RBS Modification

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2017.7.20~2017.7.22 we designed the experiment and prepare the material

2017.7.23~2017.7.24 Waiting for the company finishing the synthesis of primers we needed

2017.7.25 dissolve part Part:BBa_R0040 and BBa_J364005; chemical transfer those two part(DH5a);

2017.7.26 pick up single clone from plate and liquid cultivate over night

2017.7.27 extract two plasmids by plasmid extraction kit; use EB/DNA agarose electrophoresis to analyze the plasmid we got

2017.7.28 PCR amplify the DNA pieces we need from and digest the pieces and plasmid Part:BBa_R0040 by corresponding restriction enzyme

2017.7.29 anneal synthesis RBS single strands and detect the

concentration

2017.7.30 ligate 3 pieces which contain promoter ,RBS, and reporter protein separately in 1 reaction. chemical transfer the ligation product(DH5a);

2017.7.31 no single clone can be detect on the plate

2017.8.01~2017.8.10 we optimize the ligation condition and try to get enough transformant we need but end to fail

2017.8.11 re-design the experiment

2017.8.12 Waiting for the company finishing the synthesis of primers we needed

2017.8.13 use PCR to linerize and modify the plasmid Part:BBa_I13521

2017.8.14 digest the linerized plasmids with corresponding restriction enzyme （fail）

2017.8.15 re-design the experiment

2017.8.16 use PCR to linerize and modify the plasmid Part:BBa_I13521 and digest the linerized plasmids with corresponding restriction enzyme

2017.8.17 religate the linerized plasmids and chemically transfer it （DH5α）

2017.8.18 pick up several single clones from plate and liquid cultivate over night

2017.8.19 sequence the plasmid by Tsingke（fail）

2017.8.19~2017.8.23 waiting for the sequence result

2017.8.24~20.17.9.05 we thought we failed because our sample was contaminated, so we tried several times again with different approach. But at last we found that it's the plasmid Part:BBa_I13521 we got from somewhere else that had something wrong.

2017.9.05~2017.9.21 re-design the experiment and extract plasmid Part:BBa_I13521 from reliable source

2017.9.22 waiting for the synthesis primers

2017.9.23~2017.9.27 we try to construct the modified plasmids with overlap PCR, we tried several times and optimize the condition but got no result

2017.9.28 re-design the experiment

2017.9.29 waiting for the synthesis primers

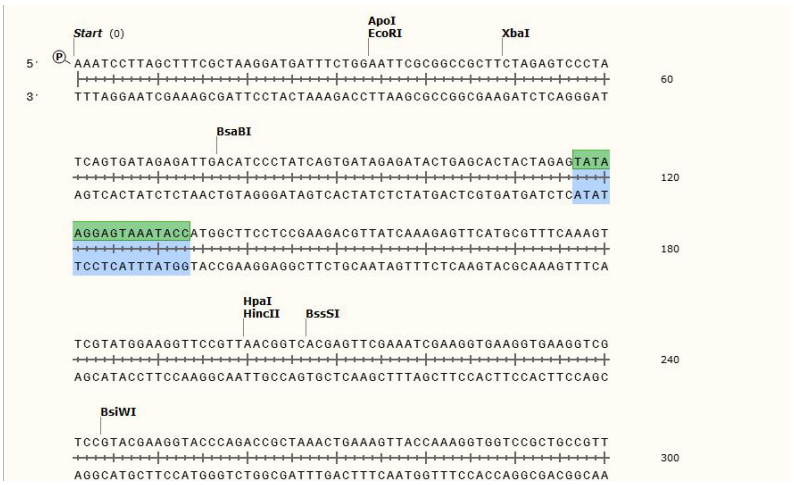
2017.9.30 PCR amplify the DNA pieces we need, including insert and backbone

2017.10.01 use agrose gels to analyze these pieces and purify it

2017.10.02 use ClonExpress® II One Step Cloning Kit to ligate the insert and the backbone and chemical transfer the ligation product(DH5α)

2017.10.04 pick up single clone from plate and liquid cultivate over night

2017.10.09 sequence the strain we get by tsingqe（success）



The successful sequence result of P1.

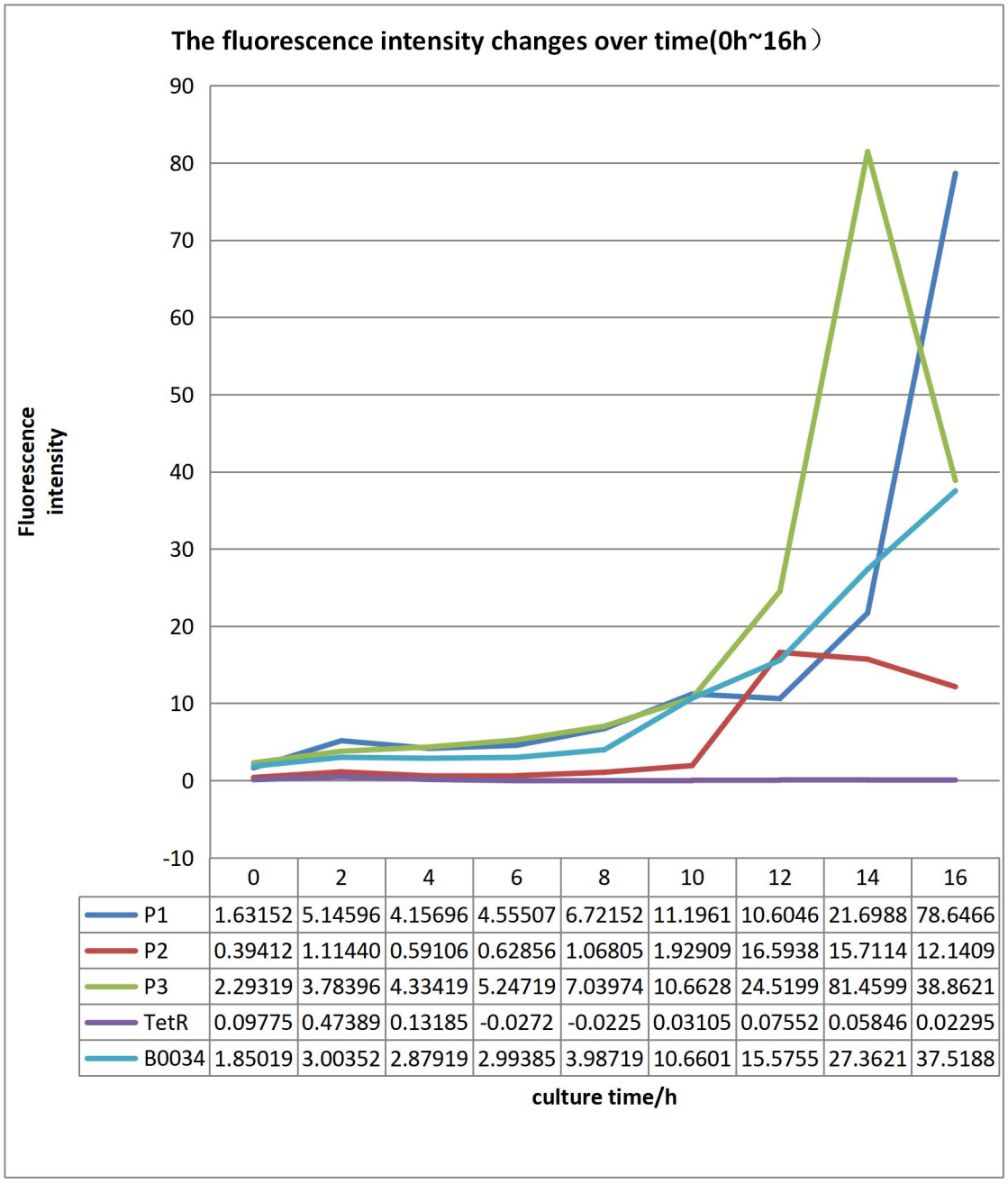
The successful sequence result of P2.

The successful sequence result of P3

2017.10.10~2017.10.17 observe the performance of the constructed strains and test the fluorescence intensity 40 hours after inoculated, founding that P1, P3 is much stronger than B0034 but P2 did not meet the expectation.

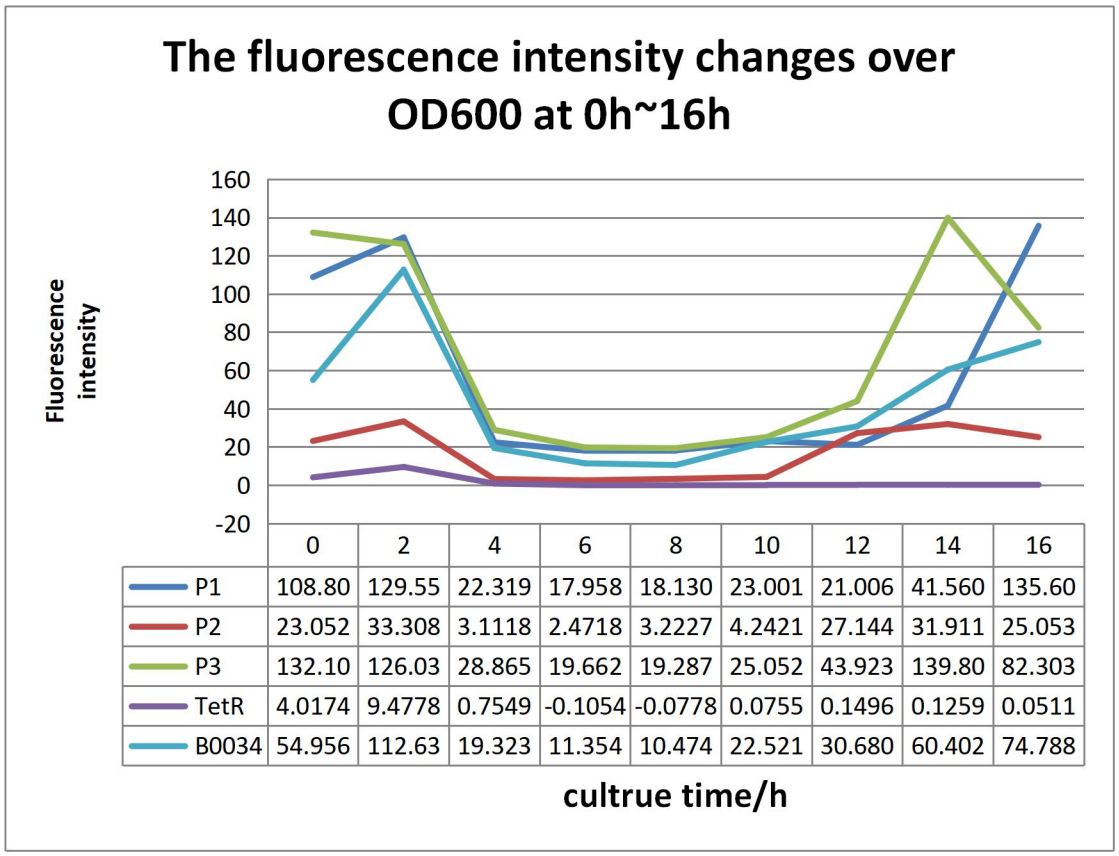
2017.10.18~2017.27 measures the change of fluorescence intensity of the bacteria liquid over time and OD600.

The fluorescence intensity changes over time at 0h~16h. P1 represent the strain containing part: BBa_K2276007. P2 represent the strain containing part: BBa_K2276008. P3 represent the strain containing part: BBa_K2276010. Tet R represent the strain that only has tetR repressible promoter without RBS and protein coding sequence. B0034 represent the strain that containing Part: BBa_I13521.



	0	2	4	6	8	10	12	14	16
P1	1.63152	5.14596	4.15696	4.55507	6.72152	11.1961	10.6046	21.6988	78.6466
P2	0.39412	1.11440	0.59106	0.62856	1.06805	1.92909	16.5938	15.7114	12.1409
P3	2.29319	3.78396	4.33419	5.24719	7.03974	10.6628	24.5199	81.4599	38.8621
TetR	0.09775	0.47389	0.13185	-0.0272	-0.0225	0.03105	0.07552	0.05846	0.02295
B0034	1.85019	3.00352	2.87919	2.99385	3.98719	10.6601	15.5755	27.3621	37.5188

The fluorescence intensity changes over OD600 at 0h~16h. P1 represent the strain containing part: BBa_K2276007. P2 represent the strain containing part: BBa_K2276008. P3 represent the strain containing part: BBa_K2276010. Tet R represent the strain that only has tetR repressible promoter without RBS and protein coding sequence. B0034 represent the strain that containing Part: BBa_I13521.


The fluorescence intensity changes over OD600 at 0h~16h
Fluorescence intensity
160
140
120
100
80
60
40
20
0
-20
0 2 4 6 8 10 12 14 16
P1 108.80 129.55 22.319 17.958 18.130 23.001 21.006 41.560 135.60
P2 23.052 33.308 3.1118 2.4718 3.2227 4.2421 27.144 31.911 25.053
P3 132.10 126.03 28.865 19.662 19.287 25.052 43.923 139.80 82.303
TetR 4.0174 9.4778 0.7549 -0.1054 -0.0778 0.0755 0.1496 0.1259 0.0511
B0034 54.956 112.63 19.323 11.354 10.474 22.521 30.680 60.402 74.788
cultrue time/h