lab note

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7.04

positive transformation of the parts which will be used in the future: R0080(Para), B0032(RBS), K145001(T7polymerase) and B0015(Terminator).

7.05

1 pick two clones each plate and shave at 37℃ to amplify the bacteria

2 The first challenge: we don't have the sequence of the plasmid PSD-MBD(containing the lpp-ompa-mbp) offered by Summers. After reading papers for the detailed information of this plasmid, I selected and ordered the sequence primers.

3 Miniprep of the plasmid.

4 DNA overnight double digestion of the plasmids.

20uL reaction system:

Buffer2 2uL
SpeI 1.5uL
PstI 1.5uL
RBS 5uL
ddH2O 10uL

Buffer3 2uL
XbaI 1.5uL
PstI 1.5uL
T7polymerase 5uL
ddH2O 10uL

Buffer 2uL
EcoRI 1.5uL
XbaI 1.5uL
terminator 5uL
ddH2O 10uL

7.06

1 Electrophoresis enzyme digestion reaction system in 1.5% agarose gel.

2. Excise the gel slice and extract the fragments.

3 ligation of insert DNA fragments into plasmid vector DNA for 1 hour

T7polymerase(P/X) 6uL
RBS-vector (P/S) 2uL
10x ligation buffer 1uL
T4 DNA Ligase 1Ul

4 transform the products of ligation and E0240

5.pick three clones and shake in 37℃ for 8h.

6 Miniprep of the E0240 and double digestion(E,X) in 37℃

7.07

1 Electrophoresis the enzyme digestion reaction system in 1.5% agarose gel.

2. Purify the fragments E0240(E X).

3 miniprep of the plasmid of rbs-T7polymerase-PSB1A2

4 double digestion rbs-T7polymerase-PSB1A2(X,P)and send to sequence to verify the plasmid.

5 Electrophoresis enzyme digestion reaction system in 1.5% agarose gel.

6 Excise the gel slice and extract the fragments of rbs-T7polymerase (insert).

7 ligation the rbs-T7polymerase to the vector with Para.

7-08

1 transformation the Para rbs-T7poly

2 the sequence results showed that rbs-T7polymerase is right but there is no result of OMPA-MBP.

7-09

1 pick several clones and amplify the bacteria.

2 miniprep the Para- rbs-T7poly

3 enzyme digestion of Para-rbs-T7poly (E,P) to verify

7-10

1. Electrophoresis enzyme digestion reaction system in 1.5% agarose gel. But the results is not right.
2. Ligation again.
3. transformation the Para rbs-T7poly
4. get the sequence of PSD-MBD, and it is right!

7-11

1. miniprep the Para-rbs-T7poly.
2. Enzyme digestion to verify

7-12

1 Electrophoresis enzyme digestion reaction system in 1.5% agarose gel. But the results is not right.

2 give up the arabinose promoter.

7-13

1 the PCR primer is arrived and begin to get the lpp-omp-mbp sequence with PCR.

SuperMix(trans)	10uL
Commercial For(with NdeI site)	1uL
Commercial Rev(with XhoI site)	1uL
Template plasmid P	0.5uL
ddH2O	up to 20 uL

SuperMix(trans)	10uL

Standard For(with prefix)	1uL
Standard Rev(with suffix)	1uL
Template plasmid P	0.5uL
ddH2O	up to 20 uL

2 Electrophoresis PCR reaction system in 1.5% agarose gel. Since the MBP is a tandem structure, the reverse primer can pair with the template at two different places, there are two bands. The larger band is selected and purified.
3 Enzyme digestion of Standard fragmets with XbaI and PstI and the Commericl fragments with NdeI and XhoI.
4 another pair of primers for PSD-MBD is ordered.

7-14
1 to verify whether the band purified from PCR is right, the fragments are digested with BamHI ,which only exists in the right fragments.
2 Electrophoresis enzyme BamHi digestion system in 1.5% agarose gel. But there is no band! We plan to do another PCR when the new primers come.

7-16
1 the new primer comes!
2 get the lpp-omp-mbp sequence with PCR. The PCR is carried out with two enzyme, the PFU and the Taq.
3 Electrophoresis PCR reaction system in 1.5% agarose gel. Now there is one and only one band of the PFU PCR system. The fragments is purified.
4 double digestion of both the carrier plasmid and the PCR fragments. The commercial plasmid PET21a and commercial fragments are digested with NdeI and XhoI. The standard plasmid with RBS and standard fragments are digested with XbaI and PstI.
5 ligation the lpp-ompa-mbp fragments to the vectors.
6 transform the ligation products.

7-17
pick the clones and shake to amplify the bactira.

7-18
1 miniprep of the lpp-ompa-mbp-PET21A and rbs-lpp-ompa-mbp-PSB1A2.
2 bacteria PCR is done to verify if the fragments have been ligated into the vector.
3 Electrophoresis PCR reaction system in 1.5% agarose gel. And I am very happy to see that the results are right!
4 the plasmid are sent to sequence for further valediction.

7-19
1 Double enzyme digestion of the rbs-lpp-ompa-mbp with XbaI and PstI.
2 Electrophoresis enzyme system in 1.5% agarose gel. And the productions of the enzyme digestion are purified.

3 ligation(rbs-lpp-ompa-mbp+T7promoter as vector)
4 transform both the lpp-ompa-mbp-PET21A to BL21 for test and the PT7-rbs-lpp-ompa-mbp to trans10.

7-20
1 the results of the sequence of the lpp-ompa-mbp-PET21a andrbs-lpp-ompa-mbp show that there are right clones.
2 amplify the PT7-rbs-lpp-ompa-mbp.
3 miniprep the plasmid and the plasmids are sent to sequence.

7-21
1 pick one positive clone of the lpp-ompa-mbp-PET21a(BL21) and shake in the 37℃.for10h
2 dilute 20ul in 5ml LB and shake the bacteria in 37℃ for 2~3h until the OD reaches to 0.6~0.8.
3 Add IPTG into the bacteria and shake in 30℃ for 4~5h.
4 send the bacteria to carry out Western blot.

7-23
1 The sequence results show that the number 1 and 4 PT7-rbs-lpp-ompa-mbp are right.
2 double digest the PT7-rbs-lpp-ompa-mbp(ES) and the terminator B0015(E X).

7-24
1 Electrophoresis enzyme system in 1.5% agarose gel. The results are right.
2 there was an accident when the gel is purified.
3 again double digest the PT7-rbs-lpp-ompa-mbp(ES) and the terminator B0015(E X).

7-25
1 Electrophoresis enzyme system in 1.5% agarose gel. The results are right and the gel is purified to get the fragments.
2 ligation(PT7-rbs-LOM+Terminator-PSB1AK3)
3 transformation the PT7-rbs-LOM to BL21 and PT7-rbs-LOM-Terminator-PSB1AK3 to trans5alpha.
4 pick the clones and bacteria PCR is done.

7-26
1 Electrophoresis PCR system in 1.5% agarose gel. The right plasmid are sent to sequence to verify.
2 induction the bacteria of BL21 with the plasmid lpp-ompa-mbp-PET21a and PT7-rbs-LOM to prepare for SDS-PAGE and Western blot.

7-27—7.31
1 induction the bacteria
2 carry out the experiment of SDS-PAGE.
8.1-8.6
1 make samples with the mercury concentration gradient of

10^-7,5*10^-7,10^-6,5*10^-6,10^-5M.
2 do the experiment of detection the concentration of mercury with dithizone to make the standard curve.

8.16
digestion of rbs-T7poly with E,X as vector and promoter P1-3 G1-3with E,S as insert.
8.17
1 Electrophoresis enzyme system in 1.5% agarose gel. The results are not right.
2 ligation.

8.18
1 PCR to get PBAD.
2 Electrophoresis PCR reaction system in 1.5% agarose gel.
3 digestion with E,S
4 ligation

8.19
1 transformation Psal-rbs-T7poly and PBAD-rbs-T7poly and PET21a-lpp-ompa-mbp(lead).

8.20
1 pick the clones and doing bacteria PCR to verify.
2 Electrophoresis enzyme system in 1.5% agarose gel. The results are right and they are sent to sequence.

8-21
1 positive cloning.
2 the sequence results show that PBAD Psal are right.

8.22
1 sequence results show that PET21a-lpp-ompa-mbp(lead) is right.
2 double enzyme digestion of lpp-ompa-mbp(lead) with E,S and B0015 terminator with E,X and RBS with S, P.
8.23
1 ligate lpp-ompa-mbp(lead) with B0015.
2 transformation.
8.24
1 pick the clones and amplify the bacteria.
2 enzyme digestion of lpp-ompa-mbp(lead)-Terminator with X,P.
3 Electrophoresis enzyme system in 1.5% agarose gel. The right bands are purified.
4 ligation the lpp-ompa-mbp(lead)-Terminator with the vector containing RBS.
8.25
1 transformation
2 amplification.
3 miniprep of the plasmids of rbs-lpp-ompa-mbp(lead)-terminator.

4 digestion of rbs-lpp-ompa-mbp(lead)-terminator with X,P

8.26

1 Electrophoresis enzyme system in 1.5% agarose gel. The right bands are purified. The plasmids are sent to sequence.

2 ligate the rbs-lpp-ompa-mbp(lead) with T7promoter.

8.27

1 transformation.

2 amplification.

3 miniprep of the plasmids of PT7-RBS-lpp-ompa-mbp(lead)-terminator. The plasmids are then sent to sequence to verify.

8.29

The sequence of PT7-RBS-lpp-ompa-mbp(lead)-terminator are right.

9.3

1 double digestion of Pbad-T7poly and Psal-T7poly with E,P and Pt7-rsb-lom-terminator with E,S , Pt7-rsb-MBP-terminator with EX.

2 Electrophoresis enzyme system in 1.5% agarose gel. The right bands are purified.

9.4

1 ligation(Pbad-T7poly psb3k3 Psal-T7poly psb3k3 lom-mbp).

2 transformation.

9.5

1 amplification.

2 miniprep of the plasmids Pbad-T7poly psb3k3 Psal-T7poly psb3k3 lom-mbp The plasmids are then sent to sequence to verify.

9.7-9.9

1 The results show that the sequence of Pbad-T7poly psb3k3 Psal-T7poly psb3k3 are not right and lom didn’t ligated into the mbp backbone.

2 digestion again.ligation-transformation-amplification-miniprep-sequence

9.11

The sequence are right.

double transformation the Pbad-T7poly psb3k3 and PT7-GFP , Psal-T7poly psb3k3,PT7-GFP.

9.12

1 Induction the Pbad-T7poly psb3k3 and PT7-GFP with arabinose, Psal-T7poly psb3k3,PT7-GFP with sal.

2 miniprep the MBP-LOM and digest the MBP-LOM and Dsba-MBP.

9.13

1 Electrophoresis enzyme system in 1.5% agarose gel. The right bands are purified.

2 the GFP doesn’t work.

3 ligation the Dsba-MBP as insert and MBP-LOM as vector.

9.14-9.19

1double transformation the Pbad-T7poly psb3k3 and PT7-GFP , Psal-T7poly psb3k3,PT7-GFP.

Induction the Pbad-T7poly psb3k3 and PT7-GFP with arabinose, Psal-T7poly psb3k3,PT7-GFP

with sal. However, the GFP doesn’t work at all, indicating the failure of PBAD and PSAL.
2 complete the construction of Dsba-MBP-MBP-LOM.

9.20-9.23
Construction the Ptet-rbs-T7polymerase and change the backbone.
9.24-9.26
Double transformation.
Fortunately, the Ptet works and GFP can be detected with fluoresce!
9.27-10.5
Change the backbone to PSB1C3 to the parts.
Ligate the Dsba-MBP-MBP-LOM device with Ptet-T7polymerase.

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