WEEK 12

6.16 Beimeng Zhang

Construction of engineering bacteria

Construction of pYB1a-aroGfbr-pheAfbr new point mutant /aroG A202T, D146N/pheA Δ304, G305K, Δ306.

The experiment process is as follows:

1. Inoculation, PYB1a-aroG-pheA to extract plasmid.
2.4 overlap fragment PCR.
3. Enzyme digestion of plasmid vector (XhoI, BglII), gelatinization and recovery.
4. Overlap connection 1/2; 3/4, electrophoresis verification.
5. Gibson connects the 12/34/ vector, completes the construction, transforms, bacteria P, picks two bacteria for sequencing.

aroG-1 fragment amplification:

PCR system（25μL）		PCR Condition		
template	2μL	98 ℃	5 min	
2xHigh fidelity mix	12.5μL	98 ℃	30 s	X30
aroG-F	1μL	60 ℃	30 s	
aroG-over-1-R	1μL	72 ℃	30 s	
DDW	8.5μL	72 ℃	10 min	

aroG-1 fragment amplification:

PCR system（25μL）		PCR Condition		
template	2 μL	98 ℃	5 min	
2xHigh fidelity mix	12.5 μL	98 ℃	30 s	X30
aroG-over-2-F	1 μL	60 ℃	30 s	
aroG-over-1-R	1 μL	72 ℃	30 s	
DDW	8.5 μL	72 ℃	10 min	

pheA-1 Fragment construction：

PCR system（25μL）		PCR Condition		
template	2 μL	98 ℃	5 min	
2xHigh fidelity mix	12.5 μL	98 ℃	30 s	X30
aroG-over-3-F	1 μL	63 ℃	30 s	
pheA-over-3-R	1 μL	72 ℃	30 s	
DDW	8.5 μL	72 ℃	10 min	

pheA-2 Fragment construction：

PCR system（25μL）	PCR Condition

template	2 μL	98 ℃	5 min	
2xHigh fidelity mix	12.5 μL	98 ℃	30 s	X30
pheA-over-4-F	1 μL	63 ℃	30 s	
pheA-R	1 μL	72 ℃	30 s	
DDW	8.5 μL	72 ℃	10 min	

Overlap Link 1/2：

aroG-1/aroG-2 50μl system		Condition		
aroG-1	1μL	98℃	5min	
aroG-2	1μL	98℃	30s	X30
aroG-F	1μL	60℃	30s	
aro-over-2-R	1μL	72℃	1min	
2X High fidelity mix	25μL	72℃	10min	
DDW	21μL			

Overlap Link 3/4：

aroG-1/aroG-2 50μlsystem		Condition		
aroG-3	1μL	98℃	5min	
aroG-4	1μL	98℃	30s	X30
pheA-R-	1μL	63℃	30s	
aro-over-3-F	1μL	72℃	1min	
2X High fidelity mix	25μL	72℃	10min	
DDW	21μL			

Gibson Connecting vectors, fragments（10μL system）：

overlap 1/2 Snippet	0.3 μL dilute with water to 2.3 μL
overlap 3/4 Snippet	0.7 μL dilute with water to 1.5μl
Carrier	1.5 μL
Exanase	1 μL
Cell buffer	2 μL
DDW	1.7 μL

Then transfer to BW competence, select two strains to extract plasmids for sequencing.

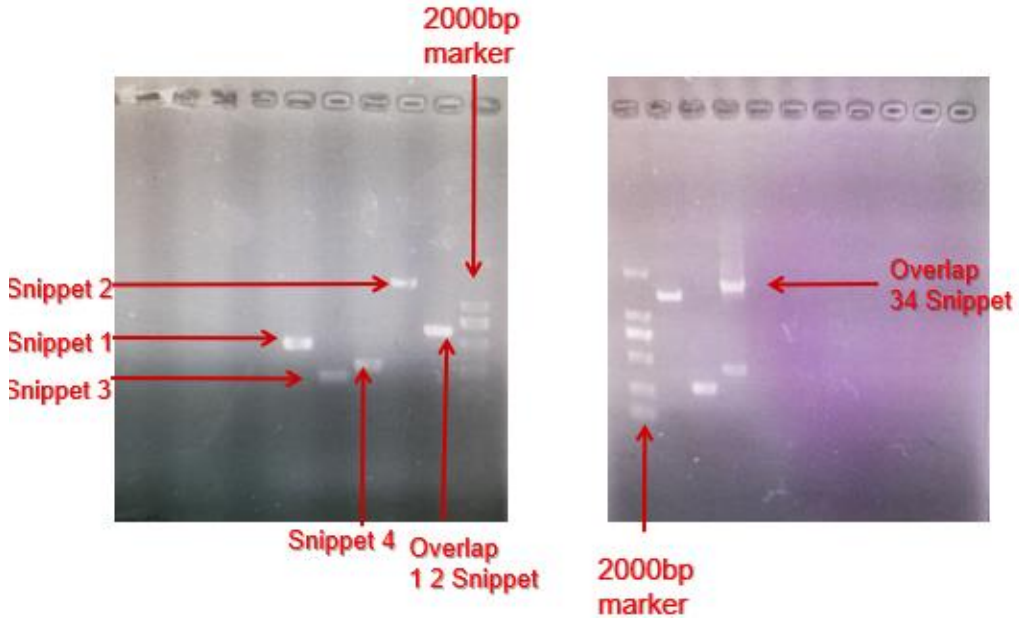

Figure 1 Electrophoresis gel diagram of fragments and ligation products

6.18 Yi Dong

Perform PCR product

Perform PCR product recovery on the products of fragments 1, 2, 3, 4 and the overlap ligation of fragments 1 and 2, and gel recovery of the products after the overlap of fragments 3 and 4.

After confirming the current system and performing error-prone PCR experiments:

PCR System（25μL）		PCR Condition	
PYB1a-PobR-Cmr	1.1 μL（10ng）	95 ℃	2 min
2Xmix Random system	25 μL	94 ℃	30 s
Mut Enhencer	3 μL	50 ℃	1 min
pobR-mut-R	1 μL	72 ℃	1 min
pobR-mut-F	1 μL	72 ℃	7 min
DDW	18.9 μL		

X30

Megawhop PCR:

PCR System（50μL）		PCR Condition	
PYB1a-PobR-Cmr	5.4 μL（50ng）	95 ℃	5 min

2Xmix Random system	25 μL	95 ℃	30 s	X20
pobR-mut	4.7 μL （500 ng）	55 ℃	30 s	
DDW	14.9 μL	72 ℃	2 min30 s	
		72 ℃	10 min	

20 ng Mega pcr undigested product was transferred to 50 μL BW competent culture. The incubation time of the transformation coating is 12 h. No colony growth was seen after 12 h. The first bacteria grows at 20 h, as shown in the picture at 36 h.

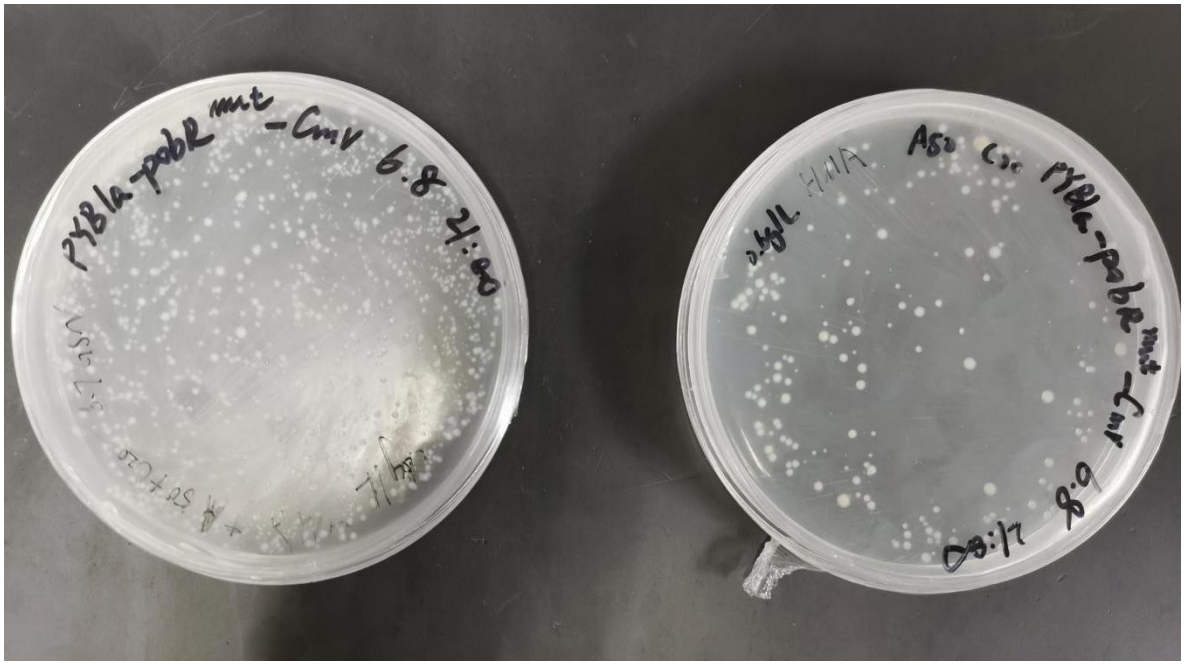

Figure 2 Growth status of bacteria after 36h of transformation and coating

6.13 Two plates of mega products with a conversion concentration of 33
6.14 Ten plates of mega products converted to a concentration of 25
6.15 Four plates of mega products converted to a concentration of 25

Several colonies grew after 20 hours of culture after transformation, and a single colony was picked for photocopy experiment.

50 μg/mL amphenicol+ 20 μg/mL chloramphenicol	50 μg/mL amphenicol+ 0.6 g/L 4HB + 20 μg/mL chloramphenicol	50A + 0.6 g/L HMA+20 μg/mL chloramphenicol
50 μg/mL amphenicol+20 μg/mL chloramphenicol	50 μg/mL amphenicol+ 0.6 g/L HMA + 20 μg/mL chloramphenicol 15 sets of photocopying in one set of two plates	
50 μg/mL amphenicol+20 μg/mL chloramphenicol	50 μg/mL amphenicol+0.6 g/L HMA+20 μg/mL chloramphenicol 2 sets of photocopying in one set of two plates	

The first set of three plates, one set of 3 sets of photocopying, a total of 100 clones.
The second set of two plates, one set of 15 sets, a total of 876 clones.
The third set of two plates, one set of two copies, a total of 73 clones.
A total of 6 bacteria were sent for re-screening this week.

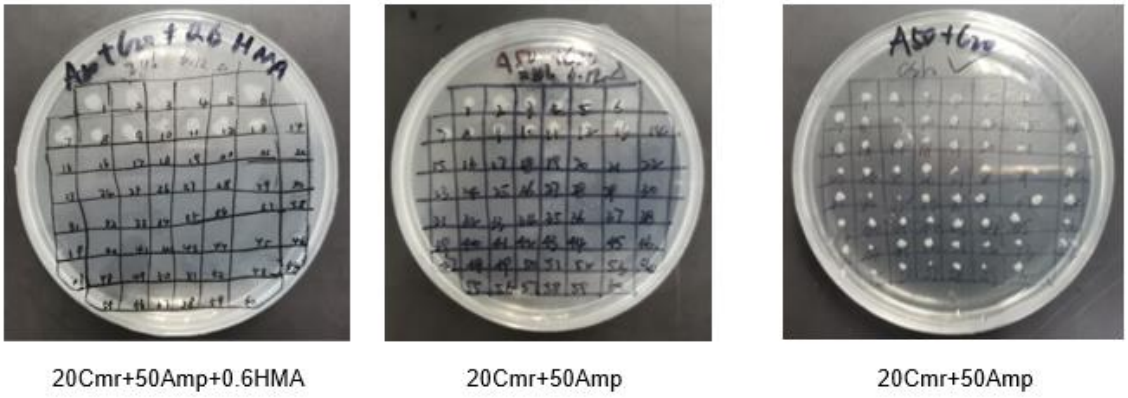

Figure 3 Part of the photocopying result

6.20 Fengqianrui Chen

Re-screening verification

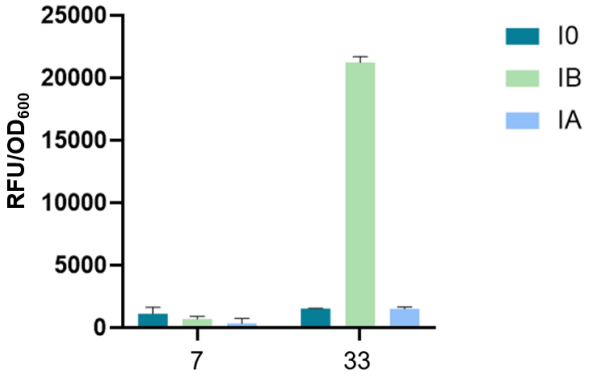

Figure 4 Re-screening verification of No. 7 and No. 33 bacteria

Bacteria No. 7 responds very weakly to HMA and 4HB; Bacteria No. 33 is a false positive strain.

Verification of 5 possible strains (of which No. 1 is No. 7 in the above picture).

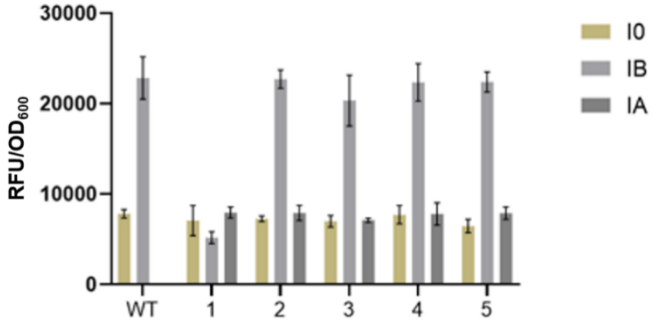

Figure 5 Re-screening verification diagram

No strains that meet the requirements appeared, and the No. 1 strain did not respond to 4HB and HMA, which is presumed to be a miscellaneous bacteria.

6.22 Shuning Guo

Preparation of electrocompetence

Verification of electrocompetence for non-resistance board, 50 μg/mL amphenicol board, and 20 μg/mL chloramphenicol board.

Bacteria grow on non-resistant plates, 50 μg/mL amphenicol plates, and 20 μg/mL chloramphenicol plates, and contamination by bacteria.

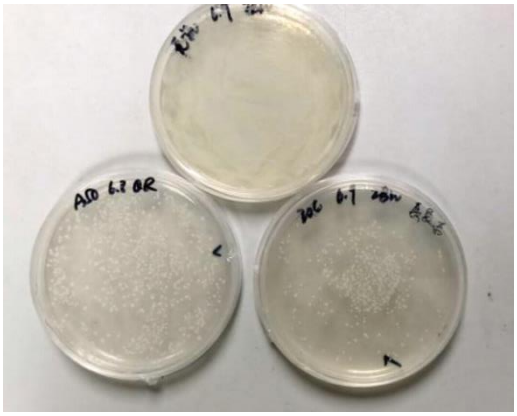

Figure 6 Reasults for non-resistance board, 50 μg/mL amphenicol board, and 20 μg/mL chloramphenicol board

Bacteria grow on non-resistant plates, 50 μg/mL amphenicol plates, and 20 μg/mL chloramphenicol plates, and contamination by bacteria.

Use BW original bacteria, 10% glycerin to coat non-anti-resistant board, 50 μg/mL amphenicol board, 20 μg/mL chloramphenicol board to verify the results.

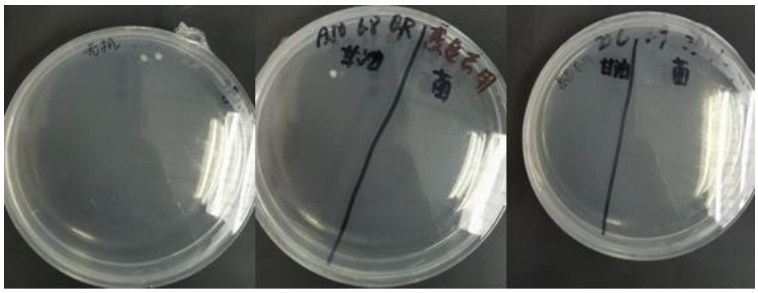

Figure 7 BW electrocompetence verification for non-resistance board, 50 μg/mL amphenicol board, and 20 μg/mL chloramphenicol board

10% glycerol has bacteria growth on non-resistant plates and 50 μg/mL amphenicol resistant plates, and BW seeds are verified as pollution-free

Reconfigure 10% glycerin to make BW competence, and apply it to non-anti-resistant board, ampicillin board, chloramphenicol board, streptomycin board, ampicillin+kanamycin board, and bacteria grow.

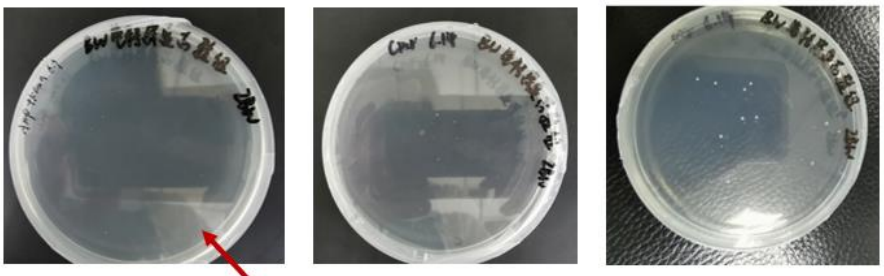

Figure 8 50 μg/mL ampicillin+50 μg/mL kanamycin+50 μg/mL chloramphenicol+50 μg/mL streptomycin board BW electrocompetence verification BW original bacteria do not grow on the resistant plate, and the colony is contaminated by bacteria during verification.

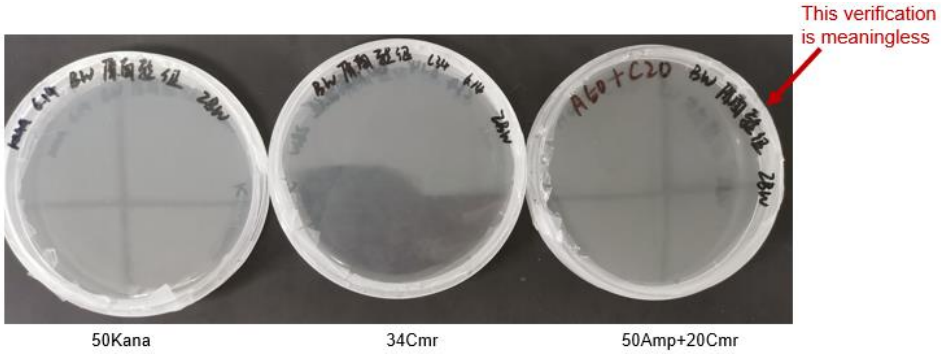

Figure 9 50 μg/mL kanamycin; 34 μg/mL chloramphenicol; 50 μg/mL ampicillin+20 μg/mL chloramphenicol board BW electrocompetence verification