Design and construction of the PobR mutant library

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	5 μL/50 ng	98℃	3 min	
2×Mut Random system	25 uL	98℃	30 s	×20
Mut Enhencer	3 uL	50℃	30 s	
pobR-mut-F	1 uL	72℃	30 s	
pobR-mut-R	1 uL	72℃	2 min	
DDW	19 uL			

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	×20
Megaprimer	600 ng/6 μL	55℃	30 s	
DDW	39 μL	72℃	2 min 30 s	
		72℃	10 min	

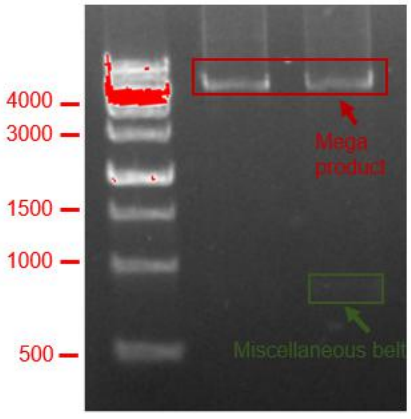


Figure 1 0528 mega results

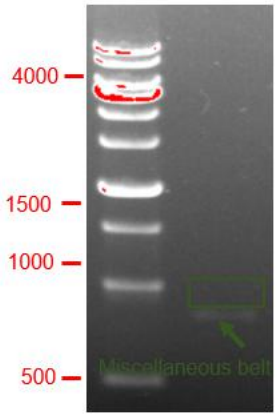


Figure 2 0601 mega results

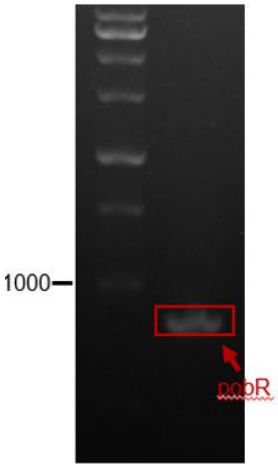
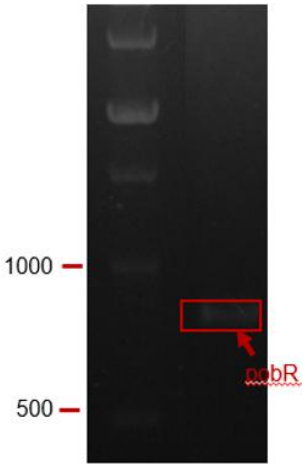


Figure 3 0528 Error-prone PCR results



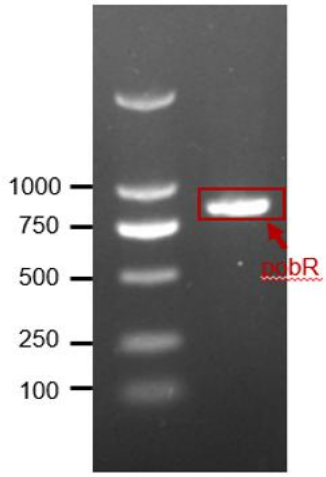


Figure 4 0601 Error-prone PCR results

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 (50 μg/mL ampicillin) 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 (50 μg/mL ampicillin+20 μg/mL chloramphenicol+0.6 mg/mL HMA) plate, and parallel three groups of experiments.

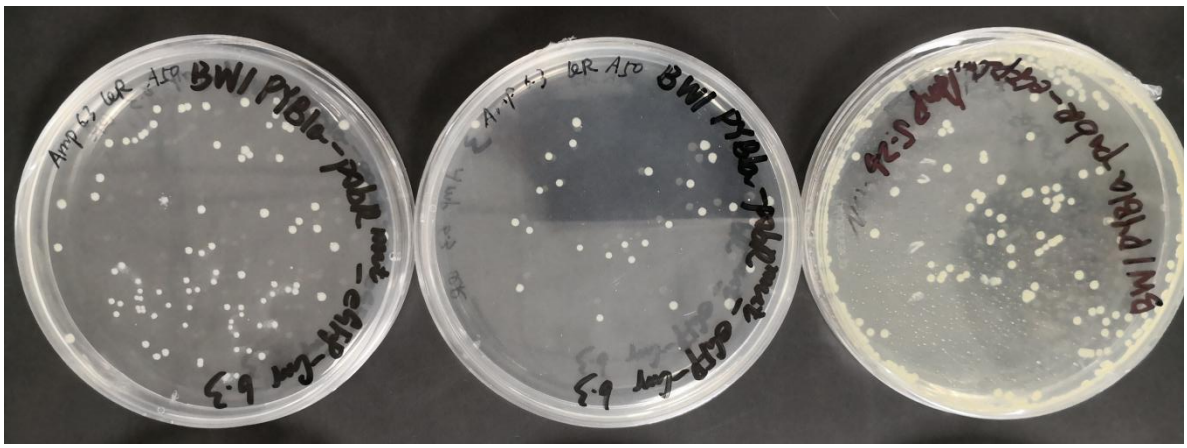


Figure 5 Centrifugal discard 60 μL supernatant, resuspend 100μL bacterial solution (56 ng DNA) to coat (50 μg/mL ampicillin) plate

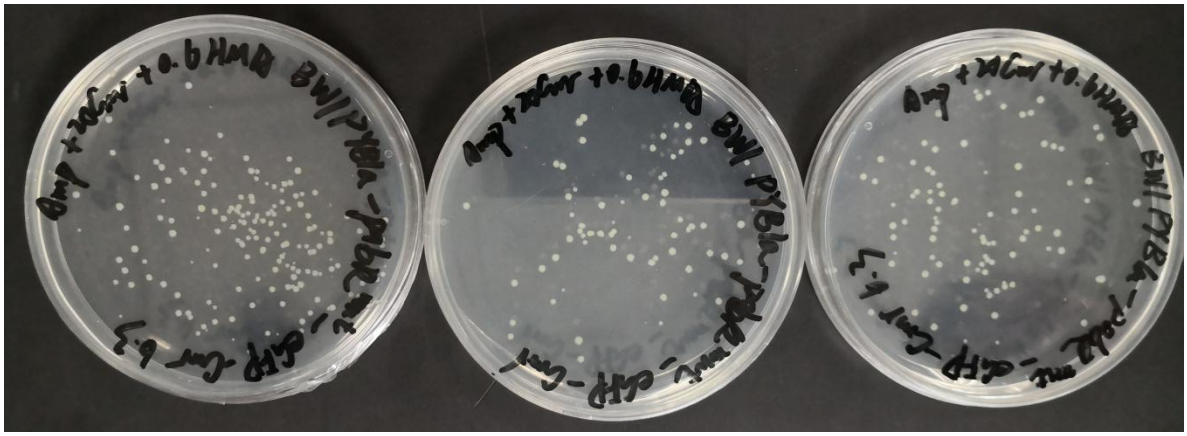


Figure 6 Centrifugation to discard the 400 μL supernatant, resuspend 100 μL of bacterial solution (280 ng DNA) coated (50 μg/mL ampicillin+20 μg/mL chloramphenicol+0.6 mg/mL HMA) plate results

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.

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	×30
Megaprimer(108 ng/μL)：4.6 μL (500 ng)	55℃	30 s	
DDW：14.9 μL	72℃	2 min 30 s	
	72℃	10 min	

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：

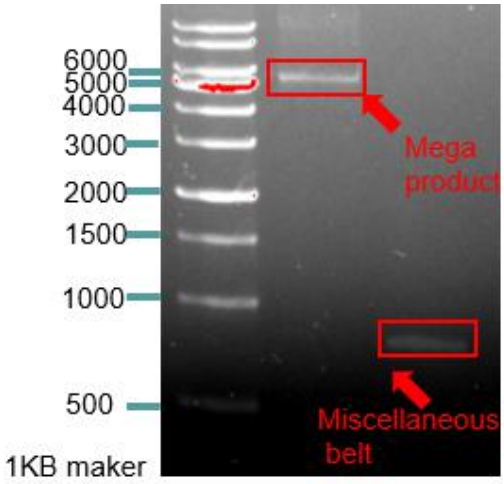


Figure 7 0606 megawhop PCR result graph

8.3 Chuyao Xu

F4-A9 sequencing results

Sequence F4-A9 and analyze its mutation sites

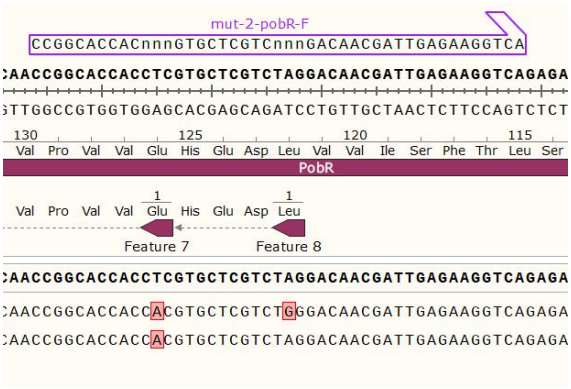


Figure 8 F7-D12 connection result sequence diagram

D12 mutation site: 122 Leu → Pro, 126 Glu → Val

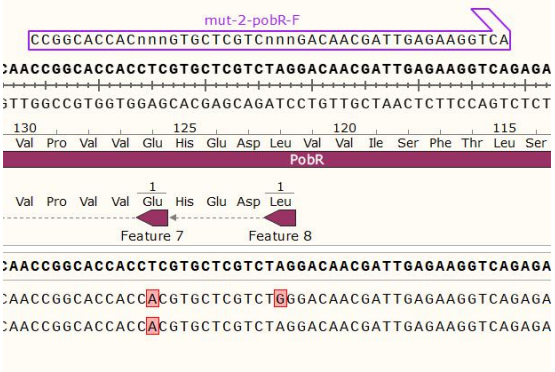


Figure 9 Sequence diagram of F4-A9 sequencing results

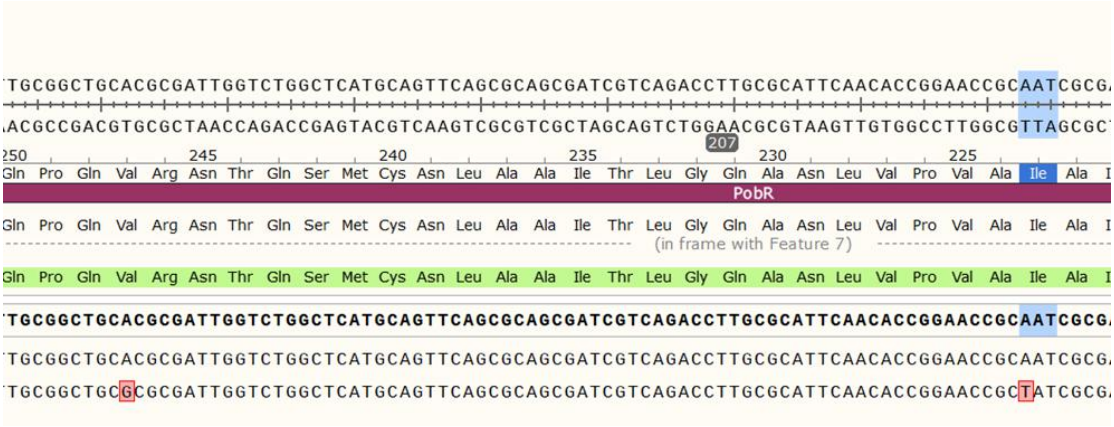


Figure 10 Sequence diagram of F4-A9 sequencing results

A9 mutation site: 126 Glu→Val; 223 Ile synonymous mutation; 247 Val→Ala

8.13 Shuhan Liu

Sequencing results

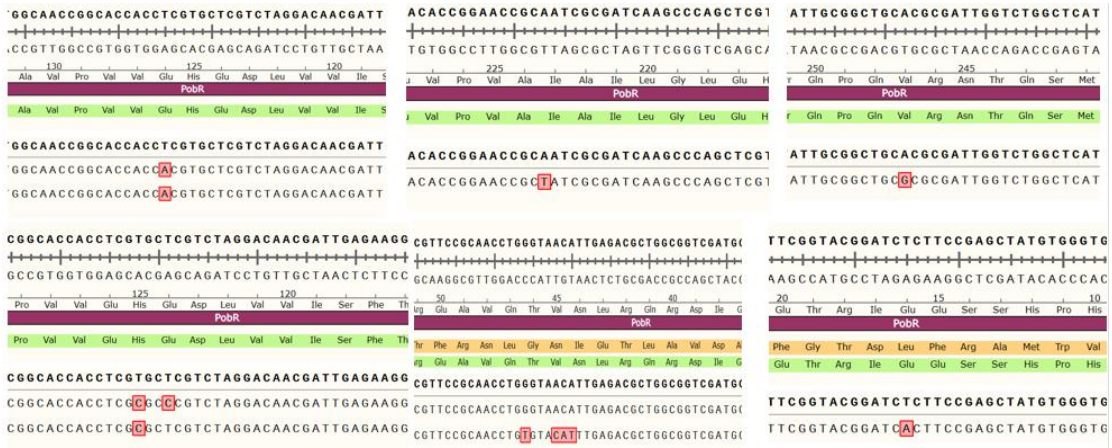


Figure 11 Sequencing of the subclone of F5-B7. Above, subclone C9; below, subclone C7

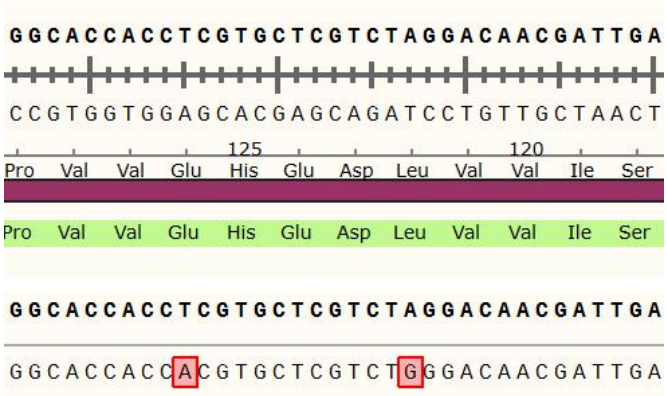


Figure 12 Sequencing of F7-E8-3

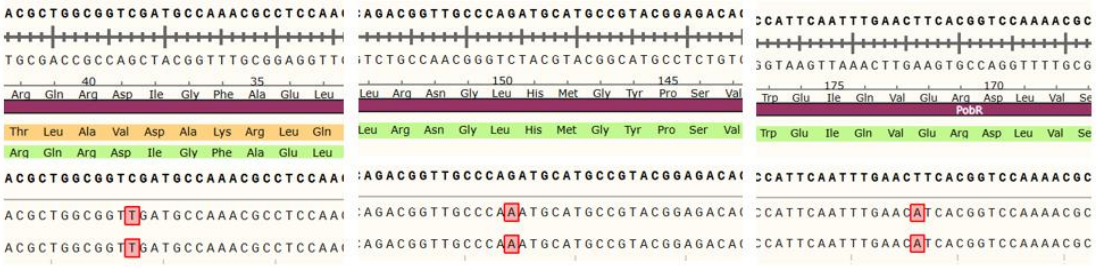


Figure 13 Sequencing of F5-A10-1

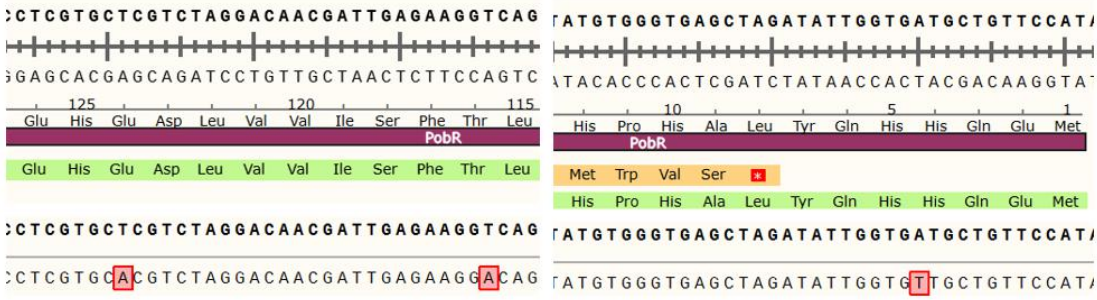


Figure 14 Sequencing of F6-F4-3

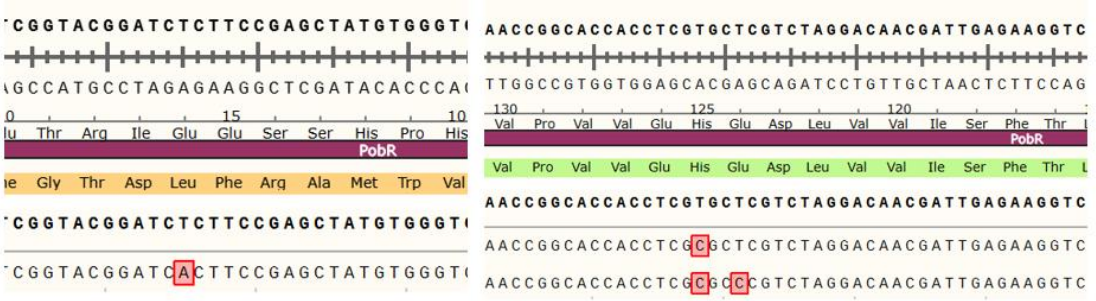


Figure 15 Sequencing of F5-B7-2