2/7/2010

1. Dissolve J23101, J23116, J23103 parts from the well and transformed them into trans 5α.

3/7/2010

1. Anneal PmerT with Forward and reverse primers.
2. Ligase PmerT with E0840.
3. Transform.

4/7/2010

1. Miniprep pc plasmids and PmerT-E0840.

5/7/2010

1. Ligase RBS and *merR*, then transform.
2. Digest pc plasmids with EcoRI and SpeI.

6/7/2010

1. Miniprep RBS-merR plasmid.
2. Digest the plasmid with XbaI and PstI.

7/7/2010

1. Ligase pc with RBS-merR, then transform.
2. Confirm the product by PCR.

8/7/2010

1. Miniprep pc-RBS-merR plasmid.

9/7/2010

1. Confirm the product by PCR.

10/7/2010

1. Digest Psb3K3 backbone with EcoRI and PstI.
2. Help ZTZ with his Pbad-E0840.

11/7/2010

1. Miniprep pc-RBS-merR plasmid and digest them with EcoRI and PstI.

12/7/2010

1. Ligase pc-RBS-merR with Psb3K3 backbone.
2. Transform.
3. For I've been confused about all the plasmids I can only PCR them all to test which ones are correct.

13/7/2010~22/7/2010

Keep on the construction of ps-RBS-merR~

A little frustrated, but things will be fine!

23/7/2010
Get one clone, J23103-RBS-merR.
Strategy change.
Order primers that consist of pc and RBS sequences.
Use J23103 –RBS-merR as template.
Try to get other clones by PCR.

24/7/2010
1. Purify PCR products.
2. Digest them with EcoRI and PstI.

25/7/2010
1. Ligase pc-RBS-merR with Psb1A2 and Psb3K3 backbone.
2. Transform.

26/7/2010
1. Miniprep plasmids.
2. Send them for sequencing.

27/7/2010
Cannot wait for the sequencing results, start function test.
1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

28/7/2010
Test GFP expression level.
For the first time, there are only 5 Hg concentration gradients and no repeats.
Get data, sort of weird.
No significantly differences.
Happy anyway.

29/7/2010
This time 10 gradients and 3 repeats.
The GFP of J23103 is extraordinarily higher than others. And no differences can be observed between the others.

30/7/2010~30/7/2010
Try again~

31/7/2010
Sequencing results come. Bad news: It seems that the products were polluted by merR.

1/8/2010

Construct parts: PmerT and PpbrA. To lift spirit.

1. Digest them and Psb1C3.
2. Ligase them.
3. Transform.

2/8/2010

1. Incubate.
2. Miniprep.
3. Sequencing.

3/8/2010

Parts are right.

4/8/2010~12/8/2010

Home, sweet home.

Though, I've spent most of the time at EXPO.

13/8/2010

Restart from the beginning.

1. PCR with my specialized primers and J23103 template.
2. Purify the products.
3. Digest.

14/8/2010

It's time to speed up a little.

1. Ligase with backbones Psb3K3 and Psb1A2.
2. Transform. 18 plates~~~

15/8/2010

1. Pick 6 candidates from each plate. Colony PCR. So many PCR tubes~
2. Miniprep those have right bands.
3. Send some for sequencing.

16/8/2010

Digest for Psb1A2 and Psb3K3 backbones.

It's difficult to prepare the Psb3K3 backbone. I hate low-copy plasmids.

17/8/2010

Sequencing results come. Very, very bad news: All I got were tubes of merR and a tube of J23103.

It seems that merR pollution is still going on, and I put too much template in the PCR system.

18/8/2010~21/8/2010

Restart. I need to hurry.

22/8/2010

J23108 turns out to be right, on both backbones.

23/8/2010

J23101, J23109, J23112 have right candidates on Psb3K3 backbone.

Transform the plasmids into competent cells.

24/8/2010

1. Pick some from plates, incubate.
2. Miniprep, CAREFULLY.
3. Digest with E, P.

25/8/2010

1. Ligase with Psb1A2. Transform.
2. J23114, J23117 has right candidates on Psb3K3 backbone, do the same to change the backbone.

26/8/2010

1. Miniprep and get J23101, J23109, J23112 on Psb1A2 backbone.
2. Digest J23114, J23117 with E, P.

27/8/2010

1. J23116 has right candidates on Psb1A2 backbone. For I've got many right ones, I can skip the transformation, the incubation, the miniprep, and digest one tube of plasmids directly. Good.

28/8/2010

1. Ligase and transform.

29/8/2010

Miniprep.

30/8/2010

Managed to get all clones I need before term begins.

1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

31/8/2010~2/9/2010

Preliminary experiment.

Test GFP expression level.

There are 5 Hg concentration gradients and no repeats.

3/9/2010

Test GFP expression level of J23112(K) and J23101(K).

There are 20 Hg concentration gradients and 3 repeats.

4/9/2010
Test GFP expression level of J23112(A) and J23101(A).
There are 20 Hg concentration gradients and 3 repeats.

5/9/2010
Test GFP expression level of J23109(K) and J23109(A).
There are 20 Hg concentration gradients and 3 repeats.

6/9/2010
Test GFP expression level of J23108(K) and J23108(A).
There are 20 Hg concentration gradients and 3 repeats.

7/9/2010
Test GFP expression level of J23117(K).
There are 20 Hg concentration gradients and 3 repeats.

8/9/2010
Test GFP expression level of J23117(A).
There are 20 Hg concentration gradients and 3 repeats.

9/9/2010
Test GFP expression level of J23116(K) and J23116(A).
There are 20 Hg concentration gradients and 3 repeats.

10/9/2010
Lessons come.

11/9/2010
Test GFP expression level of J23116(K) and J23116(A).
There are 20 Hg concentration gradients and 3 repeats.

15/9/2010
Arranged data the past few days, not very satisfying, maybe because I'm not familiar with the function testing.
And I want to work J23100 out.

16/9/2010
1. PCR.
2. Purify.
3. Digest.

17/9/2010

1. Ligase.
2. Transform.

18/9/2010
Miniprep.
Send for sequencing.

19/9/2010
The result of J23100 is terrible. Emmm. Fine.
Some of the plasmids are used up.
Transform them all.

20/9/2010
Miniprep.
Some failed.

21/9/2010
Miniprep.
1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

22/9/2010
Test GFP expression level of J23108(K) and J23108(A). 5 gradients.
Data is OK.

23/9/2010
Test GFP expression level of J23108(K) and J23108(A).
There are 20 Hg concentration gradients and 3 repeats.
Weird thing happen.
Yesterday the data show that gfp had been induced to emit. But today's data are not. Why?

24/9/2010
Test GFP expression level of J23116(K) and J23116(A).
The same thing happen.

25/9/2010
1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

26/9/2010
Test GFP expression level of J23108(K) and J23108(A).
There are 20 Hg concentration gradients and 3 repeats.
Data are OK.
Because the E.Coli are freshly prepared? Maybe.

27/9/2010~30/9/201
Deal with data.

31/9/2010
1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

1/10/2010
Holiday. Time to get data.
Test GFP expression level of J23101(K) and J23101(A).
There are 20 Hg concentration gradients and 3 repeats.
1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

2/10/2010
Test GFP expression level of J23103(K) and J23103(A).
There are 20 Hg concentration gradients and 3 repeats.
1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

3/10/2010
1. Digest Psb1C3 for backbone. A lot.
2. Digest my pc-RBS-merRs.

4/10/2010
1. Ligase.
2. Transform.

5/10/2010
Miniprep.
Get standardized parts.

6/10/2010
Test GFP expression level of J23103(K) and J23103(A).
There are 20 Hg concentration gradients and 3 repeats.
Data bad. Do again tomorrow.
1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

7/10/2010
Test GFP expression level of J23103(K) and J23103(A).
There are 20 Hg concentration gradients and 3 repeats.
1. Prepare cells with PmerT-E0840 into competent cells.

2. Transform pc-RBS-merR plasmids with different resistance into them.

8/10/2010

Test GFP expression level of J23108(K) and J23108(A).

There are 20 Hg concentration gradients and 3 repeats.

The J23108(A) data is bad, do tomorrow.

1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

9/10/200

Test GFP expression level of J23109(A).

There are 20 Hg concentration gradients and 3 repeats.

1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

10/10/2010

Test GFP expression level of J23109(K) and J23109(A).

There are 20 Hg concentration gradients and 3 repeats.

1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

11/10/2010

Test GFP expression level of J23112(K).

There are 20 Hg concentration gradients and 3 repeats.

1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

12/10/2010

Test GFP expression level of J23112(A).

There are 20 Hg concentration gradients and 3 repeats.

1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

13/10/2010

Test GFP expression level of J23114(K).

There are 20 Hg concentration gradients and 3 repeats.

1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

14/10/2010

Test GFP expression level of J23114(A).

There are 20 Hg concentration gradients and 3 repeats.

1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

15/10/2010

Test GFP expression level of J23117(K).

There are 20 Hg concentration gradients and 3 repeats.

1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

16/10/2010

Test GFP expression level of J23117(A).

There are 20 Hg concentration gradients and 3 repeats.

1. Prepare cells with PmerT-E0840 into competent cells.
2. Transform pc-RBS-merR plasmids with different resistance into them.

17/10/2010~18/10/2010

Finish my parts.

19/20/2010~

Arrange data, write report and notes for wiki, upload parts' information.