WEEK 1

3.31 Jiaqi Zhang

pYB1a-PobR constructing experiment

E.coli T1 with pYB1a-GFP was inoculated and plasmid mini kit was used to extract the plasmid pYB1a-GFP. The vector and *pobR* gene were amplified used specific primers. The PCR system and procedure are showed below.

pYB1a-GFP：1uL	pobR：1uL	98℃	3min	
2*High Fidelity Master：25uL	pobR-Gibson-F ：1uL	98℃	30s	
pYB1a-Gibson-F：1uL	pobR-Gobson-R ：1uL	60℃	30s	x30
pYB1a-Gibson-R：1uL	2*High Fidelity Master：25uL	72℃	2min	
ddw：22uL	ddw：22uL	72℃	5min	

Figure 1 The PCR system and procedure of pYB1a-GFP vector and gene *pobR*

The amplified vectors were digested by DpnI and verified by electrophoresis. The amplified gene *pobR* was verified by electrophoresis as well.

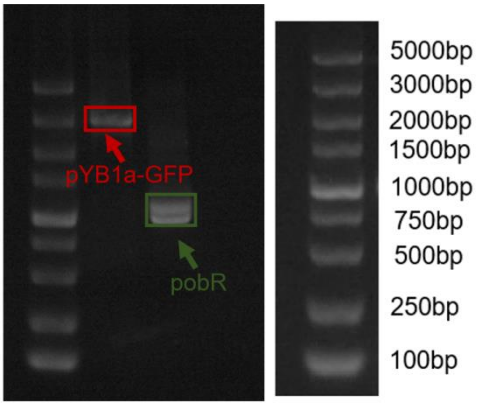

Figure 2 The electrophoresis pictures of pYB1a-GFP vector and gene *pobR*

The fragments of pYB1a-GFP vector and gene *pobR* were linked by the method of Gibson. After adding substrates, the mixture was inoculated under 30℃ for 30 minutes. The system is showed below.

pYB1a-GFP：1uL

pobR：1uL

Exnase multis：1uL

5x CE Multis Buffer：2uL

ddW:5uL

Figure 3 The system of Gibson ligation of pYB1a-GFP vector and gene *pobR*

After ligation, the plasmid was transferred into T1 receptive which plate coating were performed, and colonies were selected and inserted into LB liquid medium.

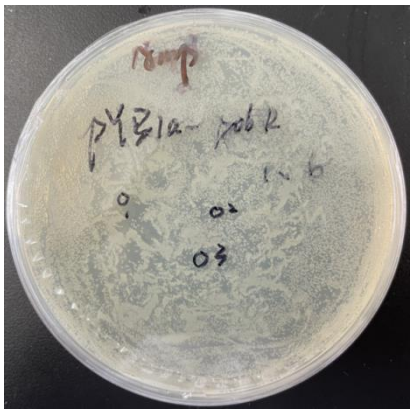

Figure 4 The picture of solid medium of recombinant T1 / pYB1a-PobR-GFP

4.1 Zishu Yang

pYB1a-PobR-eGFP-Chl constructing experiment

The *Chl* gene was amplified by using specific primers. The PCR system and procedure are showed below.

Chl-F：1uL	98℃	2min	
Chl-R：1uL	98℃	30s	x30
2*High Fidelity Master：25uL	55℃	30s	
dd水：22uL	72℃	30s	
pSB1c：1uL	72℃	3min	

Figure 5 The PCR system and procedure of *Chl* gene

The amplified fragments were purified and concentration was 53 ng/μL.

XhoI、BglⅡ double enzymes were used to digest pYB1a-PobR-eGFP plasmid in order to gain desired vector. The following is the system of digestion system.

Bgl Ⅱ： 1 uL

Xho Ⅰ:1 uL

10x Green Buffer：5 uL

pYB1a-pobR-eGFP：30 uL

ddW：13 uL

Figure 6 The XhoI、BglⅡ double enzyme digestion system of pYB1a-PobR-eGFP plasmid

After digestion under 37℃ for 2 h, digested pYB1a-PobR-eGFP was verified by electrophoresis which was showed below.

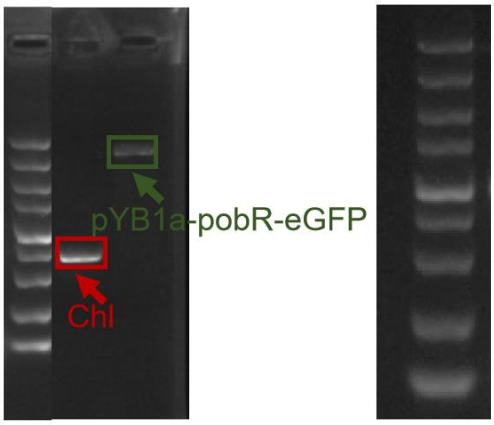

Figure 7 The electrophoresis pictures of digested pYB1a-PobR-eGFP

The fragments of pYB1a-PobR-eGFP vector and gene C*hl* were linked by T4 ligase. After adding substrates, the mixture was inoculated under 16℃ for 2 hours. The system is showed below.

Chl：4uL

pYB1a-pobR：4uL

T4 ligase：1uL

T4 ligase Buffer：1uL

Figure 8 The The system of T4 ligation of pYB1a-PobR-eGFP vector and gene *Chl*

4.2 Linshan Cao

pYB1a-PobR-eGFP-Chl constructing experiment

The growth of the plates was good, and single colonies were selected from each plate for colony PCR.

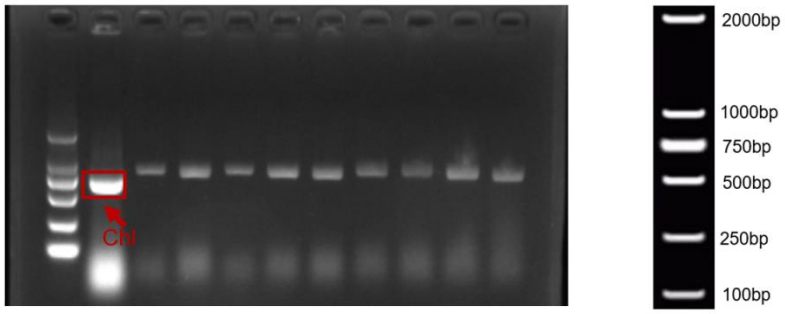

Figure 9 Colony PCR result

Lane 1 product was transferred into the liquid LB, 12 h after using plasmid mini kit, extracted plasmid was verified by using enzyme digestion.

Bgl：0.5uL

Xho：0.5uL

10xBuffer：1uL

ddW：2uL

Figure 10 Enzyme digestion system

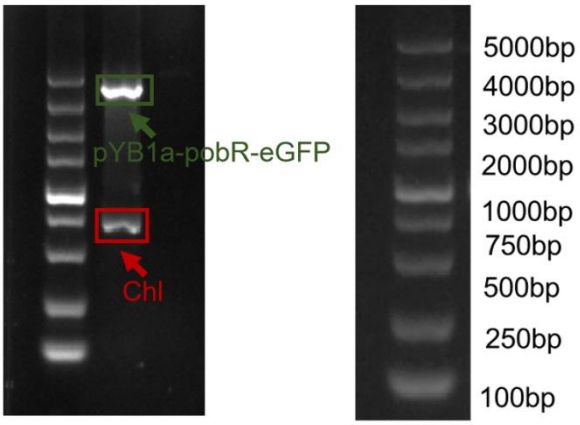

Figure 11 Electrophoresis results of enzyme digestion verification

The result showed the lane 2 product was the correct.

4.6 Zhihao Wang

Preliminary experiments of chloramphenicol screening

Our screening system: 200 μL LB + 1% bacteria solution (T1/pYB1a-pobR-eGFP-Cmr) + 10% 0.6 g/L 4HB + 0.1% ampicillin (50 mg/mL) + 0.1% chloramphenicol in different concentrations

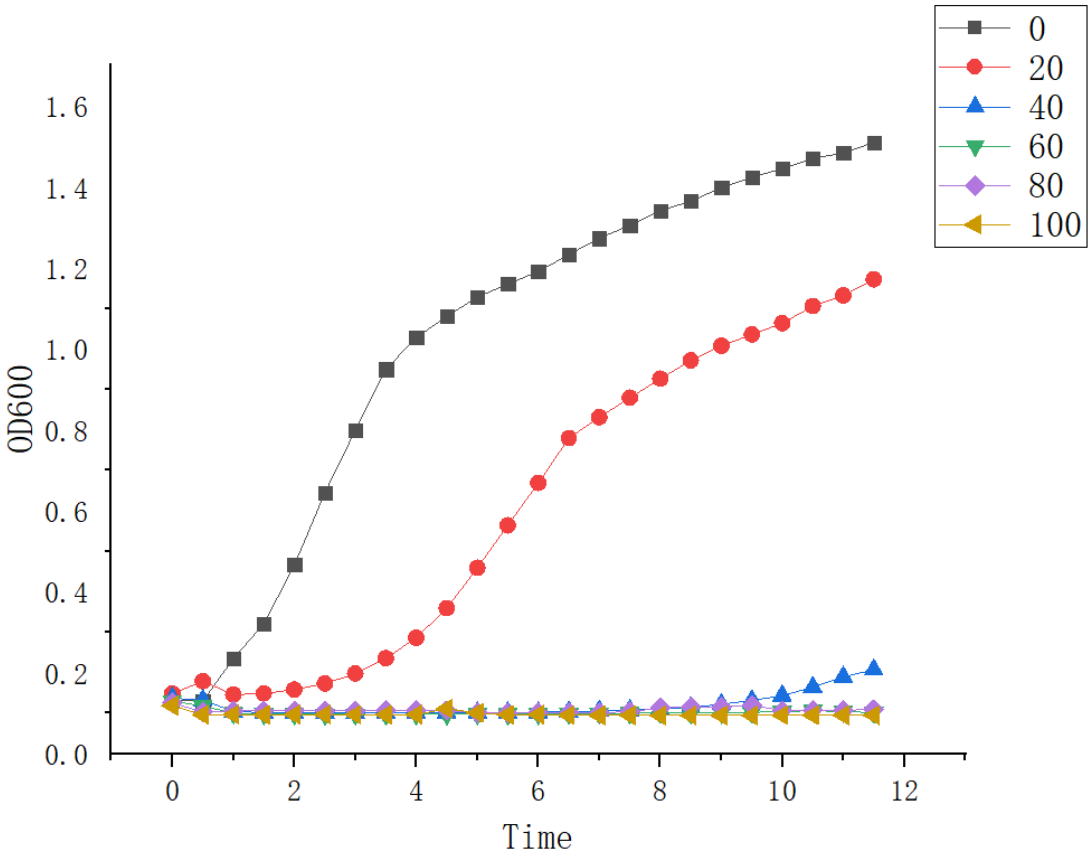

Figure 1 0.6 g/L 4HB different concentrations of chloramphenicol

The chloramphenicol concentration of 20 mg/mL made bacteria (T1/pYB1a-pobR-eGFP-Cmr) survive with 0.6 g/L 4HB's induction, so we chose this chloramphenicol concentration for a follow-up experiment.