Notebook of protein purification

Team: GreatBay_SCIE 2019

Record date: from 7.12-8.31

Expression and detection of S009, S012, S013 and S002

OD600 measurement (operator: Emma)

Strain	S002,S009, S012, S013 BL21(DE3){pET28b-CsgA-linker-Mfp5-Mfp5-7xHis}
Culturing time	16h
Dilution factor	/
Volume of sample	200uL
Average OD600nm	S002: 0.763 S009(1)(500uM, not50uM): 0.527 S012(1): 0.497 S012(2): 0.379 S013(1): 0.355 S013(2): 0.270

Preparing protein samples for PAGE (operator: Emma)

Strain	S012(1) induced
Expressed protein	pET28b-csgA-linker-mfp5-7*his
Cell pellet	2ml OD=0.68
1xsample buffer	80µl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

Strain	S012(2)
Expressed protein	pET28b-csgA-linker-mfp5-7*his
Cell pellet	2ml OD=0.47
1xsample buffer	80µl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

Strain	S013(not induced)
Expressed protein	pET28b-csgA-linker-mfp5-mfp5-7*his
Cell pellet	1ml OD=0.32
1xsample buffer	80µl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

Strain	S013(1)induced
Expressed protein	pET28b
Cell pellet	2ml OD=0.40
1xsample buffer	80μl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

Strain	S013(2)induced
Expressed protein	pET28b
Cell pellet	2ml OD=0.41
1xsample buffer	80μl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

Strain	S002(induced)
Expressed protein	pET28b
Cell pellet	1ml OD=0.94
1xsample buffer	80μl
Heating temperature	50°C
Heating time	30min
Storage location	4°C

Strain	S009(not induced)

Expressed protein	pET28b-csgA-linker-mfp3-7*his
Cell pellet	1ml OD=0.76
1xsample buffer	50μl
Heating temperature	50°C
Heating time	30min
Storage location	4°C

Strain	S009(1)(induced)
Expressed protein	pET28b-csgA-linker-mfp3-7*his
Cell pellet	2ml OD=0.35
1xsample buffer	80μl
Heating temperature	50°C
Heating time	30min
Storage location	4°C

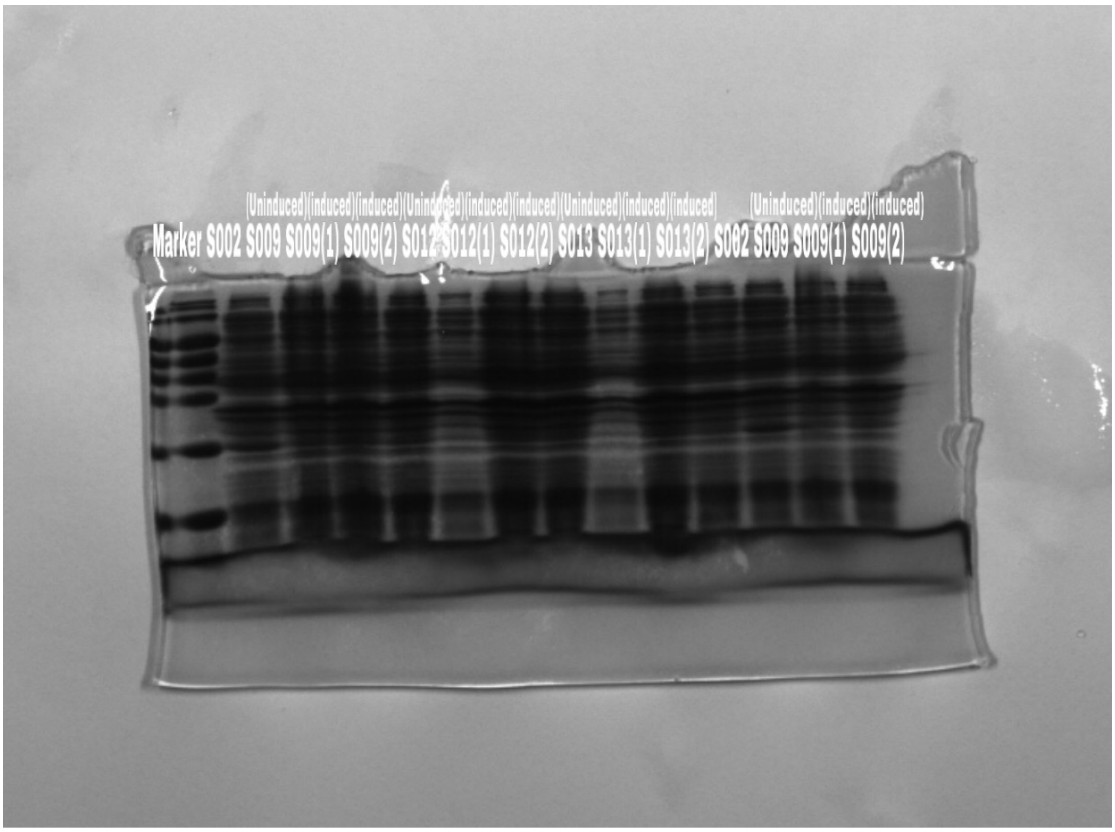
Strain	S012(not induced)(backup)
Expressed protein	pET28b-csgA-linker-mfp5-7*his
Cell pellet	1ml OD=0.44
1xsample buffer	50μl
Heating temperature	50°C
Heating time	30min
Storage location	4°C

Strain	S012(1)
Expressed protein	pET28b-csgA-linker-mfp5-7*his

Cell pellet	2ml OD=0.68
1xsample buffer	80µl
Heating temperature	50°C
Heating time	30min
Storage location	4°C

PAGE (operator: Emma)

Volume of samples	5 ul
Volume of marker	5ul
Voltage for stacking gel	100V
Voltage for resolving gel	120V
Total time length	1h20min
Staining time	30min



7.15(Mon)

Purification of S009 S012 S013（Mini prep)

Samples from Cell pellets prepared in 0711 (25ml culture, incubating conditions: 500um IPTG 37 4h)

Purification:

1. Add 500u lNondenatured lysate to 25ml（approximately 0.16g）cell pellet
2. slight vertex，add lysozyme
3. bath for30min
4. stall in -80°C Freezing and thawing for twice to help lysis
5. centrifuge（14800g，10min）
6. add 100ul resin to remaining supernanant
7. centrifuge (1000g 30s)
8. Take 20ul sample (label as 009-1，012-1，013-1)
9. Add 40ul Non-denaturing detergent into 20ul samples
10. Centrifuge (1000g 30min)
11. Take 20ul sample（label as 009-2，012-2，013-2)
12. Repeat step 9&10
13. Take 20ul sample (label as 009-3，012-3，013-3)
14. Add 20ul non-denatured elution buffer
15. suspense the gel
16. Centrifuge (1000g 30s)
17. Collect sample, each for 20ul（label as 009-E1,012-E1,013-E1)
18. Repeat step14-17 twice, take 20ul sample for each (label as009-E2,012-E2, 013-E3 and 009-E3,012-E3,013-E3)

Sample order of page:

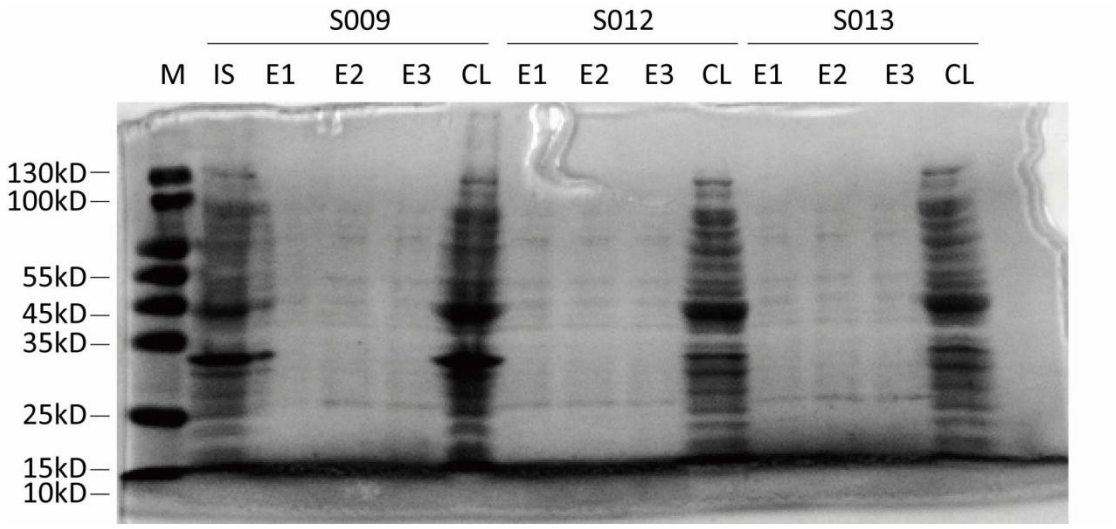
M IS009 009E1 009E2 009E3 CLS009 IS012 012E1 012E2 012E3 CLS012 013E1 013E2 013E3 CLS013

E1：solution obtained after the first elution of protein purification

E2：solution obtained after the second elution of protein purification

E3：solution obtained after the third elution of protein purification

IS：cell debris



7.16(Tue)

Large scale protein expression and purification of S012 and S013(Start)

Inoculation (operator: Emma Aislinn)

From: Liquid starter

Strain	S012
Antibiotics	Kan
Medium	LB
Volume	50ml in 250ml flasks
Dilution factor	1:200
Temperature	37
Number	9(no S012(8))

Strain	S013
Antibiotics	Kan
Medium	LB
Volume	50ml in 250ml flasks
Dilution factor	1:200

Temperature	37
Number	9(no S013(9))

OD600 measurement (operator: Emma Aislinn)

Strain	S012
Culturing time	3h
Dilution factor	
Volume of sample	200ul
Average OD600nm	0.257
Temperature	37

Strain	S013
Culturing time	3h
Dilution factor	
Volume of sample	200ul
Average OD600nm	0.261
Temperature	37

Induction (operator: Emma Aislinn Amy)

Strain	S012(1/2/3/4/5)
Inducer	50μL 500uM IPTG
Induction time	19h
Temperature	25

Strain	S012(6/7/9/10)
Inducer	50μL 0.5M IPTG

Induction time	19h
Temperature	25

Strain	S013(1/2/3/4/5)
Inducer	50μL 500uM IPTG
Induction time	19h
Temperature	25

Strain	S013(6/7/8/10)
Inducer	50μL 0.5M IPTG
Induction time	19h
Temperature	25

7.17(Wed)

Large scale protein expression and purification of S012 and S013

OD600 measurement (operator: Emma Aislinn)

Strain	S012
Culturing time	19h
Dilution factor	
Volume of sample	200ul
Average OD600nm	0.555
Temperature	25

(S012(7) was discarded because of extremely low concentration.)

Strain	S013

Culturing time	19h
Dilution factor	
Volume of sample	200ul
Average OD600nm	0.654
Temperature	25

Preparing cell pellet (operator: Emma Aislinn Amy)

Strain	S012(50uM)
Induction time length	19h
Centrifuge rate	5000g
Time	10min*5
Volume	50ml*5
Weight	1.2g
Storage location	-80
Strain	S012(500uM)
Induction time length	19h
Centrifuge rate	5000g
Time	10min*4
Volume	50ml*4
Weight	g
Storage location	-80
Strain	S013(50uM)
Induction time length	19h
Centrifuge rate	5000g
Time	10min*5
Volume	50ml*5
Weight	g
Storage location	-80
Strain	S013(500uM)
Induction time length	19h
Centrifuge rate	5000g
Time	10min*4
Volume	50ml*4
Weight	g
Storage location	-80

Protein Purification

1. Add 8ml Nondenatured lysate to each cell pellet. (total: 4 tubes)
2. Add 80ul to each cell pellet. (Total: 4 tubes)
3. Ice bathing for 1h
4. -80 Celsius 15min -> room temperature 40min (repeat for 4 times)
5. centrifuge (10000g30min)
6. take 20ul bacterial lysis supernatant into1.5ml centrifuge tube
7. take 4 tubes of 1ml 50%Resin
8. centrifuge（1000g 10s) , then remove the liquid
9. repeat step8 for three times
9. add 4 tubes of resin into 4 tubes of bacterial lysate respectively

7.18(Thu)

Large scale protein expression and purification of S012 and S013

Sample order of page (operator: Jerry)

12

M 50CL 50FT 50W1 50E1 50E2 50E3 50E8 500CL 500FT 500W1 500E1 500E2 500E3 500E8

13

M empty（50C lost）50FT 50W1 50E1 50E2 50E3 50E8 500CL 500FT 500W1 500E1 500E2 500E3 500E8

7.22(Mon)

Large scale protein expression and purification of S012 and S013

Inoculation (operator: Jerry)

From
☑Agar plates
☐Glycerol stock
☐Liquid starter

Strain	E.coli BL21(DE3) Rosetta
Antibiotics	Cm, Km
Medium	LB
Volume	3mL in 15mL tube, single colony
Temperature	37
Number	1

Buffer preparation:

FP151 Lysis Buffer

Total: 100mL

- 10mM Tris-HCL 1mL
- 100mM $NaHPO_4$ 1.4196g

Extraction Buffer

Total: 500mL

- 8M GdnHCL 382.12g
- 0.3M NaCl 8.766g
- 0.05M K_2HPO_4 4.35425g

7.23(Tue)

Preparation of protein purification of S016

Preparing cell pellet (operator: Jerry)

Strain	S016
Induction time length	4h
Centrifuge rate	10000g
Time	30min
Volume	200ml
Weight	
Storage location	-80

OD600 measurement

Strain	S016
Culturing time	2h
Dilution factor	1:10 with LB
Volume of sample	100uL
Average OD600nm	0.68
Temperature	37

Induction

Strain	S016
Inducer	1M IPTG
Induction time	4h
Temperature	37

Inoculation (operator:Jerry)

From
□ Agar plates
□Glycerol stock
♪ ?Liquid starter

Strain	E.coli BL21(DE3) Rosetta
Antibiotics	Cm, Km
Medium	LB
Volume	0.5mL in flask
Dilution factor	1:100
Temperature	37
Number	2

7.25(Thu)

Expression and purification of S016(fp151) 1st

Preparing cell pellet(operator: Emma)

Strain	S016
Induction time length	
Centrifuge rate	10000g
Time	20min
Volume	
Weight	
Storage location	-80

2.suspension (Operator: Emma)

25% (v/v) acetic acid, 2mL

3.centrifuge

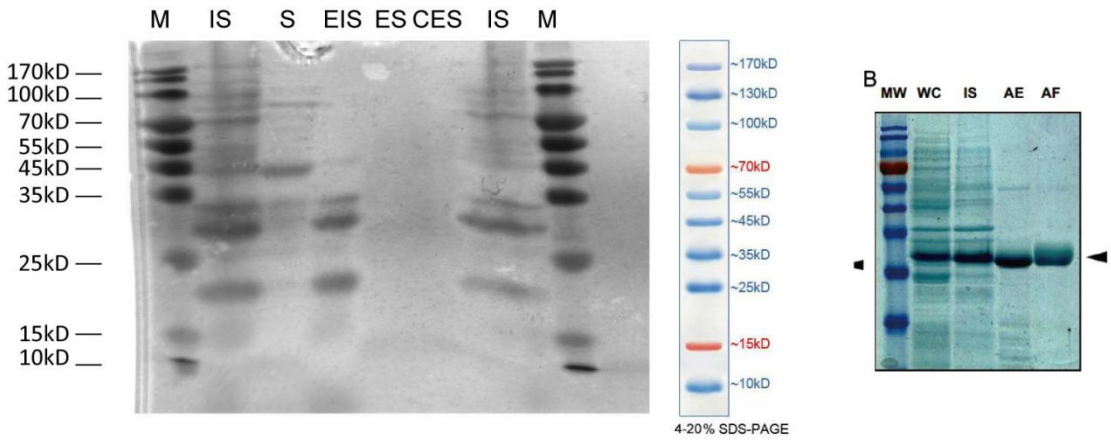
Collect the supernatant

Preparing protein samples for PAGE (operator: Wendy)

Strain	S016
Expressed protein	fp151
Cell pellet	Insoluble cell lysate/ Soluble cell lysate/ Insoluble extract/ Soluble extract/ Soluble extract (concentrated)
1xsample buffer	20 µl/ 10 µl/ 20 µl/ 10 µl/ 10 µl
Heating temperature	100°C
Heating time	10min
Storage location	

PAGE (operator: Wendy)

Volume of samples	5 ul
Volume of marker	5 ul
Voltage for stacking gel	100 V
Voltage for resolving gel	120V
Total time length	1.2h
Staining time	1h



IS-Insoluble cell lysate
S-Soluble cell lysate
EIS-Insoluble extract （25% acetic acid）
ES-Soluble extract（25% acetic acid，AE）
CES-Concentrated Soluble extract (5% acetic acid,150ul)

7.26(Fri)

Expression and purification of S020 and S025

Inoculation (operator: Winnie)

From
□ Agar plates
□Glycerol stock
♪ [?]Liquid starter

Strain	S020 E.coli BL21(DE3) Rosetta
Antibiotics	Cm, KAN
Medium	LB
Volume	0.5mL in flask
Dilution factor	1:100
Temperature	37
Number	10

Strain	S002 control
Antibiotics	KAN
Medium	LB
Volume	0.5mL in flask
Dilution factor	1:100
Temperature	37
Number	1

Strain	S025 E.coli BL21(DE3) Rosetta

Antibiotics	Cm, KAN
Medium	LB
Volume	0.5mL in flask
Dilution factor	1:100
Temperature	37
Number	2

Induction (operator: Emma)

Strain	S020, S025
Inducer	50μL 0.5M IPTG
Induction time	5h
Temperature	37

Preparing cell pellet (operator: Emma)

Strain	**S020, S025**
Induction time length	**5h**
Centrifuge rate	**14800g**
Time	**20min**
Volume	**200ul**
Weight	
Storage location	**-20**

配置Potassium phosphate Buffer(operator: Jerry)

300mM-NaCl
50mM-Potassium dihydrogen phosphate pH7.19
Total volume: 1L

Expression and purification of S016 (fp151) 1st

PAGE (operator: Wendy)

Volume of samples	5 ul
Volume of marker	5 ul
Voltage for stacking gel	100 V
Voltage for resolving gel	120V
Total time length	1h
Staining time	30 min

Expression and purification of S016(fp151) 2nd(Start)

Inoculation (operator: XYZ)

From
√ Agar plates
□ Glycerol stock
□ Liquid starter

Strain	S002(control), S016
Antibiotics	Cm/ Cm+Kan
Medium	LB
Volume	4mL in 15mL tube, single colony
Temperature	37
Number	1+1

7.27(Sat)

Expression and purification of S016 (fp151), S002. 2d

Inoculation (operator: Emma)

From
□ Agar plates
□Glycerol stock
√Liquid starter

Strain	S002 (control), S016 (1-4)
Antibiotics	Cm, Cm+Kan
Medium	LB
Volume	50mL in 250mL flasks
Dilution factor	1:100
Temperature	37, 220 rpm
Number	1+4

OD600 measurement (operator: XYZ)

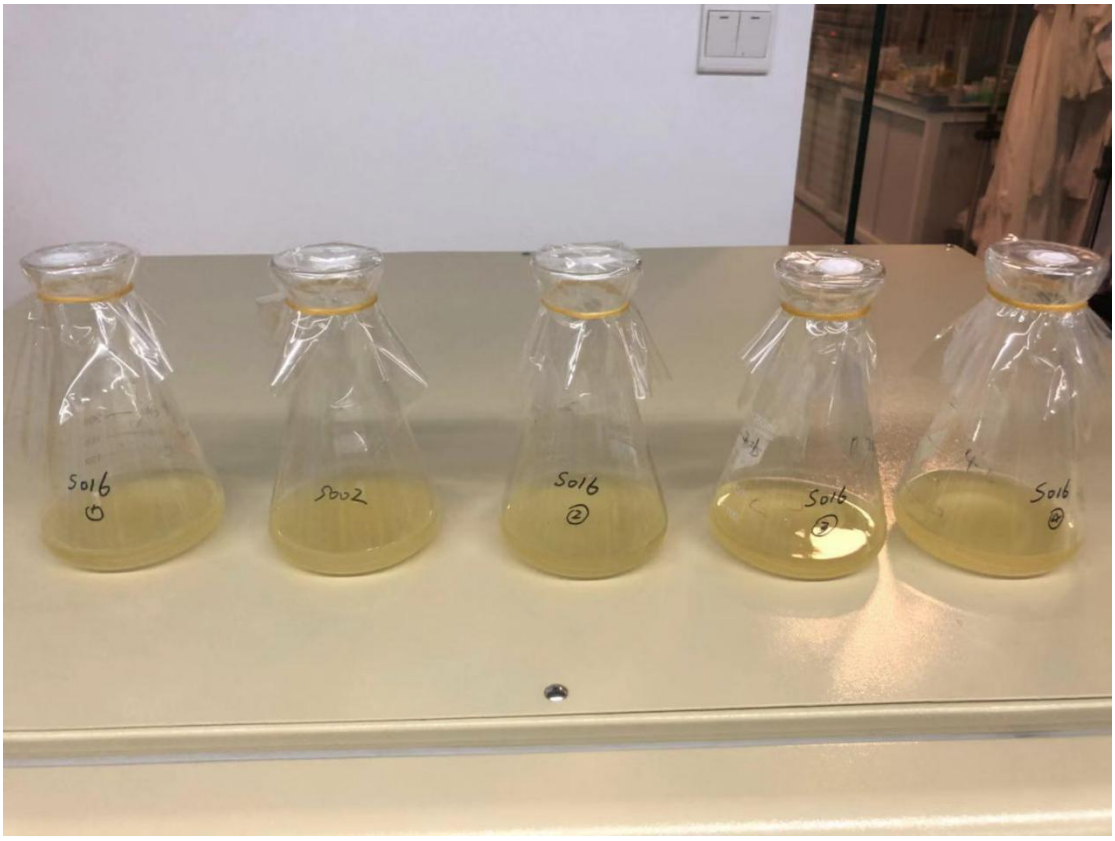
Strain	S002 (control), S016 (1-4)
Culturing time	2.5 h
Volume of sample	200uL
Average OD600nm	0.28, 0.22
Temperature	37

Induction (operator: XYZ)

Strain	S002 (control), S016 (1-4)

Inducer	50μL 1M IPTG
Induction time	8h
Temperature	37

Observing OD by naked eyes (operator: Merry)



All four flasks have roughly the same concentration of bacteria.

Preparing cell pellet (small scale, operator: Merry)

Strain	S002, S016(1), S016(3)
Induction time length	8h
Centrifuge rate	14800g
Time	2min
Volume	200ul
Weight	
Storage location	-20

Preparing cell pellet (large scale, operator: Merry)

Strain	S002, S016(1), S016(3)
Induction time length	8h
Centrifuge rate	5000g
Time	15min
Volume	100ml
Weight	62.0g
Storage location	-80

Expression and purification of S016(fp151) 1st

Preparing protein samples for PAGE (operator: Emma, Winnie)

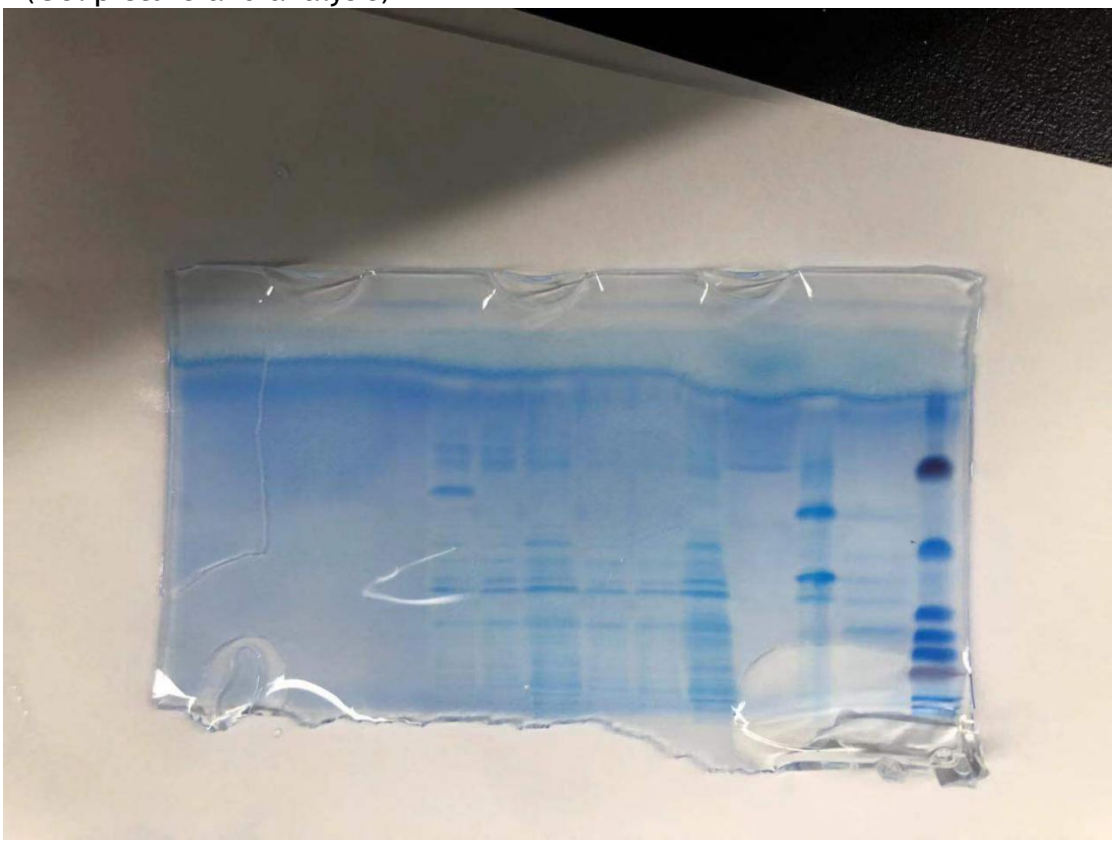
Strain	S009
Expressed protein	CsgA-linker-MFP3-7*His
Cell pellet	1ml OD=0.2
1xsample buffer	100µl
Heating temperature	100°C
Heating time	5min
Storage location	4°C/-20°C

PAGE (operator: Emma)

Volume of samples	5ul
Volume of marker	5ul
Voltage for stacking gel	100V
Voltage for resolving gel	120V

Total time length	1h
Staining time	40min

(Gel picture and analysis)



7.29(Mon)

Expression and purification of S016(fp151)

Suspension of S016 cell pellets（operator：Winnie，Ginny）

S016(30°C)	0.46g
S016(37°C)	0.13g

add lysis buffer 20ml, Ultrasonic lysis for10min

Preparing cell pellet (operator: Emma)

Strain	S016(1)(3)
Induction time length	8h
Centrifuge rate	10000g
Time	10min
Volume	100ml
Weight	62g
Storage location	-80

Collect supernatant
Add 2ml 25% acetic acid to every residue in the tube, and divide them into 665ul in 1.5ml tubes。

Ice bathing for time respectively 0.5h 1h 2h

Preparing cell pellet (operator: Emma)

Strain	S016(1)(3)(0.5h 1h 2h)(25% acetic acid added)
Induction time length	8h
Centrifuge rate	14800g
Time	10min
Volume	665ul each
Weight	Not known
Storage location	

10000g离15min。
S016 30°C 0.5h was lost

Preparing samples for PAGE (operator: Emma)

Strain	S002 IND
Expressed protein	pET28b
Cell pellet	
1xsample buffer	50μl
Heating temperature	100°C
Heating time	5min
Storage location	4°C/-20°C
Strain	S016(1) IND
Expressed protein	pET28b-fp151
Cell pellet	
1xsample buffer	50μl
Heating temperature	100°C
Heating time	5min
Storage location	4°C/-20°C
Strain	S016(3) IND
Expressed protein	pET28b
Cell pellet	
1xsample buffer	50μl
Heating temperature	100°C
Heating time	5min
Storage location	4°C/-20°C
Strain	S016(1)(3) S
Expressed protein	pET28b-fp151
Cell pellet	
5xsample buffer	16μl
Heating temperature	100°C
Heating time	5min
Storage location	4°C/-20°C
Strain	S016(0.5h 1h 2h)(30C 37C)(CES)
Expressed protein	pET28b-fp151
Cell pellet	
5xsample buffer	16μl

Heating temperature	100°C
Heating time	5min
Storage location	4°C/-20°C
Strain	S016(0.5h 1h 2h)(no 30C 0.5h)(30C 37C)(EIS)
Expressed protein	pET28b-fp151
Cell pellet	
5xsample buffer	16μl
Heating temperature	100°C
Heating time	5min
Storage location	4°C/-20°C

For Expression of S016, S017, S019, S020, S025

Inoculation (operator: Jerry)
From
□ Agar plates
√Glycerol stock
□Liquid starter

Strain	s016,s017,s019,s020,s025
Antibiotics	Cm, Km
Medium	LB
Volume	plate streaking
Temperature	37
Number	5

7.30(Tue)

Protein Purification (operators: Adonie, Ginny)

Resuspend cell pellets(S020 Rosetta/pET28b-CsgA-mfp5-7×His)
add extraction solution(ml), 10×the mass of cell pellets(g)

Expression of S017, S019, S020, S002, S025

Inoculation (operator: Emma)

From
√Agar plates
□Glycerol stock
□Liquid starter

Strain	S002
Antibiotics	Kan
Medium	LB
Volume	4ml in 15ml tubes
Dilution factor	1:1000
Temperature	37
Number	1
Strain	S017
Antibiotics	Kan Cm
Medium	LB
Volume	4ml in 15ml tubes
Dilution factor	1:1000
Temperature	37
Number	1
Strain	S019
Antibiotics	Kan Cm
Medium	LB
Volume	4ml in 15ml tubes
Dilution factor	1:1000
Temperature	37
Number	1
Strain	S020
Antibiotics	Kan Cm
Medium	LB

Volume	4ml in 15ml tubes
Dilution factor	1:1000
Temperature	37
Number	1
Strain	S025
Antibiotics	Kan
Medium	LB
Volume	4ml in 15ml tubes
Dilution factor	1:1000
Temperature	37
Number	1

7.31(Wed)

Expression of S017, S019, S020, S002

Inoculation (operator: Joseph, Ginny)

From
□Agar plates
Glycerol stock
□Liquid starter

Strain	S017, E.coli BL21(DE3) Rosetta{SM002}
Antibiotics	Cm+Kan
Medium	LB
Volume	50ml in 250ml conical flask
Temperature	25°C, 30°C, 37°C respectively
Number	3

Strain	S019, E.coli BL21(DE3) Rosetta{SM008}
Antibiotics	Cm+Kan

Medium	LB
Volume	50ml in 250ml conical flask
Temperature	25°C, 30°C, 37°C respectively
Number	3

Strain	S020, E.coli BL21(DE3) Rosetta{SM010, pET28b-csgA-linker-mfp5-7xHis}
Antibiotics	Cm+Kan
Medium	LB
Volume	50ml in 250ml conical flask
Temperature	25°C, 30°C, 37°C respectively
Number	3

Strain	S002, E.coli BL21(DE3) Rosetta{PET28b}
Antibiotics	Kan
Medium	LB
Volume	50ml in 250ml conical flask
Temperature	25°C, 30°C, 37°C respectively
Number	3

ps: the conical flasks containing S017(37°C) and S019(37°C) were contaminated.

OD600 measurement (operator: Ginny)

Strain	S017
Culturing time	1h30min
Dilution factor	no dilution
Volume of sample	200ul
Average OD600nm	0.17
Temperature	37

Strain	S019
Culturing time	1h30min
Dilution factor	no dilution
Volume of sample	200ul
Average OD600nm	0.15
Temperature	37

Strain	S020
Culturing time	1h30min
Dilution factor	no dilution
Volume of sample	200ul
Average OD600nm	0.13
Temperature	37

Strain	S017
Culturing time	1h30min
Dilution factor	no dilution
Volume of sample	200ul
Average OD600nm	0.21
Temperature	37

Preparing cell pellet (small scale, operator: Ginny)

Strain	S017, S019, S020, S002(all from 37°C, incubation time 1h)
Centrifuge rate	14800rpm
Time	2min
Volume	500ul
Weight	
Storage location	-20°C

Preparing cell pellet (small scale, operator: Diana)

Strain	S002(From 25°C, 30°C and 37°C) S019, S020, S017(From 37°C) ； 4h
Centrifuge rate	14800rpm
Time	2min
Volume	500ul
Weight	
Storage location	-20°C

Strain	S019, S020, S017(From 25°C and 30°C)； 4h
Centrifuge rate	14800rpm
Time	2min
Volume	1ml
Weight	
Storage location	-20°C

PAGE (operator: Diana)

Volume of samples	5ul/ 8ul/ 10ul
Volume of marker	5ul
Voltage for stacking gel	80V
Voltage for resolving gel	120V
Total time length	1.5h
Staining time	40min

Expression (for large scale purification) of S025

Inoculation (operator: Diana)

From
□Agar plates
Glycerol stock
□Liquid starter

Strain	S025
Antibiotics	Cm+Kan
Medium	LB
Volume	50ml in 250ml conical flask
Temperature	37°C
Number	15

OD600 measurement (operator: Diana)

Strain	S025
Culturing time	3h
Dilution factor	1: 1
Volume of sample	50uL
Average OD600nm	0.16
Temperature	37

Induction (operator: Diana)

Strain	S025
Inducer	25µL 1M IPTG
Induction time	5h
Temperature	37

Preparing cell pellet (operator: Jerry, Uranium)

Strain	S025
Centrifuge rate	5000g
Time	20min
Volume	50ml*15 tubes
Weight	
Storage location	-80

8.1(Thur)

Expression of S017, S019, S020, S002

Preparing protein samples for PAGE (operator: Diana, Wendy)

Strain	S017
Expressed protein	CsgA-linker-Mfp5-Mfp5-7xHis
Cell pellet	1ml OD=0.2
1xsample buffer	100µl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

Strain	S019
Expressed protein	CsgA-linker-Mfp3-7xHis
Cell pellet	1ml OD=0.2
1xsample buffer	100µl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

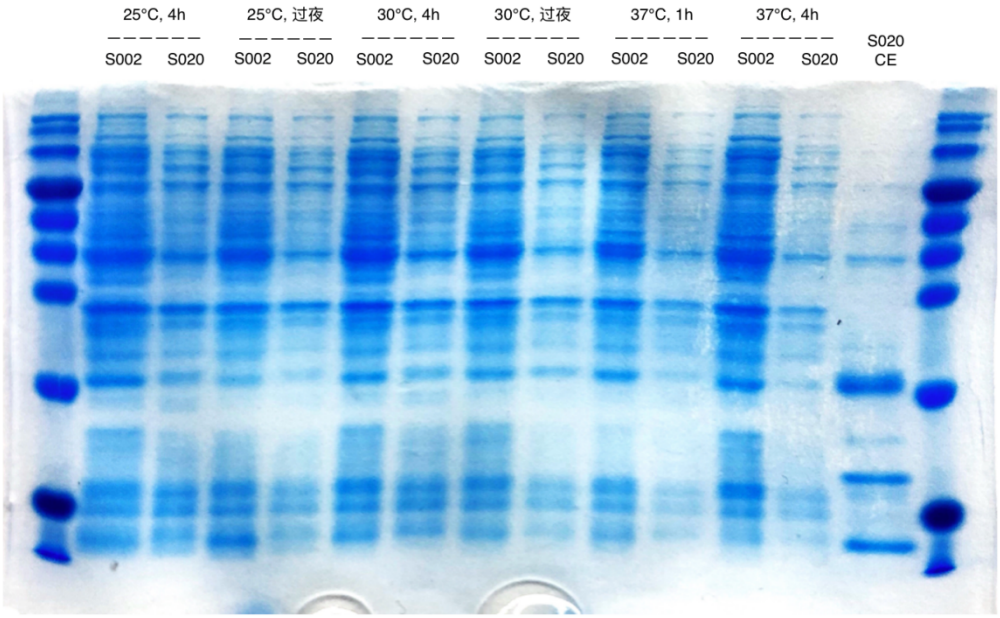
Strain	S020
Expressed protein	CsgA-linker-Mfp5-7xHis
Cell pellet	1ml OD=0.2
1xsample buffer	100µl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

Strain	S002

Expressed protein	/
Cell pellet	1ml OD=0.2
1xsample buffer	100µl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

PAGE (operator: XYZ)

Volume of samples	5 ul
Volume of marker	5 ul
Voltage for stacking gel	80 V
Voltage for resolving gel	120 V
Total time length	1h
Staining time	40min



8.2(Fri)

Large scale purification of S025

Purification（Operator：Diana）

1. purify by gel filtration columns
2. add 10ml Washing buffer，wash 4 times
3. add Elution buffer，8 times，first 4 times each for 1ml，last ones add 500ul

PAGE (operator: jerry)

Volume of samples	8 ul
Volume of marker	5 ul
Voltage for stacking gel	80 V
Voltage for resolving gel	100 V
Total time length	1h
Staining time	40min

(Gel picture and analysis)

8.6(Tue)

Inoculation (operator: Ginny)

From
□Agar plates
Glycerol stock
□Liquid starter

Strain	S017
Antibiotics	Cm+Kan
Medium	LB
Volume	50ml in 250ml conical flask
Temperature	37°C

Number	18

OD600 measurement (operator: Ginny)

Strain	S017
Culturing time	2h30min
Dilution factor	no dilution
Volume of sample	200ul
Average OD600nm	0.10
Temperature	37

Induction (operator: Ginny)

Strain	S017
Inducer	25ul 1M IPTG
Induction time	1h
Temperature	37

Preparing cell pellet (operator: Aislinn)

Strain	**S017(from 37°C)**
Induction time length	**1h**
Centrifuge rate	**5000g**
Time	**20min**
Volume	**500ul**
Weight	
Storage location	**-80**

8.7(Wed)

prepare Triton X-114 washing buffer(PH 8.0) (operator: Aislinn)

1% Triton X-114(sigma). 4ml
1mM EDTA 400ul
50mM Tris-HCL 20ml
Water 400ml-20ml-4ml-400ul
total:400ml

Protein Purification (operator: Aislinn)
Resuspend cell pellets(S017 Rosetta/pET28b-CsgA-mfp5-7×His)
add extraction solution(ml), 10×the mass of cell pellets(g)
4°C incubating24h

Large scale protein expression of S016(fp151)

Inoculation (operator: Emma Winnie)
From: Liquid starter

Strain	S016(fp151)
Antibiotics	Cm Kan
Medium	LB
Volume	50ml in conical flask
Temperature	37
Number	16

OD600 measurement (operator: Emma)

Strain	S016(fp151)
Culturing time	2.5h
Dilution factor	
Volume of sample	200uL
Average OD600nm	0.27
Temperature	37

Purification of S025（Operator：Darcy, Amy, Ginny）

1. purify by gel filtration columns
2. use washing buffer（50mM Imidazole）three times，each for 20ml
3. use 4ml washing buffer（80mM Imidazole），collect sample W1-4
4. use4ml washing buffer（100mM Imidazole），collect sampleW1-3
5. use 8ml elution buffer（300mM Imidazole），collect sample E1-8

8.5(Mon)

Expression of S020

Inoculation (operator: Wendy)

From
√Agar plates
□Glycerol stock
□Liquid starter

Strain	S020
Antibiotics	Cm+Kan
Medium	LB
Volume	4ml　in 15ml tubes
Dilution factor	1:1000
Temperature	37
Number	3

Detection of S017, S019 and S025

PAGE (operator: Wendy, Darcy)

Volume of samples	10ul
Volume of marker	10ul
Voltage for stacking gel	80 V
Voltage for resolving gel	120 V
Total time length	1h
Staining time	40min

Sample order of page:

Gel 1： M, S002 37C 4h, S017 25C 4h, S019 25C 4h, S017 25C 8h, S017 25C 8h, S017 30C 4h, S019 30C 4h, S017 30C 8h, S019 30C 8h, S017 37C 1h, S019 37C 1h, S017 37C 4h, S019 37C 4h
Gel 2： M, S002 37C 4h, S025 WC, S025 IS, S025 S, S025 W1, S025 E1, S025 E2, S025 E8

Result: Poor gel (fail)

8.6(Tue)

Expression of S017

Inoculation(operator: Ginny)

From
□ Agar plates
Glycerol stock
□Liquid starter

Strain	**S017**, E.coli BL21(DE3) Rosetta{SM002}
Antibiotics	Kan+Cm
Medium	LB
Volume	50ml in 250ml conical flask

Temperature	37
Number	18

OD600 measurement (operator: Ginny)

Strain	S017, E.coli BL21(DE3) Rosetta{SM002}
Culturing time	2h30min
Dilution factor	no dilution
Volume of sample	200ul
Average OD600nm	0.101
Temperature	37

Induction (operator: Ginny)

Strain	S017
Inducer	25ul 1M IPTG
Induction time	1h
Temperature	37

Preparing cell pellet (operator: Aislinn)

Strain	**S017(from 37°C)**
Induction time length	**1h**
Centrifuge rate	**5000g**
Time	**20min**
Volume	**500ul**
Weight	
Storage location	**-80**

Preparing cell pellet (operator: Aislinn)

Strain	**S017(from 37°C)**
Induction time length	**1h**
Centrifuge rate	**5000g**
Time	**20min**
Volume	**500ul**
Weight	
Storage location	**-80**

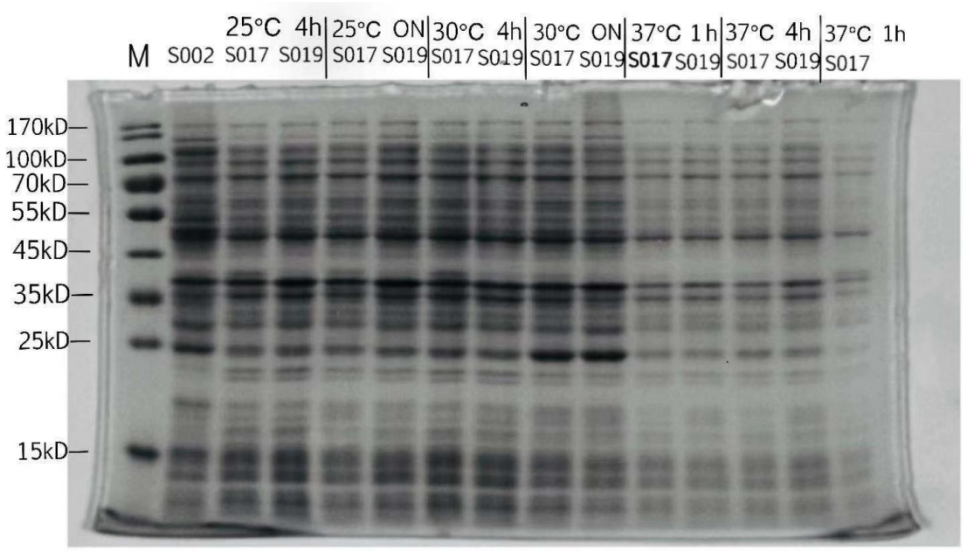
Detection of S017, S019 and S025

PAGE (operator: Jerry)

Volume of samples	**10ul**
Volume of marker	**5ul**
Voltage for stacking gel	**80 V**
Voltage for resolving gel	**120 V**
Total time length	**1h**
Staining time	**40min**

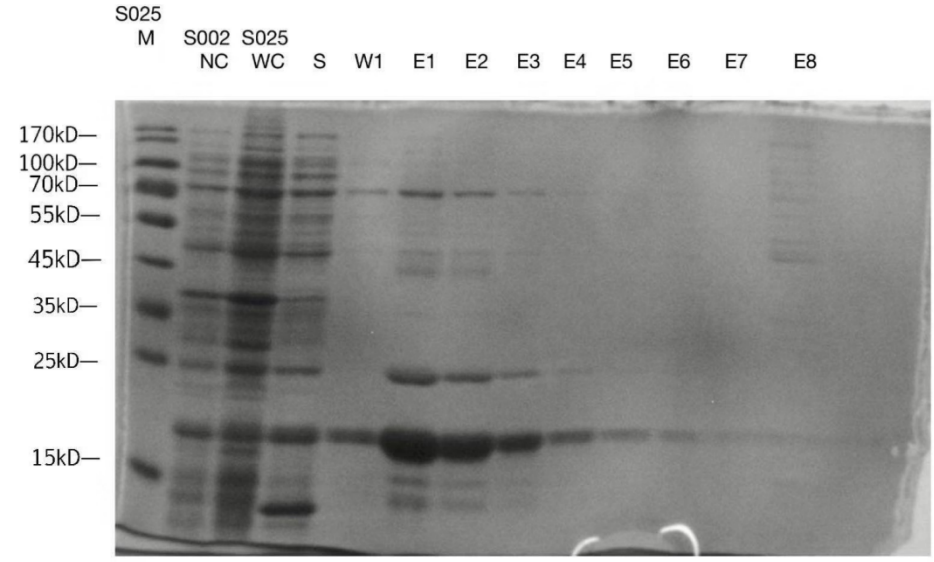
Sample order of PAGE (operator: Jerry)

胶1：M, S002 37C 4h, S017 25C 4h, S019 25C 4h, S017 25C 8h, S017 25C 8h, S017 30C 4h, S019 30C 4h, S017 30C 8h, S019 30C 8h, S017 37C 1h, S019 37C 1h, S017 37C 4h, S019 37C 4h, S017



Result:

Gel 2: M, S002 37C 4h, S025 WC, S025 IS, S025 S, S025 W1, S025 E1, S025 E2, S025 E8



Result: get target protein with high concentration

Large scale expression of S016(fp151)

Inoculation (operator: Jerry)

From

√Agar plates

□Glycerol stock

□Liquid starter

Strain	S016
Antibiotics	Cm + Km
Medium	LB
Volume	4ml in 15ml tube
Dilution factor	single colony
Temperature	37
Number	2

8.7(Wed)

Large scale expression of S016(fp151)

Inoculation (operator: Emma Winnie)

From
Agar plates
Glycerol stock
√ Liquid starter

Strain	S016(fp151)
Antibiotics	Cm Kan
Medium	LB
Volume	50ml in conical flask
Temperature	37
Number	16

OD600 measurement (operator: Emma)

Strain	S016(fp151)
Culturing time	2.5h
Dilution factor	
Volume of sample	200uL
Average OD600nm	0.27
Temperature	37

Preparing cell pellet (operator: Wendy)

Strain	S016
Centrifuge rate	5000g
Time	20min
Volume	50ml x12
Weight	
Storage location	-20

8.8(Thur)

Large scale purification of S016(fp151)

1. suspension（lysis buffer: 5ml/gram)
2. Ultrasonic lysis 30min
3. centrifuge（18000g, 20min, 4°C)
4. Collect precipitate
5. Add Triton-114 washong buffer

Purification of S017(operator: Aislinn)

1.S017 10000g centrifuge for 30min collect the supernatant
2.Add 40ul S017 Resin into collected supernatant
3.4°C incubating 3h
4.Add washing buffer

First time +50mM 50ml
Second time + 80mM 4ml collect 3 50ml tubes
Third time + 100mM 4ml collect 4 50ml tubes
Forth time 300mM elusion buffer 6ml collect 6 50ml tubes

8.9(Fri)

Large scale purification of S016(fp151)

PAGE (operator: Aislinn Winnie)

Volume of samples	**B13 5ul** **B14 8ul** **B1,B2 10ul** **B3,4,5,6,7,8,9,10,11,12 8ul** **A gel 10ul**
Volume of marker	**5ul**
Voltage for stacking gel	**80 V**
Voltage for resolving gel	**125 V**
Total time length	75min
Staining time	**1 h**

Sample order of page (operator: Aislinn)

A gel:
S025 M, W1(A1), W2(A2), W3(A3), W1(A4), W2(A5), W3(A6), E1(A7), E2(A8), E7(A9)
80mM 100mM 300mM

B gel:
M, S002induced(B13), S017induced(B14), S017 W1(B1), W2(B2), W3(B3), W1(B4), W2(B5), W3(B6), W4(B7), E1(B8), E2(B9), E3(B10), E4(B11), E5(B12) 80mM
100mM
300mM

Failed: Unclear

蛋白人们的实验记录（8.12~8.18）

8.12(Mon)

Purification of S016

Concentration of S016(operator: Amy, Ginny)

Strain	S016
Centrifuge rate	10000g
Time	30min x 4
Temperature	4°C
Medium for conc.	PBS
Volume of conc. medium	15ml
Volume of ECS*	5ml *1

Expression of S028, S033

Inoculation (operator: Wendy)

From
√Agar plates
□Glycerol stock
□Liquid starter

Strain	S028
Antibiotics	Kan
Medium	LB
Volume	4ml in 15ml tubes
Dilution factor	1:1000
Temperature	37°C
Number	3

Strain	S033
Antibiotics	Kan
Medium	LB
Volume	4ml in 15ml tubes
Dilution factor	1:1000
Temperature	37°C
Number	3

Purification of S017, S025

Concentration of S017,S025 (operator: Winnie）

Strain	S017,S025
Centrifuge rate	10000g
Time	30min x 4
Temperature	4°C
Medium for conc.	PBS
Volume of conc. medium	15ml
Volume of ECS*	s017 300ul s025 400ul

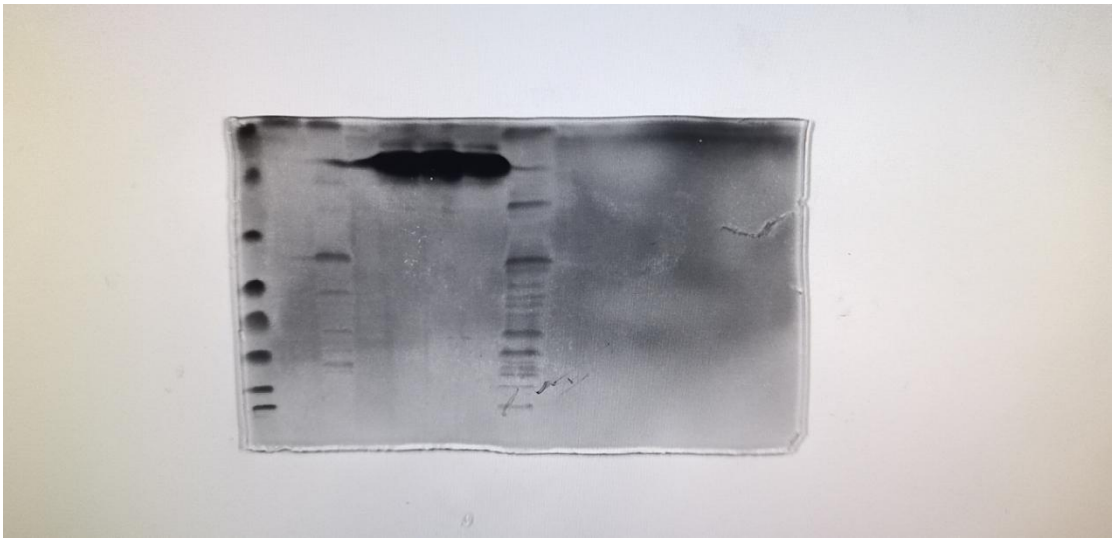
PAGE of S016, S017, S020, S025

PAGE (operator: Amy,Wendy,Ginny)

Volume of samples	10ul
Volume of marker	5ul
Voltage of stacking gel	80V
Voltage of resolving gel	120V
Total time length	1h20min
Staining time	40min

Order of samples: marker, S020, S017, S025, S016-2, S016-1, S016-3, S016 soluble

Picture:



Results:
Over the time limit (x)

8.13(Tue)

Expression of S028, S033

1. Inoculation (operator: Amy, Aislinn, Ginny)

From
□Agar plates
□Glycerol stock
√Liquid starter

Strain	S028， S033
Antibiotics	Kan
Medium	LB
Volume	50ml in 250ml conical flask
Temperature	37°C
Number	20 each

2. OD600 measurement (operator: Aislinn)

Strain	S028, S033
Culturing time	3h
Dilution factor	/
Volume of sample	200µl
Average OD600nm	S028: 0.46 S033: 0.36
Temperature	37°C

3. Induction (operator: Aislinn, Wendy)

Strain	S028, S033
Inducer	25µl 1M IPTG
Induction time	5h
Temperature	30°C

PAGE of S016, S017, S020, S025

PAGE (operator: Winnie, Jerry)

Volume of samples	8ul
Volume of marker	5ul
Voltage of stacking gel	80V
Voltage of resolving gel	120V
Total time length	1h
Staining time	

Order of samples: M, S020, S017, S016, S025

Picture:

Results: Lots of unwanted proteins in S017 and S016; S025 is too weak. Continue to do further purification of S016; Re-express S025.

Expression of S025

Inoculation (operator:Wendy)

From
☑ Agar plates
☐Glycerol stock
☐Liquid starter

Strain	S025
Antibiotics	Kan
Medium	LB
Volume	4ml
Temperature	37°C
Number	3

8.14（Wed）

Expression of S025

1.Inoculation (operator: Aislinn)

From
□Agar plates
□Glycerol stock
√Liquid starter

Strain	S025
Antibiotics	Kan
Medium	LB
Volume	50ml in 250ml conical flask
Temperature	37°C
Number	20

2.OD600 measurement (operator: Aislinn)

Strain	S025
Culturing time	3h
Dilution factor	/
Volume of sample	200μl
Average OD600nm	0.17
Temperature	37°C

3.Induction (operator: Aislinn)

Strain	S025
Inducer	25μl 1M IPTG
Induction time	5h
Temperature	37°C

4. Preparing cell pellet (induced) (operator: Aislinn)

Strain	S025
Centrifuge rate	8000g
Time	20min
Temperature	4°C
Volume	
Weight	
Storage location	-80°C

8.15(Thur)

Purification of S028 and S033

Purification (Operator: Aislinn)

train	S028
Expressed protein	Mfp5-Mfp5
Washing	6 times
Washing volume	1ml
Elution	6 times
Elution volume	1 ml

Purification (Operator: Ginny)

strain	S033
Expressed protein	mfp5
Washing	6 times
Washing volume	1ml
Elution	6 times
Elution volume	1 ml

PAGE of S016, S028, S033

Preparing protein samples for PAGE (operator: Aislinn, Ginny)

Strain	S028,S033,S016
Expressed protein	pET28b-CsgA-linker-mfp5
Cell pellet	
1xsample buffer	16μl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

8.16(Fri)

Expression of S029

Inoculation (operator: Wendy，Jerry)

From
√Agar plates
□Glycerol stock
□Liquid starter

Strain	S029
Antibiotics	Kan Cm
Medium	LB
Volume	5ml in 15ml tubes
Dilution factor	1:1000
Temperature	37°C
Number	3

8.17（Sat）

Expression of S029

1. Inoculation (operator: Wendy)

From
□Agar plates
□Glycerol stock
√Liquid starter

Strain	S029
Antibiotics	Kan Cm
Medium	LB
Volume	50ml in 250ml conical flask
Temperature	37°C
Number	24

2. OD600 measurement (operator: Wendy)

Strain	S029
Culturing time	2.5h
Dilution factor	/
Volume of sample	200µl
Average OD600nm	0.3
Temperature	37°C

3. Induction (operator: Wendy)

Strain	S029
Inducer	25µl 1M IPTG
Induction time	overnight
Temperature	30°C

8.18 (Sun)

Purification of S029

OD600 measurement

Strain	S029
Culturing time	16h
Dilution factor	\
Volume of sample	200uL
Average OD600nm	0.8
Temperature	30

Preparing cell pellet

Strain	S029
Induction time length	S029
Centrifuge rate	8000g
Time	10min
Volume	1.1L
Weight	8.5g

Lysis of cell pellets

8.5g cell pellets were resupended with 85ml extraction solution and incubated at 4, 120rpm.(0818 14:00 start)
Preparing whole cell samples for PAGE

Strain	S029
Expressed protein	Fp151-7*His
Cell pellet	0.5ml OD=0.8
1xsample buffer	100μl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

PAGE

Volume of samples	5 ul
Volume of marker	5 ul
Voltage for stacking gel	80 V
Voltage for resolving gel	100 V
Total time length	1h
Staining time	30min

M S020 S033 S017 S028 S016 S025 S002ind S029indWC
*S028 or S016 or S025 samples were lost during PAGE which may due to the unprepared gel at 4du overnight.

(Gel picture and analysis)

PAGE of all purified proteins

the same as above

Expression of S035/S036

Inoculation

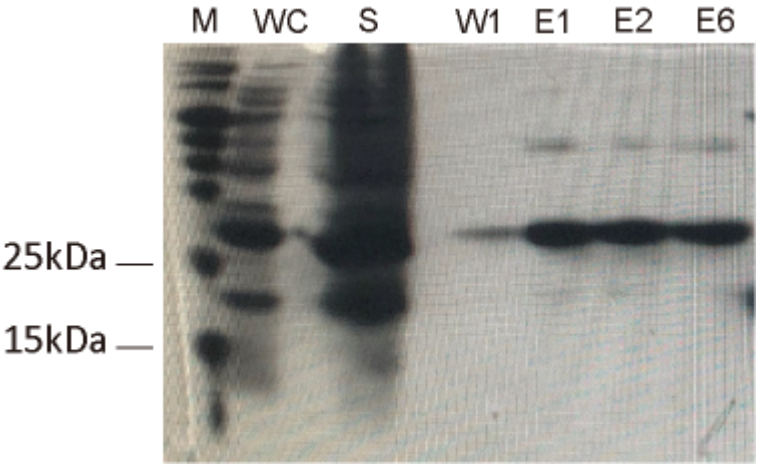
From
√ Agar plates
□ Glycerol stock
□ Liquid starter

Strain	S035/S036, cp19k-mfp5,co-cp19k-mfp5
Antibiotics	Cm+Kan
Medium	LB
Volume	3mL in 15mL tube, single colony
Temperature	37
Number	1/1

8.19(Mon)

Purification of S029

PAGE



Analysis:

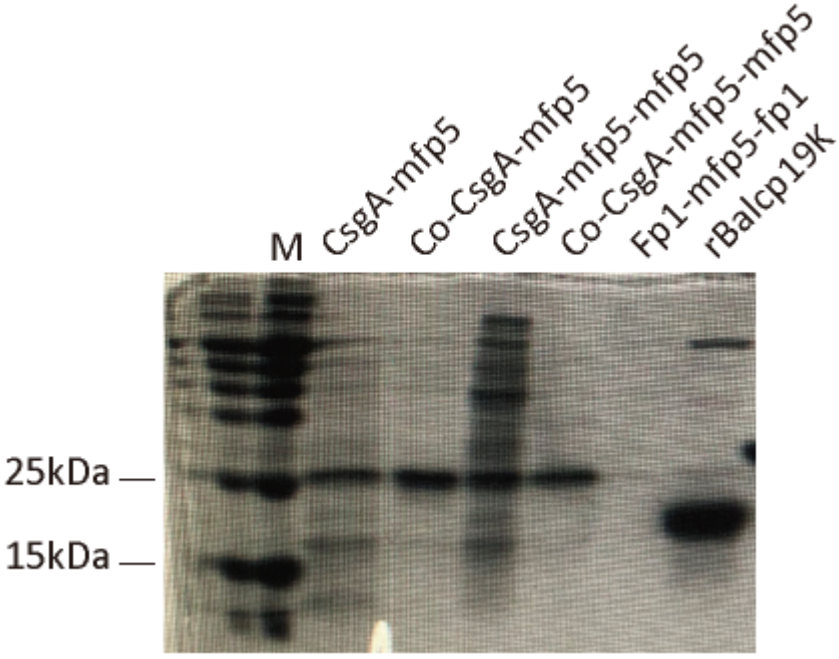
1. Target band (~25kDa) appears on Lane 3 (solube parts) which means the cell pellets were lysed completely.
2. Target band can be detected of Sample W1 which means protein can be eluted under 100mM imidazole.
3. Target band can be detected of Sample E6 which means elution is not sufficient.
4. Non-specific bands in 55kDa might be dimers.

Next plan:

1. Further elution should be done.
2. Dialyze with PBS to remove high concentration imidazole.

PAGE of all purified proteins

PAGE



Analysis:

1. Fp151 is extremely weak.Next plan: purified agian, undergoing.
2. Concentrated rBalcp19K is strong. Next plan: concentration detection.
3. CsgA-mfp5 and CsgA-mfp5-mfp5 is not purified enough.

8.24(Sat)

Concentration (operator:Ginny)

Strain	S029
Centrifuge rate	10000g
Time	30min x 2 times
Temperature	4°C
Medium for conc.	PBS
Volume of conc. medium	5ml
Volume of ECS*	200ul x 2 tubes

Preparing protein samples for PAGE (operator:Ginny)

Strain	S029, S035, S036
Expressed protein	Co-fp151, SM014, SM015
Cell pellet	-20
5xsample buffer	4µl
Heating temperature	100°C
Heating time	5min
Storage location	4°C

1. PAGE (operator:Ginny)

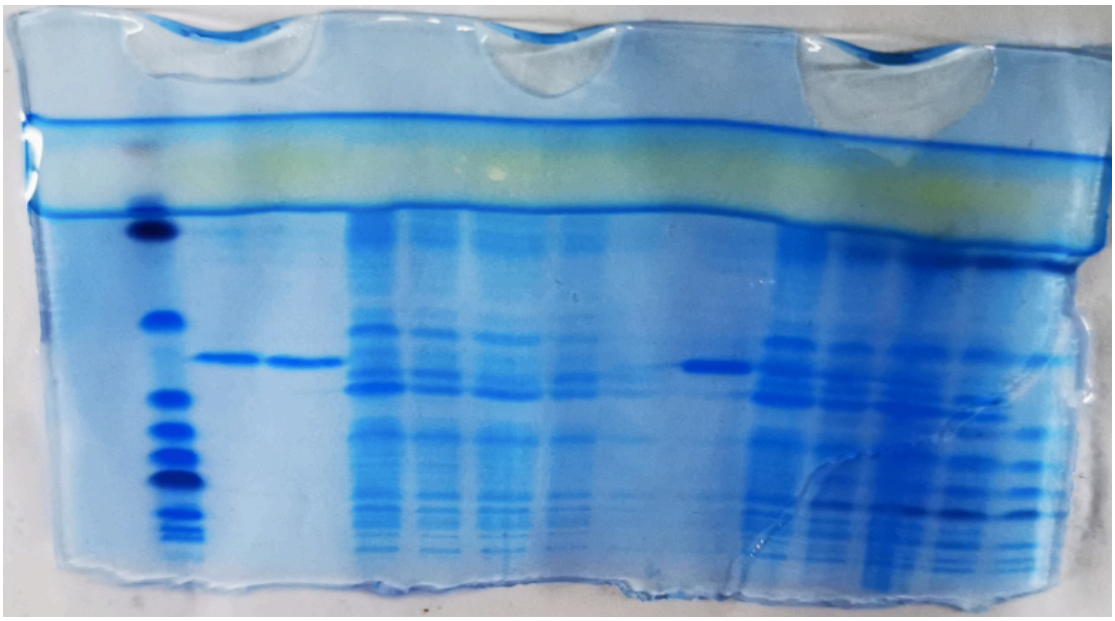
Volume of samples	5ul
Volume of marker	5ul
Voltage of stacking gel	100V
Voltage of resolving gel	120V
Total time length	60min
Staining time	30min

Order of samples: Marker, S035(WC), S035(WC 25°C 20h), S036(WC),

S036(WC 25°C 20h), S029-1, S029-2, S035(WC), S035(WC 25°C 20h), S036(WC), S036(WC 25°C 20h), S029-1, S029-2, Marker.

ps. the process has been repeated since the first time all of the samples flowed away.

Picture:



3. Concentration of S025 and S029(Operator:Ginny)

8.31 (Sat)

Inoculation (operator: Diana)

From

□ Agar plates
□Glycerol stock
☑Liquid starter

Strain	S017
Antibiotics	Kan, Cm
Medium	LB
Volume	100ml in 250ml flask
Temperature	37°C
Number	5
Strain	S020
Antibiotics	Kan, Cm
Medium	LB
Volume	100ml in 250ml flask
Temperature	37°C
Number	3
Strain	S002
Antibiotics	Kan, Cm
Medium	LB
Volume	100ml in 250ml flask
Temperature	37°C
Number	1