2018.08.13 - 2018.08.19 WEEK 7

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2018.08.13

Plasmid construction

The result of the sequencing shows that the plasmids contains in the colonies is wrong, we indicate that maybe the restriction enzyme do not work efficiently so we change the restriction enzyme and digest the pExoS54F again. This time we choose the restriction enzyme of Xba I and Sac I, and we set the single digestion control with the plasmid control.

Component	Volume or mass
pExoS54F	500 ng
Sal I	1 μL
Xba I	1 μL
ddH_2O	to 20 μL

Hold at 37 ℃ for 90 min, then conduct DNA gel electrophoresis, the result is shown below.

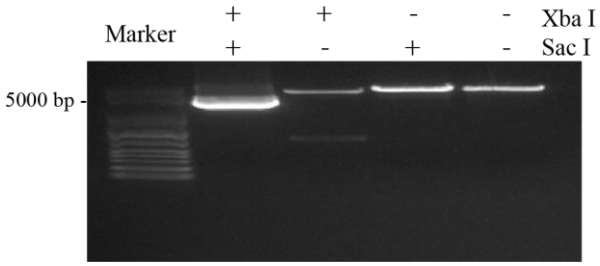

Fig.1 | Restriction Digestion

Through the result we can indicate that the restriction digestion is complete. But the single digestion of Xba I is incomplete. But we think that the double digestion is complete, so we continue the experiment.

Run the digestion product in the gel and recycle large fragments.

2018.08.14

Plasmid construction

Oligo annealing, 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
ddH_2O	to 10 μL

Digestion product and double-stranded fragment ligase.

Component	Volume or mass
T4 DNA Ligase	0.5 μL
10X T4 DNA ligase buffer	1 μL
Vector Plasmid	50 ng
Insert DNA (1:100 dilution)	1 μL
ddH_2O	to 10 μL

Chemical transformation and cover the transformation product onto the ampicillin-resistant LB agar plate.

2018.08.15

Plasmid construction

Pick out colonies (each antigen two colonies) and use the colony PCR to test whether the colony contains the right plasmid.

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

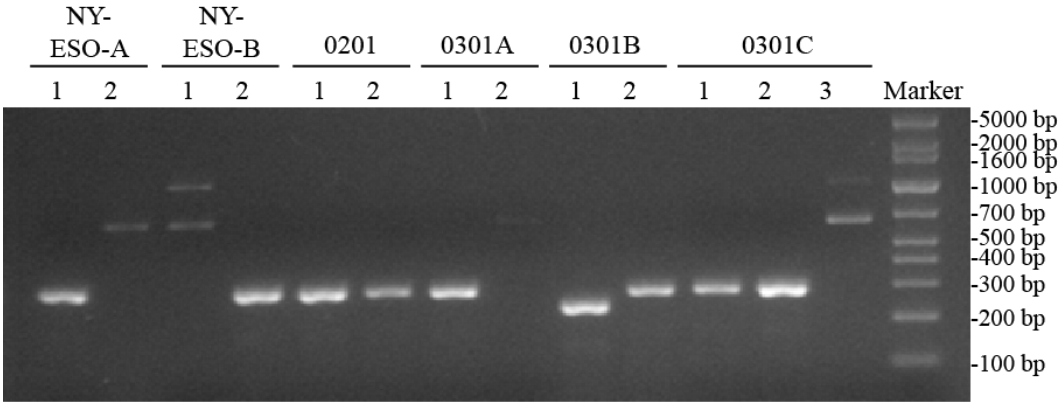

Fig.2 | Colony PCR

2018.08.16

Plasmid construction

The result shows that the plasmids are wrong again, because the bands should be between the 100 bp to 200 bp. We think that maybe we do not pick up the right colonies, so we pick up some colonies form the plate again and conduct secondry colony PCR. (Each antigen three colonies)

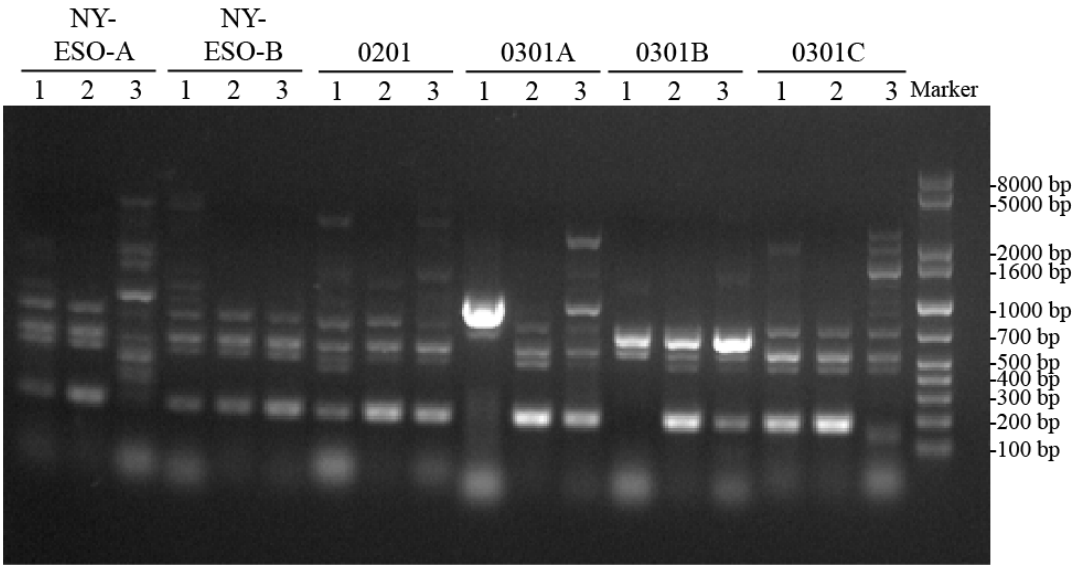

Fig.3 | Secondary Colony PCR

The result shows that the plasmids are wrong. We indicate that maybe the plasmid's digestion is still incomplete, so we conduct the whole process of plasmid construction again.

2018.08.18

Plasmid construction

Restriction digestion of enzymes Xba I and Sac I. We set the single digestion control and the plasmid control. This time we extend the digestion time for 2 hours.

Component	Volume or mass
pExoS54F	500 ng
Sal I	1 μL
Xba I	1 μL
ddH_2O	to 20 μL

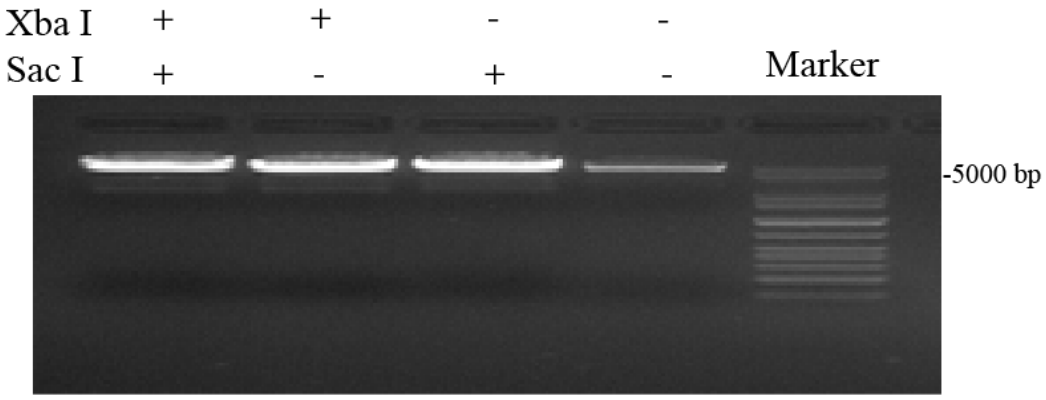

Fig.4 | Secondary Restriction Digestion

The image shows that the plasmid is digested completely both single and double, so we decide to conduct the ligation again the next week.