WEEK14

6.30 Chao Chen

Plate screening

System: 1. Perform error-prone PCR first, then Mega PCR, and finally use T4 ligation open-circle plasmid (T4 linkage system is shown in Figure 3) .2. Direct transformation without digestion of the original plasmid (system: 50 ng T4 ligation product to 50 μL commercialization experience Condition).3. Stay on ice for 30 minutes, heat shock for 45 minutes, and ice bath for 2 minutes
4. Use SOC culture medium to incubate for 1 hour, combine the two tubes into one tube and apply A50+C20+HMA plate. C20 and A50 three types of plates (A50 is used to preserve bacteria) .5. After the ideal strains are initially screened, they will enter the re-screening verification and send them for sequencing.

1. Assemble the following reaction in a sterile microcentrifuge tube:

vector DNA	100ng
insert DNA	17ng
Ligase 10X Buffer	1μl
T4 DNA Ligase (Weiss units)	0.1–1u
Nuclease-Free Water to final volume of	10μl

2. Incubate the reaction at:

room temperature for 3 hours, or
4°C overnight, or
15°C for 4–18 hours.

Figure 1 T4 connection system and procedures

After the initial screening and photocopying, it is suspected that the No. 12 bacteria may be correct.

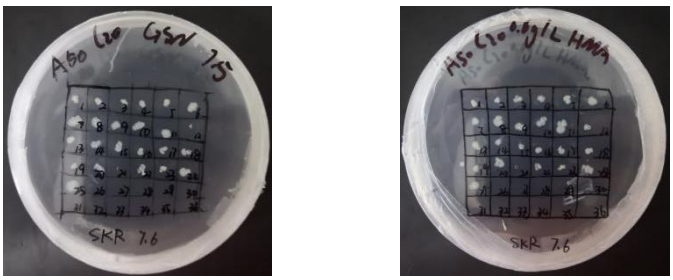

Figure 2 Preliminary screening photocopy results

7.2 Jingwen Chu

Re-screening

As of the morning of 7.7, the transformation of the 3.5 ug T4 ligation product has been completed, and the possible strains have been re-screened for verification, and no ideal strains have appeared.

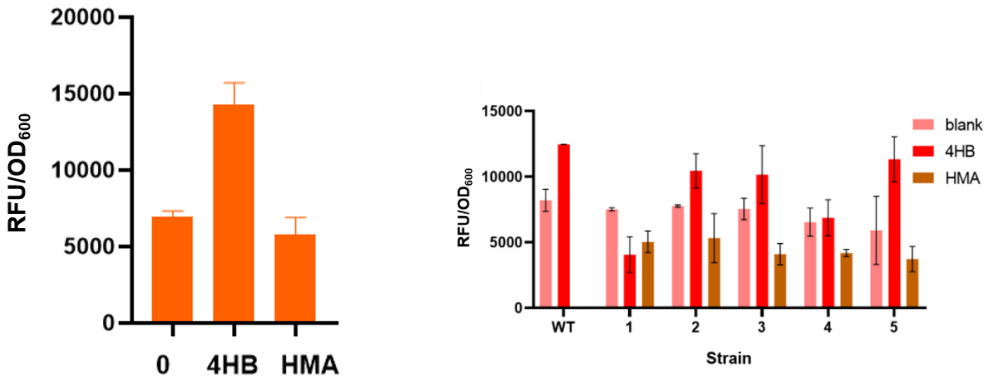

Figure 3 Re-screening result data graph

7.3 Xinlu Liu

pTarget-aspC Construction

PTarget-tyrB sequencing was carried out this week, and the sequencing results were correct, and the bacteria were kept for future use. According to its sequencing results, it is found that the mutation sites and numbers of PobR have diversified characteristics.

AspC target fragment preparation system, PCR program and primers used:

	System		Condition		
aspC-F	overlap-F-aspC-F-2	1 μL	Predenaturation	95 ℃	3 min
	overlap-R-aspC-R-2	1 μL	transsexual	95 ℃	30 s
	2*High Fidelity Mix	25 μL	annealing	60 ℃	30 s
	DDW	22 μL	extend	72 ℃	1 min
	BW genome	1 μL	Final extension	72 ℃	3 min
			Number of cycles	×30	
aspC-R	overlap-F-aspC-F-2	1 μL	Predenaturation	95℃	3 min
	overlap-R-aspC-R-2	1 μL	transsexual	95℃	30 s
	2*High Fidelity Mix	25 μL	annealing	60℃	30 s
	DDW	22 μL	extend	72℃	1 min
	BW genome	1 μL	Final extension	72℃	3 min
			Number of cycles	×30	
overlap	overlap-F-aspC-F-2	1 μL	Predenaturation	95℃	3 min
	overlap-R-aspC-R-2	1 μL	transsexual	95℃	30 s
	2*High Fidelity Mix	25 μL	annealing	60℃	30 s
	DDW	22 μL	extend	72℃	1 min
	BW genome	1 μL	Final extension	72℃	3 min
			Number of cycles	×30	

Primer	Sequence
aspC-F-over-F	AACGAAGGCGATACCATCAGCATAGGGAAT

aspC-F-over-R	GAACCTCGTCGCATTAAAAACAATGAAGCCCGCTG
aspC-R-over-F	GTTTTTAATGCGACGAGGTTCCATTATGGTTACAG
aspC-R-over-R	CAACATCTACCTGGCAGCGAACTACGGTGAAA

pTarget-aspC plasmid preparation system and PCR program:

	System		Condition		
pTarget-aspC	pTarget-aspC-F	1 uL	Predenaturation	98 ℃	3 min
	pTarget-aspC-R	1 uL	transsexual	98 ℃	30 s
	2*High Fidelity Mix	25 uL	annealing	60 ℃	30 s
	DDW	22 uL	extend	72 ℃	1 min30 s
	pTarget-613	1 uL	Final extension	72 ℃	3 min
			Number of cycles	×30	

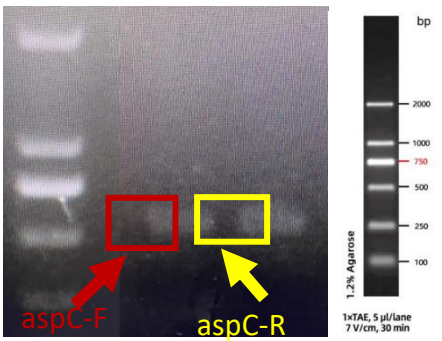

Figure 4 The aspC target fragment preparation electrophoresis result, the PCR product is purified and overlapped and connected.

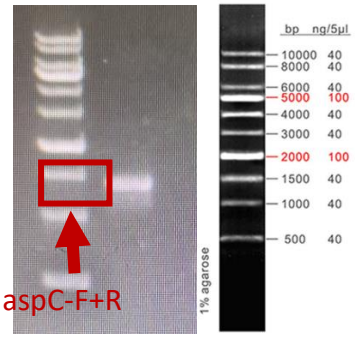

Figure 5 The aspC target fragment preparation electrophoresis results, and the PCR products are purified for later use.

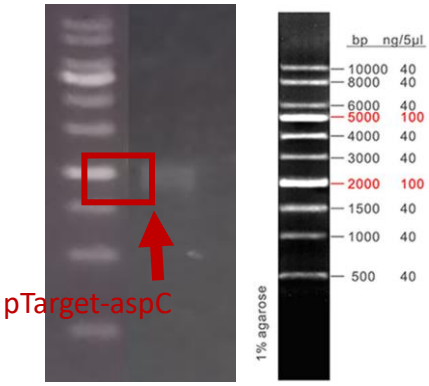

Figure 6 Prepare the electrophoresis result of pTarget-aspC, transfer it to T1

competence after digestion, spread the plate, pick the bacteria, and wait for sequencing.

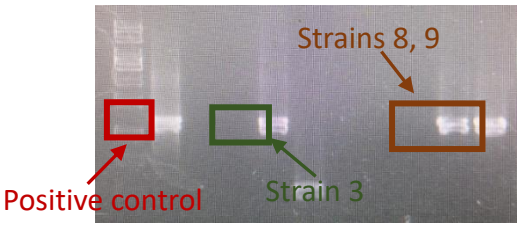

Figure 7 pYB1a-aroGfbr-pheAfbr-2 preparation colony PCR electrophoresis results: after ligation, transfer to T1 competence, plate overnight, colony PCR, select strains 3 and 9 to transfer to liquid LB, waiting to be sequenced.