2018.09.02 - 2018.09.09 WEEK 10

------ Chen Xirui, Liang Ruijuan, Ma Xinyue

2018.09.03

Plasmid construction

Re-synthesize the primer and anneal, using synthetic single-stranded fragment (forwards and rewards). Through annealing, these two single-stranded fragment will become double-stranded fragment with restriction sites.

Component	Volume or mass
T4 PNK	0.5 μL
10X T4 DNA ligase buffer	1 μL
Forward primer (100 μM)	1 μL
Reward primer (100 μM)	1 μL
ddH2O	to 10 μL

We use enzymes XbaI and SalI to cut the plasmid. To avoid self-connecting, we use a smaller system.

Component	Volume or mass
pExoS54F	200 ng
Sal I	1 μL
BamH I	1 μL
ddH2O	to 20 μL

Moreover, to ensure the enzymes work well, we set two single digestion groups as control. Set it into 37℃ for 90 min and then run an agarose gel.

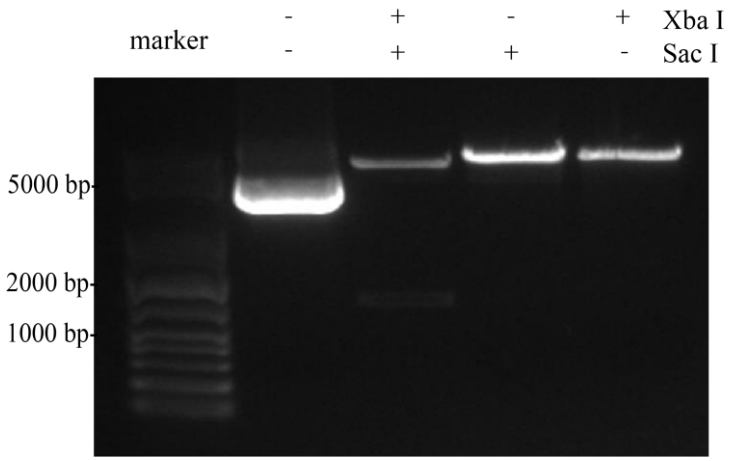

Fig.1 | Restriction Digestion

The result shows that the plasmid has been fully digested so that we continue.

Recover the digested plasmid from the gel. Digestion product and double-stranded fragment ligase.

Component	Volume or mass
T4 DNA Ligase	1 μL
10X T4 DNA ligase buffer	2 μL
Vector Plasmid	50 ng
Insert DNA (1:100 dilution)	5 μL
ddH_2O	to 20 μL

Hold for 2 hours at room temperature.

2018.09.04

Plasmid construction

Transfer the plasmid into the DH5-alpha E.coli. Add 50 μL E.coli to each ligase product.

step	time
Ice bath	30 min
42℃ activate	90 s
Ice bath	2 min
37℃ in shaker	1 h

Then, concentrate them and add 100 μL each to resuspend them and finally coat them in the solid medium with ampicillin. Set in 37℃.

2018.09.05

Plasmid construction

We found that there were several single colonies in the solid medium and we then picked up 6 colonies each in LB liquid medium containing ampicillin. Put in the 37℃ shaker for 6 hours.

Component	Volume
2× Taq Master Mix	5 μL
P1 primer	0.2 μL
Reverse primer (10 μM)	0.2 μL
template	1 μL
ddH_2O	to 20 μL

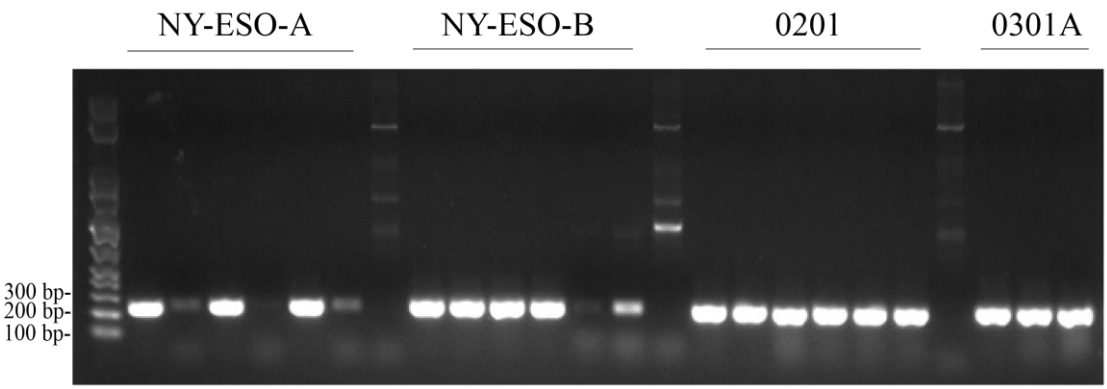

Fig.2 | Colony PCR (1)

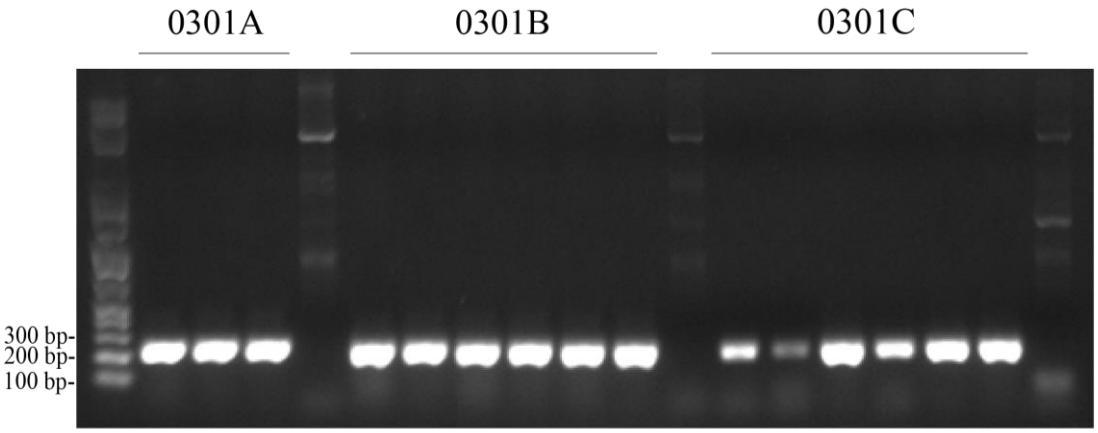

Fig.3 | Colony PCR (2)

From the results, we can primarily consider that we have successfully constructed the plasmids. Then, we sent three of each for sequencing and it is confirmed that we have got the right plasmids.

2018.09.07

Human Practice

We visited Shanghai Roche Ltd.

------ Zhang Yui, Song Zhiwei