WEEK 18

7.28 Fengqianrui Chen

Changes in the calculation method of induction strength

The calculation method of induction strength has been changed to make the calculation of induction strength more accurate.

Use the new calculation method to recalculate the data:

Background level expression intensity (I0) = (F background expression-FDH5α not transfected into plasmid)/OD background expression

4HB induction intensity (IB) = (F4HB induction-F DH5α without plasmid transfer)/OD4HB induction

HMA induction intensity (IA) = (FHMA induction-F DH5α without plasmid transfer)/ODHMA induction

Analogue induction intensity (I analogue) = (F analogue induction-F DH5α not transfected)/OD analogue induction

Note: F is the fluorescence reading of the microplate reader.

7.29 Jiameng Nie

Re-processing of re-screening data

The new induction intensity calculation method re-processes the re-screening data, and 47 strains of bacteria are screened out for re-screening verification.

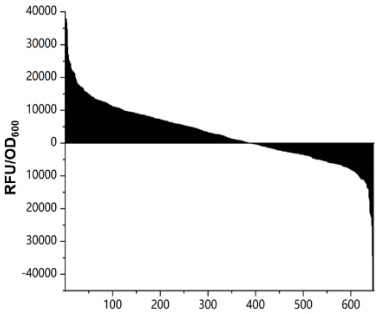

Figure 1 Re-screening and reprocessing data of strain 647

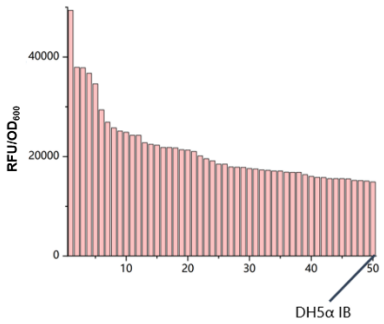

Figure 2 Re-screening re-screening diagram of 49 strains

Select the top 49 strains (including two duplicate strains) with a higher degree of induction than the DH5α control IB, and their induction is shown in the figure on the right.

1	F1-D9	8	F5-H1	15	F9-A11	22	F4-G4	29	F2-G9	36	F3-G6	43	F9-A3
2	F9-A7	9	F4-A11	16	F9-C6	23	F4-C4	30	F3-A7	37	F1-H6	44	F1-B4
3	F3-H3	10	F1-C3	17	F4-D5	24	F3-E5	31	F3-H4	38	F2-B9	45	F1-E7
4	F9-C4	11	F2-A5	18	F5-C9	25	F6-H4	32	F9-A12	39	F9-A10	46	F3-B7
5	F2-B4	12	F4-G11	19	F4-C7	26	F2-C12	33	F9-A6	40	F4-A9	47	F3-H7
6	F3-F3	13	F4-C12	20	F1-D4	27	F5-B10	34	F1-D5	41	F9-A9	48	F9-C12
7	F4-A12	14	F5-G1	21	F3-C4	28	F7-D2	35	F3-G3	42	F9-A8	49	F7-D12

Figure 3 47 strains that responded well to HMA

Note: F1-D9 and F5-H1 are the same strain; F1-E7 and F5-G1 are the same strain, which is a repeated experiment.

7.31 Kairui Su

Re-screening verification

In accordance with the new re-screening verification system, the 47 strains obtained from the re-screening were re-screened and verified, and the induction value was calculated by the new calculation method, and the 6 strains with better results were re-screened and verified.

Secondly verify whether the 47 strains screened out by the re-screening respond to HMA to rule out the possibility of PobR protein off-target.

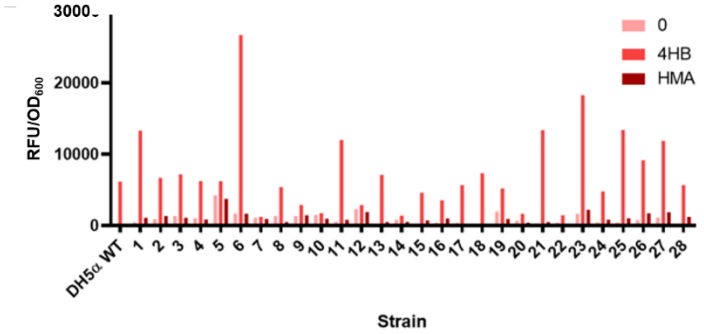

Figure 4 0726 re-screening verification data map

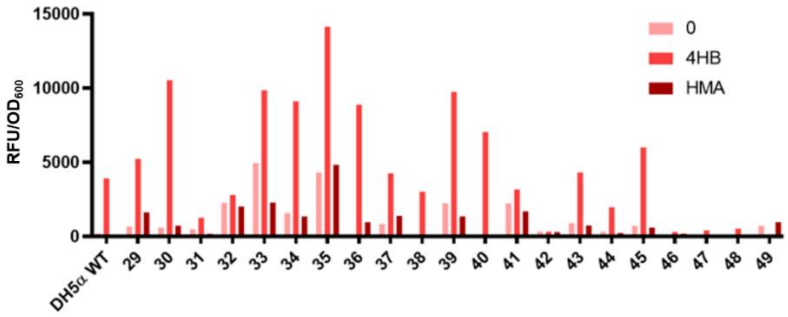

Figure 5 0726 re-screening verification data map

Note: There are two strains in the 49 sets of data that are duplicated, so there are actually 47 strains

We sorted 49 sets of data in descending order of IA/I0, and obtained 6 strains that

responded well to HMA, displayed as shown in the figure, and performed the next step to repeat the re-screening verification.

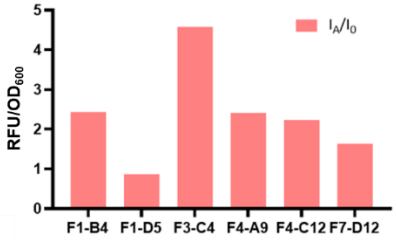

Figure 6 0726 re-screening verification of six strains that responded well to HMA

We re-screened and verified the 6 strains that responded well to HMA. The result is shown in the figure.

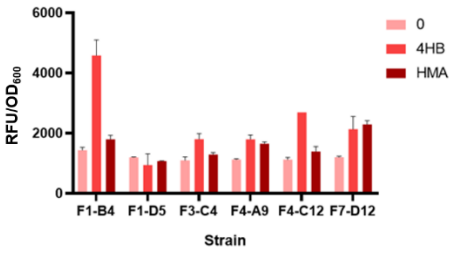

Figure 7 0727 re-screening verification data map

In this experiment, the DH5α plasmid-free bacteria did not grow, and the OD of the 4HB-induced strains was generally lower than that of the non-induced and HMA-induced strains.

Our preliminary analysis is that the 4HB concentration is too high and the acidity is strong, which is not conducive to bacterial growth.

We plan to reduce the 4HB concentration to 0.1 g/L and re-screen verification.

The two strains with higher HMA response in 0727 were re-screened and verified by reducing the 4HB concentration.

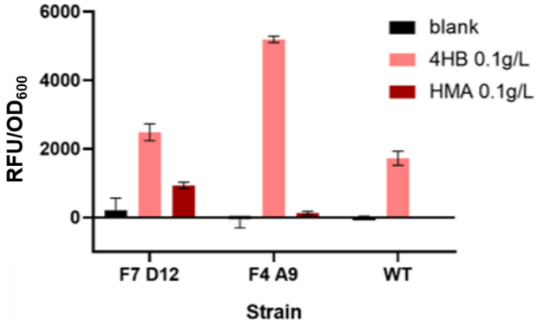

Figure 8 0728 re-screening verification data map

The growth state of the bacteria is stable (OD=0.7)

As can be seen from the figure, F7 D12 has a certain response to HMA, and F4 A9 has a weak response to HMA. It is speculated that the possible reason is that the final concentration of HMA is low, which leads to a poor response state of F4 A9.

Subsequent re-screening verification will unify the final concentration of the inducer: the final concentration of HMA is 1 g/L, and the final concentration of 4HB is 0.1 g/L.

Plan to re-screen and verify the six bacteria again.

7.31 Yifan Bian

HMA gradient verification

HMA gradient verification for strains with a higher degree of induction (F4-A9).
We performed HMA gradient verification on the bacteria with better induction degree (F4-A9) to further determine the HMA concentration range.

Control group:
200 μL LB+1‰ 50 mg/mL ampicillin
200 μL LB+1% DH5α non-transformed plasmid bacterial solution
200 μL LB+1‰ 50 mg/mL ampicillin+1% DH5αPobR^{WT} bacterial solution
196.6 μL LB+1‰ 50 mg/mL ampicillin+ 3.4 μL 6 g/L 4HB+1% DH5αPobR^{WT} bacterial solution (final concentration is 0.1 g/L)

Experimental group (200 μL system): LB+HMA+1‰ 50 mg/mL ampicillin+1% bacterial solution (HMA concentration is 0, 0.01, 0.05, 0.08, 0.1, 0.5, 0.8, 1)

Note: The concentration of HMA mother liquor used is 6 g/L and 50 g/L

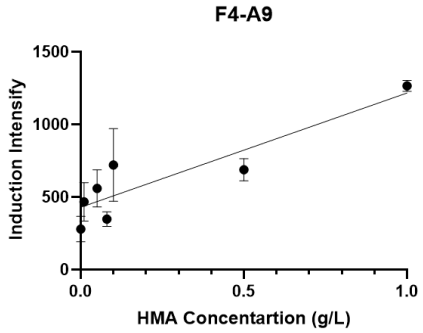

Figure 9 The degree of response of F4-A9 to HMA

In the concentration range of 0-1.0 g/L, as the concentration of HMA increases, the overall induction intensity shows an upward trend.

8.2 Zhihao Wang

Verification of HMA analog response

Re-authenticate analog verification of strains with a higher degree of induction (F7-D12, F4-A9).
Use HMA analogs to induce F4-A9, and compare the induced fluorescence value with the fluorescence values induced by LB and HMA to test its specificity.

Control group:
200 μL LB+1% DH5α non-transformed plasmid bacterial solution
200 μL LB+1‰ 50 mg/mL ampicillin+1% DH5αPobR^{WT} bacterial solution

196.6 μL LB+1‰ 50 mg/mL ampicillin+3.4 μL 50 g/L 4HB+1% DH5αPobR^{WT} bacterial solution (final concentration is 0.1 g/L)
196 μL LB+1‰ 50 mg/mL ampicillin+4 μL 50 g/L HMA+1% DH5αPobR^{WT} bacterial solution (final concentration is 1 g/L)

Experimental group (200μL system):
LB+analogs+1‰ 50 mg/mL ampicillin+1% bacterial solution (analogs include MA, HPP, Phe, Tyr, PPA, phenylethanol, phenylacetaldehyde, Trp)

Note: The concentration of the analog mother liquor used is 50 g/L.

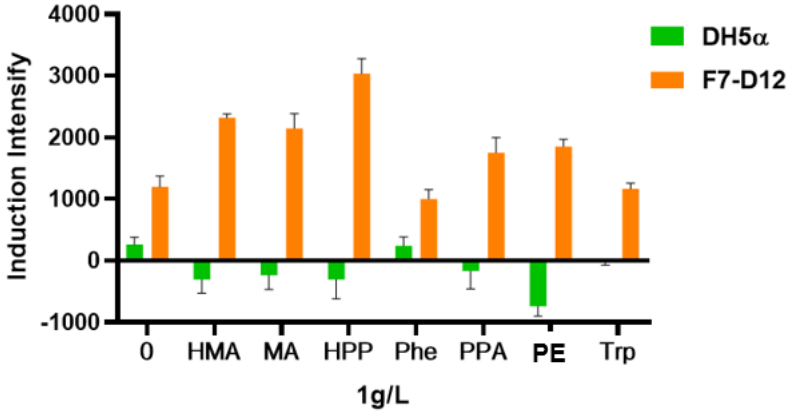

Figure 10 Correspondence of DH5αPobR^{WT} and F7-D12 to multiple analogs
Note: 1 g/L MA, HPP, Phe, PPA, phenethyl alcohol, and Trp are used to induce DH5α PobR^{WT} and F7-D12. The fluorescence results are shown in the right figure.

The enzyme labeling program of this HMA analog verification experiment was changed to Gain=80 Manual; Mirror=50% Mirror.

By comparing with the background expression, we believe that F7-D12 in this set of data still has a high response to MA and HPP.

We still found that F7-D12 did not grow in 1 g/L Tyr and phenylacetaldehyde, but the fluorescence of F7-D12 with phenylacetaldehyde was extremely high; the preliminary analysis was due to the high concentration of hydrochloric acid when Tyr used hydrochloric acid configuration, and high concentration of phenylethyl Aldehydes are harmful to cells.

8.3 Chuyao Xu

F4-A9 sequencing results
Sequence F4-A9 and analyze its mutation sites

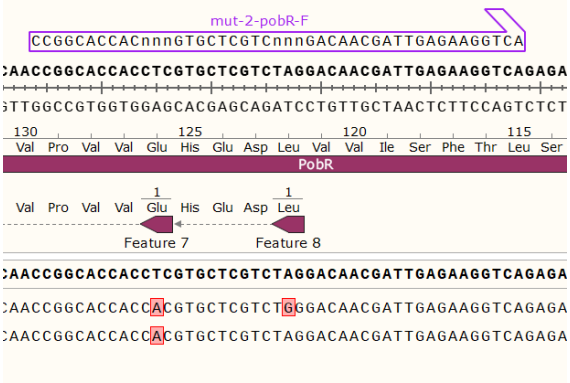

Figure 11 F7-D12 connection result sequence diagram

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

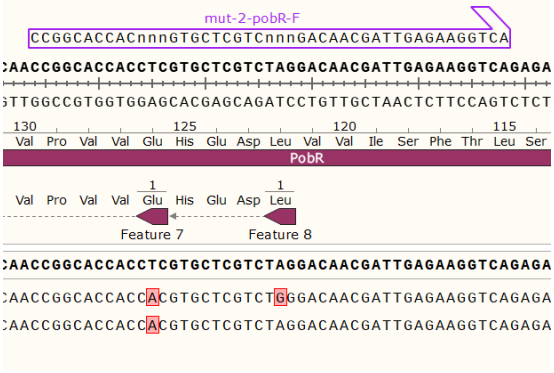

Figure 12 Sequence diagram of F4-A9 sequencing results

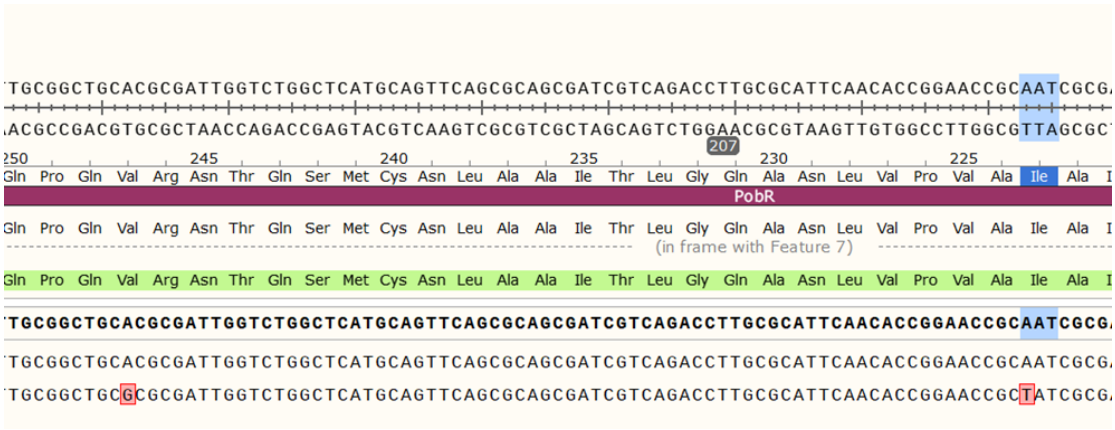

Figure 13 Sequence diagram of F4-A9 sequencing results

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