WEEK 9

5.26 Jianing Li

Determination of engineering bacteria by HPLC

MA-1: pLB1s-hmaS MA-2: pLB1s-hmaS+pYB1a-aroG^{fbr}-pheA^{fbr} MA-3: pLB1s-hmaS-tktA-ppsA+ pYB1a-aroG^{fbr}-pheA^{fbr} LY-1: pYB1a-hmaS



Figure 1 HPLC results of engineered bacteria

MA-1+HMA was the result of adding a small amount of HMA standard in the last HPLC sample. It was confirmed that the unknown substance was not HMA and the peak of 9.2 was HMA. The peak time is about 0.2 min earlier than last time. The yield of MA and HMA was the highest in MA-2, which was the same as last time.

5.27 Sunyue Cai

Screening

Our first screening system: Control group: 220 μ L LB + 0.1 % ampicillin +1 % WT bacterial solution; 200 μ L LB + 0.1 % ampicillin + 0.1 % 34 mg/mL chloramphenicol+10 % 0.6 g/L 4HB+1 % WT bacterial solution

Experimental group: $200 \ \mu L \ LB + 0.1 \ \%$ ampicillin + 0.1 % 34 mg/mL chloramphenicol + 10 % 0.6 g/L HMA, mutant bacteria were selected.

OD600												
A	0.0249	0.0267	1.1517	0.8673	0.8153	0.8996	0.6266	0.5333	0.6019	0.5803	0.5791	0.6016
в	0.0247	0.9702	1.262	0.9126	0.9352	0.9763	0.1113	0.5303	0.6146	0.6025	0.7593	0.6124
с	0.0247	0.0659	1.2068	0.9094	0.9684	0.9906	0.5837	0.2801	0.6135	0.6082	0.5647	0.6156
D	0.9496	0.9081	0.9661	0.8613	0.9619	0.9195	0.6261	0.1135	0.5249	0.5987	0.575	0.5382
E	1.0065	0.9113	0.9866	0.9664	0.9259	0.8975	0.6184	0.6111	0.5946	0.6559	0.6597	0.615
F	1.0045	1.0084	0.9763	0.9712	0.9595	0.9799	0.6278	0.6356	0.6124	0.6277	0.6626	0.5888
G	0.9518	0.9699	0.8634	0.9559	0.892	0.8785	0.6624	0.5847	0.5575	0.7163	0.6416	0.3265
н	0.4914	0.9277	0.9712	0.8652	0.9599	0.9705	0.608	0.5705	0.6264	0.5972	0.5611	0.032
eGFP	1	2	3	4	5	6	7	8	9	10	11	12
eGFP A	1 9375	2 9596	3 46331	4 15842	5 15389	6 14782	7 22363	8 12196	9 13838	10 13890	11 24458	12 15966
eGFP A B	1 9375 9131	2 9596 13749	3 46331 46277	4 15842 15368	5 15389 53010	6 14782 29622	7 22363 10142	8 12196 12628	9 13838 18075	10 13890 27851	11 24458 14087	12 15966 27095
eGFP A B C	1 9375 9131 9245	2 9596 13749 9707	3 46331 46277 47331	4 15842 15368 19966	5 15389 53010 34732	6 14782 29622 18872	7 22363 10142 13824	8 12196 12628 10482	9 13838 18075 22492	10 13890 27851 18338	11 24458 14087 16910	12 15966 27095 21833
eGFP A B C D	1 9375 9131 9245 15900	2 9596 13749 9707 14812	3 46331 46277 47331 24486	4 15842 15368 19966 14777	5 15389 53010 34732 23672	6 14782 29622 18872 15511	7 22363 10142 13824 16615	8 12196 12628 10482 9655	9 13838 18075 22492 11444	10 13890 27851 18338 11426	11 24458 14087 16910 12388	12 15966 27095 21833 39319
eGFP A B C D E	1 9375 9131 9245 15900 35167	2 9596 13749 9707 14812 15411	3 46331 46277 47331 24486 29856	4 15842 15368 19966 14777 51709	5 15389 53010 34732 23672 29683	6 14782 29622 18872 15511 13396	7 22363 10142 13824 16615 13887	8 12196 12628 10482 9655 20996	9 13838 18075 22492 11444 23514	10 13890 27851 18338 11426 21451	11 24458 14087 16910 12388 17836	12 15966 27095 21833 39319 14556
eGFP A B C D E F	1 9375 9131 9245 15900 35167 22640	2 9596 13749 9707 14812 15411 40869	3 46331 46277 47331 24486 29856 23377	4 15842 15368 19966 14777 51709 20732	5 15389 53010 34732 23672 29683 24199	6 14782 29622 18872 15511 13396 46807	7 22363 10142 13824 16615 13887 22244	8 12196 12628 10482 9655 20996 12267	9 13838 18075 22492 11444 23514 13338	10 13890 27851 18338 11426 21451 13790	11 24458 14087 16910 12388 17836 20534	12 15966 27095 21833 39319 14556 13412
eGFP A B C D E F G	1 9375 9131 9245 15900 35167 22640 17213	2 9596 13749 9707 14812 15411 40869 23171	3 46331 46277 47331 24486 29856 23377 18587	4 15842 15368 19966 14777 51709 20732 23082	5 15389 53010 34732 23672 29683 24199 13505	6 14782 29622 18872 15511 13396 46807 13715	7 22363 10142 13824 16615 13887 22244 21080	8 12196 12628 10482 9655 20996 12267 19512	9 13838 18075 22492 11444 23514 13338 12881	10 13890 27851 18338 11426 21451 13790 24634	11 24458 14087 16910 12388 17836 20534 14407	12 15966 27095 21833 39319 14556 13412 11452

Figure 2 Raw OD₆₀₀ and Fluorescence values of initial screening

Since their OD values were generally higher, we speculated that the inhibition effect of 34 mg/mL chloramphenicol in liquid LB was poor.

5.28 Juan Luo

Liquid initial screening conditions

Since the inhibition effect of 34 mg/mL chloramphenicol in liquid LB was poor, the suitable concentration of chloramphenicol in liquid LB was explored by gradient chloramphenicol concentration.



Figure 3 Growth curves of control bacteria in different concentration without induction



Figure 4 Growth curves of control bacteria in different concentration with induction

5.29 Chao Chen



We did a re-screening verification, but none of the strains met our requirements.



Figure 6 The I0, IB and IA value of each mutant



Figure 7 The I0, IB and IA value of each mutant

5.30 Xiangxin Li

First screening of solid medium conditions

In order to determine the growth of BW bacteria in solid medium under normal conditions, the control bacteria were cultured in liquid LB in 37 °C shaking table for 10 h to OD=4 to ensure the consistent growth of the control bacteria each time. ampicillin resistant plate was coated with 200 μ L bacterial solution with different dilution ratios

of 10^8 , 10^7 , 10^6 , 10^5 , 10^4



Figure 8 Growth of control bacteria in different dilution ratios According to the results of the previous step, the plate with a dilution ratio of 10^4 was selected. The number of mono clones was about 120 under the dilution ratio.