7.20-8.27

1.Constructed the plasmid gYB2a-pobRWT-mCherry-codA-cmr

The person primarily responsible for the work: Qianwen Jin

Construction time:

1. Obtain CD fragment by PCR

The target fragment is located on the pUAM-DE-CD plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system（50μL）	
pUAM-DE-CD	10ng
CD-Gibson-0425 F	2μL
CD-Gibson-0425 R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 1

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 1332bp. We obtained the correct target fragment, and the sample is purified.

2. Obtain gYb2a-PpobA*2-mCherry-Cmr fragment by PCR

The target fragment is located on the gYb2a-PpobA*2-mCherry-SacB-Cmr plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system（50μL）	
gYb2a-PpobA*2-mCherry-SacB-Cmr	10ng
Mc-Gibson-F	2μL
Mc-Gibson-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 2

The correct target fragment is 4572bp. We obtained the correct target fragment, and the sample is purified.

3. Gibson connection

The CD fragment and gYb2a-PpobA*2-mCherry-Cmr fragment are connected by Gibson connection method, and the connection system is as follows.

Connection system（10μL）	
CD	2.4μL
gYb2a-PpobA*2-mCherry-XX-Cmr	1μL
5 x Cell Buffer	2μL
Exnase II	1μL
DDW	3.6μL

Table 3

4. Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 5 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system（10μL）	
gYb2a-PpobA*2-mCherry-CD-Cmr	1μL
CD-Gibson-0420-F	0.4μL
CD-Gibson-0420-R	0.4μL
2 x Mix	5μL
DDW	3.2μL

Table 4

The PCR products were detected by agarose gel electrophoresis, and the results were as follows

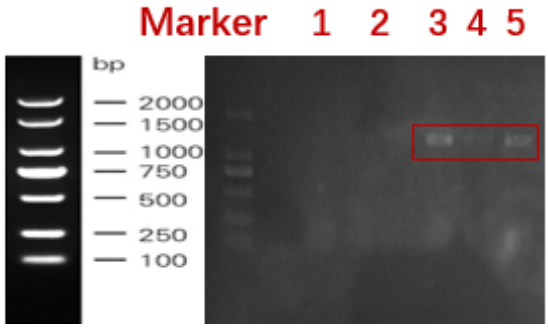


Figure 1

The correct target fragment is about 1300bp, and the length of the colony PCR sample in lanes 3,4 and 5 is inferred from the gel electrophoresis image is correct. The above 3 strains were expanded and the plasmids were put forward.

5. Enzyme digestion verification

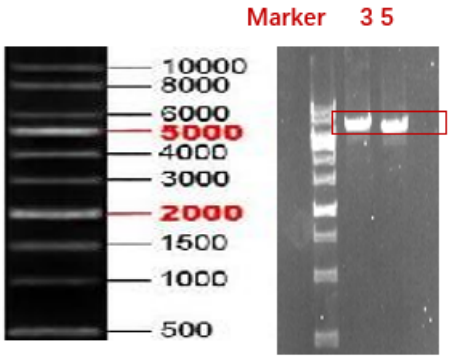
Use the enzyme KpnI to cut the plasmid gYb2a-PpobA*2-mCherry-CD-Cmr at the same time to

verify whether the plasmid is constructed correctly. The following is the system of digestion.

Digestion system（10μL）	
gYb2a-PpobA*2-mCherry-CD-Cmr	100ng
KpnI	0.2μL
custsmart	1μL
DDW	to 10μL

Table 5

Digested gYb2a-PpobA*2-mCherry-CD-Cmr is verified by electrophoresis which is showed below.



It is inferred from the gel electrophoresis that the length of the sample is as expected. The construction is preliminarily correct. We sequenced the constructed plasmid. The result shows that the sequence is the same as expected, indicating that our target plasmid has been constructed successfully.

8.28-9.3

2. Validation of PobRWT

The person primarily responsible for the work: Xiaoya Wei

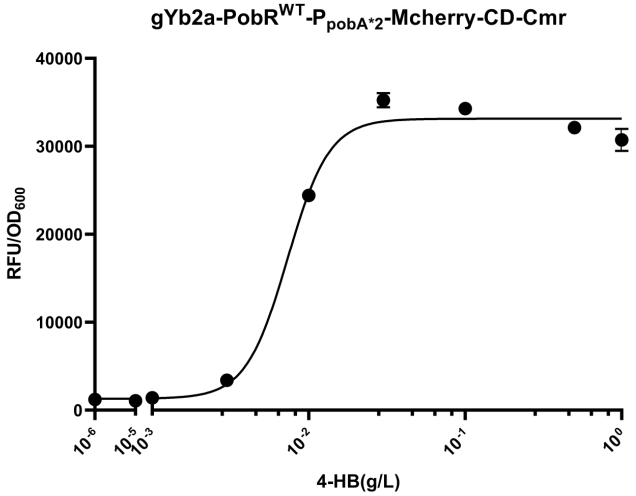
Experimental time:

$PobR^{WT}$ exhibited a high degree of ligand specificity with a narrow dynamic range.

Original wild type colonies was induced with 10-6,10-5,10-4,10-3,10-2,0.1,1,10g/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):

200μL M9 medium + 1mg/L Amp + 4HB at each concentration+ bacteria



9.5-9.7

3.Validating the feasibility of a dual selection system

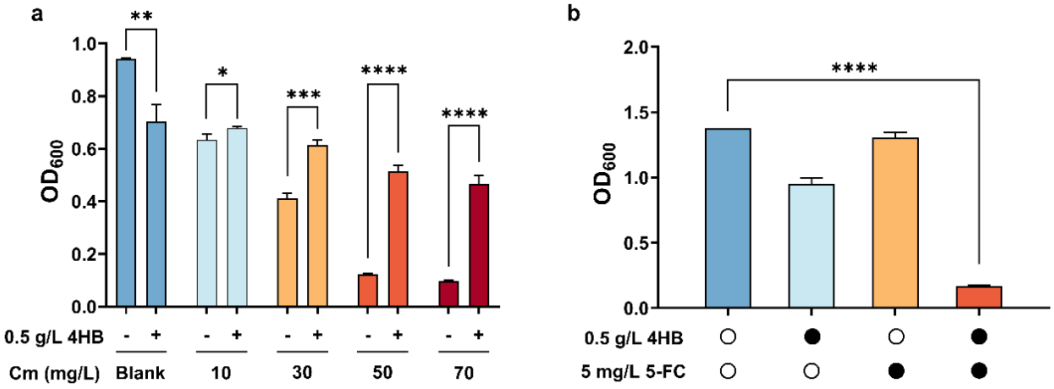
The person primarily responsible for the work: Jianing Li

Experimental time:

In our design, with the presence of 4HB, the *E. coli* BWΔ*codA* harboring the PobR^{WT} biosensor was resistant to chloramphenicol, but sensitive to 5-FC.

Our verification methods: Cultivation of bacteria harboring the PobR^{WT} biosensor in medium containing different concentrations of chloramphenicol and supplied with 0.5 g/L 4HB.

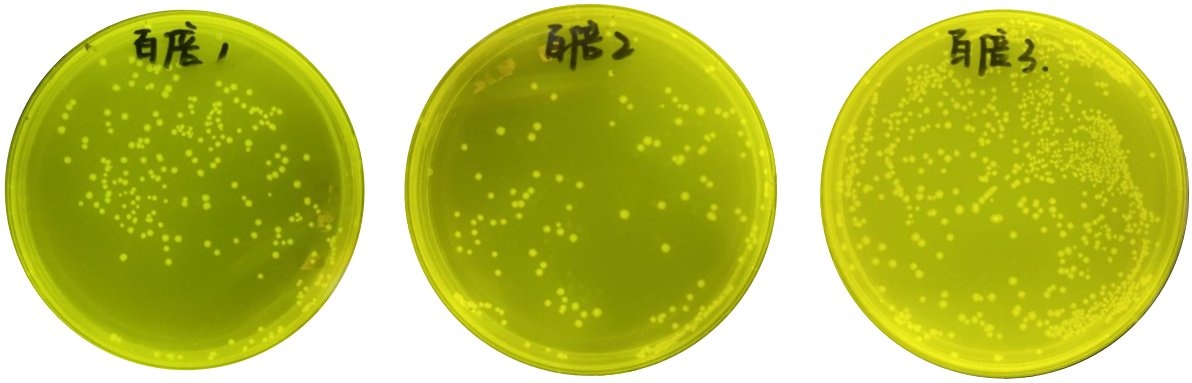
Cultivation of bacteria in medium containing either 0.5 g/L 4HB or 5 mg/L 5-FC alone, or both. The OD_{600} was measured after 12h of cultivation.



9.8-9.10

4.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.

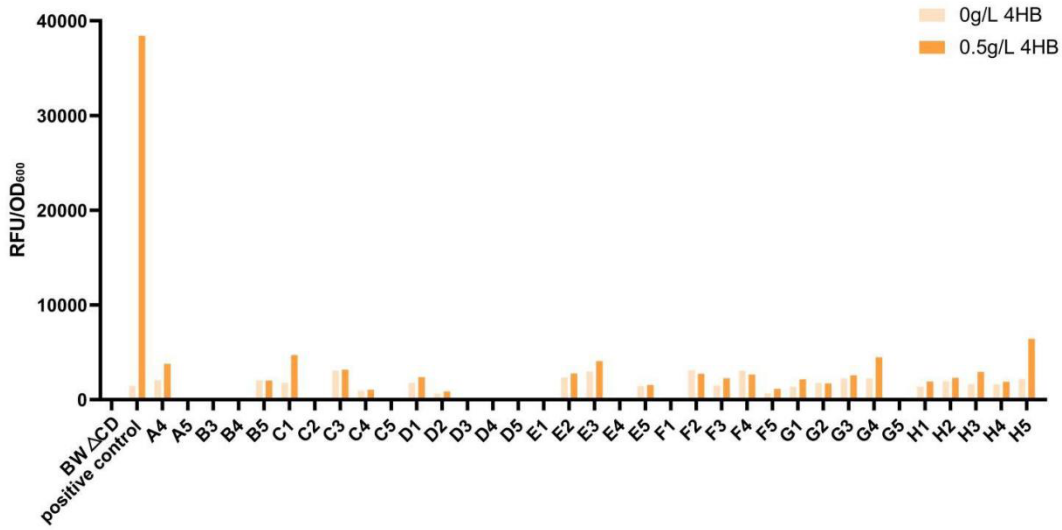


The selection capacity for each compound was more than 900,000 clones (with at least four plates about 450,000 CFU/mL.)

9.5-9.10

5. Dual selection significantly reduces the response of the sensor to natural substrates

As these mutants underwent two rounds of negative selections, when tested with increasing 4HB concentration, the obtained PobR mutants exhibited significantly reduced basal expression and robustly attenuated 4HB responsiveness compared to that of $PobR^{WT}$.



9.11-9.29

6.Selecting the PobR mutation library

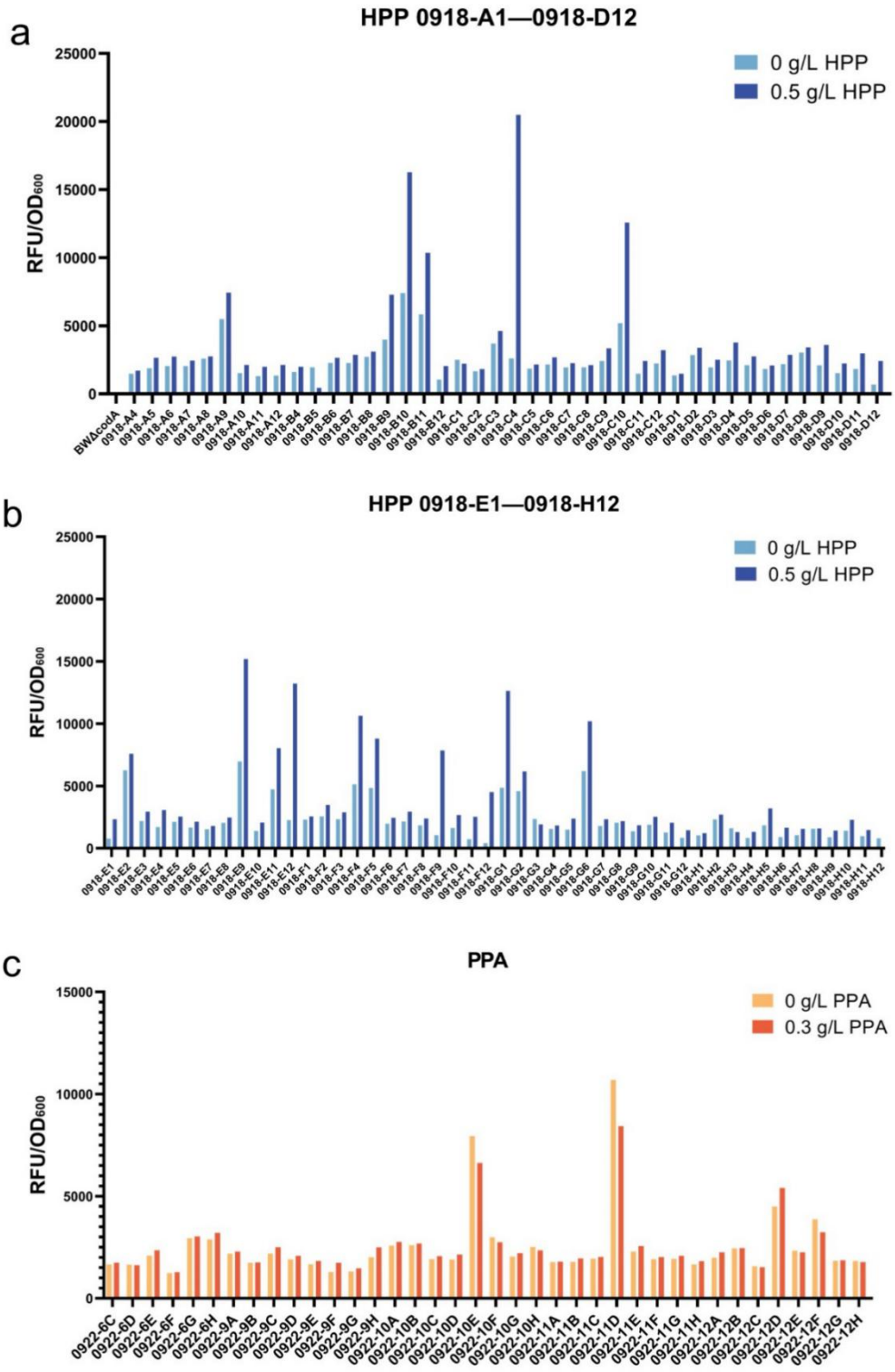
In positive selection experiments of PobR mutants using different ligands, we obtained a biosensor that the fluorescence intensity was at least 1.5-fold higher than that of the negative control.

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 0.5 g/L of HPP /0.3 g/L of PPA /0.3 g/L of 2-PE /0.3 g/L of HMA /0.5 g/L of MA /0.3 g/L of PAld /0.3 g/L of p-Coumaric to isolate single colonies. Pick the red

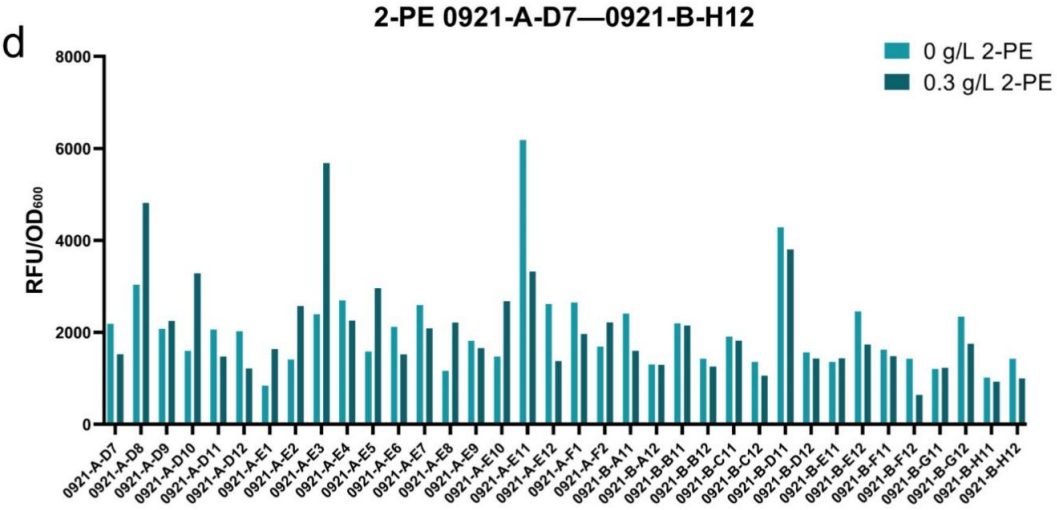
colonies from the selection medium and culture them in liquid LB containing ampicillin for 8-10 hours.

After culturing for 8-10 hours, take 2 microliters of bacterial broth to inoculate 200 microliters containing ampicillin, 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 μL M9 medium + 0.1mg/L Amp + 0.5 g/L of HPP /0.3 g/L of PPA /0.3 g/L of 2-PE /0.3 g/L of HMA /0.5 g/L of MA /0.3 g/L of PAld /0.3 g/L + bacterial broth.
Uninduced group: 200 μL M9 medium + 0.1mg/L Amp + bacterial broth.


a
HPP 0918-A1—0918-D12
0 g/L HPP
0.5 g/L HPP
RFU/OD600
25000
20000
15000
10000
5000
0
BWΔcodA
0918-A4
0918-A5
0918-A6
0918-A7
0918-A8
0918-A9
0918-A10
0918-A11
0918-A12
0918-B4
0918-B5
0918-B6
0918-B7
0918-B8
0918-B9
0918-B10
0918-B11
0918-B12
0918-C1
0918-C2
0918-C3
0918-C4
0918-C5
0918-C6
0918-C7
0918-C8
0918-C9
0918-C10
0918-C11
0918-C12
0918-D1
0918-D2
0918-D3
0918-D4
0918-D5
0918-D6
0918-D7
0918-D8
0918-D9
0918-D10
0918-D11
0918-D12
b
HPP 0918-E1—0918-H12
0 g/L HPP
0.5 g/L HPP
RFU/OD600
25000
20000
15000
10000
5000
0
0918-E1
0918-E2
0918-E3
0918-E4
0918-E5
0918-E6
0918-E7
0918-E8
0918-E9
0918-E10
0918-E11
0918-E12
0918-F1
0918-F2
0918-F3
0918-F4
0918-F5
0918-F6
0918-F7
0918-F8
0918-F9
0918-F10
0918-F11
0918-F12
0918-G1
0918-G2
0918-G3
0918-G4
0918-G5
0918-G6
0918-G7
0918-G8
0918-G9
0918-G10
0918-G11
0918-G12
0918-H1
0918-H2
0918-H3
0918-H4
0918-H5
0918-H6
0918-H7
0918-H8
0918-H9
0918-H10
0918-H11
0918-H12
c
PPA
0 g/L PPA
0.3 g/L PPA
RFU/OD600
15000
10000
5000
0
0922-6C
0922-6D
0922-6E
0922-6F
0922-6G
0922-6H
0922-9A
0922-9B
0922-9C
0922-9D
0922-9E
0922-9F
0922-9G
0922-9H
0922-10A
0922-10B
0922-10C
0922-10D
0922-10E
0922-10F
0922-10G
0922-10H
0922-11A
0922-11B
0922-11C
0922-11D
0922-11E
0922-11F
0922-11G
0922-11H
0922-12A
0922-12B
0922-12C
0922-12D
0922-12E
0922-12F
0922-12G
0922-12H

d


2-PE 0921-A-D7—0921-B-H12
0 g/L 2-PE
0.3 g/L 2-PE
RFU/OD600
8000
6000
4000
2000
0
0921-A-D7
0921-A-D8
0921-A-D9
0921-A-D10
0921-A-D11
0921-A-D12
0921-A-E1
0921-A-E2
0921-A-E3
0921-A-E4
0921-A-E5
0921-A-E6
0921-A-E7
0921-A-E8
0921-A-E9
0921-A-E10
0921-A-E11
0921-A-E12
0921-A-F1
0921-A-F2
0921-B-A11
0921-B-A12
0921-B-B11
0921-B-B12
0921-B-C11
0921-B-C12
0921-B-D11
0921-B-D12
0921-B-E11
0921-B-E12
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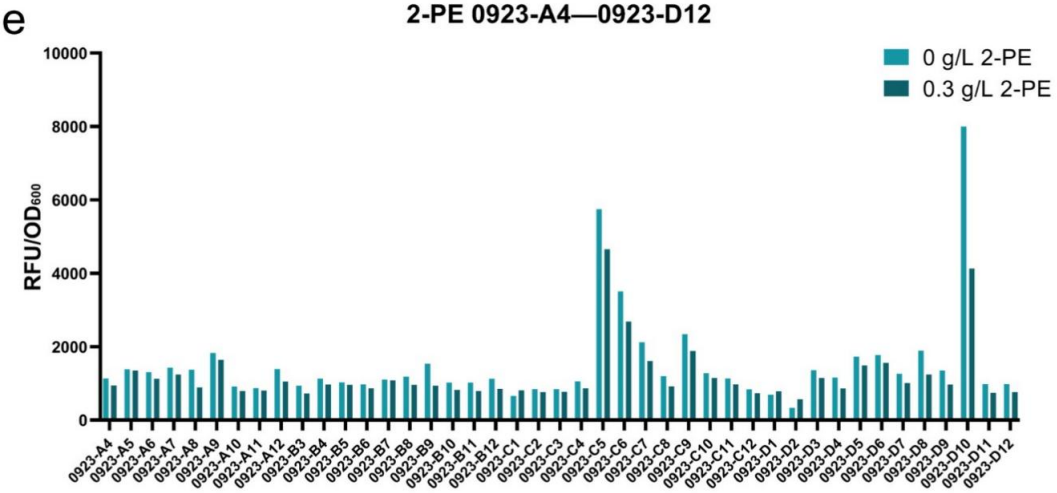
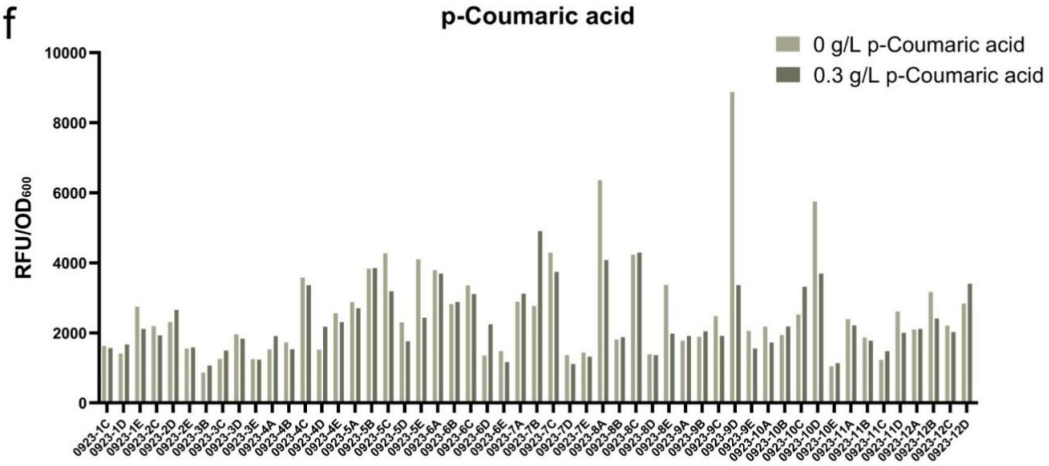
0921-B-G11
0921-B-G12
0921-B-H11
0921-B-H12

e


2-PE 0923-A4—0923-D12
0 g/L 2-PE
0.3 g/L 2-PE
RFU/OD600
10000
8000
6000
4000
2000
0
0923-A4
0923-A5
0923-A6
0923-A7
0923-A8
0923-A9
0923-A10
0923-A11
0923-A12
0923-B3
0923-B4
0923-B5
0923-B6
0923-B7
0923-B8
0923-B9
0923-B10
0923-B11
0923-B12
0923-C1
0923-C2
0923-C3
0923-C4
0923-C5
0923-C6
0923-C7
0923-C8
0923-C9
0923-C10
0923-C11
0923-C12
0923-D1
0923-D2
0923-D3
0923-D4
0923-D5
0923-D6
0923-D7
0923-D8
0923-D9
0923-D10
0923-D11
0923-D12

f


p-Coumaric acid
0 g/L p-Coumaric acid
0.3 g/L p-Coumaric acid
RFU/OD600
10000
8000
6000
4000
2000
0
0923-1C
0923-1D
0923-1E
0923-2C
0923-2D
0923-2E
0923-3B
0923-3C
0923-3D
0923-3E
0923-4A
0923-4B
0923-4C
0923-4D
0923-4E
0923-5A
0923-5B
0923-5C
0923-5D
0923-5E
0923-6A
0923-6B
0923-6C
0923-6D
0923-6E
0923-7A
0923-7B
0923-7C
0923-7D
0923-7E
0923-8A
0923-8B
0923-8C
0923-8D
0923-8E
0923-9A
0923-9B
0923-9C
0923-9D
0923-9E
0923-10A
0923-10B
0923-10C
0923-10D
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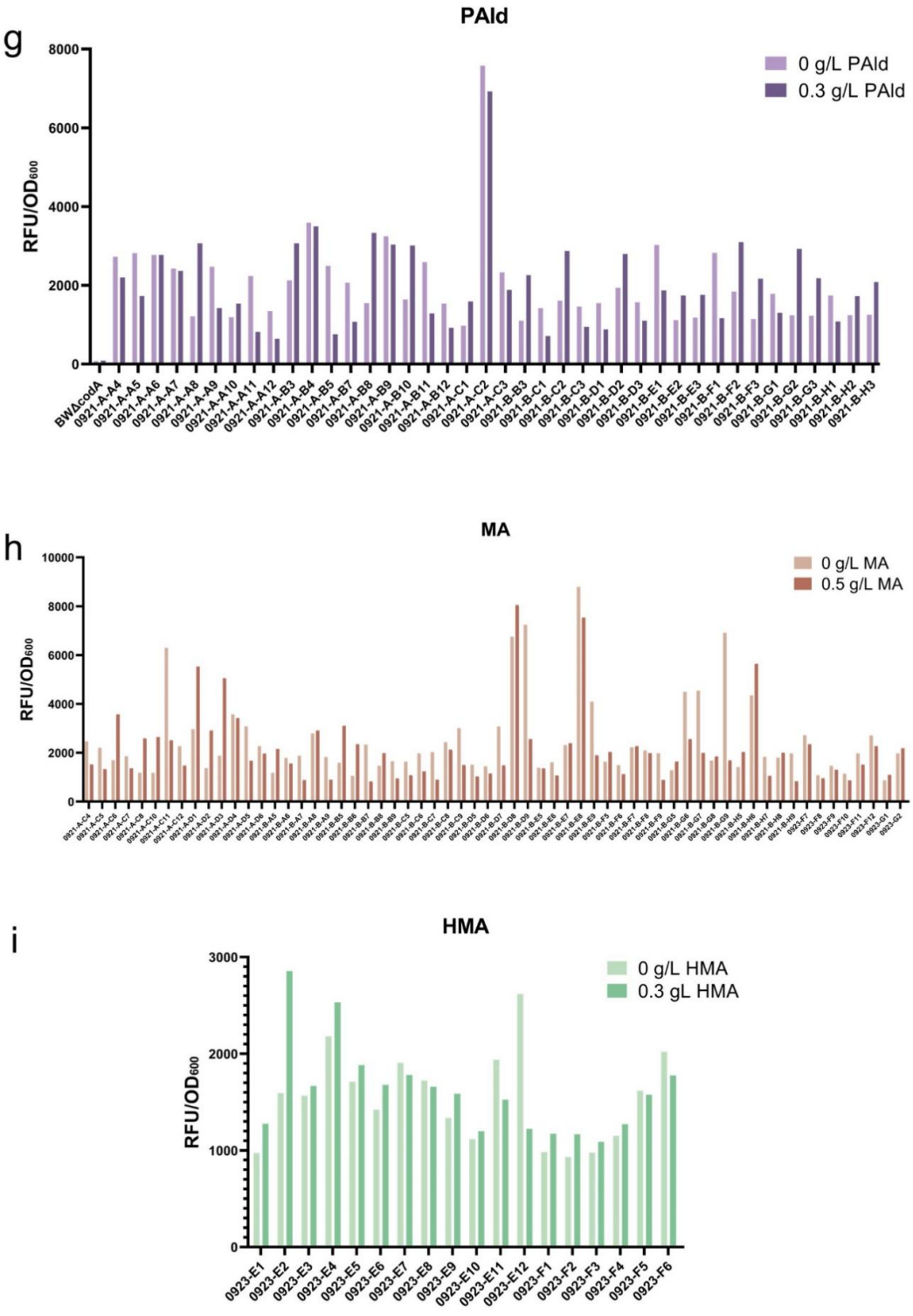
0923-12B
0923-12C
0923-12D



Among these PobR mutants, the best responsive sensor was the PobR mutant clone 0914-A8-1 with a point mutation of W177R, which showed 5.1-fold increase of mCherry fluorescence in response to HPP.

7.Probing the specificity of PobR^{W177R} to substrates and the range of response

We assessed the specificity of PobRW177R in detecting different aromatic compounds, it was induced with uninduced,0.5g/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 observed that 4HB could still trigger its activity in driving mCherry expression.