2009/06/09 (with LinMin)

11:10/ The e-coli cultivated yesterday did not grow

The material of the former culture medium went bad. Need renewing.

11:29/ Practise minipreping the plasmids

12:58/ Practise digesting the plasmids

2009/06/13 (with LinMin)

15:00/ make the culture medium, repair computer, search Parts:

Parts Code	Location	Location	Plasmid	Containment	Description
BBa_E0240	Kit Plate 1	12M	Psb1A2	GFP rbs-repoter	Medium rbs
BBa_E0840	Kit Plate 1	12O	Psb1A2	GFP rbs-repoter	Strong rbs
BBa_R0080	Kit Plate 1	12E	Psb1A2	Ara Promoter	
BBa_C0062	Kit Plate 1	4O	Psb1A2	Lux R gene	No LVA
BBa_C0051	Kit Plate 1	4E	Psb1A2	C1 repressor	With LVA
BBa_C0179	Kit Plate 2	8M	Psb1A2	lasR activator	No LVA
BBa_C0079	Kit Plate 1	14J	Psb1A2	lasR activator	With LVA
BBa_R0079	Kit Plate 1	12A	Psb1A2	LasR/Pal promoter	

16:55/ dissolve the plasmids, prepare to transform

add 15uL ddH2O

21:20/ Transform plasmids above

2009/06/14

13:10/ repair the computer

14:08/ make the LB culture medium

21:00/ search the rbs parts:

Parts Code	Location	Location	Plasmid	Containment	Description
BBa_B0030	Kit Plate 1	1H	Psb1A2	RBS	strong rbs
BBa_B0031	Kit Plate 1	2G	Psb1A2	RBS	Weak
BBa_B0032	Kit Plate 1	2I	Psb1A2	RBS	Medium
BBa_B0033	Kit Plate 1	2K	Psb1A2	RBS	Weaker
BBa_B0034	Kit Plate 1	2M	Psb1A2	RBS	
BBa_J44001	Kit Plate 1	1J	Psb1A2	RBS	
BBa_J61100	Kit Plate 1	5J	Psb1A2	RBS	
BBa_J61101	Kit Plate 1	5L	Psb1A2	RBS	
BBa_J61107	Kit Plate 1	5N	Psb1A2	RBS	
BBa_J61117	Kit Plate 1	11L	Psb1A2	RBS	
BBa_J61127	Kit Plate 1	11N	Psb1A2	RBS	

2009/06/15

21:07/ Transform plasmids above (2009/06/14)

21:48/ begin to transforming

2009/06/16

11:50/ cultivation is finished The results come out well

2009/06/19 (with Wu Shuke)
21:11/ Select clones from the plate

2009/06/20
23:00/ transform 1-12L

2009/06/21
9:50/ Transform the 2008 Parts' RBS
11:54/ finish transformaion

2009/06/22
9:14/ Transformation is failed, redoing
22:30/ re-transformation

2009/06/23
1:00/ start the cultivation
13:00/ the second transformation is failed

2009/06/24
14:59/ Prepare to make TOP10 Sensitive Cells
16:00/ Begin to make LB, etc
17:30/ Search for LuxR s

BBa_R0062	09 Kit1	6O	pSB1A2	Promoter
BBa_R0065	09 Kit1	8C	pSB1A2	Promoter
BBa_R1062	09 Kit1	8G	pSB1A2	Promoter

23:38/ Cultivate TOP10 Cells

2009/06/25
21:44/ Transform the rbs:
1003-12C; 1003-12G; 1004-2A; 1004-2C; 1004-3G

2009/06/26
22:36/ Cultivate:
1004-1C; 1004-3C; 1004-4C; 1004-2G
23:07/ Prepare to make 1-2I Sensitive Cells
23:15/ Cultivate 1-2I Cells

2009/06/27
15:58/ Miniprep the plasmids:
1004-1C; 1004-3C; 1004-4C; 1004-2G Failed
16:09/ Make the 1-2I Sensitive Cells Failed

2009/06/28

20:18/ Miniprep the plasmids:

1004-1C; 1004-2C; 1004-3C; 1004-4C; 1004-2G

21:37/ Make the 1-2I Sensitive Cells

2009/06/29

17:40/ Check the 1-2I Sensitive Cells Huge Success! The protocol is added to ftp

20:09/ Prepare to make DH5A Sensitive Cells

2009/07/03

18:00/ Search Parts:

BBa_J37032	1003	6C	pSB1A2	GFP controlled by LuxRP

22:44/ Cultivate 1003-6C Failed. The plasmid is bad.

2009/07/03-2009/07/10

Searching for papers on T3P and T3Pol, which is basic for the second logic gate. Contact with the writer to get the plasmids.

During this time, a review on T7 and T3 systems is summarized, which has been sent to ftp.

2009/07/06

19:51/ Miniprep the plasmids:

2-4O	LuxR+RBS	Amp+
1-18P	P(cat)	Kan+

21:08/ Digest the plasmids of 2-4O and 1-18P:

Digestion System Insert: 20μL;
Plasmids: 5μL;
Pst1: 1μL;
Xbal1:1ul
Buffer 1*M: 2μL;
dd H2O: 11μL.

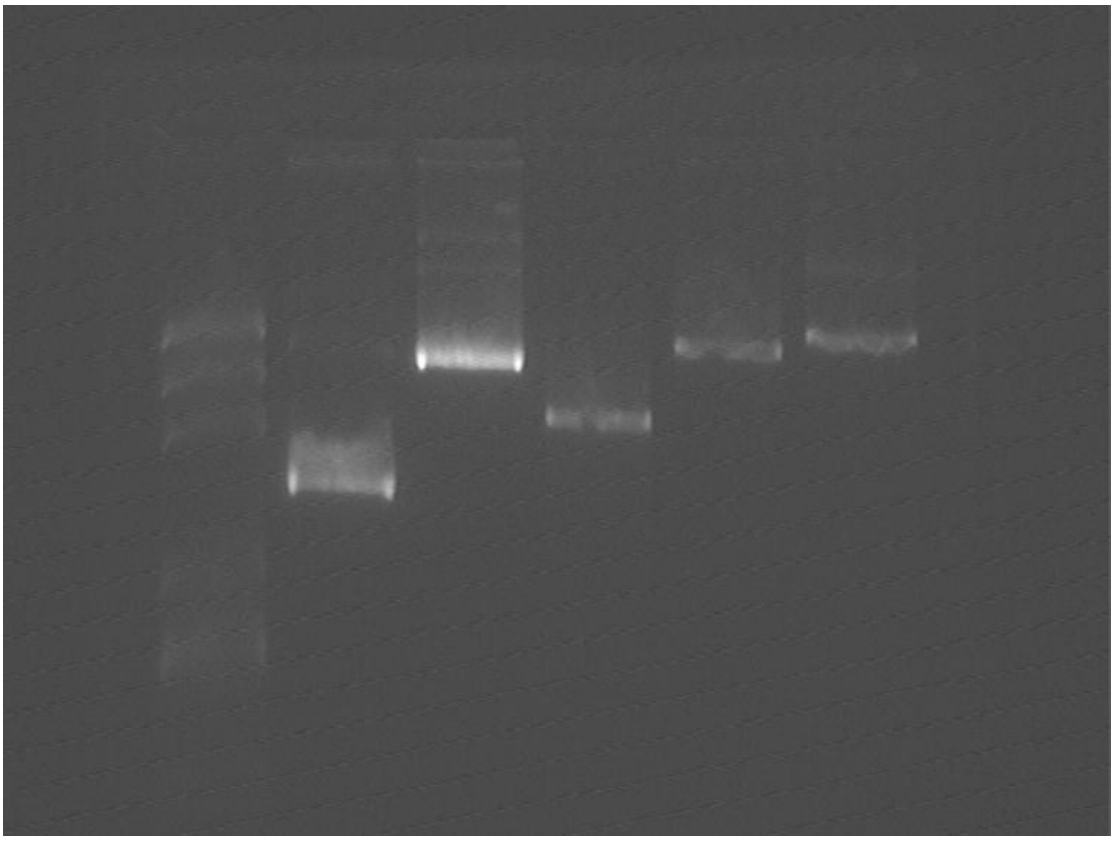
Digestion System Vector: 20μL;
Plasmids: 5μL;
Pst1: 1μL;
Spe1:1ul
Buffer 1*H: 2μL;
dd H2O: 11μL.

2009/07/07

0:34/ The digestion reaction begins

7:46/ add 0.4uL cip and 2ul buffer to the vector system

8:07/ the result come out as follows:



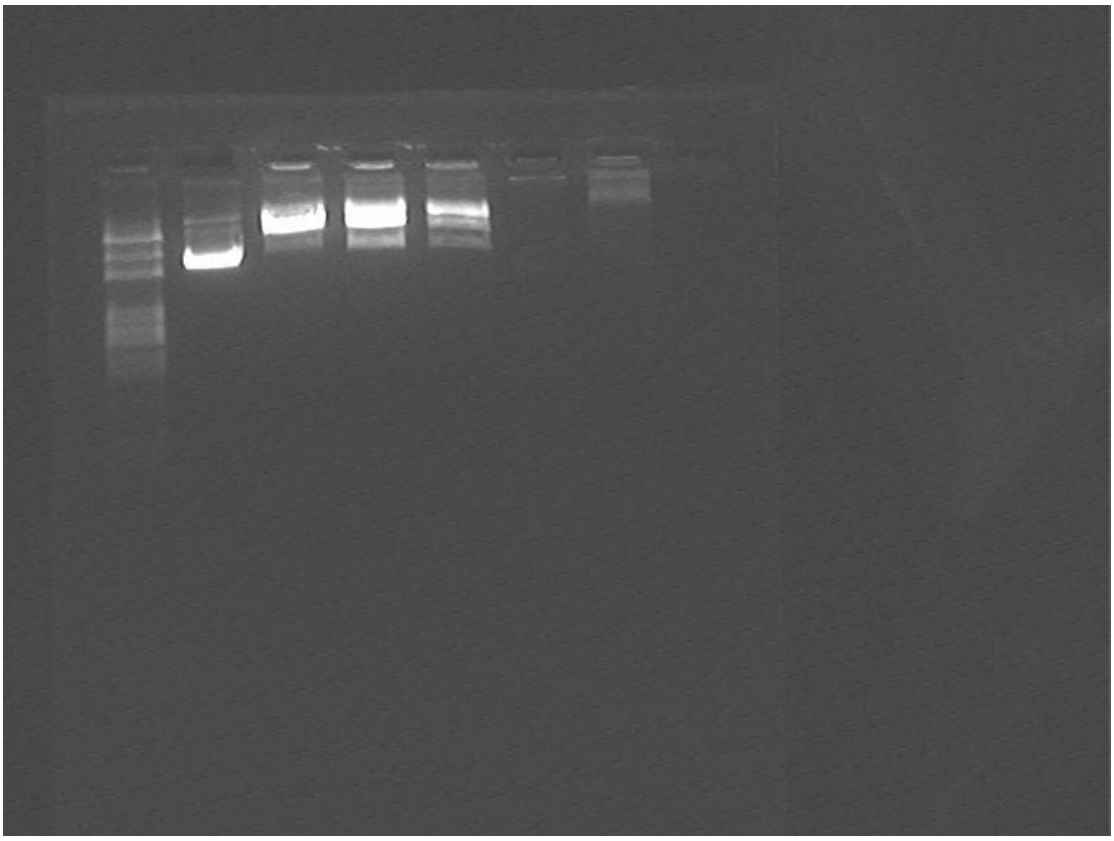
18:03/ Recycle the fragments.
19:40/ Ligate the 2-4O insert to 1-18P vector:
Insert: 3μL;
Vector: 1μL;
Buffer 10*: 1μL;
Ligase: 1uL;
dd H2O: 4μL.
19:48/ Ligation start
23:44/ Begin the transformation of the ligation plasmid

2009/07/08
16:57/ Cultivate the 2-4O->1-18P

2009/07/09
9:06/ Miniprep the 2-4O->1-18P plasmids
11:52/ Digest the plasmids of 2-4O->1-18P and 1-23L:

Digestion System Insert: 20μL;	Digestion System Vector: 20μL;
Plasmids: 5μL;	Plasmids: 5μL;
EcoR1: 1μL;	EcoR1: 1μL;
Spe1:1ul	Xbal1:1ul
Buffer 1*H: 2μL;	Buffer 1*M: 2μL;
dd H2O: 11μL.	dd H2O: 11μL.

12:13/ Reaction Start
17:32/ Stop the digestion



20:08/ Prepare to Ligation

20:17/ Ligation start