WEEK 19

7.29 Jiaqi Zhang

Gene knockout

We tried to knockout *tyrR* on the bases of BL21*ΔtyrB*. At the same time, the construction of BL21*ΔtyrB* has come to final verify phase: we removed pTarget-tyrB from the strain (Figure 1), used solid and liquid photocopying to verify pTarget has been removed from the strain. For the construction of strain BL21*ΔtyrB*, Ptarget-tyrR and the donor DNA (overlap *tyrR*) have been prepared (Figure 3).

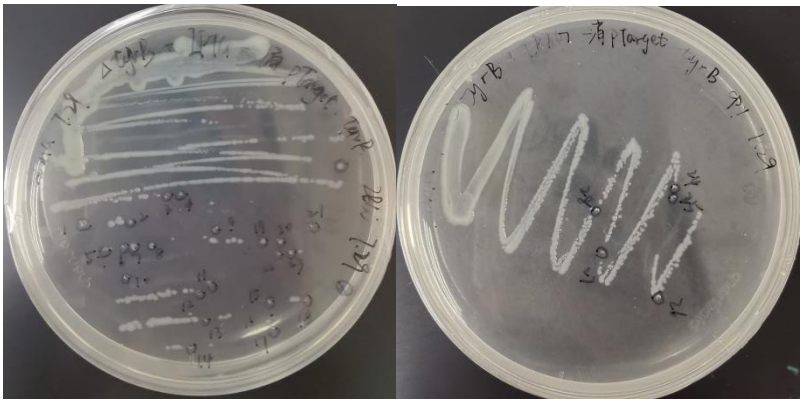

Figure 1 The growth of strain under the pressure of 50 μg/mL chloramphenicol

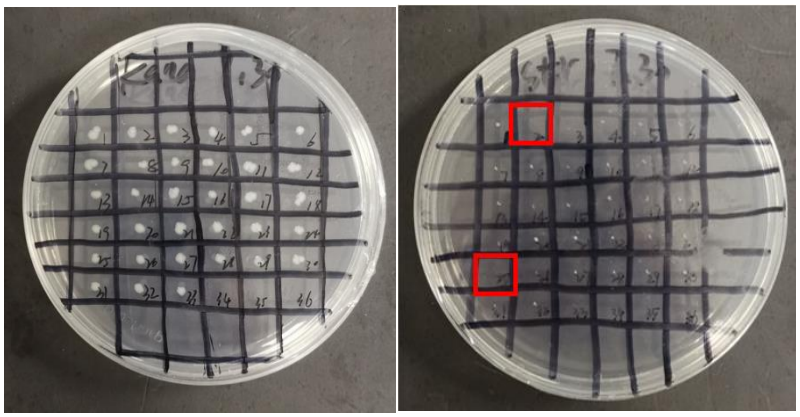

Figure 2 Solid photocopying (lift: kanamycin selection medium; right: streptomycin selection medium)

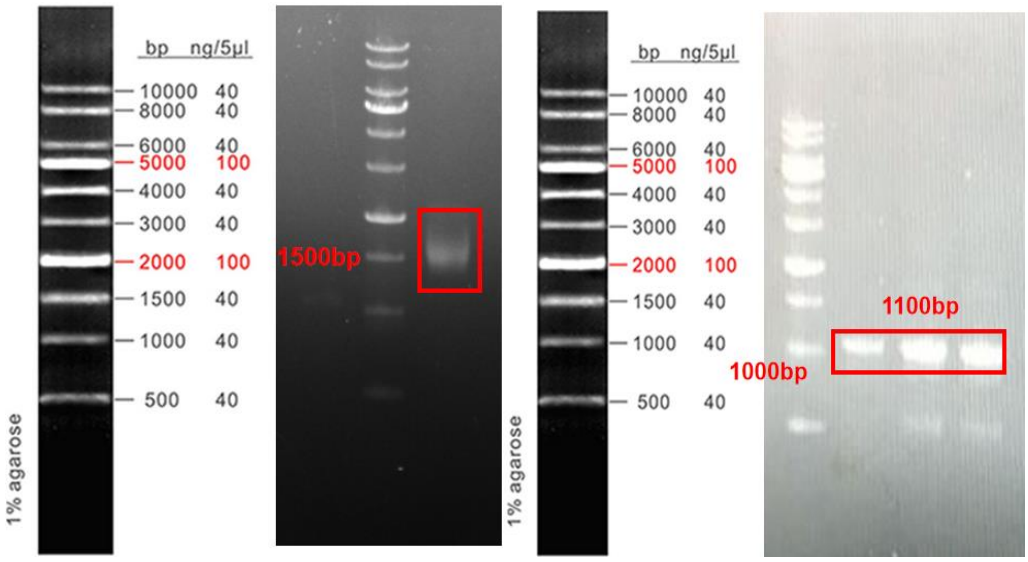

Figure 3 Left: agarose gel electrophoresis assay for pTarget-tyrR, right: agarose gel electrophoresis assay for overlap-*tyrR*

Agarose gel electrophoresis assay for pTarget-tyrR showed that removed pTarget-tyrB success. Extracted the target DNA from gel because the extra unwanted DNA fragments were produced in PCR progress (concentration of target DNA after gel extraction: 94.87 ng/μL).

7.30 Juan Luo

HMA ligand specificity test

Have HMA ligand specificity test for the preliminarily screened biosensors and DH5α*PobR*^{WT}.

Used HMA analogues (MA, HPP, Phe, PPA, Trp) to induce F4-A9, F7-D12 and DH5α*PobR*^{WT} to test the ligand specificity of F4-A9, F7-D12. The influence of solution sterilization on experimental results was considered (0730 sterilized; 0731 did not sterilize).

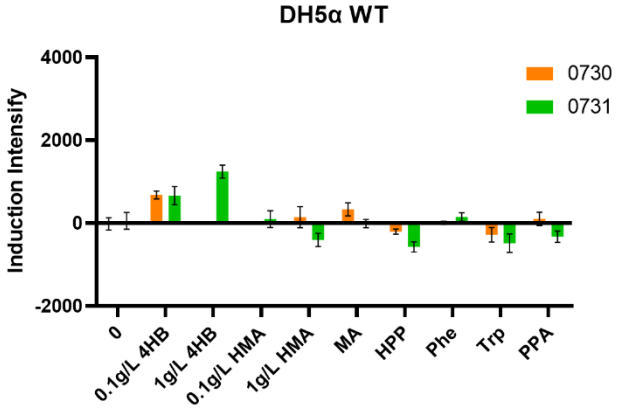

Figure 4 Comparation of the induction intensity of HMA analogues to DH5α*PobR*^{WT} in two experiments (0730, 0731)

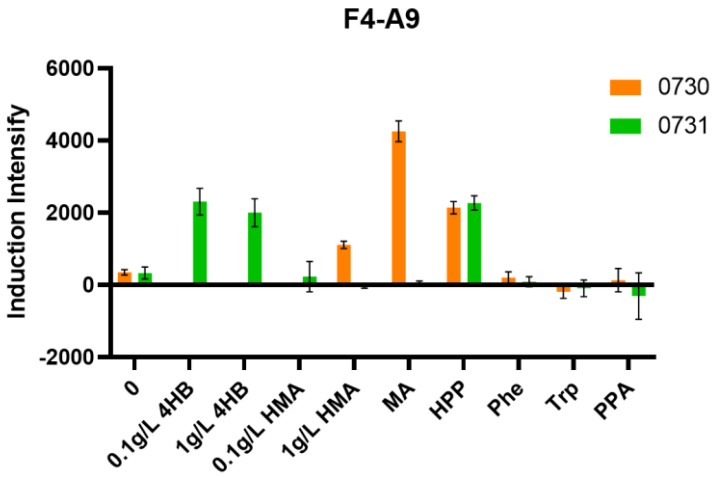

Figure 5 Comparation of the induction intensity of HMA analogues to F4-A9 in two experiments (0730, 0731)

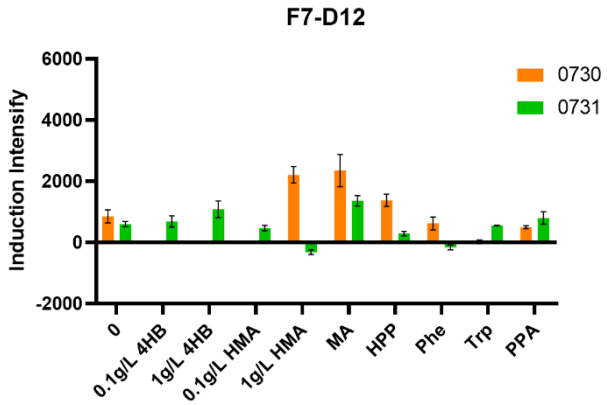

Figure 6 Comparation of the induction intensity of HMA analogues to F7-D12 in two experiments (0730, 0731)

The induction intensity of MA and HPP to F7-D12 and F4-A9 shows significant difference in two experiments. This may caused by the inactivation of some analogues after sterilize. At the same time, the induction intensity of HMA showed abnormal decrease compared with results before. We suspected that the pH adjustment process has an effect on HMA or the culture was polluted by other cells. Therefore, we plan to filtrate the HMA and use directly without sterilization.

8.1 Sunyue Cai

Fluorescence re-screening

We re-screened the clones with higher HMA induction by detecting their RFU:
We re-screened the 49 clones with higher HMA induction in “screening” by detecting their RFU after adjusting the pH of 4HB and HMA to about 7 (Figure 7, 8);
We re-screened the 6 clones with higher HMA induction in “re-screening” by detecting their RFU after adjusting the pH of 4HB and HMA to about 7 (Figure 9);
Experimental system:

Control
- 200μL LB+**1%DH5α without plasmid**
- 200μL LB+1‰ Amp+**1% DH5αPobRWT**
- 200μL LB+1‰ Amp+ **0.1g/L 4HB**+**1% DH5αPobRWT**

Three groups repetition

Experiment
- 200μL LB+1‰ Amp+**1%DH5αPobRmut**
- 200μL LB+1‰ Amp+**0.1g/L 4HB**+ **1%DH5αPobRmut**
- 200μL LB+1‰ Amp+**1g/L HMA**+ **1%DH5αPobRmut**

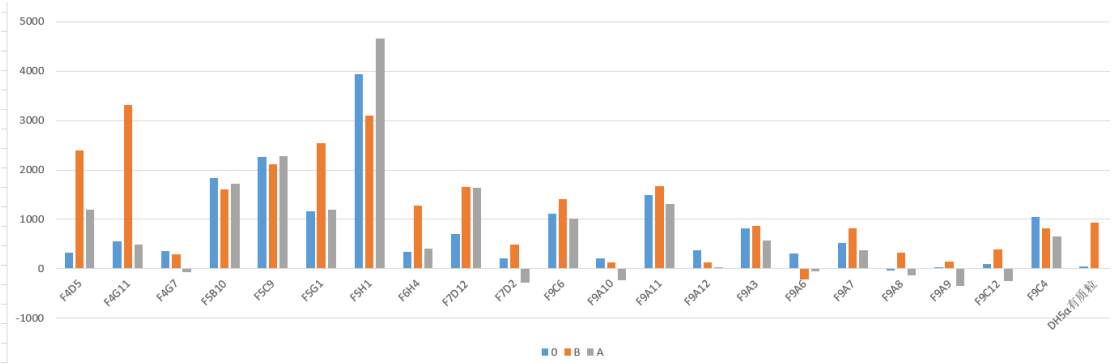

Figure 7

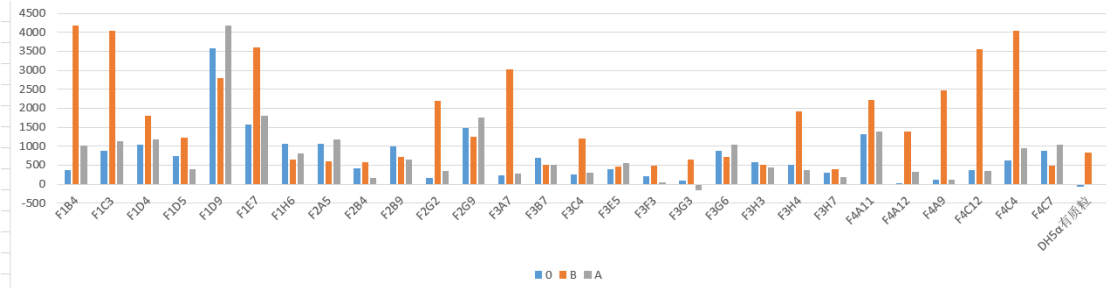

Figure 8

Figure 7, 8 The induction intensity of 49 clones in re-screening.

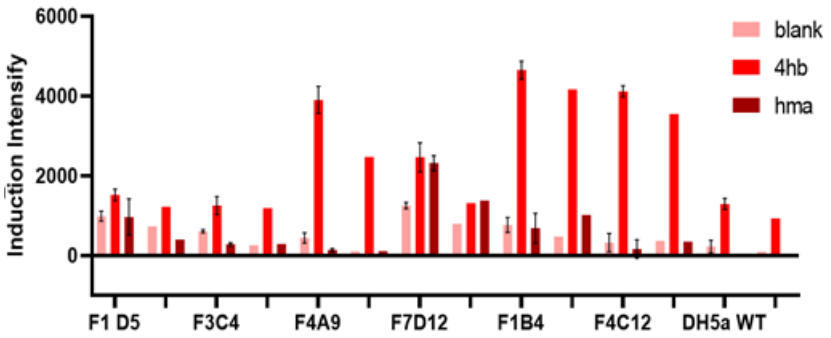

Figure 9 The induction intensity of 6 clones in re-screening

From the chart we found that the growth of experimental groups which did not add inducer and which add 4HB as inducer were normal, the induction intensity was similar to that of the last time. However, the experimental groups added HMA as inducer appeared flocculent sediments at the bottom of the 96-well-plate and the average OD_{600} is low. We speculate that the sediments are death cells. The reason may

cause this is that HMA will change its structure to another compound which is toxic for cells.

8.2 Beimeng Zhang

Site-directed mutagenesis of the PobR CDS

Site-directed mutagenesis PCR (50 μL×3)

Reaction system:

247spm	126spm	122spm
247spm-F: 1 μL	126spm-F: 1 μL	122spm-F: 1 μL
247spm-R: 1 μL	126spm-R: 1 μL	122spm-R: 1 μL
pYB1a-PobR-eGFP-Cmr: 1 μL	pYB1a-PobR-eGFP-Cmr: 1 μL	pYB1a-PobR-eGFP-Cmr: 1 μL
2×HF Mix: 25 μL	2×HF Mix: 25 μL	2×HF Mix: 25 μL
DDW: 22 μL	DDW: 22 μL	DDW: 22 μL

Reaction procedure

98 ℃ 5 min

98 ℃ 30 s
60 ℃ 30 s
72 ℃ 3 min
} ×24

72 ℃ 5 min

16 ℃ 1 h

Agarose gel electrophoresis assay showed correct bands, the concentration of the product after purified:

247spm (PCR): 32.416 ng/μL

126spm (PCR): 91.342 ng/μL

122spm (PCR): 33.478 ng/μL

8.3 Shuhan Liu

Gradient HMA concentration induction assay

To further study the influence of HMA concentration to cell growth, use F4-A9, F7-D12 to have gradient HMA concentration induction assay (Figure 10).

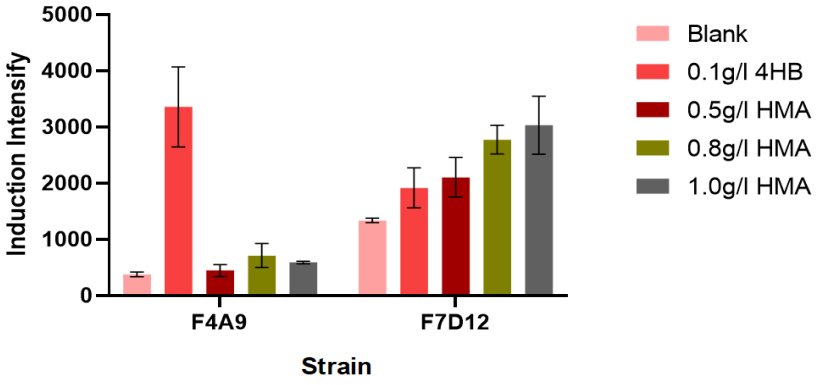

Figure 10 Gradient HMA concentration induction assay of F4-A9, F7-D12

8.3 Shuning Guo

Site-directed mutagenesis of the PobR CDS

1. DpnI digestion

Reaction system:

Spm247	Spm126	Spm122
DpnI: 0.2 μL	DpnI: 0.2 μL	DpnI: 0.2 μL
10×cutsmart: 1 μL	10×cutsmart: 1 μL	10×cutsmart: 1 μL
247spm (PCR) (32 ng/μL)：8.8 μL	126spm (PCR) (91 ng/μL): 4.4 μL	122spm (PCR) (33 ng/μL): 8.8 μL
DDW: 0	DDW: 4.4 μL	DDW: 0

Reaction procedure: 37 ℃ 2 h

70 ℃ 20 min

2. Transformation

System:

	Spm247	Spm126	Spm122
Competent cells	DH5α: 100 μL	DH5α: 100 μL	DH5α: 100 μL
Digestion product	10 μL (282 ng)	10 μL (400.4 ng)	10 μL (290.4 ng)
plate	Amp50	Amp50	Amp50

Culture at 37 ℃ for 12 h.