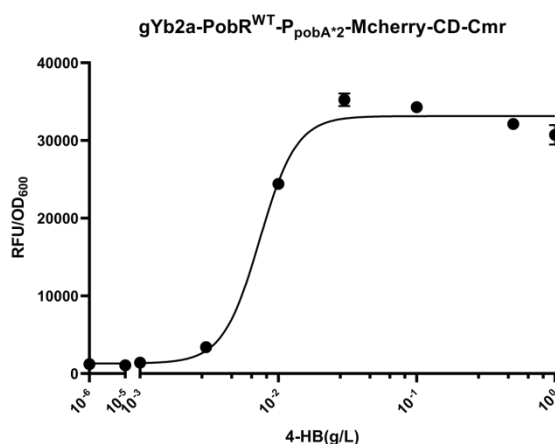


9.18 Xinyao Yuan

Select Original wild type colonies , insert them into liquid LB for a period of time and then streak to isolate single colonies. It was induced with 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} , 0.1, 1, 10 g/L 4HB, and after 12 incubations, the optical density at 600 nm (OD₆₀₀) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), and the fluorescence response curve was plotted.

Experimental group (three groups in parallel)

200 μ L M9 medium + 0.1 mg/L ampicillin(Amp) + 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} , 0.1, 1, 10 g/L 4HB+ bacterial broth



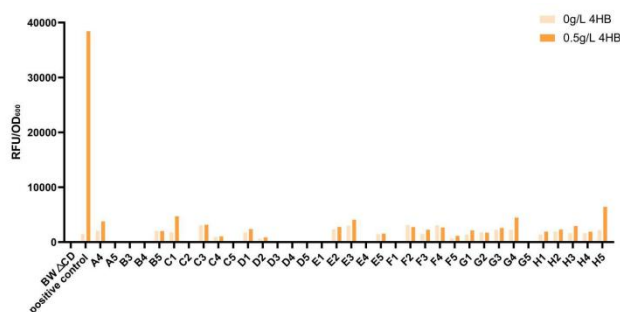
1.Negative screening: a total of two rounds of screening.

9.19 Yuetong Zhu

The first round of screening: the library was transferred to M9 medium with final concentration 0.5 g/L 4HB and 50 mg/L 5-FC to OD=0.5-0.7

9.21 Yuetong Zhu

The second round of screening: take the first round of screening bacterial liquid, transfer 1% of the inoculum to the M9 medium supplemented with 0.5 g/L 4HB, at the same time, increase the concentration of 5-FC to 200 mg/L, and cultivate to OD= 0.7-0.8.



2.Positive screening

9.22 Yuetong Zhu

Take 500 μ L of the second-round screening bacterial solution and spread it on the solid medium supplemented with 0.1 mg/L of ampicillin(Amp), 0.09 g/L of chloramphenicol(Cm) and 0.5 g/L of

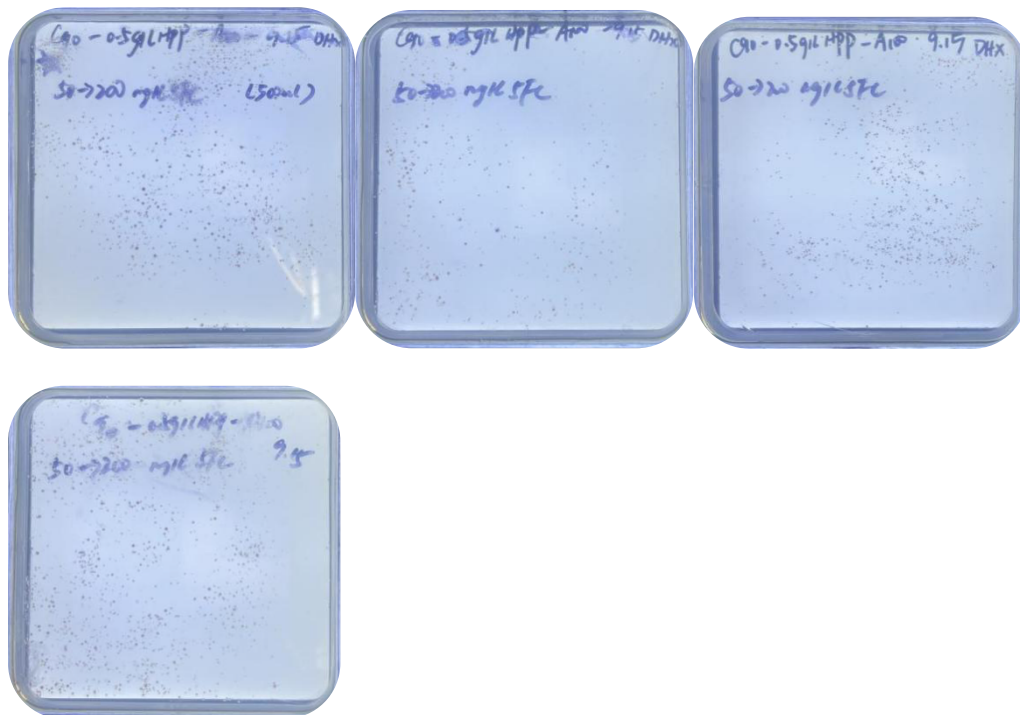
HPP to isolate single colonies. Pick the red colonies from the selection medium and culture them in liquid LB containing ampicillin for 8-10 hours.

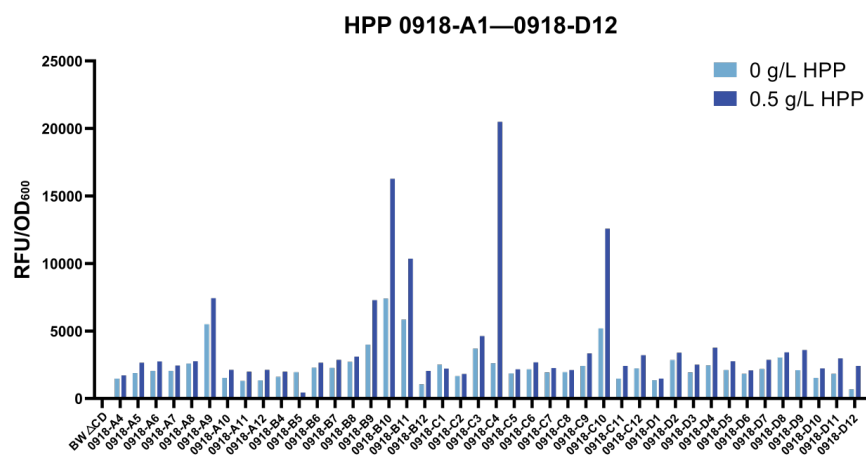
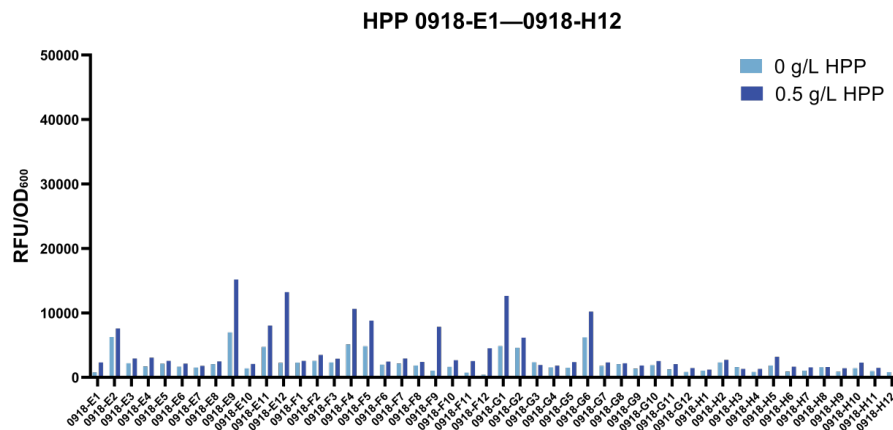
9.23 Qianwen Jin

After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, **0.5 g/L of HPP**, and ampicillin only. in M9 medium. 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).

Induction group: 200 μ L M9 medium + 0.1 mg/L Amp + **0.5 g/L HPP** + bacterial broth.

Uninduced group: 200 μ L M9 medium + 0.1 mg/L Amp + bacterial broth.



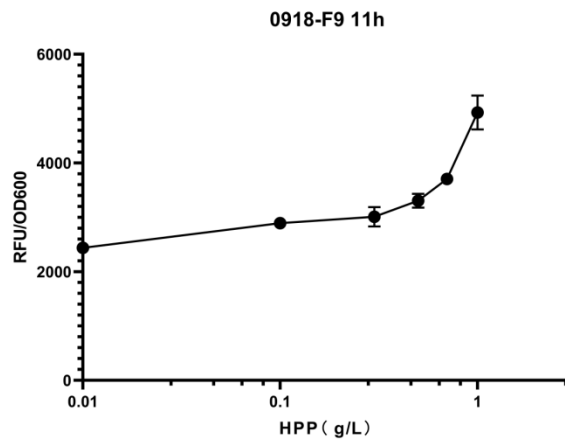
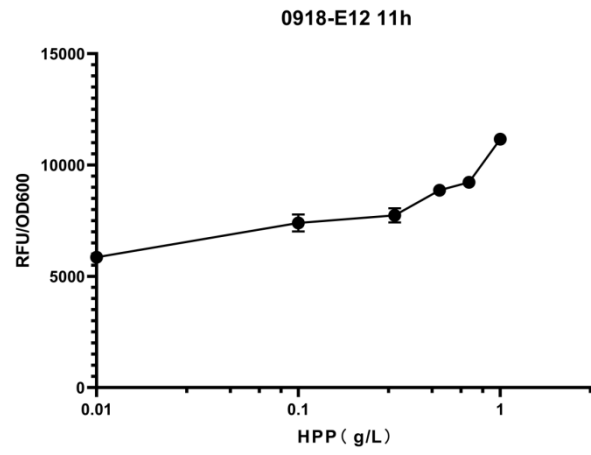
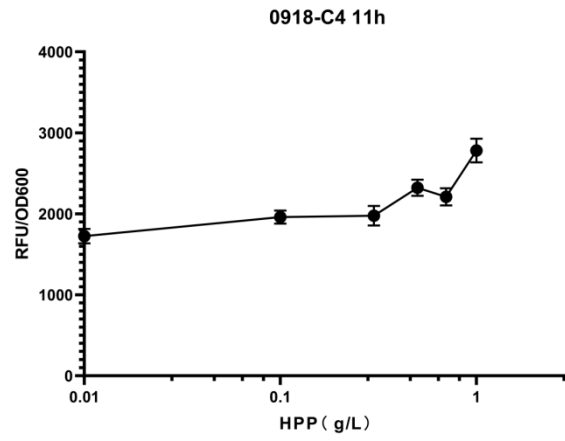


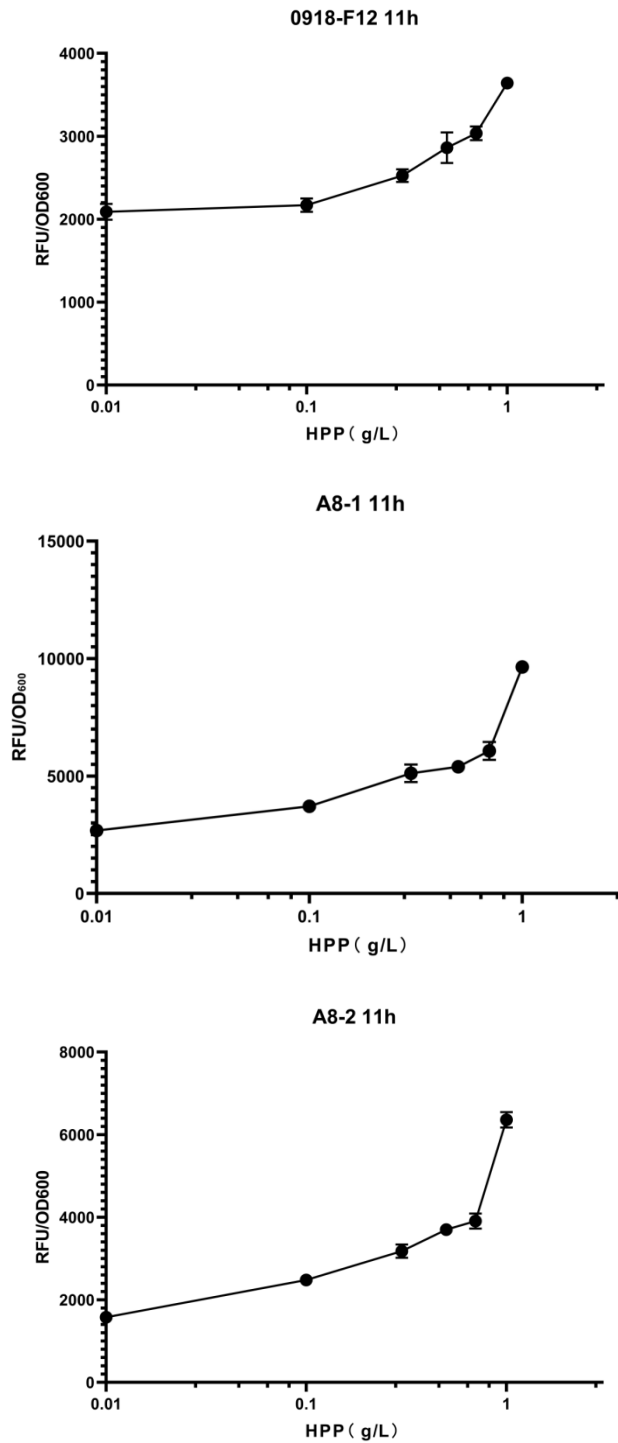
9.18 Bohui Yangyang

Select colonies with higher response multiples, insert them into liquid LB for a period of time and then streak to isolate single colonies. It was induced with **0, 0.01, 0.1, 0.3, 0.5, 0.7, 1 g/L HPP**, and after 12 incubations, the optical density at 600 nm (OD₆₀₀) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), and the fluorescence response curve was plotted. At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Experimental group (three groups in parallel)

200 μ L M9 medium + 0.1 mg/L Amp + **0, 0.01, 0.1, 0.3, 0.5, 0.7, 1 g/L HPP** + bacterial broth





1. **Negative screening: a total of two rounds of screening.**

9.19 Xiaoya Wei

The first round of screening: the library was transferred to M9 medium with final concentration 0.5 g/L 4HB and 50mg/L 5-FC to OD=0.5-0.7

9.21 Xiaoya Wei

The second round of screening: take the first round of screening bacterial liquid, transfer 1% of the inoculum to the M9 medium supplemented with 0.5 g/L 4HB, at the same time, increase the concentration of 5-FC to 200 mg/L, and cultivate to OD= 0.7-0.8.

2. Positive screening

9.22 Chengjie Dong

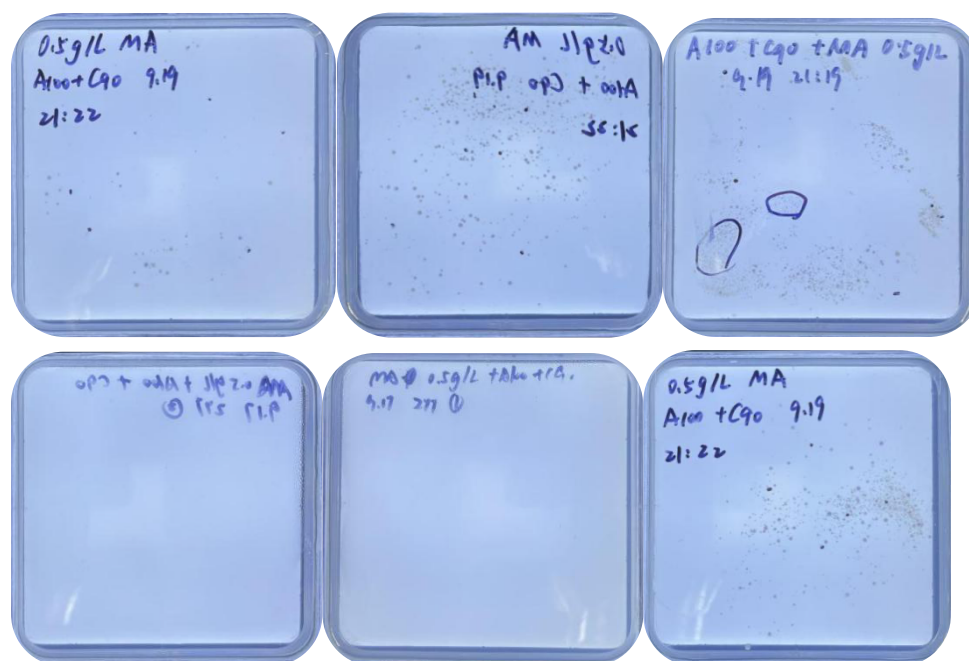
Take 500 μ L of the second-round screening bacterial solution and spread it on the solid medium supplemented with 0.1 mg/L of Amp, 0.09 g/L of Cm and **0.5 g/L of MA** to isolate single colonies. Pick the red colonies from the selection medium and culture them in liquid LB containing ampicillin for 8-10 hours.

9.23 Qianwen Jin

After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, **0.5 g/L MA**, and ampicillin only. in M9 medium. 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).

Induction group: 200 μ L M9 medium + 0.1 mg/L Amp + **0.5 g/L MA** + bacterial broth.

Uninduced group: 200 μ L M9 medium + 0.1 mg/L Amp + bacterial broth.



11p20 AM + opJ + oolA
Pl:ls Pl.p

11p20 AM + opJ + oolA
6.5 g/L MA 9.20 27/
9.21 10:00 032

opJ + oolA + AM 11p20
7.5 g/L AM JK 2.0
52 00/01 14.8

AM 11p20
Pl.p. opJ + oolA
55:15

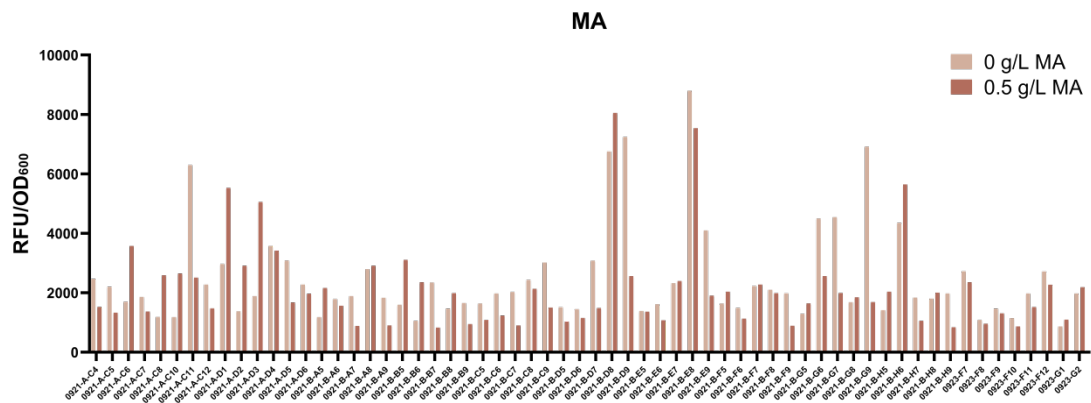
MA + 5g/L + 13h
9.17 277 0

MA + 5g/L + AM + 19
9.17 277 3

20g/L MA + 19h + 19
11p20 9.20
p-51 20m p-30

11p20 AM + opJ + oolA
Pl:ls Pl.p

11p20 AM + opJ + oolA
Pl:ls Pl.p

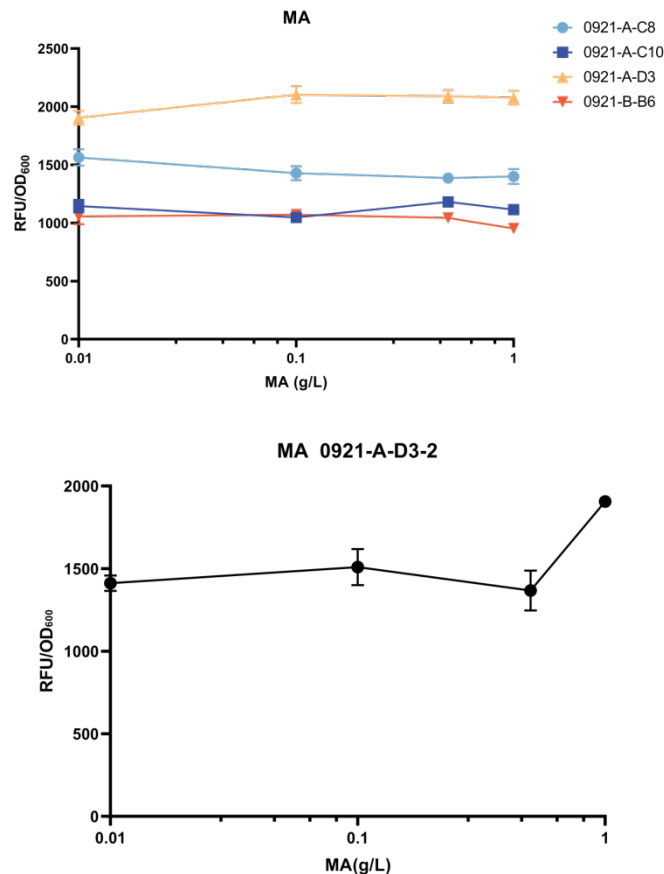


9.21 Jianing Li

Select colonies with higher response multiples, insert them into liquid LB for a period of time and then streak to isolate single colonies. It was induced with **0, 0.01, 0.1, 0.5, 1 g/L MA**, and after 12 incubations, the optical density at 600 nm (OD₆₀₀) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), and the fluorescence response curve was plotted. At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Experimental group (three groups in parallel)

200 μ L M9 medium + 0.1 mg/L Amp + **0, 0.01, 0.1, 0.5, 1 g/L MA** + bacterial broth



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

9.22 Jiale Li

The first round of screening: the library was transferred to M9 medium with final concentration 0.5 g/L 4HB and 50mg/L 5-FC to OD=0.5-0.7

9.24 Xinyao Yuan

The second round of screening: take the first round of screening bacterial liquid, transfer 1% of the inoculum to the M9 medium supplemented with 0.5 g/L 4HB, at the same time, increase the concentration of 5-FC to 200 mg/L, and cultivate to OD= 0.7-0.8.

3. Positive screening

9.25 Xinyao Yuan

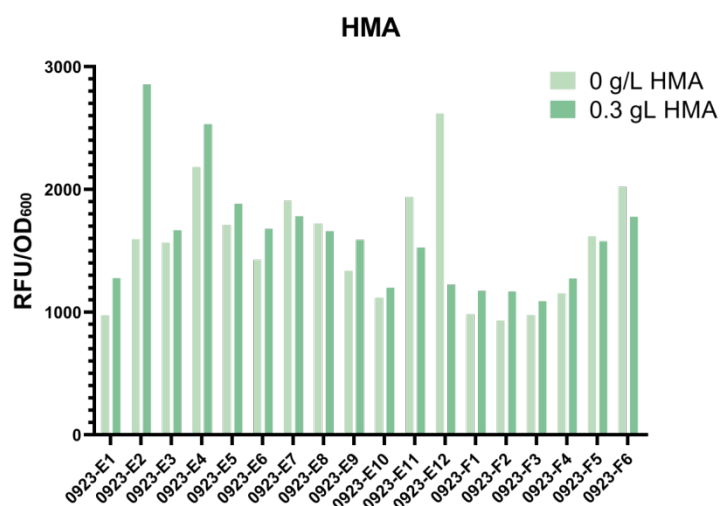
Take 500 μ L of the second-round screening bacterial solution and spread it on the solid medium supplemented with 0.1 mg/L of Amp, 0.09 g/L of Cm and 0.5 g/L of HMA to isolate single colonies. Pick the red colonies from the selection medium and culture them in liquid LB containing ampicillin for 8-10 hours.

9.26 Yuzhu Wang

After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, 0.5 g/L HMA, and ampicillin only. in M9 medium. 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).

Induction group: 200 μ L M9 medium + 0.1 mg/L Amp + **0.5 g/L HMA** + bacterial broth.

Uninduced group: 200 μ L M9 medium + 0.1 mg/L Amp + bacterial broth.

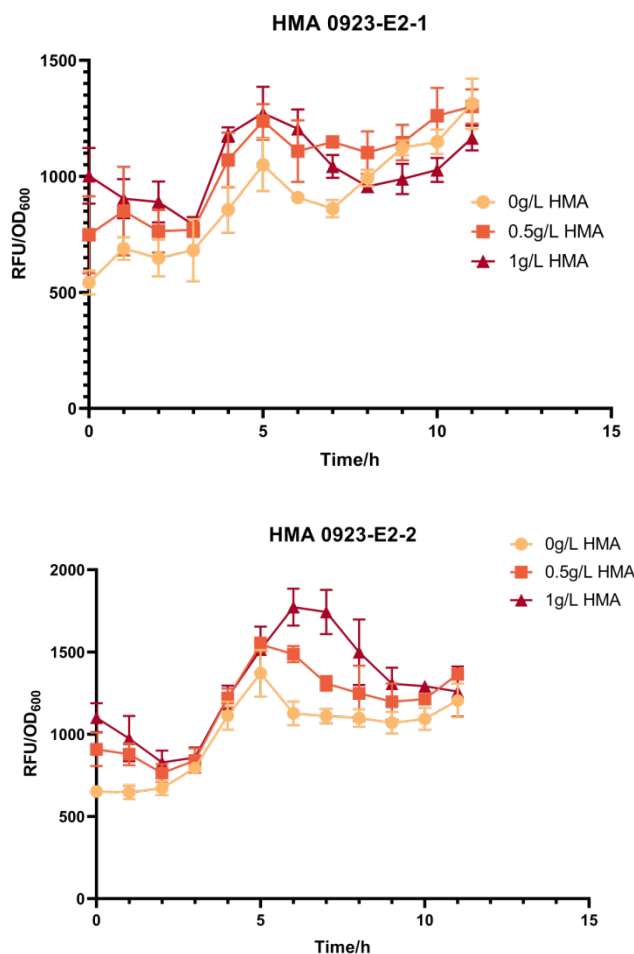


9.23 Xinyao Yuan

Select colonies with higher response multiples, insert them into liquid LB for a period of time and then streak to isolate single colonies. It was induced with 0, **0.01, 0.1, 0.5, 1 g/L HMA**, and after 12 incubations, the optical density at 600 nm (OD₆₀₀) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), and the fluorescence response curve was plotted. At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Experimental group (three groups in parallel)

200 μ L M9 medium + 0.1mg/L Amp +**0, 0.01, 0.1, 0.5, 1 g/L HMA** + bacterial broth



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

9.24 Xiaoya Wei

The first round of screening: the library was transferred to M9 medium supplemented with 0.5 g/L 4HB and 50 mg/L 5-FC to OD=0.5.

9.26 Fenglin Tao

The second round of screening: take the first round of screening bacterial liquid, transfer 1% of the inoculum to the M9 medium supplemented with 0.5 g/L 4HB, at the same time, increase the concentration of 5-FC to 200 mg/L, and cultivate to OD= 0.7-0.8.

2.Positive screening

9.27 Fenglin Tao

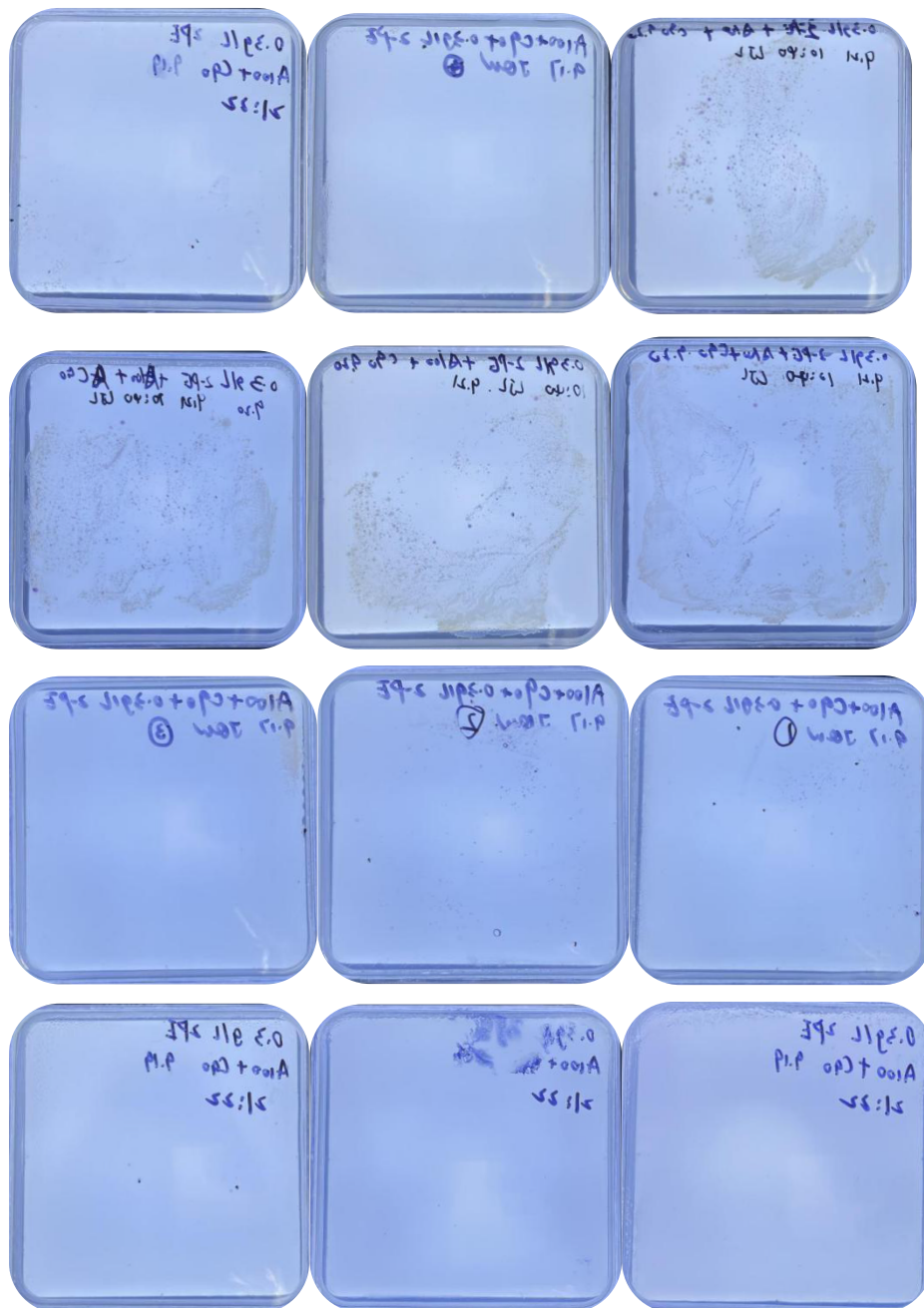
Take 500 μ L of the second-round screening bacterial solution and spread it on the solid medium supplemented with 0.1 mg/L of Amp, 0.09 g/L of Cm and 0.3 g/L 2-PE to isolate single colonies. Pick the red colonies from the selection medium and culture them in liquid LB containing ampicillin for 8-10 hours.

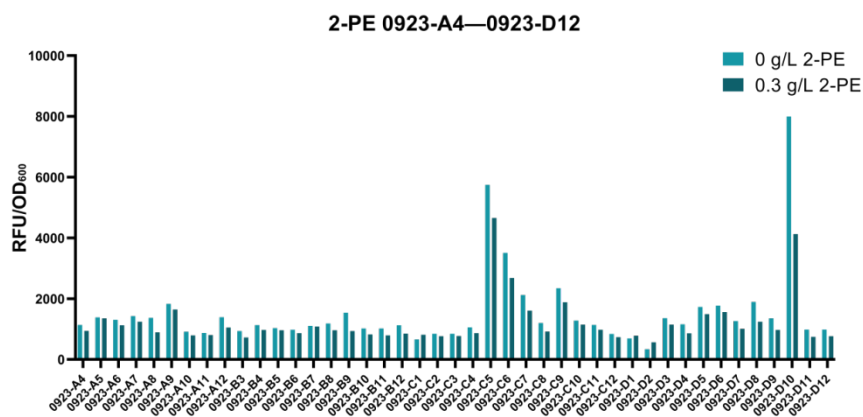
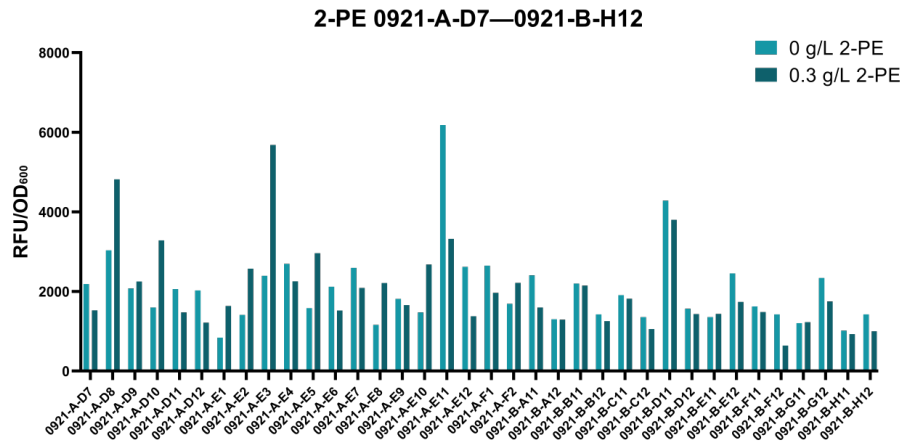
9.28 Fenglin Tao

After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, 0.3 g/L 2-PE, and ampicillin only. in M9 medium. 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).

Induction group: 200 μ L M9 medium + 0.1 mg/L Amp + **0.3 g/L 2-PE** + bacterial broth.

Uninduced group: 200 μ L M9 medium + 0.1 mg/L Amp + bacterial broth.



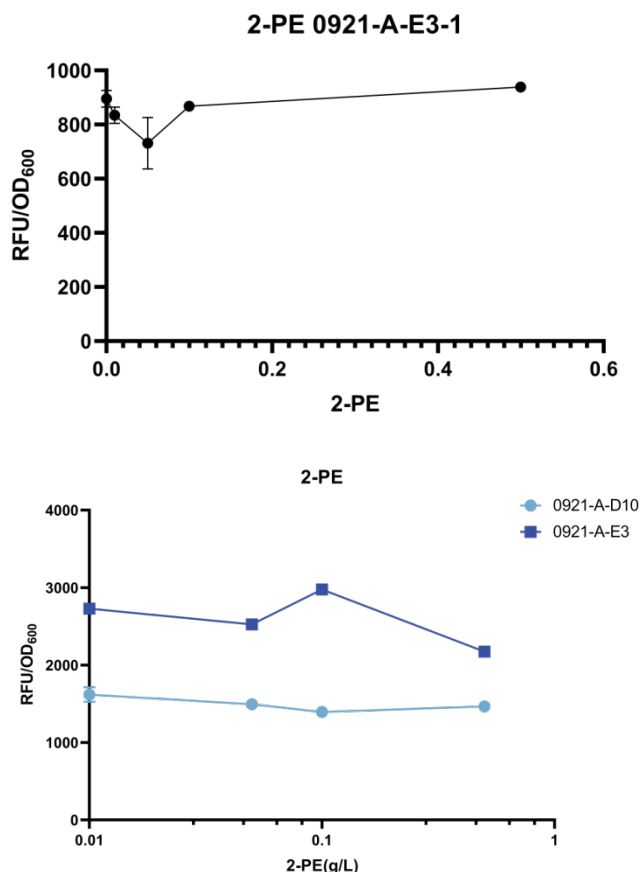


9.21 Jianing Li

Select colonies with higher response multiples, insert them into liquid LB for a period of time and then streak to isolate single colonies. It was induced with **0, 0.01, 0.05, 0.1, 0.5 g/L 2-PE**, and after 12 incubations, the optical density at 600 nm (OD₆₀₀) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), and the fluorescence response curve was plotted. At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Experimental group (three groups in parallel)

200 μ L M9 medium + 0.1 mg/L Amp + **0, 0.01, 0.05, 0.1, 0.5 g/L 2-PE** + bacterial broth



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

9.22 Bohui Yangyang

The first round of screening: the library was transferred to M9 medium with final concentration 0.5 g/L 4HB and 50 mg/L 5-FC to OD=0.5-0.7

9.24 Bohui Yangyang

The second round of screening: take the first round of screening bacterial liquid, transfer 1% of the inoculum to the M9 medium supplemented with 0.5 g/L 4HB, at the same time, increase the concentration of 5-FC to 200 mg/L, and cultivate to OD= 0.7-0.8.

2. Positive screening

9.25 Bohui Yangyang

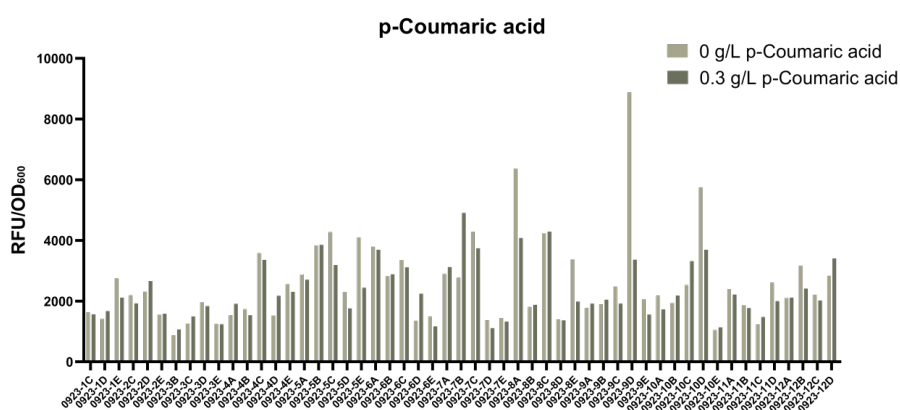
Take 500 μ L of the second-round screening bacterial solution and spread it on the solid medium supplemented with 0.1 mg/L of Amp, 0.09 g/L of Cm and 0.3 g/L p-coumaric acid to isolate single colonies. Pick the red colonies from the selection medium and culture them in liquid LB containing ampicillin for 8-10 hours.

9.26 Jiale Li

After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, 0.3 g/L p-coumaric acid, and ampicillin only. in M9 medium. 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).

Induction group: 200 μ L M9 medium + 0.1 mg/L Amp + 0.3 g/L p-coumaric acid + bacterial broth.

Uninduced group: 200 μ L M9 medium + 0.1 mg/L Amp + bacterial broth.

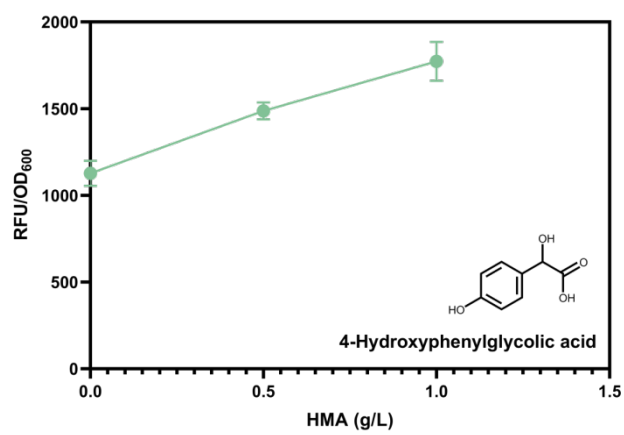
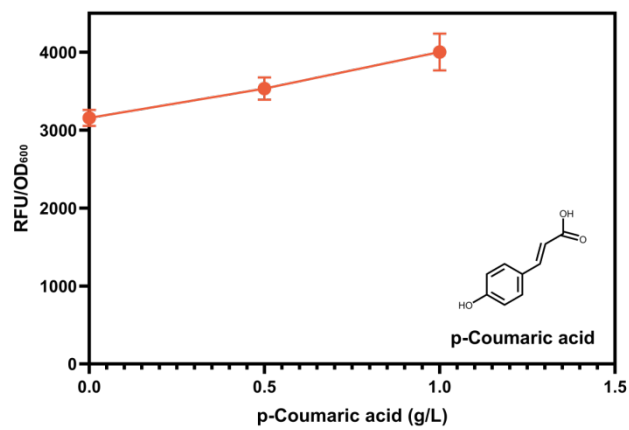


9.23 Jiale Li

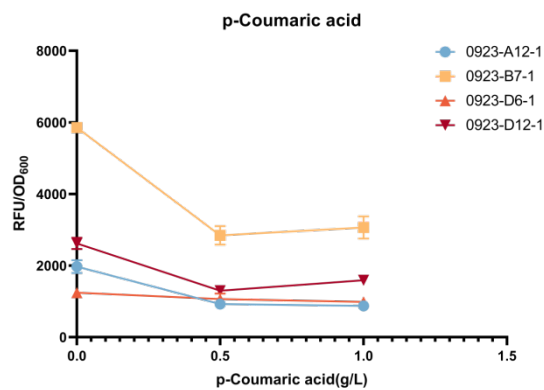
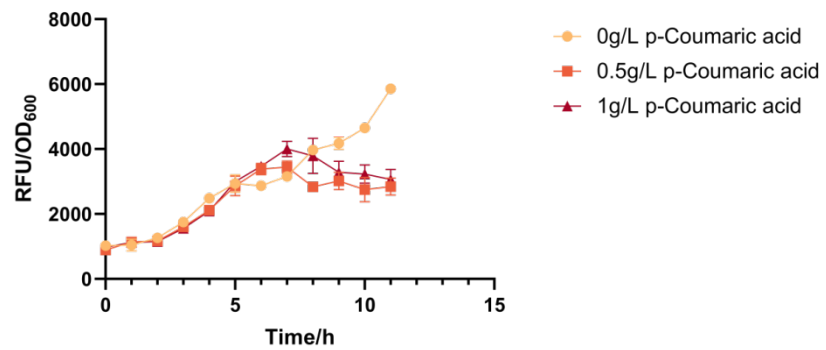
Select colonies with higher response multiples, insert them into liquid LB for a period of time and then streak to isolate single colonies. It was induced with **0,0.5,1 g/L p-coumaric acid**, and after 12 incubations, the optical density at 600 nm (OD₆₀₀) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), and the fluorescence response curve was plotted. At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Experimental group (three groups in parallel)

200 μ L M9 medium + 0.1 mg/L Amp + **0,0.5,1 g/L p-coumaric acid** + bacterial broth



p-Coumaric acid 0923-B7-1



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

9.24 Jiale Li

The first round of screening: the library was transferred to M9 medium with final concentration 0.5 g/L 4HB and 50 mg/L 5-FC to OD=0.5-0.7

9.26 Linshan Cao

The second round of screening: take the first round of screening bacterial liquid, transfer 1% of the inoculum to the M9 medium supplemented with 0.5 g/L 4HB, at the same time, increase the concentration of 5-FC to 200 mg/L, and cultivate to OD= 0.7-0.8.

2.Positive screening

9.27 Linshan Cao

Take 500 μ L of the second-round screening bacterial solution and spread it on the solid medium supplemented with 0.1 mg/L of ampicillin, 0.09 g/L of chloramphenicol and 0.3 g/L PAld to isolate single colonies. Pick the red colonies from the selection medium and culture them in liquid LB containing ampicillin for 8-10 hours.

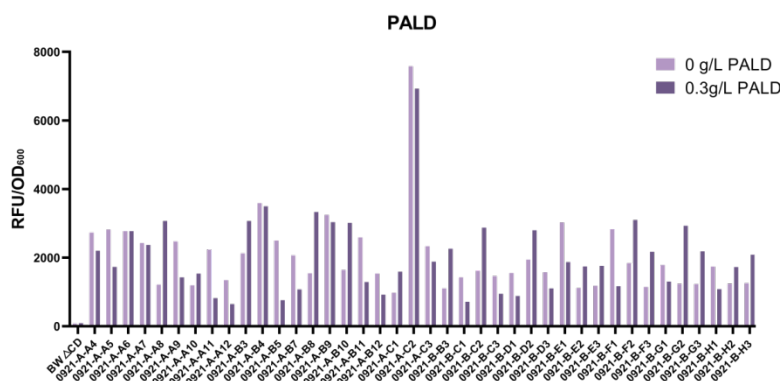
9.28 Xiaoya Wei

After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, 0.3 g/L PAld, and ampicillin only. in M9 medium. 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).

Induction group: 200 μ L M9 medium + 0.1 mg/L Amp +0.3 g/L PAld+ bacterial broth.

Uninduced group: 200 μ L M9 medium + 0.1 mg/L Amp + bacterial broth.



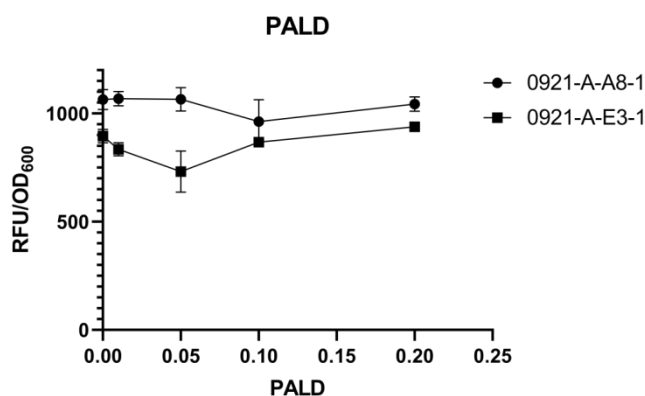


9.21 Xiaoya Wei

Select colonies with higher response multiples, insert them into liquid LB for a period of time and then streak to isolate single colonies. It was induced with 0,0.01,0.05,0.1,0.2 g/L PALd , and after 12 incubations, the optical density at 600 nm (OD600) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), and the fluorescence response curve was plotted. At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Experimental group (three groups in parallel)

200 μ L M9 medium + 0.1 mg/L Amp + 0,0.01,0.05,0.1,0.2 g/L PALd + bacterial broth



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

9.22 Jianing Li

The first round of screening: the library was transferred to M9 medium with final concentration 0.5 g/L 4HB and 50 mg/L 5-FC to OD=0.5-0.7

9.24 Jianing Li

The second round of screening: take the first round of screening bacterial liquid, transfer 1% of the inoculum to the M9 medium supplemented with 0.5g/L 4HB, at the same time, increase the concentration of 5-FC to 200mg/L, and cultivate to OD= 0.7-0.8.

2.Positive screening

9.25 Yuzhu Wang

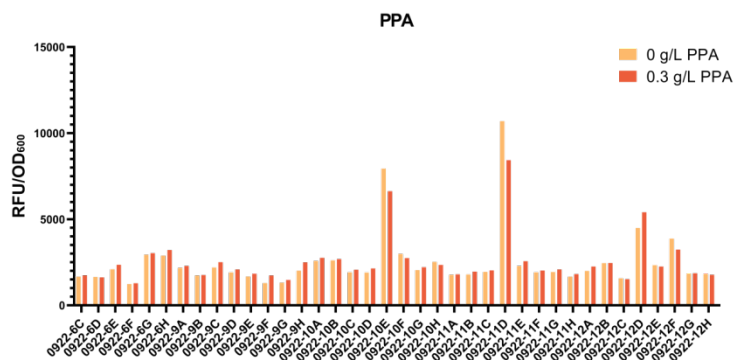
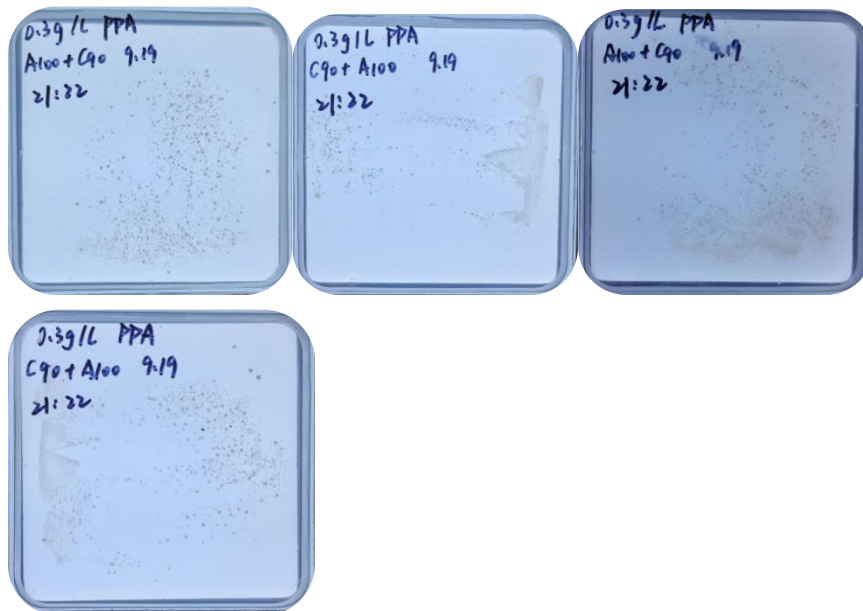
Take 500 μ L of the second-round screening bacterial solution and spread it on the solid medium supplemented with 0.1 mg/L of ampicillin, 0.09 g/L of chloramphenicol and **0.3 g/L PPA** to isolate single colonies. Pick the red colonies from the selection medium and culture them in liquid LB containing ampicillin for 8-10 hours.

9.26 Yuzhu Wang Jianing Li

After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, **0.3 g/L PPA**, and ampicillin only. in M9 medium. 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).

Induction group: 200 μ L M9 medium + 0.1 mg/L Amp +**0.3 g/L PPA**+ bacterial broth.

Uninduced group: 200 μ L M9 medium + 0.1 mg/L Amp + bacterial broth.

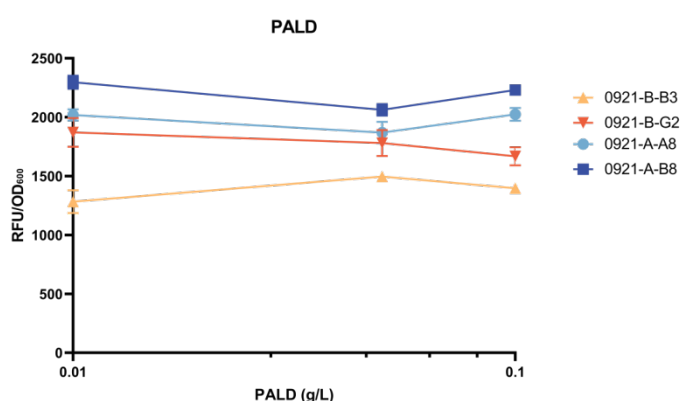


9.21 Qianwen Jin

Select colonies with higher response multiples, insert them into liquid LB for a period of time and then streak to isolate single colonies. It was induced with 0,0.01,0.05,0.1,0.5 g/L PALD, and after 12 incubations, the optical density at 600 nm (OD₆₀₀) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), and the fluorescence response curve was plotted. At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Experimental group (three groups in parallel)

200 µL M9 medium + 0.1 mg/L Amp + 0,0.01,0.05,0.1,0.5 g/L PALD + bacterial broth



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

9.22 Qianwen Jin

The first round of screening: the library was transferred to M9 medium with final concentration 0.5 g/L 4HB and 50 mg/L 5-FC to OD=0.5-0.7

9.24 Xinyao Yuan

The second round of screening: take the first round of screening bacterial liquid, transfer 1% of the inoculum to the M9 medium supplemented with 0.5 g/L 4HB, at the same time, increase the concentration of 5-FC to 200 mg/L, and cultivate to OD= 0.7-0.8.

2.Positive screening

9.25 Xinyao Yuan

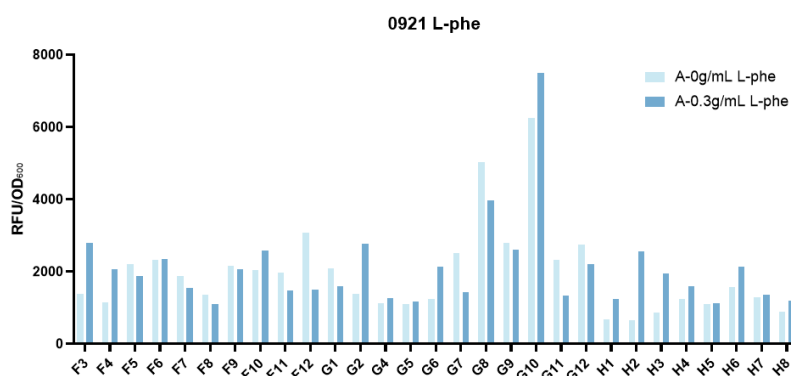
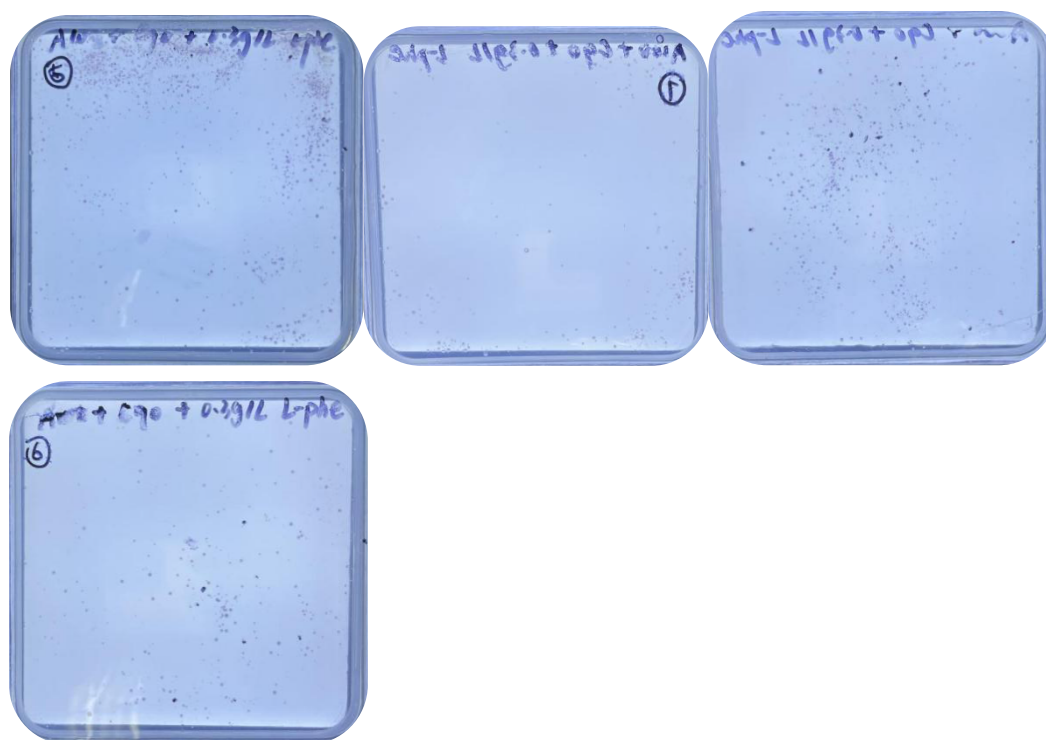
Take 500 µL of the second-round screening bacterial solution and spread it on the solid medium supplemented with 0.1 mg/L of ampicillin, 0.09 g/L of chloramphenicol and 0.3 g/L L-Phe to isolate single colonies. Pick the red colonies from the selection medium and culture them in liquid LB containing ampicillin for 8-10 hours.

9.26 Bohui Yangyang

After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, 0.3 g/L L-Phe, and ampicillin only. in M9 medium. 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).

Induction group: 200 μ L M9 medium + 0.1 mg/L Amp + **0.3 g/L L-Phe** + bacterial broth.

Uninduced group: 200 μ L M9 medium + 0.1 mg/L Amp + bacterial broth.

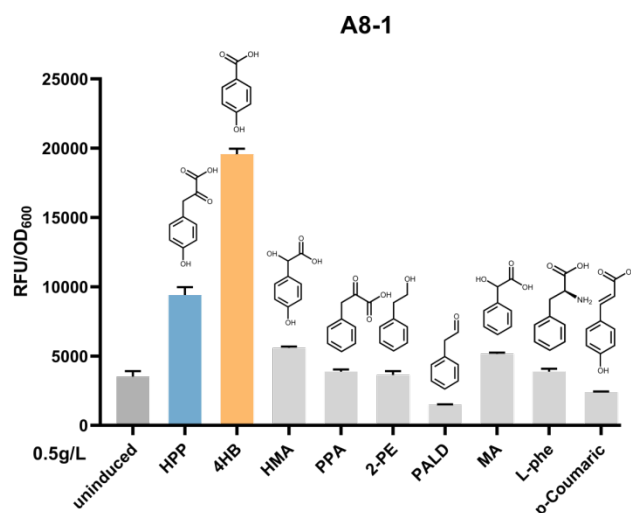


9.29 Jianing Li

To verify the specificity of the mutant strain, we select monoclonal, insert it into liquid LB for a period of time and then streak to isolate single colonies. It was induced with **uninduced, 0.5 g/L of HPP, 4HB, HMA, PPA, 2-PE, MA, PALD, L-Phe and p-coumaric acid**, and after 12 incubations, the optical density at 600 nm (OD₆₀₀) and red fluorescence were measured (552 nm as excitation wavelength and 600 nm as emission wavelength), and the fluorescence response curve was plotted. At the same time, plasmids were extracted from the bacteria that were characterized and sent for sequencing to analyze the mutation sites.

Experimental group (three groups in parallel)

200 μ L M9 medium +0.1mg/L Amp +uninduced,0.5 g/L of
HPP,4HB,HMA,PPA,2-PE,MA,PALD,L-Phe and p-coumaric acid + bacterial



To test the capacity of our mutant library, we used the dilution coating plate method to determine the total library volume. We diluted 50ul of the bacterial solution to 5000ul (1:100), from which 50ul of the solution was aspirated and applied to a plate with a final concentration of 0.1mg/L Amp, and the total amount of the mutant library was estimated based on the number of colonies versus the dilution multiple.



Based on our calculation, the original density of the two-round selected bacteria was 450,000 CFU/mL