2018.09.10 - 2018.09.16 WEEK 11

------ Ma Ningjia，Song Zhiwei

2018.09.10

Pseudomonas aeruginosa

We used electroporation to introduce the recombinant plasmids into the attenuated Pseudomonas aeruginosa. The transformed strains was PAK-JΔ9, and the plasmids to be transformed were pExoS54-NYA, pExoS54-NYB, pExoS54-0201, pExoS54-0301A, pExoS54-0301B, pExoS54-0301C.
In the evening, we melt 20 μL of PAK-JΔ9 Pseudomonas aeruginosa on the ice. Transfer to 2 mL of liquid LB medium. Inoculate overnight at 37°C shaker at 200 rpm.

2018.09.11

Pseudomonas aeruginosa

Before the electroporation, we prepared competent bacteria.
Add 100 ng of plasmid to 100 μL of competent cells, mix gently, and then pulse the competent cells with a preset electro-rotation procedure (2.5 kV).
Incubate for 12 hours at 37°C.

Human Practice

We visited doctors at Lung Hospital affiliated to Tongji University.

------ Chen Xirui, Sun Qi, Zhao Anqi

2018.09.12

Pseudomonas aeruginosa

Pick a single colony and incubate overnight in a liquid LB medium containing Carbenicillin.

2018.09.13

Pseudomonas aeruginosa

Colony PCR identification following the instruction in the table above.

Component	**Volume**
Forward primer	0.5 μL
Reverse primer（2 μM）	2.5 μL
2×PCR mix (Taq)	10 μL
Template	1 μL
ddH_2O	6 μL

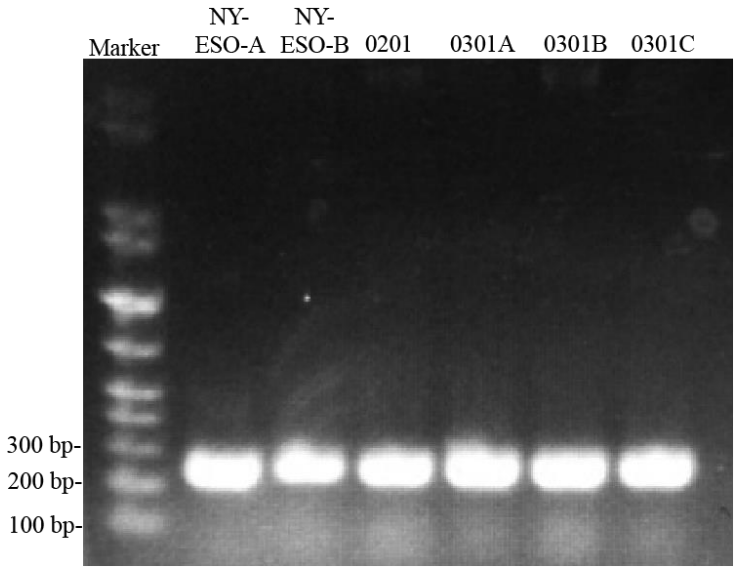

Fig.1 | Colony PCR of P.A.

From the electrophoresis results of colony PCR, we can see that the band of our target DNA fragment is in the corresponding position. Therefore, it is indicated that our recombinant plasmids have been successfully transferred into P. aeruginosa.

Plate 100 μL of the cells we picked up yesterday onto LB agar plates with Carbenicillin. Incubate overnight in a liquid LB medium containing Carbenicillin

2018.09.14

Pseudomonas aeruginosa

Creating bacterial glycerol stocks. Freeze the glycerol stock tube at -80°C.

2018.09.15

Pseudomonas aeruginosa

To confirm our procedure is right, we melted Pseudomonas aeruginosa from -80°C and transferred bacteria to LB medium. Inoculate overnight at 37°C shaker at 200 rpm.

2018.09.16

Pseudomonas aeruginosa

Colony PCR identification as what we did on 13th this weekend. And finally, we believe that our procedure is right.