WEEK 15

7.7 Juan Luo

Engineering bacteria

MA-4, MA-5 construction, *tyrB* knockout

1.MA-4, MA-5 construction

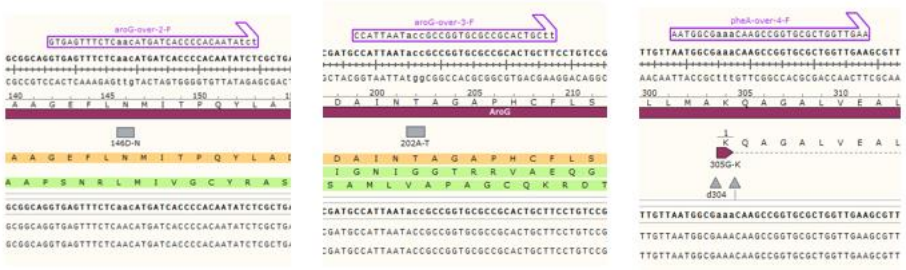

Figure 1 Sequencing results of pYB1a-aroGfbr-pheAfbr-2

Complete the construction of MA-4 and MA-5, and transfer into BW competent cells according to the above system:

Strains	Characteristics
MA-4	pLB1s-hmaS+pYB1a-aroGfbr-pheAfbr-2
MA-5	pLB1s-hmaS-tktA-ppsA+pYB1a-aroGfbr-pheAfbr-2

Solution:

1. Pick out the colonies that have grown on the petri dish for re-screening, and wash the surface of the petri dish with LB containing chloramphenicol (20 μg/mL) and HMA (0.6 g/L) after 20 hours of incubation. Collect the washing solution and incubate for 12 hours (one test tube per plate), then use a 96-well plate to measure the fluorescence value of each tube, and compare it with the fluorescence value induced by the negative control T1 original bacteria and the positive control T1 original bacteria plus 4HB to select the fluorescence The test tube with higher value, after dilution, plate it (50 μg/mL ampicillin+20 μg/mL chloramphenicol+0.6 g/L HMA), and re-select bacteria for verification.

2. Follow-up use the magnesium chloride filtered by the filter membrane, and pay attention to the coating method and time to ensure that the coating is dry.

Later, we will remedy the conversion coating:

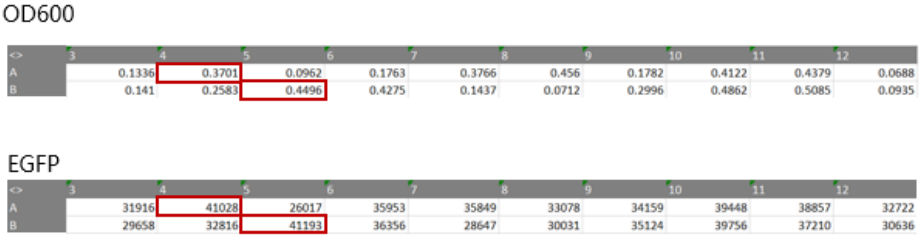

Figure 2 The results of enzyme labeling after recollection of transformed coated test tubes

Then select the bacteria at positions A4 and B5 for fluorescence microscopy verification.

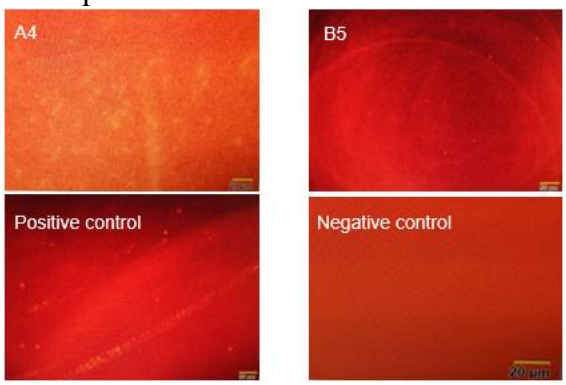

Figure 3 Fluorescence verification result of fluorescence microscope

There is fluorescence in bacteria A4 and B5, but the result image is not clear and lacks contrast with the bright field image. Therefore, the fluorescence microscope will be re-verified to determine the proportion of the fluorescent bacteria to the total bacteria. The next step is to determine Dilution multiples of the coated plate are used as a pavement.

Photocopying: A total of 58 single colonies obtained from the coating plate were obtained by photocopying before July 7 to enter the re-screening.

7.8 Fengqianrui Chen

Re-screening

A total of 524 colonies obtained from the transformation coating plate (A50+C20+0.6 g/LHMA) were re-screened;

Control group: ①220 μL LB+0.1% 50 mg/mL ampicillin+1% T1PobR^{WT} bacterial solution; ②200 μL LB+0.1% 50 mg/mL ampicillin+10% 0.6 g/L 4HB+1% T1PobR^{WT} bacterial solution ①×3 ②×3

Experimental group: 200 μL LB+0.1% 50 g/mL ampicillin+10% 0.6 g/L HMA+ plate to pick out a single colony

On July 8th, 58 bacteria were re-screened, and 8 bacteria with higher fluorescence value (yellow background) were selected for re-screening verification (A1B1C1 is a negative control, A2B2C2 is a positive control).

On July 9th, 120 bacteria were re-screened, and 8 bacteria with higher fluorescence value (yellow background) were selected for re-screening verification (A1B1C1 is a negative control, A2B2C2 is a positive control).

<>	1	2	3	4	5	6	7	8	9	10
A	11024	9371	28506	25185	15464	18485	24736	22319	23551	17891
B	11483	9698	29301	31145	16134	17552	24439	23240	23234	16102
C	11830	8459	37995	15739	13669	22926	22543	19775	21249	
D			24369	35555	30457	22055	21912	27429	52343	
E			25027	22109	22858	24172	35509	23024	21699	
F			33046	23013	19854	24302	21541	24663	21647	
G			23627	5170	24447	24027	27072	22424	27676	
H			28602	25361	27956	36689	26148	22030	25093	

Figure 4 Plate number: 0705 0706 F-1 fluorescence value of coated plate

The positive control value is low due to operational errors, so select other re-screened control data to process the data.

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	22367	19893	13052	20859	29102	16415	18970	14826	18956	16480	18560	17396
B	18457	26295	14714	52871	16093	15246	15778	15915	25378	18032	19334	19656
C	19263	23252	16436	17257	14470	15343	15767	14300	19288	19190	16470	21333
D			13997	19939	15448	14982	17187	12247	17540	16089	14913	18462
E			14783	16275	15883	16832	17313	16771	14775	18308	16067	19728
F			19615	15507	13213	13577	15289	14637	14217	19870	127	18318
G			18519	18496	17456	16082	17040	17295	31740	16977	18164	21386
H			18061	17680	15523	17799	18721	15599	15553	17015	16747	18024

Figure 5 Board number: 0707 coated board F-2 fluorescence value

<>	1	2	3	4	5	6	7
A	49136	46599	33400	31371	27369	30476	33670
B	37503	50945	31556	30969	27842	30137	33963
C	43198	44693	32059	31443	27346	30347	28851
D			31463	29081	27573	27160	29395
E			30458	31098	36569	31641	30066
F			38315	30688	24954	28501	26887
G			31430	29128	28245	38633	28046
H			40784	35205	26871	29208	32420

Figure 6 Board number: 0707 coated board F-3 fluorescence value

166 bacteria were re-screened on July 10, of which 8 (framed in red) were re-screened on July 8 with higher fluorescence expression, and 11 bacteria with higher fluorescence value (yellow background) were selected for re-screening verification (A1B1C1 is the negative control, A2B2C2 is the positive control).

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	26853	29304	33132	28822	26182	32437	30104	30308	32161	26823	39702	31383
B	22336	33395	29253	28337	26072	28599	29155	28607	32347	29102	31403	29234
C	26505	30276	29134	39728	23647	27128	34044	23897	26827	29853	28994	31510
D	26241	26597	27786	28213	32949	26907	25014	26531	28257	27831	24882	26790
E	26993	23674	31359	25599	21991	24955	23293	25159	24608	21927	26260	27888
F	29528	25038	28398	29008	18368	25883	25884	23622	23308	28394	25951	26389
G	27544	29687	30927	49565	26106	29878	23420	25234	23836	26353	36335	25982
H	29025	25480	27966	29311	27748	28128	26366	22682	26188	26753	27986	29605

Figure 7 Plate number: 0708 coated plate F-4 fluorescence value

<>	1	2	3	4	5	6	7	8	9	10	11
A	20297	17952	22048	21463	18226	19584	22258	19335	21055	26503	19236
B	16795	25087	19722	23452	21284	20031	21106	21179	17387	31686	21479
C	17952	20942	19124	18984	19856	19230	20672	16574	35482	21503	19609
D	26431		19958	23957	19426	17950	20039	17814	19217	22813	24619
E	27394		20388	26124	18354	18793	20739	20461	19495	31765	
F	25403	24452	19282	16554	17885	17914	19153	16270	20008	23449	
G	32149	25926	19738	17751	18367	18958	20058	17326	18945	19802	
H	50624	25642	20323	19385	17811	19370	22175	18535	20644	21106	

Figure 8 Plate number: 0708 coated plate F-5 fluorescence value

7.12 Sunyue Cai

Re-screening verification

A total of 42 bacteria with relatively high fluorescence intensity obtained from the re-screening were verified by re-screening;

Use a microplate reader to verify whether the re-screened strains correspond to HMA, and at the same time exclude false positives caused by PobR protein off-target:

Control group (three groups in parallel)

220 μL LB+0.1% 50 μg/mL ampicillin

220 μL LB+0.1% 50 μg/mL ampicillin+1% T1PobR^{WT} bacterial solution

200 μL LB+0.1% 50 μg/mL ampicillin+10% 0.6 g/L 4HB+1% T1PobR^{WT} bacterial solution

Experimental group (three groups in parallel)

220 μL LB+0.1% 50 μg/mL ampicillin+1% BWPobR^{mut} bacterial liquid

200 μL LB+0.1% 50 μg/mL ampicillin+10% 0.6 g/L 4HB+1%BWPobR^{mut} bacterial liquid

200 μL LB+0.1% 50 μg/mL ampicillin+10% 0.6 g/L HMA+1%BWPobR^{mut} bacterial liquid

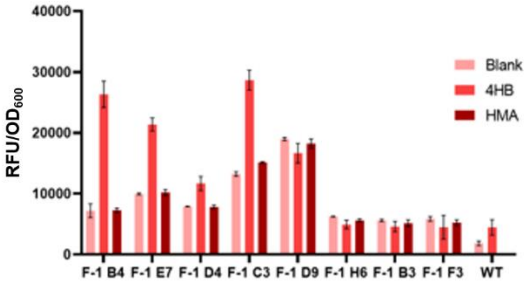

Figure 9 0709 8 strains undergoing re-screening verification

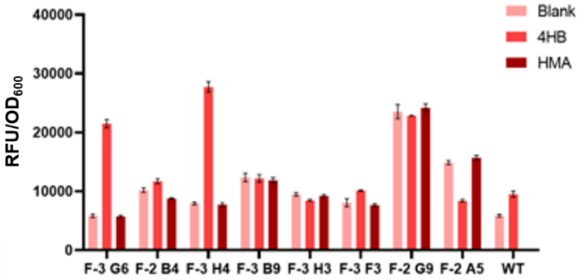

Figure 10 0710 8 strains undergoing re-screening verification

Part of the strains that have been re-screened and verified before are re-screened and verified (deep-well plate 14 h) (corresponding to data re-screening verification T9), and the results are compared with the first re-screening verification results as follows:

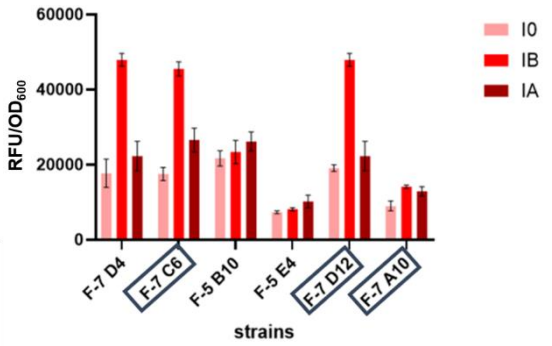

Figure 11 Verification of the first re-screening of 6 strains

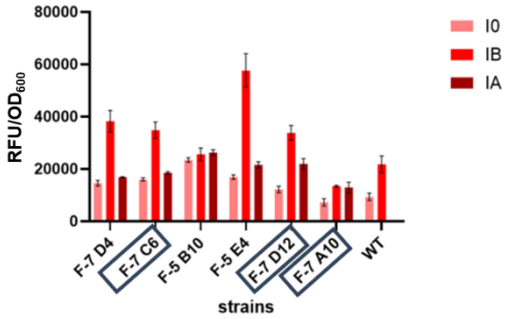

Figure 12 Verification of the second re-screening of 6 strains

Except for strain F-5 E4, the induction intensity of each strain in the two re-screening verifications was similar, indicating that the re-screening verification results were more repeatable and the results were more credible.

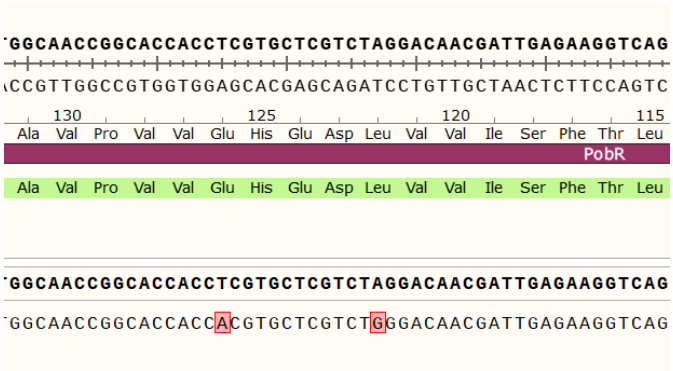

Figure 13 F7-D12 sequencing diagram

Then transfer to BW Competence for verification.

7.13 Hongda Fu

Data collation

Purpose: To compare the data performance of each strain in two re-screening methods

(fluorescence value screening and chloramphenicol screening) and two re-screening verification methods (deep-well plate and enzyme-labeled overnight), so as to verify the existing re-screening And re-screening to verify the accuracy of the method, and compare the 4HB induction intensity of each wild strain to determine the accuracy of each wild strain as a control strain.

Methods: Compare the HMA induction intensity (IA) of the same strain in fluorescence rescreening, chloramphenicol rescreening, enzyme-labeled overnight, and deep-well plate overnight, and compare the 4HB induction intensity (IB) of different wild strains.
1. The HMA induction intensity (IA) part of the experimental results comparing the data obtained by the same strain in re-screening bacteria, re-screening bacteria liquid, re-screening and verifying enzyme label overnight, and re-screening and verifying deep-well plate overnight are as follows:

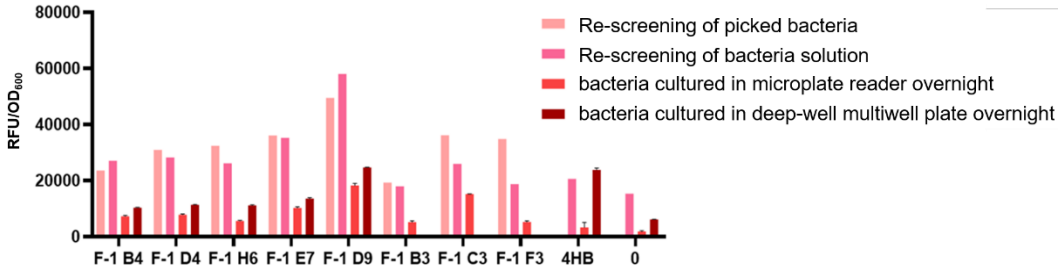

Figure 14 Re-screening of 6 strains

The fluorescence induction intensity of different strains is uneven, indicating that the results obtained by several methods are quite different.

2. Comparing the 4HB induction intensity (IB) of different wild strains, the experimental results are as follows:

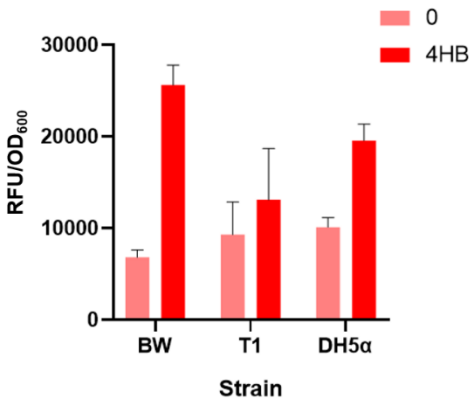

Figure 15 The experimental results

The fluorescence induction intensity of different strains is different. The 4HB induction intensity of BW bacteria is significantly higher than that of the other two strains. Therefore, DH5α should be used as a control afterwards, but the use of BW bacteria as experimental strains in subsequent experiments should also be considered to obtain more obvious result.