2018.08.20 - 2018.08.26 WEEK 8

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2018.08.20

Plasmid construction

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

This time we extend the ligation time and hold at room temperature for 4 hours.

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

2018.08.21

Plasmid construction

Pick out colonies (each antigen four 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

Conduct DNA gel electrophoresis, the result is shown below.

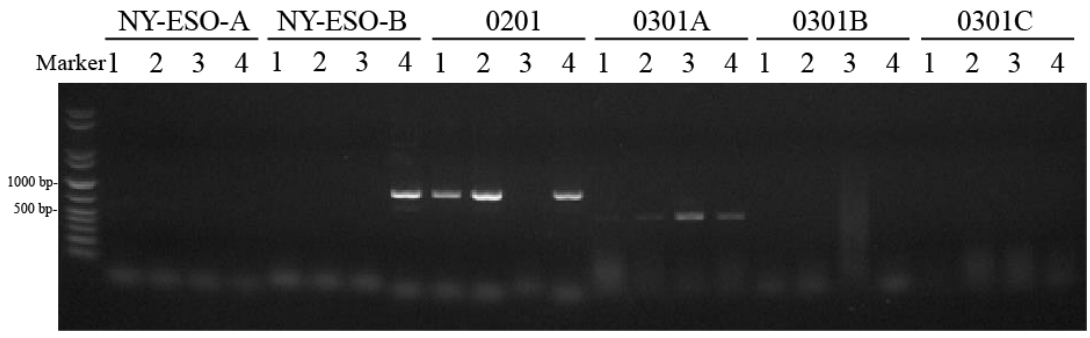

Fig.1 | Colony PCR

The results are still wrong, so we reply the process from ligation to transformation.
Digestion product and double-stranded fragment ligase. This time we double the amount of insert DNA.

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)	2 μL
ddH_2O	to 10 μL

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

2018.08.22

Plasmid construction

Pick out 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

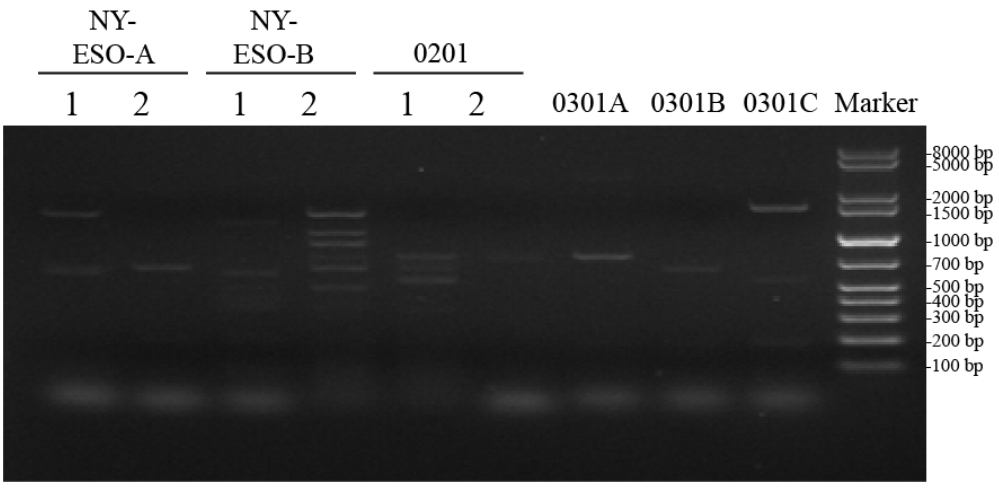

Fig.2 | Colony PCR

The results are wrong again.

2018.08.24

Plasmid construction

Restriction digestion of enzymes Xba I and Sac I. We set the single digestion control and the plasmid control. The digestion time is 2 hours.

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

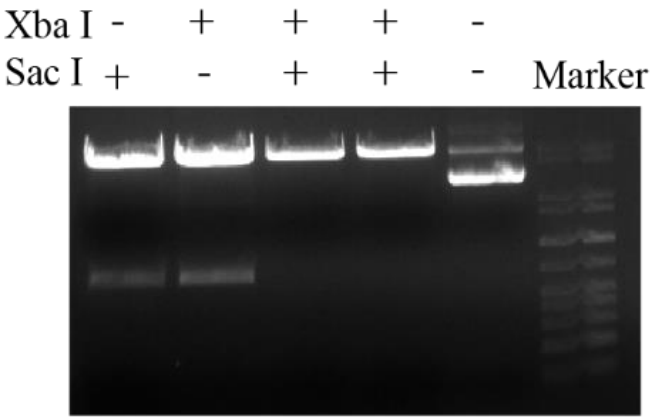

Fig.3 | Restriction Digestion

The image shows that the plasmid is digested completely both single and double.

2018.08.25

Plasmid construction

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

Hold at room temperature for 4 hours.

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

2018.08.26

Plasmid construction

Pick out colonies (each antigen four 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

Conduct DNA gel electrophoresis, the result is shown below.

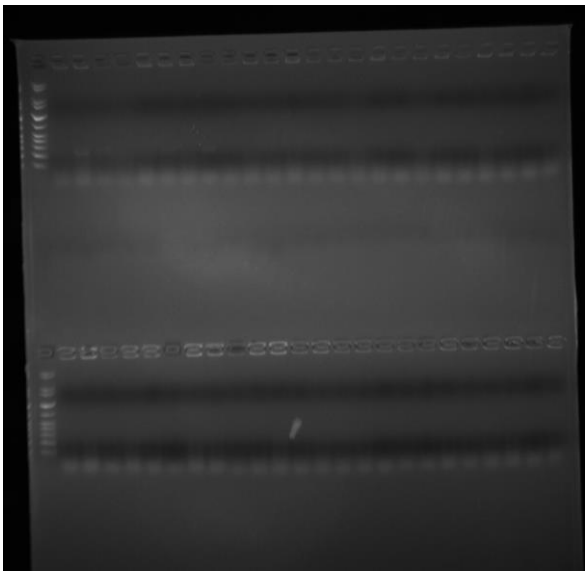

Fig.4 | Colony PCR

The results are still wrong.