【Part of experiments on LacZ and T7P-C1s, bi-stable】

2009/08/16

20:11/ Prepare the ligation system

3uL insert: (T7P+C1; RBS = 1-1H, 2G, 2I, 2K, 2M, 1J, 5J)

1uL vector:1-1M (plas)

1uL 10* buffer

4uL ddH2O

1uL ligase

20:28/ start the reaction

2009/08/17

15:31/ X-gal(DMF): 50uL spread on the plate A+K+

15:38/ begin to dry at 37C

15:47/ begin to transformation

17:45/ (transformation): spread on the plate to cultivate (not exposed to light)

2009/08/18

14:22/ pick up colonies on seven T7P+C1 plates, cultivate in 5mL LB(A+)

14:23/ pick up lacZ colonies for sequencing

2009/08/19

14:30/ mini-prep the plasmid

16:22/ prepare the enzyme digestion

2.5uL plas(*7)

0.5uL EcoR1

0.5uL Pst1

1uL Buffer H

5.5uL ddH2O

16:49/ start digestion

22:45/ prepare the di-transformation

2009/08/20

1:17/ begin to cultivate

16:13/ transform the T7P+C1; RBS = 1-1H, 2G, 2I

20:00/ cultivate

2009/08/22

16:18/ pick up colonies T7P-C1*7, cultivate

22:11/ pick cultivation into LB(K+) -> check

2009/08/23

13:49/ mini-prep the plasmids: T7P-C1*7

15:47/ preserve the bacteria: T7P-C1*7

19:50/ transform: bi-stable(K+) -> BL21
21:28/ cultivate the plate

2009/08/24
16:05/ pick the bi-stable colony and cultivate
22:41/ cultivate lacZ for sequencing

2009/08/25
14:47/ Make bi-stable sensitive cells
20:18/ Succeed! Transformation: T7P+C1*7
22:14/ Cultivation

2009/08/26
0:06/ Cultivate 1-18C-LacZ
16:08/ mini-prep 1-18c-lacZ, cultivate T7P+C1/Bi-stable
21:40/ T7P/Bi-stable: 2.3.4.6.7 +iptg(1%) induce
23:55/ +PBS, check by flow cytometry

2009/08/27
1:43/ Spread 2.3.4.6.7 on plates
13:38/ mini-prep the plasmids: T7P/Bi-stable
15:46/ start the enzyme digestion
20:27/ stop the reaction
21:56/ Spread 1-2G, 1-1J, 1-2K, 1-2M on plates
22:22/ begin to cultivate

2009/08/28
21:11/ cultivate T7P-C1/BS*7, lacZ

2009/08/29
10:50/ mini-prep the plasmid:T7P-C1/BS
22:51/ Cultivate

2009/08/30
13:19/ Cultivate T7P+C1/BS

2009/08/31
11:47/ 100uL BS(red) ->10mL LB(K+)
23:30/ Prepare the PCR: BS, 1-18P, 1-18P/T7, -
- Template: 0.5uL
- For: 0.5uL
- Rev: 0.5uL
- ddH2O: 15.2uL

23:52/ Start the PCR reaction