9.18

Restricted enzyme digestion of pet28a backbone

Take 1 pcr tube ,add ragents under 0celsius

Plasmid	2ul
10X buffer	5ul
NdeI	1ul
NcoI	1ul
ddH2O	upto50ul

Make 0.1% agarose electrophoresis gel

30ml TAE buffer

0.3g agarose

1ul yeagreen DNA stain

Result:no target band spotted.

9.19

Restricted enzyme digestion of CYC1-B4GalT1

Take 1 pcr tube ,add ragents under 0 celsius

Plasmid	2ul
10X buffer	5ul
BamHI	1ul
XhoI	1ul
ddH2O	upto50ul

Make 0.1% agarose electrophoresis gel

30ml TAE buffer

0.3g agarose

1ul yeagreen DNA stain

Result: target band is very faint

9.20

Restricted enzyme digestion of pGADT7 and pGBKT7backbone

Take 1 pcr tube ,add ragents under 0 celsius

Plasmid	2ul	
10X buffer	5ul	
BamHI	1ul	EcoRI
XhoI	1ul	BamHI
ddH2O	upto50ul	

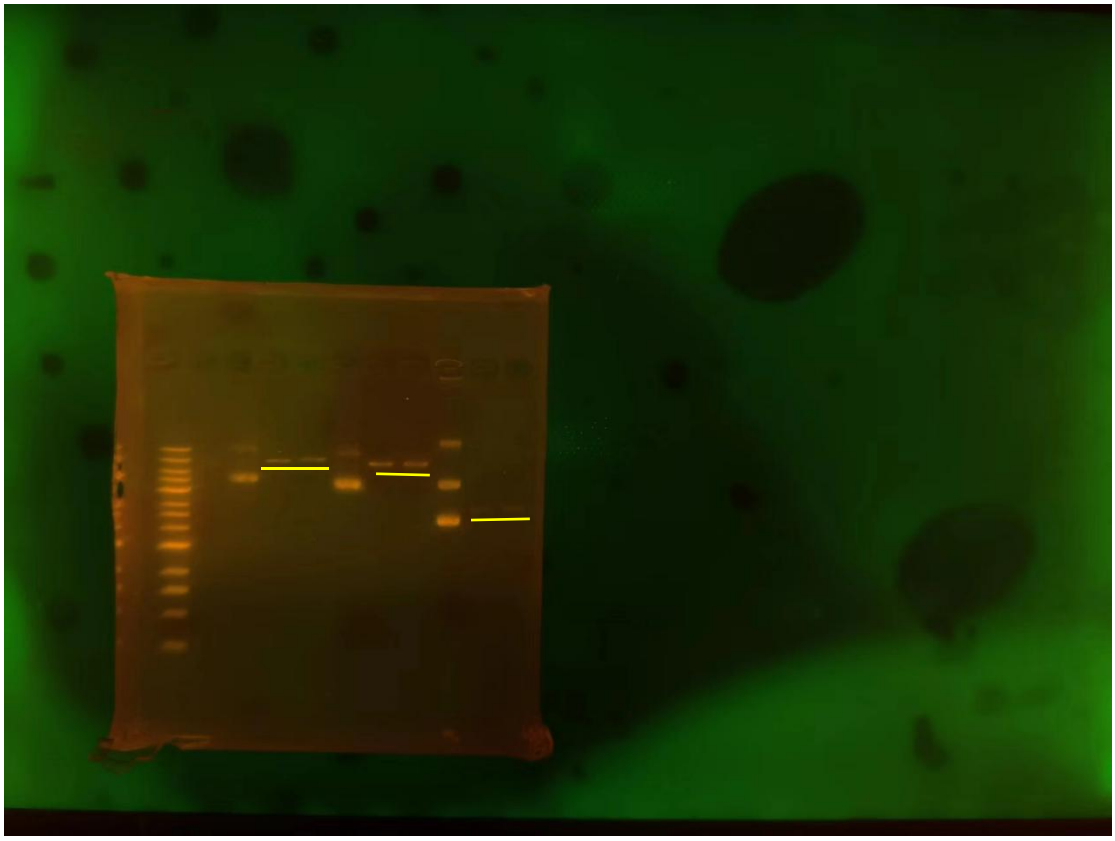
Make 0.1% agarose electrophoresis gel

30ml TAE buffer

0.3g agarose

1ul yeagreen DNA stain

Result: the target band was clear.



9.21

Extract digested pGADT7 and pGBKT7backbone and CYC1-B4GalT1

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. However, it is strongly recommended that fresh TAE buffer or TBE buffer be used as running buffer. Do not reuse running buffer as its pH will increase and reduce yields.
2. When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a wide, clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose.
3. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 mL microcentrifuge tube.
4. Add 1 volume XP2 Binding Buffer.
5. Incubate at 50-60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes. If the size of the gel block is large or the concentration of the gel is high, the incubation time can be appropriately extended until the gel is completely melted.
6. Insert a HiBind® DNA Mini Column in a 2 mL Collection Tube.

7. Add no more than 700 µL DNA/agarose solution to the HiBind® DNA Mini Column. Centrifuge at 10,000 × g for 1 minute at room temperature, and discard the filtrate. Then place the column back into the collection tube.
8. If the volume of the DNA/agarose solution exceeds 700 µL, repeat Steps 7-9 until all of the sample has been transferred to the column.
9. Discard the filtrate and insert the HiBind® DNA Mini Column back to the collection tube. Add 300 µL XP2 Binding Buffer into the binding column. Centrifuge at maximum speed (≥13,000 × g) for 1 minute at room temperature and discard the filtrate.
10. Insert the HiBind® DNA Mini Column back to the collection tube. Add 700 µL SPW Buffer (the buffer must be properly diluted with 100% ethanol) into the binding column. Centrifuge at 10,000 × g for 1 minute at room temperature and discard the filtrate.
11. Insert the HiBind® DNA Mini Column back to the collection tube. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.
12. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube. Add 15-30 µL Elution Buffer or deionized water directly to the center of the column membrane. Let sit at room temperature for 2 minutes. Then centrifuge at 13,000 × g for 1 minute in order to elute DNA.

9.22

PCR amplify CYC1-LALBA,FIG1-GalE,PpltR-lacO-Bvn1-Ma, PampC-lacY, 2samples each

Add reagents under 0 celcius:

2Xbuffer	12.5ul
dNTPs	0.5ul
Template	0.25ul
Enzyme P505	0.5ul
primerF	1ul
primerR	1ul
DDH2O	up to 25

Pcr program : 95C 5min

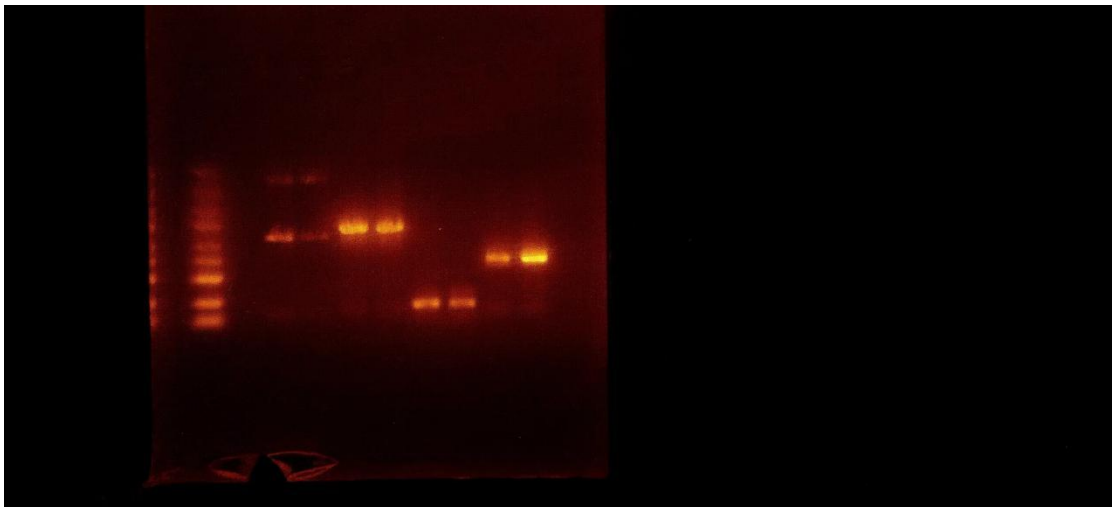
Repeat30*:

95C	60sec
54C	30sec
72C	90sec

72C 5min

4C wait

Perform a 0.1% electrophoresis



9.23

Reamplify FIG1-GalE,PampC-lacY,,PpltR-lacO-Bvn1-Ma,CYC1-LALBA,CYC1-B4GalT1, 2samples each

Add reagents under 0 celcius:

2Xbuffer	12.5ul
dNTPs	0.5ul
Template	0.25ul
Enzyme P505	0.5ul
primerF	1ul
primerR	1ul
DDH2O	up to 25

Pcr program : 95C 5min

Repeat30*:

95C 60sec

57C 30sec

-0.1C per turn

72C 90sec

72C 5min

4C wait

9.24

Extract PCR productions.

Use 40ul of elution buffer ,20ul for 2 times.

The samples of 10 samples are

18.12,4.77,54.8234.31,26.04,25.14,27.15,55.82,7.45,5.92

9.25

Overlapping PCR to synthesizePampC-lacY-PpltR-lacO-Bvn1-Ma andFIG1-GalE-CYC1-LALBA,two samples each

Add reagents under 0 celcius:

2Xbuffer	25ul
dNTPs	1ul
Template	0.5ng
Enzyme P505	1ul
DDH2O	up to 47

Pcr program : 95C 5min

Repeat15*:

95C	60sec
54C	30sec
72C	90sec

72C 5min

4C wait

Then add

PrimerF	2ul
PrimerR	2ul

95C 5min

Repeat30*:

95C	60sec
69C	30sec
-0.5C per turn	
72C	90sec

72C 5min

4C wait

Result: PampC-lacY-PpltR-lacO-Bvn1-Mahas accurateband

FIG1-GalE-CYC1-LALBA has 4 bands ,the correct band is faint.

9.26

Gel extraction from D8 production.

Result:Concentraation is very low

9.27
reamplifyFIG1-GalE-CYC1-LALBA

Add reagents under 0 celcius:

2Xbuffer	12.5ul
dNTPs	0.5ul
Template	0.25ul
Enzyme P505	0.5ul
primerF	1ul
primerR	1ul
DDH2O	up to 25

95C 5min

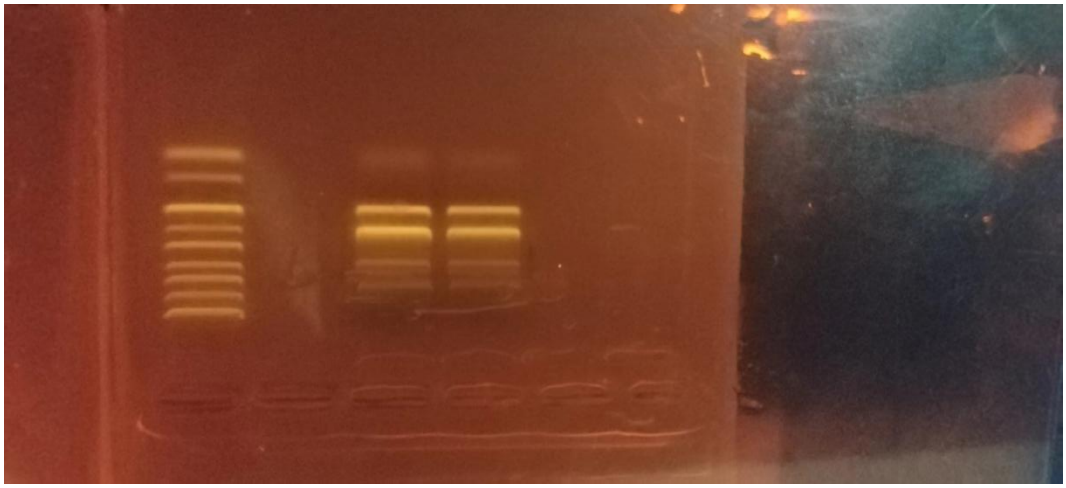
Repeat25*:

	95C	60sec
	54C	30sec
	72C	90sec

72C 5min

4C wait

Run a gel electrophoresis



9.28
Gel extraction from 9.27 production

Mix the 2 bands together

The final concentration are 8.37