WEEK 11

6.9 Chao Chen

Re-screening verification

He conducted a re-screening verification of a total of 21 strains (two of which were obtained from the preliminary screening experiment, and the remaining 17 have undergone a re-screening verification). No strains that meet the requirements have been screened, and they have been tested last Thursday. The 3 strains reported were sent for sequencing. The re-screening verification system is as follows.

Control Group	Test Group
220 µL LB+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}

Two of the mutant strains were obtained from the preliminary screening experiment on the plate. The methods of obtaining: 1. Transfer 10 µL of digestion product into 50 µL BW competent, add 600 µL LB medium to 37℃ shaker for 45 min, then take 500 µL bacterial solution to spread the plate and grow details as following. 2. Select 2 of the colonies and culture on a shaker at 37°C. 3. Verify it in accordance with the re-screening verification system (2 µL bacterial solution).

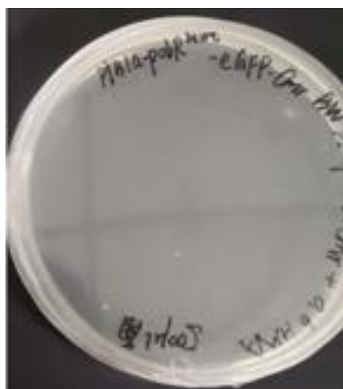


Figure 1 500 µL bacterial solution 50 µg/mL ampicillin+20 µg/mL chloramphenicol+0.6 g/L HMA growth

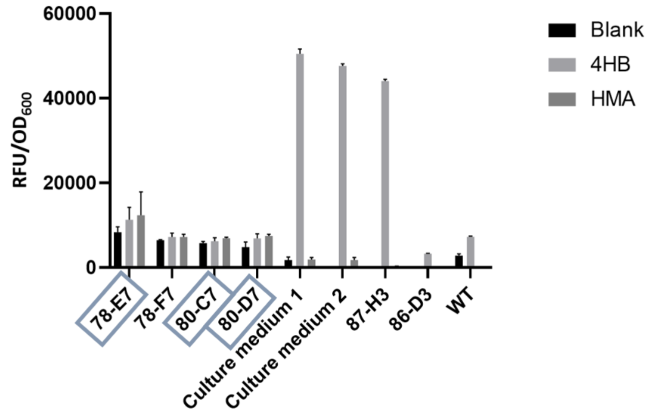


Figure 2 Partial results of re-screening verification

There are no strains that meet the standards and the labeled strains have been sent for sequencing.

6.11 Jingwen Chu

Preliminary experiment of flat plate screening

Then, the plate screening feasibility experiment was carried out, and the BW control bacteria (only responding to 4HB) were quantified and plated:

LB+0.1 g/L 4HB+50 μg/mL ampicillin+20 μg/mL chloramphenicol, LB+50 μg/mL ampicillin+20 μg/mL chloramphenicol, LB+50 μg/mL ampicillin.

Calculate the number of colonies in each plate and compare them. If the number of colonies on the plate containing 4HB is similar to that on the 50 μg/mL ampicillin plate, the plate screening is feasible. The specific operation is as follows: BW control bacteria are cultured on a shaker at 37°C for 8 hours, and LB is used to quantify the OD600 of the bacterial solution. For 4, use the gradient dilution method to dilute the bacterial solution (OD600 = 4) by 104, and then count the plate after coating 100 μL.

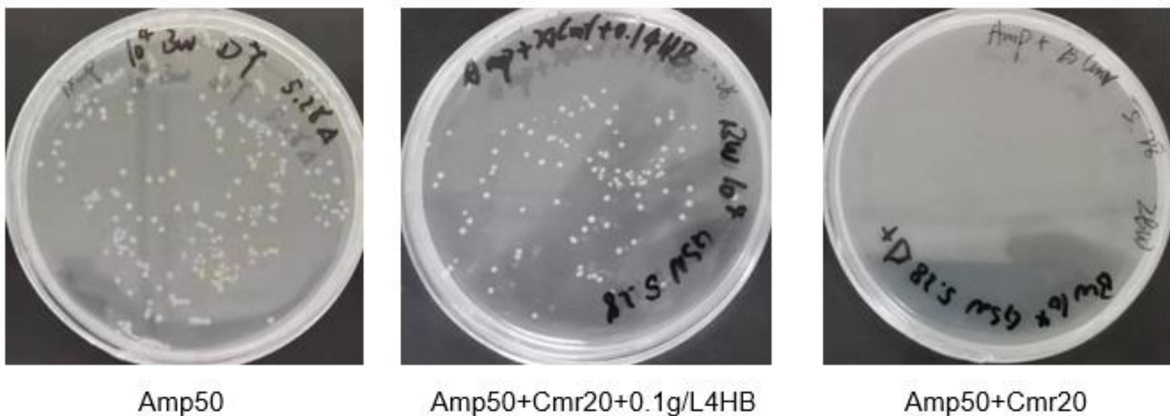


Figure 3 Results of re-screening verification

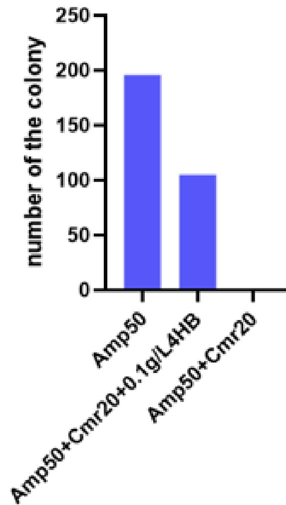


Figure 4 Coated board data chart

Therefore, it is feasible to use plates for preliminary screening.

Estimate the mutation library capacity: first add 10 μL of digestion product (336 ng) to 50 μL of competence, then add 600 μL LB (total system 660 μL) to culture for 45 min, then experiment:

1. Centrifuge and discard 60 μL of supernatant, resuspend 100 μL bacterial solution (56 ng) and spread on (*A50*) plate, and parallel three groups of experiments.
2. Centrifuge and discard 400 μL of supernatant, resuspend 100 μL bacterial solution (280 ng) and spread on (*A50*+*C20*+0.6 mg/mL HMA) plate, and parallel three groups of experiments.

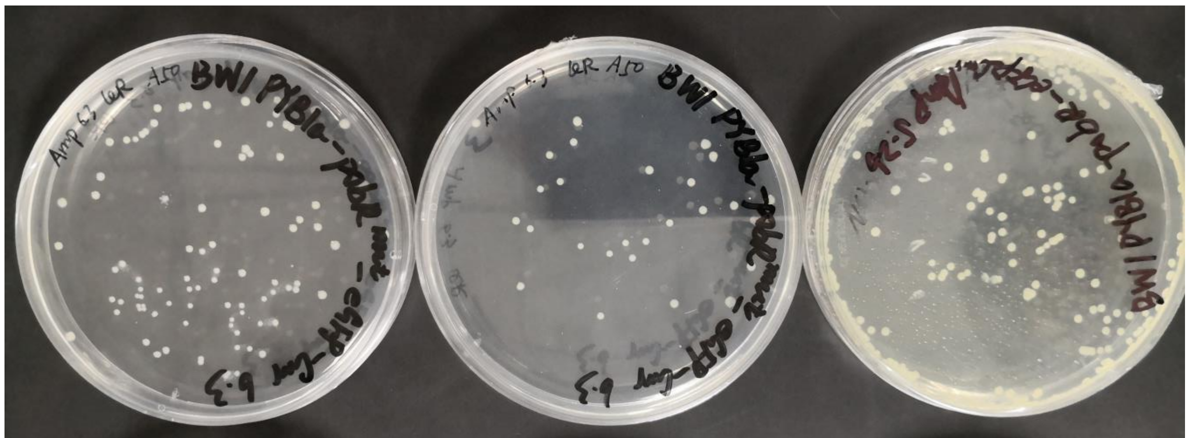


Figure 5 Centrifugal discard 60 μL supernatant, resuspend 100μL bacterial solution (56 ng DNA) to coat (*A50*) plate

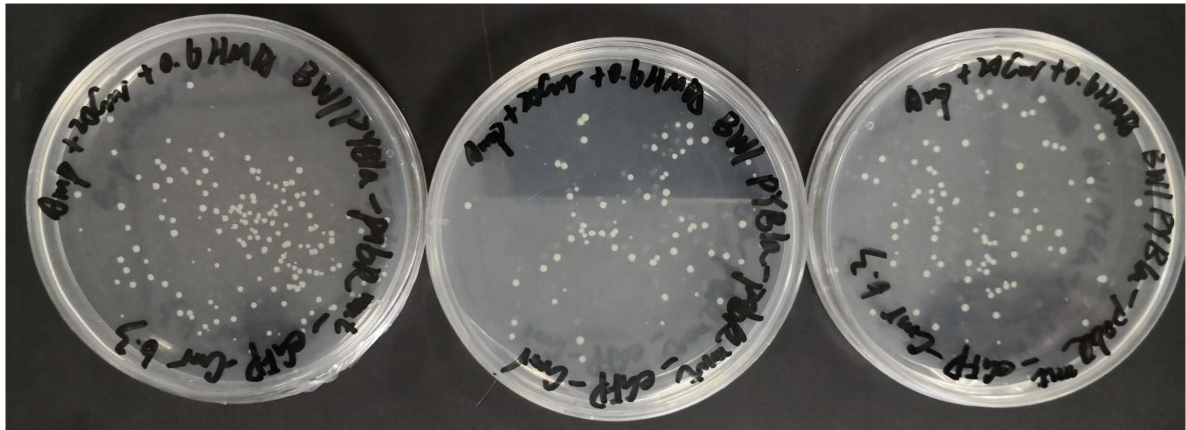


Figure 6 Centrifugation to discard the 400μL supernatant, resuspend 100 μL of bacterial solution (280 ng DNA) coated (*A50*+*C20*+0.6 mg/mL HMA) plate results

Too many colonies may have false positive bacteria. Therefore, 24 colonies were selected for preliminary photocopy verification, and the experiment was planned to be repeated to observe the follow-up results.

Verify whether the lack of digestion will affect the subsequent screening with 20 μg/mL chloramphenicol containing plates, and re-estimate the mutation library capacity. Experimental method: ① directly transform 10ng DNA into 50 μL competence without digestion, and plate all the plates after centrifugation (50 μg/mL ampicillin+20 μg/mL chloramphenicol); ② transform 20 ng or 200 ng DNA into 50 μL competence after DpnI digestion (unpurified), and then centrifuge them all Coated board (50 μg/mL ampicillin).

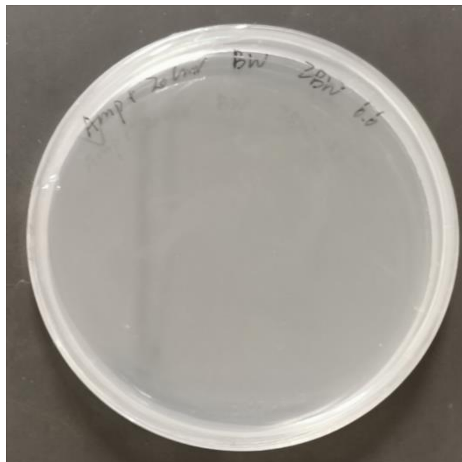
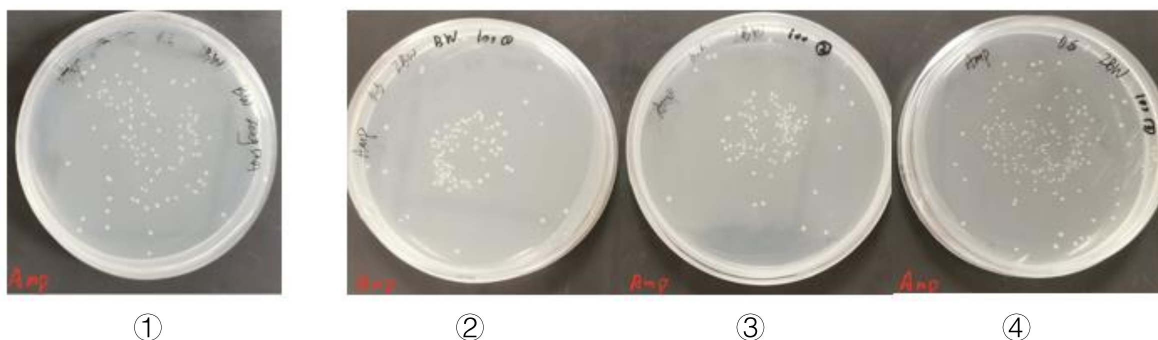


Figure 7 Directly transform 10 ng DNA to 50 μL competent without digestion, and plate all after centrifugation

The results show that no digestion has no effect on subsequent screening.



① ② ③ ④

Figure 8 DpnI digested (unpurified) and transformed 20 ng or 200 ng DNA to 50 μL competent

① Transform 20 ng DNA ②, ③, ④ Transform 200 ng DNA

According to the plate count results, there is no significant difference in the number of colonies obtained from transforming 20 ng or 200 ng DNA to 50 μL competent. Therefore, the transformation efficiency has reached a plateau when 4 ng DNA per μL is competent, so the quality of DNA used for each transformation can be appropriately reduced afterwards.

Now take the transformation of 20 ng DNA into 50 μL competence as an example to calculate the mutation library capacity (M):

$$M = a\frac{nm}{b}$$

Among them: n=total volume of megaPCR purified product (30 μL)

m=concentration of megaPCR purified product (30.8 ng/μL)

a=Number of plate colonies (114)

b=The quality of transformed DNA (20 ng)

The capacity of the mutant library obtained by megaPCR is M=5267 clones.

Subsequently, the mutants that only responded to HMA but not to 4HB were screened out, and mutants that were off-target by PobR were excluded. Select 24 colonies obtained from the pre-screened pre-experimental coating plate (0.6 g/L HMA 20 μg/mL chloramphenicol) for photocopying. The specific method is as follows:

0.6 g/L HMA+*20* μg/mL chloramphenicol (coated) and photocopy it on ①0.6 g/L 4HB+20 μg/mL chloramphenicol to verify whether PobR is responsive to 4HB (I_B), ② 0.6 g/L HMA+20 μg/mL chloramphenicol, to verify whether PobR is responsive to HMA (I_A), ③20 μg/mL chloramphenicol, verify whether the PobR mutant can inhibit the promoter (I_0).

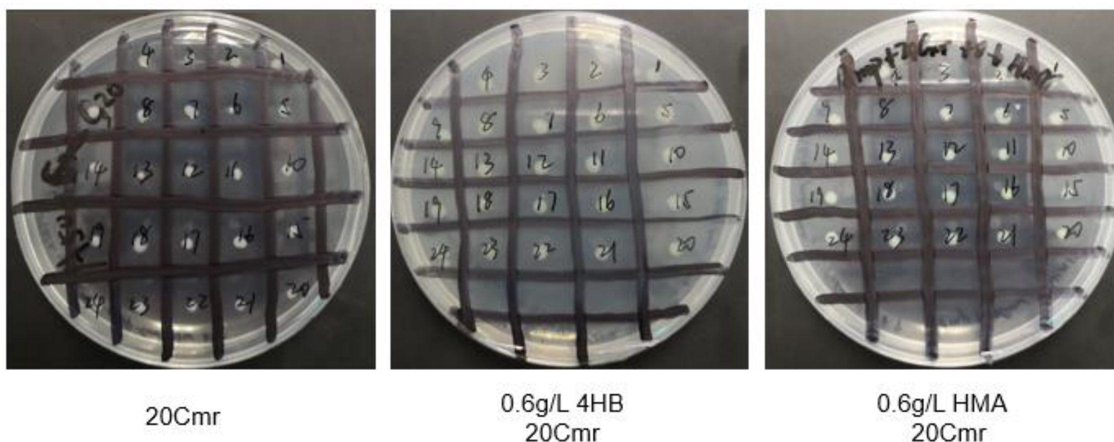


Figure 9 Part of the result of photocopying

Except for the 24 colonies that did not grow on the plate containing HMA, the other 23 colonies grew on the three plates (20 μg/mL chloramphenicol; 0.6 g/L 4HB, 20 μg/mL chloramphenicol; 0.6 g/L HMA, 20 μg/mL chloramphenicol), so Analysis of the 24 colonies were all false positive colonies (PobR protein off-target).

6.13 Ruilei Zhang

Megawhop PCR system modification

We have subsequently modified the megawhop PCR system, and the modified version is as follows:

PCR System（50 μL）	PCR Condition	
pYB1a-PobR-eGFP-Cmr (9.3 ng/μL)：5.5 μL(50 ng)	95 ℃ 5 min	
2xHigh fidelity mix：25 μL	95 ℃ 30 s	X30
Megaprimer(108 ng/μL)：4.6 μL(500 ng)	55 ℃ 30 s	
ddW：14.9 μL	72℃ 2min30s	
	72℃ 10min	

When adding the sample, add the sample according to the 100 μL system, mix and aspirate 50 μL in another PCR tube before running the program, and then centrifuge the two PCR tubes and run the program. After running, the electrophoresis results are as follows:

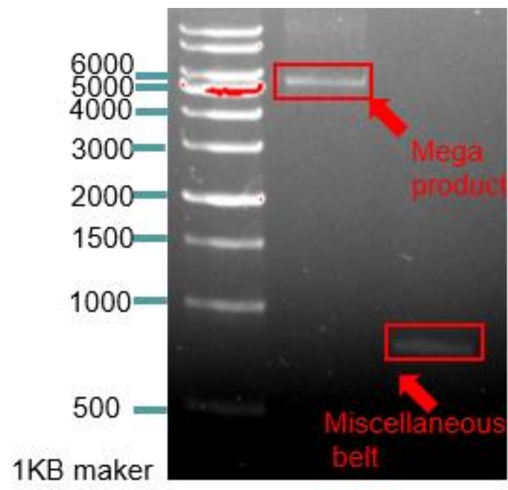


Figure 10 0606 megawhop PCR result graph