WEEK 2

4.7 Yifan Bian

Optimization of induction conditions

Our screening system: 200 μL LB+1% bacteria solution (T1/pYB1a-pobR-eGFP-Cmr) +10% 4HB in different concentrations + 0.1% ampicillin (50 mg/mL) + 0.1% chloramphenicol (20 mg/mL).

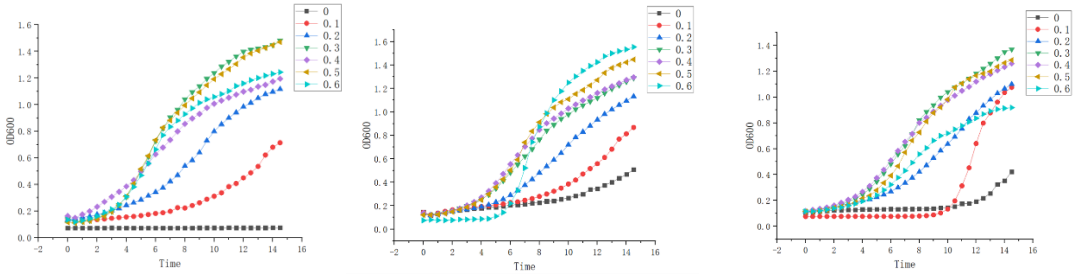

Figure 1 20 mg/mL chloramphenicol different concentrations of 4HB (Three groups of repeats); Bacteria (T1/pYB1a-pobR-eGFP-Cmr) could survive with a low concentration of 4HB, which allowed us to expand our screening; Since there were much rising in the fluorescence values after 8.5 hours, the fluorescence values of 8.5 hour were selected. for fitting

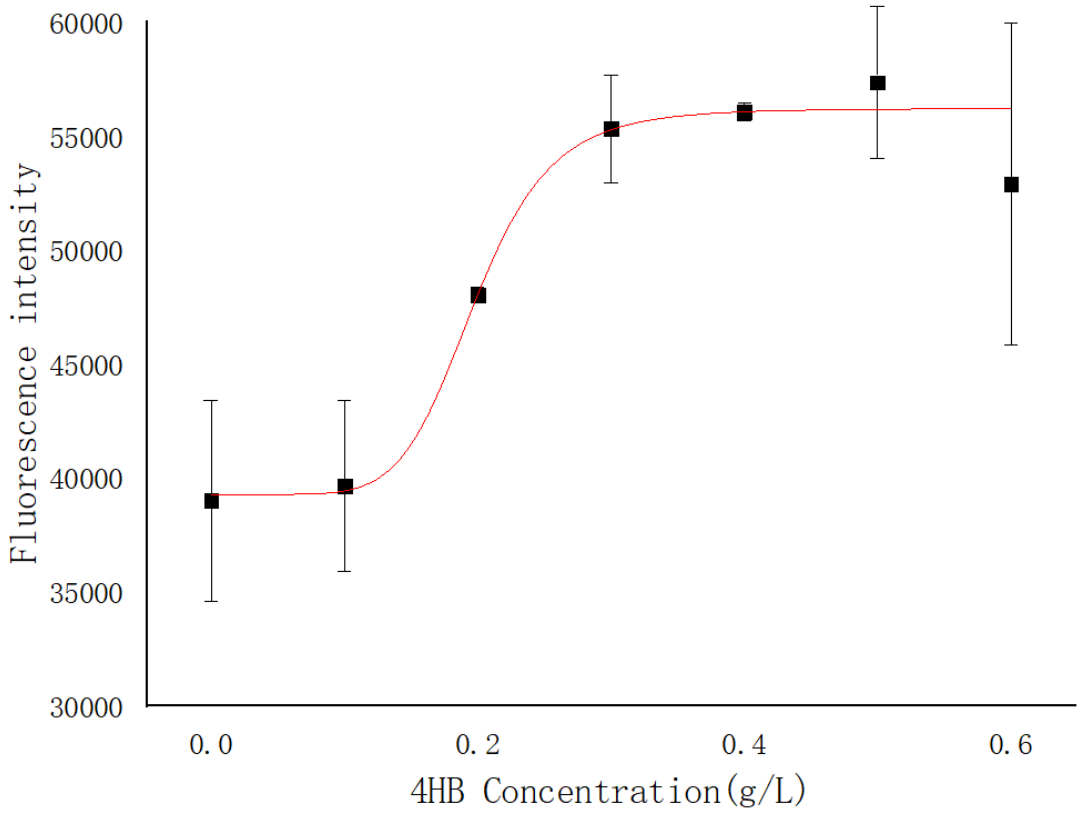

Figure 2 Changes of fluorescence values in different 4HB concentrations in 8.5 hour

4.12 Kairui Su

Screening of random mutation

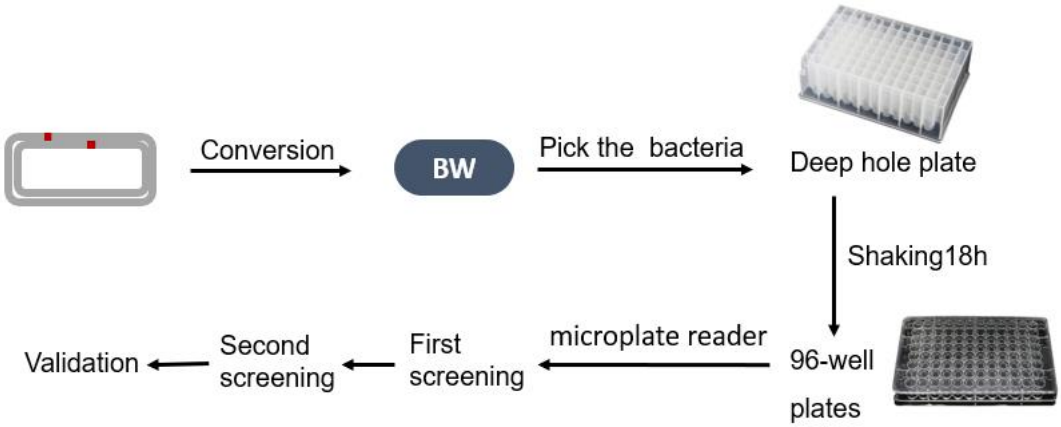

Figure 3 The main process of screening of random mutation

We conducted a round of random mutation screening, among which only 16-F4 and 18-B4 entered the re-screening verification step. Except for blank control, their OD_{600} values were about 1.5.

	1	2	3
F WT	16701	20966	47720
G 16-F4	24234	44331	25861
H 18-B4	35381	36124	34627
	mut	mut+4HB	mut + HMA

WT

WT+4HB

Figure 4 Re-screening validation data of 16-F4 and 18-B4

The fluorescence intensity of 18-B4 mutation was similar to that after induction, indicating that 18-B4 was probably mutated so that the inhibitory effect of PobR disappeared basically. The fluorescence value of 16-F4 was higher than that of uninduced, indicating that the strain could respond to HMA but the response effect was poor. The response of both strains to 4HB was weakened to varying degrees.