实验记录

2018 年 7 月 24　9:11

date: 7.23

1. 50%的甘油进行分装，每管 500ul
2. 插枪头

date: 7.24

1. 倒 A 抗平板
2. Pet-21a 甘油菌平板划线活化，加 A 抗扩大培养。一共 3 管每管 10ml。1 管 NC 对照。
3. PUC57-UGT 引物设计
4. UGT 甘油菌活化

date: 7.25

1.
2. UGT 片段 PCR（PUC57backbone）

vloume 50ul

Master mix	25μ
F	2μ
R	2μ
Template	4μ
dd water	17μ

program：

98	98	52-60	72	72	4
2min	10s	15s	15s	5min	hold

30 cycles

3. extract pET21a from E.coli
4. UGT carring E.coli purification
5. pET-21acarring E.coli purification

date: 7.26

1. UGT restriction endonuclease digest

Quick cut buffer	10μ
UGT pcr product	20μ
BamHI	1μ
EcoRI	1μ
dd water	3μ
pET-21a plasmid	5μ

date: 7.27

1. extract PUC57 plasmid

date: 7.28

1. add EcoRI site on UGT fragment

volume 10μ

Master mix	5μ
F	0.4μ
R	0.4μ
Template	0.8μ
dd water	3.4μ

gradient PCR in 55-68°C. 8 columns total.

2. add EcoRI and NdeI site on UGT fragment

Master mix	5μ
F	0.4μ
R	0.4μ
Template	0.8μ
dd water	3.4μ

gradient PCR in 55-65°C. 8 columns total.

4.PCR at best temperature

volume100μ

Master mix	50μ
F	4μ
R	4μ
Template	8μ
dd water	34μ

8 columns total. 4 single restriction endonuclease digest and 4 double restriction endonuclease digest

date: 7.30

1. single and double restriction endonuclease digest of UGT and pET21a

Quick cut buffer	10μ
UGT pcr product	20μ
BamHI	1μ
EcoRI	1μ
dd water	3μ
pET-21a plasmid	5μ

2. UGT 与 pET-21a single and double restriction endonuclease digest and ligation volume40μ

10X ligation buffer	4μ
vector	10μ
Insert sequence	20μ
T4 DNA ligase	2μ
dd water	4μ

date: 7.31

1. pET-21a plasmid extract. 4 column total.
2. pET-21a double restriction endonuclease digest verification
3. UGT-pET-21a EcoRI single restriction endonuclease digest：

10X ligation buffer	4μ
vector	9μ
Insert sequence	25μ
T4 DNA ligase	2μ

4. UGT-pET-21a EcoRI/NdeI double restriction endonuclease digest：

10X ligation buffer	4μ
vector	9μ
Insert sequence	25μ
T4 DNA ligase	2μ

date: 8.1

1. double restriction endonuclease digest of UGT fragment.

volume：30μl

PCR product	25μ
EcoRI	1μ
NdeI	1μ
buffer	3μ

pET-21a single restriction endonuclease digest

volume：20μl

pET-21a	10μ
EcoRI	1μ
NdeI	1μ
buffer	2μ
dd water	6μ

2. UGT-pET-21a vector construction

10X ligation buffer	4μ
Vector	5μ
Insert frangment	25μ
T4 DNA ligase	2μ

date: 8.2

1. UGT-pET-21a single restriction endonuclease digest and ligation

10X ligation buffer	5μ
Vector	20μ
Insert sequence	20μ
T4 DNA ligase	2μ
dd water	3μ
total	50μ

2. Colony PCR

supermix	25μ
F	2μ
R	2μ
Template	4μ
dd water	17μ
total	50μ

5 columns total. One of them is negative control

3.Colony PCR of the double restriction endonuclease digest and ligation bacteria

supermix	25μ
F	2μ
R	2μ
Template	4μ
dd water	17μ
total	50μ

date: 8.3

1. Transfer the plasmids (single and double) into *E.coli*
2. Extract plasmids from the double restriction endonuclease digest and ligation transferred E.coli.
3. Transfer the third branch of ligation product
4. medium PCR

supermix	5μ
F	0.4μ
R	0.4μ
Template	1μ
dd water	3.2μ

total	10μ

10 columns total

date: 8.5

1. Colony PCR of the UGT-transfered E.coli.

volume：20μ

2x super pcr mix	10μ
template	1μ
Primer1	0.8μ
Primer2	0.8μ
ddH2O	7.4μ

The template is diluted into 1/10 concentration

PCR program：

98°C	3min	
98°C	30s	35cycle
60°C	30s	35cycle
72°C	5s	35cycle
72°C	2min	
4°C	hold	

date: 8.7

1. BL21 colony PCR

supermix	5μ
F	1μ
R	1μ
Template	1μ
dd water	2μ
total	10μ

10 column total. No lanes.

date: 8.8

1. single and double restriction endonuclease digest

Quick cut buffer	3μ
UGT pcr product	25μ
NdeI	1μ
EcoRI	1μ
pET-21a plasmid	5μ
total	30μ

Quick cut buffer	3µ
UGT pcr product	25µ
EcoRI	2µ
pET-21a plasmid	5µ
total	30µ

date: 8.9

1. redo UGT PCR

MCLAB mix	20μ
F	4μ
R	4μ
Template	2μ
dd water	20μ
total	20μ

date: 8.10

1. restriction endonuclease digest and ligation then transfer into *E.coli*
2. pick 8 colonies then sequence
3. verify by PCR with redesign primers

super mix	5μ
F	1μ
R	1μ
Template	1μ
dd water	2μ
total	10μ

8 columns total

result: 3 of them are correct plasmid. Amplify them for plasmid extracting.

date: 8.11

1. sequence the plasmid by Qingke company

date: 8.12

1. sequence the plasmid by Sangon Biotech, get same result of Qingke (correct).

date: 8.13

1. BL21transfer

date: 8.14

1. BL21 transferred strain PCR

positive. Store the strain in glycerol in -80 °C

2. Amplify the strain in 250ml LB medium.

date: 8.16

1. Lyse the bacteria by ultrasonic

2. Run the SDS-PAGE electrophoresis and stained with coomassie brilliant blue. The control group protein is too much so can get any useful information.