WEEK 22

8.18 Shuning Guo

Site-directed mutagenesis of the PobR CDS

Picked 2 clones from each of the plate and inoculated into 5 mL LB medium and culture at 37 ℃ for 8 h.

Then, 700 μL culture were used for storage at -20 ℃ and other culture were store at 4 ℃ for further experiments.

8.19 Ziwei Pan

Gene knockout

We tried to knock *aspC* out using BL21*ΔtyrB* eletrocompetent cells prepared last week. At the same time, we re-tested the transform efficiency of BL21*ΔtyrR* chemical competent cells.

Reaction system (10μL)	
aspC-700-F/*aspC*-700-R	0.2μL/0.2μL
2×Rapid Taq Master Mix	5μL
template	1μL
ddw	3.6μL

Reaction process		
98℃	2min	
98℃	30s	×30
52℃	30s	
72℃	1min	
72℃	3min	

Figure 1 The reaction system and process of colony PCR to test *aspC* knockout

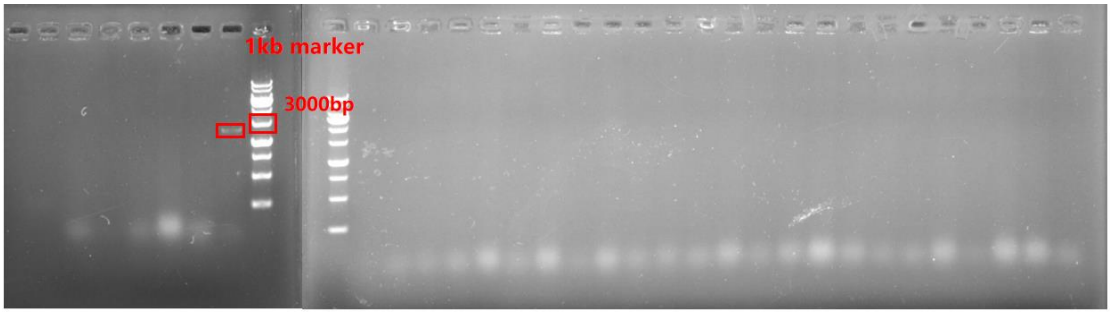

Figure 2 Picked 23 and 6 clones from the plates respectively to have colony PCR

From the result we can see that no correct bands were shown, negative control did not show band either. The reaction system and process of colony PCR needed to be improved.

Reaction system (10μL)		Reaction progress		
aspC-F-Over-F/*aspC*-R-Over-R	0.2μL/0.2μL	98℃	3min	
template	1μL	98℃	30s	×30
		58℃	30s	
2×Rapid Taq Master Mix	5μL	72℃	1min 50s	
ddw	3.6μL	72℃	3min	

Figure 3 The improved reaction system and process of colony PCR to test *aspC* knockout

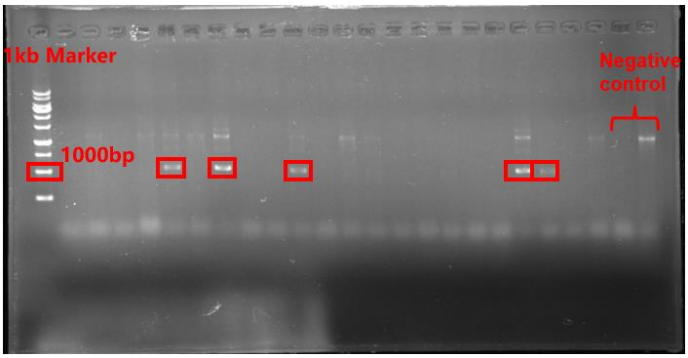

Figure 4 Agarose gel electrophoresis assay of improved colony PCR
5 clones showed correct bands (1100 bp) in agarose gel electrophoresis.

Figure 5 Inoculated 5 clones with correct bands into 5mL LB medium from EP tube and culture at 30 ℃ for 20 h

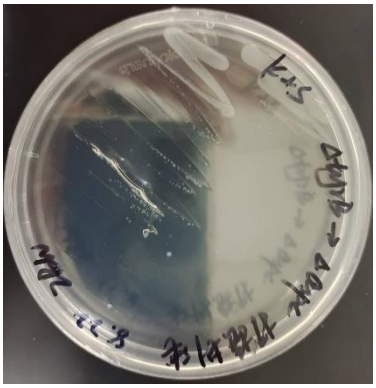

Figure 6 Used streak plate method to isolate monoclones of the clone grew well in 5 mL LB

5 clones showed correct band in the agarose gel electrophoresis assay of improved colony PCR. However, only 1 of the 5 clones can growth in the LB. Therefore, we speculated that the cultures were polluted when culture in EP tube. We used streak plate method to isolate monoclones of the clone growth well in 5 mL LB to reduce the influence of pollution.

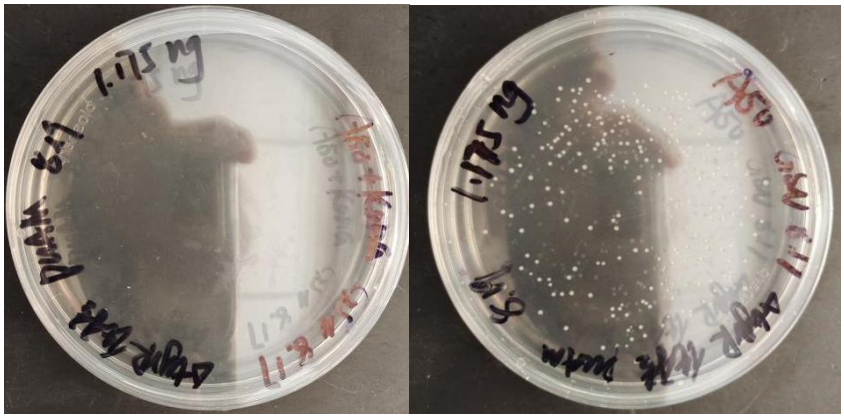

Figure 7 Transformation efficiency test of BL21*ΔtyrR* chemical competent cells
1.175 ng pUAM were mixed with 50 μL BL21*ΔtyrR* chemical competent cells and plated on LB mediums with kanamycin and ampicillin (left) or only ampicillin at 37 ℃ for 12 h to test the efficiency.
From the result we can see that the clones grew much better on the LB medium with only ampicillin. Therefore, we speculated that the Pcas9 plasmid was lost during chemical transformation because the Pcas9 is heat sensitive.

8.20 Beimeng Zhang

Site-directed mutagenesis of the PobR CDS
Sequencing result:
Two clones of spm125 have expected mutation on the plasmids, spm247/16/124 do not.

8.21 Juan Luo

Gradient HMA & 4HB concentration induction assay
Because the IA of F5-B7-2, F5-A10-1, F7-E8-3 does not saturate induced by 1.0 g/L HMA, we further tested their operational ranges of HMA by using higher concentration HMA (Figure 8~10). At the same time, we used gradient 4HB concentration induction assay to test the operational range of 4HB to DH5αWT to test its operational range of 4HB (Figure 11).

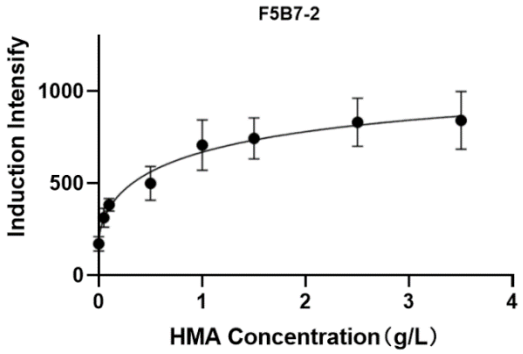

Figure 8 Gradient HMA concentration induction assay of F5-B7-2

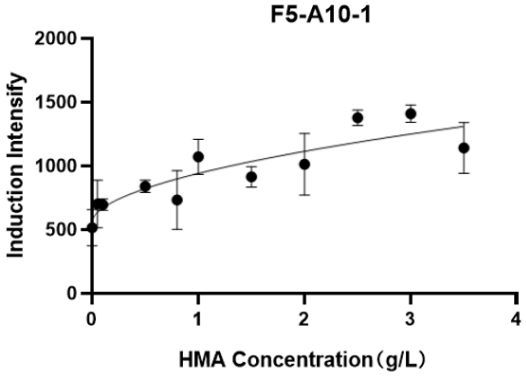

Figure 9 Gradient HMA concentration induction assay of F5-A10-1

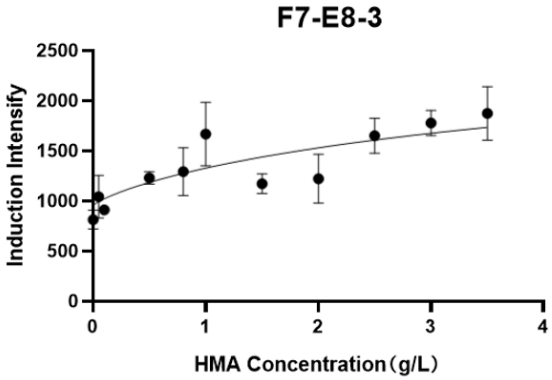

Figure 10 Gradient HMA concentration induction assay of F7-E8-3

From the result we can see that the IA of F5-B7-2 saturate induced by 3.5 g/L HMA. the IA of F5-A10-1 did not saturate induced by 1.0 g/L HMA. the IA of F7-E8-3 did not saturate induced by 3.5 g/L HMA.

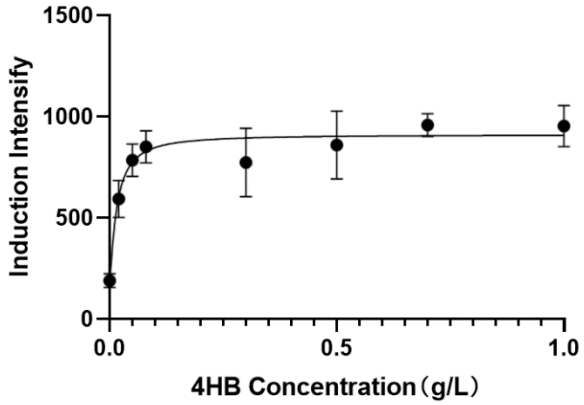

Figure 11 Gradient 4HB concentration induction assay of DH5αWT

From the result we can see that the IB of DH5αWT saturate induced by 0.1 g/L 4HB.

8.21 Baitong Shen

Site-directed mutagenesis of the PobR CDS

DpnI digestion

Reaction system:

Spm247	Spm124	Spm16
DpnI: 0.2 μL	DpnI: 0.2 μL	DpnI: 0.2 μL
10×cutsmart: 1 μL	10×cutsmart: 1 μL	10×cutsmart: 1 μL
Spm247 (PCR) (38 ng/μL): 1.3 μL	Spm124 (PCR) (29 ng/μL): 1.7 μL	Spm16 (PCR) (55 ng/μL): 1 μL
DDW: 7.5 μL	DDW: 7.1 μL	DDW: 7.8 μL

Reaction procedure:

37 ℃ 2 h

70 ℃ 20 min

8.22 Zhongyue Li

Chloramphenicol ALE pre-experiment

Transformed pYB1a-PobR$^{F7\text{-}E8\text{-}3}$-eGFP-Cmr, pYB1a-PobR$^{F5\text{-}A10\text{-}1}$-eGFP-Cmr, pYB1a-PobR$^{F5\text{-}B7\text{-}2}$-eGFP-Cmr into BL21 competent cells respectively.

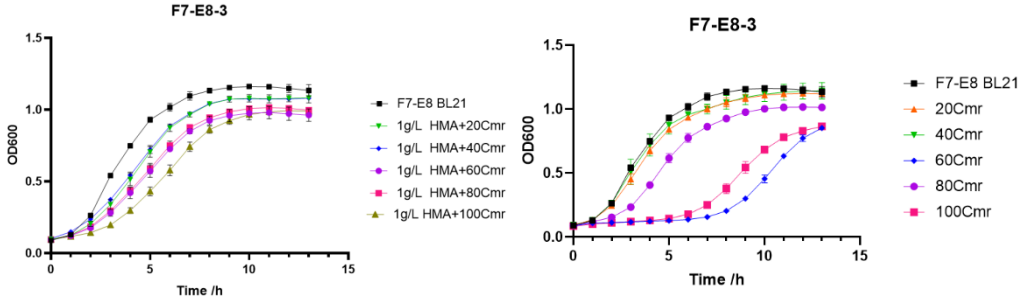

Figure 12 Growth curve of F7-E8-3 under the pressure of different concentrations of

chloramphenicol, left, added 1 g/L HMA as inducer; right, no inducer

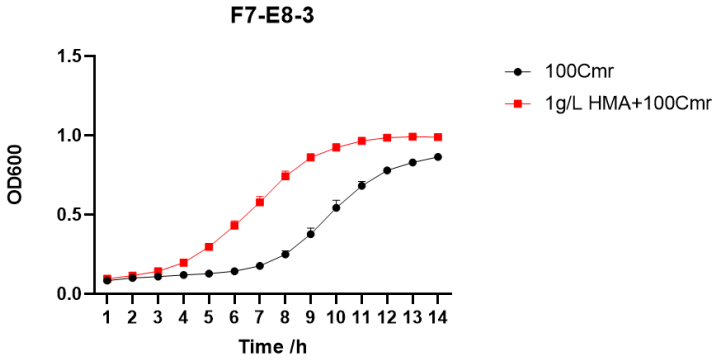

Figure 13 Comparation of the growth curve of F7-E8-3 with or without inducer under the pressure of 100 μg/mL chloramphenicol

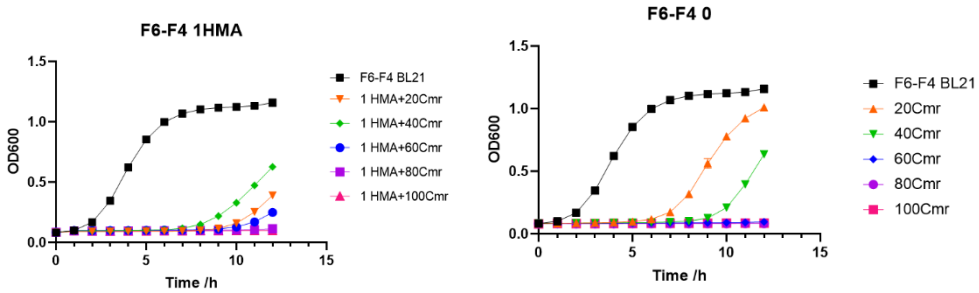

Figure 14 Growth curve of F6-F4-3 under the pressure of different concentrations of chloramphenicol, left, added 1 g/L HMA as inducer; right, no inducer

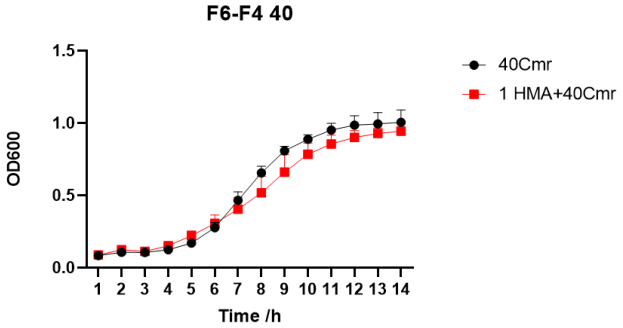

Figure 15 Comparation of the growth curve of F6-F4-3 with or without inducer under the pressure of 40 μg/mL chloramphenicol

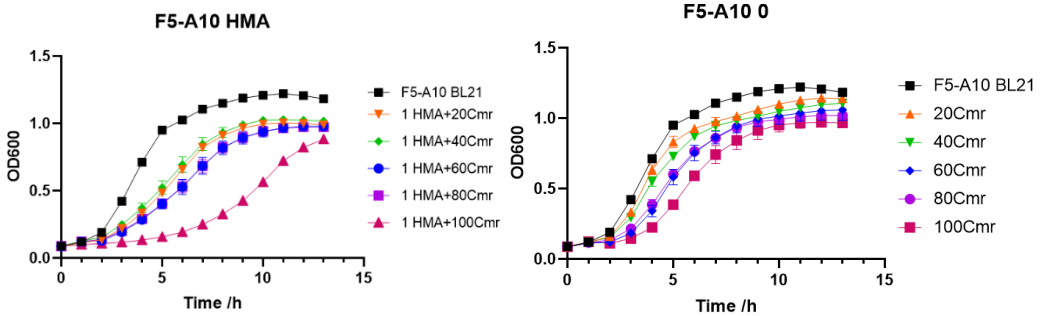

Figure 16 Growth curve of F5-A10-1 under the pressure of different concentrations of chloramphenicol, left, added 1 g/L HMA as inducer; right, no inducer

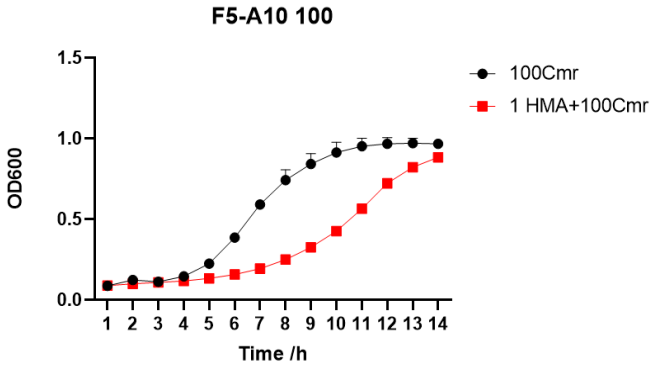

Figure 17 Comparation of the growth curve of F5-A10-1 with or without inducer under the pressure of 100 μg/mL chloramphenicol

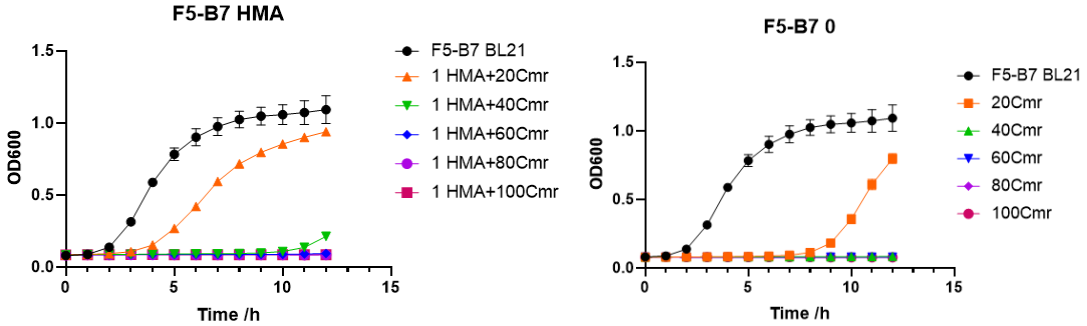

Figure 18 Growth curve of F5-B7-2 under the pressure of different concentrations of chloramphenicol (1_{st}), left, added 1 g/L HMA as inducer; right, no inducer

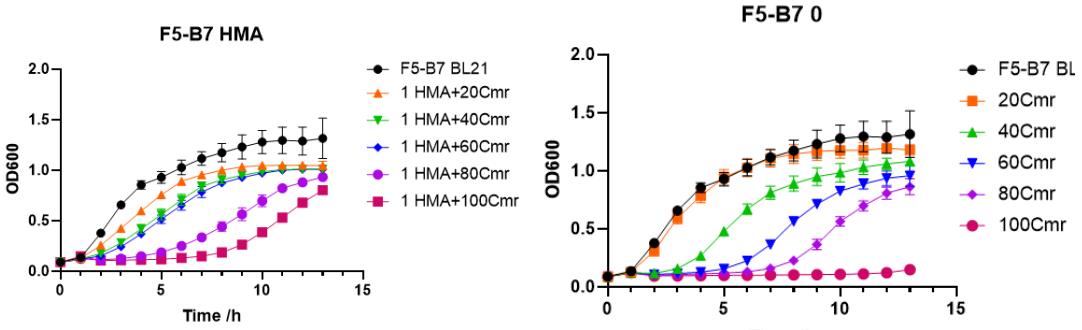

Figure 19 Growth curve of F5-B7-2 under the pressure of different concentrations of chloramphenicol (2_{nd}), left, added 1 g/L HMA as inducer; right, no inducer

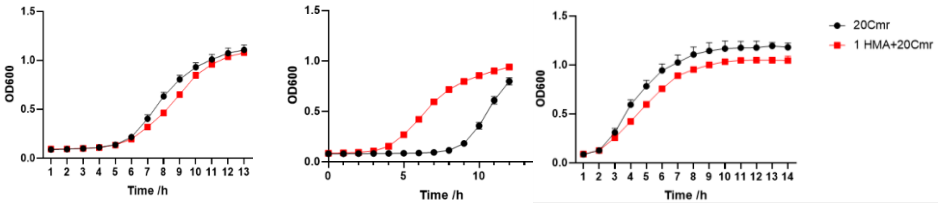

Figure 20 Comparation of the growth curve of F5-B7-2 with or without inducer under the pressure of 20 μg/mL chloramphenicol in three times (from left to right: 1_{st}, 2_{nd}, 3_{rd})

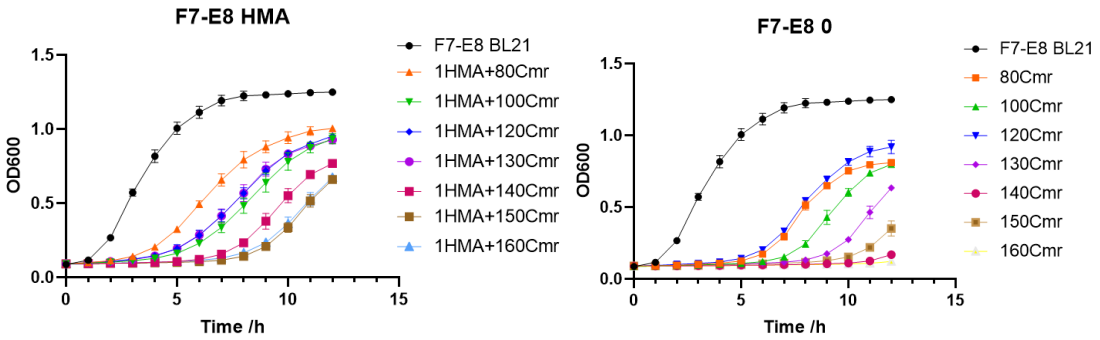

Figure 21 Growth curve of F7-E8-3 under the pressure of different concentrations of chloramphenicol (1_{st}), left, added 1 g/L HMA as inducer; right, no inducer

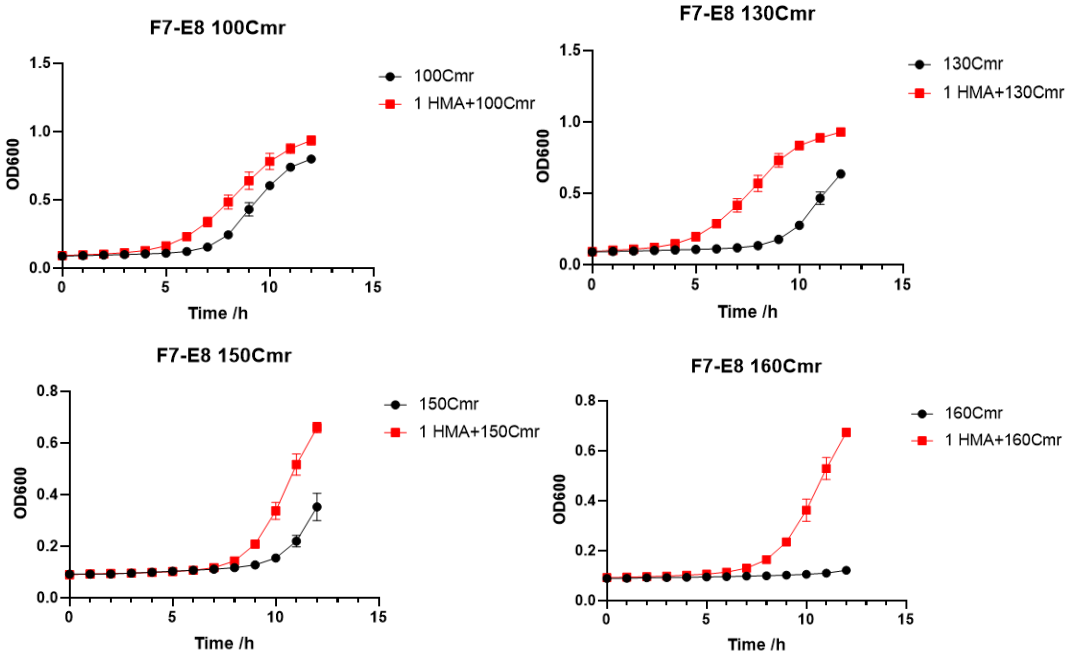

Figure 22 Comparation of the growth curve of F7-E8-3 with or without inducer under the pressure of different chloramphenicol concentration (1_{st})

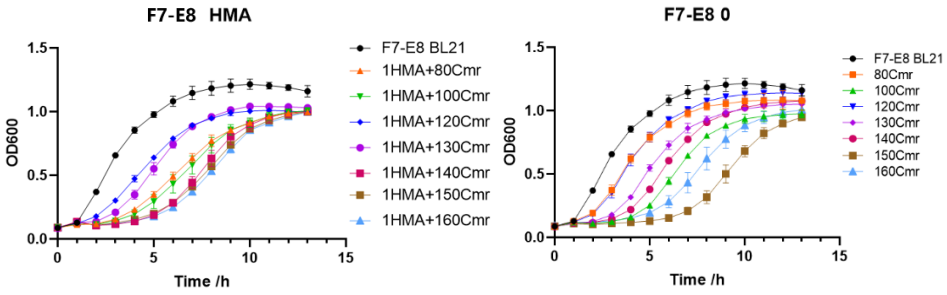

Figure 23 Growth curve of F7-E8-3 under the pressure of different concentrations of chloramphenicol (2_{nd}), left, added 1 g/L HMA as inducer; right, no inducer

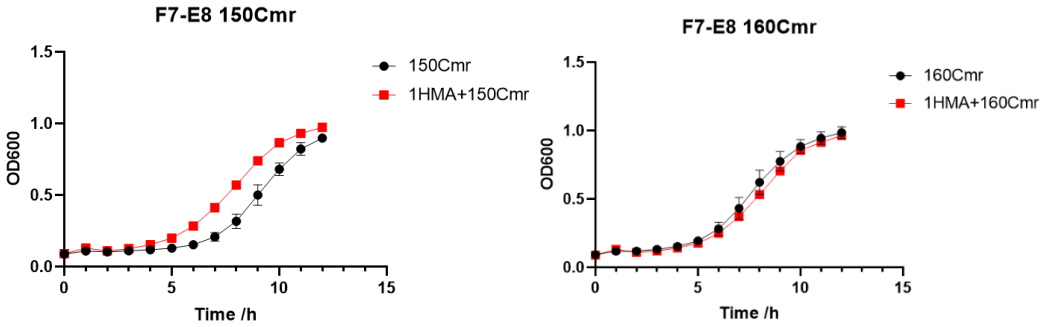

Figure 24 Comparation of the growth curve of F7-E8-3 with or without inducer under the pressure of different chloramphenicol concentration (2_{nd})

The data from different times' experiments shows significant differences. Therefore, the experimental system still needs to be adjusted to improve the stability.

8.22 Xinlu Liu

Site-directed mutagenesis of the PobR CDS

Transformation

System:

	Spm247	Spm124	Spm16
Competent cells	DH5α: 100 μL	DH5α: 100 μL	DH5α: 100 μL
Digestion product	10μL (50ng)	10μL (49.3ng)	10μL (55ng)
plate	Amp50	Amp50	Amp50

Culture at 37 ℃ for 12 h.

8.23 Shuning Guo

Site-directed mutagenesis of the PobR CDS

Picked 2 clones from each of the plate and inoculated into 5mL LB medium and cultured at 37℃ for 8h.

Then, 700 μL culture were used for storage at -20℃ and other culture was store at 4℃ for further experiments.

8.24 Shuhan Liu

Ligand specificity test

Used HMA analogues to induce F7-E8-3, F6-F4-3 and DH5α*pobR*^{WT} to test the ligand specificity of the mutants.

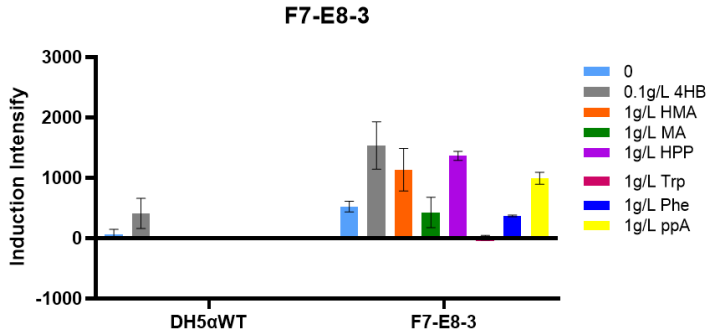

Figure 25 The induction intensity of HMA analogues to F7-E8-3

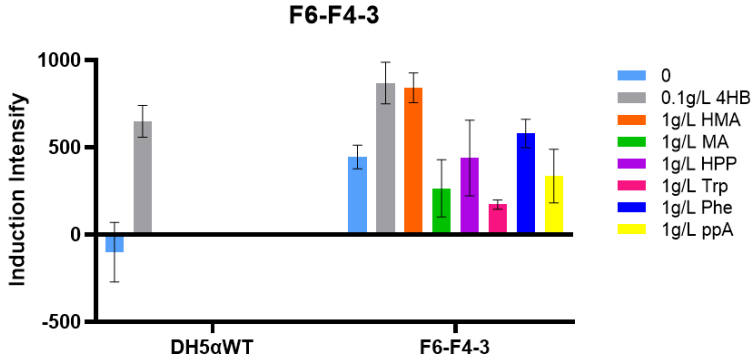

Figure 26 The induction intensity of HMA analogues to F6-F4-3

From the result we can see that F7-E8-3 can be induced by 4HB, HMA, HPP; F6-F4-3 can be induced by HMA and Phe.

8.24 Beimeng Zhang

Site-directed mutagenesis of the PobR CDS

DNA sequencing result showed that clones named as spm124-1/spm124-2/spm16-1/spm247-2 have expected mutation on the plasmids, spm16-2 does not have mutation on its plasmid, spm247-1 has two mutation sites on its plasmid.