Lab note

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6.30

1. Dissolve primers: primers for *mbp* fragments (standardization): SD_ForA, SD_ForC, SD_Rev. primers for *mbp* fragments (commercialization): NdeI_A(For), NdeI_C(For), XhoI(Rev).
2. Miniprep: plasmids provided by Anne O Summers and I-120.
3. PCR reaction for *mbp* fragments using dissolved primers.

5x PHF buffer II	4uL
ddNTP Mixture	1.6uL
For	1uL
Rev	1uL
Template plasmid	0.5uL
phusionHF	0.2uL
ddH2O	up to 20 uL

4. Electrophoresis PCR reaction system in 1% agarose gel.
5. Excise the gel slice and extract the *mbp* fragments.

7.1

1. DNA double digestion of *mbp* fragments for 3 hours: NdeI/XhoI for commercialization and XbaI/PstI for standardization.

20uL reaction system:

Buffer4	2uL
NdeI	1.5uL
XhoI	1.5uL
mbp(com)	15uL

Buffer3	2uL
XbaI	1.5uL
PstI	1.5uL
mbp(SD)	15uL

2. DNA Purification.

7.17

1. PCR reaction for *mbp* fragments using dissolved primers.

Primers Reverse is the new ones with strepp tag.

10x easypfu buffer	2uL
ddNTP Mixture	2uL
For	1uL
Rev	1uL
Template plasmid	0.5uL
phusionHF	0.2uL
ddH2O	up to 20 uL

2. Electrophoresis PCR reaction system in 1% agarose gel.
3. Excise the gel slice and extract the *mbp* fragments.

7.18
1. DNA double digestion of *mbp* fragments and *merr* plasmids.
2. ligation of insert DNA fragments into plasmid vector DNA for 1 hour

mbp(NdeI/XhoI)	6uL
pET-21a	2uL
10x ligation buffer	1uL
T4 DNA Ligase	1uL

mbp (XbaI/PstI)	6uL
B0034 (*rbs*)	2uL
10x ligation buffer	1uL
T4 DNA Ligase	1uL

merr (XbaI/PstI)	6uL
B0034 (*rbs*)	2uL
10x ligation buffer	1uL
T4 DNA Ligase	1uL

3. Transformation

7.19
1. Miniprep of *rbs-merr*
2. Digestion and identification by Electrophoresis

Buffer3	1uL
XbaI	0.75uL
PstI	0.75uL
rbs-merr	7.5uL

7.20
Miniprep of 12 colonies of *mbp*-commercialization.
No colonies formation of *rbs-mbp*.

7.21
1. DNA double digestion of colonies of *mbp*-pET-21a and *rbs-merr*.
10uL reaction system:

Buffer4	1uL
NdeI	0.75uL
XhoI	0.75uL
mbp-pET-21a	7.5uL

Buffer3	1uL
XbaI	0.75uL

PstI 0.75uL
rbs-mbp 7.5uL
2. Electrophoresis in 1% agarose gel.
Sequence *mbp*_ commercialization
(The result is confirmed to be incorrect.)
3. ligation of *rbs-mbp*-1 and 2 into plasmid vector T7 for 1 hour
rbs-mbp(XbaI/PstI) 6uL
T7 vector(SpeI/PstI) 2uL
10x ligation buffer 1uL
T4 DNA Ligase 1uL
4. Transformation

7.22
No colonies formation of T7-*rbs-merr*. Do ligation and transformation again.

7.23
Picking colonies of T7-*rbs-merr* and shaking at 37℃ overnight.

7.24
Problems existed in our experiment: The PCR reaction product cannot be verified. New primers forward (3C and 3Plus) for *mbp* fragments are designed.
1. PCR reaction for *mbp* fragments using dissolved primers.
For 1uL
Rev 1uL
Easymix 10uL
template 0.2uL
ddH2O up to 20 uL
2. Miniprep of T7-*rbs-merr*
2. Digestion and identification by Electrophoresis
EcoRI Buffer 1uL
EcorRI 0.75uL
PstI 0.75uL
T7-*rbs-merr* 7.5uL
The band was not correct.

7.25
1. Electrophoresis PCR reaction product last day in 1% agarose gel.
2. Excise the gel slice and extract the *mbp* fragments.
3. DNA double digestion of *mbp* fragments for 3 hours: XbaI/XhoI for commercialization and XbaI/PstI for standardization.
20uL reaction system:
Buffer4 2uL
XbaI 1.5uL
XhoI 1.5uL

Product 15uL

Buffer3 2uL
XbaI 1.5uL
PstI 1.5uL
Product 15uL
2. DNA Purification.
3. ligation for 1 hour
mbp(XbaI/XhoI) 6uL
pET-21a 2uL
10x ligation buffer 1uL
T4 DNA Ligase 1uL

mbp (XbaI/PstI) 6uL
B0034 (*rbs*) 2uL
10x ligation buffer 1uL
T4 DNA Ligase 1uL

7.26
Transformation of ligation mixure.
Name of plates were: 3C-com, 3Plus-com, 3C-SD, 3plus-SD
*com means commercialization and SD means standardization

7.27-7.28
No colonies formation of 3C-com and 3plus-com
1. Picking 18 colonies from 3C-SD and 3plus-SD(*mbp*_SD) and shaking at 37℃ for 10 hours.
2. Miniprep *mbp*_SD
3. Digestion and identification by Electrophoresis
EcoRI Buffer 1uL
EcorRI 0.75uL
PstI 0.75uL
*mbp*_SD 7.5uL
Sequence.
(The result is confirmed to be incorrect.)

7.30
Template of pASK-*mbp* is verified to have mutagenesis; therefore we decide to construct *mbp* by linking 2 *merr* fragments together.
1. PCR reaction for six *merr* fragments: N_B and B_X; SD(XbaI)_B and B_SD(PstI); SD(EcoRI)_B and B_SD(PstI).
10x buffer 5uL
ddNTP Mixture 5uL
For 1uL
Rev 1uL

Template plasmid 0.5uL
Polymerase 1uL
ddH2O up to 50 uL
2. Electrophoresis PCR reaction system in 1% agarose gel to identification.
3. DNA purification from reaction mixture.
4. DNA double digestion for 3 hours.
20uL reaction system:
N_B:
Buffer3 2uL
NdeI 1.5uL
BamHI 1.5uL
Product 15uL
B_X:
Buffer3 2uL
BamHI 1.5uL
XhoI 1.5uL
Product 15uL
SD(Xbal)_B:
Buffer3 2uL
XbaI 1.5uL
BamHI 1.5uL
Product 15uL
B_SD(PstI):
Buffer3 2uL
BamHI 1.5uL
PstI 1.5uL
Product 15uL
SD(EcoRI)_B:
EcoRI Buffer 2uL
EcorRI 1.5uL
BamHI 1.5uL
Product 15uL
5. DNA purification after the double digestion.
6. ligation of 2 *merr* fragments into the vector for 6 hours.
Insert1 3uL
Insert2 3uL
Vector 2uL
10x ligation buffer 1uL
T4 DNA Ligase 1uL
mbp-Com: Inserts: N_B and B_X; vector: pET-21a(NdeI/XhoI)
mbp-SD: Inserts: SD(Xbal)_B and B_SD(PstI), vector:B0034(SpeI/PstI)
mbp: SD(EcoRI)_B and B_SD(PstI), vector: pSB1K3 (EcoRI/PstI)
7. Transformation

7.31

No colonies formation of *mbp*-Com.

1. Picking colonies from *mbp*-SD and *mbp*, shaking at 37℃ for 10 hours.
2. Miniprep of *mbp*-SD and *mbp*
3. Miniprep of B0034
4. Double digestion for 3 hours.

Buffer2 2uL
SpeI 1.5uL
PstI 1.5uL
B0034(*rbs*) 5uL
ddH2O up to 20 uL

8.1

1. *mbp* Digestion and identification by Electrophoresis

EcoRI Buffer 1uL
EcorRI 0.75uL
PstI 0.75uL
mbp 7.5uL

The band is not correct.

2. *mbp*-SD Double Digestion

Buffer3 2uL
XbaI 1.5uL
PstI 1.5uL
*rbs-mbp*_SD 5uL
ddH2O up to 20uL

3. Electrophoresis *rbs-mbp*-SD in 1% agarose gel.
4. Excise the gel slice and extract the *rbs-mbp*-SD fragments.

8.2

1. Ligation of *rbs-mbp*_SD plasmid vector T7 for 1 hour.

rbs-mbp(XbaI/PstI) 6uL
T7 vector(SpeI/PstI) 2uL
10x ligation buffer 1uL
T4 DNA Ligase 1uL

2. Transformation
3. Ligation SD-Com again and transformation.

8.3

1. Picking colonies from T7-*rbs-mbp*_SD and *mbp*-Com, shaking at 37℃ for 10 hours.
2. Colonies PCR:

For 0.5uL
Rev 0.5uL
Easymix 5uL
template 0.2uL

ddH2O up to 10 uL
Identification by Electrophoresis
Sequence.
(The result is confirmed to be incorrect.)
3. Do ligation of T7-*rbs-merr* and transformation again, using the new B0034.

8.4
1. Picking colonies of T7-*rbs-merr* and shaking at 37℃ overnight.
2. Miniprep of T7-*rbs-merr*
3.Double Digestion and identification by Electrophoresis
EcoRI Buffer 1uL
EcorRI 0.75uL
PstI 0.75uL
T7-*rbs-merr* 7.5uL
Sequence.

8.5-8.8
Attend the iGEM-China meet up in Shang Hai!

8.11
- ✓ The sequence of T7-*rbs-merr* is correct.
- ✓ The sequence of *mbp* is correct.
- ✓ The sequence of *mbp*-Com is correct

1. Positive transformation of *mbp*
2. DNA double digestion of *mbp* fragments for 1 hour:

Buffer3 2uL
Xbal 1.5uL
PstI 1.5uL
Product 10uL
ddH2O up to 20 uL
2. ligation of *mbp* fragments into plasmid vector for 1 hour
mbp (Xbal/PstI) 6uL
B0034 (s/p) 2uL
10x ligation buffer 1uL
T4 DNA Ligase 1uL
3. Transformation

8.13
Test the mercury inductive expression system.

8.15
1. Picking 15 colonies from *rbs-mbp*_SD, shaking at 37℃ for 10 hours.
2. Colonies PCR:
For 0.5uL

Rev 0.5uL
Easymix 5uL
template 0.2uL
ddH2O up to 10 uL
Identification by Electrophoresis.
Sequence.
3. DNA double digestion of *rbs-mbp* fragments for 1 hour:
Buffer3 2uL
Xbal 1.5uL
PstI 1.5uL
Product 10uL
ddH2O up to 20 uL
4. ligation of *mbp* fragments into plasmid T7 vector for 1 hour
rbs-mbp (Xbal/PstI) 6uL
T7 vector (s/p) 2uL
10x ligation buffer 1uL
T4 DNA Ligase 1uL
5. Transformation

- Start the work of Human Practice

8.17
1. Picking 6 colonies from T7-*rbs-mbp*_SD, shaking at 37℃ for 10 hours.
2. Colonies PCR:
For 0.5uL
Rev 0.5uL
Easymix 5uL
template 0.2uL
ddH2O up to 10 uL
Identification by Electrophoresis.
Sequence.

8.18-8.20
Edit the Human practice questionnaire.

8.21

- ✓ The sequence of T7-*rbs-mbp*_SD is correct

1. Positive transformation of T7-*rbs-mbp*_SD into BL-21a
2. DNA double digestion of T7-*rbs-mbp* fragments for 1 hour:
EcorRI Buffer 2uL
EcoRI 1.5uL
SpeI 1.5uL
Product 10uL
ddH2O up to 20 uL
3. Ligation of fragments into plasmid 1-23L(terminator) for 1 hour

T7-*rbs-mbp* (EcoRI/ SpeI) 6uL
1-23L (EcoRI/Xbal) 2uL
10x ligation buffer 1uL
T4 DNA Ligase 1uL
4. Transformation

8.22
1. Picking colonies from T7-*rbs-mbp*-Terminator, shaking at 37℃ for 10 hours.
2. Colonies PCR:
For 0.5uL
Rev 0.5uL
Easymix 5uL
template 0.2uL
ddH2O up to 10 uL
Identification by Electrophoresis.

8.23
T7-*rbs-mbp*-Terminator: Digestion and identification by Electrophoresis
EcoRI Buffer 1uL
EcorRI 0.75uL
PstI 0.75uL
Product 7.5uL
Sequence.

8.24
Get the plasmid of Lpp-OmpA-*mbp* and start the assembly work.
1. DNA double digestion of T7-*rbs-mbp*-Terminator fragments for 1 hour:
EcorRI Buffer 2uL
EcoRI 1.5uL
SpeI 1.5uL
Product 10uL
ddH2O up to 20 uL
2. DNA double digestion of Lpp-OmpA-*mbp* for 1 hour:
EcorRI Buffer 2uL
EcoRI 1.5uL
XbaI 1.5uL
Product 10uL
ddH2O up to 20 uL
3. Ligation for 1 hour:
T7-*rbs-mbp* -terminator (EcoRI/ SpeI) 6uL
LOM (EcoRI/Xbal) 2uL
10x ligation buffer 1uL
T4 DNA Ligase 1uL
4. Transformation

8.25

Start the function test of *mbp*: inductive expression by IPTG

8.26

✓ The sequence of T7-*rbs*-*merr*-terminator is correct.

Assembly plasmids: Digestion and identification by Electrophoresis

EcoRI Buffer	1uL
EcorRI	0.75uL
PstI	0.75uL
Product	7.5uL

Sequence.

9.1-10.1

Pass the bench work to other guys.

1. Finished the wiki of Cytosol Expression.
2. Focus on the human practice work:

Lab contact and PI interviews.

Questionnaires sent out to NIBS, PKU, PUHSC.

Dig information out of books and reports

10.4

Chang Backbone of *mbp* and T7-*rbs*-*mbp*-Terminator:

1. Double digestion: inserts (E/S)
2. Electrophoresis in 1% agarose gel.
3. Excise the gel slice and extract target fragments.
4. Ligate inserts to pSB1C3 (E/S)
5. Transformation

10.5

1. Miniprep
2. Identification by Electrophoresis.

10.6-10.13

1. Writing the essay of Human practice.
2. Writing the Biosafety question required by iGEM committee.

10.14

Chang Backbone of *rbs*-*mbp*:

1. Double digestion: inserts (E/P)
2. Electrophoresis in 1% agarose gel.
3. Excise the gel slice and extract target fragments.
4. Ligate inserts to pSB1C3 (E/P)

5. Transformation

10.15

1. Picking colonies from *rbs*, shaking at 37℃ for 10 hours.
2. Colonies PCR:

For	0.5uL
Rev	0.5uL
Easymix	5uL
template	0.2uL
ddH2O	up to 10 uL

Identification by Electrophoresis.

3. Digestion:

Buffer3	1uL
BamHI	0.75uL
rbs-mbp	5uL
ddH2O	up to 10uL

identification by Electrophoresis

Sequence.

10.18

- ✓ The sequence resuLt of *mbp* (pSB1C3) is correct.
- ✓ The bench work is finished!

10.19-10.26

Revise the wiki of human practice.