WEEK 13

6.22 Shuhan Liu

Photocopying

11 groups of 660 bacteria were carried out, and 2 strains meeting the requirements were screened out.

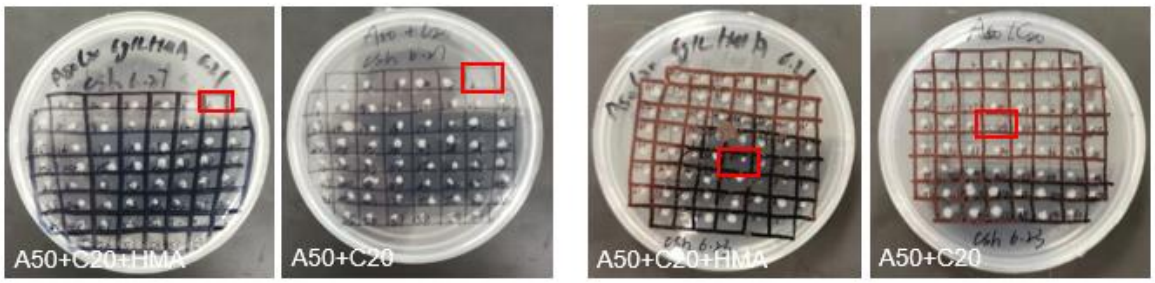

Figure 1 Photocopy of partial results

Photocopy 660 bacteria, of which the 26th on June 23rd and the 6th on June 28th are ideal for re-screening and certification, and the rest are false positive bacteria.

6.26 Sunyue Cai

Re-screening verification

No ideal strains appear.

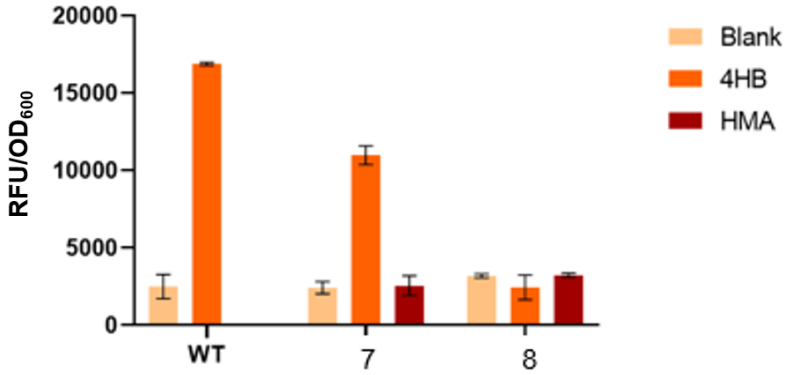

Fig 2 Re-screening verification data of two strains showing good growth conditions in photocopying

A total of two bacteria were re-screened and verified, and they were all false positive bacteria. Bacteria No. 8 did not respond to 4HB and HMA, and ideal strains did not appear.

6.27 Beimeng Zhang

Verification of the efficiency of commercialization to competence

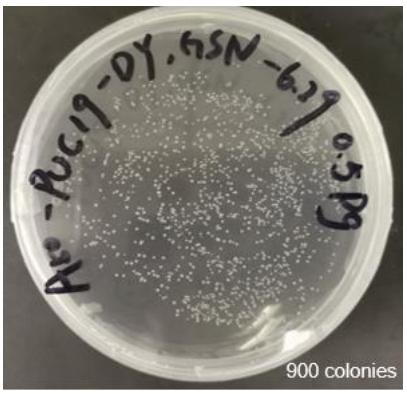

Figure 3 PUC19 plasmid verification DH5a

PUC19 plasmid: 5 pgDNA is transferred to 50 μL competent, 500 μL LB is added, and 0.5 pgDNA (A50) is applied.

The conversion efficiency is $1.8*10^9$/ug.

Figure 4 50 μg/mL ampicillin board verification

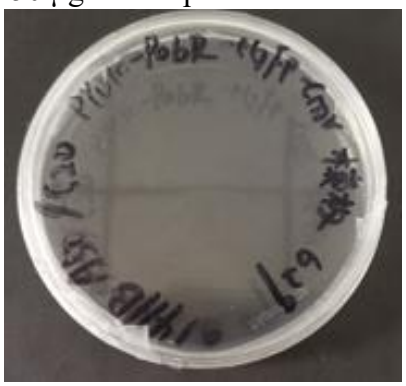

Figure 550 μg/mL ampicillin + 20 μg/mL chloramphenicol + 0.6 g/L 4HB board verification

pYB1a-PobR-Cmr-eGFP 50 pg DNA is transferred to 50 μL competence, 500 μL LB is added, and 5 pg DNA is applied.

19 colonies appeared on the 50 μg/mL ampicillin plate (transformation efficiency is $3.8*10^6$);

no colonies appeared on the 50 μg/mL ampicillin+20 μg/mL chloramphenicol+0.6 g/L 4HB plate.

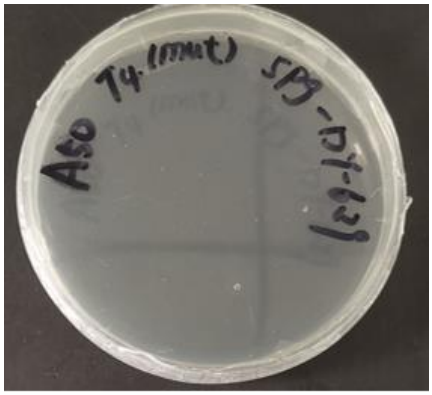

Figure 6 T4 connection Mega digestion product verification DH5a

T4 connects Mega product 50 pg DNA to 50 μL competent, add 500 μL LB, apply 5 pg DNA (50 μg/mL ampicillin). No colonies appeared on the 50 μg/mL ampicillin plate.

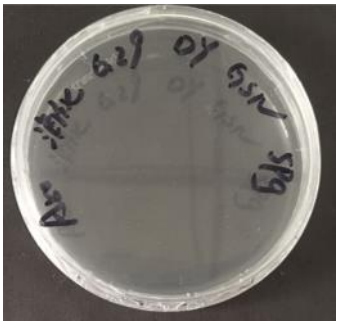

Figure 7 Mega digestion product verification DH5a

Mega digestion and purification product (9.254 ng/μL) 50 pg DNA to 50 μL competent, add 500 μL LB:

(1) Apply 5 pg DNA: ①50 μg/mL ampicillin, ②50 μg/mL ampicillin+20 μg/mL chloramphenicol , ③50 μg/mL ampicillin+20 μg/mL chloramphenicol +0.6 g/L HMA

No colonies appeared on all the plates

6.29 Baitong Shen

Construction of engineering bacteria

Construction of pYB1a-aroGfbr-pheAfbr new point mutant, preparation for *tyrB* knockout

1. Construction of a new point mutant of pYB1a-aroGfbr-pheAfbr (construction of pYB1a-aroGfbr-pheAfbr-2)

It is expected to divide the target gene into four fragments for PCR, use primer design to introduce mutation sites, then use overlap to connect 12 and 34 fragments respectively, use XhoI and BglII to linearize the vector, and then use Gibson for multi-fragment ligation

The primers are as follows:

aroG-F-2	gcctggtgccgcgcggcagcctcgagATGAATTATCAGAACGACGAT

aroG-over1-R	GATCATgttGAGAAACTCACCTGCCGctg
aroG-over2-F	GTGAGTTTCTCaacATGATCACCCCACAATAtct
aroG-over-2-R	CGCACCGGCggtATTAATGGCATCGATAGCCA
aroG-over-3-F	CCATTAATaccGCCGGTGCGCCGCACTGCtt
pheA-over-3-R	CACCGGCTTGtttCGCCATTAACAACGTGgttt
pheA-over-4-F	AATGGCGaaaCAAGCCGGTGCGCTGGTTGAA
pheA-R	ccgaattcaccactagtaccagatctTCAGGTTGGATCAACAGG

The PCR system is as follows:

Snippet 1		Snippet 2		Snippet 3		Snippet 4	
aroG-F-2	1 μL	aroG-over-2-F	1 μL	aroG-over-3-F	1 μL	pheA-over-4-F	1 μL
aroG-over1-R	1 μL	aroG-over-2-R	1 μL	pheA-over-3-R	1 μL	pheA-R	1 μL
2*High Fidelity Mix	25 μL	2*High Fidelity Mix	25 μL	2*High Fidelity Mix	25 μL	2*High Fidelity Mix	25 μL
DDW	22 μL	DDW	22 μL	DDW	22 μL	DDW	22 μL
pYB1a-aroG-pheA	1 μL	pYB1a-aroG-pheA	1 μL	pYB1a-aroG-pheA	1 μL	pYB1a-aroG-pheA	1 μL

PCRCondition:

PCR conditions for fragment 1, 2, 4		PCR conditions for fragment 3		
98 ℃	2 min	98 ℃	2 min	
98 ℃	30 s	98 ℃	30 s	X30
60 ℃	30 s	60 ℃	30 s	
72 ℃	30 s	72 ℃	30 s	
72 ℃	3 min	72 ℃	3 min	

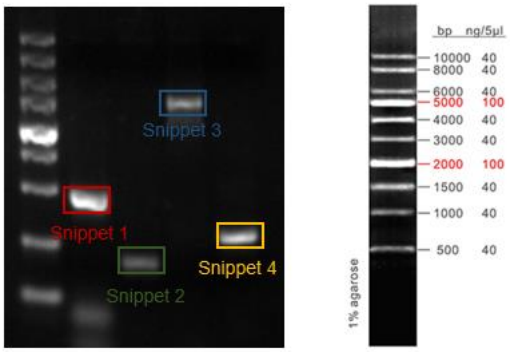

Figure 8 Detection gel image of fragment 1, 2, 3, 4

Fragments 1, 3 are gel-recovered, and fragments 2, 4 are directly purified.

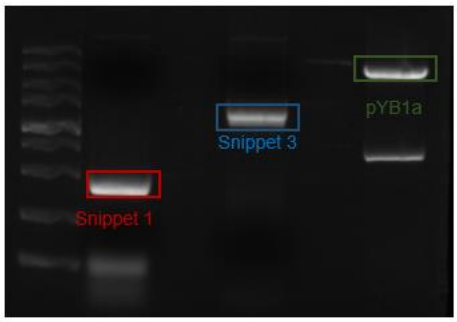

Figure 9 The detection glue after the glue is recycled2）*tyrB* knockout preparation

After redesigning the primers, use the following system for fragment amplification ：

pTarget-tyrB PCR System		PCR Condition		
tyrB-target-F-2	1 μL	98 ℃	3 min	
tyrB-target-R-2	1 μL	98 ℃	30 s	X30
2*High Fidelity Mix	25 μL	60 ℃	30 s	
DDW	22 μL	72 ℃	1 min	
pTarget-613	1 μL	72 ℃	3 min	

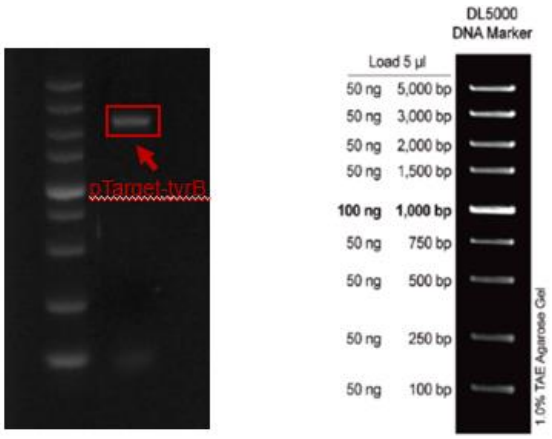

Figure 10 pTarget-tyrB detection gel map

After the PCR product was purified, it was digested with DpnI, and then all transferred to T1 competence. After 12 hours, the growth status is as follows:

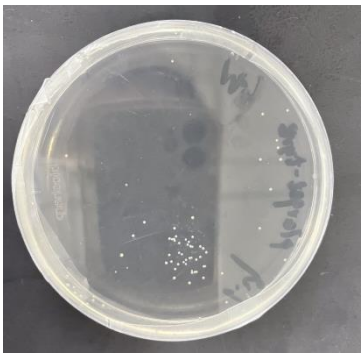

Figure 11 T1 Competent Growth State after 12h

Pick 3 of the colonies and use liquid LB to expand the culture, extract the plasmid, and sequence.