WEEK 23

8.25 Linshan Cao

Gene knockout

We picked clones from the plate which were used to isolate monoclones of the clone grew well in 5mL LB after knocking *aspC* out to do further verification (Figure 1, 2, 3). At the same time, we changed the medium to 2×YT medium to provide more nutrition during culture and avoid cell death during culture.

2×YT medium:

Component concentration : 1.6% (W/V) Tryptone, 1% (W/V) Yeast Extract, 0.5% (W/V) NaCl

Amount of configuration : 1L

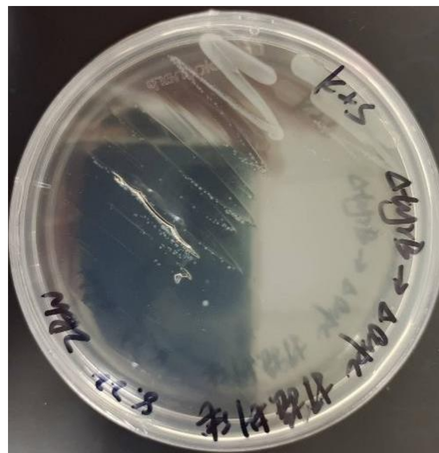


Figure 1 Used streak plate method to isolate monoclones of the clone grew well in 5 mL LB

Reaction system (10μL)	
aspC-F-Over-F/*aspC*-R-Over-R	0.2μL/0.2μL
template	1μL
2×Rapid Taq Master Mix	5μL
ddw	3.6μL

Reaction progress		
98℃	3min	
98℃	30s	×30
58℃	30s	×30
72℃	1min 50s	×30
72℃	3min	

Figure 2 The reaction system and process of colony PCR to test *aspC* knockout using clones picked from the plate

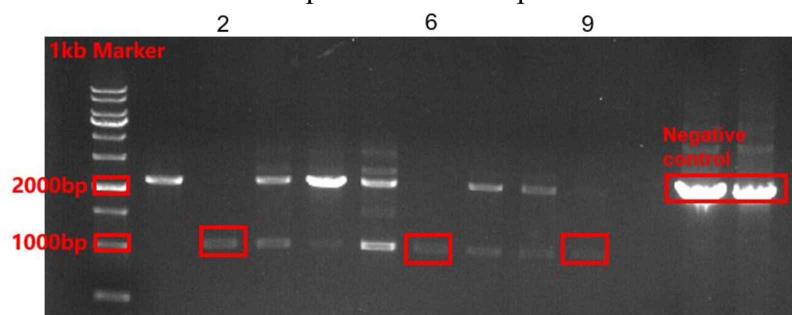


Figure 3 Agarose gel electrophoresis assay of improved colony PCR using clones picked from the plate

Correct bands (1100 bp) were shown in agarose gel electrophoresis assay. We chose

the clones 2, 6, 9 which did not show other unwanted bands to do further tests.

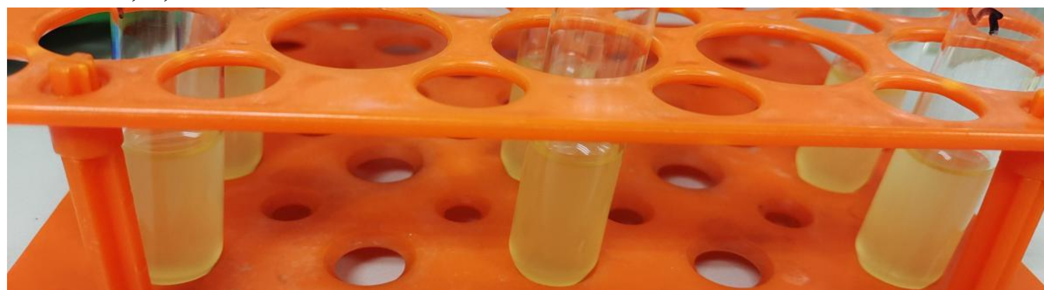


Figure 4 Expanded propagation of the clones 2, 6, 9 using 5 mL 2×YT medium and added 1 mM IPTG to remove Ptarget

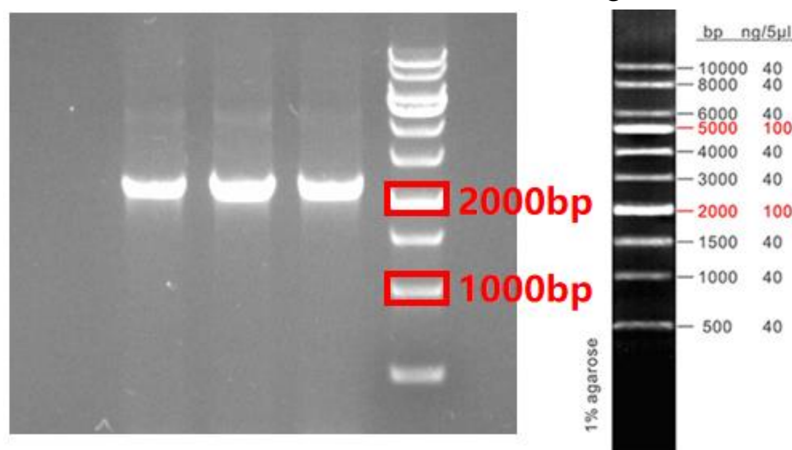


Figure 5 Agarose gel electrophoresis assay of colony PCR using clones 2, 6, 9 after culturing in 2×YT without adding IPTG

Clones 2, 6, 9 could grow in 2×YT. However, agarose gel electrophoresis assay of colony PCR using these three clones did not show correct band (1100 bp) after culturing in 2×YT. Therefore, we suspected that the cultures were polluted.

8.28 Xin Xin

Chloramphenicol ALE pre-experiment

Used clones which have better induction of HMA to do the chloramphenicol ALE pre-experiment again (Figure 6~14).

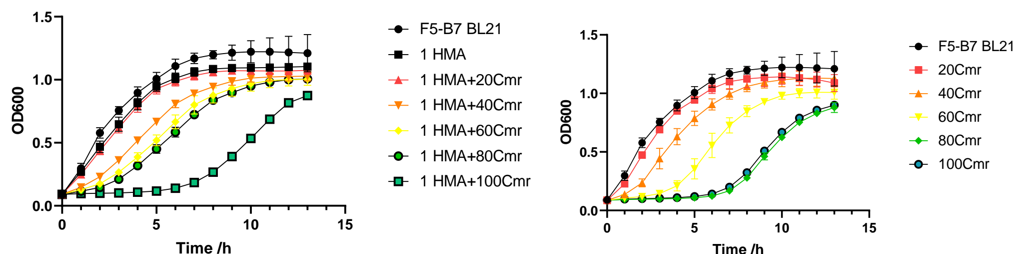


Figure 6 Growth curve of F5-B7-2 under the pressure of different concentrations of chloramphenicol (1_{st}), left, added 1 g/L HMA as inducer; right, no inducer

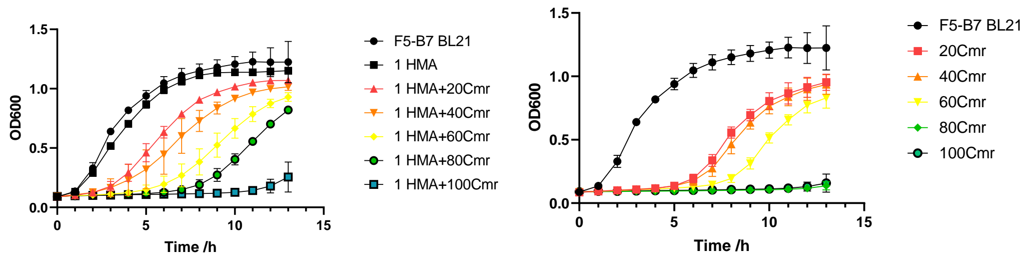


Figure 7 Growth curve of F5-B7-2 under the pressure of different concentrations of chloramphenicol (2_{nd}), left, added 1 g/L HMA as inducer; right, no inducer

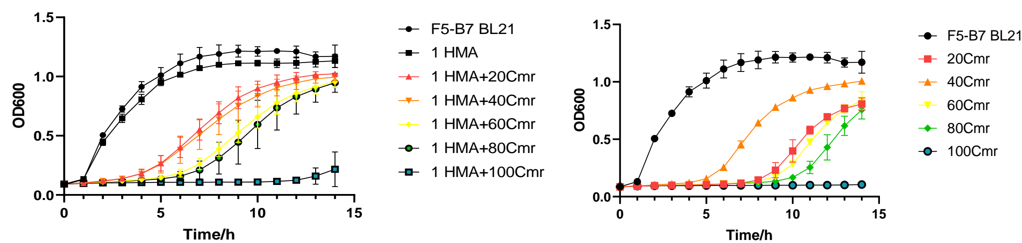


Figure 8 Growth curve of F5-B7-2 under the pressure of different concentrations of chloramphenicol (3_{rd}), left, added 1 g/L HMA as inducer; right, no inducer

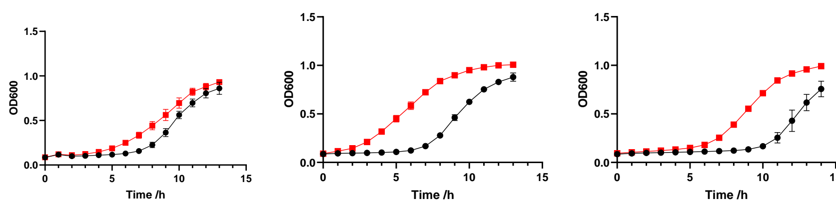


Figure 9 Comparation of the growth curve of F5-B7-2 with or without inducer under the pressure of 80 μg/mL chloramphenicol in three times (from left to right: 1_{st}, 2_{nd}, 3_{rd})

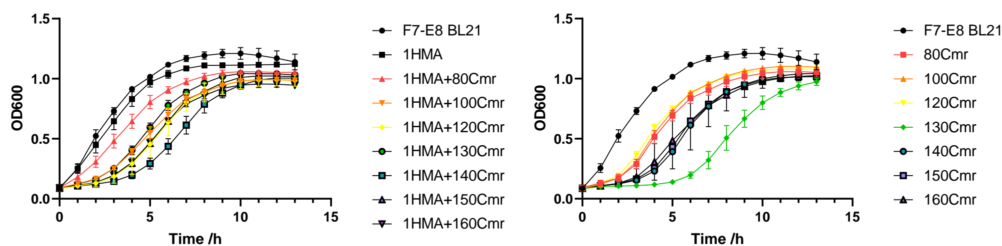


Figure 10 Growth curve of F7-E8-3 under the pressure of different concentrations of chloramphenicol (1_{st}), left, added 1 g/L HMA as inducer; right, no inducer

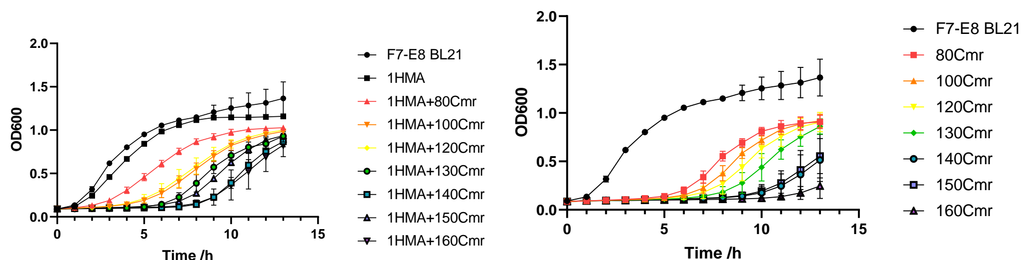


Figure 11 Growth curve of F7-E8-3 under the pressure of different concentrations of

chloramphenicol (2$_{nd}$), left, added 1 g/L HMA as inducer; right, no inducer

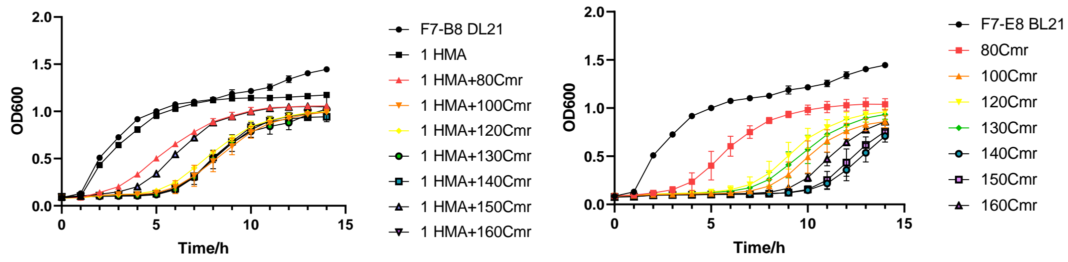


Figure 12 Growth curve of F7-E8-3 under the pressure of different concentrations of chloramphenicol (3$_{rd}$), left, added 1 g/L HMA as inducer; right, no inducer

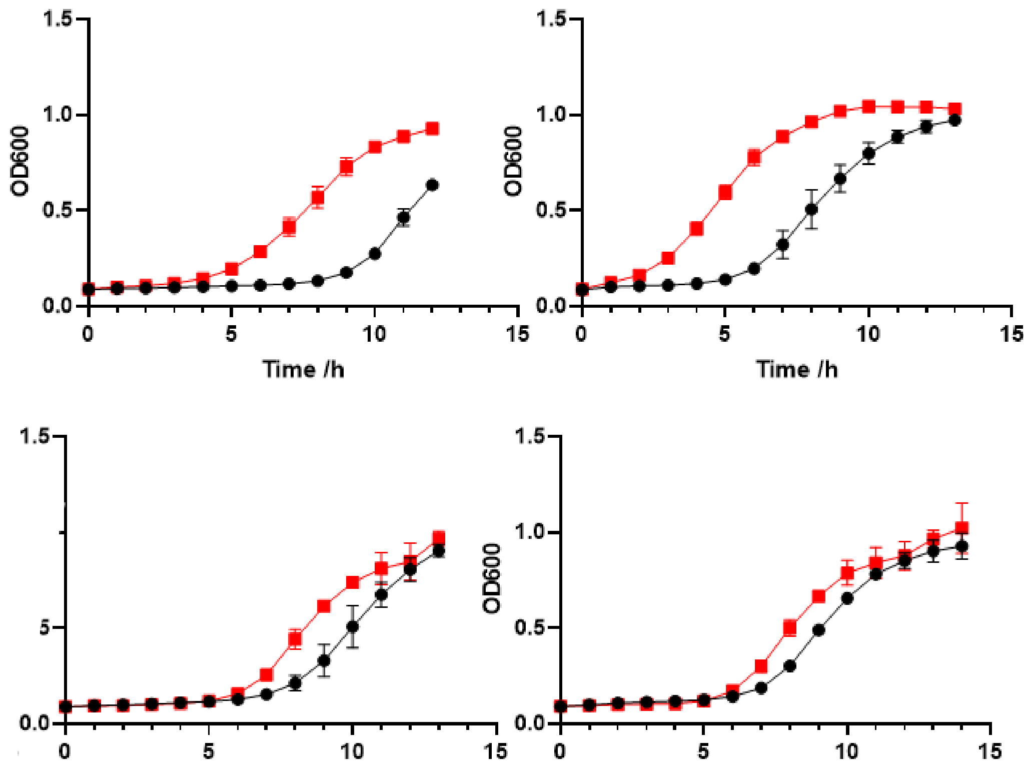
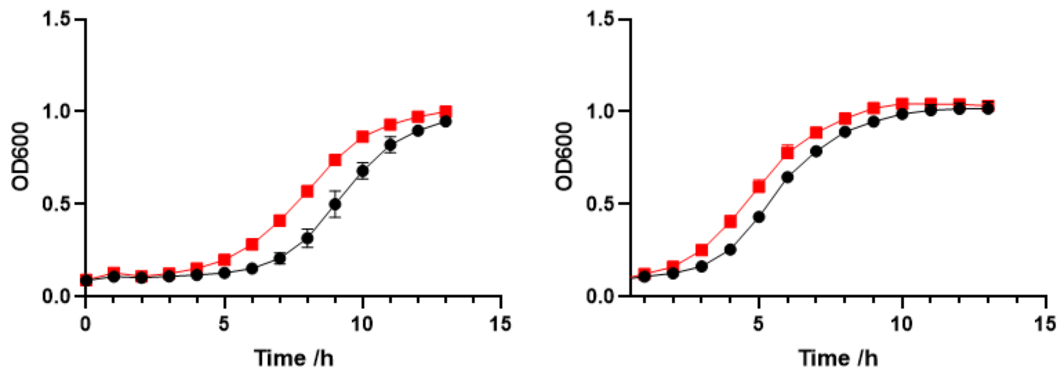


Figure 13 Comparation of the growth curve of F7-E8-3 with or without inducer under the pressure of 130 μg/mL chloramphenicol in four times



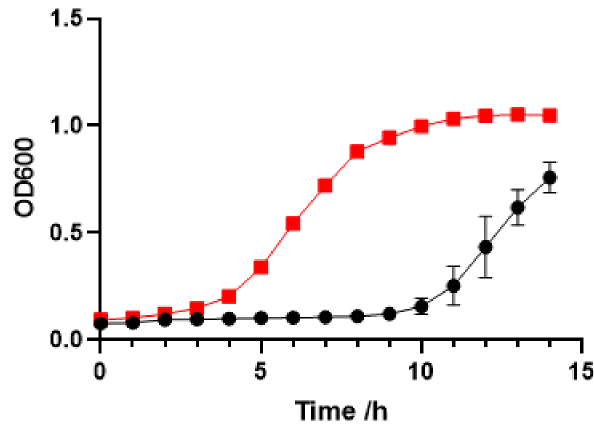


Figure 14 Comparation of the growth curve of F7-E8-3 with or without inducer under the pressure of 150 μg/mL chloramphenicol in three times

From the result we can see that the length of lag phases was different in each time. We speculated the reason is the difference of cell viability when inoculating. Therefore, we use new experimental method to detected the growth of cells under the chloramphenicol pressure：

Compared with the previous experiment, the difference was that the strains were precultured in different ways:

1. F5-B7 BL21 and F7-E8 BL21 were streaked, and single colonies were selected after 12h culture;
2. transfer to 5 mL LB culture for 8 h (OD≈0.5);
3. Continue to transfer 10% bacterial solution to 5 mL LB for 3 h (OD between 0.11-0.12);
4. Add to the plate for overnight enzyme labeling experiment.

Then, we re-did the chloramphenicol ALE pre-experiment of the two clones again using new method (Figure 15~18).

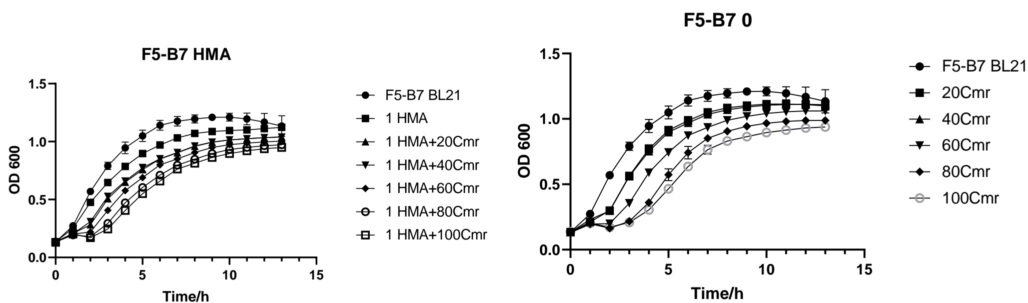


Figure 15 Growth curve of F5-B7-2 under the pressure of different concentrations of chloramphenicol, left, added 1 g/L HMA as inducer; right, no inducer

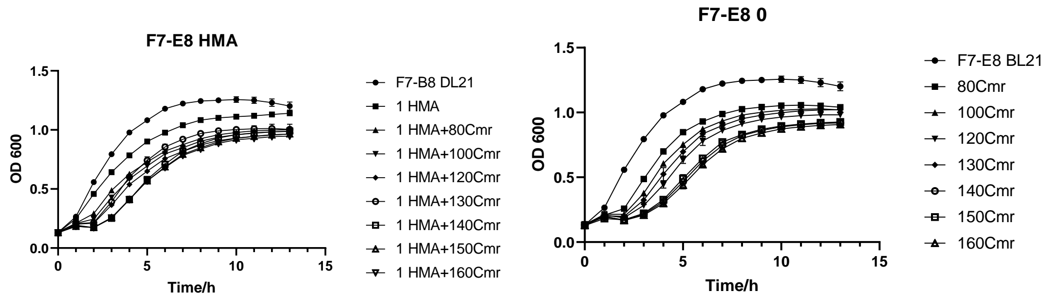


Figure 16 Growth curve of F7-E8-3 under the pressure of different concentrations of chloramphenicol, left, added 1 g/L HMA as inducer; right, no inducer

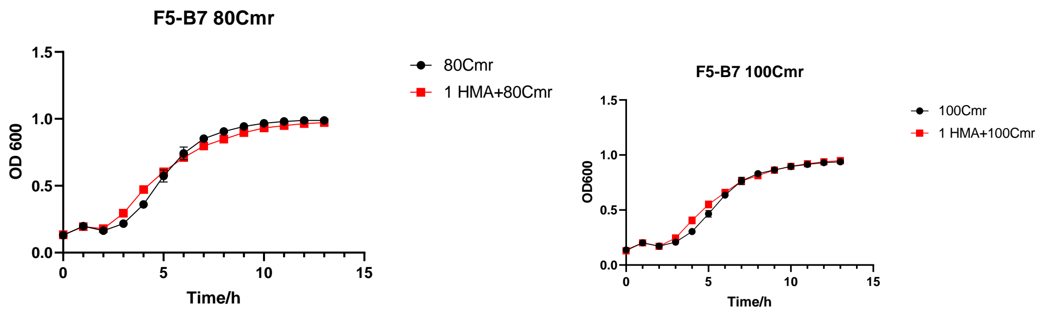


Figure 17 Comparation of the growth curve of F5-B7-2 with or without inducer under the pressure of 80 μg/mL (left) or 100 μg/mL (right) chloramphenicol

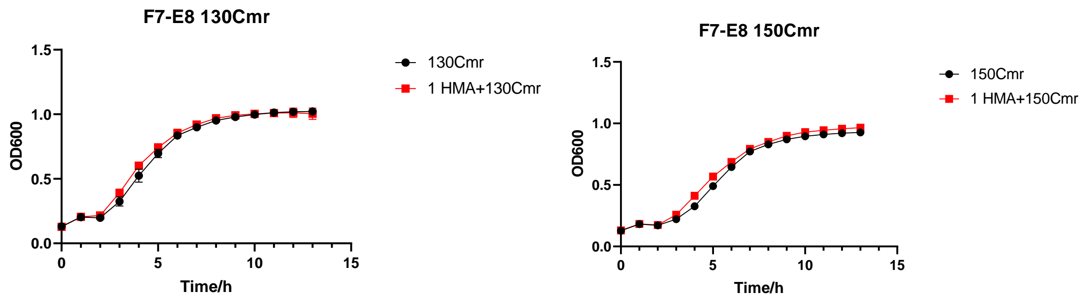


Figure 18 Comparation of the growth curve of F7-E8-3 with or without inducer under the pressure of 130 μg/mL (left) or 150 μg/mL (right) chloramphenicol

From the result we can see that the cells grew faster than before and no remarkable lag phase was shown. However, the growth of cells did not show any difference with or without inducer. This was suspected causing by the too large inoculum size. Therefore, gradient inoculum size experiment should be down.

8.30 Baitong Shen

Fluorescence re-screening of single amino acid mutants

We used re-screening method to detected the induction intensity of HMA for five PobR mutants with single amino acid mutation (Figure 19).

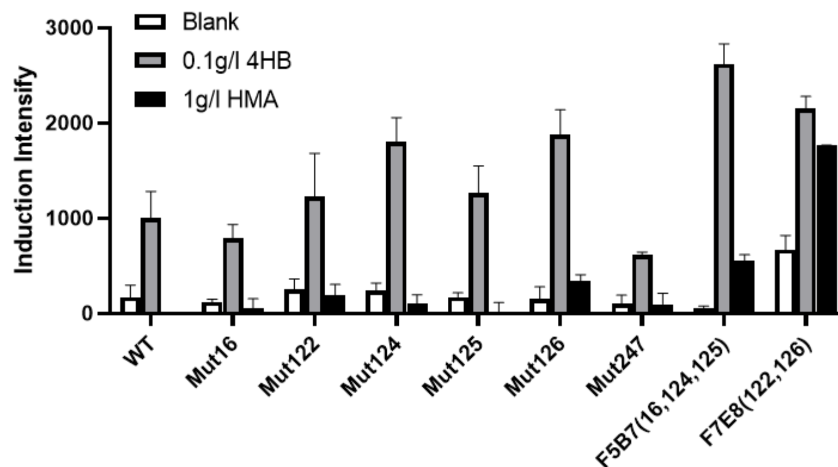


Figure 19 The induction intensity of HMA for five PobR mutants with single amino acid mutation

After analyzing the result, E124G, E126V showed a higher IB than $PobR^{WT}$; E126V might influence the HMA sensitivity of PobR. What needs to be emphasized is that all five single amino acid mutants did not increase the IB or IA to the level of double (P5-B7) or triple mutants (P7-E8). This result supported that the increased induction level of P5-B7 and P7-E8 is caused by the synergistic effect of multiple amino acid mutations.