E-PN Production Protocol

Materials

Genetic Materials

- E.coli ΔfimA
- Plasmid GspilA-T4PAS-p24Ptac

Hard Materials

- 10 cm diameter culture plates
- standard LB medium (Agar)
- M9 medium (Agar)
- glycerol
- IPTG
- Chloramphenicol
- Waring blender
- ethanolamine buffer
- Triton X-100 detergent
- Omega membrane 100 K 76 mm, Pall Corporation (a stirring filtration unit that had a 100 kDa molecular weight cut o! membrane filter made from poly(ether sulfone))
- nitrogen gas (69 kPa)

Preparation of M9 medium (Agar)

Materials

- 5X M9 salts
- Agar (16g/L)
- 1mM thiamine hydrochloride
- 0.4% glucose
- 0.2% casamino acids
- 2mM MgSO4
- 0.1mM CaCl2
- 0.5% glycerol
- 0.5mM IPTG
- Chloramphenicol(CmR)

1. Prepare for the solutions

	A	B
1	Type of Substanc	Volume/Mass
2	M9 Salts	11.3g
3	Agar	16g
4	ddH20	872.4ml

2. **Setrilization under 121 degree celcius for 15 mintues**

3. After the sterilization, cool down the solution to about 55 degrees Celsius, and add the following items

	A	B
1	Type of substanc	volume
2	10 mg/mL thiam	34mL
3	20% glucose	20mL
4	10% Casamino a	20mL
5	1M MgSO4	2mL
6	1M CaCl2	100µL
7	10% glycerol	50mL
8	1M IPTG	500 µL
9	CmR	340µL

Procedure

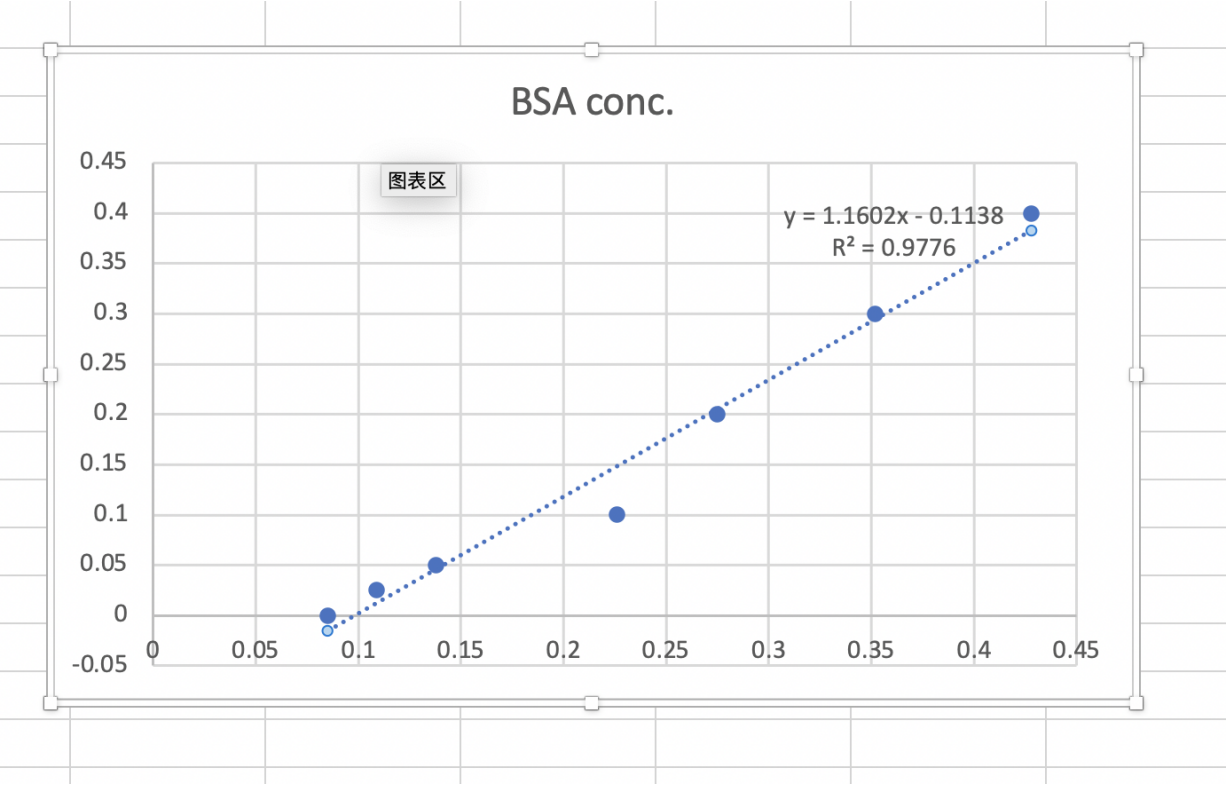
1. CRITICAL Prepare the competent cell of *E.coli fimA.* Then transform the GspilA-T4PAS-p24Ptac into *E.coli fimA.* The designated name is BIOT-CmR.
2. Culture BIOT-CmR on 10 cm diameter culture plates of standard LB medium amended with chloramphenicol and solidified with agar.
3. After overnight growth at 30 °C, cells were scraped from the surface and suspended in 6 mL of M9 medium.
4. Twenty plates of M9 medium supplemented with 0.5% glycerol, 0.5 mM IPTG, and chloramphenicol were spread-plated with 300µL of the suspended cells.
5. The plates were incubated at 30 °C for 48 h.
6. The cells were harvested from the plates with 1 mL of M9 medium (500 µL to scrape, 500 µL to wash) for each plate.

7. The 20 mL suspension of cell scrapings was centrifuged at 4000 rpm for 15 min at 4 °C to pellet the cells.
8. The supernatant was discarded, and the cells were resuspended in 30 mL of 150 mM ethanolamine buffer (pH 10.5) and poured into a Waring blender.
9. The tubes were washed three times with 20 mL of the ethanolamine buffer, which was also added to the blender.
10. The 90 mL suspension was blended for 2 min on low speed to shear the e-PNs from the cells.
11. The contents of the blender were transferred to a centrifuge bottle along with a wash of the blender with 10 mL of ethanolamine bu!er.
12. The blended material was centrifuged at 5000g for 30 min at 4 °C to remove the cells. The supernatant containing the e-PNs was collected.
13. Triton X-100 detergent was added at a final concentration of 6 mM to solubilize any remaining cellular debris
14. The mixture was shaken at 100 rpm at room temperature for 45 min and then added to a stirring filtration unit that had a 100 kDa molecular weight cut off membrane filter made from poly(ether sulfone) (Omega membrane 100 K 76 mm, Pall Corporation) to collect the e-PNs on the filter.
15. Additional ethanolamine buffer was added to dilute the sample to yield a final Triton X-100 concentration of 2 mM.
16. The sample was filtered under nitrogen gas (69 kPa).
17. The sample on the filter was washed four times with 100 mL of water.
18. The e-PNs were collected from the filter by scraping the surface into 500 µL of water.
19. The scraping procedure was repeated two more times to yield a suspension of e-PNs in 1.5 mL of water.

Results

We used BIOT-CmR to produce E-PNs.

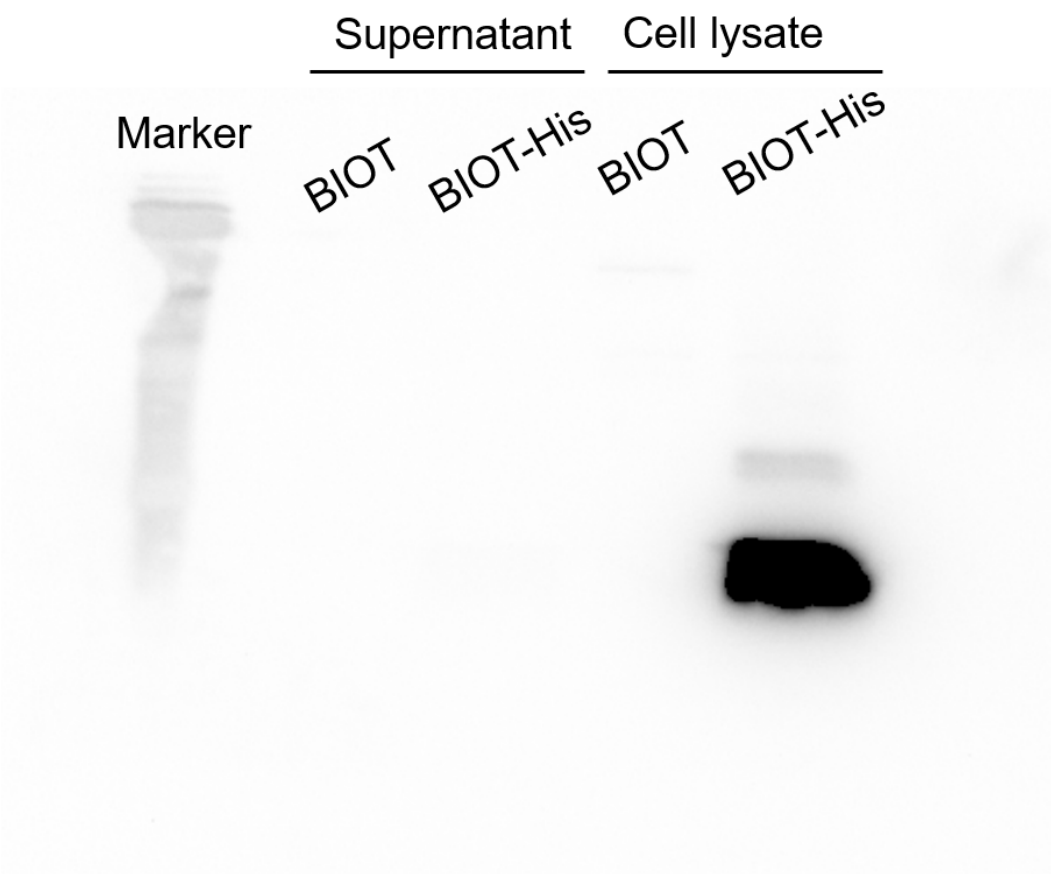
	A	B	C
1	BSA conc.	flu.	BIOT.flu.
2	0	0.085	0.435
3	0.025	0.109	
4	0.05	0.138	
5	0.1	0.226	
6	0.2	0.275	
7	0.3	0.352	
8	0.4	0.428	
9	0.5	Overflow	



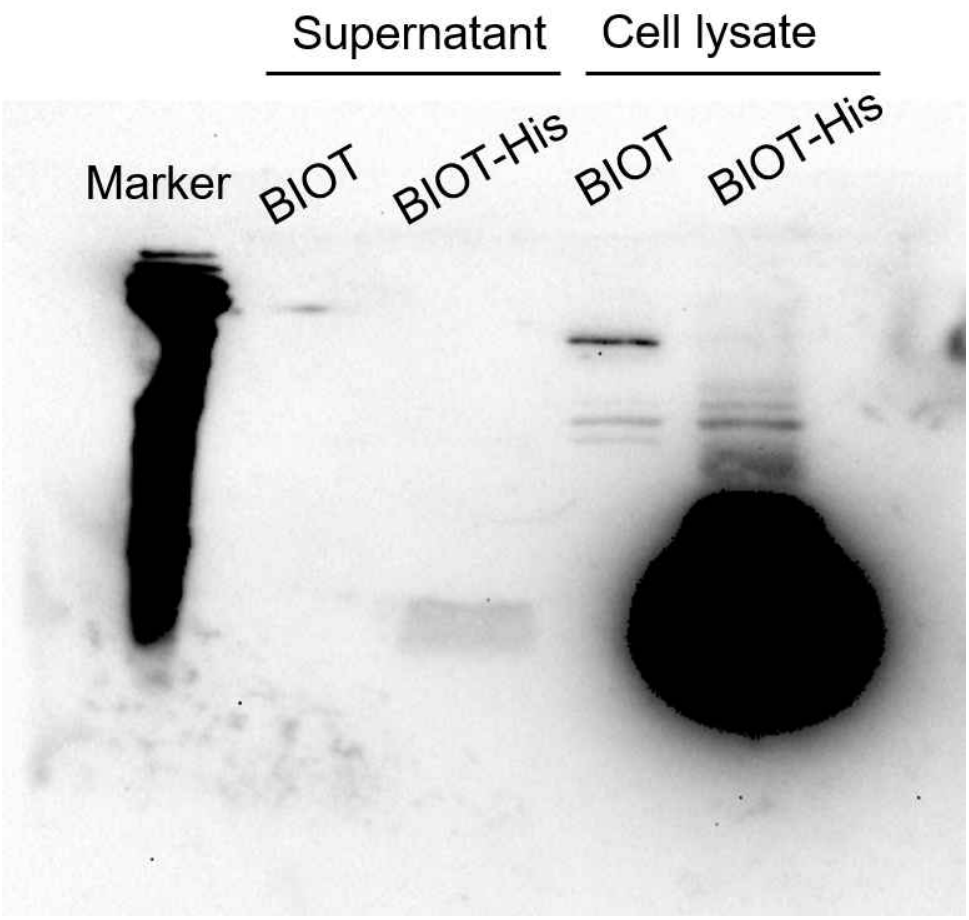
From the determination of BSA experienment, we could infer that the total protein we gained was 1ml. After caculations, we could infer that he final concentration was 0.4mg/ml, which was 400ng/ul.

Detect protein expression by Western Blot

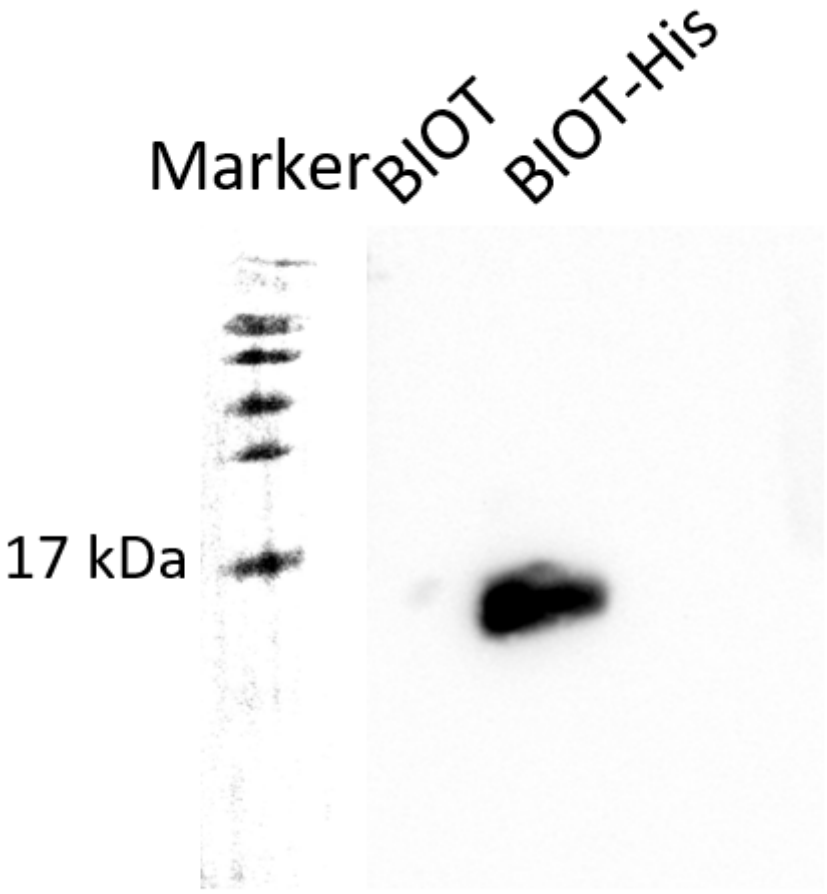
Pre-test results



Sample loading sequence:
BIOT-supernatant, BIOT-His-supernatant, BIOT-lysate, BIOT-His-lysate
Antibody: Anti-His-tag；exposure: 10s



Sample loading sequence:
BIOT-supernatant, BIOT-His-supernatant, BIOT-lysate, BIOT-His-lysate
Antibody: Anti-His-tag; exposure: 300s.



> Western Blot of the cell lysis of BIOT and BIOT-His(express e-PN and e-PN-His respectively); Antibody: Anti-His tag; e-PN-His: 7.7 kDa; Exposure: 2s.

It can be seen that the bottom band is the characteristic BIOT-His band, which has a very high concentration in the cell lysate and a low concentration in the supernatant.