tube and quickly freeze them by liquid nitrogen.
Store the S.c in -80℃ refrigerator.

Result:
OD600=1.126
Get 13X100μl competent S.cerevisiae BY4742, stored in -80℃ refrigerator.

2019年8月10日星期六

Purpose:
Transfer the pYES2/pYES2-CNS1/pYES2-CNS2/pYES2-CNS1.2/pYES2-CNS3 plasmid into competent S.cerevisiae AH109 by setting voltage and time gradients.

Procedure:
Take the competent S.cerevisiae AH109 out of -80℃ refrigerator and recover it in ice.
Measure the concentration of plasmids.
Set voltage and time gradients for pYES2 as follows.

Table3

	time	1200V	1350V	1500V	E	F
1	1ms	1	4	7		
2	2ms	2	5	8		
3	4ms	3	6	9		

Set 1500V & 2ms for pYES2-CNS1/pYES2-CNS2/pYES2-CNS1.2/pYES2-CNS3.
Add 50μl competent S.cerevisiae BY4742 and 1μl pYES2 into electroporation cup.
Electroporate each group.
Add 1ml 4℃ 1mol/L sterile sorbitol solution in each electroporation cup and wait for 1min.
Test the result by spread plate for 3-6 days (50μl mixed liquids on SD-Ura solid culture medium and culture at 30℃).

Result:
The concentration of pYES2 is
213.60 ng/μl. pYES2-CNS1:
83.71 ng/μl. pYES2-CNS2:
126.50 ng/μl.
pYES2-CNS1.2: 68.32 ng/μl.
pYES2-CNS3: 20.70 ng/μl.

There are so many colonies on the culture medium and we think that the SD-Ura medium can not screen the yeast.

TEDA: yegfp-pyes2

2019 年 8 月 5 日星期一

- purpose: extract plasmid of pYES2 and pYES2-cns3 ●

participator: yj jtt

- process:

1. bacteria culture. inoculate 1ul Glycerin E.coli into 50ml tube with 15ml LB. We cultiate two tubes for each one. cultivate Glycerin E.coli for 16 hours
2. plasmid extract. concentrate 5 ml bacteria solution into 1.5 ml tube. Then extract plasmid according to the instruction.

2019 年 8 月 6 日星期二 double enzyme digestion

- purpose: Get the linearized vector pYES2
- participator: yj czz
- pro res :
  prepae thezymedigstiosystem

pYES2双酶切体系（DNA浓度 261.85..

	A	B
`	体系	ul
ↄ	Xba	ul
ω	Hind	ul
^	质粒pYES	ul
ɲ	* buffer	ul
ɔ	ddH	ul

2. hold in PCRAmplifier in 37℃ for 2 hours
3. gel elecphoresis
4. gel kit

- results:

1. Nucleic acid gel electrophoresis image

p ES2酶 . pn

lan1、5: r 10000 lan2-5: enzyme-digst d produc s

there is a strip around the place of 7000bp and there is also a strip around the place of 150bp

2. concentration of DNA after gel kit is around 20ng/ul

- conclusion:

there is a strip around the place of 7000bp and there is also a strip around the place of 150bp. Therefore we can see that our enzyme double digestion of pYES2 sucessed.

Gradient PCR

The first Gradient PCR

- perpose: to seek suitable annealing tempreture of yeGFP's PCR
- participator: yj
- progress:

1. prepare the PCR system

gradient PCR system

	A	B
[illegible]	total volume	ul
[illegible]	MIX	. ul
[illegible]	R	ul
[illegible]	F	ul
[illegible]	puc-yegfp	ul
[illegible]	ddH	. ul

2. put PCR tubes in PCRAmplifier and set PCR grogram as follows:

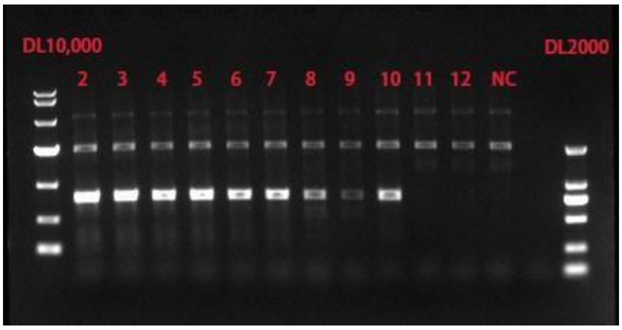
gradient PCR program

	A	B	C
1	98℃	2min	
2	98℃	10s	30cycles
3	62-74℃	15s	
4	72℃	10s	
5	72℃	5min	
6	4℃	forever	

3. gel eletrophoresis

- res ls:

TDU1. j



lane1:DL10000 lane2-13: PCR products(62-74℃) lane15:DL2000 our interest strip (yeGFP) is 770 bp. we can see that the strip which around 770bp in lane2 is the lightest, so 62℃ is more suitable

- conclusion: the suitable annealing suitable maybe lower than 62℃, so we should do another gadient PCR to explore the anealing tempreture below 62℃.

The second Gradient PCR

- perpose: to seek suitable annealing tempreture of yeGFP's PCR
- participator: yj
- progress:

1. prepare the PCR system

Gradient PCR system(the same like t..

	A	B
1	total volume	ul
2	MIX	. ul
3	R	ul
4	F	ul
5	puc-yegfp	ul
6	ddH	. ul

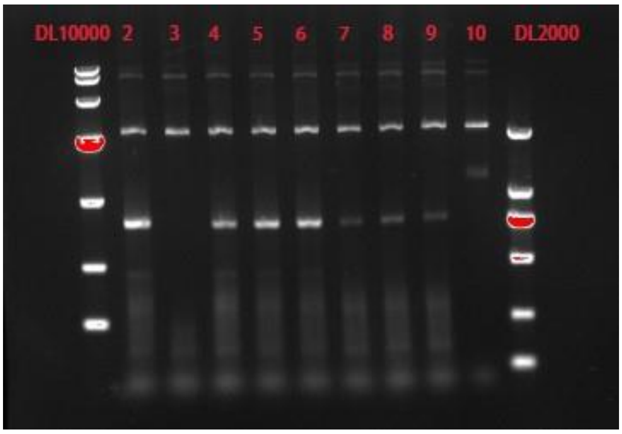
2. put PCR tubes in PCRAmplifier and set PCR grogram as follows:

Table1

	A	B	C
1	98℃	2min	
2	98℃	10s	30cycles
3	55-63℃	15s	
4	72℃	5s	
5	72℃	5min	
6	4℃	forever	

- result:

image. pn



lane1:DL10000 lane2-10: PCR products(63-55℃) lane11:DL2000

our interest strip (yeGFP) is 770 bp. we can see that the strips which around 770bp in lane2/lane5/lane6/lane are lighter.

The third Gradient PCR

1. prepare the PCR system

   (same as the PCR system last time)

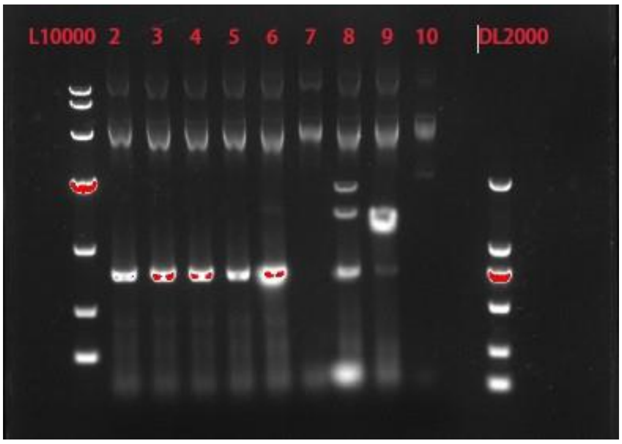
2. put PCR tubes in PCRAmplifier and set PCR grogram as follows

Table2

	A	B	C
1	98℃	2min	
2	98℃	10s	30cycles
3	55-63℃	15s	
4	72℃	7s	
5	72℃	5min	
6	4℃	forever	

- result:

image.pn



lane1:DL10000 lane2-10: PCR products(63-55℃)

lane11:DL2000 our interest strip (yeGFP) is 770 bp. we can see that the strips which around 770bp in lane3/lane4/lane6/lane are lighter.

Conclusion:

the best suitable annealing temperature is 62.5℃ and the best extension time is 7 seconds.

amplify PCR

- participator: yj
- progress:

1. prepare the PCR system

Amplify PCR system(PUC-yeGFP)

	A	B
1	total volume	ul
2	MIX	. ul
3	R	ul
4	F	ul
5	puc-yegfp	ul
6	ddH	. ul

2. put PCR tubes in PCRAmplifier and set PCR grogram as follows:

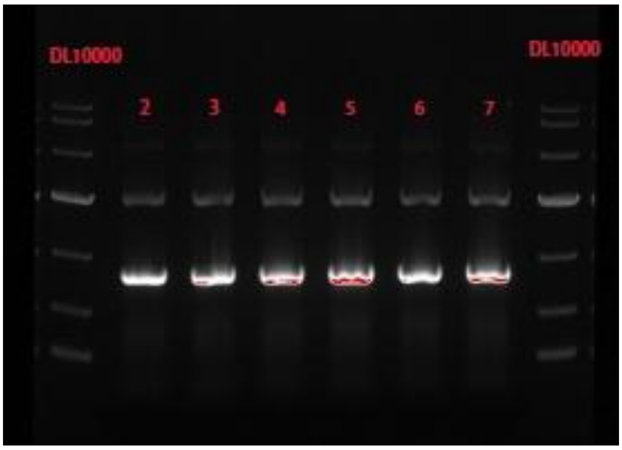
Amplify PCR program(PUC-yeGFP)

	A	B	C
1	98℃	2min	
2	98℃	10s	30cycles
3	62.5℃	15s	
4	72℃	7s	
5	72℃	5min	
6	4℃	forever	

3. Gel eletrophoresis

- res ls:

image. pn



lane1:DL10000 lane2-7: PCR products
lane11:DL10000 our interest strip (yeGFP) is 770 bp. we can see that the strips around 770bp are of high light. We get lineared yeGFP fragment. 2019年8月7日星期三

TEDA Ligase

- purpose: connect yeGFP fragment to lineared pYES2
- anticipator: yj
- progress:

1. prepare the TEDA ligase system

Table3

	A	B	C	D	E
1		1	1	1	4(NC)
2	TEDA buffer	4uL	4uL	4uL	0uL
3	骨架（pYES2)	230ng	230ng	230ng	230ng
4	片段（yeGFP)	46ng	60ng	90ng	60ng
5	ddH2O	add to 20ul	add to 20ul	add to 20ul	add to 20ul

2. Put PCR tubes in PCRAmplifier at 30℃ for 40 minutes and 4℃ for 2 minutes.
3. Pure the PCR system by gel kit.

transformation

- purpose: Transfer the plasmid we construct into the competent cell DH5α
- participator: yj
- progress:
  a. Add 10ul plasmid solusion into 50ul competent cell DH5α and set the mixed liquor on ice for 30 minutes
  b. Put the mixed solution in water at 42℃ for 1 minutes for water bathing.
  c. Instantly, put the solution on ice for 2 minutes
  d. Add the solution into 500ul LB medium and shake cultivate for 1 hour.
  e. Get 100ul of the bacteria solution to spread LB plate(add ampicillin in advance)
  f. Put the plate at 37℃ for overnight culture.

2019 年 8 月 8 日星期四

colony PCR

- purpose: Transfer the plasmid we construct into the competent cell DH5α
- participator: yj
- progress:

1. Pick a monoclonal strain on a tablet and mark it

2. Prepare the PCR system as follows:

Colony PCR system(pYES2-yeGFP)

	A	B
1	*T Mix	. ul
2	foward primer	ul
3	reverse primer	ul
4	DNA(colony sulution)	ul
5	ddH O	. ul

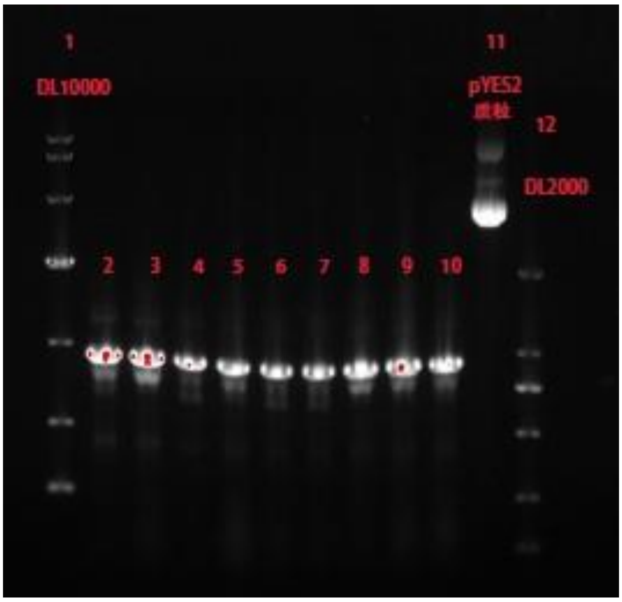
3. put PCR tubes in PCRAmplifier and set PCR grogram as follows:

Table4

	A	B	C
1	98℃	2min	
2	98℃	10s	30cycles
3	62.5℃	10s	
4	72℃	7s	
5	72℃	2min	
6	4℃	forever	

- res ls:

image. pn



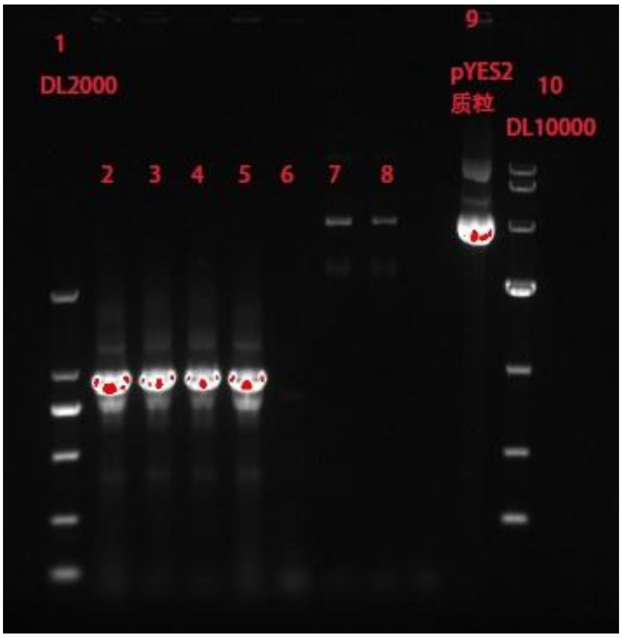
lane1:DL10000 lane2-5: colony PCR products of group 1

lane6-10:colony PCR products of group 2

lanelane11: pYES2 plasmid

lane12:DL10000

image.pn



lane1:DL10000 lane2-5: colony PCR products of group3 lane7-8: colony PCR profucts of group 4(negative control group)
lane19: pYES2 plasmid lane12:DL10000

interest strip (yeGFP) is 770 bp.
We can see that the strips around 770bp are of high light.
The results of colony PCR are all positive and results of negative control are negative

- next step:

cultivate the colony of lane3and lane4 and extract the plasmid of the two bacteria samples. Then DNA sequence is needed.

2019 年 8 月 9 日星期五

- The bacteria from the colony of lane3 and lane4 grows well in LB mudium(with ampicillin)
- extract the plasmid from cultured DH5α (lane6 sample) ● send the plasmid to do DNA sequence

2019 年 8 月 11 日星期日

- conclusion: the result of DNA sequence showed that the construct of pYES2-yeGFP succeed.

yeGFP-pyES2-1_cye-F_TSS20190819-028-3128_F0
2.ab1

泰达：promoters-cns3

2019 年 8 月 26 日星期一梯

度 PCR

- **提出：**寻求适合 PCR 的退火温度
- **参加者：** yj
- **进**

1。 准 PCR系

梯度PCR系统

	一种	乙

不同引物PCR的影

表格1

	一种	乙	C	d

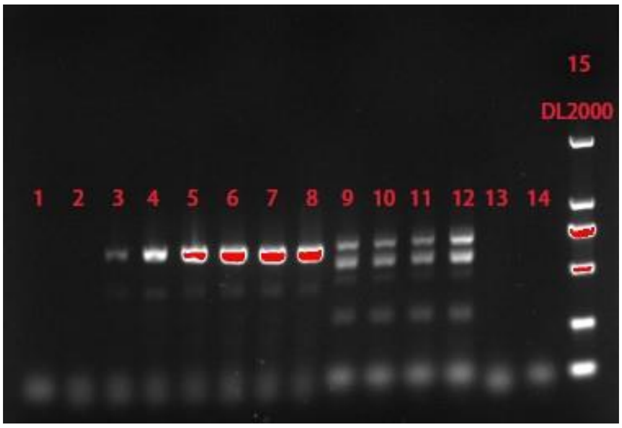
2.将PCR试管放PCRAmpli中，并按照以下步骤设置图

梯度PCR程序

	一种	乙	C

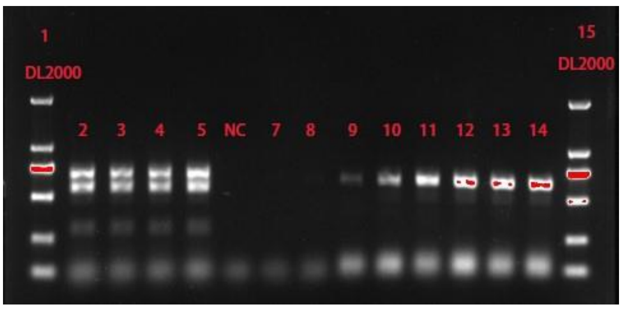
- **结果:**

image.pn



1-8道：TPS1（56-66℃）9-12道：PDA1（66-50℃）13-14道：NC道15：DL2000

image.pn



泳道1：DL2000泳道2-5：PDA1（50-56℃）泳道6-8：NC泳道7-14：TEF1（66-56℃）泳道15：DL2000

Therefore,we can see that the best annealing tempreture for the three genes:

TPS1——56℃

TEF1——56℃

PDA1——56℃

amplify PCR

- participator: yj
- progress:

1. prepare the system

Table2

	A	B

(different primers to PCR different gnen)

2. put PCR tubes in PCRAmplifier and set PCR grogram as follows:

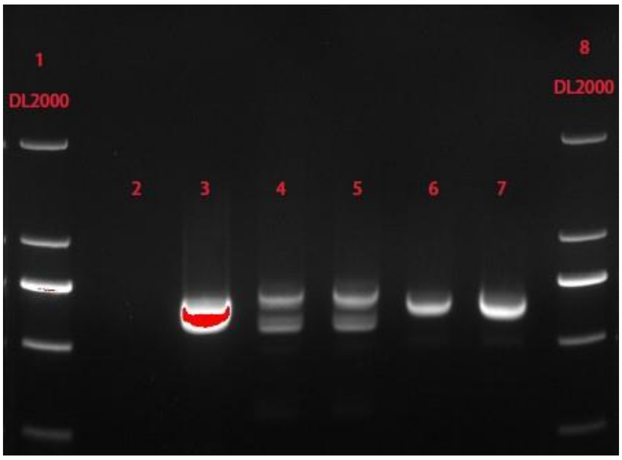
Table4

	A	B	C

3. gel kit

- results:

image.pn



lane1:DL2000 lane2-3: TPS1 lane4-5: PDA1 lane6-6: TEF1 lane8: DL2000

We can see that there is no strip on lane2. The two strips on lane4 can not be distinguished well, so we recover both, and name them as PDA-S and PDA-X, so as the lane5.

single enzyme digestion

- purpose: Get the linearized vector pYES2-CNS3
- participator: yj
- progress:

1. prepare the enzyme digestion system

pYES2 double enzyme digestion syst..

	A	B

2. hold in PCRAmplifier in 37℃ for 3 hours
3. gel elecphoresis

Digest with alkaline phosphatase

- purpose: Catalyze the removal of 5 ’phosphate groups from linearized pYES2-CNS3

to aviod the connect of itself ● participator: yj

- progress:

1. prepare the RSAP digestion system

Table3

	A	B

2. hold in PCRAmplifier in 37℃ for 30 minutes and then 65℃ for 5 minutes.
3. gel elecphoresis

2019 年 8 月 29 日星期四

TEDA

- purpose: connect the promoter to the backbone.

- participator: y
- +j

- progress:

1. prepare the RSAP digestion system

Table5

	A	B	C	D	E	F

transformation

- purpose: Transfer the plasmid we construct into the competent cell DH5α

- participator: yj

- progress:
  a. Add 10ul plasmid solusion into 50ul competent cell DH5α and set the mixed liquor on ice for 30 minutes
  b. Put the mixed solution in water at 42℃ for 1 minutes for water bathing.
  c. Instantly, put the solution on ice for 2 minutes
  d. Add the solution into 500ul LB medium and shake cultivate for 1 hour.
  e. Get 100ul of the bacteria solution to spread LB plate(add ampicillin in advance)
  f. Put the plate at 37℃ for overnight culture.

- results: there are little colony grow on the plate. The transformation failed!

我们认为可能是因为未完成单酶消化的步骤或未完全完成用碱性磷酸酶的消化，所以线性化的载体与其自身连接并导致大量 PLNCX。

- **下一步：**将单一酶的消化时间延长到 3 小时，以确保有更多的消化载体。然后重复用碱性磷酸酶消化的步骤。

但是，菌落的最终结果表明这里没有阳性结果。

下一个：

尽管 TEDA 的方法在 pYES2-yeGFP 的构建中是成功的，但在启动子-CNS3 的构建中却没有。我们尚未找到原因，因此我们选择了另一种方法来完成

double enzyme digest - promoters-cns3

2019年8月25日星期日

Double enzyme digestion

- purpose:
  a. get lineared backbone with our interested constitutive promoters
  b. get lineared insert fragment cns3-1

- participator: yj ● purpose:

1. prepare the double enzyme digestion system

TEF1、TP1

double enzyme digestion system(TEF1/TPS1)

	A	B
1	* Green buffeer	ul
2	XbaI	ul
3	HindIII	ul
4	TEF /TPS plasmid	ug
5	ddH O	add to ul

PDA1

double enzyme digestion system(PD...

	A	B
1	* Green buffeer	ul
2	XbaI	ul
3	EcoRi	ul
4	PDA plasmid	ug
5	ddH O	add to ul

cns3-1

double enzyme digestion system(cns...

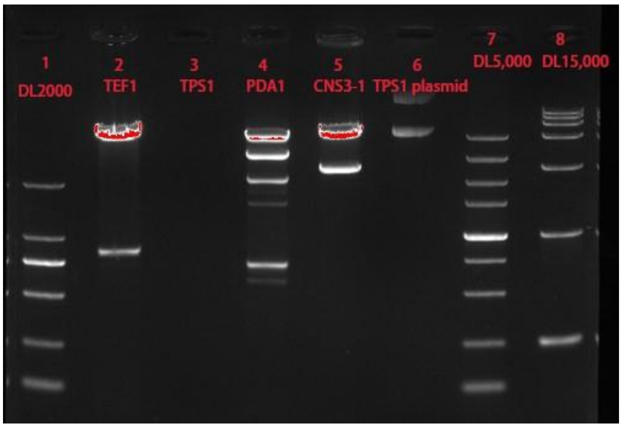
	A	B
1	* Green buffeer	ul
2	XbaI	ul
3	HindIII	ul
4	cns plasmid	ug
5	ddH O	add to ul

2. set the tubes in PCR Amplifier at 37 ℃ for 2hours

4. Test under ultraviolet light 3. gel kit

- res ls:

image. pn



lane1: DL2000 lane2: enzyem digestion product of TEF1 lane3: enzyem digestion product of TPS1 lane4: enzyem digestion product of PAD1 lane5: enzyem digestion product of CNS3-1 lane6: TPS1 plasmid lane7: DL5000 lane8: DL15000

The length of our target strand :

Table1

	A	B
1	TEF	bp
2	TPS	bp
3	PDA	bp
4	CNS -	bp
5	TPS plasmid	bp

- conclusion:

Therefore, we can see that we get the lineared TEF1、CNS3-1 But the enzyme digestion of TPS1 and PDA1 failed.

Gradient PCR

- purpose: seek suitable PCR annealing tempreture of CNS3-2
- anticipator: yj ● progress:

1. prepare the PCR system as follow:

Table2

	A	B
1	mix	. ul
2	foward primer	ul
3	reverse primer	ul
4	NTA-CNS plasmid	ul
5	ddH O	. ul

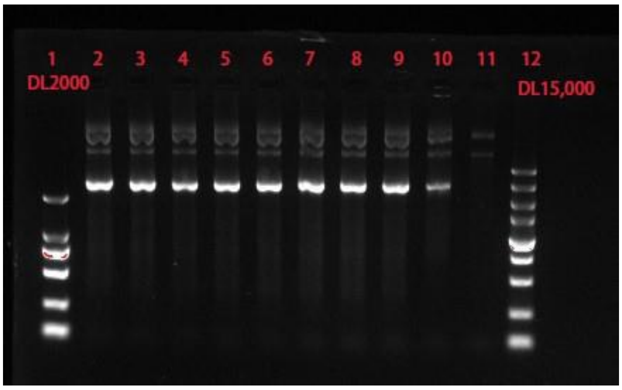
2. Put the tubes in PCR Amplifier and set PCR program as follow:

Gradient PCR (CNS3-2)

	A	B
1	tempreture (℃)	time
2	98	2min
3	98	10s
4	48-58	15s
5	72	27s
6	72	5min
7	4	forever

3. Test under ultraviolet light ● result:

image.pn



lane1: DL2000
lane2-11: gradient PCR
product(58℃-48℃) lane12: DL15000

We can see that the are strips are the place of 2500bp

● conclusion: we choose 57.3℃ as the annealing tempreture

PCR amplification

● purpose: get the lineared insert fragment CNS3-2 ● anticipator: yj ● progress:

1. prepare the PCR system as follow:

Table3

	A	B
[illegible]	mix	. ul
[illegible]	foward primer	ul
[illegible]	reverse primer	ul
[illegible]	NTA-CNS plasmid	ul
[illegible]	ddH O	. ul

2. Put the tubes in PCR Amplifier and set PCR program as follow:

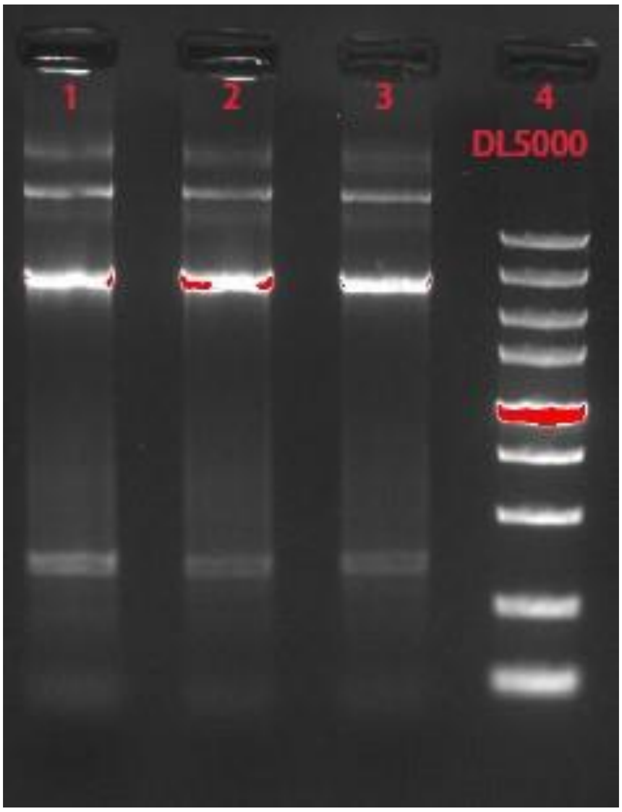
Table4

	A	B
1	tempreture (℃)	time
2	98	2min
3	98	10s
4	57.3	15s
5	72	27s
6	72	5min
7	4	forever

3. Test under ultraviolet light
4. gel kit and quatificate

- results:

image. pn



lane1-3: PCR product of CNS3-2
lane4: DL5000
the strips are 2500bp are our interest strand

- transformation: we get the lineared insert fragment CNS3-2

2019 年 8 月 26 日星期一

Connect

- purpose: connect CNS3-1 to TEF1 backbone
- anticipator: yj ● progress:

1. prepare the connection system as follows:

connection system(TEF1-CNS3)

	A	B
1	* buffer	ul
2	cns -	ul(ng)
3	TEF	ul(ng)
4	T ligase	ul
5	ddH O	ul

2. Put the tubes in PCR Amplifier at 16℃ for overningt connection

Transformation

- purpose: Transfer the plasmid we construct into the competent cell DH5α
- participator: yj
- progress: detail mentioned before.
- results: sample lost!!!

Double enzyme digestion(cns3-2)

- purpose: get insert fragment cns3-2 with enzyme digest site Xbal and EcoRI
- participator: yj
- progress:

1. prepare the double enzyme digestion system as follows:

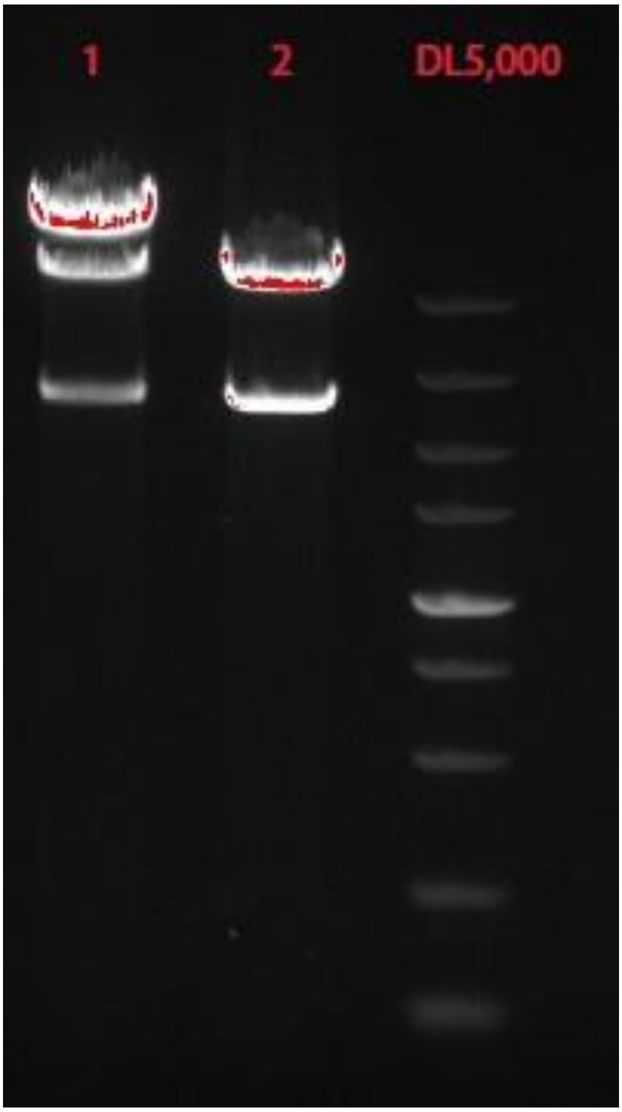
enzyme digest system(cns3-2)

	A	B
1	* Green buffeer	ul
2	XbaI	ul
3	EcoRi	ul
4	CNS -	ug
5	ddH O	add to ul

2. set the tubes in PCR Amplifier at 37 ℃ for 3 hours
4. Test under ultraviolet light
3. gel kit

- results:

image. pn



lane1-2: enzyme digestion product of CNS3-2 lane3: DL5,000

2019 年 8 月 27 日星期二

connect

- purpose:

connect CNS3-1 to TEF1 backbone connect CNS3-1 to TPS1 backbone connect CNS3-2 to PDA1 backbone

● anticipator: yj ●

progress:

1. prepare the connection system as follows:

TPS1-CNS3

connection system(TPS1-CNS3)

	A	B
1	*Buffer	ul
2	T ligase	ul
3	TPS backbone	. ul(ng)
4	CNS -	. ul(. ng)
5	ddH O	. ul

TEF1-CNS3

connection system(TEF1-CNS3)2

	A	B
1	*Buffer	ul
2	T ligase	ul
3	TEF backbone	. ul(ng)
4	CNS -	. ul(. ng)
5	ddH O	. ul

PDA-CNS3

connection system(PDA1-CNS3)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD
1	10*Buffer	2ul																												
2	T4 ligase	1ul																												
3	PDA1 backbone	3.0ul(50ng)																												
4	CNS3-2	3.5ul(63.7ng)																												
5	ddH2O	10.5ul																												

2. Put the tubes in PCR Amplifier at 16℃ for overningt connection

Transformation

- purpose: Transfer the plasmid we construct into the competent cell DH5α
- participator: yj
- progress: detail mentioned before.

2019 年 8 月 28 日星期

三 colony PCR

- purpose: check if the plasmid was transfered into DH5α
- participator: yj
- progress:

1. Pick a monoclonal strain on a tablet and mark it 2.Prepare the PCR system as follows:

Colony PCR system(pYES2-yeGFP)

	A	B
1	*T Mix	. ul
2	foward primer	ul
3	reverse primer	ul
4	DNA(colony sulution)	ul
5	ddH O	. ul

3. set PCR grogram as follows:

Table5

	A	B	C
1	98℃	2min	
2	98℃	10s	30cycles
3	57.3℃	15s	
4	72℃	27s	
5	72℃	2min	
6	4℃	forever	

- results:

image.pn

lane1: DL10K lane2: PDA1 lane3: PDA1 PC lane4: TPS1 lane5: TPS1 PC lane6:DL2K We can see that there are our interest strip on lane 2 and lane4

- conclusion:

the results of colony PCR of PDA1 and TPS1 is positive
because there is no TEF1 colony on plate, so we repeat the construction of TEF1-CNS3

- next step: cultivate the colony of lane6 and lane9 and extract the plasmid of the two bacteria samples. Then DNA sequence is needed.

2019 年 8 月 29 日星期四

- The bacteria from the colony grows well in LB mudium(with ampicillin) • conclusion:

the result of lane9 is false positive

- extract the plasmid from cultured DH5α • send the plasmid to do DNA sequence

2019 年 8 月 30 日星期五

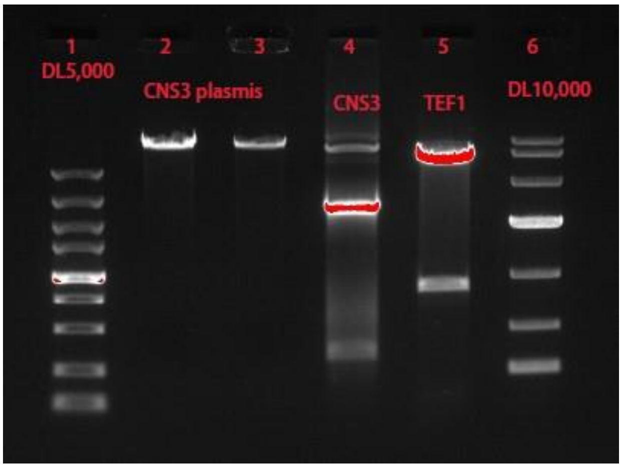
- the result of DNA sequence showed that the construct of PDA1-CNS3 and TPS1-CNS3 succeed.

2019 年 8 月 31 日星期六

Double enzyme digestion of TEF1 and CNS3-1

1. prepare the system(as above)
2. 37 ℃ for 3 hours ● results:

image.pn



lane1:DL5000
lane2-3:CNS3 plasmid lane4: enzyme digestion product of CNS3 lane5: enzyme digestion product of TEF1

- conclusion: the enzyme digestion is successful

2019年9月1日星期日

connect

- purpose:
  connect CNS3-1 to TEF1

backbone
- anticipator: yj
- progress:

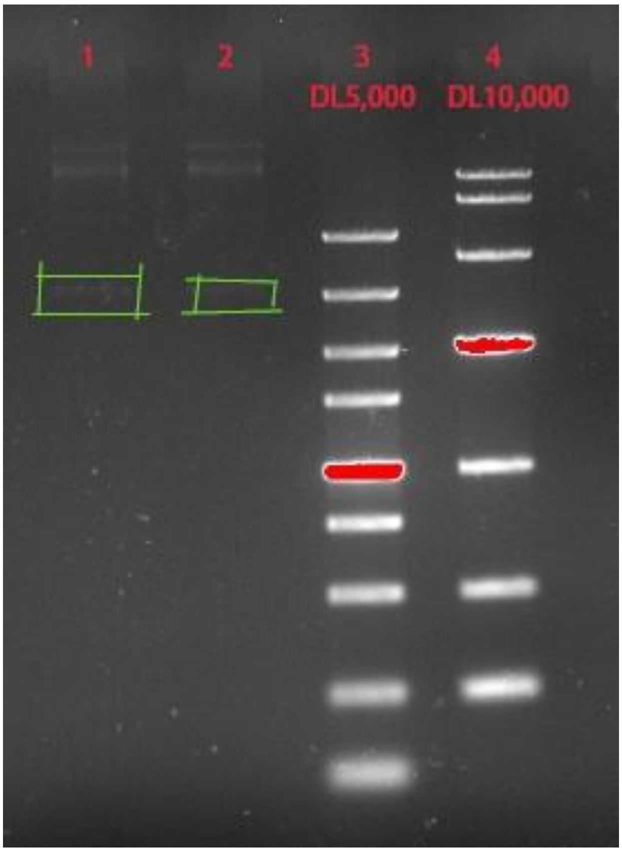
1. prepare the connection system(as above)
2. Put the tubes in PCR Amplifier at 16℃ for overningt connection

---

2019年9月2日星期一

- results:

image.pn



lan1-2: conneaoduct EF1-CNS3
lane3: DL5000 lane4:
DL10000

Transformation

- purpose: Transfer the plasmid(TEF1-CNS3) we construct into the competent cell DH5α
- participator: yj
- progress: detail mentioned before.

2019年9月3日星期二

colony PCR

- purpose: check if the plasmid was transfered into DH5α

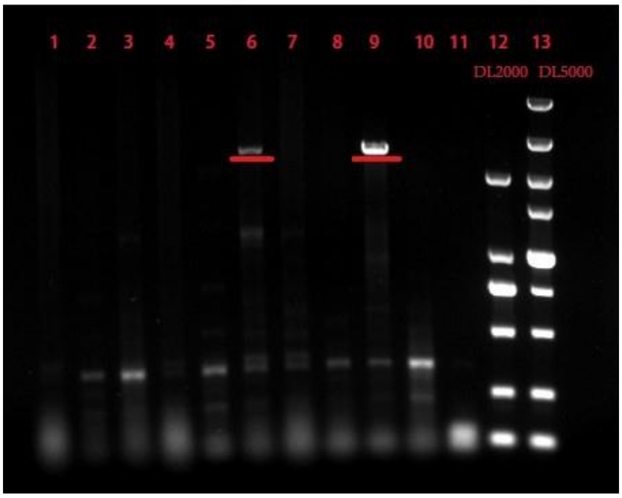
- participator: yj

- progress:

1. Pick a monoclonal strain on a tablet and mark it
2. Prepare the PCR system (as above)
3. set PCR program (as above)

- results:

image.pn



lan1-11: colony roduc s ofEF1-CNS3
lane12: DL2000
lane13:DL5000
We can see that the results of lane6 and lane9 are positive

- next step:

cultivate the colony of lane6 and lane9 and extract the plasmid of the two bacteria samples. Then DNA sequence is needed.

2019年9月4日星期二

- The bacteria from the colony of lane6 grows well in LB mudium(with ampicillin), but that from lane9 doesn't.
- conclusion:

the result of lane9 is false positive

- extract the plasmid from cultured DH5α (lane6 sample) ● send the plasmid to do DNA sequence

2019年11月3日星期日

- the result of DNA sequence showed that the construct of TEF-CNS3 succeed.

TEF_YIN1_TSS20190930-028-4984_C09.ab1

double enzyme digest - promoters-cns3

2019年8月25日星期日

Double enzyme digestion

- purpose:
  a. get lineared backbone with our interested constitutive promoters
  b. get lineared insert fragment cns3-1

- participator: yj ● purpose:

1. prepare the double enzyme digestion system

TEF1、TP1

double enzyme digestion system(TEF1/TPS1)

	A	B
1	* Green buffeer	ul
2	Xbal	ul
3	HindIII	ul
4	TEF /TPS plasmid	ug
5	ddH O	add to ul

PDA1

double enzyme digestion system(PD...

	A	B
1	* Green buffeer	ul
2	Xbal	ul
3	EcoRi	ul
4	PDA plasmid	ug
5	ddH O	add to ul

cns3-1

double enzyme digestion system(cns...

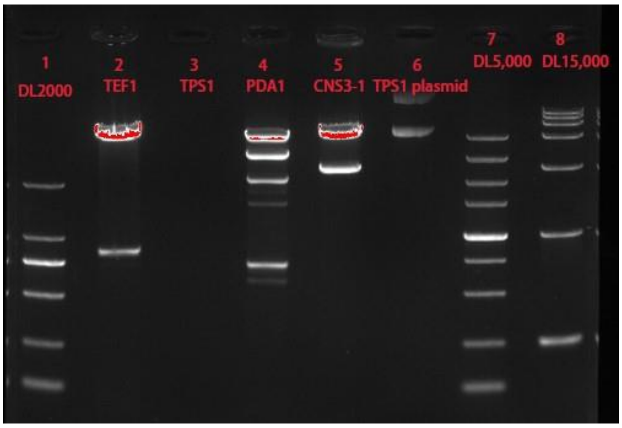
	A	B
1	* Green buffeer	ul
2	XbaI	ul
3	HindIII	ul
4	cns plasmid	ug
5	ddH O	add to ul

2. set the tubes in PCR Amplifier at 37 ℃ for 2hours

4. Test under
ultraviolet light 3.
gel kit

- res ls:

image. pn



lane1: DL2000 lane2: enzyem digestion product of TEF1 lane3: enzyem digestion product of TPS1 lane4: enzyem digestion product of PAD1 lane5: enzyem digestion product of CNS3-1 lane6: TPS1 plasmid lane7: DL5000 lane8: DL15000

The length of our target strand :

Table1

	A	B
1	TEF	bp
2	TPS	bp
3	PDA	bp
4	CNS -	bp
5	TPS plasmid	bp

- conclusion:

Therefore, we can see that we get the lineared TEF1、CNS3-1 But the enzyme digestion of TPS1 and PDA1 failed.

Gradient PCR

- purpose: seek suitable PCR annealing tempreture of CNS3-2

- anticipator: yj ● progress:

1. prepare the PCR system as follow:

Table2

	A	B
1	mix	. ul
2	foward primer	ul
3	reverse primer	ul
4	NTA-CNS plasmid	ul
5	ddH O	. ul

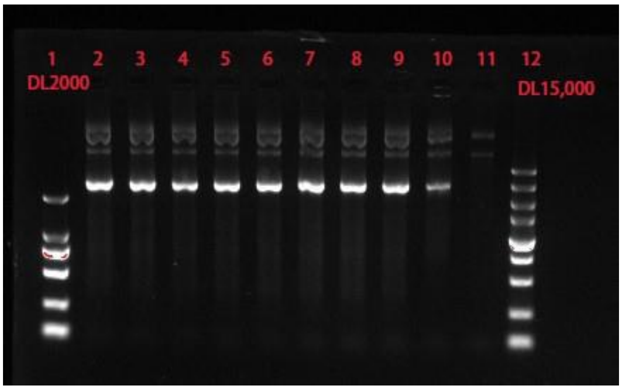
2. Put the tubes in PCR Amplifier and set PCR program as follow:

Gradient PCR (CNS3-2)

	A	B
1	tempreture (℃)	time
2	98	2min
3	98	10s
4	48-58	15s
5	72	27s
6	72	5min
7	4	forever

3. Test under ultraviolet light ● result:

image.pn



lane1: DL2000
lane2-11: gradient PCR
product(58℃-48℃) lane12: DL15000

We can see that the are strips are the place of 2500bp

- conclusion: we choose 57.3℃ as the annealing tempreture

PCR amplification

- purpose: get the lineared insert fragment CNS3-2 ● anticipator: yj ● progress:

1. prepare the PCR system as follow:

Table3

	A	B
1	mix	. ul
2	foward primer	ul
3	reverse primer	ul
4	NTA-CNS plasmid	ul
5	ddH O	. ul

2. Put the tubes in PCR Amplifier and set PCR program as follow:

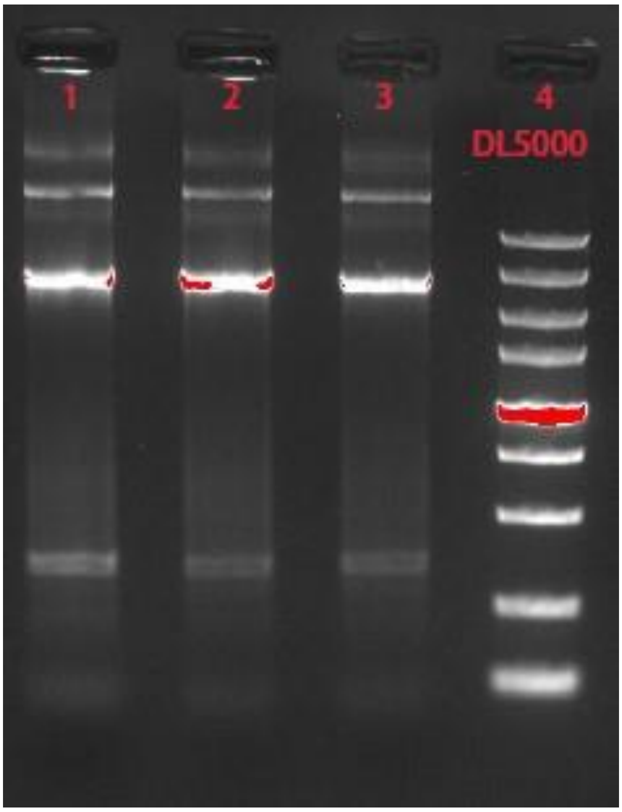
Table4

	A	B
1	tempreture (℃)	time
2	98	2min
3	98	10s
4	57.3	15s
5	72	27s
6	72	5min
7	4	forever

3. Test under ultraviolet light
4. gel kit and quatificate

- results:

image. pn



lane1-3: PCR product of CNS3-2
lane4: DL5000
the strips are 2500bp are our interest strand

- transformation: we get the lineared insert fragment CNS3-2

2019 年 8 月 26 日星期一

Connect

- purpose: connect CNS3-1 to TEF1 backbone
- anticipator: yj ● progress:

1. prepare the connection system as follows:

connection system(TEF1-CNS3)

	A	B
`	* buffer	ul
◡	cns -	ul(ng)
ฆ	TEF	ul(ng)
⊃	T ligase	ul
ภ	ddH O	ul

2. Put the tubes in PCR Amplifier at 16℃ for overningt connection

Transformation

- purpose: Transfer the plasmid we construct into the competent cell DH5α
- participator: yj
- progress: detail mentioned before.
- results: sample lost!!!

Double enzyme digestion(cns3-2)

- purpose: get insert fragment cns3-2 with enzyme digest site Xbal and EcoRI
- participator: yj
- progress:

1. prepare the double enzyme digestion system as follows:

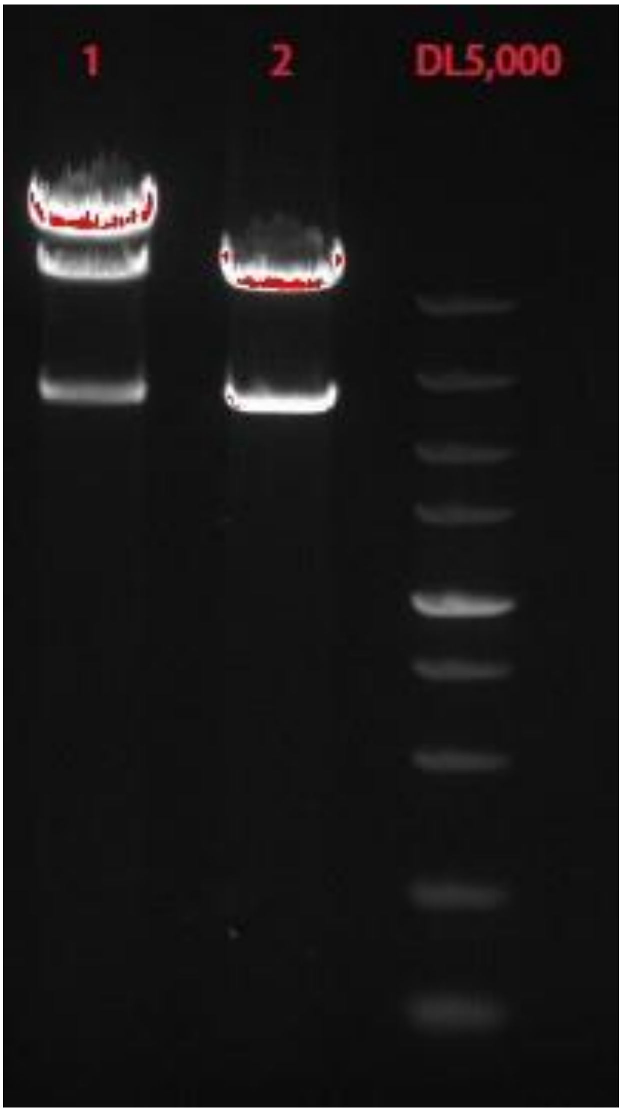
enzyme digest system(cns3-2)

	A	B
.	* Green buffeer	ul
	XbaI	ul
	EcoRi	ul
	CNS -	ug
	ddH O	add to ul

2. set the tubes in PCR Amplifier at 37 ℃ for 3 hours
4. Test under ultraviolet light
3. gel kit

- results:

image. pn



lane1-2: enzyme digestion product of CNS3-2 lane3: DL5,000

2019年8月27日星期二

connect

- purpose:

connect CNS3-1 to TEF1 backbone connect CNS3-1 to TPS1 backbone connect CNS3-2 to PDA1 backbone

● anticipator: yj ●

progress:

1. prepare the connection system as follows:

TPS1-CNS3

connection system(TPS1-CNS3)

	A	B
1	*Buffer	ul
2	T ligase	ul
3	TPS backbone	. ul(ng)
4	CNS -	. ul(. ng)
5	ddH O	. ul

TEF1-CNS3

connection system(TEF1-CNS3)2

	A	B
1	*Buffer	ul
2	T ligase	ul
3	TEF backbone	. ul(ng)
4	CNS -	. ul(. ng)
5	ddH O	. ul

PDA-CNS3

connection system(PDA1-CNS3)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD
1	10*Buffer	2ul																												
2	T4 ligase	1ul																												
3	PDA1 backbone	3.0ul(50ng)																												
4	CNS3-2	3.5ul(63.7ng)																												
5	ddH2O	10.5ul																												

2. Put the tubes in PCR Amplifier at 16℃ for overningt connection

Transformation

- purpose: Transfer the plasmid we construct into the competent cell DH5α
- participator: yj
- progress: detail mentioned before.

2019 年 8 月 28 日星期

☰ colony PCR

- purpose: check if the plasmid was transfered into DH5α
- participator: yj
- progress:

1. Pick a monoclonal strain on a tablet and mark it 2. Prepare the PCR system as follows:

Colony PCR system(pYES2-yeGFP)

	A	B
[illegible]	*T Mix	. ul
[illegible]	foward primer	ul
[illegible]	reverse primer	ul
[illegible]	DNA(colony sulution)	ul
[illegible]	ddH O	. ul

3. set PCR grogram as follows:

Table5

	A	B	C
1	98℃	2min	
2	98℃	10s	30cycles
3	57.3℃	15s	
4	72℃	27s	
5	72℃	2min	
6	4℃	forever	

- results:

image. pn

lane1: DL10K lane2: PDA1 lane3: PDA1 PC lane4: TPS1 lane5: TPS1 PC lane6:DL2K We can see that there are our interest strip on lane 2 and lane4

- conclusion:

the results of colony PCR of PDA1 and TPS1 is positive
because there is no TEF1 colony on plate, so we repeat the construction of TEF1-CNS3

- next step: cultivate the colony of lane6 and lane9 and extract the plasmid of the two bacteria samples. Then DNA sequence is needed.

2019 年 8 月 29 日星期四

- The bacteria from the colony grows well in LB mudium(with ampicillin) ● conclusion:

the result of lane9 is false positive

- extract the plasmid from cultured DH5α ● send the plasmid to do DNA sequence

2019 年 8 月 30 日星期五

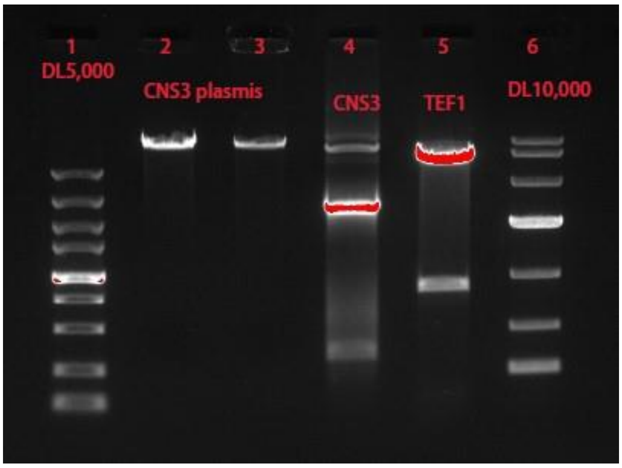
- the result of DNA sequence showed that the construct of PDA1-CNS3 and TPS1-CNS3 succeed.

2019 年 8 月 31 日星期六

Double enzyme digestion of TEF1 and CNS3-1

1. prepare the system(as above)
2. 37 ℃ for 3 hours ● results:

image.pn



lan1:DL5000
lane2-3:CNS3 plasmid lane4: enzyme digestion product of CNS3 lane5: enzyme digestion product of TEF1

- conclusion: the enzyme digestion is successful

2019 年 9 月 1 日星期日

con nect

- purpose: connect CNS3-1 to TEF1 backbone
-

anticipa
tor: yj

●

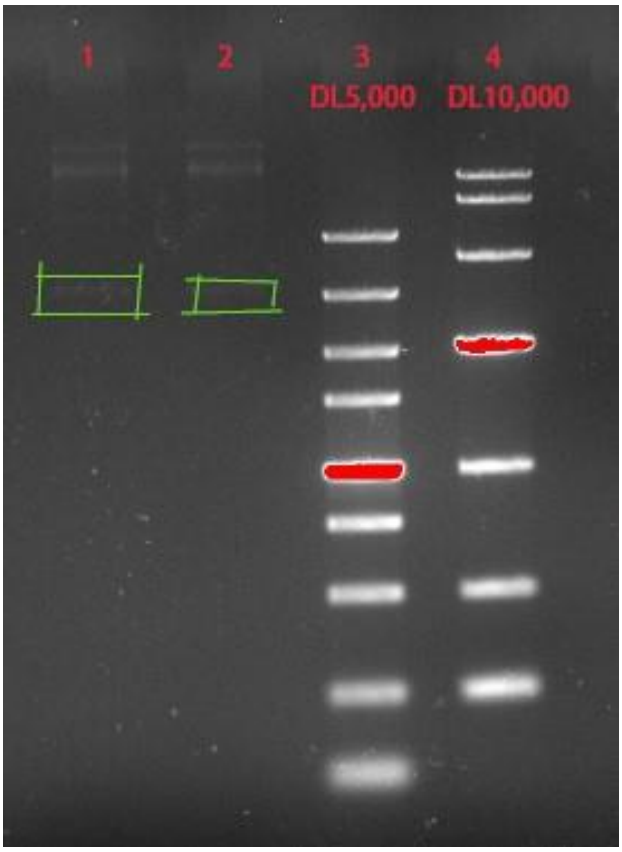
progress:

1. prepare the connection system(as above)
2. Put the tubes in PCR Amplifier at 16℃ for overningt connection

2019年9月2日星期
一

- results:

image.pn



lan1-2: connecoduct EF1-CNS3
lane
3:
DL50
00
lane
4:

DL10
000

Transformation

- purpose: Transfer the plasmid(TEF1-CNS3) we construct into the competent cell DH5α

- participator: yj

- progress: detail mentioned before.

2019
年9
月3
日星
期二

co lo ny PC R

- purpose: check if the plasmid was transfered into DH5α

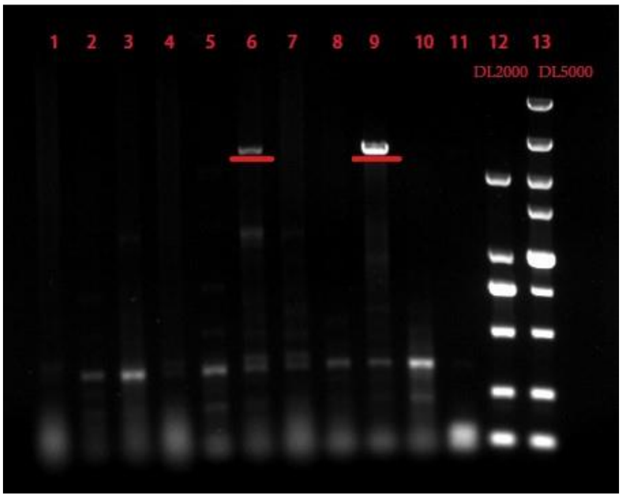
- participator: yj

- progress:

1. Pick a monoclonal strain on a tablet and mark it
2. Prepare the PCR system (as above)
3. set PCR program (as above)

- results:

image.pn



lan1-11: colony roducs ofEF1-CNS3
lan
e12
:
DL2
000
lan
e13
:DL
500
0
We can see that the results of lane6 and lane9 are positive

- next step:

cultivate the colony of lane6 and lane9 and extract the plasmid of the two bacteria samples. Then DNA sequence is needed.

2019 年 9 月 4 日星期
三

- The bacteria from the colony of lane6 grows well in LB mudium(with ampicillin), but that from lane9 doesn't.
- conclusion:

the result of lane9 is false positive

- extract the plasmid from cultured DH5α (lane6 sample) ● send the plasmid to do DNA sequence

2019年11月3日星期日

- the result of DNA sequence showed that the construct of TEF-CNS3 succeed.

TEF_YIN1_TSS20190930-028-4984_C09.ab1

Protein detection

19.09.05

Aim: To test the existence of Cns3\ Cns1\Cns1-Cns2

Process:

Samples: only\yh\mf *1\Cns1\Cns1-Cns2

1. To test the pollution situation of the cultures via light microscope.
2. Using yeast lysis kit to break the engineered yeast. (The kit is from Sangon Biotech, C50013)
3. WB to test the existence of Cns3.

Results:

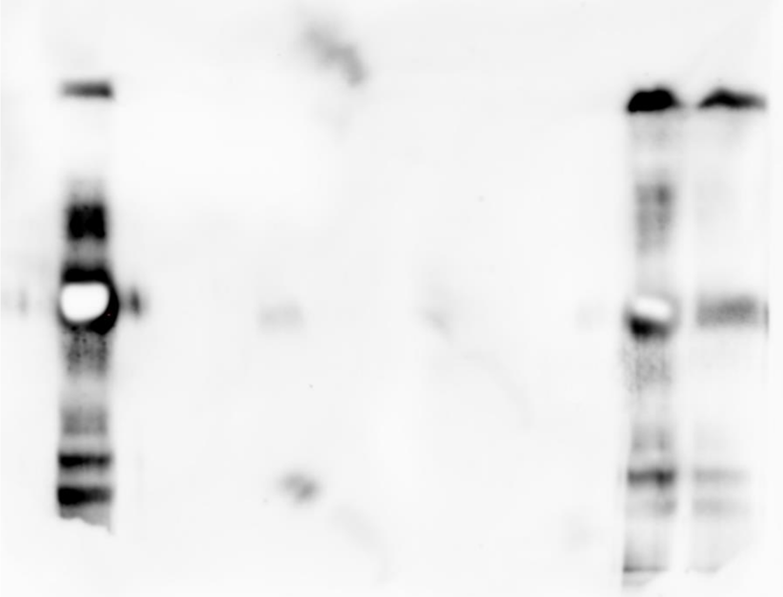
1. the total yeast of sample only is little. The sample yh has been polluted. The sample mf is not polluted.
2. the protein concentration of mf: 41.63g/ml; yh: 41.63g/ml; Cns1:38.60mg/ml; Cns1-Cns2: 58.02mg\ml.
3. WB: two bands in the WB. They seem like Cns1 and Cns2. But repeat this experiments can make a more reliable results. Besides, we do not detected Cns3.

Conclusions:

Maybe repeat can work.

19.09.09
Aim: gain the endo-protein of yeast.
Process: Using yeast lysis kit to break the engineered yeast. (The kit is from Sangon Biotech, C50013)
Results:
The protein concentration are all about 50mg/ml.

19.09.10
Aim: to detect the existence of Cns3\Cns1\Cns2
Process: WB.
Results: No band at all!



Next step:
Do it again.

19.09.18
Aim: the last time to detect the existence of Cns3.
Process: using the yeast lysis liquid last time, then, WB.
Results: no target band.

Next step:

We think it may not be our problem that there is no band. Maybe the yeast did not express enough protein to be targeted when inducing two days.
So, after discussion, we decided to do qPCR and HPLC to verify the existence and function of Cns1\2\3.

qPCR

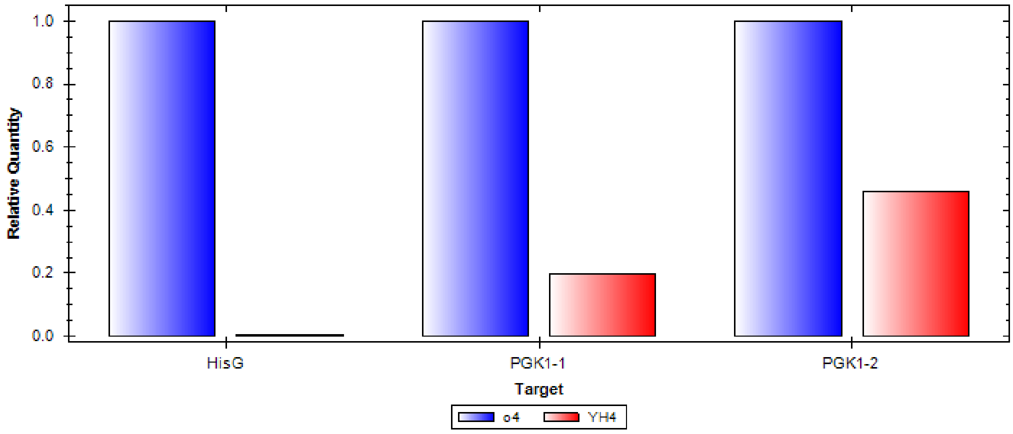
09.28 qPCR preparation
We prepare all the reagents for the total RNA extraction and RT-PCR.
Three kinds of kit we use:
Total RNA extraction: Sangon Biotech B518627
RT: Takara RR036Q
QPCR: Takara RR820Q

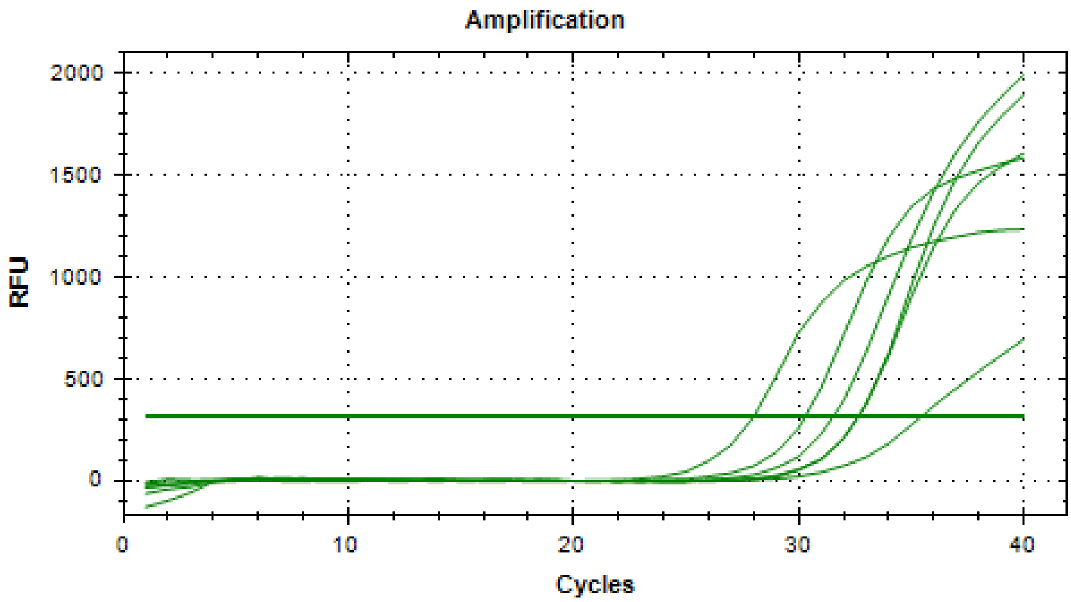
09.30 RNA extraction
Samples: yh4 only4
Process: steps as the instruction of the kit.
Results: RNA concentration: yh4:26ug/ul; only4: 23ug/ul

10.01 RT-QPCR 1
Samples: RNA of yh4 and only4
Process: 1. RT first. Steps as the instruction tell.
2. QPCR. Steps as the instruction tell. The program is the same as the recommended process as the instruction.

	1	2	3	4	5	6
A	yh4-pCns3	yh4-pPGK1.1	Yh4-pPGK1.2	Only4-pCns3	Only4-pPGK1.1	Only4-Ppgk1.2

Results: successfully get the positive result of yh4 and only4.



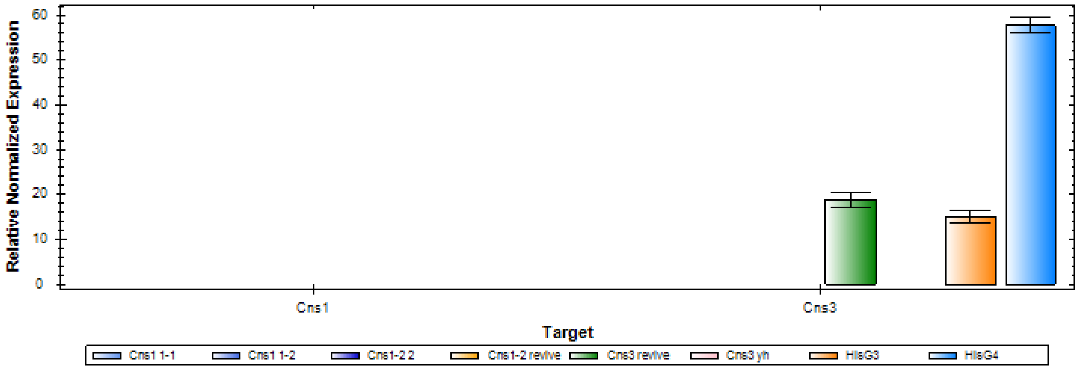


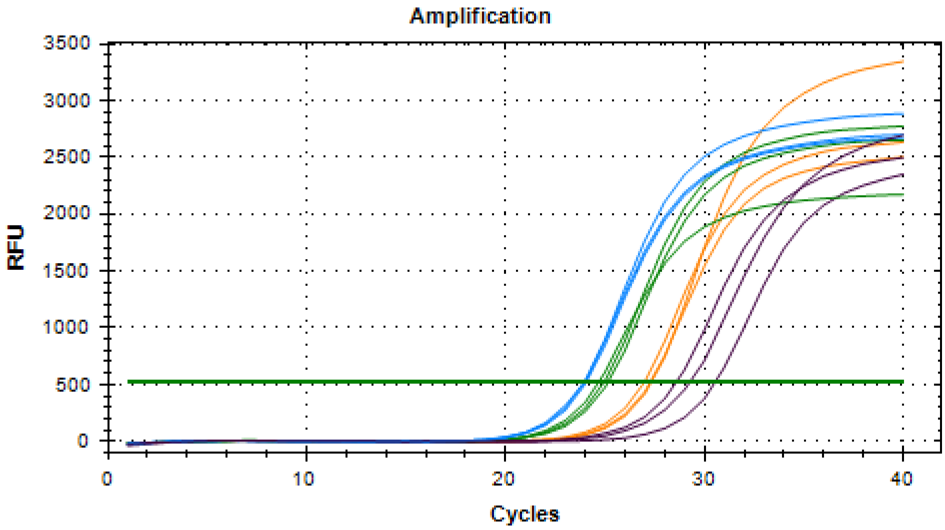
Conclusion: the pre-test is successful.

10.04 qPCR2

Samples: Cns3 only3 only4 yh4 Cns1-2 Cns1linker2 Cns1

Results:



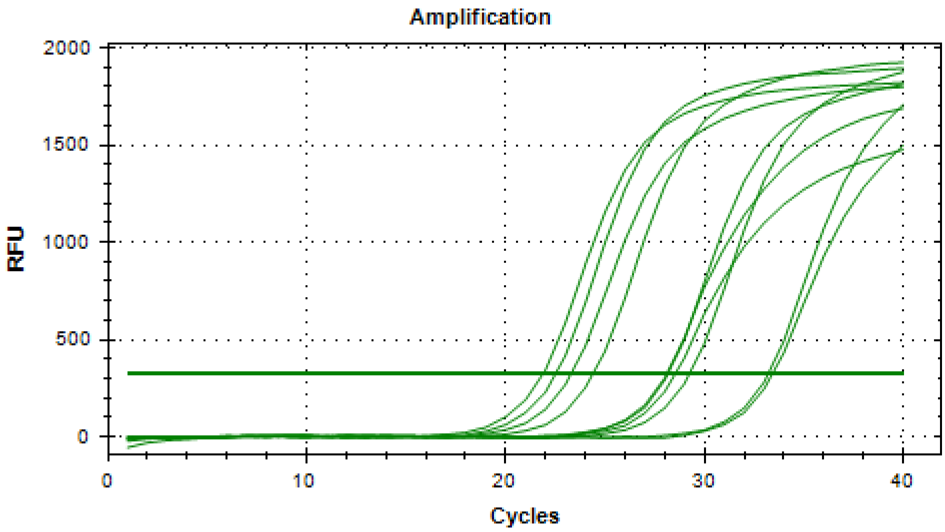


Cns3 only3 only4 been detected successfully. But samples about Cns1 and Cns2 are all failed. It may because of the primers.
Next step:
We just plan to choose another primer to detect the samples.

10.06 qPCR3

Samples: Cns1-2 Cns1linker2 Cns1, yh4

Results:



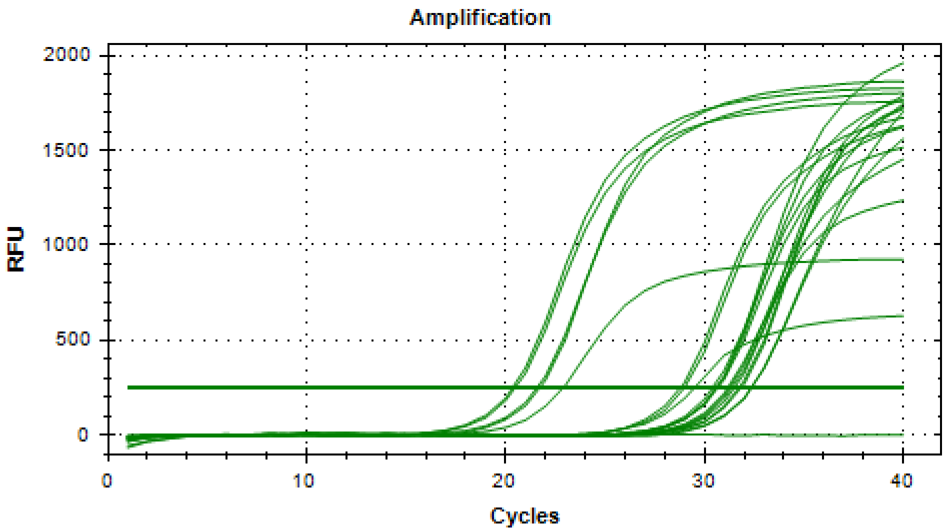
Well	Fluor	Target	Content	Sample	Cq	Cq Mean	Cq Std. Dev
A01	SYBR	cns2	Unkn	cns1-2	33.41	33.41	0.000
A02	SYBR	cns2	Unkn	cns1-2	33.20	33.20	0.000
A03	SYBR	cns2	Unkn	cns1-2r	28.10	28.10	0.000
A04	SYBR	cns2	Unkn	cns1-2r	28.52	28.52	0.000
A05	SYBR	cns1	Unkn	cns1 1-1	21.87	21.87	0.000
A06	SYBR	cns1	Unkn	cns1 1-1	22.50	22.50	0.000
A07	SYBR	cns1	Unkn	cns1 1-2	23.28	23.28	0.000
A08	SYBR	cns1	Unkn	cns1 1-2	24.38	24.38	0.000
A09	SYBR	HisG	Unkn	YH4	29.24	29.24	0.000
A10	SYBR	HisG	Unkn	YH4	28.15	28.15	0.000
A11	SYBR		Unkn		N/A	0.00	0.000
A12	SYBR		Unkn		N/A	0.00	0.000
B01	SYBR		Unkn		N/A	0.00	0.000
B02	SYBR		Unkn		N/A	0.00	0.000
B03	SYBR		Unkn		N/A	0.00	0.000
B04	SYBR		Unkn		N/A	0.00	0.000
B05	SYBR		Unkn		N/A	0.00	0.000
B06	SYBR		Unkn		N/A	0.00	0.000
B07	SYBR		Unkn		N/A	0.00	0.000
B08	SYBR		Unkn		N/A	0.00	0.000
B09	SYBR		Unkn		N/A	0.00	0.000
B10	SYBR		Unkn		N/A	0.00	0.000
B11	SYBR		Unkn		N/A	0.00	0.000
B12	SYBR		Unkn		N/A	0.00	0.000

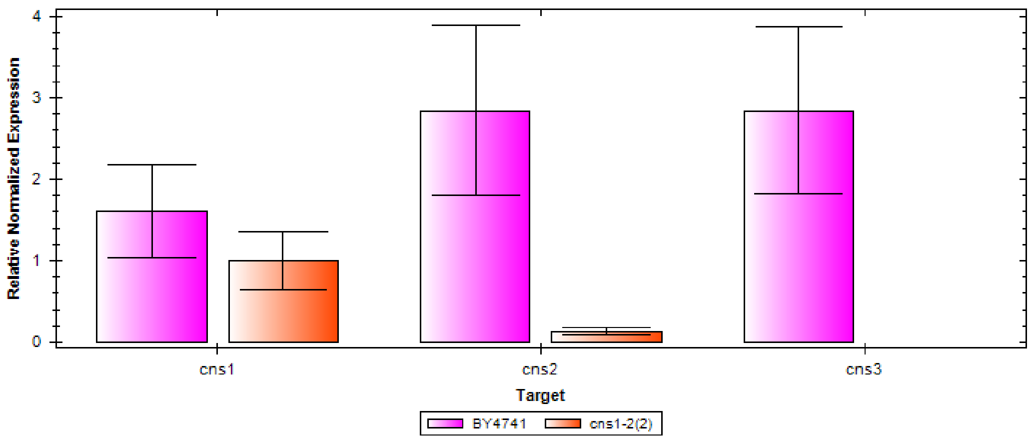
10.07 qPCR4

Next step:
Cns3\Cns3 only\cCns1-2r\Cns1 to perform qPCR

10.09

Samples: Cns1-2r\Cns1 BY4741
Results:





Cns1 √
Cns2 √

TF group

TF GROUP NOTEBOOK

Time line:
7.22-7.29: To prepare for the whole project we did gradient PCR to find the best annealing temperature. And we started to construct of the recombined plasmids(pYES2-yeGFP& pMET3-GAL4). We also tested the growth curve of AH109& BY4741.

7.30-8.6: In the last week we failed and didn't get the recombined plasmid. So we repeated the experiments and get a positive colony PCR results, but the sequences result showed there was no target sequence(yeGFP) on the plasmid.

8.7-8.14: We modified the primers and repeated the experiments. After a positive double enzyme verification result, we sequenced the plasmid(pMET3-GAL4).

8.15-8.22: the plasmid was pYES2-yeGFP actually. Then we tried to transform the recombined plasmid into the yeast. Then we verified it

and get the positive result. However, there was no fluorescence when we put the plate under UV.

8.23-8.30:We then developed the yeast which contain the recombined plasmid. Also we changed the proportion of glucose and galactose in the medium. At the same time, the construction of pMET3-GAL4 continued.

8.31-9.6: We finally get the plasmid pMET3-GAL4. Then we tried to get the pMET3-GAL4-pGAL1-yeGFP plasmid.

7.22 Mon.

1.[Dissolving primers] participator: Wang Mingyao purpose: dissolving primers (primers for pMET3 and Gal4) for latter experiments process:

Primers' name	the Volume of dd water
Primer GAL4 (F)	26μl
Primer GAL4(R)	32μl
Primer pMET3(F)	41μl
Primer pMET3(R)	37μl

use 10 μl of products respectively with adding 90 μl ddH20 for latter genome PCR

others are stored in the -20℃ refrigerator.

2.[Cross inoculation of Glycids and cultivate Saccharomyces cerevisiae BY4742]

participator: Luo Yaxin, Ji Jiayi purpose: refresh the glycids for latter plasmid(pYES2-NTA) extraction process: 300 μl 50% glycerin+ 200 μl ddH20+ 500 μl glycids fluid

3.[gradient temperature PCR of yeGFP]

participator: Luo Yaxin purpose: detect the best temperatures of the primers(yeGFP-F&yeGFP-R) process:

Primer yeGFP-F	1μl
Primer-yeGFP-R	1μl
dd water	20μl
Ⅰ-5™ 2×High-Fidelity Master Mix	25μl
yeGFP-PUC57	3μl

temperature(℃)	time
98	2min
98	10s
60-65	15s
72	2min
72	5min
4	∞

number	temperature(℃)
1	60.0
2	60.4
3	61.0
4	61.9
5	63.1
6	64.0

result: the best annealing temperature is 61.9℃.

7.24 Wed.

1.[Saccharomyces cerevisiae BY4742 genome PCR]

participator: Wang MIngyao

purpose: get the PCR products of pMET3 and GAL4 from the genome of Saccharomyces cerevisiae process:

Reagents	Volume
5×Prime STAR GXL Buffer	10μl
dNTP Mixture	4μl
primerF	3μl
primerR	3μl
Template	1μl
Primer STAR GXL DNA Polymerase	1μl
Sterilized distilled water	18μl

Temperature(℃)	time
98	2min
98	10s
60	15s
68	30s
68	5min
4	forever

temperature	time
98	2min
98	10s
55	15s
68	30s
68	5min
4	forever

result:

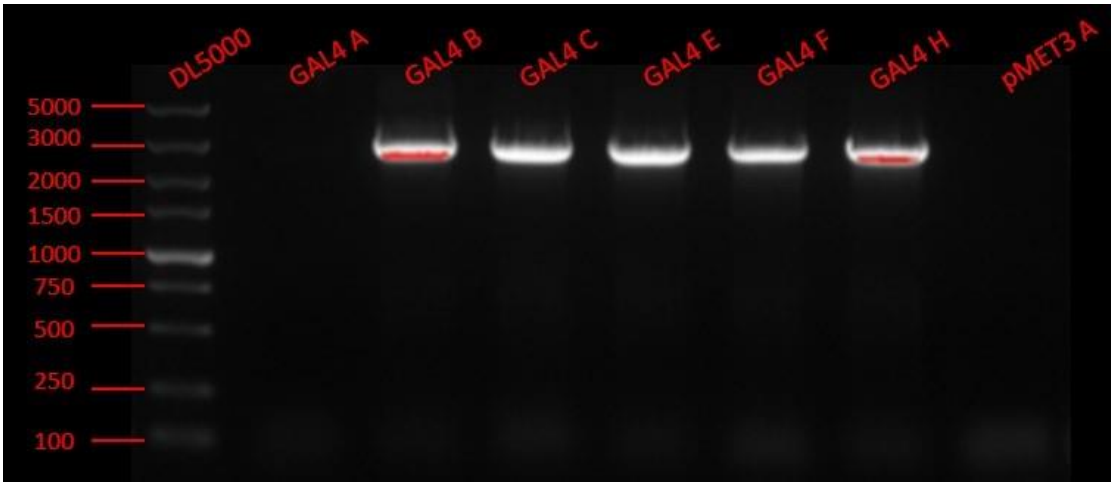


Fig 1. **Saccharomyces cerevisiae BY4742 genome PCR result-1**

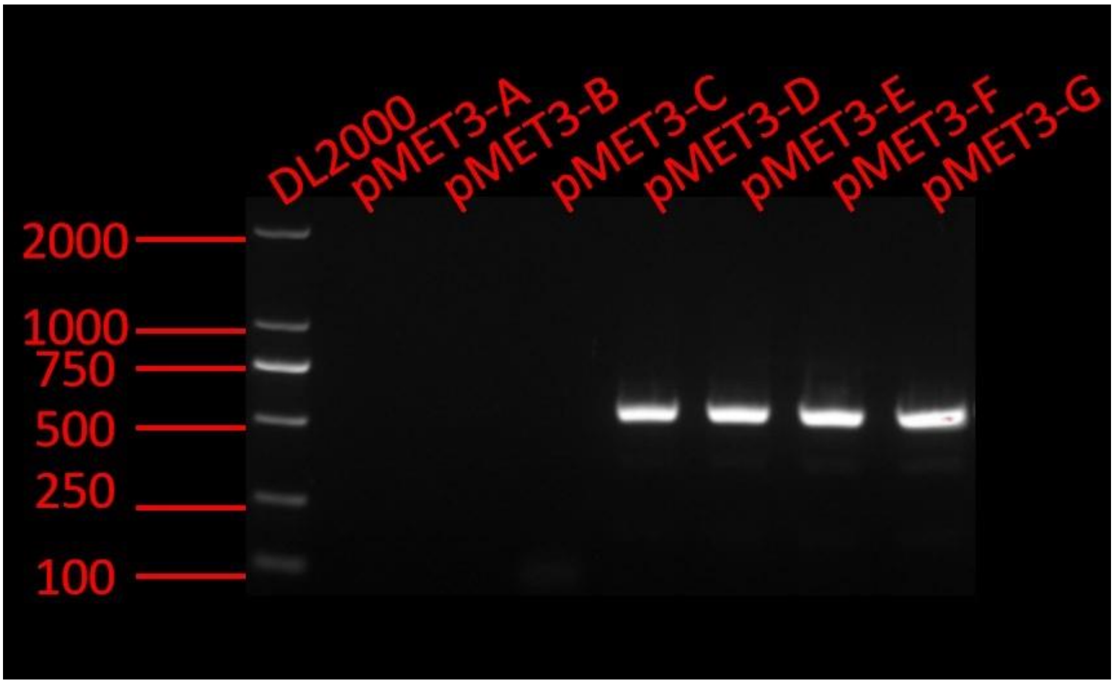


Fig 2. **Saccharomyces cerevisiae BY4742 genome PCR result-2**

2.[DNA deoxyribonucleic acid and gel extraction] participator: Wang Mingyao, Zhou Hao, Xu Yilong, Li Xinrui purpose: prove the results of former PCR and extract the DNA fragments of pMET3 and GAL4 respectively process: prepare the gel: 0.7g agarose+70mL 1×TAE+7μl EB

3.[double enzyme digestion of yeGFP and pYES2-NTA]
participator: Luo Yaxin, Ji Jiayi purpose: create viscous ends for later connection reactions
systems:

Reagents	Volume
*Not*I	1μl

*Xba*I	1μl
10×buffer	2μl
yeGFP/pYES2	3.4μl/5μl
Sterilized distilled water	12.6μl/11μl

for 37℃, 40min.
result:

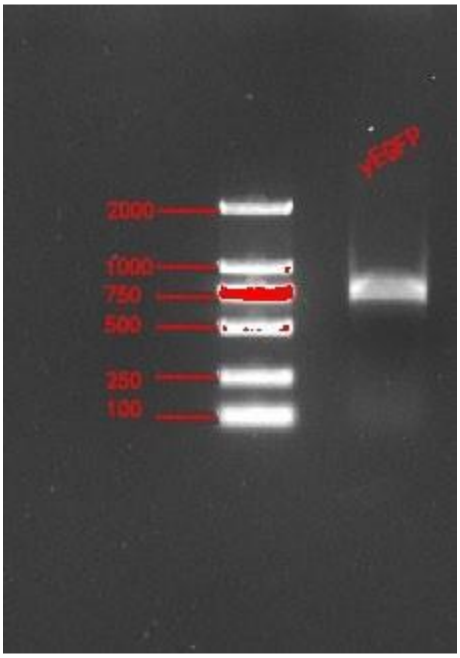


Fig 3. double enzyme digestion of yeGFP, the target fragments should be 731bp.

7.25 Thur.
1.[detect the content of pYES2-NTA by Onedrop Spectrophotometer] participator: Zhou Hao, Jin Qihan process: as the handbook of Onedrop result:

Sample	Content(ng/μl)
Sample1	94.4
Sample2	80.55

2.[Gradient PCR of pMET3 and GAL4] participator: Zhou Hao, Wang MIngyao, Xu Yilong, Li Xinrui purpose: to find the best annealing temperature for the PCR reactions of pMET3 and GAL4 process: the general response system&reaction procedures are settled as before
↓ the gradient annealing temperatures are settled with the sets of samples:

A: 65.0℃

B: 63.4℃

C: 60.9℃

D: 57. 1℃

E: 52.6℃

F: 48.8℃

G: 46.4℃

H: 45.0℃

↓

DNA deoxyribonucleic acid to detect the best temperature for GAL4/pMET3 PCR reactions result:

DNA fragment	best temperature
GAL4	63.4℃
pMET3	45.0℃

3.[gel extraction of pMET3&GAL4 and detect their contents by Onedrop] participator: Xu Yilong, Zhou Hao, Wang Mingyao purpose: to extract pMET3/GAL4 fragments for later experiments process: as the guidebook result:

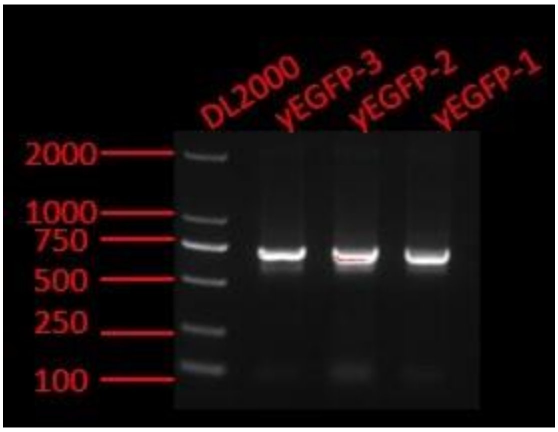


Fig 4. gel extraction of pMET3&GAL4 result

4.[connection of pYES2-yeGFP]

participator: Luo Yaxin, Ji Jiayi

purpose: get a restructuring plasmid of
pYES2-yeGFP process:

pYES2	4.5μl
yeGFP	2.3μl
T4 ligase	0.5μl
buffer	1μl
Sterilized distilled water	1.7μl

7.26 Fri.
1.[Double Enzyme Digestion of GAL4/pMET3/yeGFP/pYES2-NTA] participator: Zhou Hao, Wang Mingyao, Xu Yilong, Liu Yanmu
purpose: create viscous ends for later connection reactions
process: confirm the restrictive endonucleases:

pMET3: *Spe* Ⅰ, *Eco*R Ⅰ

GAL4: *Xba* Ⅰ, *Not* Ⅰ
↓

Reagents	Volume
Qc Spe Ⅰ	1μl
Qc EcoR Ⅰ	1μl
10×Qc buffer	3μl
pMET3 sample	3μl
dd water	22μl

Reagents	Volume
Qc Not Ⅰ	1μl
Qc Xba Ⅰ	1μl
10×Qc buffer	3μl
GAL4 sample	3μl
dd water	22μl
Qc Not Ⅰ	1μl
Qc Xba Ⅰ	1μl
10×Qc buffer	3μl
yeGFP sample	4μl

dd water	21μl

Qc Spe Ⅰ	1μl
Qc EcoR Ⅰ	1μl
10×Qc buffer	5μl
pYES2-NTA sample	10μl
dd water	33μl

Qc Spe Ⅰ	1μl
Qc EcoR Ⅰ	1μl
10×Qc buffer	5μl
pYES2-NTA sample	10μl
dd water	33μl

↓

37℃ double enzyme digestion reaction for 1h

75℃ enzyme inactivation reaction for 30min

↓ extract the products of reactions result:

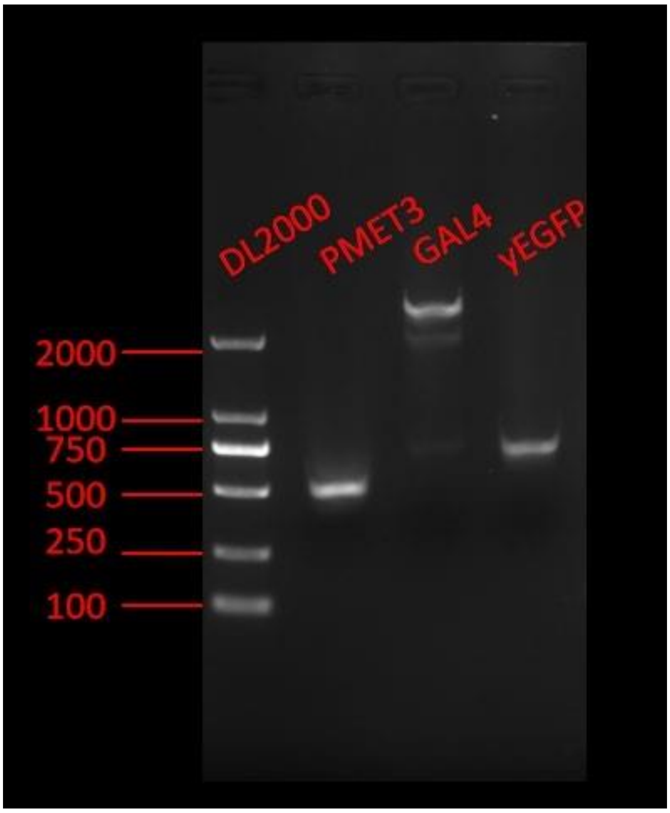


Fig 5. Double Enzyme Digestion of GAL4/pMET3/yeGFP/pYES2-NTA result 1

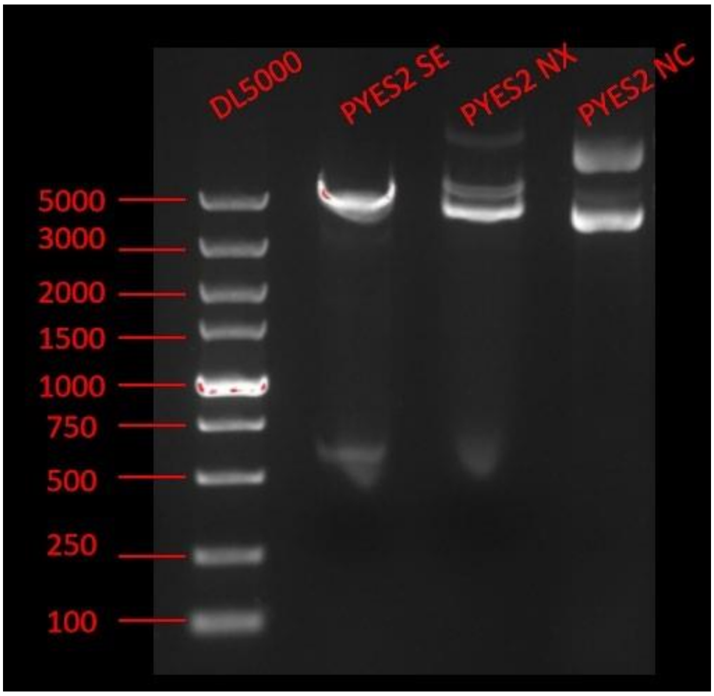


Fig 6. Double Enzyme Digestion of GAL4/pMET3/yeGFP/pYES2-NTA result 2

2.[connect pYES2-pMET3(*Eco*R Ⅰ /*Spe* Ⅰ)&pYES2-GAL4(*Not* Ⅰ/*Xba* Ⅰ)&pYES2-yeGFP(*Not* Ⅰ/*Xba* Ⅰ)

participator: Wang Mingyao, Xu Yilong purpose: as the title

process:

10xT4 buffer	1μl
pYES2-NTA	3.5μl
pMET3	5μl
T4 Ligase	0.5μl

10xT4 buffer	1μl
pYES2-NTA	1.5μl
GAL4	7μl
T4 Ligase	0.5μl

10xT4 buffer	1μl
pYES2-NTA	3.5μl
yeGFP	5μl
T4 Ligase	0.5μl

3.[transformation of the r*eco*mbination plasmid(pYES2-yeGFP)] participator: Luo Yaxin purpose: transform the plasmid into DH5a.
process: as the protocol.

7.27 Sat.

1.[yeGFP PCR& double enzyme digestion(*Xba* Ⅰ/*Not* Ⅰ)]

participator: Luo Yaxin purpose: create viscous ends for later connection reactions

Mixture	25μl
Primer-F	2μl
Primer-R	2μl
Template	2μl
dd water	19μl

98	2min	
98	10s	30cycles
61.9	15s	
72	15s	
72	5min	
4	forever	

best annealing temperature：61.9℃

↓

Qc Not Ⅰ	2μl
Qc Xba Ⅰ	2μl
10×Qc buffer	3μl
yeGFP sample	1μl
dd water	22μl

↓

37℃ double enzyme digestion for 1h

75℃ enzyme inactivation reaction for 30min
↓ extract the products of
reactions

2.[Flat plate line vaccination of Glycids &Liquid culture of transformed DH5α] participator: Zhou Hao, Wang Mingyao purpose: expand cultivation for later plasmids' extraction

3.[connection of pYES2-yeGFP]
participator: Luo Yaxin, Ji Jiayi
purpose: get a restructuring plasmid of
pYES2-yeGFP process:

pYES2	6μl
yeGFP	8.3μl
T4 ligase	1μl
buffer	2μl
Sterilized distilled water	2.7μl

7.28 Sun.
1.[extraction of pYES2-NTA] participator: Xu Yilong purpose: extract adequate samples of pYES2-NTA for later experiments process: as the handbook result:

7.29 Mon.
1.[transform DH5α with pYES2-pMET3/GAL4/yeGFP and dilution plating] participator: Zhou Hao, Wang Mingyao purpose: expand contents of the connection products process: as handbook result: get available clone

2.[test of AH109/BY4741 growth curve] [7.26-7.28] participator: Luo Yaxin, Ji Jiayi purpose: get the growth curve of AH109/BY4741 for later experiments results:

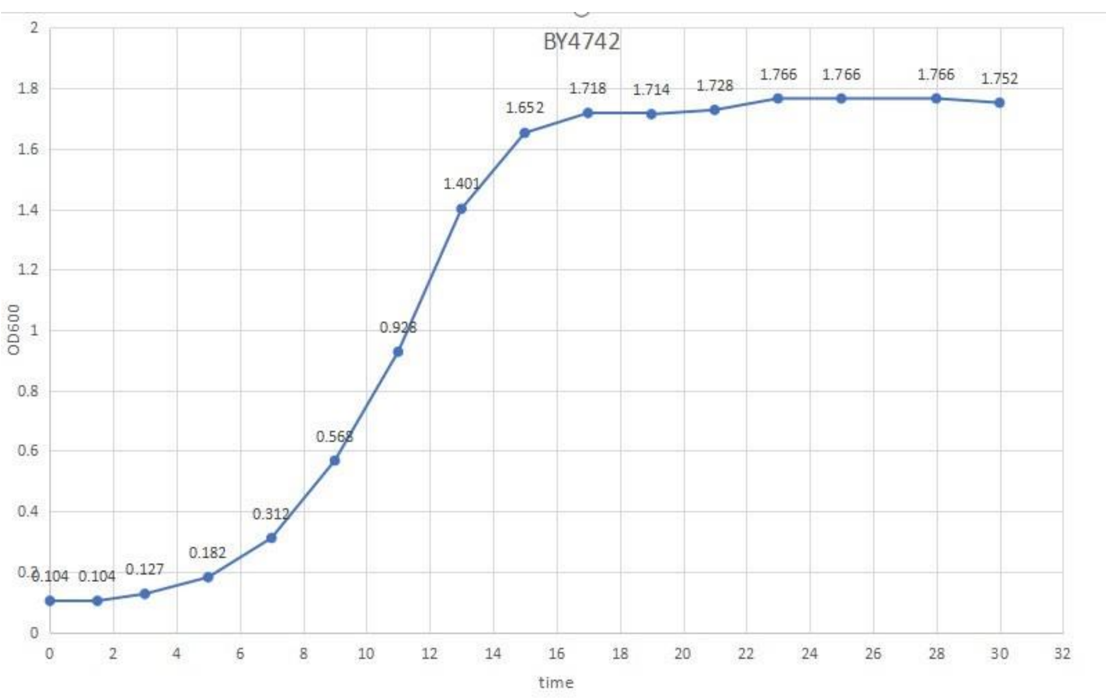


Fig 7. The growth curve of BY4742

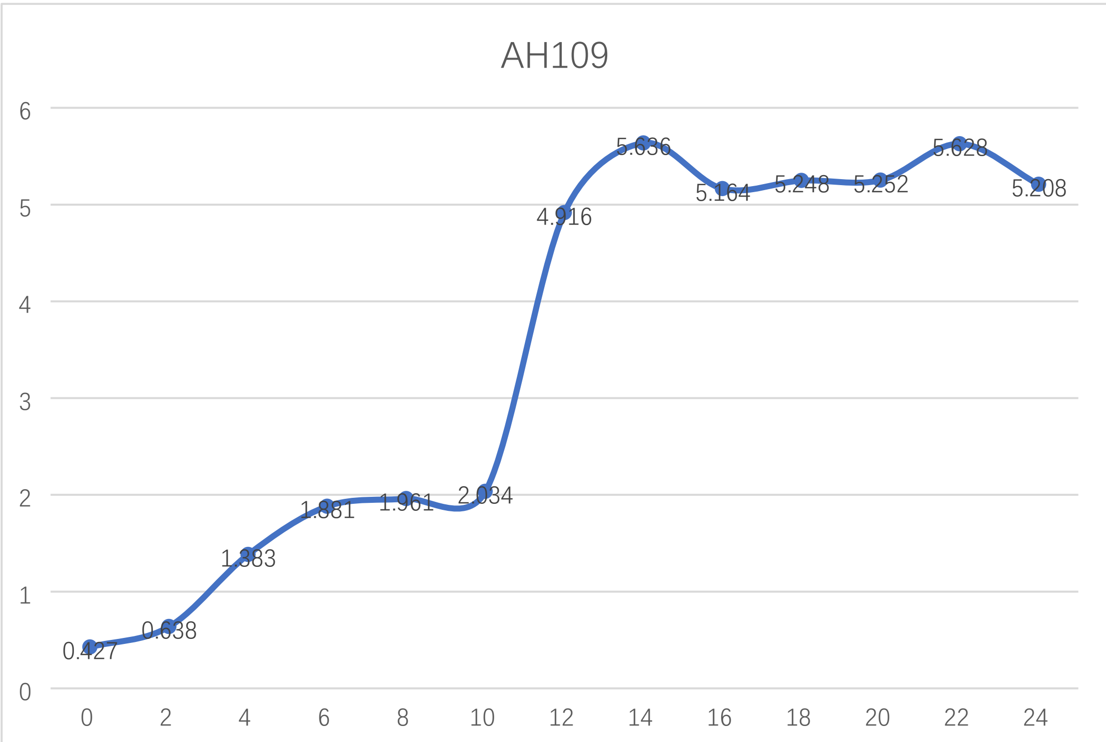


Fig 8. The growth curve of AH109

7.30 Tues.

1.[clone cultivation and germ PCR]

participator: Zhou Hao, Wang Mingyao

purpose: prove the results of connections

primer1	1μl
primer2	1μl
dd water	2μl
（1/100）clone	1μl
2X T5 Mix	5μl

2.[DH5α(pYES2-NTA) liquid extraction and cultivation]

participator: Luo Yaxin, Ji Jiayi purpose: get more available pYES2-NTA plasmid for later experiment

7.31 Wed.

1.[double enzyme digestion verifying reaction] participator: Wang Mingyao, Zhou Hao purpose: verify the result of the connections of pMET3/GAL4/yeGFP-pYES2 process:

Reagents	Volume
Qc Spe Ⅰ	1μl
Qc EcoR Ⅰ	1μl
10×Qc buffer	2μl
pMET3 sample	6/6/5μl
dd water	10/10/11μl

Reagents	Volume
Qc Not Ⅰ	1μl
Qc Xba Ⅰ	1μl
10×Qc buffer	2μl
GAL4 sample	1.5/2/5μl

dd water	14.5/14/11μl
Qc Not Ⅰ	1μl
Qc Xba Ⅰ	1μl
10×Qc buffer	3μl
yeGFP sample	2/4/5.5μl
dd water	14/12/10.5μl

Result:

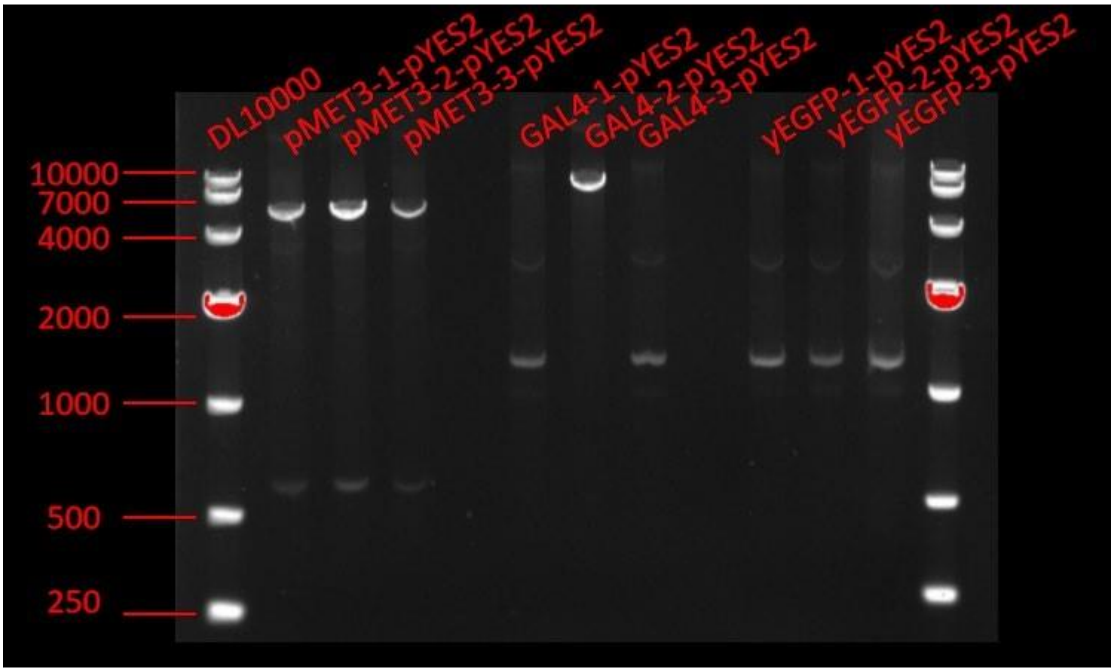


Fig 9. The double enzyme digestion verification result

2.[PCR of yeGFP] participator: Luo Yaxin
purpose: prepare for the later double enzyme digestion. process:

Primer yeGFP-F	1μl
Primer-yeGFP-R	1μl

dd water	8.5μl
Ⅰ-5™ 2×High-Fidelity Master Mix	12.5μl
yeGFP-PUC57	2μl

temperature(℃)	time
98	2min
98	10s
61.9	15s
72	2min
72	5min
4	∞

3.[double enzyme digestion of pMET3, Gal4, yeGFP and PYES2]

Aim: pMET3, Gal4, yeGFP and PYES2, PCR products restriction enzyme digestion. Progress: pMET3, PYES2: *Spe*I, *Eco*RI,

	pMET3(55ng/μl)	PYES2(219ng/μl)
*Eco*RI	1μl	1μl
*Spe*I	1μl	1μl
10X Quick cut buffer	3μl	3μl
DNA volume	8μl	2μl
dd water	17μl	23μl
Sum	30μl	30μl
Remarks	*Eco*RI: 37℃ 5min *Spe*I: 37o 30min	*Eco*RI: 37℃ 15min *Spe*I: 37o 5min

Gal4: *Not*I, *Xba*I,

	Gal4(60ng/μl)
*Not*I	1μl
*Xba*I	1μl
10X Quick cut buffer	3μl

DNA volume	7.5μl
dd water	17.5μl
Sum	30μl
Remarks	*Not*I: 37℃ 10min *Xba*I: 37℃ 10min

yeGFP, PYES2: *Not*I, *Xba*I,

	yeGFP(182ng/up)	PYES2(219ng/μl)
NotI	1μl	1μl
XbaI	1μl	1μl
10X Quick cut buffer	3μl	5μl
DNA volume	22μl	18μl
dd water	3μl	7μl
Sum	30μl	30μl
Remarks	NotI: 37℃ 10min XbaI: 37℃ 10min	NotI: 37℃ 5min XbaI: 37℃ 10min

Result: Remaining detection.
Conclusion: None
Next step: pMET3, Gal4, yeGFP and PYES2, PCR products will be tested through electrophoresis and nucleotide concentration detection.

8.1 Thur.
1.[double enzyme digestion reaction] participator: Luo Yaxin
purpose: prepare for the following steps

Reagents	Volume
Qc Spe Ⅰ	1μl
Qc EcoR Ⅰ	1μl
10×Qc buffer	3μl
pMET3 sample	16μl
dd water	9μl

Reagents	Volume
Qc Not Ⅰ	1μl

Qc Xba Ⅰ	1μl
10×Qc buffer	2μl
GAL4 sample	1/2μl
dd water	5/4μl

2.[gel extraction of pMET3&GAL4]

participator: Luo Yaxin, Ji Jiayi
purpose: get purified fragments.
process: as the protocol.
Result: 42.72ng/μl 36.82 ng/μl 56.73 ng/μl

3.[ligation of GAL4-pYES2]

participator: Luo Yaxin
purpose: [repeating experiment*1] process:

10x T4 buffer	2μl
T4 Ligase	1μl
template	3μl
fragment	9μl
dd water	5μl

16℃ for 12h then stored at 4℃

4.[ligation of pMET3-pYES2]

participator: Wang Mingyao
purpose: [repeating experiment*1] process:

10x T4 buffer	4μl
T4 Ligase	2μl
template	23μl
fragment	11μl

16℃ for 12h then stored at 4℃

5.[liquid cultivation of DH5α transformed germ clone(by pMET3pYES2、GAL4-pYES2、yeGFP-pYES2)]

participator: Xu Yilong

purpose: provide more ingredients for later extraction of pMET3-pYES2、 GAL4-pYES2 and yeGFP-pYES2 process: 10μl【A+】 + 10ml LB+ 1 clone

[next step]: extract plasmids and verify the ligation

8.2 Fri.

1.[extraction of pMET3-pYES2、GAL4-pYES2、yeGFP-pYES2] participator: Xu Yilong purpose: extract adequate samples for later experiments process: as the handbook result: as expected

2.[detect the concentration of pMET3-pYES2、GAL4-pYES2、yeGFPpYES2 by Onedrop Spectrophotometer]
participator: Xu Yilong
purpose: detect the content so as to calculate the enzyme digestion system process: as the handbook result:

pMET3-1-pYES2	153.58ng/μl
pMET3-2-pYES2	178.14ng/μl
pMET3-3-pYES2	192.94ng/μl
GAL4-2-pYES2	343.06ng/μl
yeGFP-1-pYES2	84.84ng/μl
yeGFP-2-pYES2	139.40ng/μl
yeGFP-3-pYES2	168.49ng/μl

3.[double enzyme digestion to verify ligation of pMET3-pYES2、GAL4pYES2、yeGFP-pYES2]
participator: Zhou hao, Xu Yilong purpose: verify the results of pMET3-pYES2、GAL4-pYES2、yeGFP-pYES2 connection before sequencing process:

	10X loading buffer	SpeI	Hind III	NotI	XbaI	sample	dd water
pMET3-1pYES2	2μl	1μl	1μl	\	\	6.5μl	10.5μl
pMET3-2pYES2	2μl	1μl	1μl	\	\	5.5μl	11.5μl

pMET3-3pYES2	2μl	1μl	1μl	\	\	5μl	11μl
pYES2	2μl	1μl	1μl	\	\	4.5μl	11.5μl
pYES2	\	\	\	\	\	4.5μl	15.5μl
yeGFP-1pYES2	2μl	\	\	1μl	1μl	11.5μl	4.5μl
yeGFP-2pYES2	2μl	\	\	1μl	1μl	7μl	9μl
yeGFP-3pYES2	2μl	\	\	1μl	1μl	5.5μl	10.5μl
GAL4-2pYES2	2μl	\	\	1μl	1μl	2.5μl	13.5μl

37℃ reaction for 1h and 60℃ inactivation for 15min

4.[gel electrophoresis of former reaction] participator: Zhou hao, Xu Yilong purpose: verifying the cutting result and choose appropriate sample for sequencing process: as the protocol result:

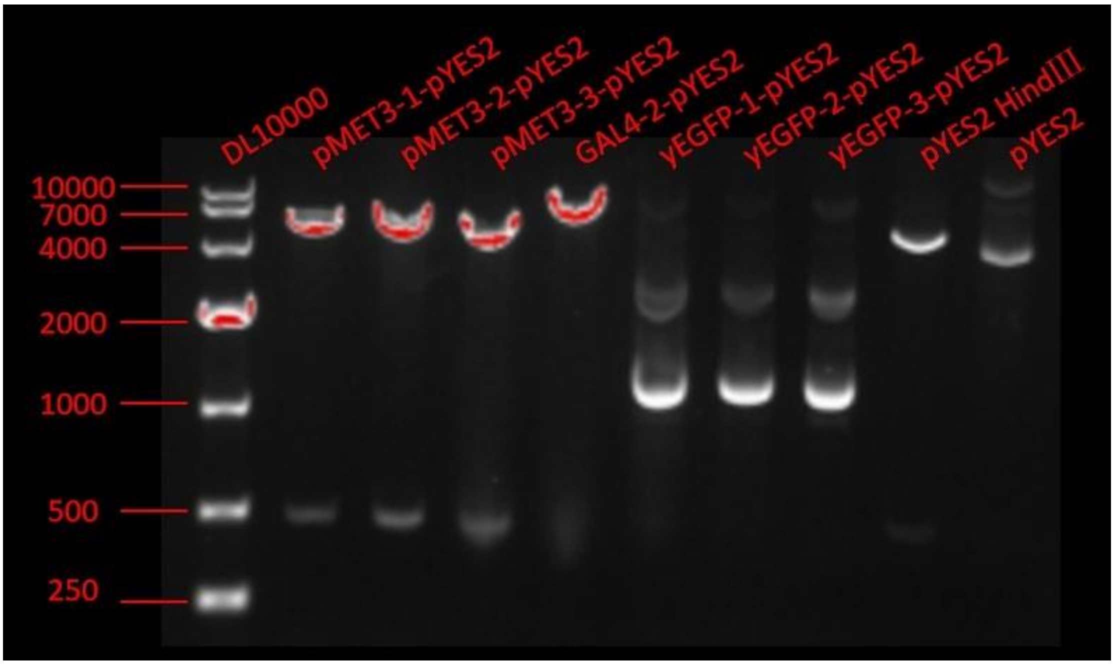


Fig 10. double enzyme digestion to verification result, for the recombined

plasmid pMET3-pYES2、GAL4-pYES2、yeGFP-pYES2

[next step]: repeat parts of experiments because the result of gel electrophoresis is unsatisfactory

5.[transformation of pYES2-yeGFP]
participator: Luo Yaxin, Ji Jiayi purpose:
transform the recombined plasmid into DH5a
process: as the protocol

8.3 Sat.
1.[extraction of yeGFP-pYES2] participator: Xu Yilong
purpose: extract adequate samples for later experiments result: as the handbook

2.[detect the content of yeGFP-pYES2 by Onedrop Spectrophotometer] participator: Xu Yilong purpose: detect the content so as to calculate the enzyme digestion system process: as the handbook result:

98.97ng/μl	90.14ng/μl	42.49ng/μl	6.43ng/μl	51.81ng/μl	242.76ng/μl	51.81ng/μl	109.49ng/μl	185.63ng/μl	70.18ng/μl

3.[liquid cultivation of DH5α transformed germ clone(by pMET3-pYES2)] participator: Wang Mingyao, Xu Yilong purpose: provide more ingredients for later extraction of pMET3-pYES2 process: 10μl【A+】+10ml LB+1 clone

4.[double enzyme digestion verifying reaction of pMET3-yeGFP]
participator: Wang Mingyao, Xu Yilong purpose: verify the results of pMET3-yeGFP connection before sequencing process:

Qc Buffer	2μl
Qc *Not* Ⅰ	1μl
Qc *Xba* Ⅰ	1μl
pMET3-yeGFP	5/9/4μl
dd water	11/7/12μl

37℃ reaction for 1h and 60℃ inactivation for 15min
3.[gel electrophoresis of former reaction] participator: Wang Mingyao, Xu Yilong purpose: verifying the cutting result and choose appropriate sample for sequencing process/result:

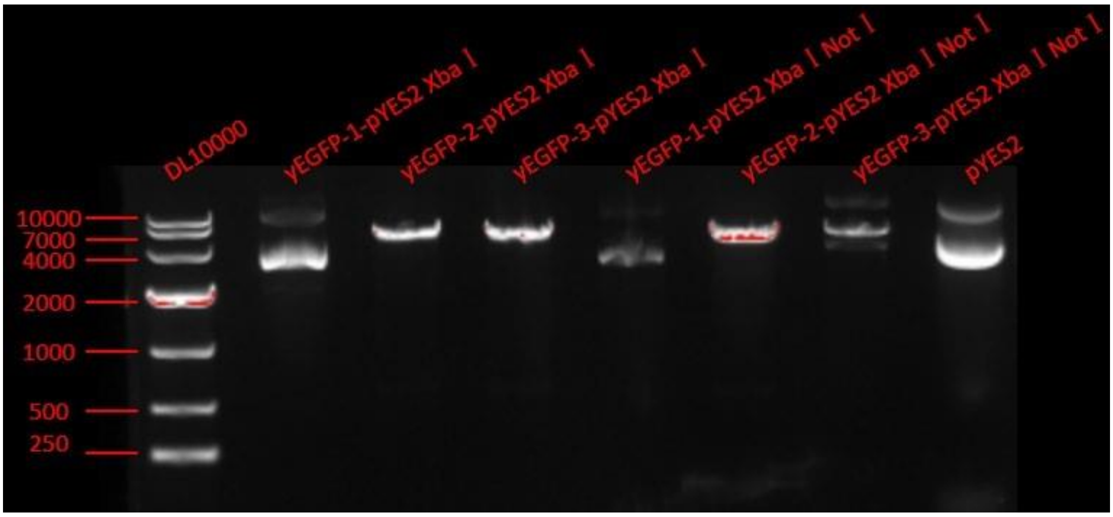


Fig 11. double enzyme digestion verifying reaction of pMET3-yeGFP result

8.4 Sun.

1.[extraction of pMET3-pYES2] participator: Wang Mingyao, Xu Yilong purpose: extract adequate samples for later experiments process: as the handbook

2.[detect the concentration of pMTE3-pYES2 by Onedrop Spectrophotometer] participator: Wang Mingyao, Xu Yilong purpose: detect the concentration so as to calculate the enzyme digestion system process: as the handbook result:

156.85ng/μl	152.92ng/μl	230.65ng/μl	238.64ng/μl	247.91ng/μl	211.64ng/μl	189.80ng/μl

3.[double enzyme digestion verifying reaction of pMET3-pYES2 and GAL4-2-pYES2] participator: Wang Mingyao, Zhou Hao, Xu Yilong purpose: verify the results of pMET3-yeGFP connection before sequencing process:

	10X loading buffer	*Spe*I	*Hin*dⅢ	*Not*I	*Xba*I	sample	dd water
pMET3-1pYES2	2μl	1μl	1μl	\	\	4μl	12μl
pMET3-N1-pYES2	2μl	1μl	1μl	\	\	5μl	11μl
pMET3-2pYES2	2μl	1μl	1μl	\	\	4μl	12μl

pMET3-N2-pYES2	2μl	1μl	1μl	\	\	6μl	10μl
pMET3-3pYES2	2μl	1μl	1μl	\	\	4μl	12μl
pMET3-N3-pYES2	2μl	1μl	1μl	\	\	5μl	11μl
GAL4-2pYES2	2μl	\	\	1μl	1μl	2.5μl	13.5μl

37℃ reaction for 1h and 60℃ inactivation for 15min

4.[gel electrophoresis of former ligation] participator: Zhou Hao, Xu Yilong purpose: verifying the cutting result and choose appropriate sample for sequencing process: as the handbook result:

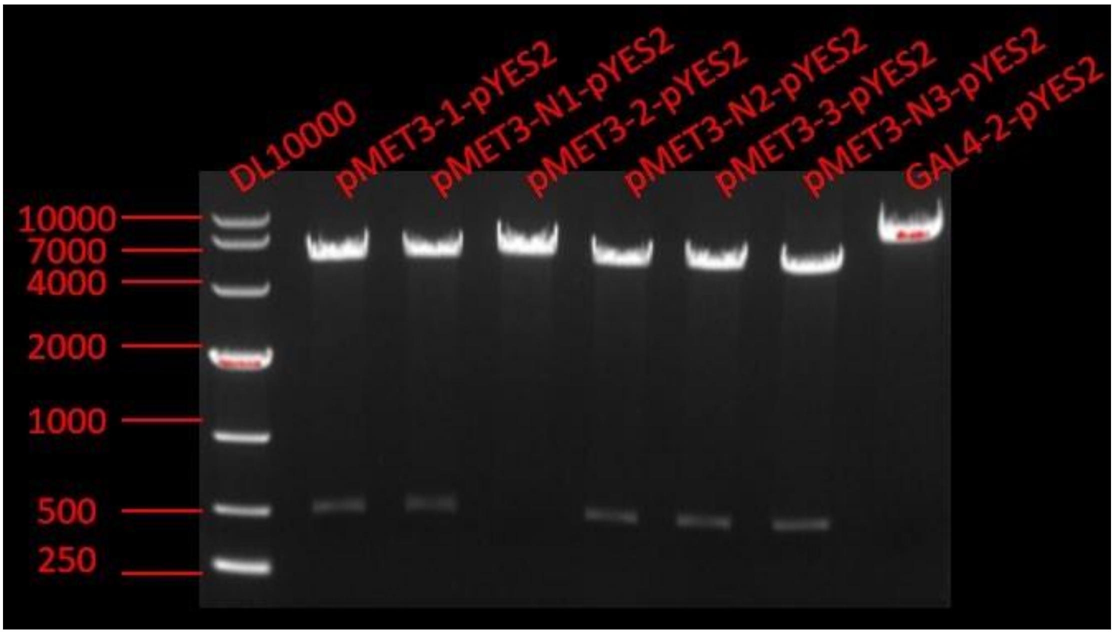


Fig 12. double enzyme digestion verifying reaction of pMET3-pYES2 and GAL4-2-pYES2 result

8.5 Mon.

1.[extraction of yeGFP-pYES2] participator: Xu Yilong purpose: extract adequate samples for later experiments process: as the handbook

2.[detect the content of pMTE3-pYES2 by Onedrop Spectrophotometer] participator: Xu Yilong, Ke Yitao purpose: detect the content so as to calculate the enzyme digestion system process: as the handbook result:

165.40ng/μl	179.56ng/μl	138.22ng/μl	85.84ng/μl

3.[double enzyme digestion verifying reaction of yeGFP-pYES2 (*Not*I *Xba*I)] participator: Zhou hao purpose: verify the results of pMET3-yeGFP ligation before sequencing process:

	10X loading buffer	*Not*I	*Xba*I	sample	dd water
yeGFP-1-pYES2	2μl	1μl	1μl	6μl	10μl
yeGFP-2-pYES2	2μl	1μl	1μl	5.5μl	10.5μl
yeGFP-3-pYES2	2μl	1μl	1μl	7μl	9μl

Result:

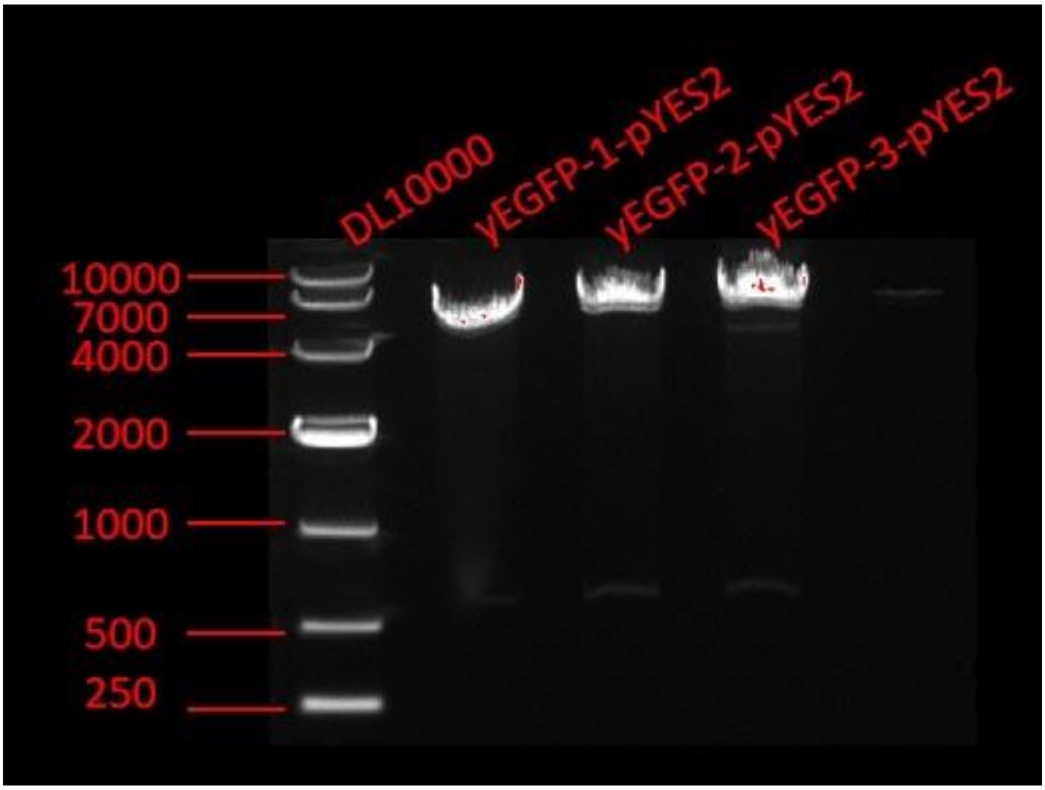


Fig 13. double enzyme digestion verifying reaction of yeGFP-pYES2 result

4.[double enzyme digestion of pMET3, GAL4-pMET3, pMET3-pYES2, yeGFP, GAL4]

participator: Zhou Hao purpose: prepare for the ligation of pMET3-GAL4-pYES2 and pMET3-yeGFPpYES2 process:

	10X loading buffer	NotI	XbaI	SpeI	EcoRI	sample	dd water
pMET3 fragment	2μl	\	\	1μl	1μl	3μl	13μl

GAL4pYES2	2μl	\	\	1μl	1μl	2.5μl	13.5μl
GAL4pYES2(NC)	2μl	\	\	\	\	4.5μl	11.5μl
pMET3-2pYES2(NC)	2μl	\	\	\	\	4.5μl	13.5μl
pMET3-2pYES2	2μl	1μl	1μl	\	\	4.5μl	11.5μl
yeGFP fragment	2μl	1μl	1μl	\	\	1μl	15μl
GAL4-ABC fragment	2μl	1μl	1μl	\	\	3μl	13μl

5.[gel electrophoresis of former digested products]
participator: Zhou Hao, Xu Yilong purpose: verify if the result of enzyme digestion is as expected process: as the handbook result:

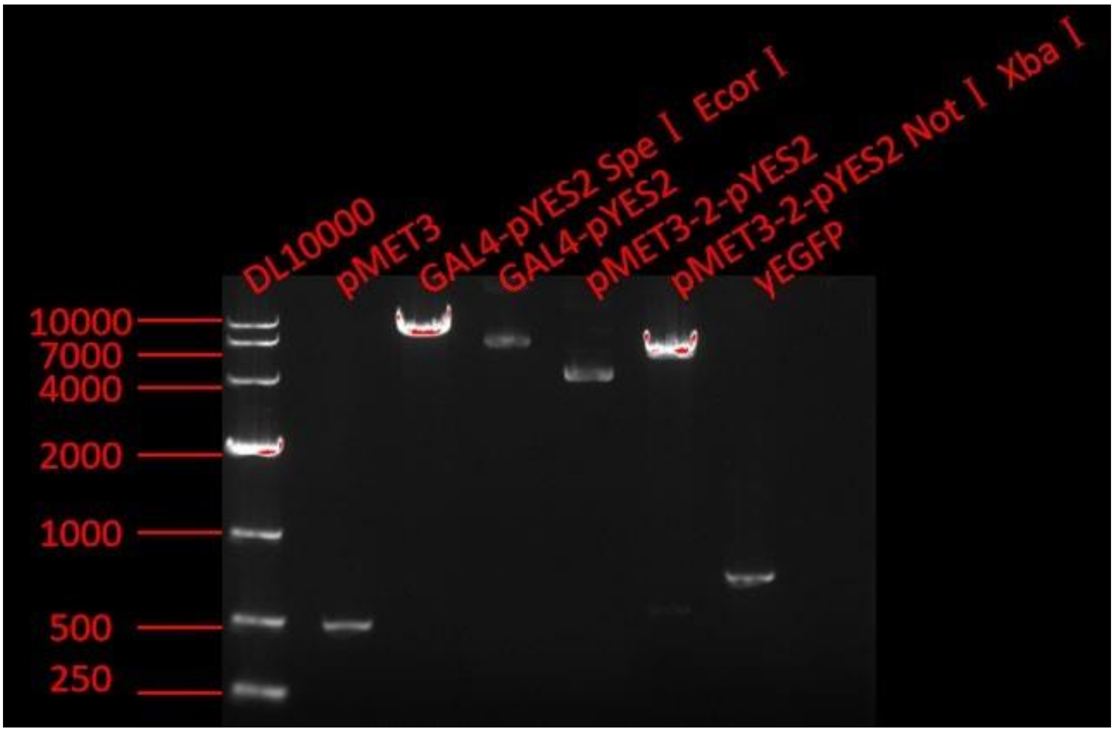


Fig 14. **double enzyme digestion result**

6.[Gel extraction of former digested products] participator: Zhou Hao, Xu Yilong
purpose: collection of digested products
process: as the handbook result: as expected

7.[detect the concentration of gel extraction products]
participator: Xu Yilong purpose: detect the concentration so as to calculate the ligation system process: as the handbook
result:

GAL4-pYES2(*Spe*I *EcoR*I)	pMET3-2pYES2	yeGFP ligation	pMET3 ligation
11.72μl	11.55μl	0.07μl	-2.13μl

The Elution Buffer used in blank determination is *Not* from that used in gel extraction, and the pMET3 ligation's concentration is low, so the result was negative.

8.6 Tues.
1.[Colony PCR] participator: Yang Yuanhan, Ji Jiayi, Xu Yilong purpose: detect which colony contented yeGFP process: as the handbook[we used 2X Accurate Taq Master Mix (dye plus) from Accurate Biotechnology]

2X Accurate Taq Master Mix (dye plus)	5μl
primer F	0.2μl
primer R	0.2μl
Template	0.2μl
dd water	4.4μl

94℃	1min	
98℃	10s	30 cycles
62℃	30s	
72℃	1min	
72℃	2min	

We finally collected 30 tubes.

2.[Gel electrophoresis of former PCR products] participator: Yang Yuanhan, Ji Jiayi, Xu Yilong purpose: detect which colony has yeGPF-pYES2 so as to choose colony for solution culture process: as the handbook

3.[solution culture of pMET3-pYES2] participator: Zhou Hao, Xu Yilong
purpose: because the gene sequencing of pMET3-pYES2 had succeed, we wanted to obtain more pMET3-pYES2.
process: as the handbook
result: as expected

4.[PCR of GAL4, yeGFP and pMET3]
participator: Xu Yilong purpose: get abundance fragments for ligation process: as the process in 22/7/2019, and the annealing temperature is showed below

GAL4	63.4℃
yeGFP	62℃
pMET3	45.0℃

8.7 Wed.
1.[Gel electrophoresis of GAL4/yeGFP/pMET3] participator: Xu Yilong
purpose: detect whether the PCR is succeeded process: as the handbook result:

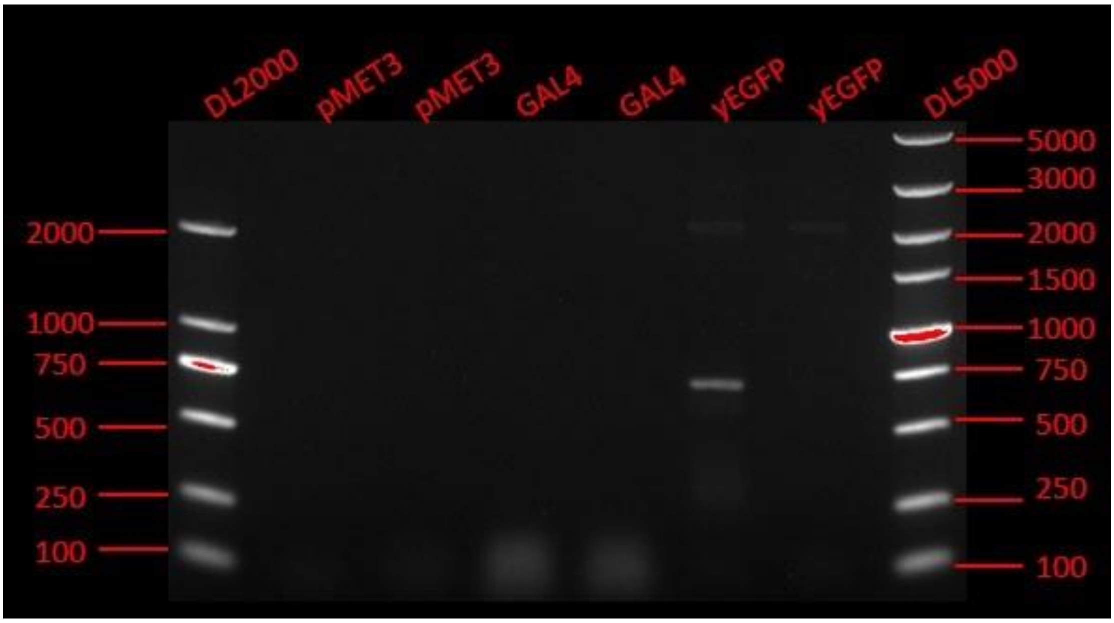


Fig 15. The PCR results of

GAL4/GFP/pMET3

2.[PCR of GAL4, yeGFP and pMET3]
participator: Ke Yitao purpose: get abundance fragments for ligation
process:

Reagents	Volume	
primerF	1.5μl	
primerR	1.5μl	
Template	1μl	
Primer STAR GXL DNA Polymerase	15μl	It is mixed with 5XPrime STAR GXL Buffer and dNTP Mixture
Sterilized distilled water	31μl	

the annealing temperature is the same as that in 6/8/2019。

3.[extraction of yeGFP-pYES2, pMET3-pYES2, pGAL4-pYES2]
participator: Yang Yuanhan, Luo Yaxin, Zhou Hao, Zhao Zirui
purpose: obtain aboundance samples for downstream experiments process: as the handbook result: as expected

4.[detect the concentration of three kinds of plasmid]
participator: Yang Yuanhan, Luo Yaxin, Zhou Hao, Zhao Zirui purpose: calculate the double enzyme digestion system process: as the handbook result:

5.[gel electrophoresis of PCR products] participator: Xu Yilong
purpose: verify the result of PCR
process: as the handbook result:

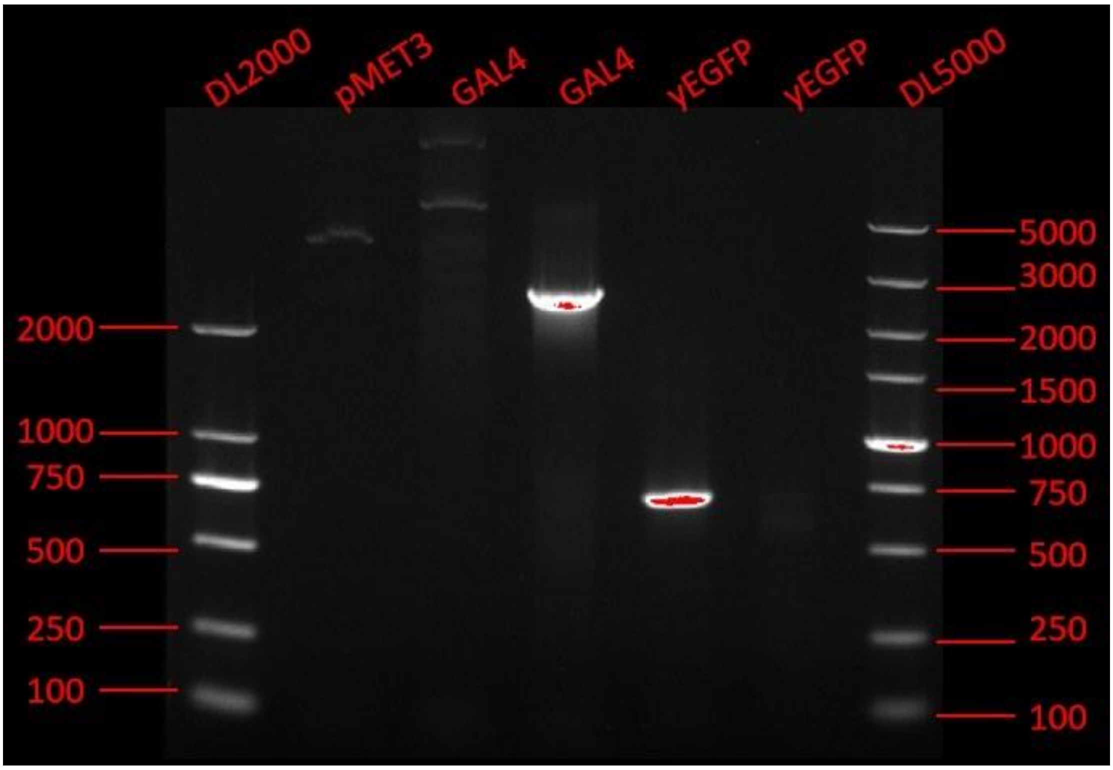


Fig 16. The verification of PCR results

6.[gel extraction of yeGFP and GAL4]
participator: Xu Yilong, Zhao Zirui
purpose: obtain pure fragments for
ligation process: as the handbook
result: as expected

7.[detect the concentration of gel extraction products]
participator: Xu Yilong, Zhao Zirui purpose: detect the concentration so as to calculate the ligation system process: as the handbook result:

8.[PCR of yeGFP] participator: Yang Yuanhan purpose: obtain abundant yeGFP fragments (use pYES2 only as negative control) process: as the protocol, the system is the small as NO.2
result: as expected

9.[gel electrophoresis of PCR products(yeGFP)] participator: Xu Yilong
purpose: verify the result of PCR process:
as the handbook result:

There is a fragment in the third line, suggesting primers of yeGFP can also amplify a 750bp fragment, just like the yeGFP fragment , so the result of colony PCR is unreliable.

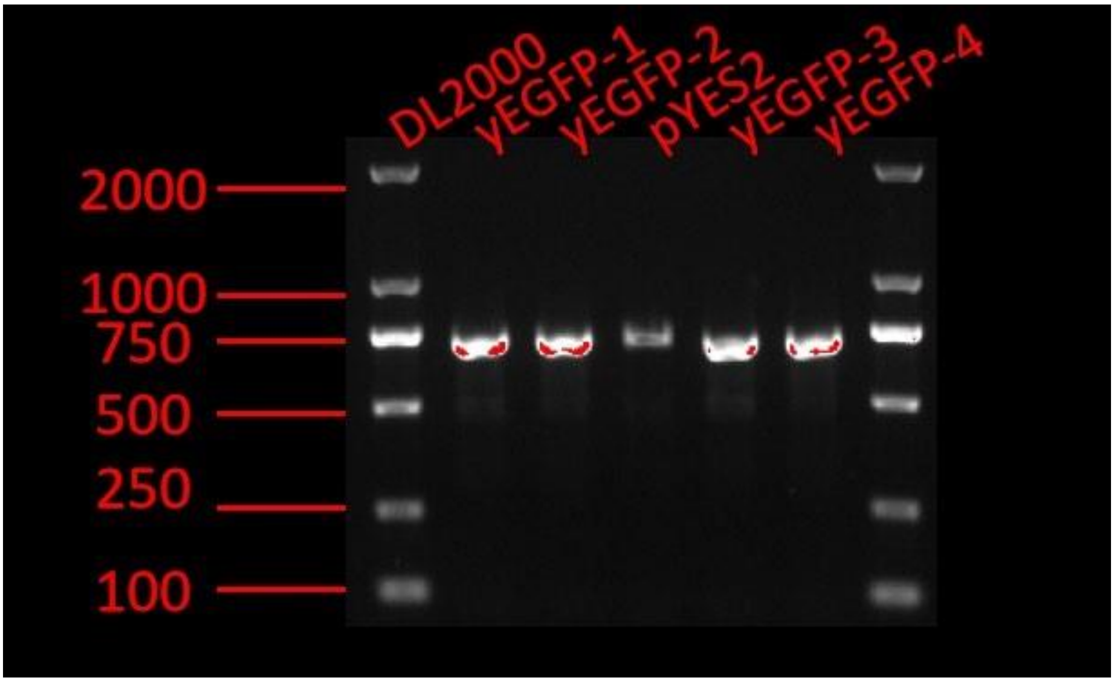


Fig 17. gel electrophoresis of PCR products, showed the primers can also amplify a 750bp fragment, which may lead to the colony PCR results fault

10.[purification of PCR products(yeGFP)] participator: Yang Yuanhan purpose: obtain pure products process: as the protocol result: as expected

8.8 Thur.

1.[gel electrophoresis of purified products and gel extraction] participator: Xu Yilong, Zhao Zirui purpose: obtain pure products process: as the protocol result:

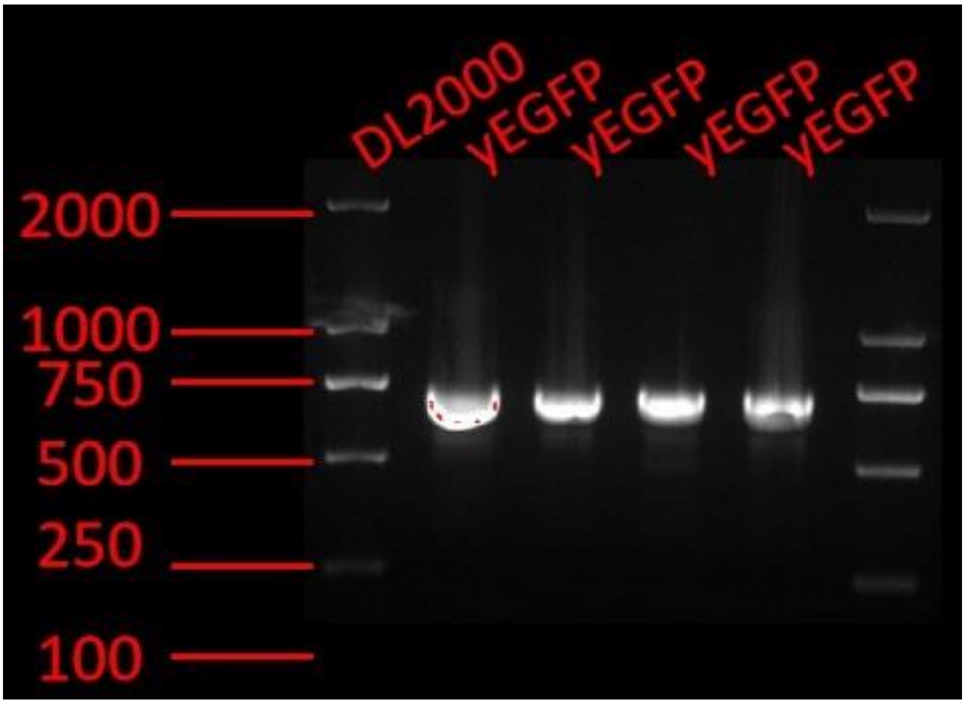


Fig 18. Purified fragments gel electrophoresis result

2.[detect the concentration of yeGFP fragment]
participator: Xu Yilong, Zhao Zirui purpose: detect the concentration to calculate response system process: as the protocol result: 76.02ng/μl

3.[double enzyme digestion of yeGFP fragment(*Not*I *Xba*I), pYES2(*Not*I *Xba*I)] participator: Yang Yuanhan purpose: prepare for ligation process:

yeGFP	26.3μl
*Not*I	1μl
*Xba*I	1μl
Buffer	4μl
dd water	7.7μl

pYES2	10μl
*Not*I	1μl
*Xba*I	1μl
Buffer	3μl
dd water	15μl

4.[gel electrophoresis of digestion products and gel extraction]
participator: Yang Yuanhan purpose: obtain pure products process: as the handbook result:

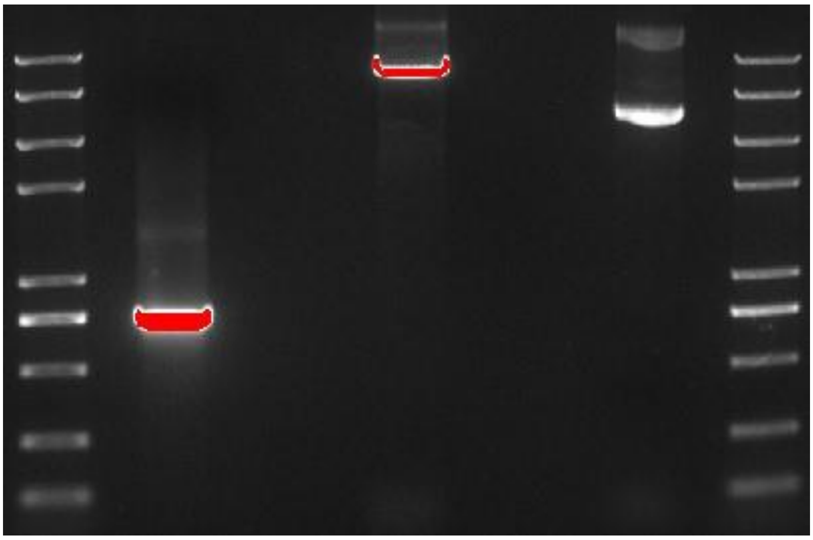


Fig 19. double enzyme digestion of

yeGFP fragment(***Not*** I ***Xba*** I),

pYES2(***Not*** I ***Xba*** I) result

5.[detect the concentration of digestion products] participator: Yang Yuanhan purpose: prepare for the calculate of ligation system process: as the handbook result:

yeGFP	34.24μl
pYES2	6.28μl

6.[ligation of yeGFP and pYES2]
participator: Yang Yuanhan

purpose: obtain yeGFP-pYES2
process:

yeGFP	1.3μl
pYES2	7.5μl
T4 ligase	1μl
dd water	8.2μl
Buffer	2μl

Put at 16℃ overnight

7.[double enzyme digestion of yeGFP fragment, GAL4 fragment and pMET3-pYES2]

participator: Zhou Hao purpose: prepare for the ligation of pMET3-yeGFP-pYES2 and pMET3-GAL4pYES2 process:

	dd water	10Xbuffer	*Not*I	*Xba*I	templet
pMET3-pYES2	26μl	4μl	2μl	2μl	3μl/6μl
GAL4	19μl	4μl	2μl	2μl	3μl/6μl
yeGFP	16μl	4μl	2μl	2μl	8μl/16μl

8.[gel electrophoresis of products] participator:

Zhou Hao, Zhao Zirui, Xu Yilong purpose: detect the result of double enzyme digestion process:
as the handbook result:

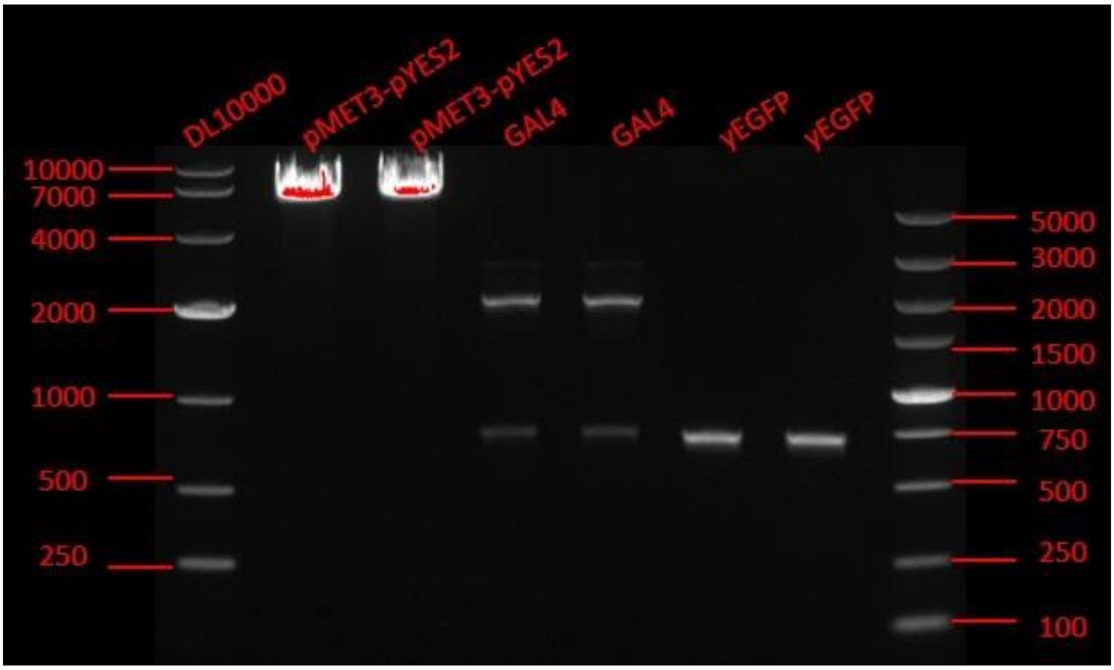


fig 20. double enzyme digestion of yeGFP fragment, GAL4 fragment and pMET3-pYES2 result

9.[gel extraction of former products] participator: Zhou Hao, Zhao Zirui purpose: obtain samples for ligation process: as the handbook result: as expected

10.[detect the concentration of digestion products] participator: Zhou Hao, Zhao Zirui purpose: detect the concentration so as to calculate the ligation system process: as the handbook result:

pMET3-pYES2	31.88ng/μl
GAL4	11.84ng/μl
yeGFP	8.26ng/μl

11.[ligation of yeGFP, GAL4 and pMET3-pYES2] participator: Zhou Hao, Zhao Zirui purpose: obtain pMET3-yeGFP-pYES2 and pMET3-GAL4-pYES2 process:

	10XT4 buffer	T4	pMET3pYES2	GAL4	dd water
GAL4 ligation	2μl	1μl	3μl	11.5μl	2.5μl
yeGFP ligation	2μl	1μl	3μl	5.5μl	8.5μl

16℃ overnight

8.9 Fri.

1.[transform yeGFP-pYES2 into trans5α and plated] participator: Ke Yitao purpose: transform yeGFP-pYES2 into trans5α so as to obtain enough plasmids by solution culture process: as the handbook, 37℃ overnight
result: no colony

2.[transform yeGFP-pMET3-pYES2 and GAL4-pMET3-pYES2 into trans5α and plated] participator: Zhou Hao purpose: transform yeGFP-pMET3-pYES2 and GAL4-pMET3-pYES2 into trans5α so as to obtain enough plasmids by solution culture process: as the handbook, 37℃ overnight
result: as expected

8.10 Sat.

1.[liquid cultivation of DH5α transformed germ clone(by yeGFP-pMET3pYES2 and GAL4-pMET3-pYES2)]

participator: Zhou Hao purpose: verify whether the transformation was succeed and try to provide more ingredients for later extraction of yeGFP-pMET3-pYES2 and GAL4-pMET3-pYES2 process: as the handbook

result: as expected

2.[transform yeGFP-pYES2 into trans5α and plated] participator: Yang Yuanhan , Xu Yilong purpose: transform yeGFP-pYES2 into trans5α so as to obtain enough plasmids by solution culture

process: as the handbook, 37℃ overnight

result:

3.[double enzyme digestion of pYES2 and yeGFP-pUC] participator: Yan Yuanhan, Ke Yitao purpose: obtain vector and yeGFP fragment process:

*Xba*I	2μl
*Not*I	2μl
pYES2	13.7μl
buffer	5μl
dd water	27.3μl

*Xba*I	2μl
*Not*I	2μl
pUC-yeGFP	38.5μl
buffer	5μl
dd water	2.5μl

4.[PCR of yeGFP, gel electrophoresis and gel extraction] participator: Yang Yuanhan, Zhao zirui, Xu Yilong purpose: obtain enough yeGFP fragments and send them to gene sequencing to verify the yeGFP process: as the process mentioned above

result:

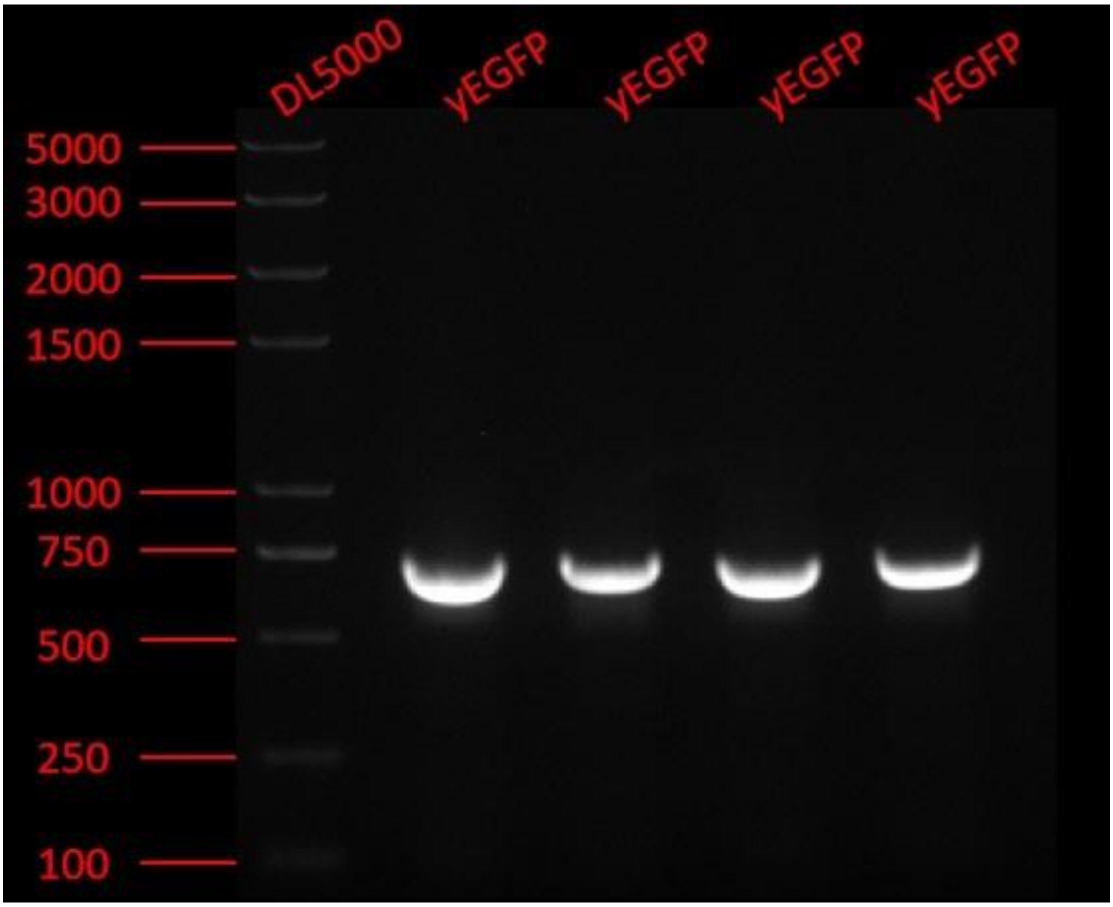


fig 21. The PCR result of yeGFP

8.11 Sun.

1.[gel electrophoresis of PCR production(yeGFP fragment), gel extraction and detecting the concentration] participator: Xu Yilong, Ke Yitao purpose: prepare for downstream experiments such as the ligation of yeGFPpYES2 process: as the handbook result: the concentration was too low to do downstream experiments, and a*not*her group of samples ' concentration was also very abnormal, we suspected that the Elution buffer was impure.

2.[double enzyme digestion and single enzyme digestion of former middle extraction plasmids and gel electrophoresis] participator: Xu Yilong purpose: detect whether there were some impurity among those pYES2 process: as the handbook

3.[extraction of yeGFP-pMET3-pYES2 and GAL4-pMET3-pYES2] participator: Zhao Zirui, Xu Yilong purpose: obtain enough plasmids for downstream experiments process: as the handbook result: as expected

4.[detect the concentration of yeGFP-pMET3-pYES2 and GAL4-pMET3pYES2 by Onedrop Spectrophotometer]
participator: Xu Yilong, Zhao Zirui purpose: detect the concentration of yeGFP-pMET3-pYES2 and GAL4pMET3-pYES2 so as to calculate the response system process: as the handbook

5.[extraction of pUC57-yeGFP] participator: Zhou Hao, Zhao Zirui purpose: obtain enough plasmids for downstream experiments process: as the handbook
result: as expected

6.[detect the concentration of pUC57-yeGFP by Onedrop Spectrophotometer] participator: Zhou Hao, Zhao Zirui purpose: detect the concentration so as to calculate the response system process: as the handbook

7.[double enzyme digestion of yeGFP-pMET3-pYES2 and GAL4-pMET3pYES2 and gel electrophoresis] participator: Xu Yilong purpose: verify the result of ligation process: result:

8.[double enzyme digestion of pMET3-pYES2 and pUC57-yeGFP] participator: Zhou Hao purpose: obtain vector and gene of interest for ligation process: result:

9.[gel electrophoresis of former products]
participator: Zhou Hao purpose: verify the result of digestion and obtain samples process: as the handbook
result:

10.[gel extraction of yeGFP fragment and pMET3-pYES2 vector]
participator: Zhou Hao, Zhao Zirui purpose: obtain samples for ligation process: as the handbook, but we used (TAE?) as a substitute for Elution buffer
result: as expected

11.[detect the concentration of products and detect which bottle of Elution buffer had impurity] participator: Zhang Chenming purpose: prepare for calculation of ligation response and detect impure Elution buffer process: as the handbook

12.[ligation of yeGFP fragment and pMET3-pYES2 vector] participator: Zhou Hao, Zhang Chenming purpose: obtain yeGFP-pMET3-pYES2 process:

10X T4 buffer	2μl
T4 ligase	1μl
pYES2-pMET3 vector	2μl
yeGFP fragment	1μl
dd water	4μl

16℃ overnight

13.[select single colony contained pUC57-yeGFP and pYES2, and solution culture of them] participator: Ke Yitao purpose: obtain abundant bacterium for plasmids extraction process: as the protocol result: as expected

8.12 Mon.

1.[transform the pMET3-yeGFP-pYES2 into DH5α and coat]

participator: Zhang Chenming, Wang Mingyao purpose: transform the yeGFP-GAL4-pYES2 into DH5α process: as the protocol [because there were only LB solid medium without ampicillin, we add some ampicillin on the medium and store some bacterial liquid for LB solid medium with ampicillin]

result: as expected

2.[gel electrophoresis of pMET3-yeGFP-pYES2] participator: Wang Mingyao purpose: verify the result of ligation process: as the handbook result: as expected

3.[extraction of pUC57-yeGFP and pYES2]

participator: Ke Yitao purpose: as the title process: as the handbook

result: the gel electrophoresis was strange

4.the same content as NO.13 in 11/8/2019

5.[double enzyme digestion of pUC57-yeGFP and pYES2]

participator: Ke Yitao purpose: obtain abundance fragments and vectors for ligation of pYES2yeGFP process:

result: as expected

6.[gel electrophoresis, gel extraction of yeGFP fragment and pYES2 vector and detect the concentration of them] participator: Ke Yitao purpose: obtain pure products process: as the handbook result: as expected

7.[ligation of pYES2-yeGFP]
participator: Ke Yitao, Xu Yilong
purpose: obtain pYES2-yeGFP
process:

8.13 Tues.
1.[select colonies of yeGFP-GAL4-pYES2 from plates and liquid cultivation] participator: Wang Mingyao purpose: obtain enough bacterium for plasmids extraction process: as the handbook result: as expected

2.[extraction of pUC57-yeGFP and pYES2, gel electrophoresis and detect the concentration] participator: Xu Yilong purpose: obtain abundance sample process: as the handbook result: as expected

3.[transform pYES2-yeGFP into DH5α and coated] participator: Yang Yunhan, Ji Jiayi purpose: obtain abundance plasmids process: as the handbook result: negative

4.[transform pYES2-yeGFP into DH5α and coated]
participator: Xu Yilong, Zhao Zirui purpose: obtain abundance plasmids process: as the handbook, we use BL21 instead of DH5α result: negative

8.14 Wed.
1.[Save glycerin bacteria and extraction of pMET3-yeGFP-pYES2]
participator: Wang Mingyao, Xu Yilong purpose: obtain abundance plasmids for double enzyme digestion verification process: as the handbook result: as expected

2.[double enzyme digestion of pMET3-yeGFP-pYES2 with *Xba*I and *Spe*I] participator: Wang Mingyao purpose: prepare for the verification of the result of ligation process: as the protocol

3.[gel electrophoresis of digestion products] participator: Xu Yilong purpose: verify the result of ligation process: as the handbook
result: positive

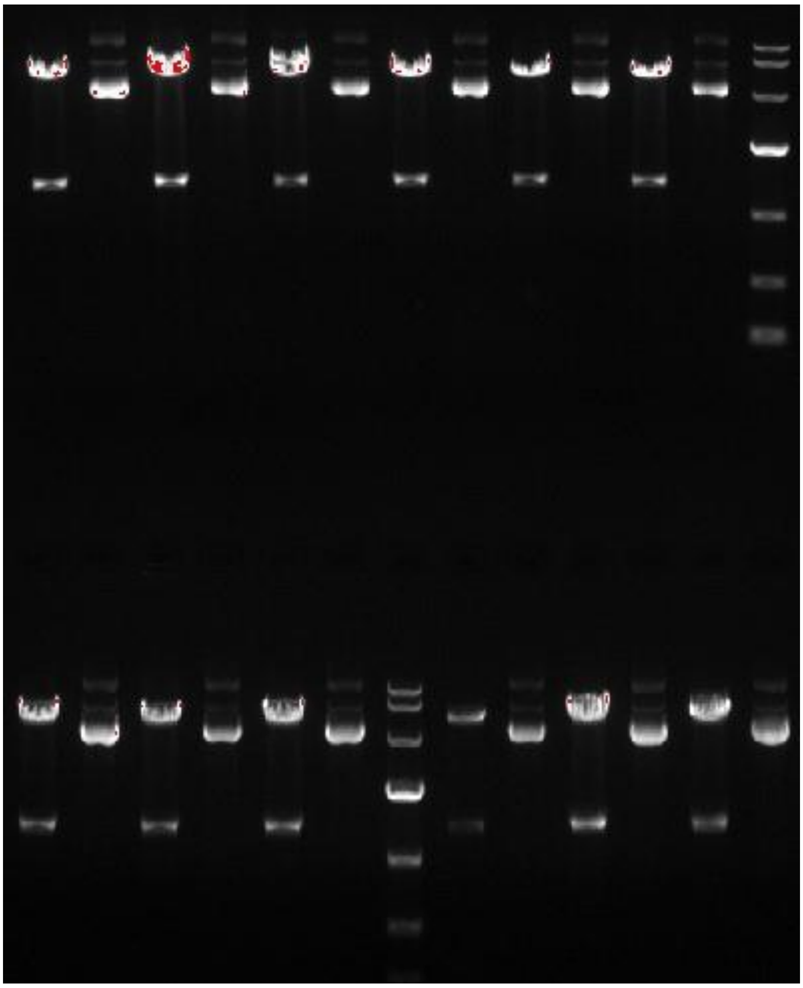


Fig 22.

4.[streaking glycerin bacterium] participator: Wang Mingyao, Xu Yilong purpose: obtain colonies process: as the protocol result: as expected

5.[double enzyme digestion of pMET3-yeGFP-pYES2 and pYES2 with *Not*I and *Xba*I] participator: Ke Yitao purpose: obtain enough yeGFP fragment process: as the protocol

6.[gel electrophoresis the products from NO.4 and NO.5 and gel extraction] participator: Ke Yitao purpose: verify result

and obtain purified fragments process: as the handbook
result: as expected

7.[ligation of yeGFP fragment and pYES2 vector] participator: Ke Yitao purpose: obtain yeGFP-pYES2 process: as the handbook

8.16 Fri.

1.In 15/8/2019 and early 16/8/2019，we do the same thing as the handbook to ligate yeGFP fragment and pYES2 vector to obtain pYES2-yeGFP, but we finally find that **the pYES2-pMET3-yeGFP obtained in 14/8/2019 was pYES2-yeGFP**.

2. [extraction plasmids of pYES2-pMET3-yeGFP ①-1 and ①-2 (they are pYES2-yeGFP actually)] participator: Luo Yaxin purpose: obtain enough pYES2-yeGFP for transformation process: as the handbook result: as expected

3.[select colony form plate of pYES2-pMET3 and liquid cultivation] participator: Wang Mingyao purpose: obtain pYES2-pMET3 process: as the handbook result: as expected

4.[transform pYES2-yeGFP to BY4741]
participator: Yang Yuanhan purpose: prepare for functional verification process: as the protocol

8.19 Mon.

1.[PCR of GAL4] participator: Luo Yaxin purpose: get enough fragments for later experiments process:

genome	3μl
primer-F	1μl
primer-R	1μl
MCLAB	12.5μl
ddH20	7.5μl

temperature	time	
98	2min	
98	10s	×30 times
60	15s	
68	30s	
68	5min	
4	forever	

2.[double enzyme digestion of GAL4]
participator: Luo Yaxin purpose: prepare for the following experiments process:

Reagents	Volume
Qc *Not* I	1μl
Qc *Xba* I	1μl
10×Qc buffer	2μl
GAL4 sample	3μl
dd water	13μl

37℃ for 40 min.

8.21 Wed.

1.[extraction of pMET3-pYES2] participator: Zhao Zirui purpose: obtain enough plasmids for downstream experiments process: as the handbook result: as expected

2.[gel extraction of GAL4 fragment and pMET3-pYES2 vector]
participator: Zhao Zirui purpose: verify the result of digestion and obtain samples, prepare for downstream experiments such as the ligation of pMET3-GAL4-pYES2 process: as the guidebook result: as expected

3.[ligation of GAL4 fragment and pMET3-pYES2 vector] participator: Yang Yuanhan, Zhao Zirui
purpose: obtain target plasmid pMET3-GAL4-pYES2
process: as the handbook

Reagents	Volume
pMET3-pYES2	1.2μl
GAL4	1.9μl
T4 ligase	1μl
10× Buffer	2μl
dd water	13.9μl

result: as expected

8.22 Thur.

1.[transform the pMET3-yeGFP-pYES2 into DH5α and coat] participator: Yang Yuanhan, Liu Yanmu, Zhao Zirui purpose: obtain more plasmid for downstream experiments process: as the protocol result: as expected

2.[extraction of pYES2-yeGFP from BY4741] participator: Yang Yuanhan, Luo Yaxin purpose: vivificate whether there is the recombined plasmid in the BY4741.
process: as the protocol

8.23 Fri.

1.[gel extraction of GAL4 fragment] participator: Zhao Zirui, Liu Yanmu purpose: verify the result of PCR and obtain samples, prepare for downstream experiments process: as the handbook result:

8.24 Sat.

1.[extraction of pMET3-yeGFP-pYES2]
participator: Zhao Zirui, Li Xinrui purpose: obtain enough plasmids for downstream experiments process: as the handbook result: as expected

2.[gel extraction of GAL4 fragment and pMET3-pYES2 vector]
participator: Zhao Zirui purpose: verify the result of digestion and obtain samples, prepare for downstream experiments such as the ligation of pMET3-GAL4-pYES2 process: as the handbook result: as expected

8.26 Mon.

1.[double-enzyme verification of pYES2-pMET3-GAL4] participator: Luo Yaxin purpose: verify the recombined plasmid process:

plasmid	10μl
10×buffer	2μl
NdeI	1μl
*Xba*I	1μl
dd water	6μl

37℃, 30min.

8.27 Tues.

1.[gradient and colony PCR for yeGFP]

Participator: Chen Xi, Luo Yaxin process:

as the protocol

Prepare for the following system: 8 tubes

	10μl for each tube
template DNA	0.4μl
primer F	0.4μl
primer R	0.4μl
2x T5 Super Mix	5μl
ddH20	3.8μl

Run gradient PCR as followed and perform Agarose gel electrophoresis on the product:

Pre degeneration	98℃	3min	
degeneration	98℃	10s	
annealing	45.6–55.6℃	10s	
extend	72℃	10s	30 cycle
final extend	72℃	2min	
final hold	4℃	∞	

Run the colonies PCR reaction as follows and perform Agarose gel electrophoresis on the product:

	10μl for each tube
template DNA	0.4μl
primer F	0.4μl
primer R	0.4μl
2x T5 Super Mix	5μl
ddH20	3.8μl

Pre degeneration	98℃	3min	
degeneration	98℃	10s	
annealing		10s	
extend	72℃	10s	30 cycle
final extend	72℃	2min	
final hold	4℃	∞	

results:

8.29 Thur.

1.[Gradient PCR of pYES2-yeGFP]

participator: Wang Mingyao purpose: verify the optimum temperature of oligoes process:

PrimeSTAR Max premix(2X)	5μl
P(PMGGY)-F	1μl
P(PMGGY)-R	1μl
template	0.4μl
dd water	2.6μl

65℃-75℃

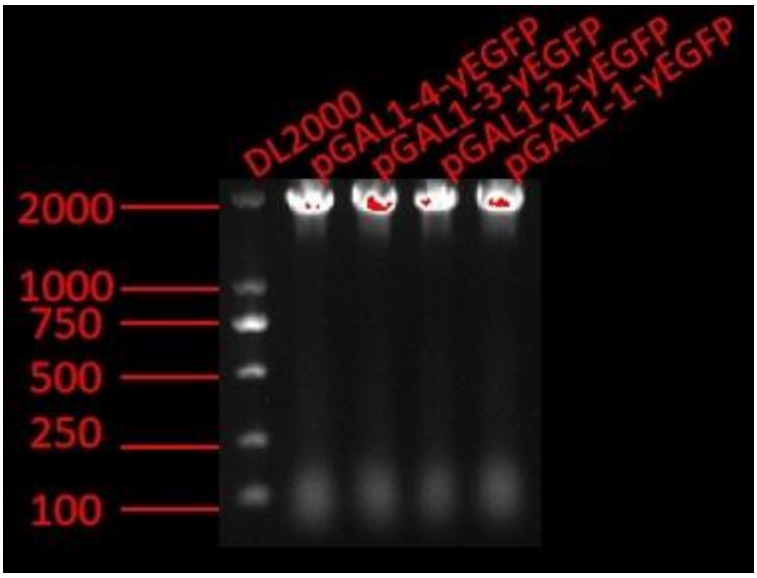


Fig 23. Gradient PCR of pYES2-yeGFP result

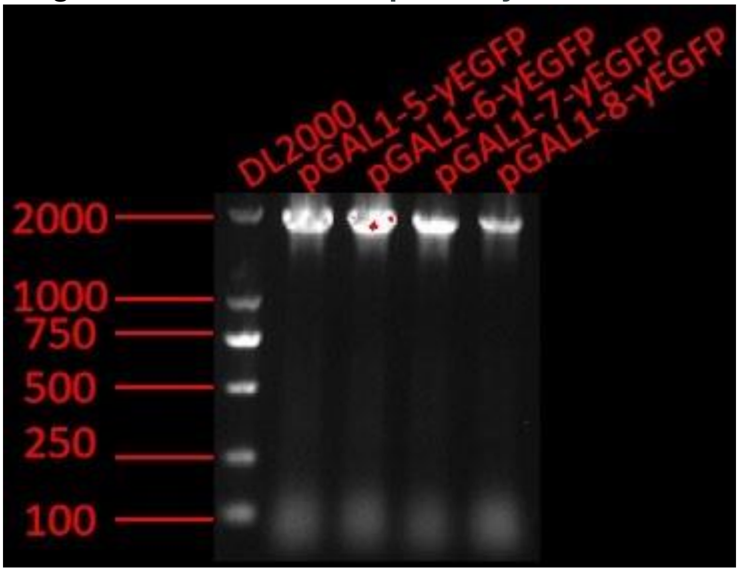


Fig 24. Gradient PCR of pGAL1-yeGFP result

result: the best temperature is 65℃

8.30 Fri.

1.[double-enzyme reaction of pYES2-pMET3，yeGFP and GAL4]

participator: Zhou Hao

purpose: as the title

process:

	10X loading buffer	*EcoR*I	*Xba*I	sample	dd water
pYES2-pMET3	2μl	1μl	1μl	5μl	11μl

yeGFP fragment	2μl	1μl	1μl	10μl	6μl
GAL4 fragment	2μl	1μl	1μl	10μl	11μl

result: as expected

2.[ligation of pYES2-pMET3 with yeGFP] participator: Zhou Hao purpose: as the title process:

Reagents	Volume
pMET3-pYES2	3μl
yeGFP	10μl
T4 ligase	1μl
10× Buffer	2μl
dd water	4μl

result: as expected

3.[ligation of pYES2-pMET3 with GAL4] participator: Zhou Hao purpose: as the title process:

Reagents	Volume
pMET3-pYES2	3μl
GAL4	5μl
T4 ligase	1μl
10× Buffer	2μl
dd water	9μl

result: as expected

8.31 Sat.

1.[double enzyme digestion to verify ligation of pYES2-pMET3-yeGFP and pYES2-pMET3-GAL4] participator: Zhou hao purpose: verify the results of pYES2-pMET3-yeGFP/pYES2-pMET3-GAL4 connection before sequencing process:

	10X loading buffer	*Spe*I	*Hin*d Ⅲ	*Not*I	*Xba*I	sample	dd water

pYES2pMET3yeGFP	2μl	1μl	1μl	\	\	6.5μl	10.5μl

	10X loading buffer	*Spe*I	*Hin*d Ⅲ	*Not*I	*Xba*I	sample	dd water
pYES2pMET3GAL4	2μl	/	/	1μl	1μl	6.5μl	10.5μl

The image below shows the result as"Trans2K Plus2 pYES2-pmet3-yeGFP(1, 2, 3, 5)cut pYES2-pmet3-yeGFP(1, 2, 5) pYES2-pmet3 pYES2-pmet3-gal4 pYES2-pmet3-gal4cut Trans2K Plus2"（from the left side to the right side） And we can see that the GAL4 fragment in the second panel counting from the right side.

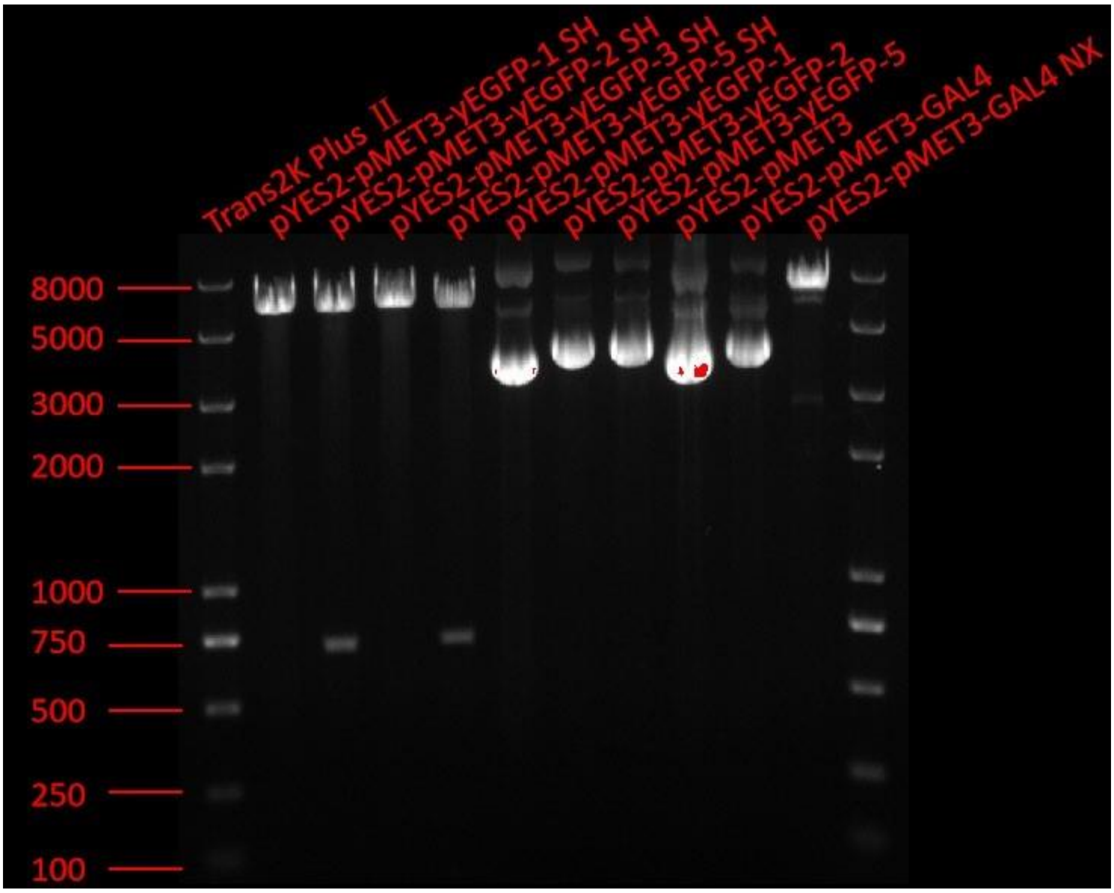


Fig 25. Double enzyme digestion to

verification recombined plasmid

37℃ reaction for 1h and 60℃ inactivation for 15min
result: as expected

2.[transformation of pYES2-yeGFP] participator: Zhou Hao purpose: transform the recombined plasmid into DH5a process: as the protocol

9.1 Sun.
1.[liquid cultivation of DH5α transformed germ clone (by yeGFP-pMET3pYES2 and GAL4-pMET3-pYES2)] participator: Wang Mingyao
purpose: verify whether the transformation was successes and try to provide more ingredients for later extraction of pYES2-pGAL1-yeGFP/pYES2-pMET3GAL4
process: as the handbook
result: as expected

9.2 Mon.
1.[pYES2-pGAL1-yeGFP Gradients PCR] participator: Wang Mingyao
purpose: confirm the best temperature to obtain the ″pGAL1-yeGFP″ fragment from pYES2-pGAL1-yeGFP process:

PrimeSTAR Max permix(2X)	5μl
P(PMGGY)-F	1μl
P(PMGGY)-R	1μl
template	0.4μl
dd water	2.6

60℃-65℃

result: confirm that the best PCR annealing temperature is 63.3℃

9.3 Tues.

1.[yeast chemical transformation] participator: Zhou Hao purpose: transform plasmids into yeast germ while performing active status process: as the protocol from Lin Wei (seen in the page of protocols) result: as expected

9.4 Wed.

1.[extraction of GAL4-pMET3-pYES2] participator: Wang Mingyao purpose: obtain enough plasmids for sequencing and later connection reaction process: as the handbook result: as expected

HPLC

10.11 Fri

Sample: adenosine CNS3 COR PTN

Process: prepare supernatant fraction samples and analyzed by HPLC

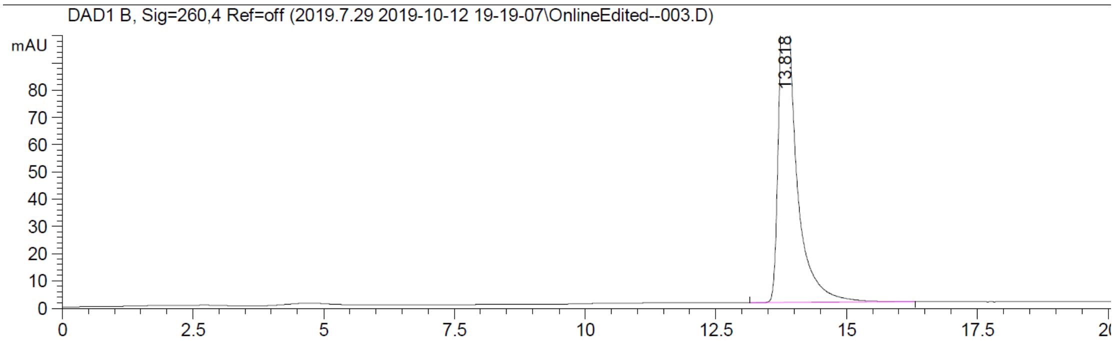
Result: Buffer was too polar to make the samples' peak separate. Try to another buffer that was used for nucleoside.

10.12 Sat

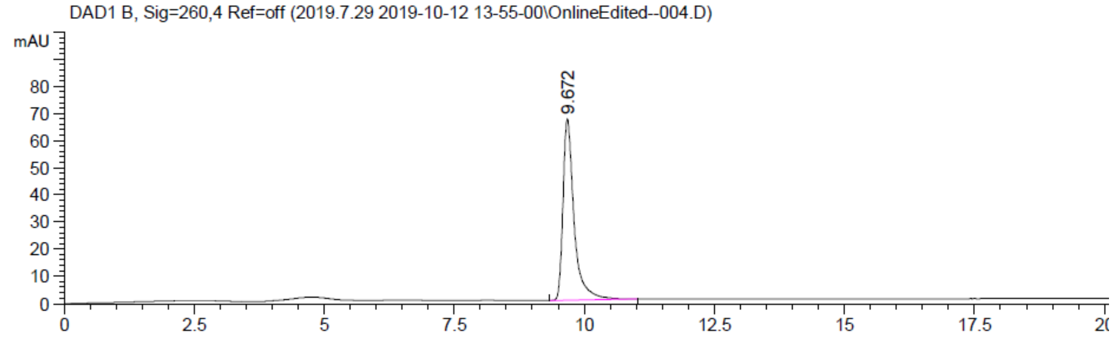
Sample: adenosine CNS3 COR PTN BY MF YH

Process: prepare supernatant fraction samples and analyzed by HPLC

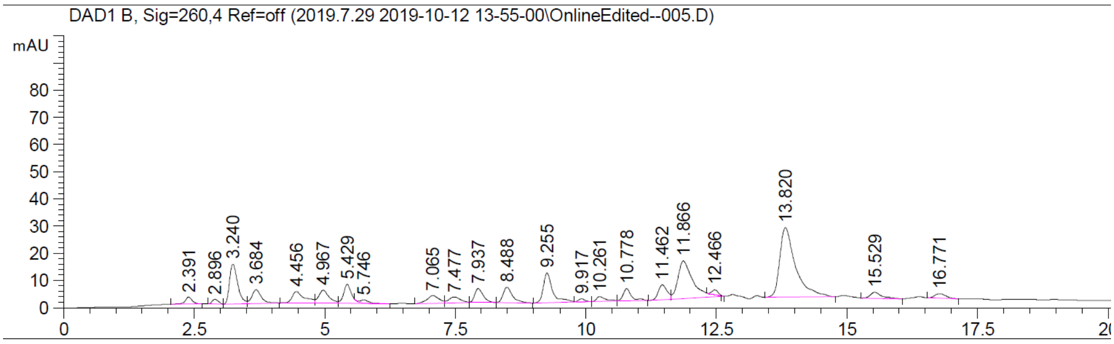
Result:



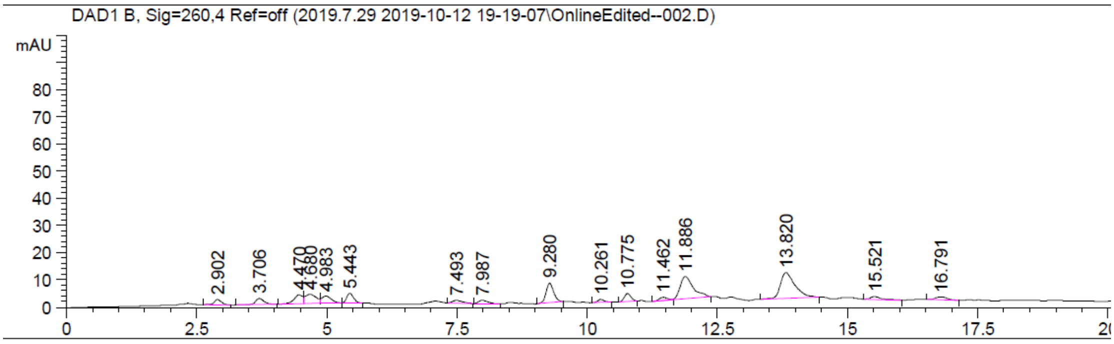
ADN



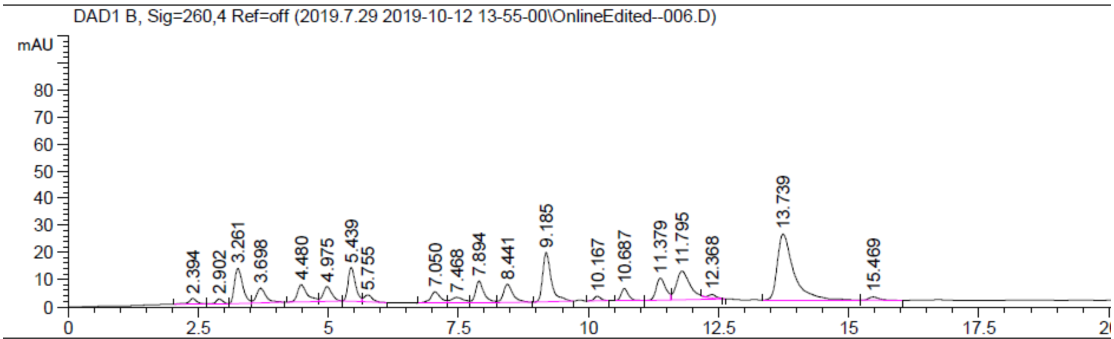
PTN



BY



CNS3



YH

There is no PTN or COR found in the sample

10.14 Mon

Sample: PTN BY YH ONLY

Process: prepare supernatant fraction samples and analyzed by HPLC

Result:

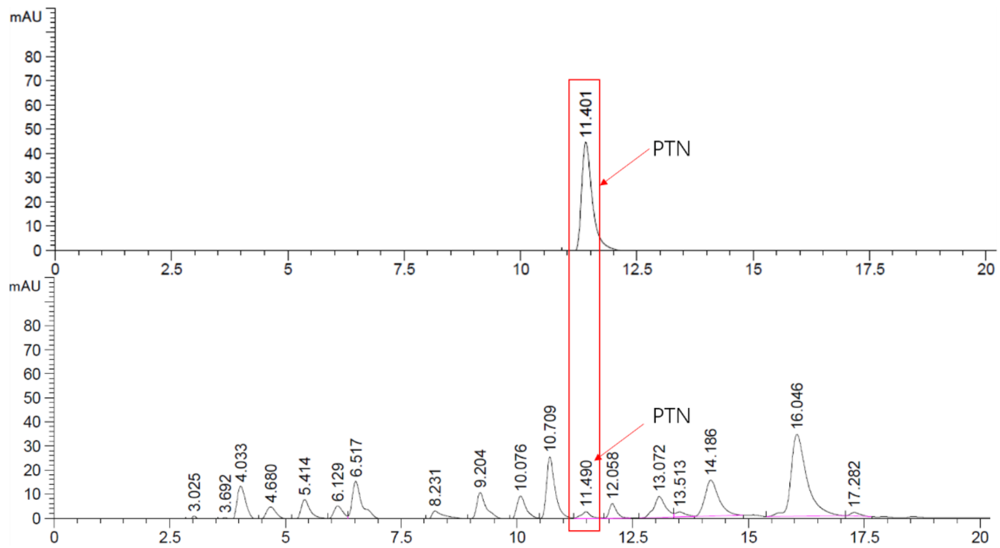


Figure 1 HPLC analysis of PTN

Top: Standard sample of 5mM tryptamine.
Bottom: Supernatant fraction of Cns3-hisG YH sample. The strain BY4741 was culturing in YPDG media for 3 days

PTN was found in the sample Cns3-hisG YH strain