

## 8.5 Qianwen Jin

### 1. *glb2a-PobR-PpobA\*2-mCherry-SacB-Cmr* Phenotypic analysis

The bacterial liquid was transferred to the liquid LB supplemented with 1% 100 mg/mL Amp, at the same time, inducing with different final concentrations of 4HB. After 12 hours, the optical density at 600 nm (OD<sub>600</sub>) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), the fluorescence response curve and growth curve were plotted.

Experimental group (three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.1g/L 4HB + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.2g/L 4HB + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.3g/L 4HB + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.4g/L 4HB + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.6g/L 4HB + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.7g/L 4HB + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.8g/L 4HB + bacterial broth.

Control group (three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + bacterial broth.

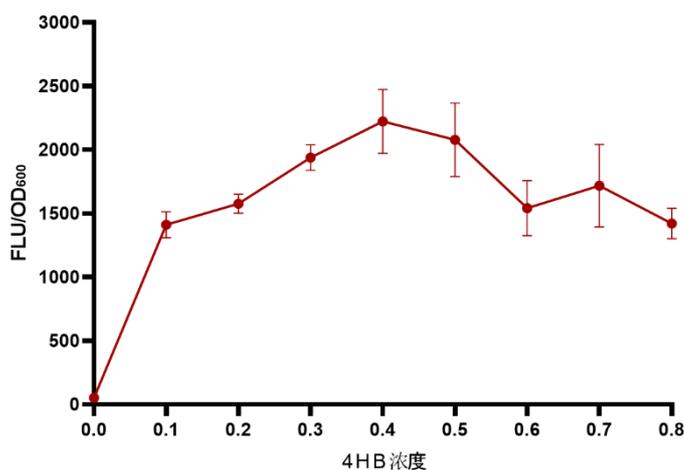


Figure 1 induced by different concentrations of 4HB

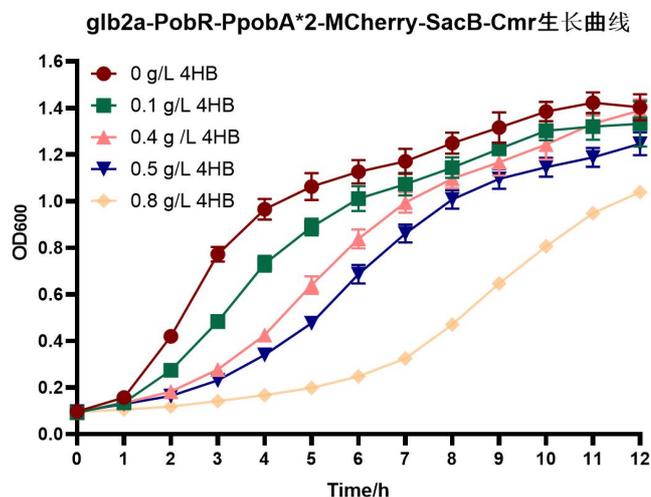


Figure 2 glb2a-PobR-PpobA\*2-mCherry-SacB-Cmr growth curve

## 8.6 Chengjie Dong

Select bacterial liquid at a certain concentration of 4HB, culture at liquid LB supplemented with different concentrations of Cmr and Sucrose. After 12 hours, measure the optical density at 600 nm (OD600) and red fluorescence (552 nm as excitation wavelength and 600 nm as emission wavelength).

Experimental group (three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 0g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 10g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 30g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 50g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 70g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 90g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 0g/L Sucrose + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 2g/L Sucrose + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 4g/L Sucrose + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 5g/L Sucrose + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 8g/L Sucrose + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration Xg/L 4HB + 10g/L Sucrose + bacterial broth.

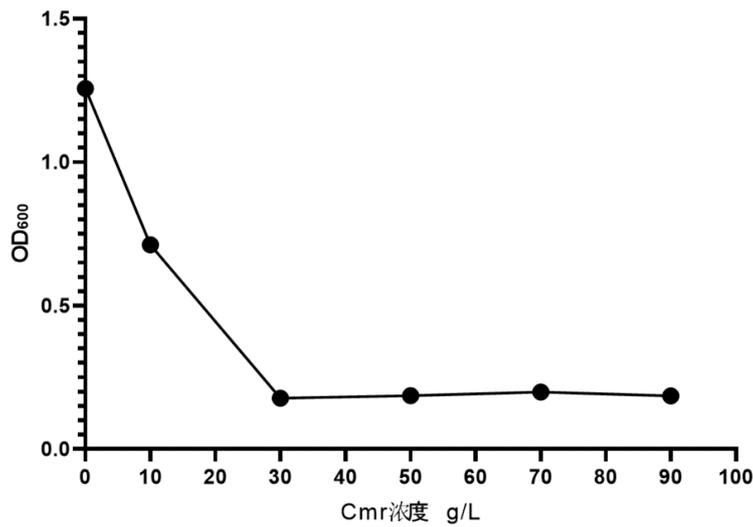


Figure 3 Growth of glb2a-PobR-PpobA\*2-mCherry-SacB-Cmr at different Cmr concentrations

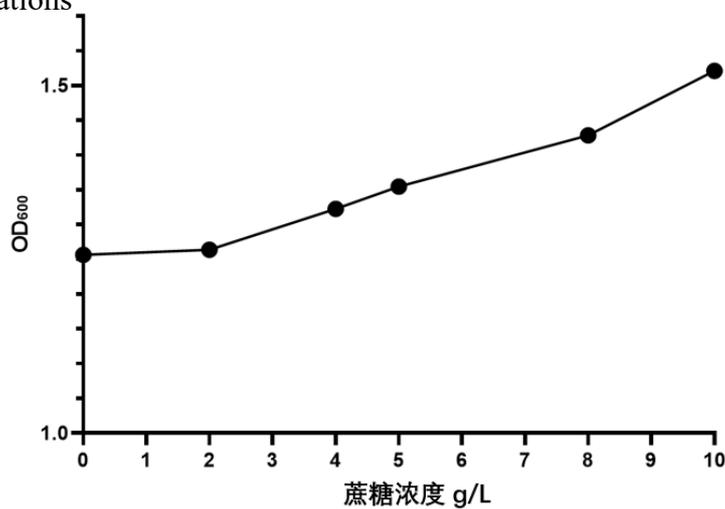


Figure 4 Growth of glb2a-PobR-PpobA\*2-mCherry-SacB-Cmr at different Sucrose concentrations

## 8.7 Linshan Cao

### 1. glb2a-PobR-PpobA\*2-mCherry-SacB-Cmr Phenotypic analysis

**The first group :** The bacterial liquid was transferred to the liquid LB supplemented with 1% 100 mg/mL Amp and final concentration 0.5g/L 4HB, as the same time, inducing with different concentrations of Cmr. After 12 hours, the optical density at 600 nm (OD<sub>600</sub>) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), the growth curve were plotted.

Experimental group (three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 0g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 5g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 10g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 20g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 30g/L Cmr + bacterial broth.

Control group (three groups in parallel):

200  $\mu$ L liquid LB + DH5 $\alpha$ .

**The second group** : The bacterial liquid was transferred to the liquid LB supplemented with 1% 100 mg/mL Amp and final concentration 0.5g/L 4HB, as the same time, inducing with different concentrations of Sucrose. After 12 hours, the optical density at 600 nm (OD<sub>600</sub>) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), the growth curve were plotted.

Experimental group (three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 1g/L Sucrose + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 2g/L Sucrose + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 3g/L Sucrose + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 4g/L Sucrose + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 5g/L Sucrose + bacterial broth.

Control group (three groups in parallel):

200  $\mu$ L liquid LB + DH5 $\alpha$ .

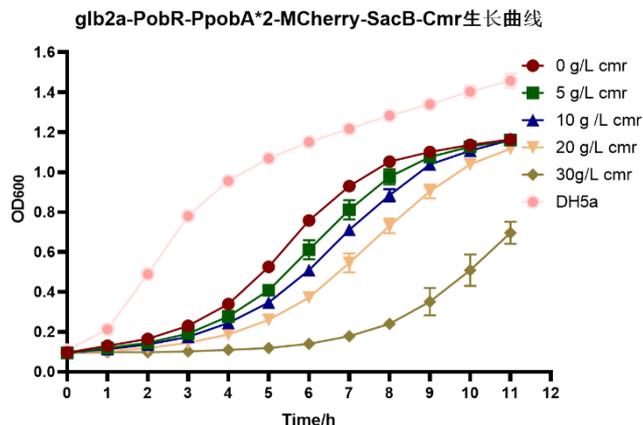


Figure 1 induced by different concentrations of Cmr

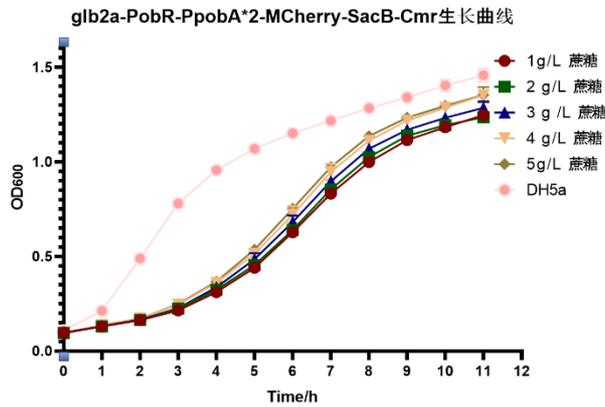


Figure 2 induced by different concentrations of Sucrose

## 8.8 Jianing Li

### 1. glb2a-PobR-PpobA\*2-mCherry-SacB-Cmr Phenotypic analysis

The bacterial liquid was transferred to the liquid LB supplemented with 1% 100 mg/mL Amp, at the same time, induction group with final concentration 0.5g/L 4HB and different concentration of Cmr ; uninduced group just with different concentration of Cmr. After 12 hours, the optical density at 600 nm (OD600) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), the fluorescence response curve and growth curve were plotted.

Induction group(three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 0g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 10g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 30g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 50g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 70g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 90g/L Cmr + bacterial broth.

Uninduced group (three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 0g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 10g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 30g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 50g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 70g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 90g/L Cmr + bacterial broth.

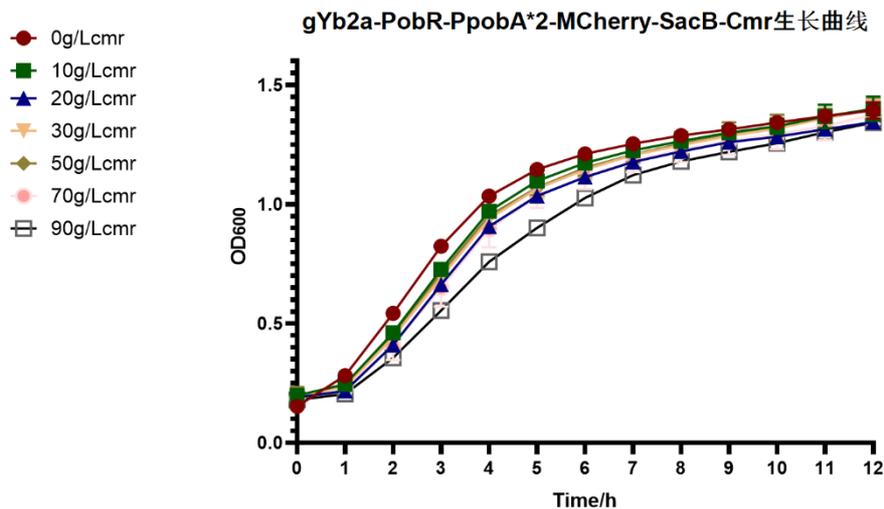


Figure 1 Induction group

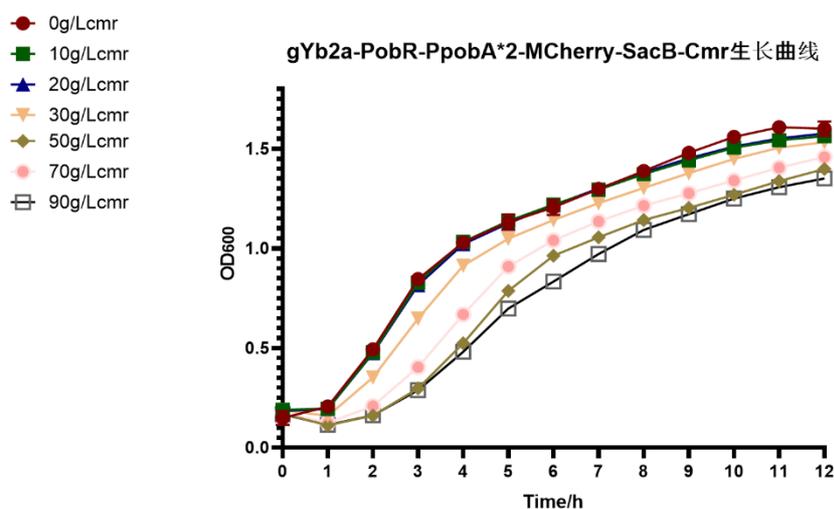


Figure 2 Uninduced group

## 8.9 Jiale Li

### 1. glb2a-PobR-PpobA\*2-mCherry-SacB-Cmr Phenotypic analysis

The bacterial liquid was transferred to the liquid LB supplemented with 1% 100 mg/mL Amp, at the same time, induction group with final concentration 0.5g/L 4HB and different concentration of Cmr ; uninduced group just with different concentration of Cmr. After 12 hours, the optical density at 600 nm (OD600) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), the fluorescence response curve and growth curve were plotted.

Induction group(three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 0g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 10g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 30g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 50g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 70g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 90g/L Cmr + bacterial broth.

Uninduced group (three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 0g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 10g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 30g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 50g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 70g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 90g/L Cmr + bacterial broth.

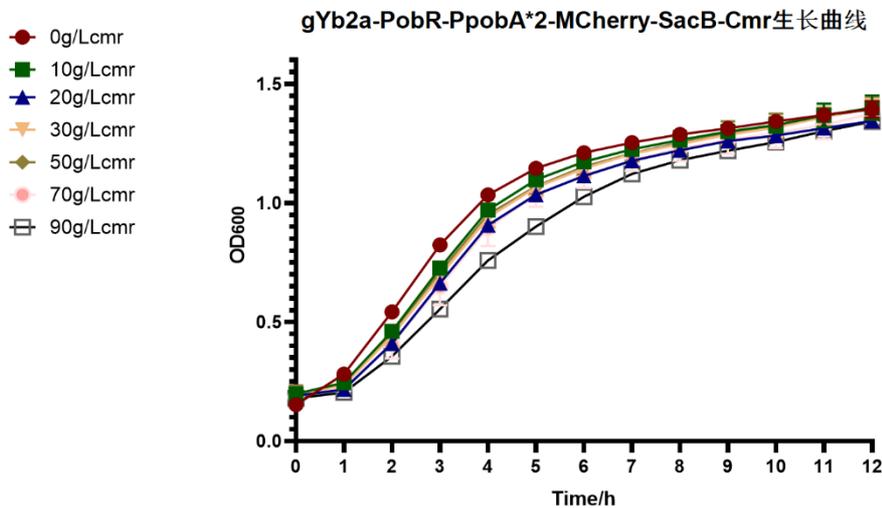


Figure 1 Induction group

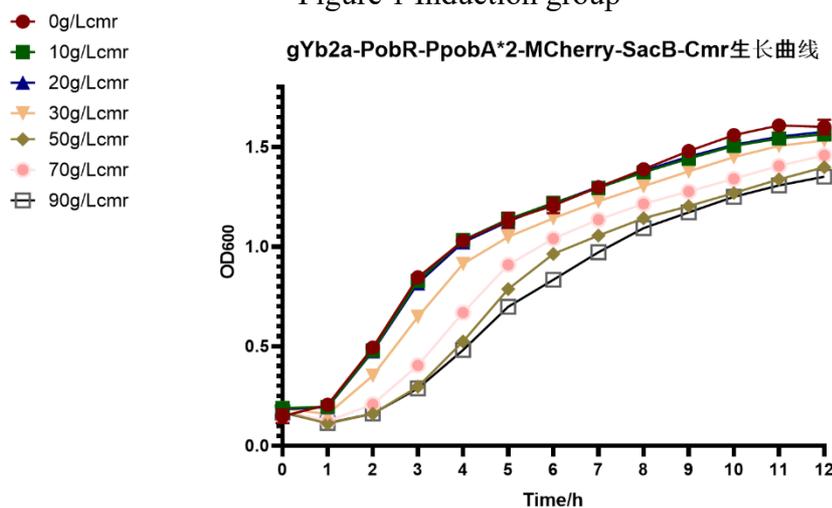


Figure 2 Uninduced group

## 8.10 Bohui Yangyang

### 1. gYb2a-PobR-PpobA\*2-Mcherry-CD-cmr Phenotypic analysis

#### The first group:

The bacterial liquid was transferred to the liquid LB supplemented with 1% 100 mg/mL Amp, at the same time, inducing with different final concentrations of 4HB. After 12 hours, the optical density at red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), the fluorescence response curve was plotted.

Experimental group (three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.1g/L 4HB + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.3g/L 4HB + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.7g/L 4HB + bacterial broth.

#### The second group:

The bacterial liquid was transferred to the liquid LB supplemented with 1% 100 mg/mL Amp, at the same time, induction group with final concentration 0.7g/L 4HB and different concentration of Cmr ; uninduced group just with different concentration of Cmr. After 12 hours, the optical density at 600 nm (OD600) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength).

Induction group (three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.7g/L 4HB + 10g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.7g/L 4HB + 30g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + final concentration 0.7g/L 4HB + 50g/L Cmr + bacterial broth.

Uninduced group (three groups in parallel):

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 10g/L Cmr + bacterial broth.

200  $\mu$ L liquid LB + 1% 100 mg/mL Amp + 30g/L Cmr + bacterial broth.

Control group : 200  $\mu$ L liquid LB + DH5 $\alpha$

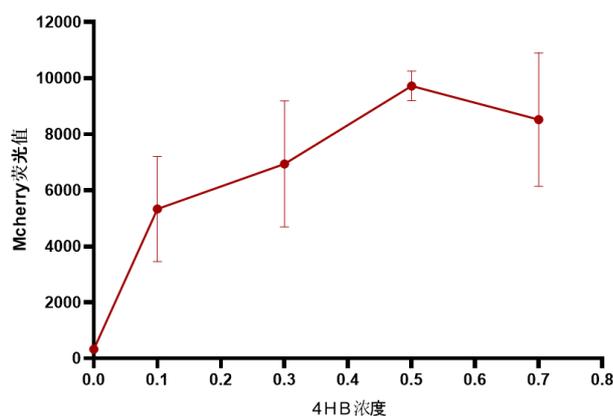


Figure 1 Response to different concentrations of 4HB

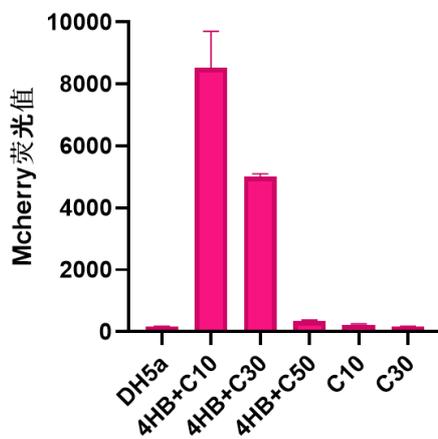


Figure 2

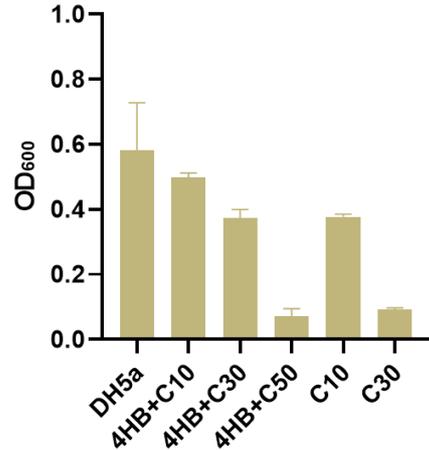


Figure 3

Figure 2 Fluorescence of induction group and uninduced group under different concentrations of cmr; Figure 3 OD<sub>600</sub> of induction group and uninduced group under different concentrations of cmr.

## 8.11 Xiaoya Wei

### 1. gYb2a-PobR-PpobA\*2-Mcherry-CD-cmr Respond to 4HB

The bacterial liquid was transferred to the M9 medium supplemented with 1% 100 mg/mL Amp, at the same time, inducing with different final concentrations of 4HB. After 12 hours, the optical density at red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), the fluorescence response curve was plotted.

Experimental group (three groups in parallel):

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.1g/L 4HB + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.3g/L 4HB + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.7g/L 4HB + bacterial broth.

Meanwhile, in order to verify the function of the CD, we transfer the bacterial liquid to the M9 medium supplemented with 1% 100 mg/mL Amp, at the same time, induction group with final concentration 0.5g/L 4HB and 5 mg/L 5FC ; uninduced

group just with 5mg/L 5FC. After 12 hours, the optical density at 600 nm (OD600) was measured.

Experimental group (three groups in parallel):

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 5mg/L 5FC + bacterial broth.

Control group (three groups in parallel):

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + 5mg/L 5FC + bacterial broth.

200  $\mu$ L M9 medium + bacterial broth.

## 8.12 Qianwen Jin

To verify the function of Cmr, we transfer the bacterial liquid to the M9 medium supplemented with 1% 100 mg/mL Amp, at the same time, experimental group with final concentration 0.5g/L 4HB and different concentration of Cmr ; control group just with different concentration of Cmr. After 12 hours, the optical density at 600 nm (OD600) was measured.

Experimental group (three groups in parallel):

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 0mg/mL Cmr + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 10mg/mL Cmr + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 30mg/mL Cmr + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 50mg/mL Cmr + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + 70mg/mL Cmr + bacterial broth.

Control group (three groups in parallel):

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + 0mg/mL Cmr + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + 10mg/mL Cmr + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + 30mg/mL Cmr + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + 50mg/mL Cmr + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + 70mg/mL Cmr + bacterial broth.

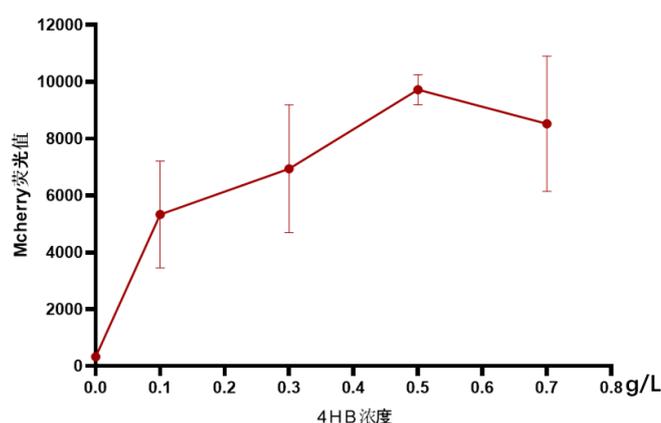
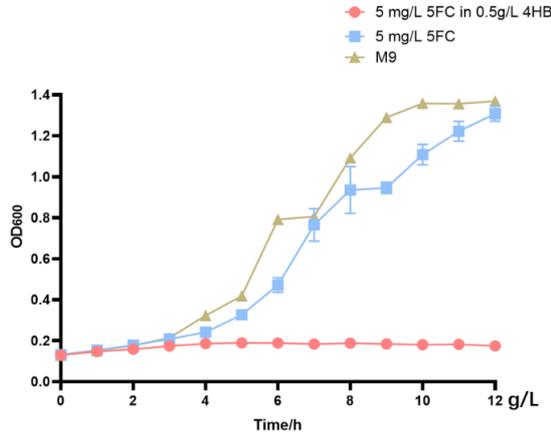


Figure 1 Response to different concentrations of 4HB



gYb2a-PobR-PpobA\*2-MCherry-CD-Cmr(BW▲CD) 生长曲线

Figure 2

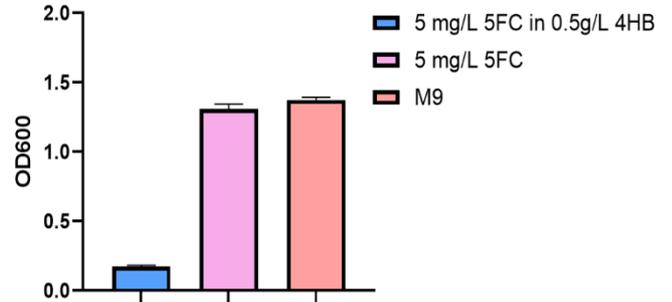


Figure 3

Figure 2 gYb2a-PobR-PpobA\*2-Mcherry-CD-cmr growth curve; Figure 3 Effect of 5FC on Bacterial Growth

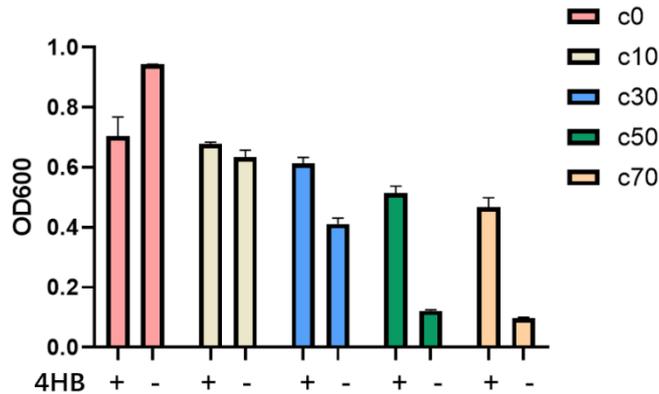


Figure 4 Response to different concentrations of Cmr

### 8.14 Chengjie Dong

Taking 2 $\mu$ L gYb2a-PobR(wild)-PpobA\*2-Mcherry-CD-Cmr bacterial solution was added into a 96-well plate, and 1%100mg/mL Amp was added. Phenotypic analysis was performed with and without final concentration 0.5g/L HMA induction. After 12 hours, optical density at 600 nm (OD600) was measured to verify the effect of CD.

Induction group:

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp +1%90mg/mL Cmr + final concentration 0.5g/L HMA+bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + 1%90mg/mL Cmr+  
final concentration 5mg/l 5FC  
+bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp +1%90mg/mL  
Cmr+final concentration 0.5g/L HMA in final concentration 5mg/l 5FC+ bacterial  
broth.

Uninduced group: 200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + 1%+90mg/mL  
Cmr+bacterial broth.

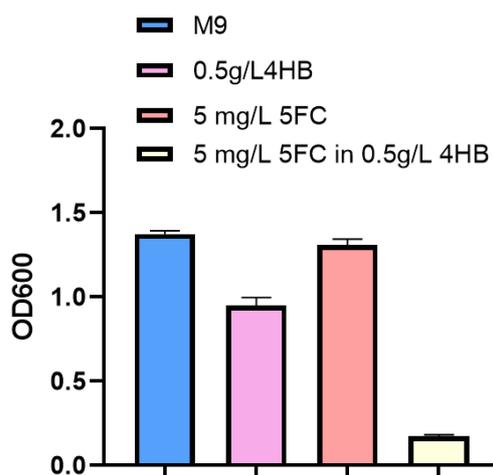


Figure1.gYb2a-PobR(wild)-PpobA\*2-Mcherry-CD-Cmr (BW $\Delta$ codA) growth situation

### 8.15 Xinyao Yuan

1. Negative screening: a total of three rounds of screening :

The first round of screening: the library was transferred to M9 medium supplemented with final concentration 0.5g/L HMA and final concentration 1mg/L 5-FC to 12h

The second round of screening : take the first round of screening bacterial liquid, transfer 1% of the inoculum to the M9 medium supplemented with final concentration 0.5g/L HMA , at the same time, add final concentration 5mg/L 5-FC to 12h

The three round of screening : take the two round of screening bacterial liquid, transfer 1% of the inoculum to the M9 medium supplemented with final concentration 0.5g/L HMA , at the same time, add final concentration 10mg/L 5-FC to 12h

### 8.16 Xinyao Yuan

2. Positive screening

Take 5  $\mu$ L of the three-round screening bacterial solution and spread it on the solid medium supplemented with 1% 100 mg/mL Amp to isolate single colonies. culture single colonies in liquid LB containing ampicillin for 8-10 hours. After culturing for

8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, and final concentration 0.5g/L 4HB in M9 medium. After 12 hours, measure the red fluorescence (552 nm as excitation wavelength and 600 nm as emission wavelength). At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Mcherry of Seven single colonies which selected from the board respectively is 3313,5209,3274,2350,39222,4005,8133

The results showed that all of them had red fluorescence, indicating that all of them responded to 4HB and made Mcherry express

Experimental group (three groups in parallel):

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + final concentration 1mg/L 5-FC+bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + final concentration 5mg/L 5-FC+bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + final concentration 10mg/L 5-FC+bacterial broth.

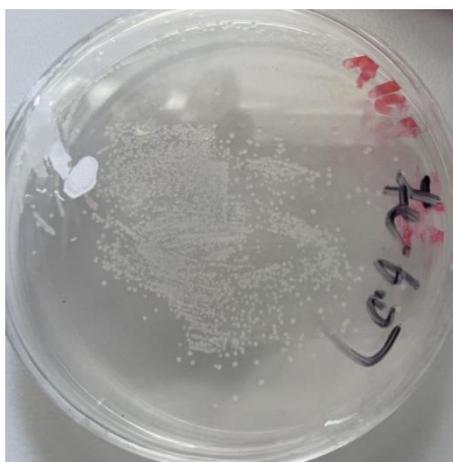


Figure 2 Three rounds of screening the growth of bacterial solution

## 8.17 Yuzhu Wang

1. Negative screening: a total of one rounds of screening :

The first round of screening: the library was transferred to M9 medium supplemented with final concentration 0.5g/L 4HB and the final concentrations were 50mg/L, 100mg/L, 200mg/L, 500mg/L, 1000mg/L 5-FC to 12h

## 8.18 Yuzhu Wang

### 2. Positive screening

Take 5ul of the one round of screening bacterial liquid at different concentrations of 5FC was diluted 1, 5 and 20 times, respectively and spread it on the solid medium supplemented with 1% 100 mg/mL Amp to isolate single colonies. culture single colonies in liquid LB containing ampicillin for 8-10 hours. After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, and final concentration 0.5g/L 4HB in M9 medium. After 12 hours, measure the red fluorescence (552 nm as excitation wavelength and 600 nm as emission wavelength). At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Induction group:

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB++bacterial broth.

Uninduced group:

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + bacterial broth. (PobR wild as positive control, BW $\Delta$ codA as negative control)



Figure 3 One round of screening for the growth of diluted bacterial solution plate

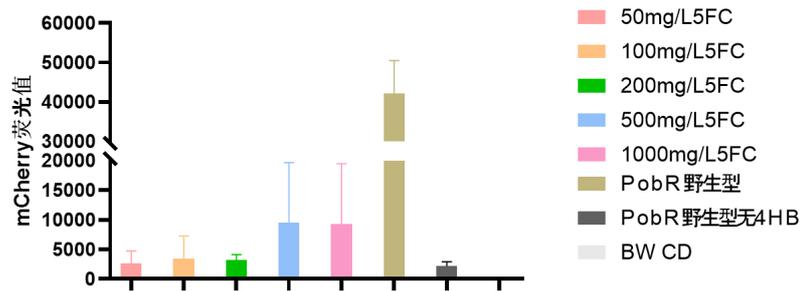


Figure 4 Mcherry of strains undergoing screening veification

### 8.19 Yuetong Zhu

Take ten single colonies with low fluorescence value were selected and cultured in liquid LB containing ampicillin for 8-10 hours and take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, and final concentration 0.5g/L 4HB in M9 medium. After 12 hours, measure the red fluorescence (552 nm as excitation wavelength and 600 nm as emission wavelength). At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

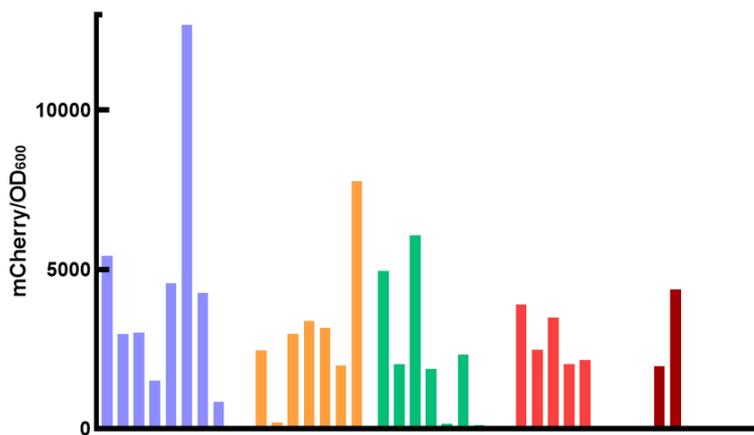


Figure 5 OD and mcherry of strains undergoing screening veification

### 8.20 Fenglin Tao

#### 1. Positive screening

Take one round of screening bacterial liquid at different concentrations of 5FC respectively and spread it on the solid medium supplemented with 1% 100 mg/mL Amp to isolate single colonies. culture single colonies in liquid LB containing ampicillin for 8-10 hours. After culturing for 8-10 hours, take 2 microliters of

bacterial broth to inoculate 200 microliters containing ampicillin, and final concentration 0.5g/L 4HB in M9 medium. After 12 hours, measure the red fluorescence (552 nm as excitation wavelength and 600 nm as emission wavelength). At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Induction group:

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + final concentration 50mg/l 5FC + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + final concentration 100mg/l 5FC + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + final concentration 200mg/l 5FC + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + final concentration 500mg/l 5FC + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L 4HB + final concentration 1000mg/l 5FC + bacterial broth.

Uninduced group: 200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + bacterial broth (PobR wild as positive control, BW $\Delta$ CD as negative control).

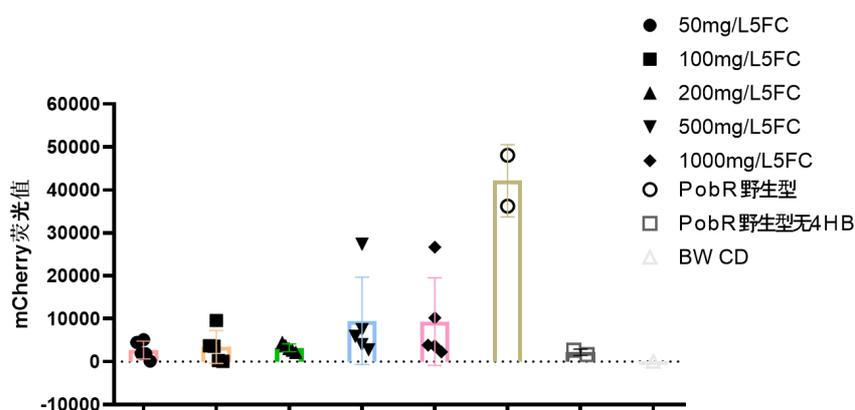


Figure 6 Mcherry of strains undergoing screening veification

### 8.21 Fenglin Tao

Take the bacterial solution at the concentration of final concentration 50mg/L 5-FC above and spread it on the solid medium supplemented with 1% 100 mg/mL Amp to isolate single colonies. culture single colonies in liquid LB containing ampicillin for 8-10 hours. After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, final concentration 0.5g/L 4HB, 1% 30 mg/mL Cmr, and the final concentration was 0.5g/L certain concentration of aromatic compounds in M9 medium. After 12 hours, measure the red fluorescence (552 nm as excitation wavelength and 600 nm as emission wavelength). At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Induction group:

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp +final concentration 20g/L HMA + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp +final concentration 40g/L HPP + bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp +final concentration 80g/L 4HB + bacterial broth.

Uninduced group: 200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + bacterial broth.

OD600 of four single colonies which selected from the board respectively is 0.268,0.225,0.186,0.53.

## 8.22 Jianing Li

### 2.Positive screening

Take the bacterial solution induced by different certain concentration of aromatic compounds and spread it on the solid medium supplemented with 1% 100 mg/mL Amp and 1% 30mg/mL Cmr to isolate single colonies. culture single colonies in liquid LB containing ampicillin for 8-10 hours. After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, 1% 30mg/mL Cmr, final concentration 0.5g/L 4HB, and different certain

concentration of aromatic compounds in M9 medium. After 12 hours, measure the red fluorescence (552 nm as excitation wavelength and 600 nm as emission wavelength).At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Induction group:

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp +final concentration 20g/L HMA +final concentration 0.5g/L 4HB+1%30mg/mL Cmr+ bacterial broth.

200  $\mu$ L M9 medium + 1% 100 mg/mL Amp +final concentration 40g/L HPP +final concentration 0.5g/L4HB+ 1%30mg/mL Cmr+bacterial broth.

Uninduced group: 200  $\mu$ L M9 medium + 1% 100 mg/mL Amp + final concentration 0.5g/L4HB+1%30mg/mL Cmr+bacterial broth[gYb2a-PobR(wild)-PpobA\*2-Mcherry-CD-Cmr)].

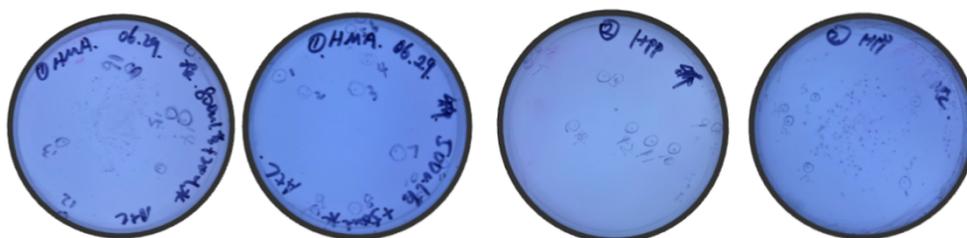


Figure 7 the growth of diluted bacterial solution plate

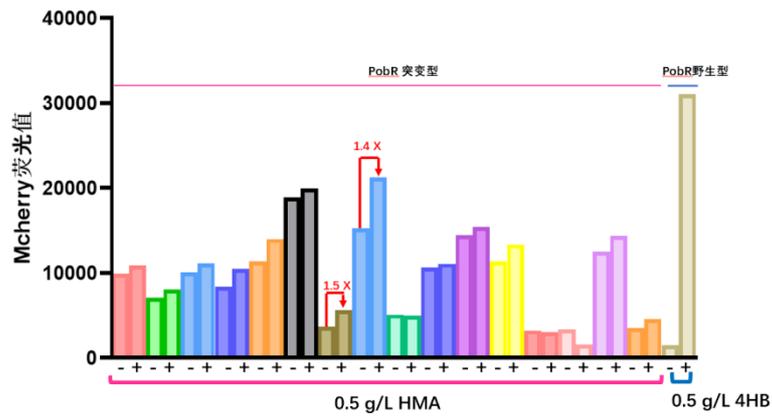


Figure 8 Mcherry of strains undergoing screening veification

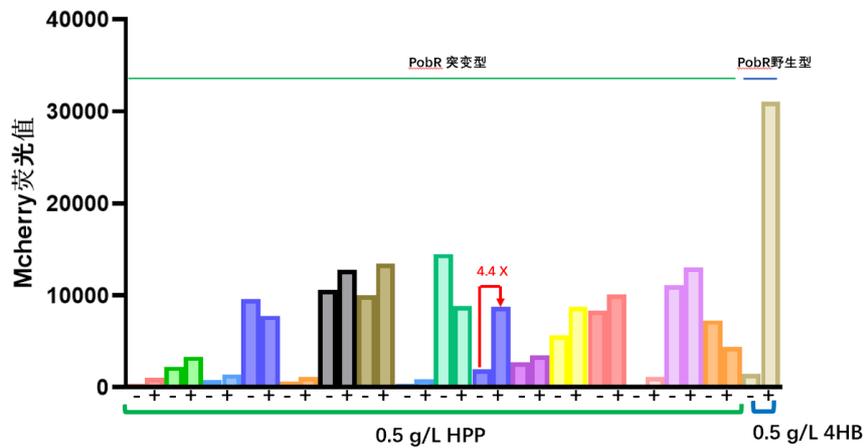
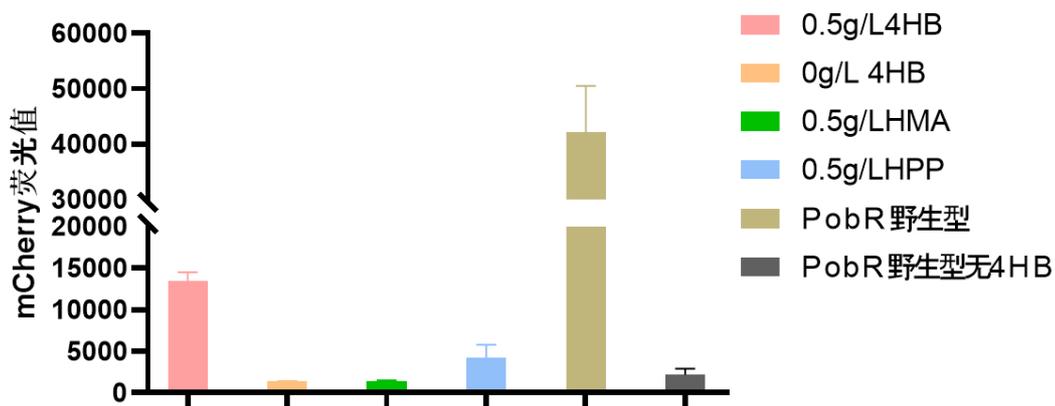


Figure 9 Mcherry of strains undergoing screening veification

### 8.26 Jiale Li

The strains obtained with 4HB and HMA were induced separately with a more pronounced HPP response (4.4-fold response).

50  $\mu$ L of the bacterial solution was added to liquid LB and incubated at 37° for 12 h. The fluorescence was observed in 96-well plates under conditions induced by final concentrations of 0.5 g/L 4HB, HMA and HPP, respectively. The specific results were as follows.



The results showed that the strain was significantly less responsive to 4HB than the wild type, but was also responsive to HMA.

### 8.27 Bohui Yangyang

The response of *E. coli* to 4HB at different concentrations of 5FC was observed under fluorescence microscopy :

Experimental group: 5FC final concentrations of 50mg/L, 100mg/L, 200mg/L, 500mg/L and 1000mg/L and 4HB final concentration of 0.5g/L were set.

Positive control: P obR wild type + 0.5 g/L 4HB

Negative control: P obR wild type + 0 g/L 4HB

Increase 5FC concentration to screen for more specific response strains

The sap induced at 0.5g/L 4HB, 1000mg/L 5FC was taken and screened with aromatic compounds (HPP/HMA) as well as Cmr.

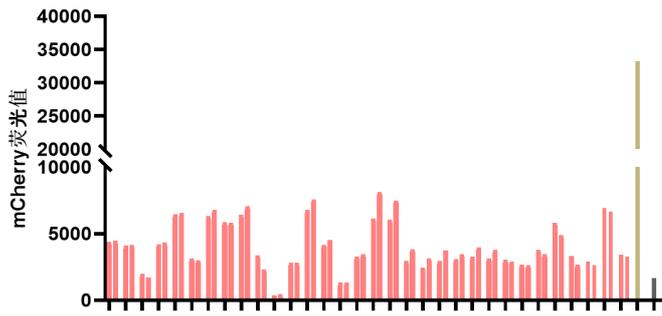
HMA group: LB 5ml. inoculum 1ml . HMA final concentration 0.5g/L. A100 5ul. Cmr for C30 6ul

5mL LB + 1% 100 mg/mL Amp + 0.1g/L HMA +1% bacterial broth +1% 30 mg/mL Chl

HPP group: 5mL LB + 1% 100 mg/mL Amp + 0.1g/L HPP +1% bacterial broth +1% 30 mg/mL Chl

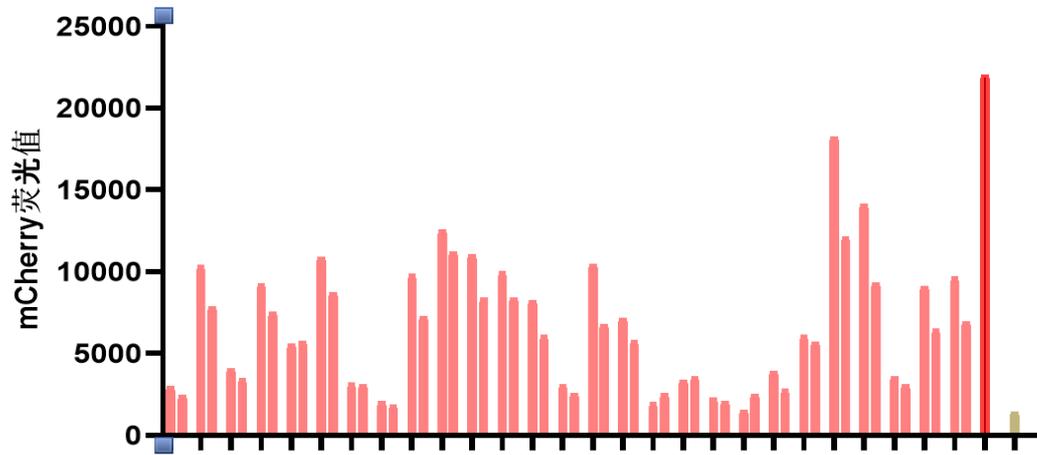
### 8.28 Xiaoya Wei

After isolation of single colonies, ampicillin and LB were added to the deep well plate for culture, and then transferred to 96-well plate for culture in M9 medium. The fluorescence of HMA group was shown as follows:



### 8.29 Yuzhu Wang

The Cmr concentration of HPP group was reduced to 70mg/L and 2.5 $\mu$ L was added. The fluorescence obtained under the same other steps was shown as below:



### 8.30 Linshan Cao

Increase the amount of Cmr added to the system and screen again.

HMA group:

5mL LB + 1% 100 mg/mL Amp + 0.1g/L HMA +5% bacterial broth +1% 100 mg/mL Chl

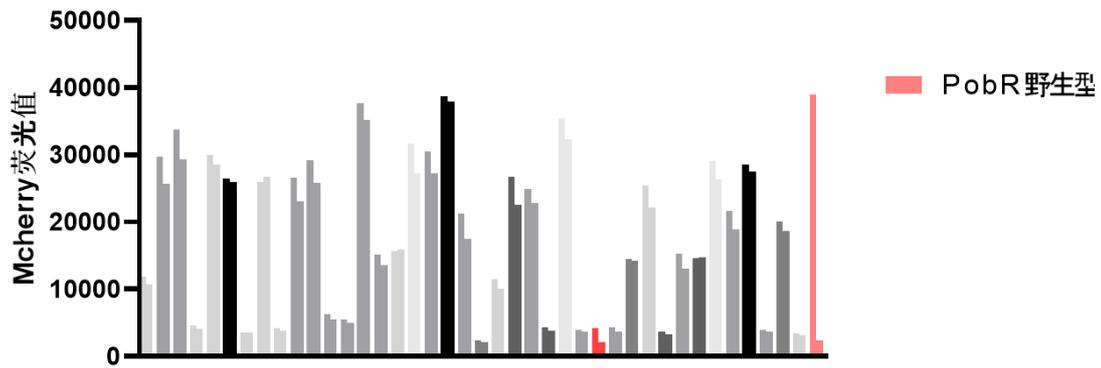
OD 1.0 after about 20h incubation

HPP group: 5mL LB + 1% 100 mg/mL Amp + 0.1g/L HPP +5% bacterial broth +1% 100 mg/mL Chl

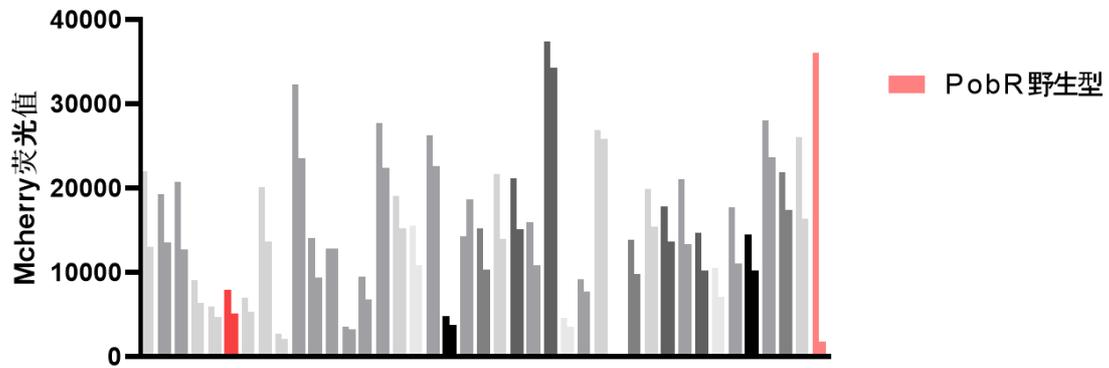
OD 1.1 after approx. 20h incubation

### 9.1 Linshan Cao

Single colonies were isolated from the above broth and cultured in deep-well plates, then characterised for fluorescence in 96-well plates with M9 medium.



HMA



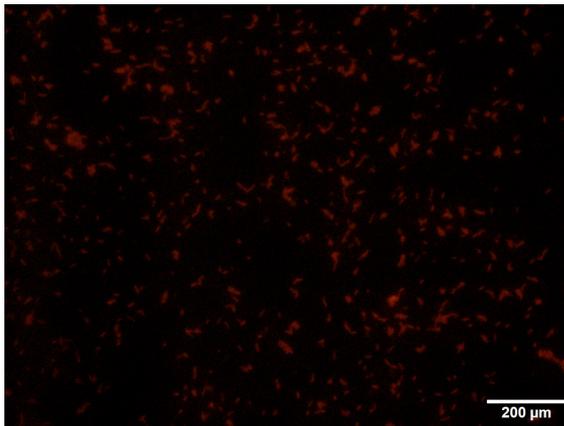
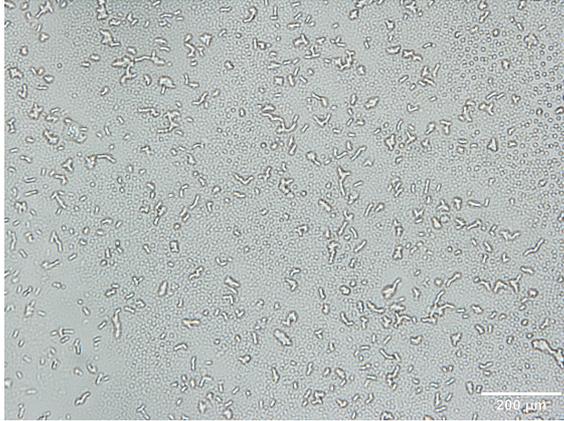
HPP

Screening of the existing fixed-point saturation mutant library A (122, 126)

Based on the experimental data, it was hypothesized that neither 50 mg/L nor 1000 mg/L 5FC could fully achieve inhibition, and the mutant library was re-screened with 50 mg/L 5FC to verify that the hypothesis was correct.

Screening system.

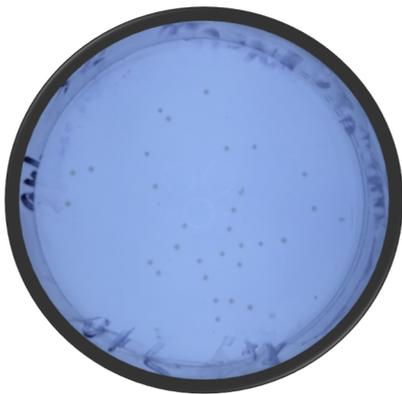
The results of the screening were as follows



#### 9.4 Qianwen Jin

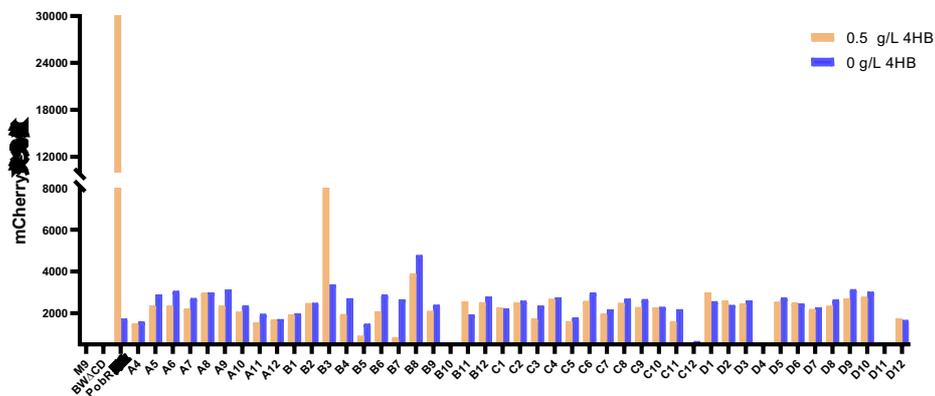
Reproduction of the PobR mutant library.

The reconstituted PobR mutant library was transformed into BW $\Delta$ CD and the coated plates were estimated to have an approximate capacity of 5,000 mutants for this mutation library.



Forty single colonies were selected for incubation in deep well plates and later

characterised by adding a final concentration of 0.5mg/L 4HB in M9 and the fluorometric results were as follows:



The current mutant library was less responsive to 4HB.

### 9.5 Fenglin Tao

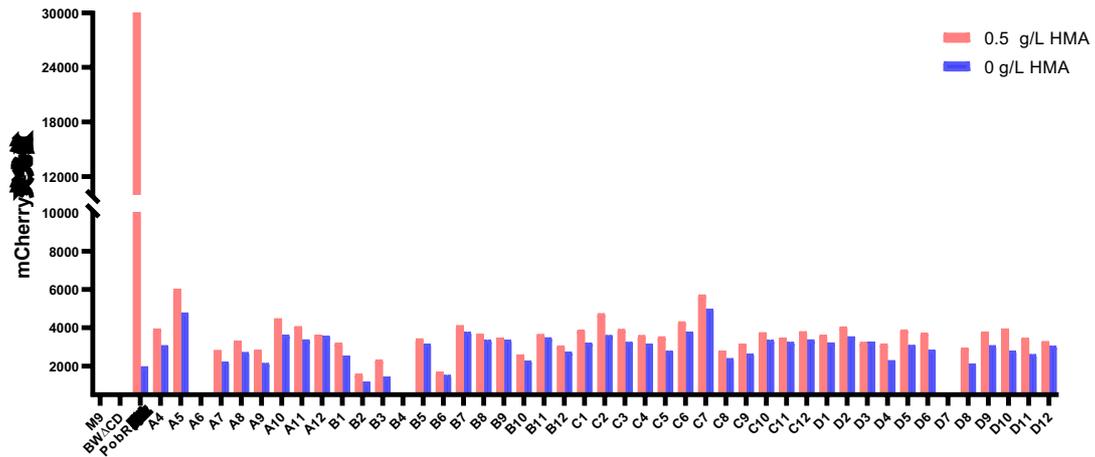
Screening for HMA-responsive strains

Screening system: M9 + final concentration 0.5g/L HMA + final concentration 60mg/L chloramphenicol

OD=0.49, fluorescence value=770 before inoculation. 5% inoculum. 37° shaker incubation until OD=0.7. Single colonies were isolated and induced by adding HMA.

The results were as follows:

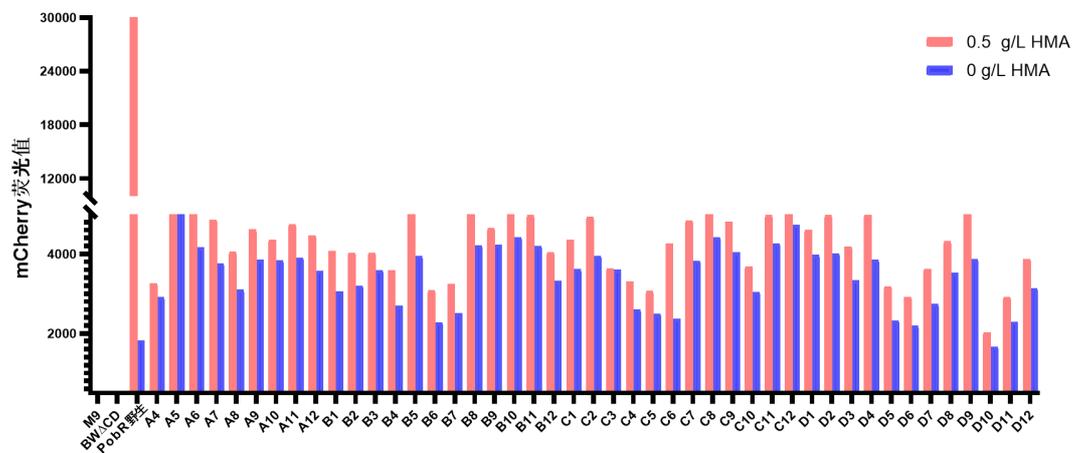




## 9.6 Qianwen Jin

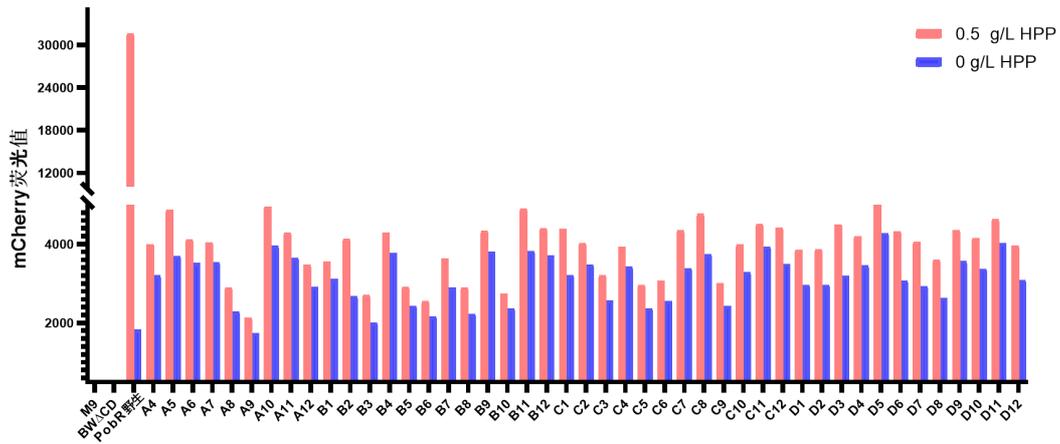
Re-screening of strains responding to HMA

The strains from the previous round of screening were transferred into M9 medium with increased chloramphenicol concentration (to 90mg/L) and then added to a final concentration of 0.5g/L HMA for screening. The fluorescence results are shown below:



Screening of strains responding to HPP

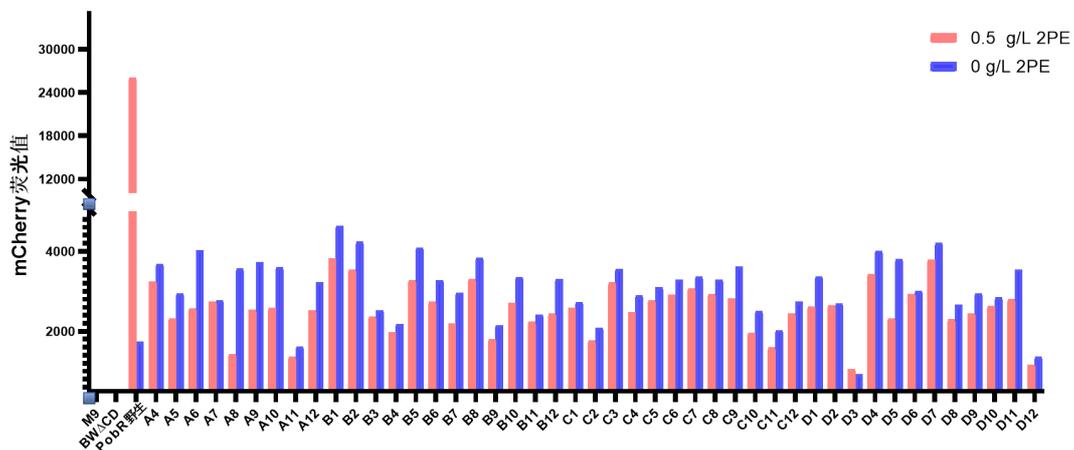
The final concentration of HPP was 0.5 g/L and the concentration of chloramphenicol was 60 mg/L. The fluorescence results were as follows.



### 9.7 Xiaoya Wei

Verify whether 0.5mg/L aromatic compound is toxic to cells

2PE was chosen as the experimental substance, and the final concentrations of 2PE in the system were both 0.5 g/L and chloramphenicol was 60 mg/L. The fluorescence results were as follows:



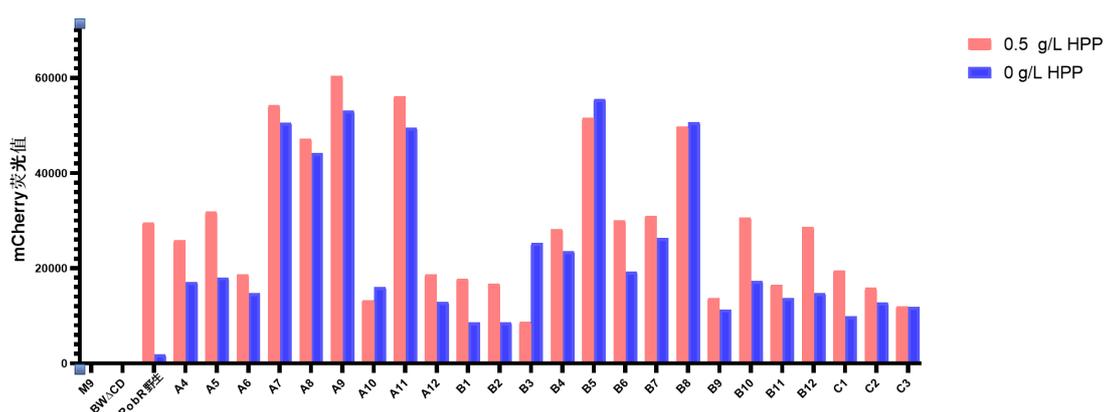
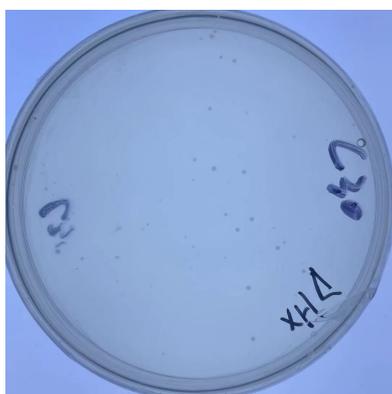
The results demonstrate that 0.5mg/L 2PE decreases the OD value of the bacterial solution and affects cell growth, therefore it is believed that aromatic compounds inhibit cell growth at a final concentration of 0.5mg/L.

### 9.8 Yuetong Zhu

Screening of strains responding to HPP

Based on previous results, it was hypothesized that positive screening in liquid LB did

not give the desired results, so this was replaced by isolating single colonies by coating the negatively screened bacterial solution on solid medium supplemented with 1% 100mg/mL Amp, 1% 90mg/mL Cmr and a concentration of aromatic compounds. Twenty-four colonies were picked from the plates and induced with a final concentration of 0.5mg/L HPP. The fluorescence results were as follows.

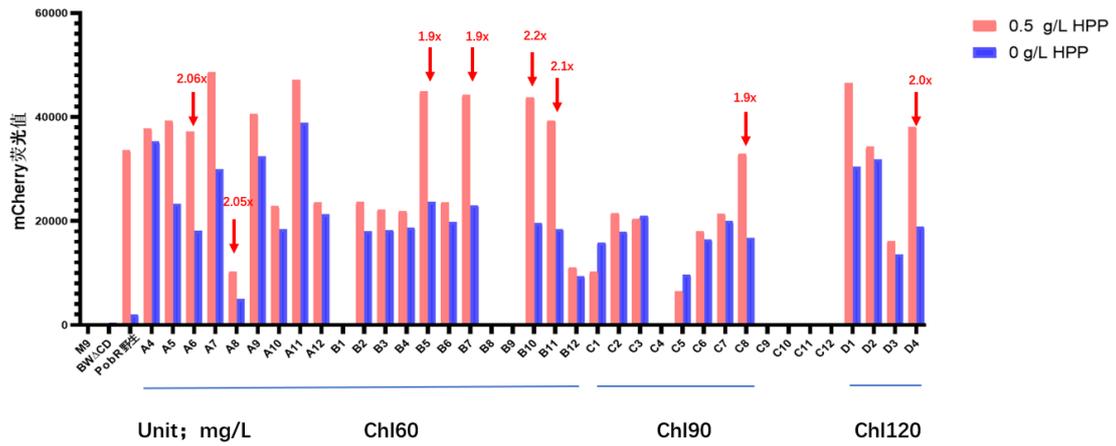


## 9.9 Jiale Li

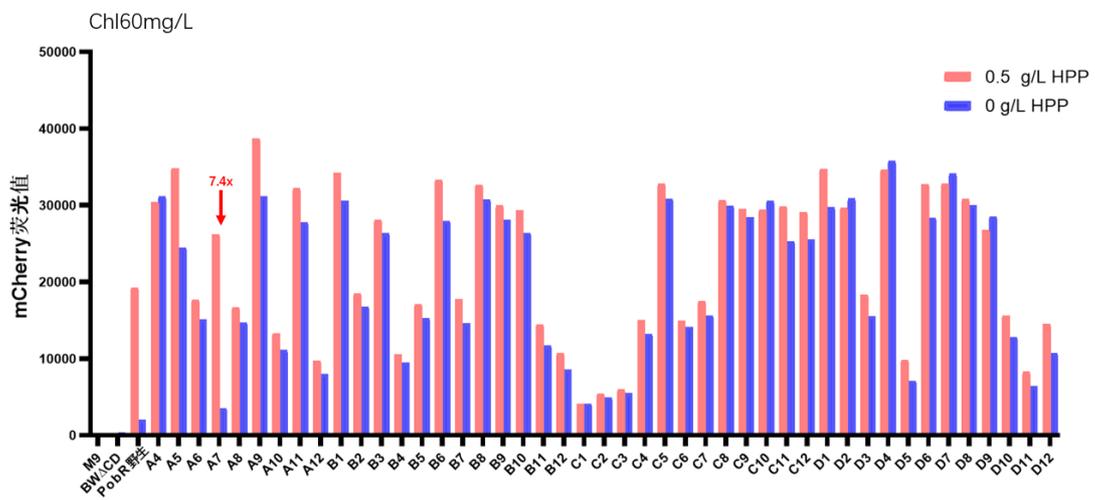
Fixed-point saturation mutagenesis did not give satisfactory results, so we performed random mutation of the full Pobr sequence, which was used to increase the body of the mutation library.

Positive screening in solid media with different concentrations of chloramphenicol  
 The solid medium was supplemented with chloramphenicol at a concentration of 60 mg/L, 90 mg/L, 120 mg/L 25  $\mu$ L and HPP final concentration of 0.5 g/L for isolation of single colonies.

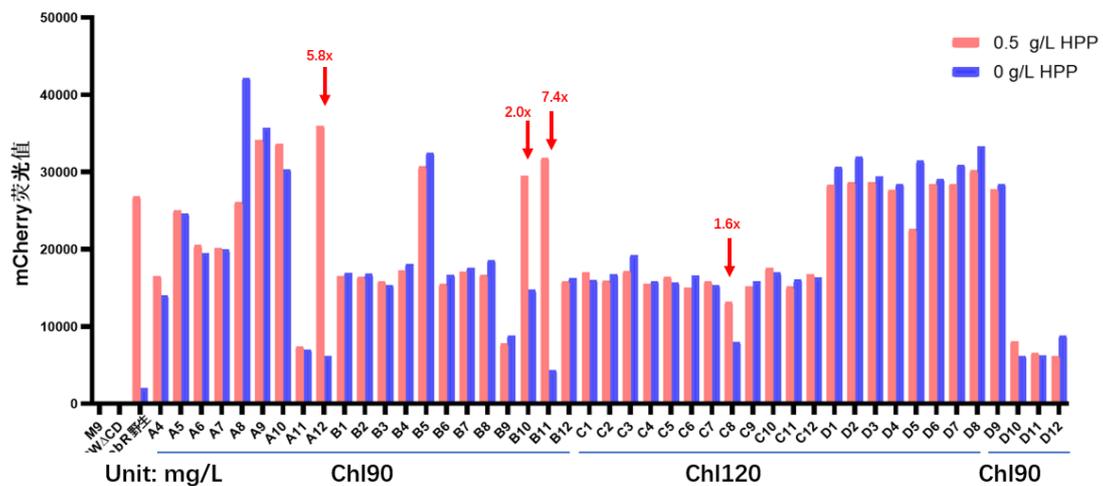
Fixed-point saturation mutation group:



Random mutation group:



Chl90mg/L and Chl120mg/L



A total of 10 strains with better results were selected from both groups.

Plasmids were extracted from 10 of these strains in the sentinel saturation mutation

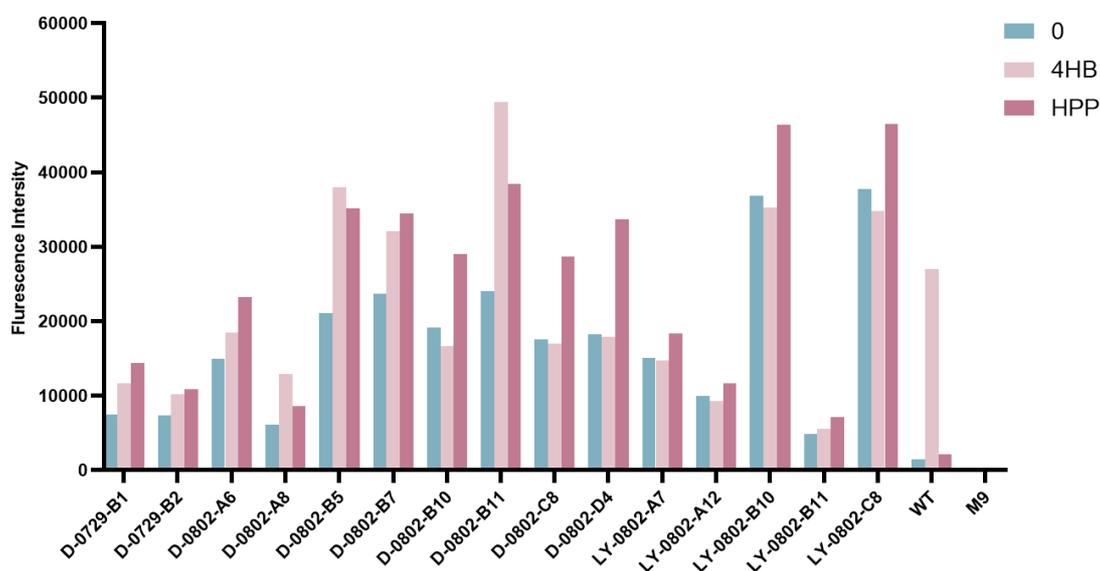
group and sent for sequencing. Six of them were sequenced successfully and the sequencing results are as follows:

| Name    | Sequencing success           | Mutation location           | Name    | Sequencing success   | Mutation location |
|---------|------------------------------|-----------------------------|---------|----------------------|-------------------|
| C30-B1  | Success(Twin Peaks)          | -----                       | C90-C8  | Success              | 124,125           |
| C30-B2  | Success                      | 80, 124, 125                | C120-D4 | Success (Twin Peaks) | -----             |
| C60-A6  | Success                      | 124, 125                    |         |                      |                   |
| C60-A8  | Success                      | 49, 124, 125                |         |                      |                   |
| C60-B5  | Success                      | 42, 124, 125                |         |                      |                   |
| C60-B7  | Success                      | the same strain with C60-B5 |         |                      |                   |
| C60-B10 | Success (Signal attenuation) | -----                       |         |                      |                   |
| C60-B11 | Success                      | the same strain with C60-B5 |         |                      |                   |

### 9.11 Chengjie Dong

#### Repeat Screening

Repeat the screening for the 10 strains selected above. The corresponding 10 strains were diluted 1000 times and coated on solid medium. The solid medium had chloramphenicol antibiotic concentrations of 60 $\mu$ g/mL, 90 $\mu$ g/mL, 120 $\mu$ g/mL and HPP final concentration of 0.5mg/L, and also contained ampicillin. 36h later colonies were picked for verification. The fluorescence results are shown below :



Re-screening of strains after one negative screening.

Since single colonies isolated in the plates generally possessed relatively high background fluorescence, the strains in 9.9 were screened negatively again after negative screening.

### 9.13 Fenglin Tao

Second round of negative sieving: the bacterial broth of week 23 was transferred twice and added to M9 medium at 1% inoculum, with final concentrations of 50, 100 and 200 mg/L 5-FC, Amp and final concentration of 0.5 g/L 4HB respectively. the OD values were 0.6226, 0.3105 and 0.1445 after incubation for about 12 h. Therefore, the 100, 200 mg/L 5-FC system, continued incubation for 12h and measured OD values of 0.8123, 0.3301.

The above 100, 200 mg/L 5-FC screening solution was applied to solid medium at an inoculum size of 1 ul. The final concentration of Chl in solid medium was 60, 90 and 120 ug/mL, and Amp antibiotics and a final concentration of 0.5 g/L HPP were added. 48 h later, no colonies grew on any of the three plates. It is thought that the inoculum was too small to cause this.

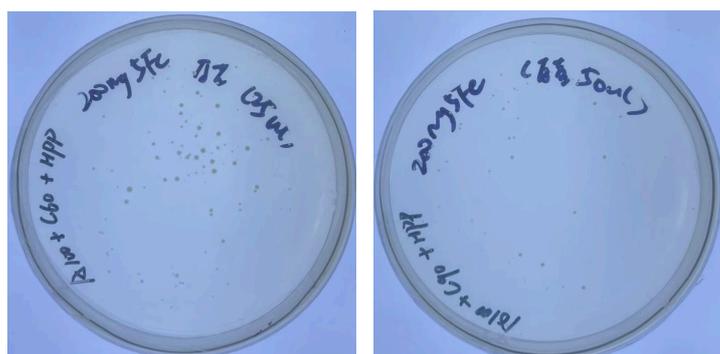
### 9.15 Fenglin Tao Qianwen Jin

Two different methods were used instead.

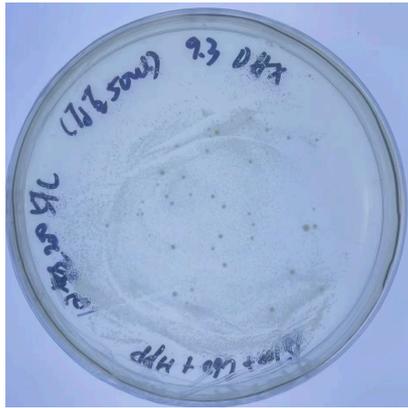
Method 1: Select the bacterial solution initially at 200 mg/L 5FC, increase the inoculum to 50 ul of the original solution and re-coat the plate. Chl final concentrations of 60, 90 and 120ug/mL in solid medium with Amp antibiotics and a final concentration of 0.5 g/L HPP. incubate at 37° and wait to observe their growth.

Method 2: The 100mg/L 5-FC system was transferred to a final concentration of 200mg/L 5-FC system at 1% inoculum and incubated for 36h to reach an OD of 0.8923. 50ul of the transferred bacterial broth was taken and coated. The solid medium was incubated at a final concentration of 60, 90 and 120ug/mL with Amp antibiotics and a final concentration of 0.5 g/L HPP. The plates were incubated at 37° and the growth was observed.

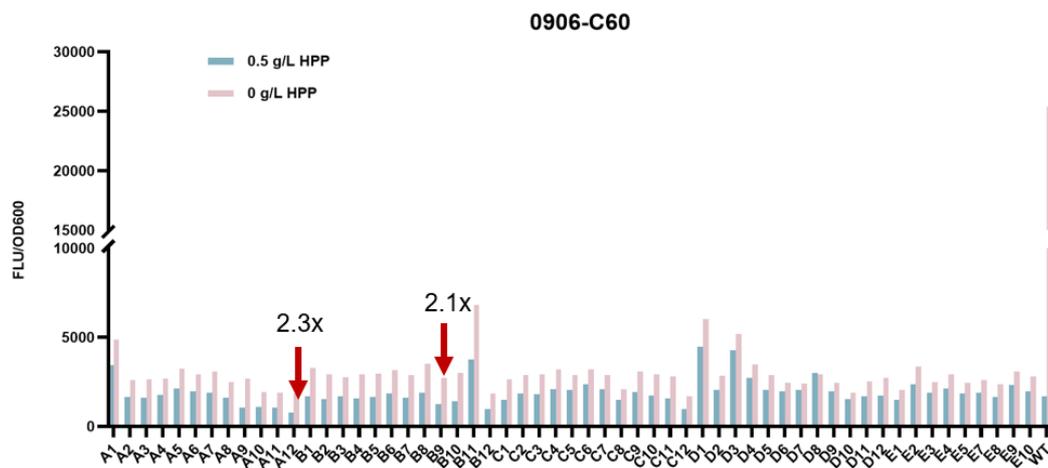
The methodological group of plates grew as follows :



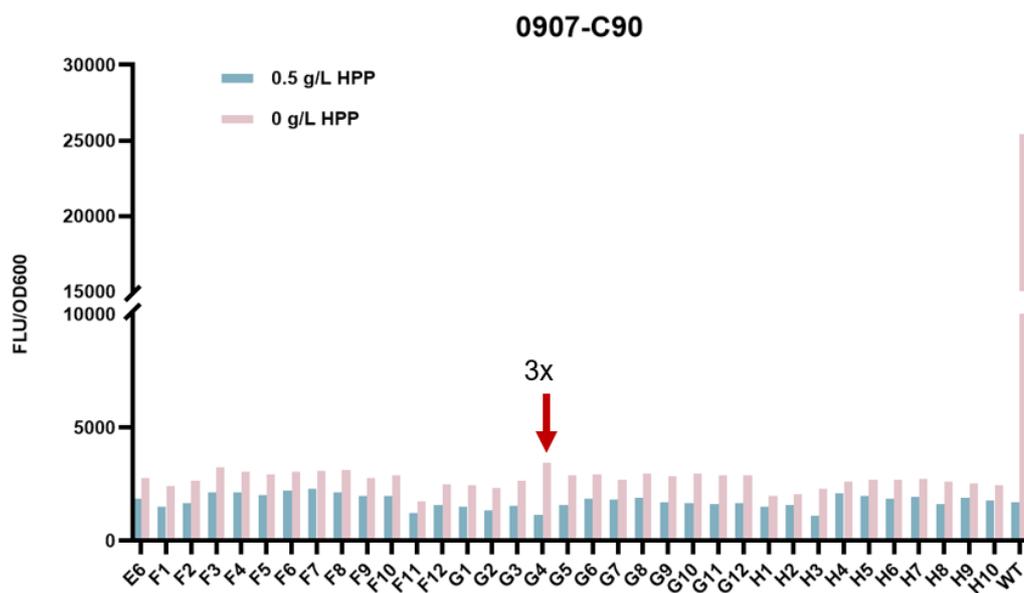
The growth of the plates in Method II group was as follows :



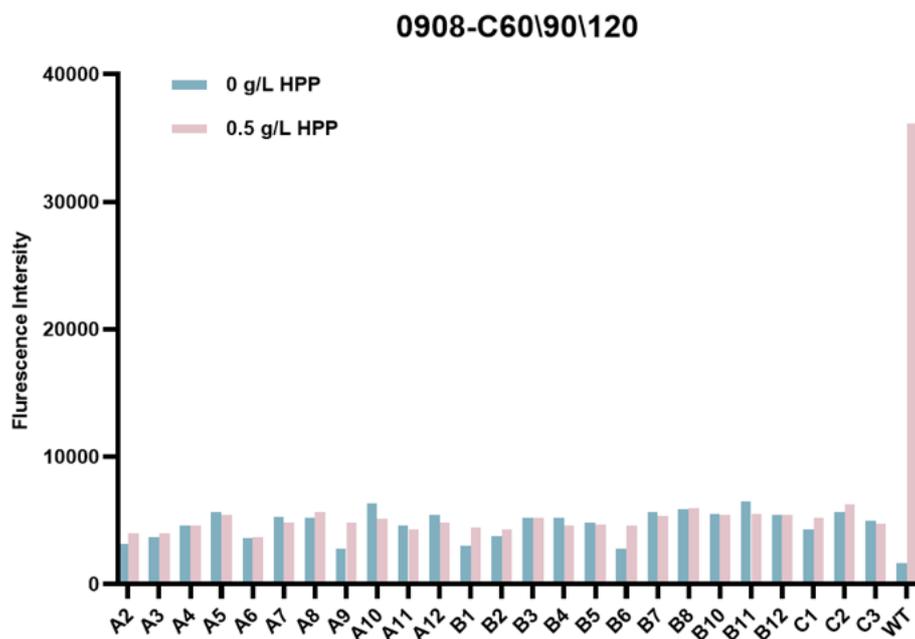
Single colonies were picked from plates at a chloramphenicol concentration of 60 mg/L in the method set for characterisation and fluorescence was as follows :



Single colonies were picked from plates at a chloramphenicol concentration of 90 mg/L in the method set for characterisation and fluorescence was as follows :



Method 2 Groups Chloramphenicol concentrations of 60 / 90 / 120 mg/L were characterised by picking colonies on solid media bacteria and the fluorescence was as follows.



There was no good response and stray bacteria were found on the plates, presumably staining from the transfer process.

### 9.17 Chengjie Dong Linshan Cao

#### Re-screening Method 1 Retained Bacterial Solution

The plates were selected with chloramphenicol concentrations of 60mg/L and 90mg/L and HPP final concentration of 0.5g/L. The amount of bacterial solution coated on the plates was increased to 300µL and 500µL each and the plates were incubated for 36 hours with the following growth.



