Screening for HMA-responsive PobR mutants

4.6 Zhihao Wang

Preliminary experiments of chloramphenicol screening

Our screening system: 200 μL LB + 1% bacteria solution (T1/pYB1a-PobR-eGFP-Cmr) + 10% 0.6 g/L 4HB + 0.1% ampicillin (50 mg/mL) + 0.1% chloramphenicol in different concentrations

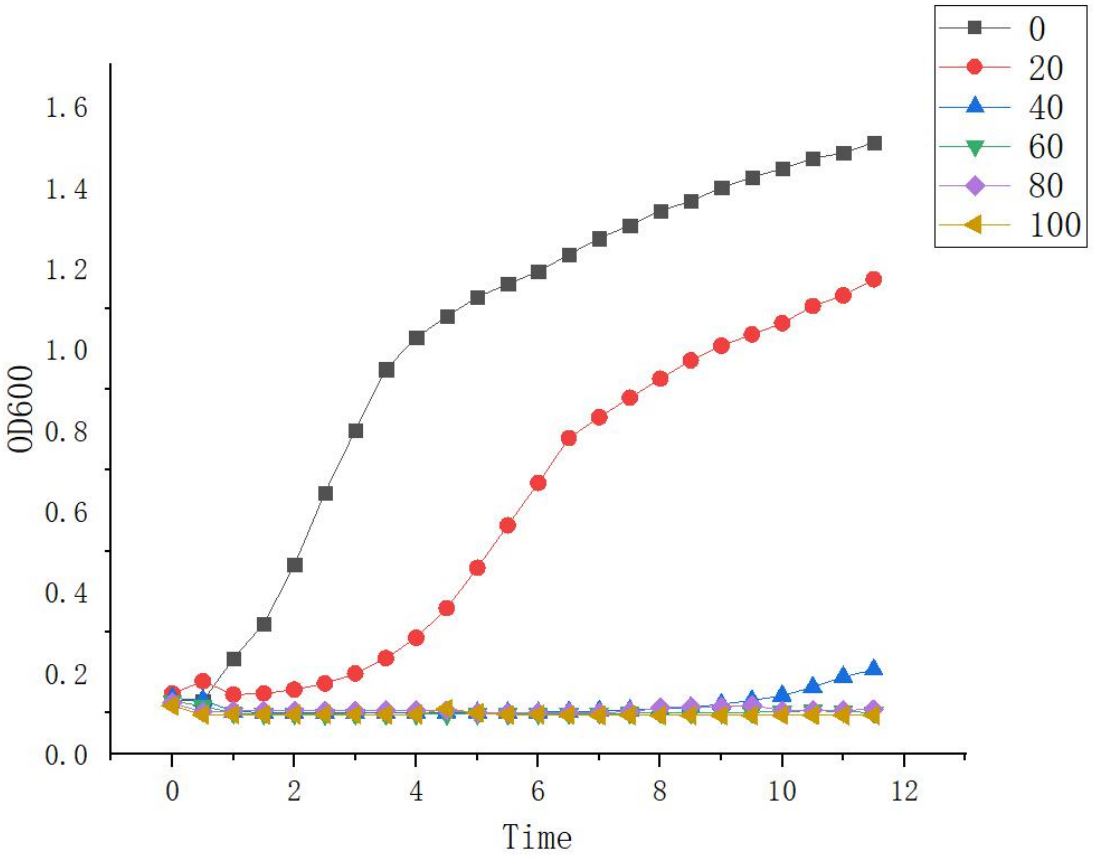

Figure 1 0.6 g/L 4HB different concentrations of chloramphenicol

The chloramphenicol concentration of 20 mg/mL made bacteria (T1/pYB1a-PobR-eGFP-Cmr) survive with 0.6 g/L 4HB's induction, so we chose this chloramphenicol concentration for a follow-up experiment.

6.11 Jingwen Chu

Preliminary experiment of flat plate screening

Then, the plate screening feasibility experiment was carried out, and the BW control bacteria (only responding to 4HB) were quantified and plated:

LB+0.1 g/L 4HB+50 μg/mL ampicillin+20 μg/mL chloramphenicol, LB+50 μg/mL ampicillin+20 μg/mL chloramphenicol, LB+50 μg/mL ampicillin.

Calculate the number of colonies in each plate and compare them. If the number of colonies on the plate containing 4HB is similar to that on the 50 μg/mL ampicillin plate, the plate screening is feasible. The specific operation is as follows: BW control

bacteria are cultured on a shaker at 37°C for 8 hours, and LB is used to quantify the OD_{600} of the bacterial solution. For 4, use the gradient dilution method to dilute the bacterial solution (OD_{600} = 4) by 104, and then count the plate after coating 100 μL.

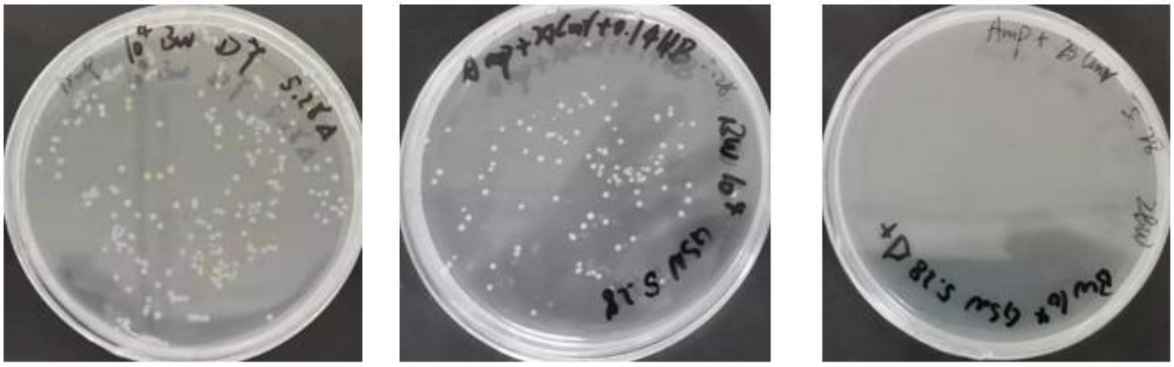

Figure 2 Results of re-screening verification

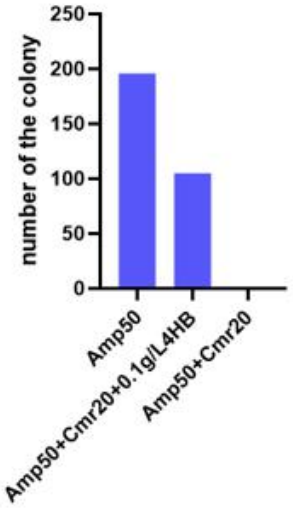

Figure 3 Coated board data chart

Therefore, it is feasible to use plates for preliminary screening.

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.

8.7 Chao Chen

Re-screening

We stabilized the system and screened the 647 clones selected last time (Figure 2), then we re-screened the clones (83 clones in total) which induction intensity of HMA is higher than the induction intensity of 4HB to DH5αPobR^{WT} (about 800) .

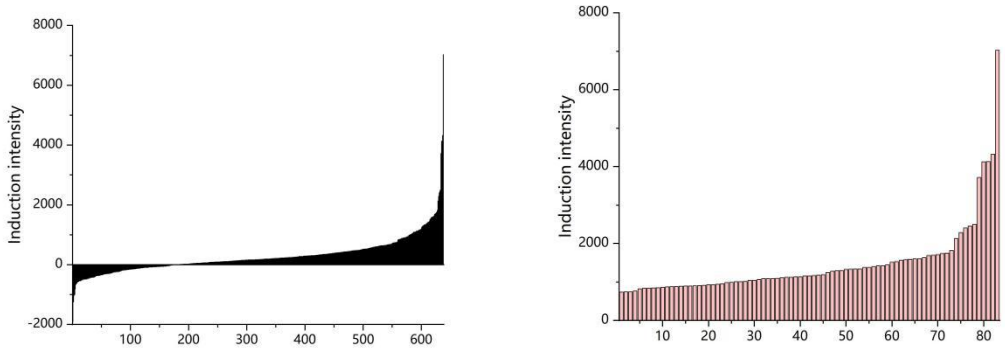

Figure 4 Left, 647 clones' induction intensity of HMA; right, 83 clones' induction intensity of HMA which were higher than 800

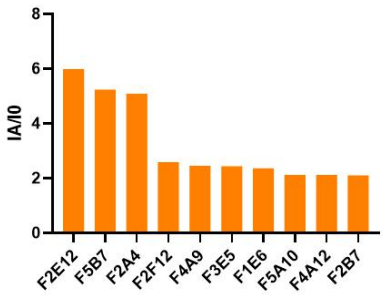

Figure 5 10 clones' ratio of IA/I0 which were highest in 83 clones