WEEK 25

9.15 Linshan Cao

pYB1a-PobR^{mut}(F5-B7)-eGFP-SacB constructing experiment

This week, we firstly carried out plasmid preservation and improvement for pYB1a-PobR^{mut}(F5-B7)-eGFP-SacB strains 1-3, 1-4 and 3-5 with correct colony PCR results. Finally, we carried out XhoI and SpeI double digestion verification. After correct results, we carried out preliminary experiment of sucrose gradient exploration.

Reaction system (10μL×3)		Reaction procedure	
PYB1a-PobR^{mut}-eGFP-SacB 1-3/1-4/3-5	2.2/3.5/2.5μL (0.2μg)	37℃	2h
XhoI	0.2μL		
SpeI	0.2μL		
rCutsmart	1μL		
DDW	6.4/5.1/6.1μL		

Figure 1 Double enzymes digestion verification

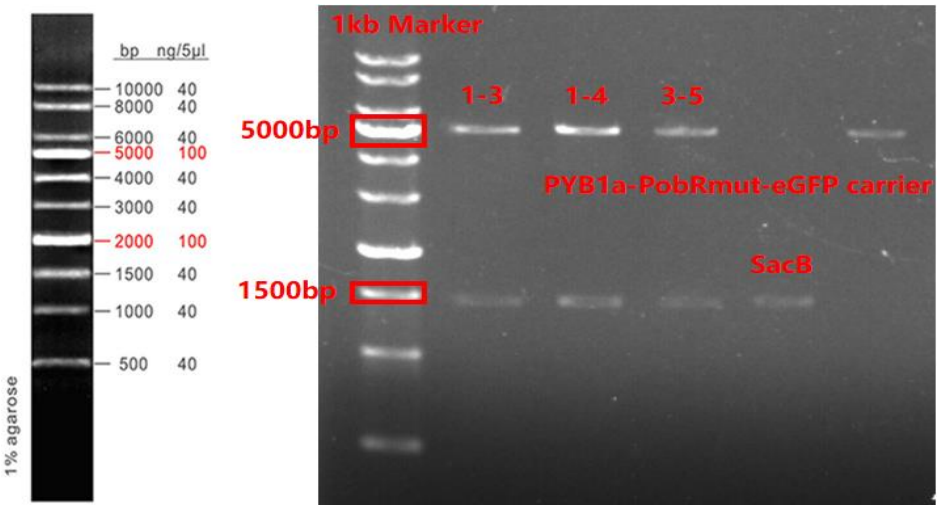


Figure 2 Electrophoresis results

The enzyme digestion was correct (1-3/1-4/3-5), and the carrier and fragment bands were correct.

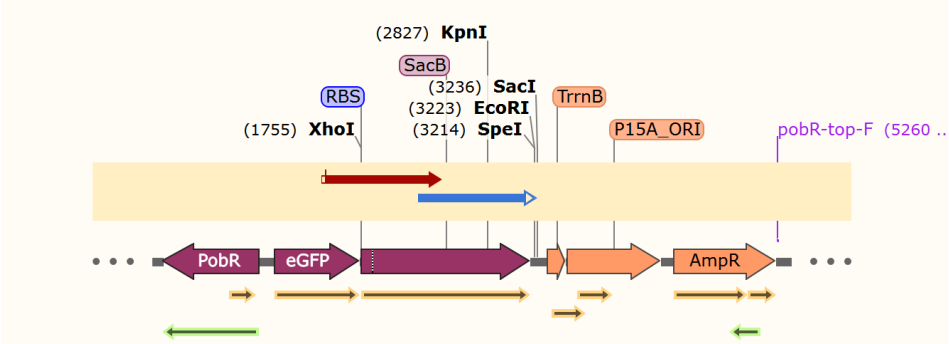


Figure 3 pYB1a-PobR^{mut}(F5-B7)-eGFP-SacB sequencing results

Objective Strains 1-3, 1-4, 3-5 were sequenced and the results were correct.

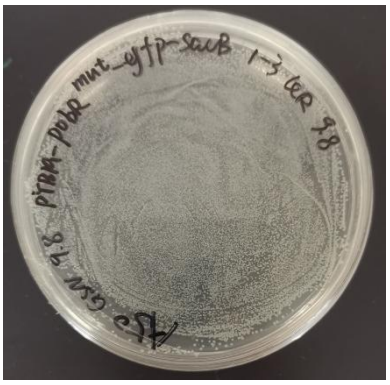


Figure 4 pYB1a-PobR^{mut}(F5-B7)-eGFP-SacB (1-3) (Δ tyrB/BL21chemical transformation receptive state)

The strains grew well. The strains were picked and shaken in tube LB and incubated overnight at 37℃ for 12 h.

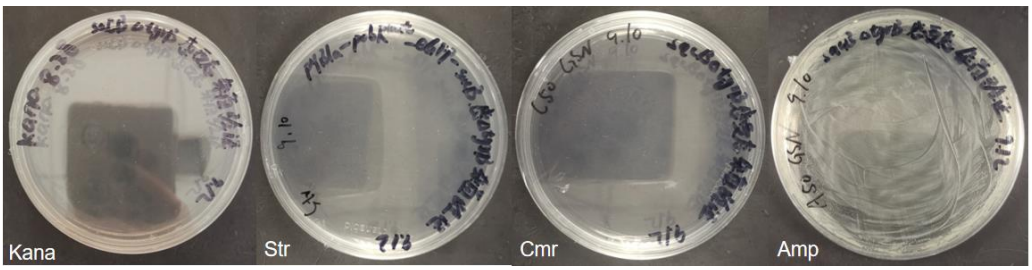


Figure 5 pYB1a-PobR^{mut}-eGFP-SacB bacterial validation (*Δ tyrB*/BL21chemical transformation receptive state)

The results were correct.

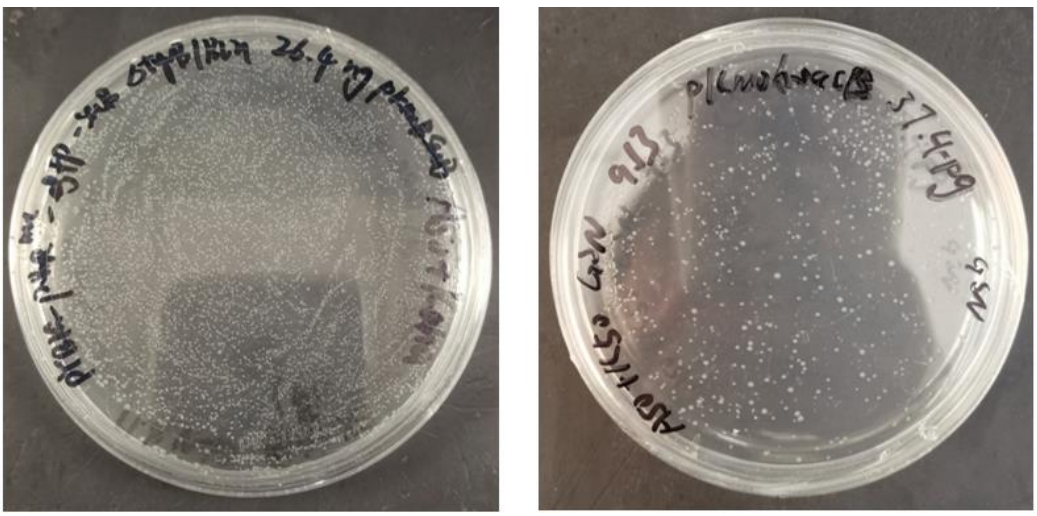


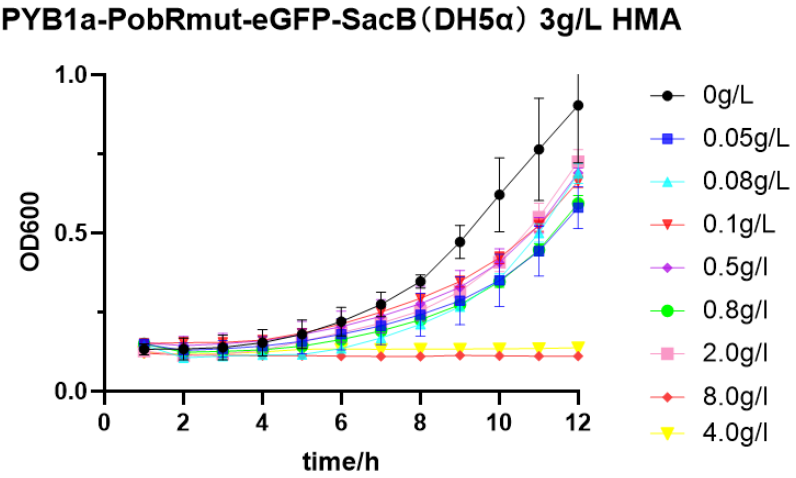
Figure 6 pYB1a-PobR^{mut}-eGFP-SacB efficiency verification (*Δ tyrB*/BL21chemical transformation receptive state)

In efficiency verification, 37.4 pg PK18MOB-SACB was transferred to complete coating, and the experienced efficiency was verified to be 1.07×10^{7}.

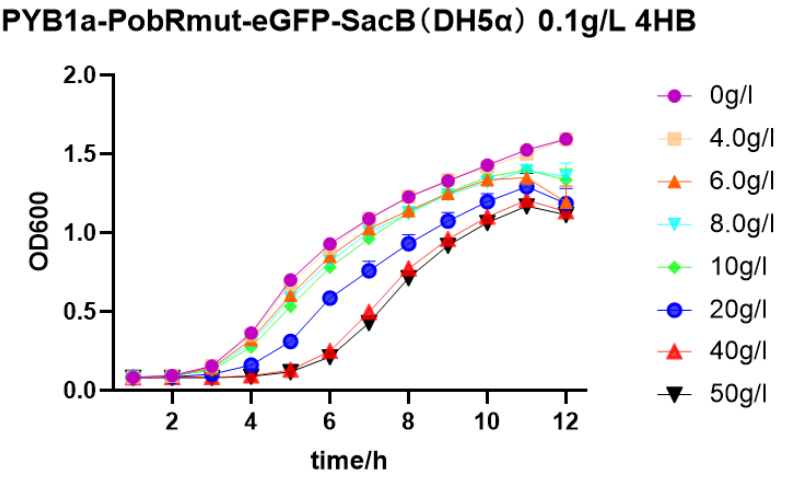
9.17 Jiameng Nie

pYB1a-PobR^{mut}(F5-B7)-eGFP-SacB sucrose gradient pretest

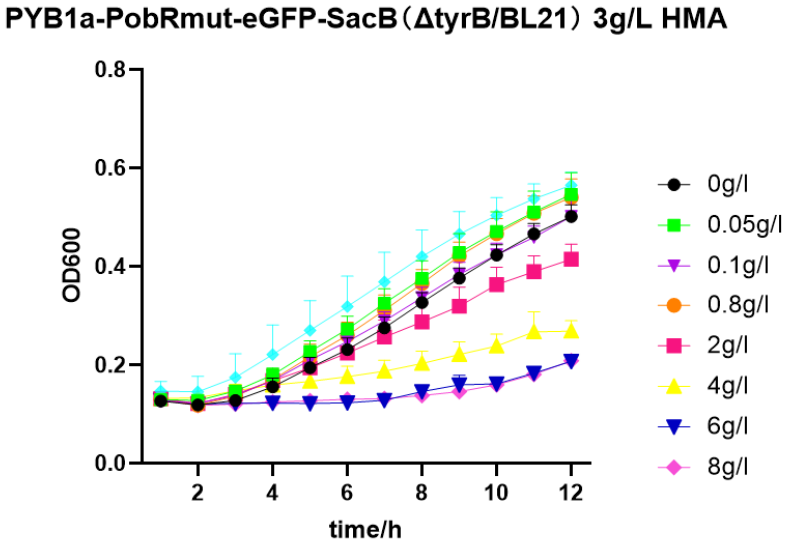
We explored the lethal sucrose concentration of pYB1a-PobR^{mut}(F5-B7)-eGFP-SacB under HMA or 4HB.



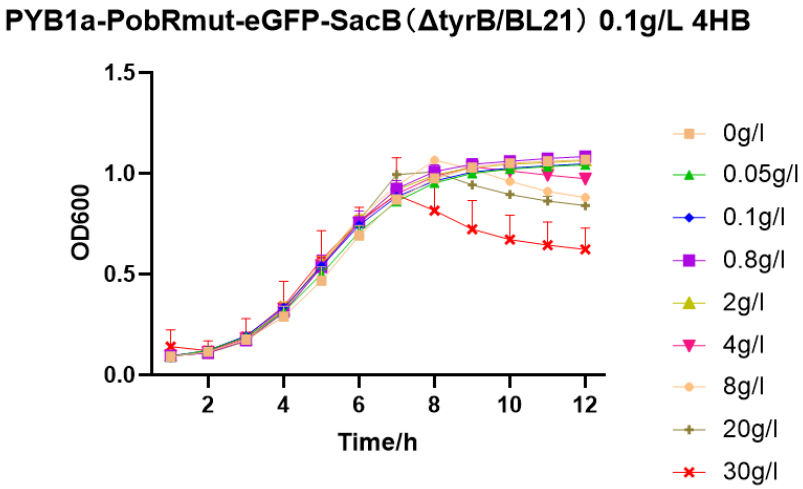
(1) pYB1a-PobR^{mut}-eGFP-SacB (DH5α) sucrose gradient pre-experiment was conducted under at 3 g/L HMA (sucrose concentration was 0, 0.05, 0.08, 0.1, 0.5, 0.8, 2, 4, 6, 8, 10, 20, 30, 40, 50 g/L in sequence)



(2) pYB1a-PobR^{mut}-eGFP-SacB (DH5α) sucrose gradient pre-experiment was conducted under at 0.1 g/L 4HB (sucrose concentration was 0, 0.05, 0.08, 0.1, 0.5, 0.8, 2, 4, 6, 8, 10, 20, 30, 40, 50 g/L, respectively)



(3) pYB1a-PobR^{mut}-eGFP-SacB (Δ*tyrB*/BL21) sucrose gradient pre-experiment was conducted under at 3 g/L HMA (sucrose concentration was 0, 0.05, 0.08, 0.1, 0.5, 0.8, 2, 4, 6, 8, 10, 20, 30, 40, 50 g/L)



(4) pYB1a-PobR^{mut}-eGFP-SacB (Δ*tyrB*/BL21)sucrose gradient pre-experiment was conducted under at 0.1 g/L 4HB (sucrose concentration was 0, 0.05, 0.08, 0.1, 0.5, 0.8, 2, 4, 6, 8, 10, 20, 30, 40, 50 g/L)

Figure 7 Growth curve of different bacteria carrying pYB1a-PobR^{mut}-eGFP-SacB under sucrose gradient

9.20 Ziwei Pan

pRB1s-HmaS generation of mutant library replacing with Anderson promoter

In the pRB1s-HmaS generation of mutant library replacing with Anderson promoter experiment we detected protein expression by SDS-PAGE and subsequent experiments were conducted.

- SDS-PAGE

5%Concentrated gel 12%separated gel

PRB1s-hmaS DH5α OD=4.2 (Supernatant & precipitation)

PRB1s-Anderson-hmaS BL21 OD=4.7 (Supernatant & precipitation)

PRB1s-Anderson-hmaS BW OD=2.0 (Supernatant & precipitation)

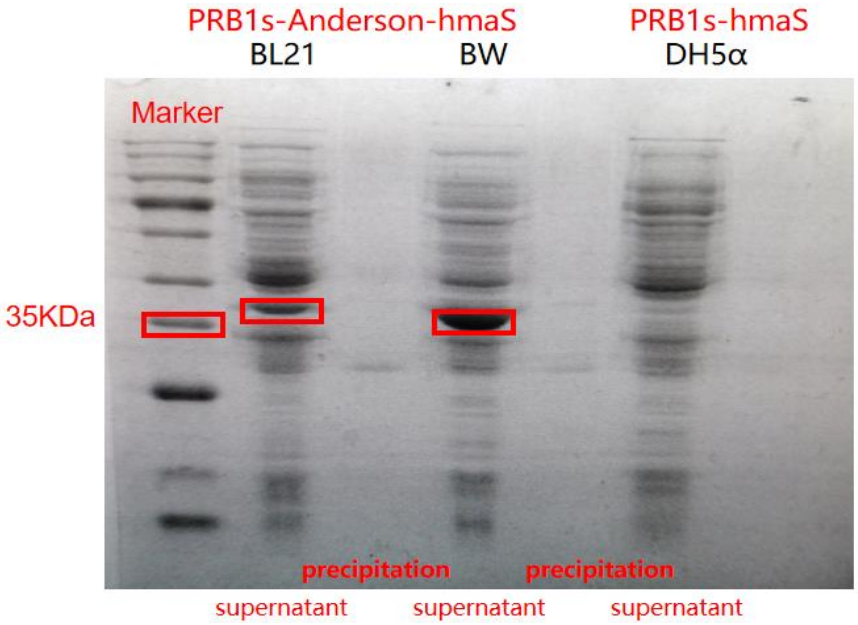


Figure 8 SDS-PAGE result

The protein size of HmaS was about 38 KDa. After exogenous introduction of *hmaS*, HMA was produced in BW and BL21, and the expression amount of BW was higher than that of BL21.

- SDS-PAGE

5%Concentrated gel & 12%separated gel

No IPTG induction was added in tube culture for 12h

PRB1s-Anderson-hmaS BL21 OD=3.0 (Supernatant & precipitation)

No plasmid BL21(protobacteria) OD=1.3 (Supernatant & precipitation)

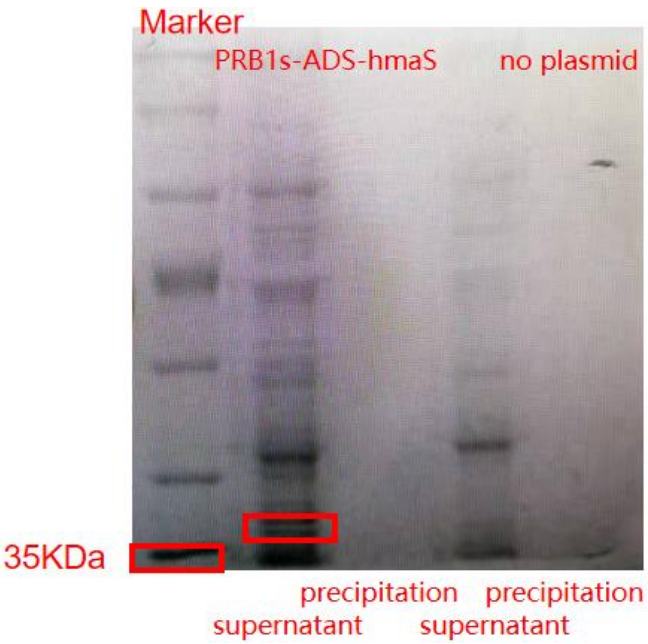


Figure 9 SDS-PAGE result

The size of HmaS protein is about 38 KDa. After exogenous introduction of *hmaS*, there was obvious protein expression at 38 KDa, which was presumed to be HMA.

Reaction system (50μL)	
Error prone-F /Error prone-R	1μL/1μL
PRB1s-ADS-hmaS(181 ng/μL)	0.1μL
2×Mix Radom System	25μL
Mut Enhencer	3μL
DDW	19.9μL

Reaction procedure		
95℃	5min	
95℃	30s	×30
60℃	1s	
72℃	1min	
72℃	7min	

Figure 10 error-prone PCR reaction system and procedure

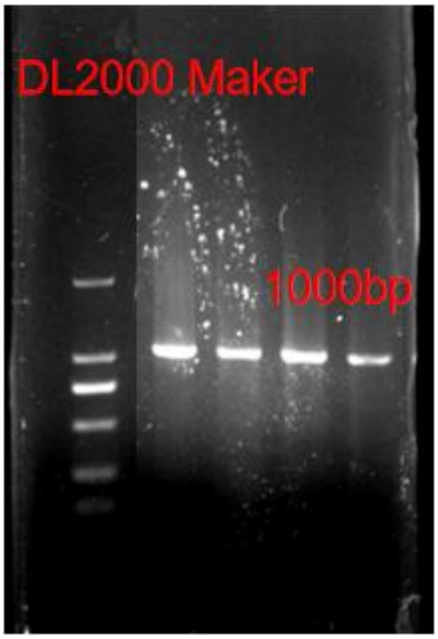


Figure 11 error-prone PCR result

The concentration of purified PCR product was 136.9 ng/μL.

Reaction system (50μL)	
Error prone-hmaS	3.6μL (500ng)
Prb1s-ADS-hmaS(181 ng/μL)	0.4μL(50ng)
2×HF Mix	25μL
DDW	21μL

Reaction procedure		
95℃	5min	
95℃	30s	×34
54-56℃	30s	
72℃	3min	
72℃	10min	

Figure 12 Error-prone PCR reaction system and procedure

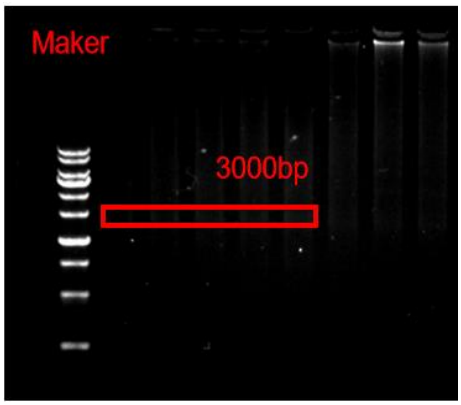


Figure 13 Error-prone PCR result

The correct size band was found at 3000 bp, but the band was not obvious. The four tubes were combined into one tube, and the purified concentration of PCR product was 113 ng/μL and 160.2 ng/μL.

Reaction system (50μL) X5	
DpnI Endonuclease	1μL
Prb1s-ADS-hmaS^{mut}(160.1 ng/μL)	0.4μL(640ng)
Cut smart	5μL
DDW	43.6μL

Figure 14 DpnI digestion system

The concentration of the product was 30.1 ng/μL after 5 tubes were combined with 1 tube.

Reaction system (50μL)	
Error prone-hmaS	3.6μL (500ng)
Prb1s-ADS-hmaS^{mut} digestion product	0.4μL(50ng)
2×HF Mix	25μL
DDW	21μL

Figure 15 T4 connection system

The concentration of the purified product was 11 ng/μL.

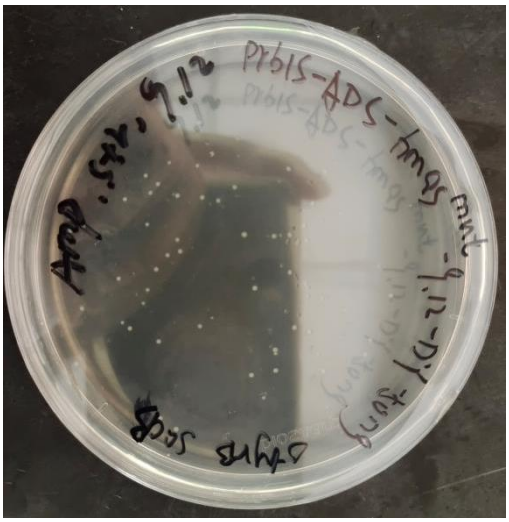


Figure 16 pRB1s-ADS-HmaS^{mut} (Δ tyrB-SacB receptive state)

About 50 strains were grown after centrifugation at 50 ng, only 1 strain was grown at 1 ng on average, the rate was not ideal. Five strains were selected and sequenced to compare whether mutations occurred in *hmaS* gene.