

# Ligand specificity and detection ranges of the two selected Pobr mutants

## 8.13 Shuhan Liu

### Gradient HMA concentration induction assay & fluorescence re-screening

We used gradient HMA concentration induction assay to further study the operational Range of F7-E8-3 and F5-B7-2.

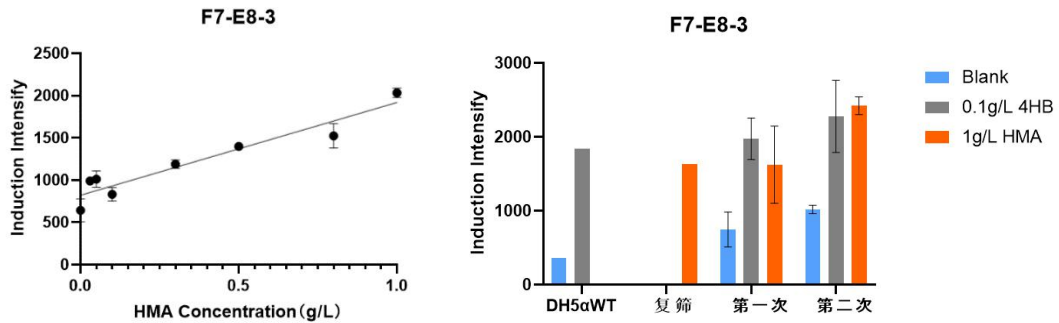


Figure 1 Left, gradient HMA concentration induction assay of F7-E8-3. Right, Data collection of F7-E8-3. The average value of IA/I0 = 2.3

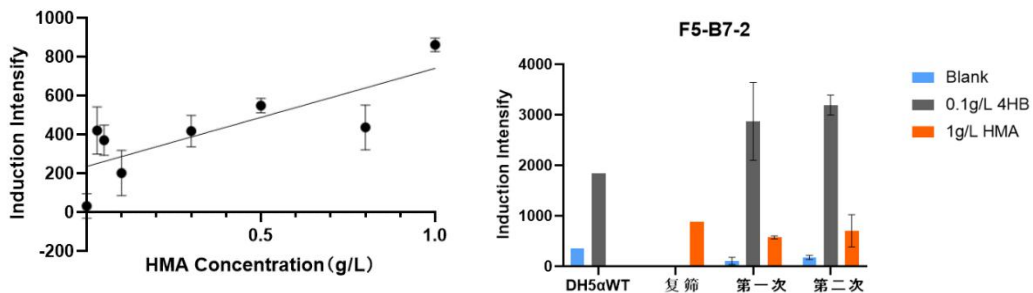


Figure 2 Left, gradient HMA concentration induction assay of F5-B7-2. Right, Data collection of F5-B7-2. The average value of IA/I0 = 3.8

## 8.14 Linxi Jiang

### Ligand specificity test

Used HMA analogues to induce F7-E8-3, F5-B7-2 and DH5α Pobr<sup>WT</sup> to test the ligand specificity of the mutants.

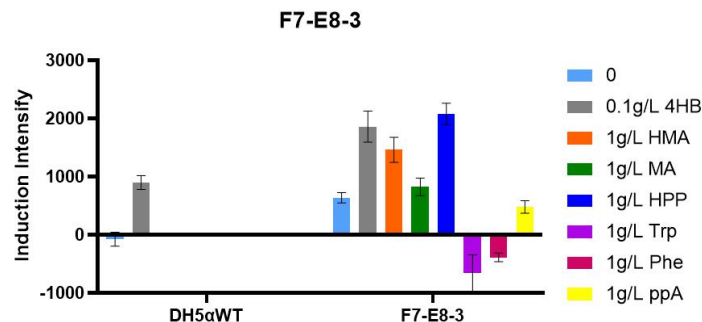


Figure 3 The induction intensity of HMA analogues to F7-E8-3

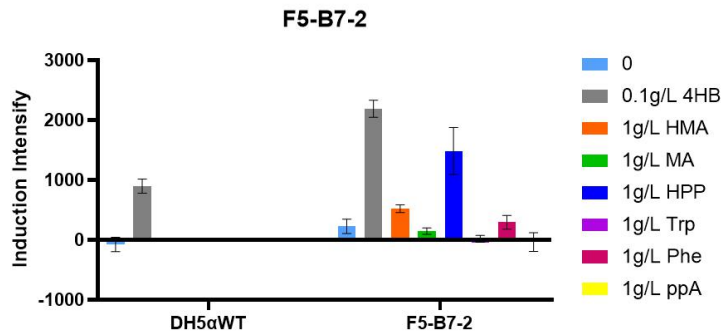


Figure 4 The induction intensity of HMA analogues to F5-B7-2

## 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 5~6). At the same time, we used gradient 4HB concentration induction assay to test the operational range of 4HB to DH5α PobR<sup>WT</sup> to test its operational range of 4HB (Figure 7).

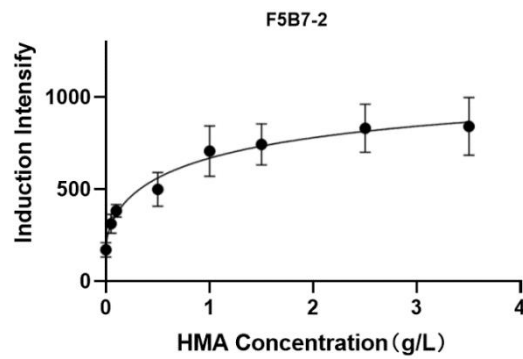


Figure 5 Gradient HMA concentration induction assay of F5-B7-2

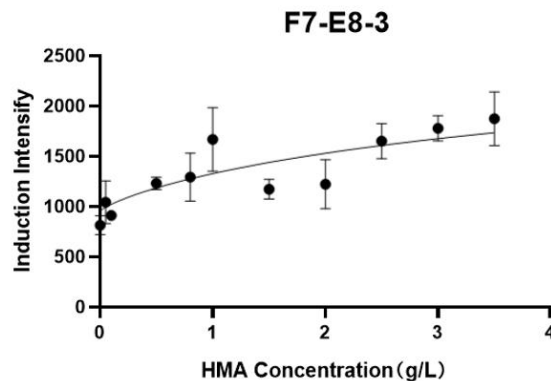


Figure 6 Gradient HMA concentration induction assay of F7-E8-3

Result analyzes: the IA of F5-B7-2 saturate induced by 3.5 g/L HMA. the IA of F7-E8-3 did not saturate induced by 3.5 g/L HMA.

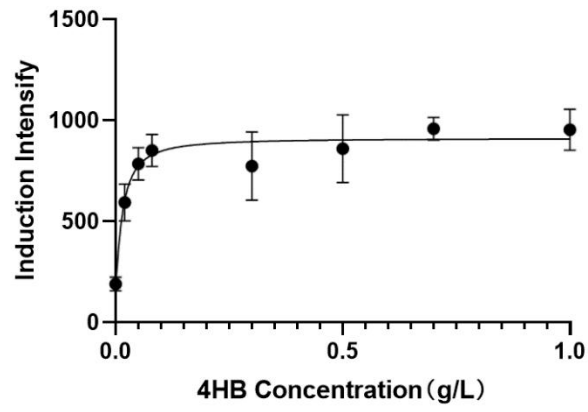


Figure 7 Gradient 4HB concentration induction assay of DH5 $\alpha$ PobR<sup>WT</sup>  
Result analyzes: the IB of DH5 $\alpha$ PobR<sup>WT</sup> saturate induced by 0.1 g/L 4HB.