WEEK 16

7.14 Jingwen Chu

Re-screening

The first and second rounds of re-screening with a total of 10 plates have been completed.

Verify whether the colony obtained after transformation coating (50 μg/mL ampicillin+20 μg/mL chloramphenicol+0.6 g/L HMA) corresponds to HMA.

Culture system (deep well plate): 200 μL LB+0.1% 50 mg/mL ampicillin+1% bacterial solution

Re-screening system (deep hole plate):

Control group:

Empty LB+0.1% 50 mg/mL ampicillin

LB+0.1% 50 mg/mL ampicillin+1% DH5αPobR^{WT} bacterial liquid

LB+0.06 g/L 4HB+0.1% 50 mg/mL ampicillin

Test group

LB+0.06 g/L HMA+0.1% 50 mg/mL ampicillin+0.1% bacterial liquid

Experimental results:

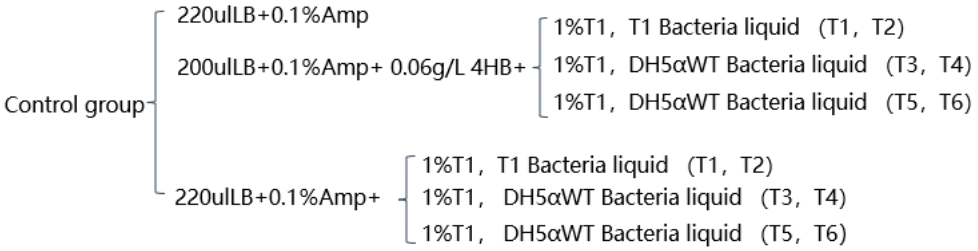
1. The first round of re-screening (selecting bacteria) and the second round of re-screening (cultivating in a deep well plate for 12 hours, taking the bacterial solution plus inducer and incubating for 12 hours according to the enzyme label) have all been completed.
2. In the first round of re-screening, a total of 43 bacteria entered the re-screening verification.
3. In the second round, a total of 43 bacteria entered the re-screening verification.
4. There are 30 overlapping bacteria in the first and second rounds of re-screening verification.

7.15 Shuhan Liu

Re-screening verification

The strains with higher fluorescence in the re-screening will enter the re-screening verification, and all current experimental data will be summarized.

Use a microplate reader to verify whether the re-screened strains correspond to HMA, and at the same time exclude false positives caused by PobR protein off-target.



Test group:

220 μL LB+0.1% 50 mg/mL ampicillin+1% DH5αmut bacterial liquid

200 μL LB+0.1% 50 mg/mL ampicillin+0.06 g/L 4HB+1% DH5αPobR^{mut} bacterial liquid

200 μL LB+0.1% 50 mg/mL ampicillin+0.06 g/L HMA+1% DH5αPobR^{mut} bacterial liquid

The strains selected in the first round of re-screening (selection of bacteria) and the second round of re-screening (deep-well plate culture) were subjected to two re-screening verifications. The following is the 43 selected in the first round of re-screening (selecting bacteria) Strain re-screening verification data is summarized.

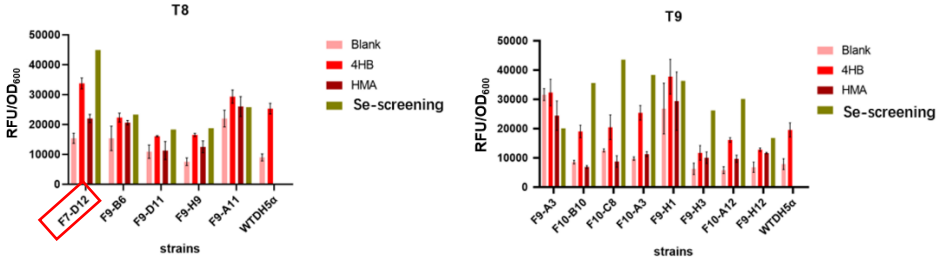


Figure 1 Part of the results of the re-screening verification experiment

7.17 Linxi Jiang

Verification of possible strains

Propose plasmid transformation of BW bacteria, perform HMA gradient verification and second re-screening verification, and response experiments of analogs.

1. In the re-screening verification, the plasmids of the strains with better response to HMA were transformed into BW for verification, painted *A50*; *A50*+*C20*; *A50*+*C20*+HMA.
2. The bacteria induced by HMA are selected for HMA gradient verification.
3. Use HMA analogs for induction to verify the specificity of the mutant strain's response.
4. This week, F-7 D12, F-7 A10, F-5 E4 plasmids were transferred to BW plate, only D12 was induced by HMA, and the other two strains were not induced.

A total of 410,000 mutant strains were screened during the experiment, and the mutation of strain F-7 D12 appeared to be a good result. Summary of F-7 D12HMA four re-screening verification results:

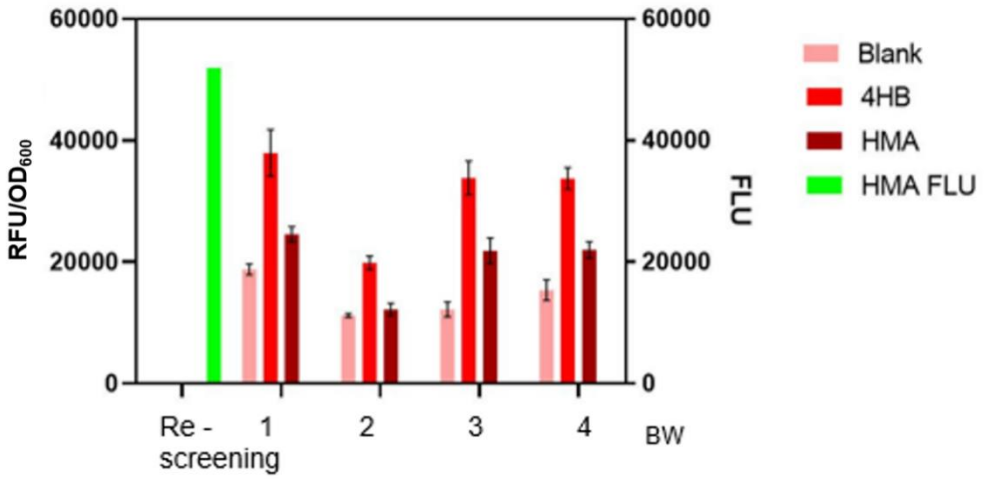


Figure 2 Verification results of four times of microtiter plate re-screening

This week, F-7 D12, F-7 A10, F-5 E4 plasmids were transferred to BW plate, only D12 was induced by HMA, and the other two strains were not induced.

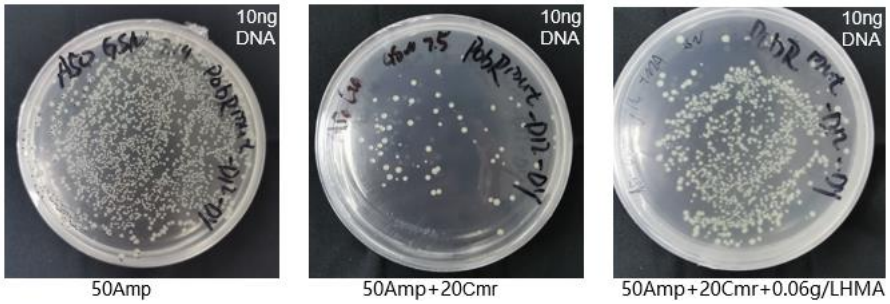


Figure 3 F-7 D12 coating results

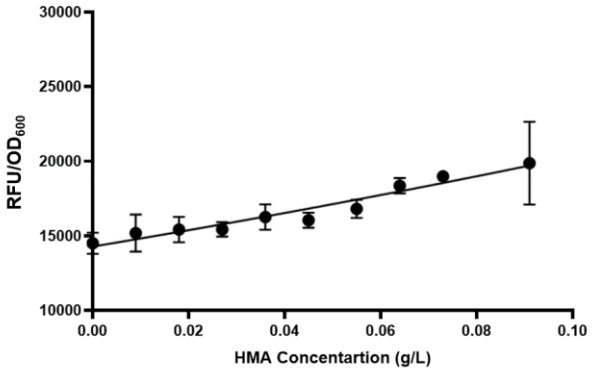


Figure 4 F-7 D12HMA gradient response results

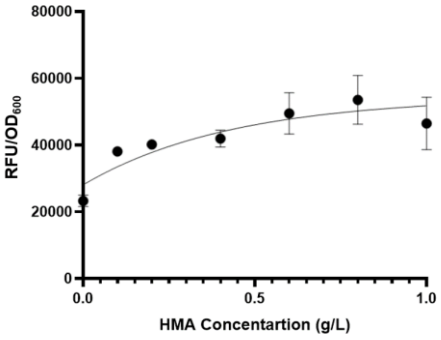


Figure 5 F-7 D12HMA saturation concentration verification result

When the concentration of HMA reaches 0.5 g/L, the response of D12 to HMA is basically saturated.

Use HMA analogs to induce D12, and compare the induced fluorescence value with the fluorescence values induced by LB and HMA to test its specificity.

Experimental group (200μL system): LB+0.1 g/L analogue+0.1% 50 mg/mL ampicillin+0.1% bacterial solution

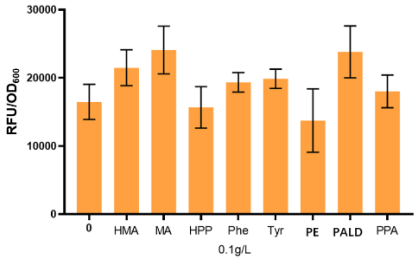


Figure 6 The results of D12 response induced by various analogs are shown in the figure

7.20 Juan Luo

D12 site-directed saturation mutation

T4 connection after PCR, re-screening and re-screening verification after transformation and coating. Strains with a higher response to site-directed mutagenesis enter the re-screening.

PCR System (50μL)		Link System (50μL)		PCR Condition		
2x mix	25 μL	T4 Ligase	5 μl	95 ℃	5 min	
pobR-mut 2-F	1 μL	Buffer	5 μl	95 ℃	30 s	30×
pobR-mut 2-R	1 μL	DNA	25 μl	63 ℃	30 s	
F7D12 Plasmid	1 μL	DDW	15 μl	72 ℃	4 min	
DDW	22 μL	Overnight connection		72 ℃	10 min	

Conversion coating:166 ng DNA was transferred to 100 μl DH5α competent, and 41.5 ng DNA were respectively coated on a 50 μg/mL ampicillin+50 μg/mL chloramphenicol+0.06 g/L HMA plate and incubated at 37°C for 12 hours.

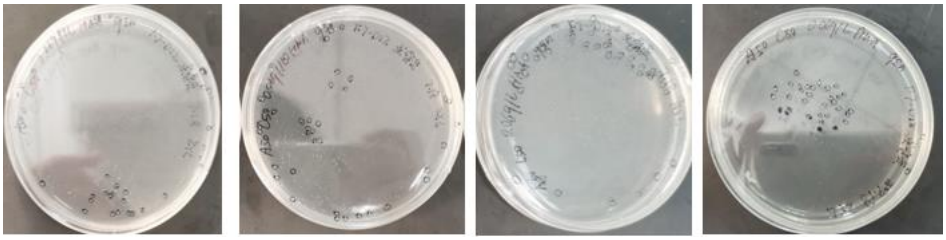


Figure 7 Growth of plate bacteria

Re-screening:

Culture system (deep well plate): 200μL LB+0.1% 50 mg/mL ampicillin+select bacteria

Re-screening system (deep hole plate)
Control group:
Empty LB+0.1% 50 mg/mL ampicillin
LB+0.1% 50 mg/mL ampicillin+1%Bacteria liquid
LB+0.1g/L 4HB+0.1% 50 mg/mL ampicillin
Test group:
LB+0.06 g/L HMA+0.1% 50 mg/mL ampicillin+0.1% bacterial liquid