WEEK 10

6.2 Sunyue Cai

Re-screening verification

We conducted a preliminary screening experiment of about 1300 monoclonals on 15 plates from No. 73 to 87, and selected 61 clones with high OD and fluorescence values for re-screening verification. The re-screening verification system is as follows:

Control Group	Test Group
220 μLLB+0.1％ 50 mg/mL ampicillin	220 μL LB+0.1 ％ 50 mg/mL ampicillin+1% $BWPobR^{mut}$
220 μL LB+0.1％50 mg/mL ampicillin+1% $BWPobR^{WT}$ Bacteria	200 μL LB+0.1 ％ 50 mg/mL ampicillin+10% 0.6 g/L 4HB+1% $BWPobR^{mut}$
200 μL LB+0.1 ％ 50 mg/mL ampicillin+10% 0.6 g/L 4HB+1% $BWPobR^{WT}$	200 μL LB+0.1 ％ 50 mg/mL ampicillin+10% 0.6 g/L HMA+1% $BWPobR^{mut}$

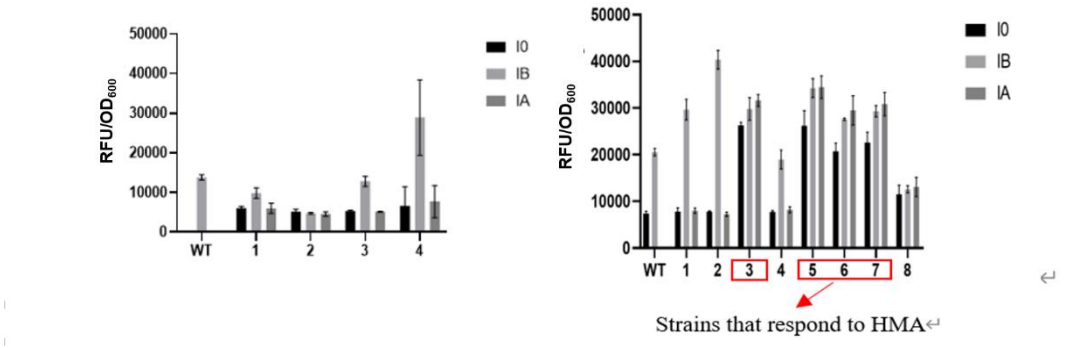


Figure 1 Data diagram of partial re-screening verification experiment

There are three parallel groups, but no strains screened meet the requirements.

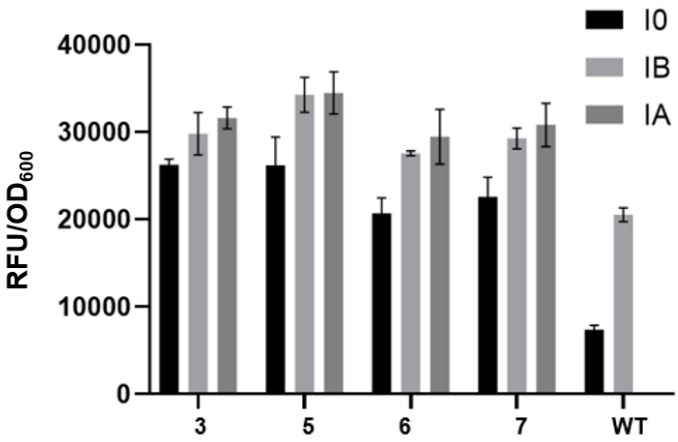


Figure 2 Re-screening verification diagrams of four strains with better responses to HMA

The above four strains have a high response to HMA. Due to the high I0 value, it is speculated that PobR protein mutants cannot inhibit the promoter. Repeated experiments are required to verify.

6.4 Jianing Li

Megawhop PCR

Our error-prone PCR system and PCR conditions are as follows:

PCR System		PCR Condition		
pYB1a-PobR-eGFP-Cmr	5μL / 50 ng	98 ℃	3 min	
2×Mut Random system	25 μL	98 ℃	30 s	X20
Mut Enhencer	3 μL	50 ℃	30 s	
pobR-mut-F	1 μL	72 ℃	30 s	
pobR-mut-R	1 μL	72 ℃	2 min	
ddW	19 μL			

Then we use cycle-pure-kit kit for PCR product purification：

PCR System		PCR Condition		
pYB1a-PobR-eGFP-Cmr	5 μL / 50 ng	95 ℃	5 min	
2x High fidelity mix	50 μL	95 ℃	30 s	X30
Megaprimer	600 ng / 6 μL	55 ℃	30 s	
ddW	39 μL	72 ℃	2 min30 s	
		72 ℃	10 min	

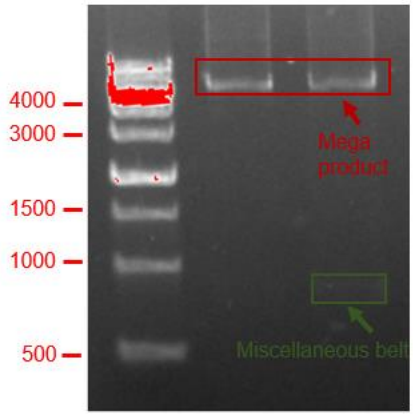


Figure 3 0528 mega results

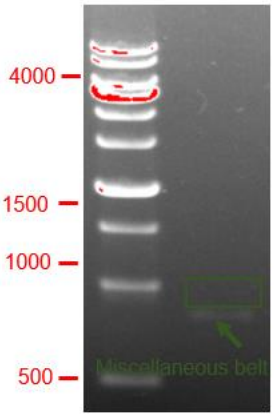
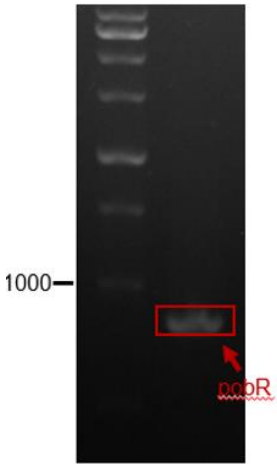


Figure 4 0601 mega results



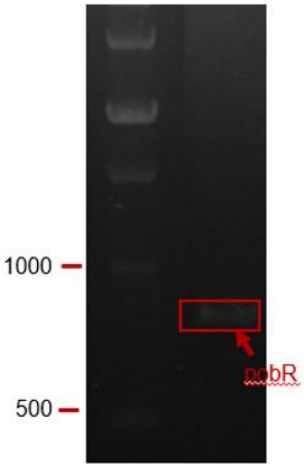


Figure 5 0528 Error-prone PCR results

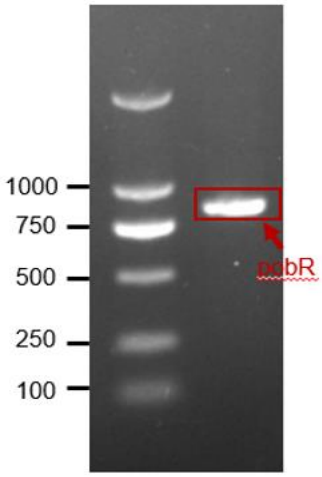


Figure 6 0601 Error-prone PCR results

6.6 Chao Chen

Preliminary test of flat plate screening

In order to determine whether the low-intensity induction can make the strain grow normally under the condition of adding 0.1% 20 mg/mL and 30 mg/mL chloramphenicol, the following experiment was done: antibiotic 1‰ + inducer 10% and 200 μL were cultured in a shaker at 37°C. After the OD600 is about 4, the bacterial solution diluted 10^4 times is coated.

Figure7 BWPobR^{WT}+1‰ 50 mg/mL ampicillin

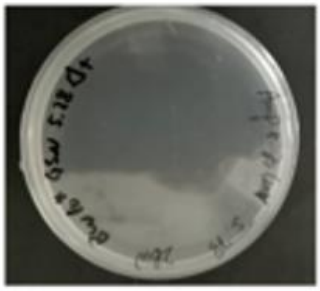


Figure 8 BWPobR^{WT}+1‰ 50 mg/mL ampicillin+1‰ 20 mg/mL chloramphenicol

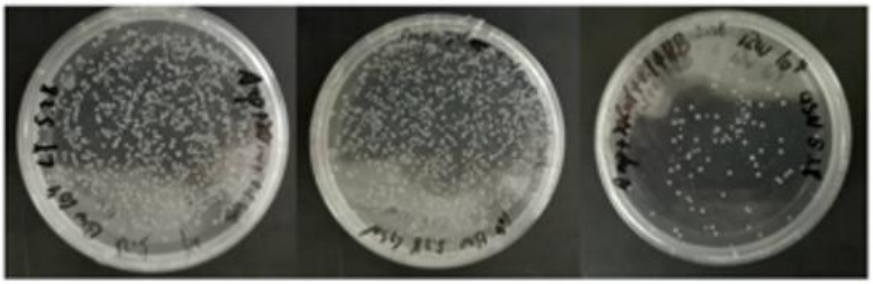


Figure 9 BWPobR^{WT}+1‰ 50 mg/mL ampicillin+1‰ 20 mg/mL chloramphenicol+0.1 g/L 4HB

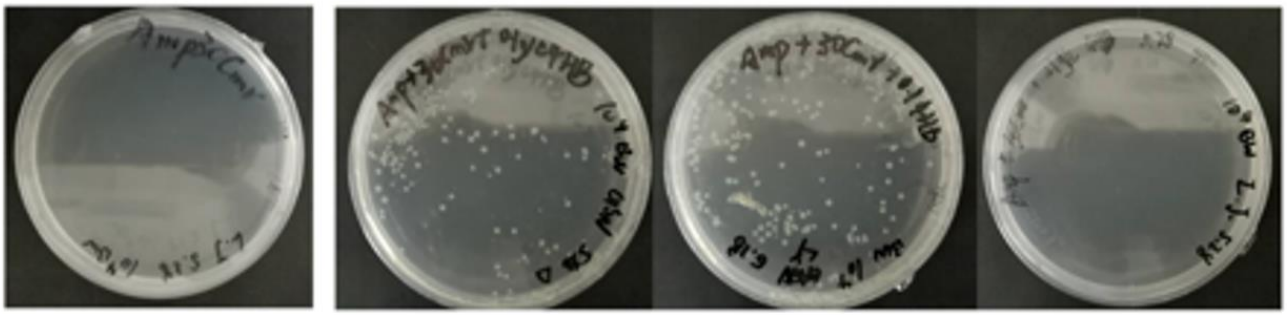


Figure 10 BWPobR^{WT}+1‰ 50 mg/mL ampicillin+1‰ 30 mg/mL chloramphenicol; 1‰ 50 mg/mL ampicillin+1‰ 30 mg/mL chloramphenicol+0.1 g/L 4HB

It is judged that under the condition of adding 0.1% 20 mg/mL and 30 mg/mL chloramphenicol, low-intensity induction can make the strain grow normally. In order to judge the random mutation library capacity and roughly judge the number of colonies that can grow in a single round of coating plate preliminary screening, the following experiments are carried out : After Megahop pcr, use Dpn endonuclease for digestion. The digestion system is as follows (4 tubes of 10 μL digestion system) digestion at 37°C for 2 h, and digestion at 75°C for 20 min.

Dpn| Enzyme	0.2 μL
CutSmart	1 μL
DNA	6 μL（55 ng/μL）
DDW	2.8 μL

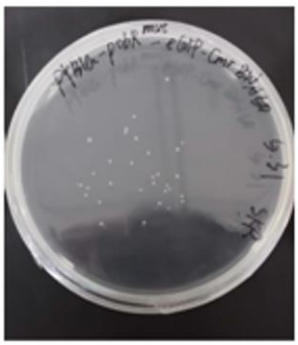


Figure12 50μL BWPobR^{WT}Bacteria+50 μg/mL ampicillin

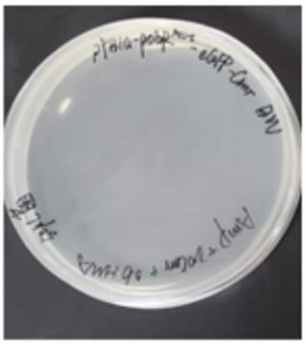


Figure13 50 μL BWPobR^{WT} Bacteria+50 μg/mL ampicillin + 30 μg/mL chloramphenicol + 0.6 g/L HMA

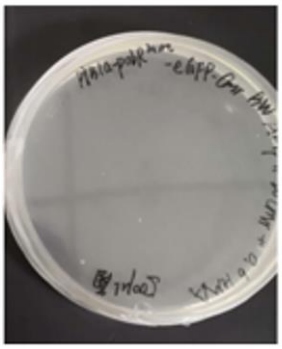


Figure14 500 μL BWPobR^{WT} Bacteria+50 μg/mL ampicillin + 20 μg/mL chloramphenicol + 0.6 g/L HMA

Transfer 10 μL digestion product into 50 μL BW competence, add 600 μL LB medium to 37°C shaker for 45 min, then take 50 μL and 500 μL bacterial solution for the preparation of coating engineering bacteria. The number of colonies was small, which was in line with expectations.