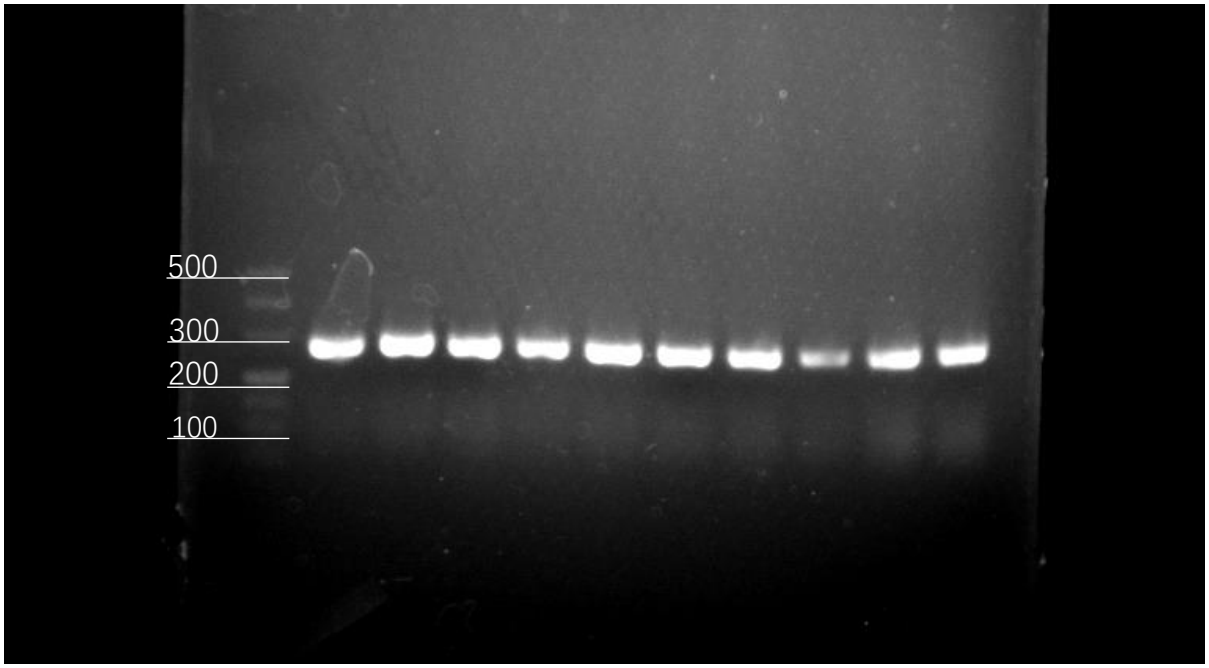
Lab Notes-Application Group

- **2017.7.29**

1.PCR-Spytag (225bp)



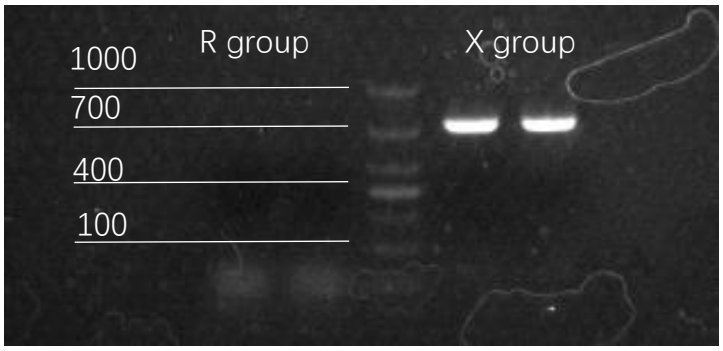
2.Pure the PCR product

Spytag1 OD=187.7ng/μl A260/A280=1.87

Spytag2 OD=315.8ng/μl A260/A280=1.83

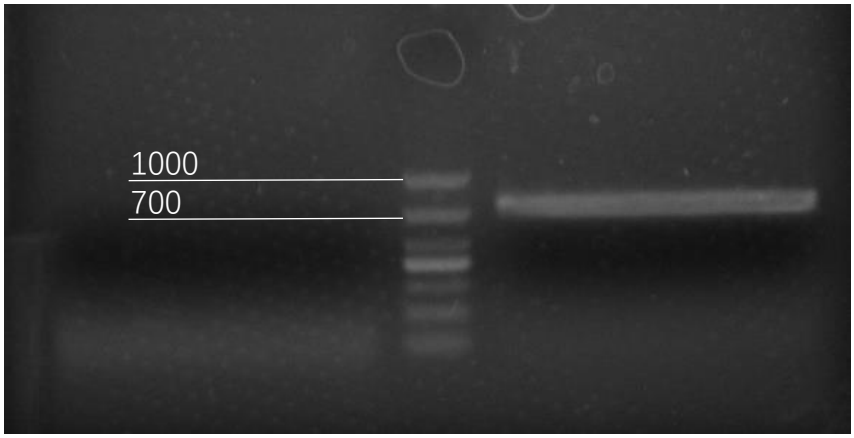
- **2017.7.31**

1.PCR-GFP Verify the primer (GFP 714bp)



Result: primer “R” is wrong; primer ”X” is unfavorable

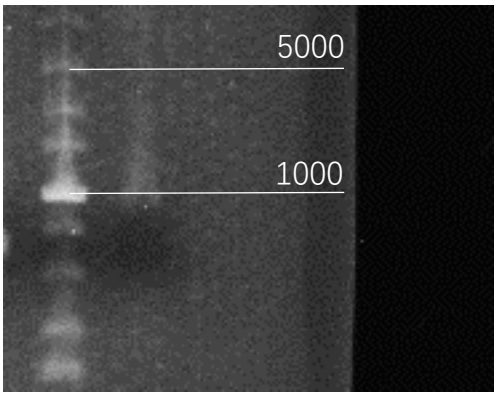
2.Pure the PCR product



GFP-PCR OD=105.7ng/μl A260/A280=1.10

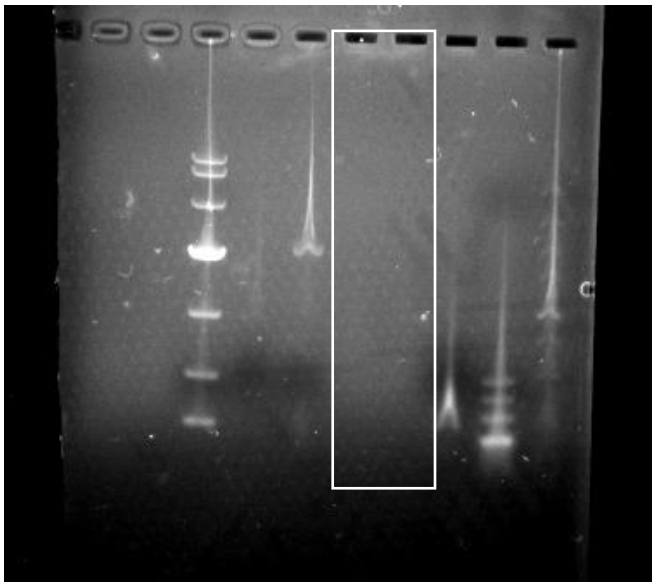
- **2017.8.1**

1.Overlap PCR, form "Spytag-GFP"



2.Pure the PCR product

Spytag-GFP OD=115.0ng/μl A260/A280=1.17



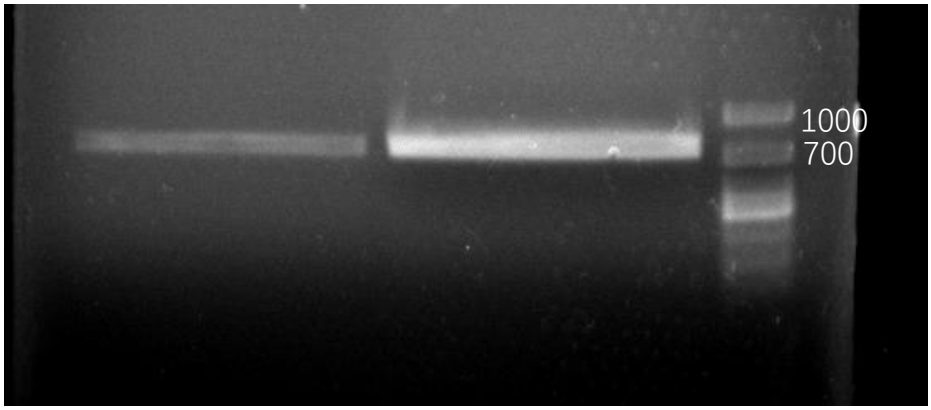
Result: the product is negative, redo those work.

1. **Prepare LB 400ml**

2. **Incubate the *E.coli* containing pET28a**

- **2017.8.2**

1. **PCR-GFP**



2.Pure the PCR product

GFP-PCR OD=27.8ng/μl A260/A280=2.46

3.Extract plasmid pET28a

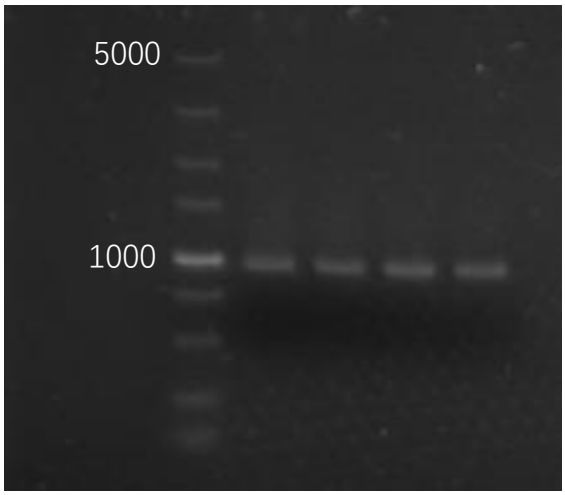
pET28a 1 OD=119.1ng/μl A260/A280=2.01

pET28a 2 OD=94.1ng/μl A260/A280=2.38

pET28a 3 OD=141.0ng/μl A260/A280=2.31

● 2017.8.3

1.Overlap PCR, form "Spytag-GFP"



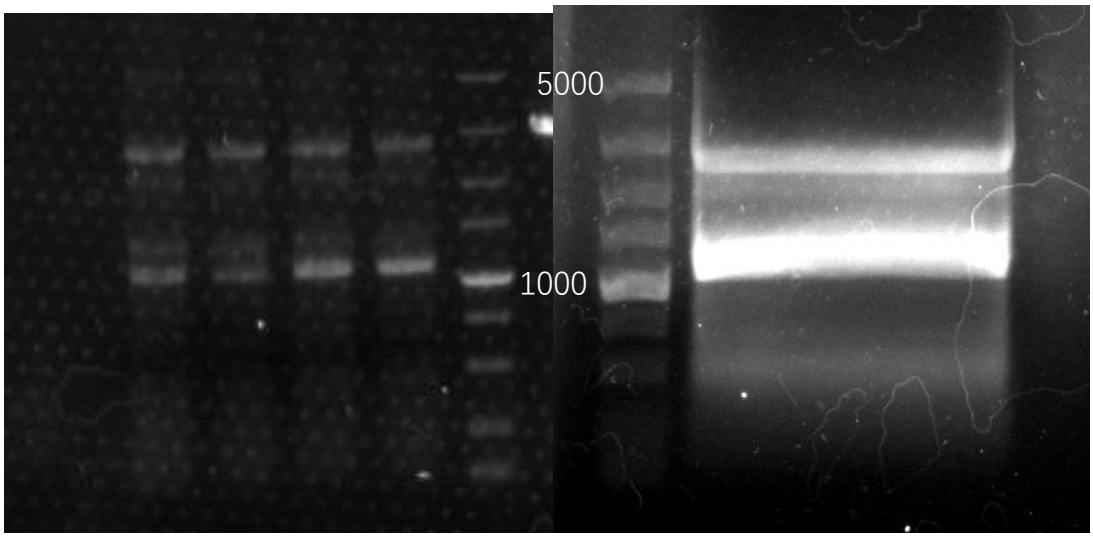
2.Pure the PCR product

Spytag-GFP1 OD=149.9ng/μl A260/A280=1.83

Spytag-GFP2 OD=151.7ng/μl A260/A280=1.86

● 2017.8.6

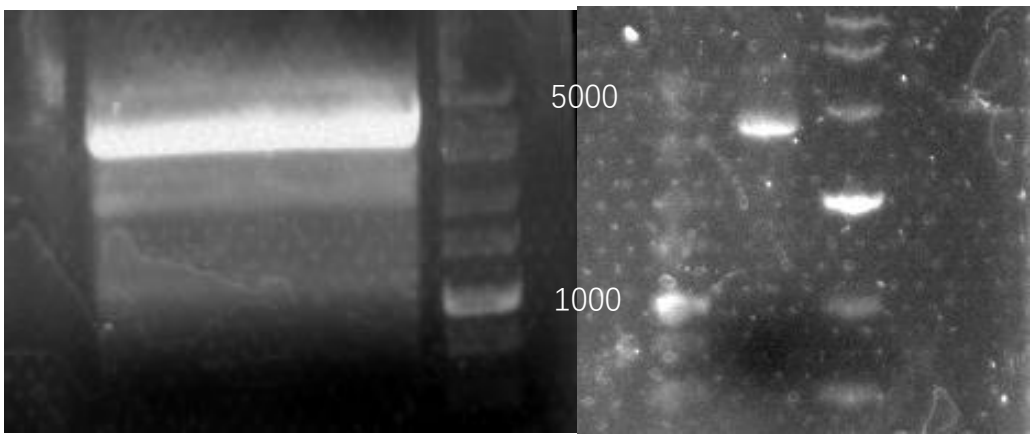
1. PCR to receive linear pET28a(5014bp)



Result: slight band. Failed, check the primer and PCR condition and find the primer has mistake.

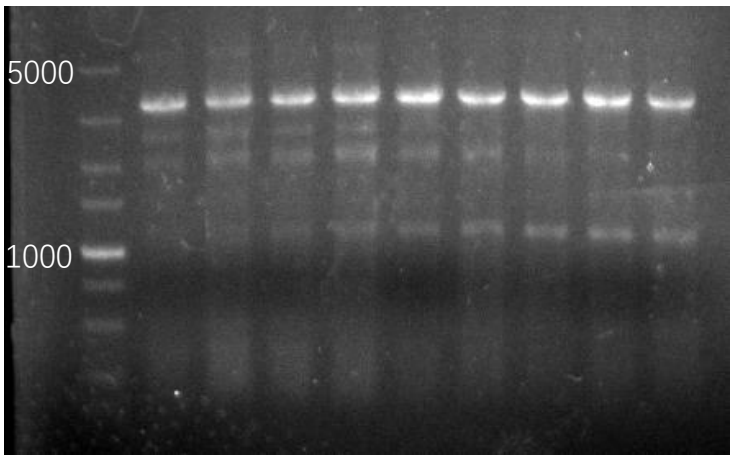
- **2017.8.10**

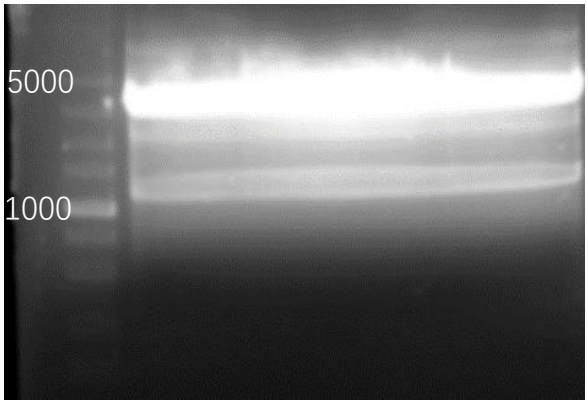
1. PCR to receive linear pET28a(5014bp)



Result: negative result, check the PCR condition

2. pET28a(5014bp) gradient PCR





Result: negative result, the plasmid is too long and addition primer should be inserted to the system.

- **2017.8.13**

19:20

1.Activating the *G.xylinus* culture media with 4% cellulase.

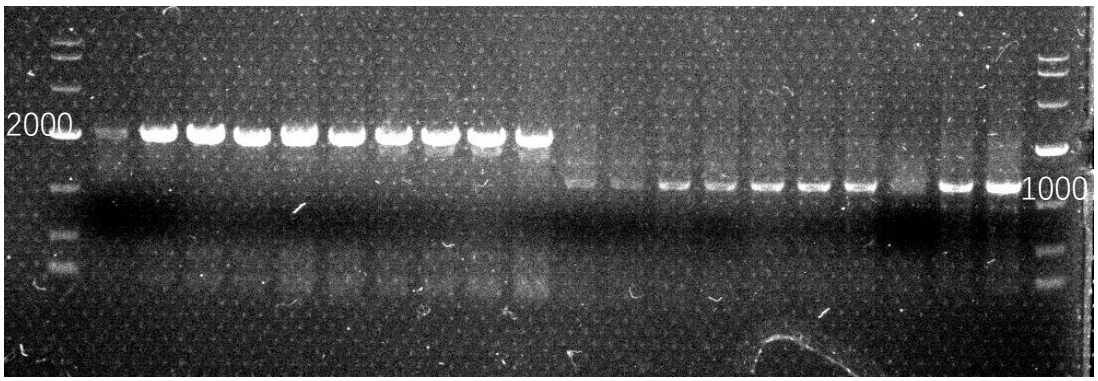
2.Inoculate the *G.xylinus* (4% of the total culture volume) into culture media.

- **2017.8.14**

1. **Inoculate the *G.xylinus* (OD=1.3) into facial mask mould with culture media, and culture for 5 days**

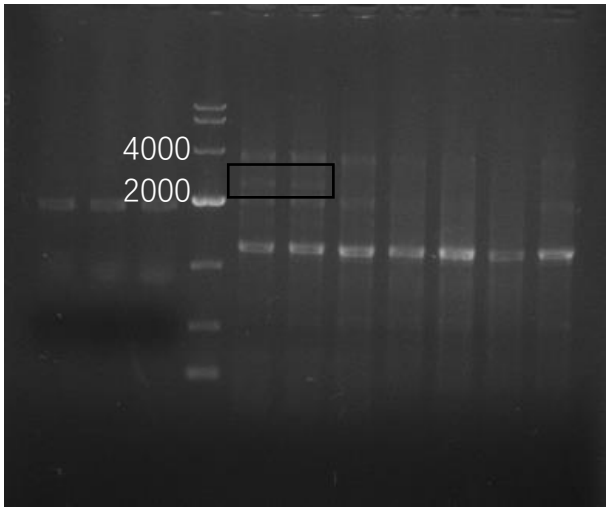
- ## 2017.8.15

1.pET28a divided into two parts to gradient PCR(2102bp,2996bp)



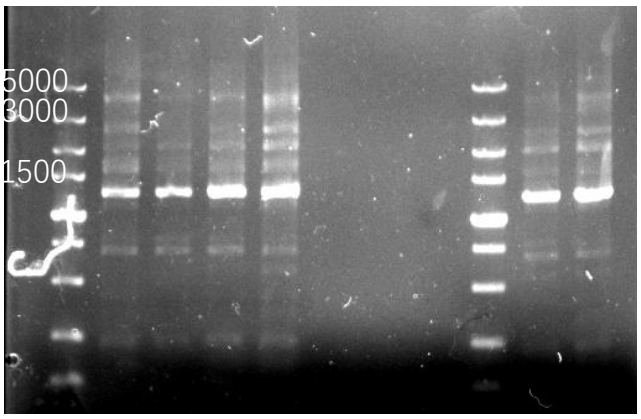
Result: 2996bp band is wrong, redo those work.

2. pET28a(2996bp) gradient PCR

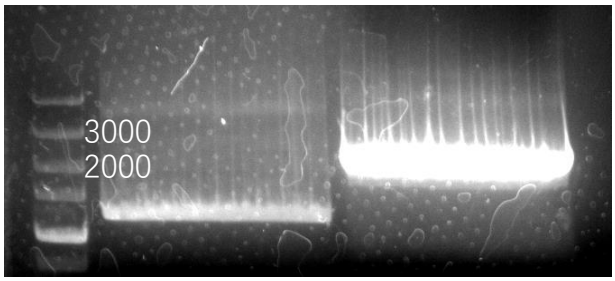


- ## 2017.8.16

1.pET28a(2996bp) gradient PCR



2. Pure the PCR product



pET28a(2996bp) OD=35.9ng/μl A260/A280=1.11

pET28a(2102bp) OD=185.4ng/μl A260/A280=1.54

3.Enzyme linked two fragments of plasmid

4.Prepare LB 200ml

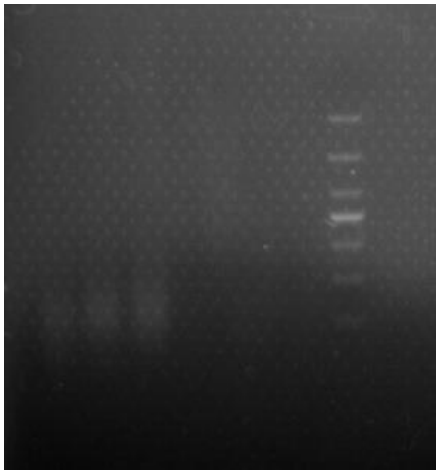
- **2017.8.17**

1. Prepare six culture dish (Kan)

2. Transformation

- **2017.8.18**

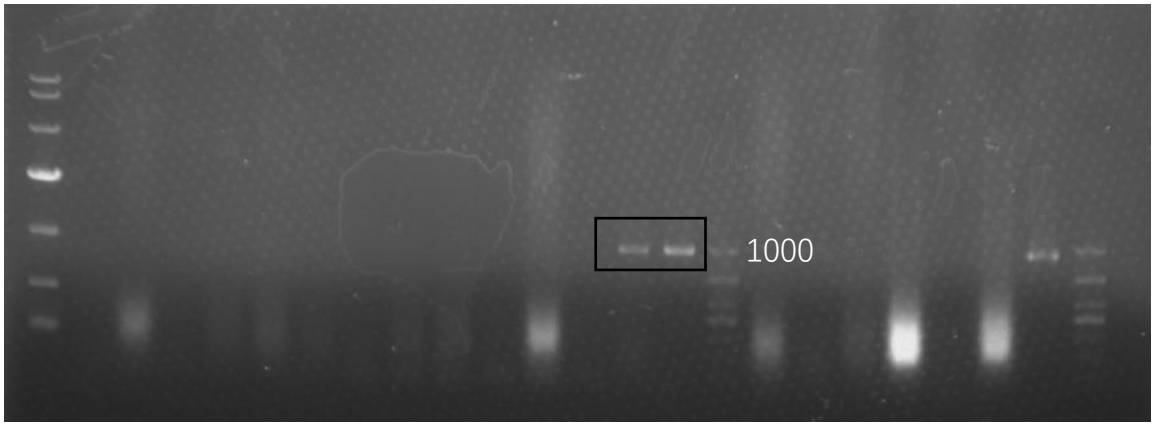
1. Colony PCR



Result: negative result, redo those work.

- **2017.8.19**

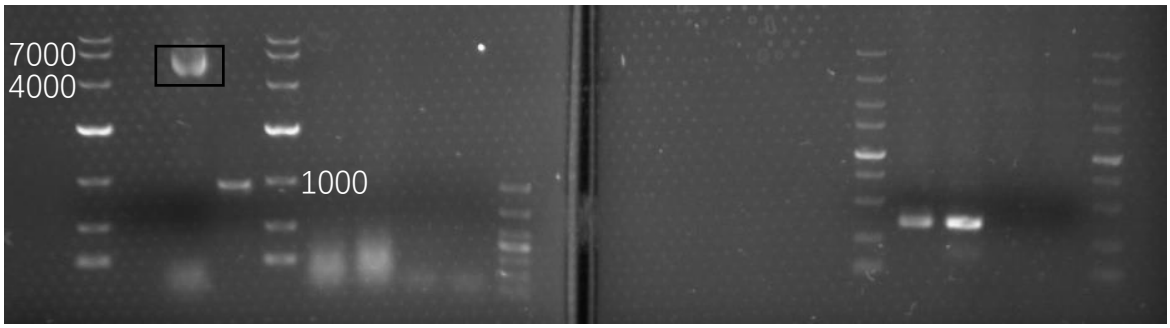
1. Colony PCR



- **2017.8.20**

1.Extract plasmid pET28a-Spy Tag-GFP (6000bp)

pET28a-Spytag-GFP OD=625.9ng/μl A260/A280=1.25

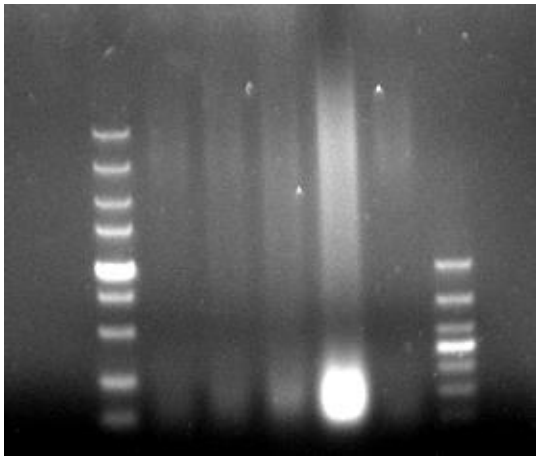


- **2017.9.5**

1.Transformation

- **2017.9.6**

1. Colony PCR pET28a-Spytag-GFP (B21 bacterium)



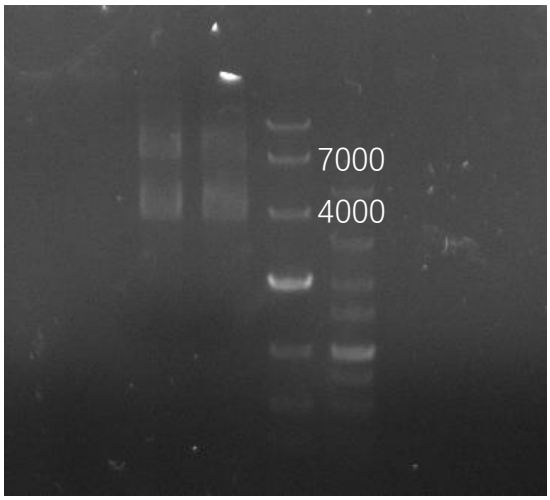
Result: negative result, plasmid validation.

- **2017.9.7**

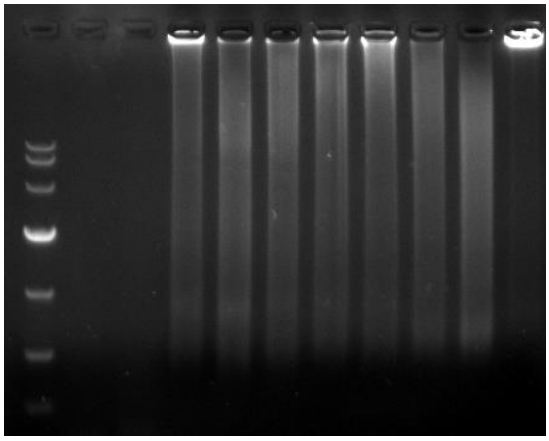
1. Extract plasmid pET28a (BL21)

28a-Spytag-GFP1 OD=94.7ng/μl A260/A280=1.70

28a-Spytag-GFP2 OD=67.8ng/μl A260/A280=1.85



2.PCR-Spytag (225bp)



Result: negative result.

- **2017.9.9**

1. Save BL21 in Glycerol Tube

- **2017.10.11**

1. **Prepare LB 750ml**

- **2017.10.13**

1. **Vaccinate colony PCR pET28a-Spytag-GFP in Triangle bottle**

- 2017.10.14

1. Activation the strain
2. Prepare LB 500ml
2. Inoculation culture 500ml

- 2017.10.15

1. Centrifugal bacteria cell and save in -80°C
2. Prepare PBS buffer 1L

- 2017.10.16

1. Cell disruption

- 2017.10.21

1. Soak processed BC membrane in protein solution

- 2017.10.25

1. Keep membrane in -20°C

- 2017.10.26

1. Dry BC membrane with freeze dryer

- 2017.10.30

1. Observe with transmission electron microscope