Notebook of molecular cloning

Team: GreatBay_SCIE 2019

Record date: from 7.12-8.31

7.12 (Fri)

Number of single colonies on plates (operator：Diana)

1 on SM011
2 on SM004, SM006
0 on SM005, SM007

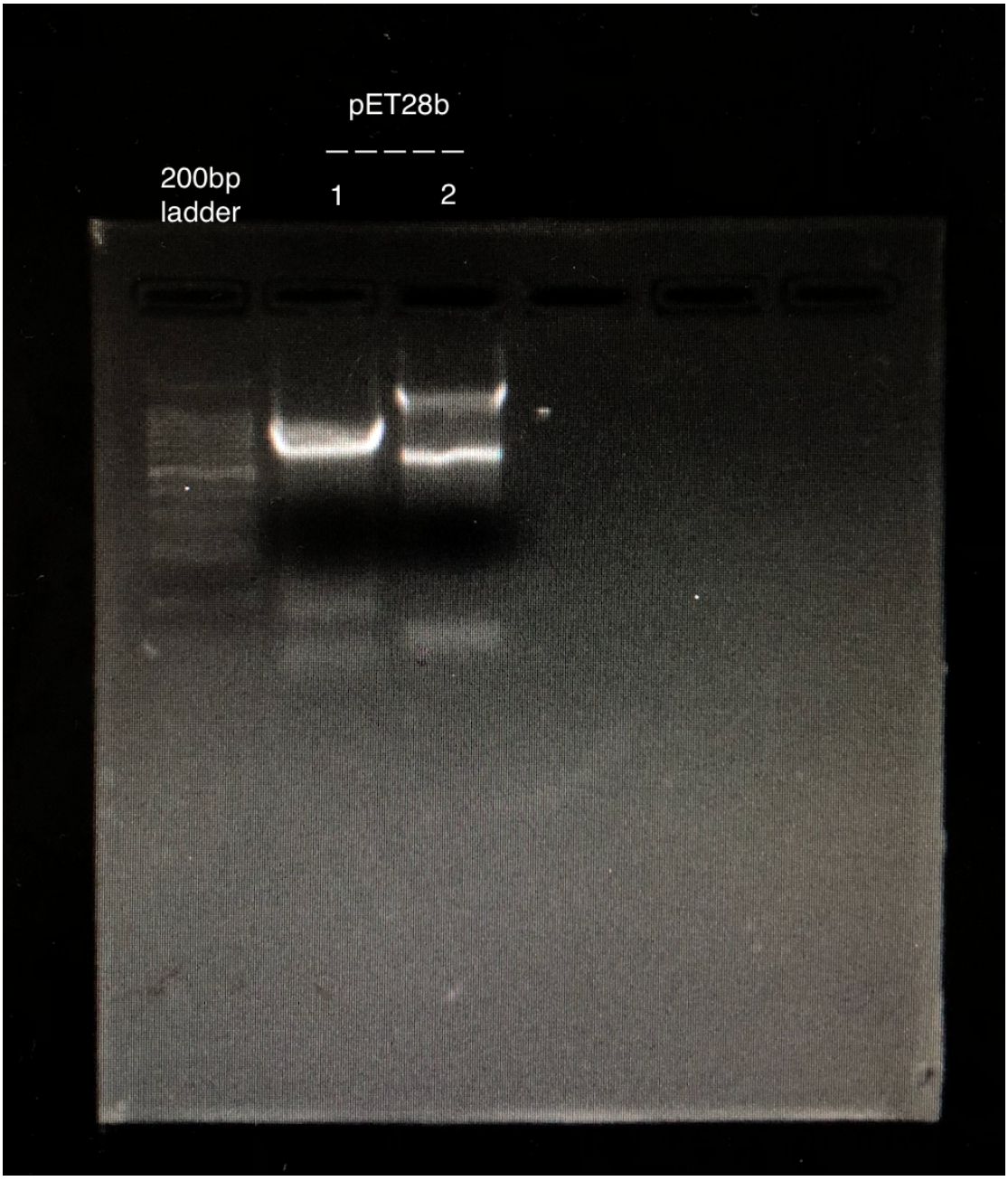
PCR Template (operator: Diana, Aislinn)

□Purified genome
√Purified plasmid
□Single colony
□Bacteria liquid
□Boiled Bacteria cell lysate

PCR		
Primer 1	SP002-F	2.5
Primer 2	SP002-R	2.5
Template	SM010	0.5 (77.9ng/ul)
H2O		19.5
DNA polymerase	rTaq	25
All		50
		50/tube×1
Product length	1900bp	

PCR		
Primer 1	SP003-F	2.5
Primer 2	SP003-R	2.5
Template	SM010	0.5
H2O		19.5
DNA polymerase	rTaq	25
All		50
		50/tube×1
Product length	3400bp	

Process		
1	94	60s
2	94	30s
3	55	30s
4	72	105s
Go to 2	35 cycle	
5	72	5min
6	16	∞



Gel extraction (operator: Diana, Aislinn)

√Gel extraction
□Liquid extraction

Elution buffer
☒EB
□H2O

Elution volume: 40ul

Name: pET28b-1,2
Concentration(A260/280nm)： 64.85ng/μL(pET28b-1), 42.95ng/ul (pET28b-2)

Reconstruction of SM005，SM007

Gibson assembly	(operator: Diana)	
Backbone		
Fragment 1	pET28b-1	1
Fragment 2	pET28b-2	1.5
Fragment 3	SM005 Fr3	0.5
Fragment 4	SM005 Fr4	1
Fragment 5	SM005 Fr5	1
H_2O		Add to 5
GA mix		5
All		10

Backbone		
Fragment 1	pET28b-1	1.3
Fragment 2	pET28b-2	2
Fragment 3	SM007 Fr3	0.7
Fragment 4	SM007 Fr4	1
H_2O		Add to 5
GA mix		5
All		10

Process		

1	50	60min
2	12	∞

Transformation (operator: Diana)

□Electroporation1μL
√Chemical transformation10μL

Host: DH5 alpha
Plasmid to construct: SM005, SM007

Recovery medium: LB
Volume of bacterial liquid to spread: 150ul
Agar plate and antibiotic to use：LB Cm
Transformation conditions：heat shock at42°C for 90s

7.13 (Sat)

Number of single colonies on plates (operator：Diana)

0 on all.

7.15 (Mon)

Construction of SM004, SM006, SM011

Colony selection (operator: Diana)

From
√Agar plates
□Glycerol stock
□Liquid starter

Strain	SM004, SM006, SM011

Antibiotics resistance	Kan
Medium	LB
Volume	Inoculating single colonies on plates
Temperature	37
Number	5

SM004: 1, 2
SM006: 3, 4
SM011: 5

PCR Template: (operator: Diana)

□Purified plasmid
□Purified genome
√Single colony
□Bacteria liquid
□Boiled Bacteria cell lysate

PCR for verification		
Primer 1	SP004-F	1
Primer 2	SP004-R	1
Template	SM004 colony	
H2O		13
DNA polymerase	Taq Mix	15
All		30
		30/tube×2
Product length	1551bp	

PCR for verification		
Primer 1	SP004-F	1
Primer 2	SP004-R	1
Template	SM006 colony	
H2O		13
DNA polymerase	Taq Mix	15
All		30
		30/tube×2
Product length	1779bp	

PCR for verification		
Primer 1	SP004-F	1
Primer 2	SP004-R	1
Template	SM011colony	
H2O		13
DNA polymerase	Taq Mix	15
All		30
		30/tube×2
Product length	1446bp	

Process		
1	94	10min
2	94	30s
3	55	30s
4	72	1min
Go to 2	35 cycle	
5	72	5min

6	16	∞

control: pET28b, 585bp

Constructing SM003, SM012, SM013

Gibson assembly	(operator: Diana)	
Backbone		
Fragment 1	pET28b-1	1.2
Fragment 2	pET28b-2	1.8

Fragment 3	SM003 Fr3	1
Fragment 4	SM003 Fr4	1
H_2O		Add to 5
GA mix		5
All		10

Backbone		
Fragment 1	pET28b-1	1.5
Fragment 2	pET28b-2	2.5
Fragment 3	SM012 Fr3	1
H_2O		Add to 5
GA mix		5
All		10

Backbone		
Fragment 1	pET28b-1	1
Fragment 2	pET28b-2	1.5
Fragment 3	SM003 Fr3	0.8
Fragment 4	SM005 Fr4	1
Fragment 5	SM004 Fr4	0.7
H_2O		Add to 5
GA mix		5
All		10

Process		
1	50	60min
2	12	∞

Transformation (operator: Diana，Uranium)

□ Electroporation1μL
√Chemical transformation10μL

Host: DH5 alpha
Plasmid to construct: SM003, SM012, SM013

Recovery medium: LB
Volume of bacterial liquid to spread：200ul
Agar plate and antibiotic to use: LB, Kan
Transformation conditions：heat shock at 42°C for 90s

Construction of SM005 and SM007

Gibson assembly	(operator: XYZ)	
Backbone		
Fragment 1	pET28b-1	1
Fragment 2	pET28b-2	2
Fragment 3	SM005 Fr3	2
Fragment 4	SM005 Fr4	2.5
Fragment 5	SM005 Fr5	2.5
H_2O		Add to 10
GA mix		10
All		20

Backbone		
Fragment 1	pET28b-1	0.5
Fragment 2	pET28b-2	1.5
Fragment 3	SM005 Fr3	1

Fragment 4	SM005 Fr4	3.5
Fragment 5	SM005 Fr5	3.5
H_2O		Add to 10
GA mix		10
All		20

Backbone		
Fragment 1	pET28b-1	1
Fragment 2	pET28b-2	2
Fragment 3	SM007 Fr3	1
Fragment 4	SM007 Fr4	6
H_2O		Add to 10
GA mix		10
All		20

Process		
1	50	60min
2	12	∞

Transformation (operator: XYZ)

□ Electroporation1μL
√Chemical transformation20μL

Host: DH5 alpha
Plasmid to construct: SM005, SM007

Recovery medium: LB
Volume of bacterial liquid to spread：300ul
Agar plate and antibiotic to use: LB, Kan
Transformation conditions：heat shock at 42°C for 90s

Culture preservation of Se004 (Start)

Inoculation (operator: Diana)

From
√Agar plates
□Glycerol stock
□Liquid starter

Strain	Se004
Antibiotics	Amp
Medium	LB
Volume	3mL in 15mL tube, single colony
Temperature	37
Number	1

7.16 (Tues)

Construction of SM004, SM006, SM011

PCR Template: (operator: Diana)

□Purified plasmid
□Purified genome
√Single colony
□Bacteria liquid
□Boiled Bacteria cell lysate

PCR for verification		
Primer 1	SP004-F	1

Primer 2	SP004-R	1
Template	SM004 colony	
H2O		13
DNA polymerase	Taq Mix	15
All		30
		30/tube×2
Product length	1551bp	

PCR for verification		
Primer 1	SP004-F	1
Primer 2	SP004-R	1
Template	SM006 colony	
H2O		13
DNA polymerase	Taq Mix	15
All		30
		30/tube×2
Product length	1779bp	

PCR for verification		
Primer 1	SP004-F	1
Primer 2	SP004-R	1
Template	SM011colony	
H2O		13
DNA polymerase	Taq Mix	15
All		30
		30/tube×2
Product length	1446bp	

Process		
1	94	10min
2	94	30s
3	55	30s
4	72	1min
Go to 2	35 cycle	
5	72	5min
6	16	∞

control: pET28b, 585bp

Inoculation (operator: Diana)

From
√Agar plates
□Glycerol stock
□Liquid starter

Strain	DH5alpha {SM004(1, 2), SM006(3, 4), SM011(5)}
Antibiotics	Kan
Medium	LB
Volume	4mL in 15mL tube, single colony
Temperature	37
Number	5

Construction of SM003, SM012, SM013

Construction of SM005, SM007

Number of single colonies on plates

SM003: 9
SM012: 6
SM013: 9
SM007: 5
SM005: 0
SM011: 0

Culture preservation of Se004 (Done)

Preparing bacterial glycerol stocks(operator: Diana)

Strain name：DH5α (pMV-Cfa[C]-Cfa[N]}
Number in stocks: S014

7.17 (Wed)

Construction of SM003, SM012, SM013

Construction of SM005, SM007

Colony selection (operator: Diana)

From
√Agar plates
□Glycerol stock
□Liquid starter

Strain	DH5alpha{SM003, SM012, SM013, SM007}
Antibiotics resistance	Kan
Medium	LB
Volume	Inoculating single colonies on plates
Temperature	37
Number	2 for each, 8 in total

PCR Template (operator: Diana)

□Purified plasmid
□Purified genome
√Single colony
□Bacteria liquid
□Boiled Bacteria cell lysate

PCR for verification		

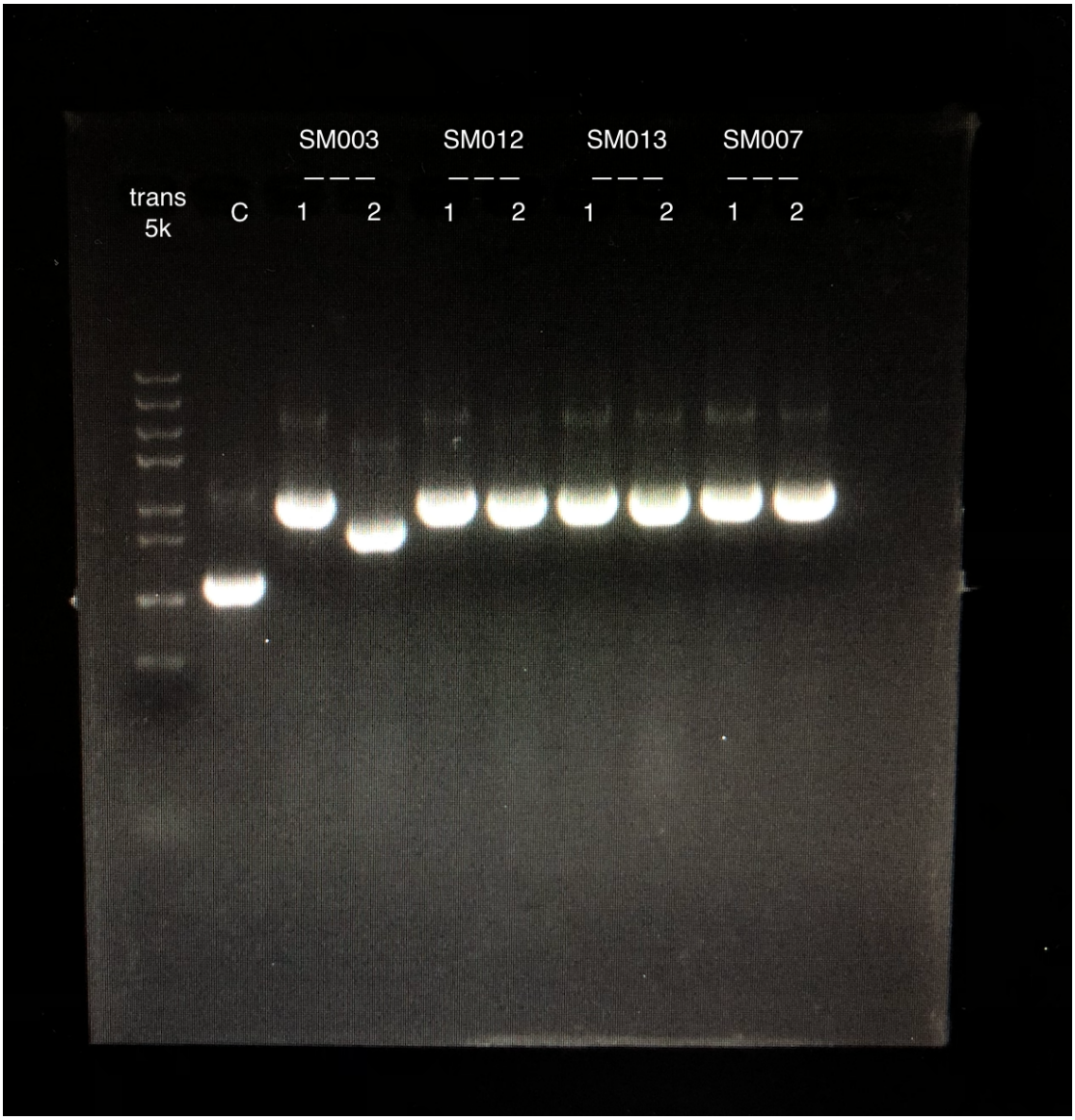
Primer 1	SP004-F	1
Primer 2	SP004-R	1
Template	SM003 colony	
H2O		13
DNA polymerase	Taq Mix	15
All		30
		30/tube×2
Product length	852bp	

Primer 1	SP004-F	1
Primer 2	SP004-R	1
Template	SM012 colony	
H2O		13
DNA polymerase	Taq Mix	15
All		30
		30/tube×2
Product length	1473bp	

Primer 1	SP004-F	1
Primer 2	SP004-R	1
Template	SM013 colony	
H2O		13
DNA polymerase	Taq Mix	15
All		30
		30/tube×2
Product length	1143bp	

Primer 1	SP004-F	1
Primer 2	SP004-R	1
Template	SM007 colony	
H2O		13
DNA polymerase	Taq Mix	15
All		30
		30/tube×2
Product length	1209bp	

Process		
1	94	10min
2	94	30s
3	55	30s
4	72	1min
Go to 2	35 cycle	
5	72	5min
6	16	∞



Construction of SM015 (Start)

PCR Template (Operator: Adonie)

- Purified plasmid
- Purified genome
- Single colony
- Bacteria liquid
- Boiled Bacteria cell lysate

Fragment 1, rTaq

Components		
Primer 1	SP020	1.5
Primer 2	SP021	1.5
Template	SM015 (pACYC-duet1-mTyr-CNK)	0.5
H2O		10.5
DNA polymerase	Taq Mix	15
All		30
		30
Product length	1973 bp	

Fragment 2, rTaq

Components		
Primer 1	SP019	1.5
Primer 2	SP022	1.5
Template	SM015 (pACYC-duet1-mTyr-CNK)	0.5
H2O		10.5
DNA polymerase	Taq Mix	15
All		30
		30
Product length	2952 bp	

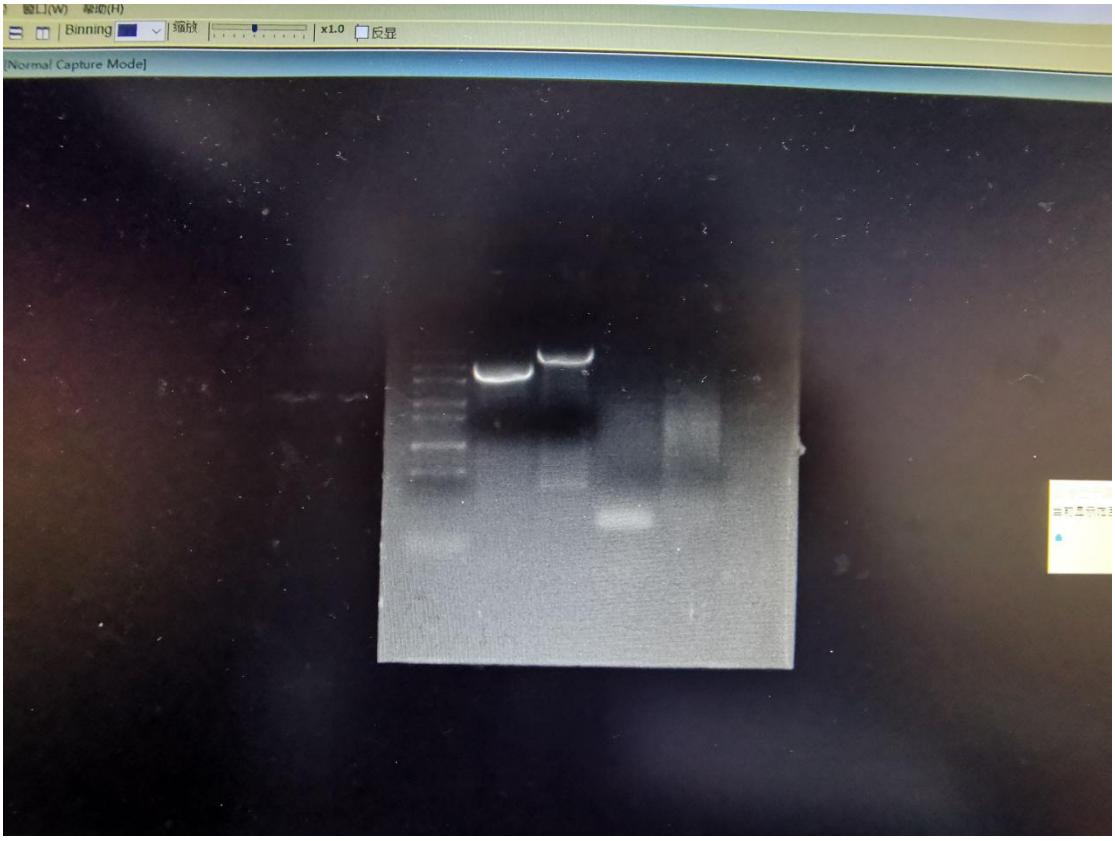
Fragment 1, FastPfu

Components		
Primer 1	SP020	1.5
Primer 2	SP021	1.5
Template	SM015 (pACYC-duet1-mTyr-CNK)	0.5

H2O		10.5
DNA polymerase	FastPfu 2x Mix	15
All		30
		30
Product length	1973 bp	

Fragment 2, FastPfu

Components		
Primer 1	SP019	1.5
Primer 2	SP022	1.5
Template	SM015 (pACYC-duet1-mTyr-CNK)	0.5
H2O		10.5
DNA polymerase	FastPfu 2x Mix	15
All		30
		30
Product length	2952 bp	



Transformation of Sy003, SM002, SM008, SM010 into Rosetta（Start）

Transformation (operator: Diana)

□Electroporation1μL
√Chemical transformation1μL

Host: Rosetta(DE3)
Plasmid to construct：Sy003, SM002, SM008, SM010

Recovery medium：LB
Volume of bacterial liquid to spread：200 μL
Agar plate and antibiotic to use：LB，Cm，Kan
Transformation conditions：heat shock at 42°C for 90s

Construction of SM004, SM006, SM011

Preparing bacterial glycerol stocks(operator: Adonie)

Strain name: SM004, SM006, SM011
Number in stocks：S015, S016, S017

Collection of cell pellet
Strain Name: SM004, SM006, SM011

- Centrifuge at 12000 rpm for 2 min and discard waste fluid.
- Cell pellets are stored at -20°C

Sequencing (operator: Diana)

Sample	Primer	Requirements
□bacterial liquid □Plasmid □PCR purified √PCR unpurified	SP004-F SP004-R	□ Forward □ Reverse √ Both

SM004 (1): 0 mismatches, 0 gaps/insertions. Correct.

SM004 (2): 0 mismatches, 2 gaps/insertions. Correct.

SM006 (3): Not of sufficient quality to be used for alignment.

SM006 (4): 1 mismatch, 1 gap/insertion(1 Mfp5 absent).

SM011 (5): 1 mismatch, 3 gaps/insertions(1 Mfp3 absent)

7.18 (Thur)

Construction of SM015

Gel extraction (operator: Diana)

√Gel extraction
□Liquid extraction
Elution buffer
☒EB
□H2O
Elution volume: 40ul
Name: SM015(1), SM015(2)
Concentration(A260/280nm)：51.8ng/ul, 41.9ng/ul

Gibson assembly	(operator: Diana)	
Backbone		
Fragment 1	SM015-1	2
Fragment 2	SM015-2	3
H_2O		Add to 5
GA mix		5
All		10
Process		
1	50	60min
2	12	∞

Transformation (operator: Uranium)

□Electroporation1μL
√Chemical transformation10μL

Host: DH5alpha，100 μL
Plasmid to construct：SM015

Recovery medium：LB，300 μL

Volume of bacterial liquid to spread：200 μL
Agar plate and antibiotic to use：LB，Cm
Transformation conditions：heat shock at 42°C for 90s

Transformation of Sy003, SM002, SM008, SM010 into Rosetta

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

Strain	Rosetta {Sy003, SM002, SM008, SM010}
Antibiotics	Kan, Cm
Medium	LB
Volume	4mL in 15mL tube, single colony
Temperature	37
Number	4

Culture preservation of Sy003

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

Strain	DH5α {Sy003}
Antibiotics	Kan
Medium	LB

Volume	4mL in 15mL tube, single colony
Temperature	37
Number	1

Culture preservation of Sy002 (Done)

Preparing bacterial glycerol stocks(operator: Diana)
Strain name：DH5α {pACYC-duet1-mTyr-CNK-7his}
Number in stocks：S018

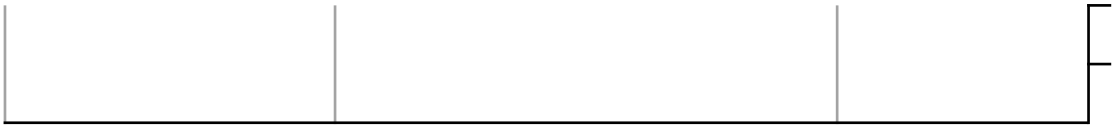
7.19 (Fri)

Construction of SM003, SM012, SM013

Construction of SM005, SM007

Sequencing (operator: Diana)

Sample	Primer	Requirements
□Bacterial liquid □Plasmid □PCR purified √PCR unpurified	SP004-F SP004-R	□ Forward □ Reverse √ Both



SM003: 1 mismatch, 3 gaps/insertions. Mismatch due to mutation.

SM012: Mfp5-Cfa[N] absent.

SM013: Cfa[C]-Mfp5-Cfa[N] absent.

SM007: 1 mfp3 absent.

Transformation of Sy003, SM002, SM008, SM010 into Rosetta（Done）

Preparing bacterial glycerol stocks(operator: XYZ)

Strain name：Rosetta{Sy003, SM002, SM008, SM010}

Number in stocks：S016, S017, S019, S020

Culture preservation of Sy003（Done）

Preparing bacterial glycerol stocks(operator: XYZ)

Strain name：DH5α{Sy003}

Number in stocks：S021

7.22 (Mon)

Construction of SM003

Colony selection (operator: Adonie)

From

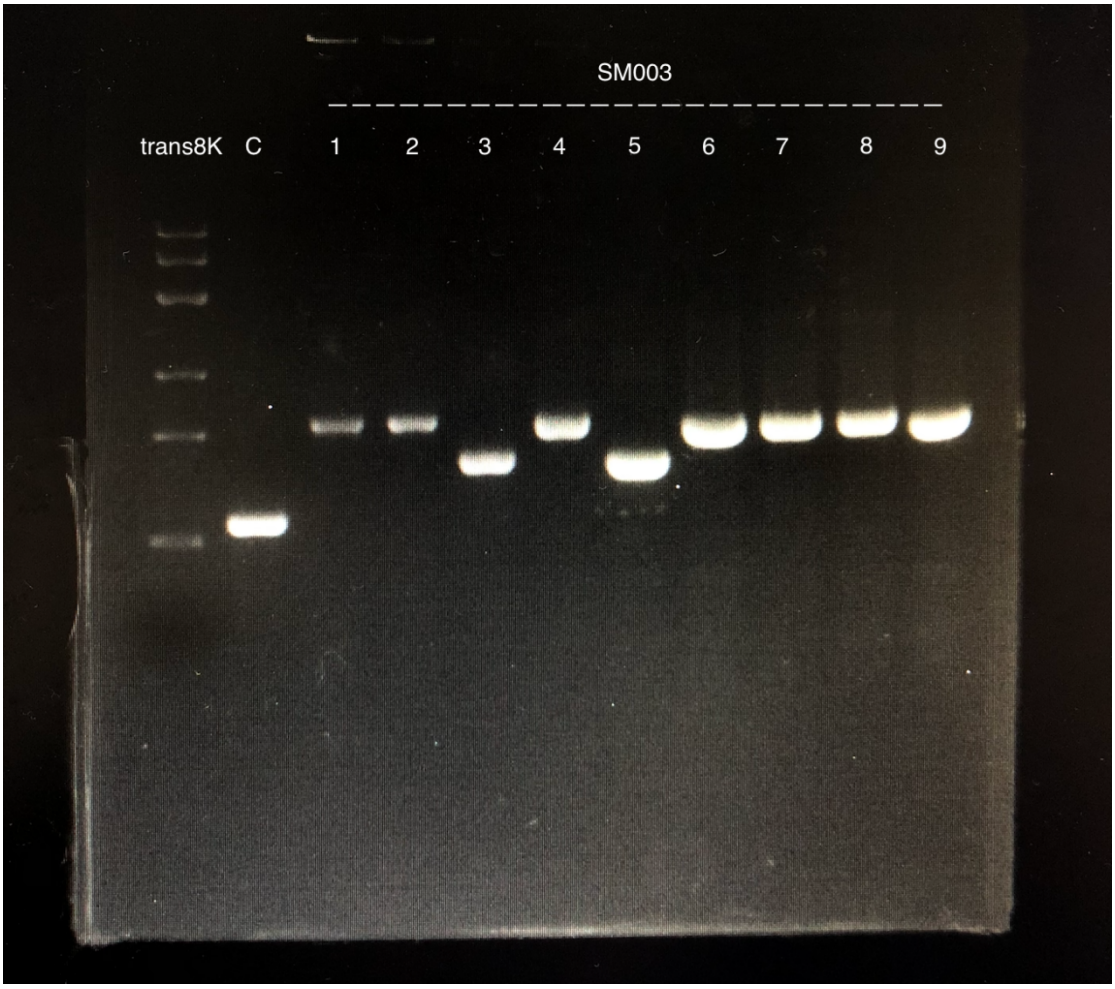
√Agar plates

□Glycerol stock
□Liquid starter

Strain	DH5alpha{SM003}
Antibiotics resistance	Kan
Medium	LB
Volume	Inoculating single colonies on plates
Temperature	4
Number	9

PCR Template (operator: Adonie)
□Purified plasmid
□Purified genome
√Single colony
□Bacteria liquid
□Boiled Bacteria cell lysate

PCR for verification		
Primer 1	SP004-F	1
Primer 2	SP004-R	1
Template	SM003 colony	
H2O		13
DNA polymerase	Taq Mix	15
All		30
		30/tube×8
Product length	852 bp	



Transformation of SM004 into BL21(DE3)（Start）

Plasmid extraction (operator: Adonie)

strain	DH5α {SM004}
plasmid	SM004

Elution buffer
√ EB
□ H2O
Elution volume: 50ul
Concentration:156.3ng/ul

Transformation (operator: Diana)
□ Electroporation1μL

√Chemical transformation 0.5μL

Host: BL21(DE3), 100 μL
Plasmid to construct：suicide plasmid（P194→Plux）

Recovery medium：LB, 300 μL
Volume of bacterial liquid to spread：400 μL
Agar plate and antibiotic to use：LB, Kan
Transformation conditions：heat shock at 42°C for 90s

Construction of SM012, SM013（Start）

Gibson assembly	(operator: Diana)	
Backbone		
Fragment 1	pET28b-1	0.5
Fragment 2	pET28b-2	1.5
Fragment 3	SM012 Fr3	1.7
Fragment 4	SM004 Fr4	1.3
H_2O		Add to 5
GA mix		5
All		10

Backbone		
Fragment 1	pET28b-1	0.5
Fragment 2	pET28b-2	1.2
Fragment 3	SM003 Fr3	1.1
Fragment 4	SM005 Fr4	1.2
Fragment 5	SM004 Fr4	1

H_2O		Add to 5
GA mix		5
All		10

Process		
1	50	60min
2	12	∞

Transformation (operator: Diana)
□Electroporation1μL
√Chemical transformation10μL

Host: DH5alpha，100 μL
Plasmid to construct：SM012, SM013
Recovery medium：LB，300 μL
Volume of bacterial liquid to spread：200 μL
Agar plate and antibiotic to use：LB，Kan
Transformation conditions：heat shock at 42°C for 90s

7.23 (Tues)

Construction of SM014（Start)

PCR Template (operator: Diana)
□Purified genome
√Purified plasmid
□Single colony
□Bacteria liquid
□Boiled Bacteria cell lysate

rTaq

PCR		

Primer 1	SP016	1.5
Primer 2	SP017	1.5
Template	Sy005	0.5(30ng/ul)
H2O		11.5
DNA polymerase	rTaq Mix	15
All		30
		30/tube×1
Product length	581bp	

Primer 1	SP015	1.5
Primer 2	SP005-Mfp5-R	1.5
Template	SM010	0.5(77.9ng/ul)
H2O		11.5
DNA polymerase	rTaq Mix	15
All		30
		30/tube×1
Product length	301bp	

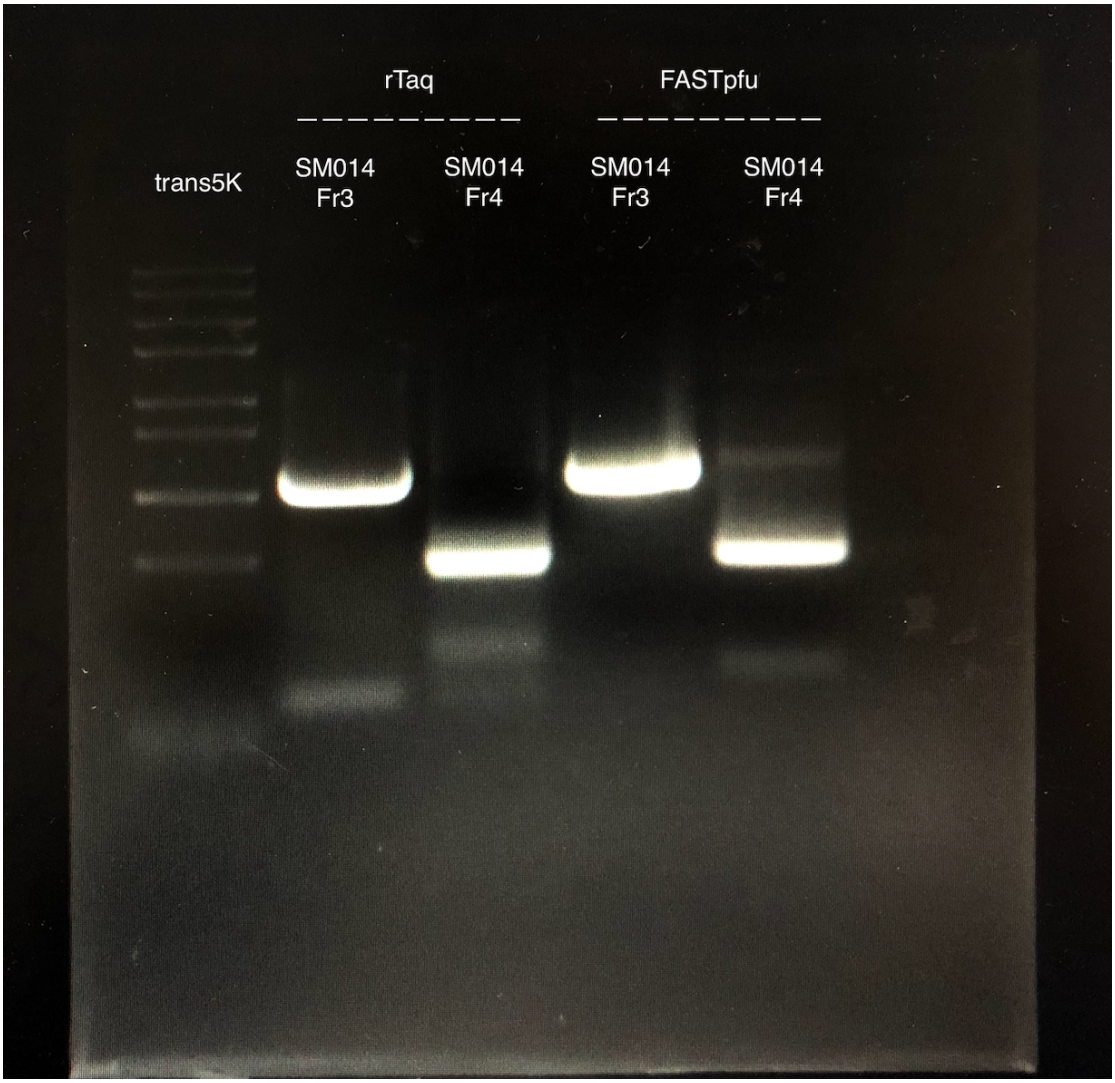
Process		
1	94	10s
2	94	30s
3	55	30s
4	72	30s
Go to 2	35 cycle	
5	72	2min
6	12	∞

FASTpfu

PCR		
Primer 1	SP016	1
Primer 2	SP017	1
Template	Sy005	0.5(30ng/ul)
H2O		18.5
DNA polymerase	FASTpfu Mix	9
All		30
		30/tube×1
Product length	581bp	

Primer 1	SP015	1
Primer 2	SP005-Mfp5-R	1
Template	SM010	0.5(77.9ng/ul)
H2O		18.5
DNA polymerase	FASTpfuMix	9
All		30
		30/tube×1
Product length	301bp	

Process		
1	98	1min
2	98	30s
3	55	30s
4	72	30s
Go to 2	32 cycle	
5	72	2min
6	12	∞



Gel extraction (operator: Adonie)

√Gel extraction
□Liquid extraction

Elution buffer
☒EB
□H2O

Elution volume: 30ul
Name：SM014 Fr3
Concentration(A260/280nm)：37.3ng/ul
Name：SM014 Fr4
Concentration(A260/280nm)：8.35ng/ul

Gibson assembly	(operator: Diana)	
Backbone		
Fragment 1	pET28b-1	0.5
Fragment 2	pET28b-2	1.3
Fragment 3	SM014 Fr3	0.5
Fragment 4	SM014 Fr4	2.7
H_2O		Add to 5
GA mix		5
All		10
Process		
1	50	60min
2	12	∞

Transformation (operator: Uranium, Diana)
□Electroporation1μL
√Chemical transformation10μL

Host: DH5alpha
Plasmid to construct：SM014

Recovery medium：LB, 300uL
Volume of bacterial liquid to spread：200 μL
Agar plate and antibiotic to use：LB，Kan
Transformation conditions：heat shock at 42°C for 90s

Construction of SM015

Gibson assembly	(operator: Diana)	
Backbone		

Fragment 1	SM015 Fr1	1.8
Fragment 2	SM015 Fr2	3.2
H_2O		Add to 5
GA mix		5
All		10
Process		
1	50	60min
2	12	∞

Transformation (operator: Uranium)
□Electroporation1μL
√Chemical transformation10μL

Host: Rosetta(DE3)
Plasmid to construct：SM015

Recovery medium：LB，300ul
Volume of bacterial liquid to spread：200 μL
Agar plate and antibiotic to use：LB，Cm
Transformation conditions：heat shock at42°C for 90s

Construction of SM003

Sequencing (operator: Diana)

Sample	Primer	Requirements
□菌液 □质粒 □PCR纯化 √PCR未纯化	SP004-F SP004-R	□ Forward □ Reverse √ Both

3: 1 mismatch, 1 insertion, no mutation, correct.

4:

5: 1 mismatch due to mutation, 2 gaps, incorrect.

Inoculation (operator: Uranium)

From

√Agar plates

□Glycerol stock

□Liquid starter

Strain	SM003
Antibiotics	Kan
Medium	LB
Volume	3mL in 15mL tube, single colony
Temperature	37
Number	1

Transformation of SM004 into BL21(DE3)

Number of single colonies on plates (operator:Diana)：3

Inoculation (operator: Uranium)

From

√Agar plates

□Glycerol stock

□Liquid starter

Strain	SM004
Antibiotics	Kan
Medium	LB

Volume	3mL in 15mL tube, single colony
Temperature	37
Number	1

Construction of SM012, SM013

Number of single colonies on plates (operator:Diana)： 0 on both plates

7.24 (Wed)

Construction of SM014

Number of single colonies on plates (operator:Diana)：0

Construction of SM015

Gibson assembly	(operator: Diana)	
Backbone		
Fragment 1	SM015 Fr1	1.8
Fragment 2	SM015 Fr2	3.2
H_2O		Add to 5
GA mix		5
All		10

Process		
1	50	60min
2	12	∞

Transformation (operator: Darcy)
□Electroporation1μL
√Chemical transformation10μL

Host: DH5alpha
Plasmid to construct：SM015
Recovery medium：LB，300ul
Volume of bacterial liquid to spread：200 μL
Agar plate and antibiotic to use：LB，Cm
Transformation conditions：heat shock at 42°C for 90s

Transformation of SM003 into BL21(DE3)

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

Strain	SM003(S022)
Antibiotics	KAN
Medium	LB
Volume	3mL in 15mL tube, single colony
Temperature	37
Number	1

Culture preservation of Se005

Inoculation (operator: Uranium)

From
√Agar plates
□Glycerol stock
□Liquid starter

Strain	SE005
Antibiotics	KAN
Medium	LB
Volume	3mL in 15mL tube, single colony
Temperature	37
Number	1

Transformation of Se005 into rosetta

Transformation (operator: Darcy)

□Electroporation1μL
√Chemical transformation10μL

Host: BL21(DE3) rosetta
Plasmid to construct：Se005
Recovery medium：LB，300ul
Volume of bacterial liquid to spread：100 μL
Agar plate and antibiotic to use：LB，Kan
Transformation conditions：heat shock at 42°C for 90s

Culture preservation of SM003（Done)

Preparing bacterial glycerol stocks (operator: Diana)

Strain name：DH5α {SM003}
Number in stocks：S022

Transformation of SM004 into BL21(DE3)（Done）

Preparing bacterial glycerol stocks (operator: Diana)
Strain name: BL21(DE3){SM004}
Number in stocks：S023

7.25 (Thur)

Construction of SM015

Number of single colonies on plates (operator:Diana)：0

Transformation of SM003 into BL21(DE3)

Plasmid extraction (operator: Diana，Adonie)

strain	DH5α {SM003}
plasmid	SM003

Elution buffer
√TB
□H2O

Elution volume：50uL
Concentration(A260/280)：80.75ng/μL

Transformation (operator: Diana)
□Electroporation1μL

√Chemical transformation10μL

Host: BL21(DE3)
Plasmid to construct：SM003

Recovery medium：LB，300ul
Volume of bacterial liquid to spread：300 μL
Agar plate and antibiotic to use：LB，Kan
Transformation conditions：heat shock at 42°C for 90s

Construction of SM012, SM013

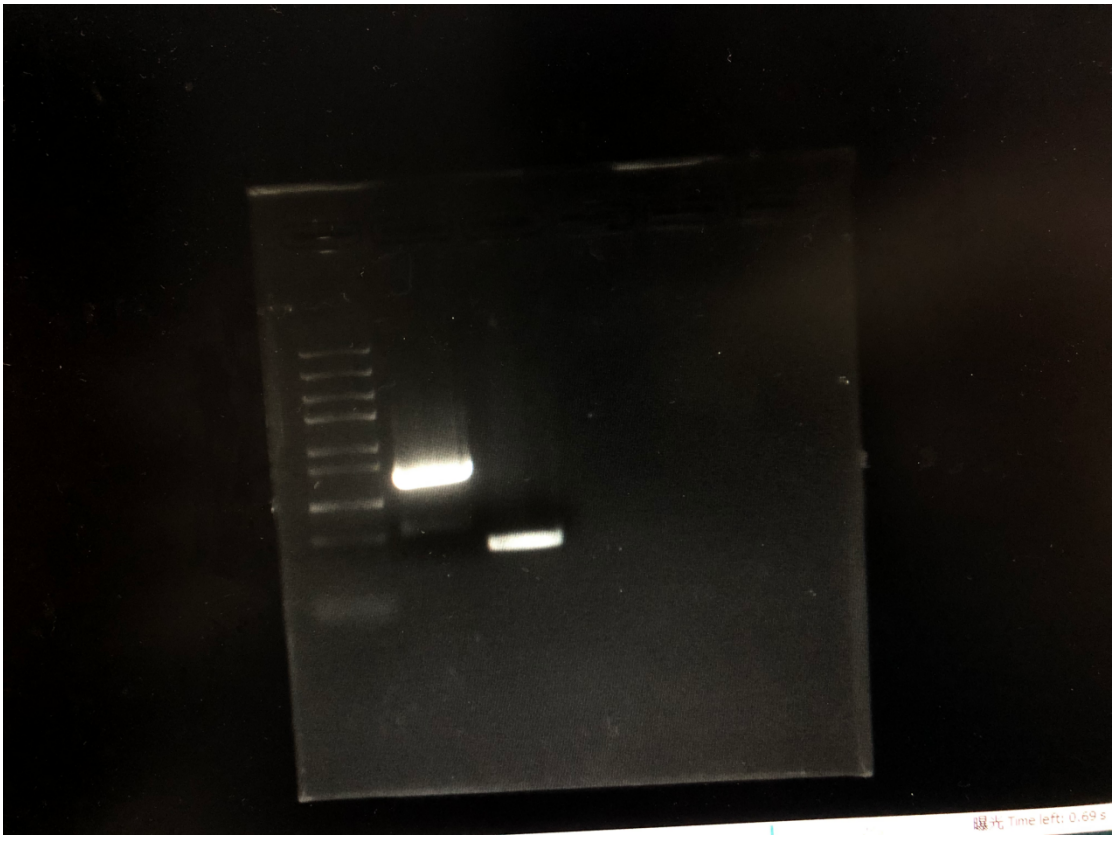
PCR Template (operator: Diana，Adonie)
□Purified genome
√Purified plasmid
□Single colony
□Bacteria liquid
□Boiled Bacteria cell lysate

PCR		
Primer 1	SP014	1
Primer 2	SP001 CsgA-Mfp5-R	1
Template	SM010	0.5
H2O		18.5
DNA polymerase	FASTpfu	9
All		30
		30/tube×1
Product length	690bp	

Primer 1	SP005 Mfp5-CfaC-F	1

Primer 2	SP001 CsgA-Mfp5-R	1
Template	SM010	0.5
H2O		18.5
DNA polymerase	FASTpfu	9
All		30
		30/tube×1
Product length	228bp	

Process		
1	98	1min
2	98	30s
3	55	30s
4	72	30s
Go to 2	35 cycle	
5	72	2min
6	12	∞



Gel extraction (operator: Adonie)

Gel extraction
□Liquid extraction

Elution buffer:
EB
□H2O

Elution volume: 30ul
Name: SM012 Fr3
Concentration(A260/280nm): 25.75ng/ul

Gibson assembly	(operator: Darcy)	
Backbone		
Fragment 1	PET28b-1	1
Fragment 2	PET28b-2	2.2
Fragment 3	SM012 Fr3	1
Fragment 4	SM004 Fr4	0.8

GA mix		5
All		10
Process		
1	50	60min
2	12	∞

Transformation (operator: Diana)
□Electroporation1μL
□Chemical transformation10μL

Host: DH5 alpha
Plasmid to construct：SM012

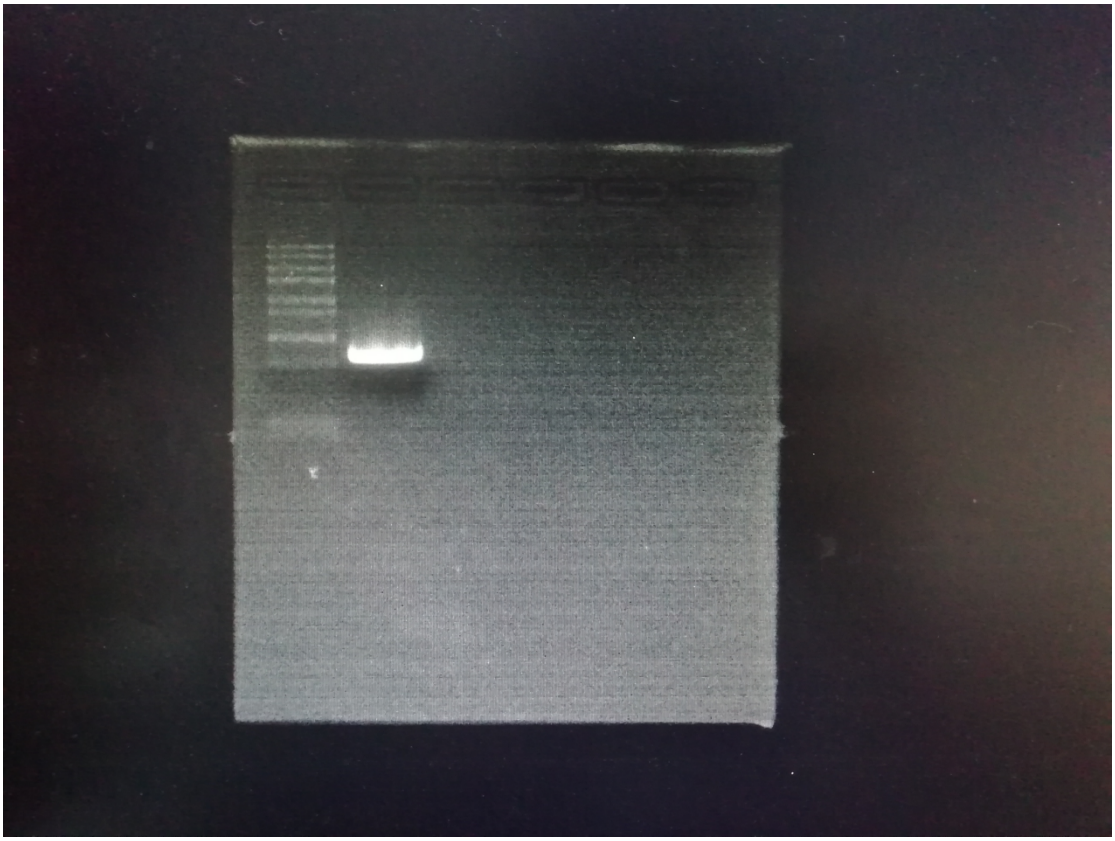
Recovery medium：LB, 300ul
Volume of bacterial liquid to spread：200 μL
Agar plate and antibiotic to use：LB，Kan
Transformation conditions：heat shock at 42°C for 90s

Construction of SM012, SM013

PCR Template (operator: Diana)
□Purified genome
√Purified plasmid
□Single colony
□Bacteria liquid
□Boiled Bacteria cell lysate

PCR		
Primer 1	SP023	1
Primer 2	SP001 CsgA-Mfp5-R	1
Template	SM010	0.5

H2O		18.5
DNA polymerase	FASTpfu	9
All		30
		30/tube×1
Product length	370bp	



Gel extraction (operator: Adonie)

Gel extraction
□Liquid extraction
Elution buffer
EB
□H2O
Elution volume：30 ul
Name：SM013 Fr (3, 4)
Concentration(A260/280nm)：17.15ng/ul

Culture preservation of Se005（Done）

Preparing bacterial glycerol stocks(operator: Diana)
Strain name：DH5α {Se005}
Number in stocks：S024

Transformation of Se005 into rosetta

Number of single colonies on plates (operator:Diana)：～100

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

Strain	BL(DE3){Se005}
Antibiotics	Cm, Kan
Medium	LB
Volume	4mL in 15mL tube, single colony
Temperature	37
Number	2

7.26 (Fri)

Construction of SM012, SM013

Number of single colonies on plates (operator:Diana)：0

Construction of SM012, SM013

Gibson assembly	(operator: Diana)	
Backbone		
Fragment 1	pET28b-1	0.7
Fragment 2	pET28b-2	1.8
Fragment 3	SM013 Fr3+4	1.5
Fragment 4	SM004 Fr4	1
H_2O		Add to 5
GA mix		5
All		10
Process		
1	50	60min
2	12	∞

Transformation (operator: Diana)
□Electroporation1μL
√Chemical transformation10μL

Host: DH5alpha
Plasmid to construct：SM013

Recovery medium：LB，300ul
Volume of bacterial liquid to spread：200 μL
Agar plate and antibiotic to use：LB，Kan
Transformation conditions：heat shock at 42°C for 90s

Transformation of SM003 into BL21(DE3)

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

Strain	BL21(DE3) {SM003}
Antibiotics	Kan
Medium	LB
Volume	3mL in 15mL tube, single colony
Temperature	37
Number	1

Transformation of Se005 into rosetta（Done)

Preparing bacterial glycerol stocks(operator: Diana)
Strain name：BL21(DE3) rosetta {Se005}
Number in stocks：S025

7.27 (Sat)

Transformation of SM003 into BL21(DE3) (Done)

Preparing bacterial glycerol stocks(operator: Emma)
Strain name：BL21(DE3) {SM003}
Number in stocks：S026

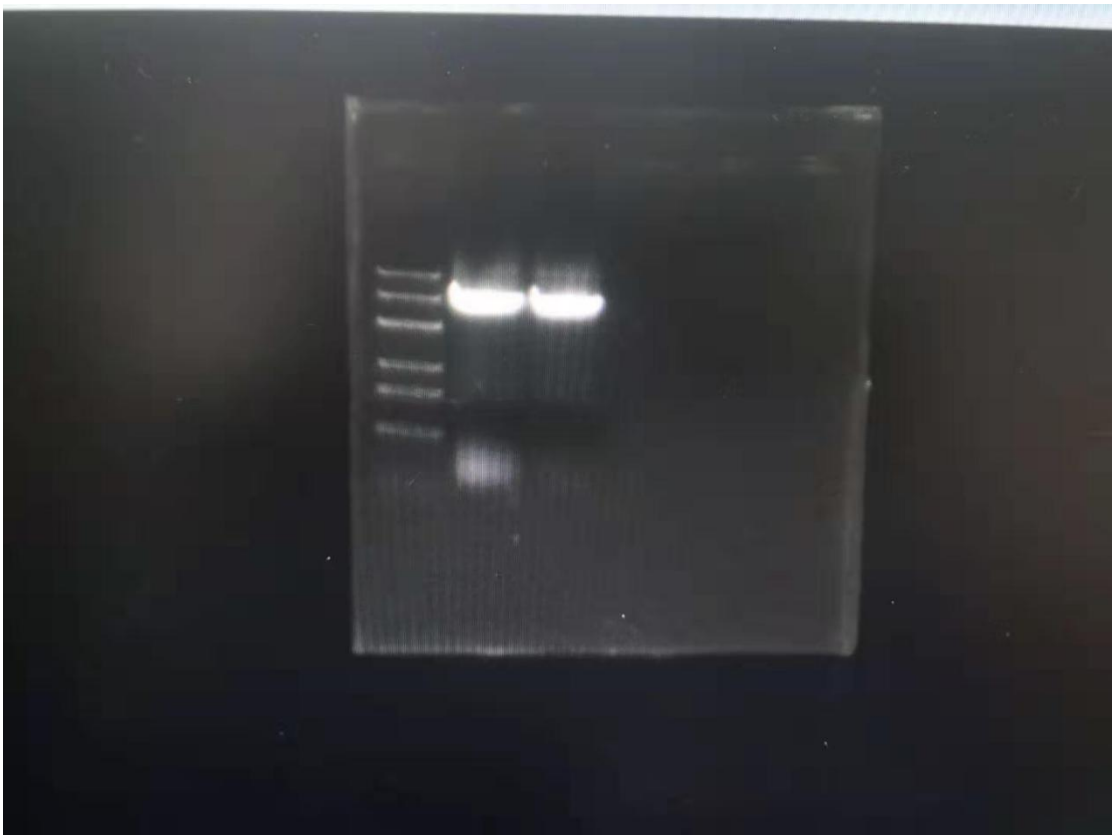
7.29 (Mon)

Contruction of SM015

PCR Template (operator: Cherie, Uranium)

□Purified genome
√Purified plasmid
□Single colony
□Bacteria liquid
□Boiled Bacteria cell lysate

PCR		
Primer 1	SP019	1
Primer 2	SP020	1
Template	SY002	1
H2O		30
DNA polymerase	FASTpfu	15
MgSO4		2
All		50
Product length	bp	4893



Gel extraction (operator: Cherie)

Gel extraction

□Liquid extraction

Elution buffer

□EB

H2O

Elution volume：50 ul

Name：SM015

Concentration(A260/280nm)：58.5ng/ul

6.3.Self-ligation of PCR segments (PCR片段自连)

Self-ligation (operator: Uranium)

10X T4 DNA Ligase Buffer NEB1

Fragment SM015 8

T4 DNA Ligase NEB0.5

T4 PNK NEB0.5

All 10

Process

1 25 120min

2 12 ∞

7.30(Tues)

Chemical transformation(化转)

Transformation of SM015 (operator: Uranium)

□Electroporation 1 μL
√Chemical transformation 10μL

Host: DH5a，100 μL
Plasmid to construct：SM015

Recovery medium：LB，300 μL
Volume of bacterial liquid to spread：200 μL
Agar plate and antibiotic to use：LB，Cm
Transformation conditions：heat shock at 42°C for 90s

7.31(Wed)

Transformation of SM015 (operator: Uranium)

1.Inoculation (接种)

Inoculation (operator: Uranium)

From
√ Agar plates
□Glycerol stock
□Liquid starter
Strain SM015
Antibiotics Cm
MediumLB
Volume purify
Temperature 37
Number 1

4.1.Simple PCR

Constuction of SM004 SM006 SM011

PCR Template (operator: Uranium)

□Purified genome
√Purified plasmid
□Single colony

□Bacteria liquid
□Boiled Bacteria cell lysate

PCR
Primer 1 SP035
Primer 2 SP034
Template SM010
H2O 30+2(MgSO4)
DNA polymerase FastPfu mix15
All 50

Product length 5960 bp
Process
1 95 1min
2 95 20s
3 55 20s
4 72 210s
Go to 2 32 cycle
5 72 2min
6 12 ∞

PCR
Primer 1 SP040
Primer 2 SP034
Template SM010
H2O 30+2(MgSO4)
DNA polymerase FastPfu mix15
All 50

Product length 6251 bp
Process
1 95 1min
2 95 20s
3 55 20s
4 72 210s
Go to 2 32 cycle
5 72 2min
6 12 ∞

PCR SM007 Fr1
Primer 1 SP029
Primer 2 SP028
Template SM008
H2O 30+2(MgSO4)
DNA polymerase FastPfu mix15
All 50

Product length 5870 bp
Process
1 95 1min

2 95 20s
3 55 20s
4 72 210s
Go to 2 32 cycle
5 72 2min
6 12 ∞

PCR SM011 Fr1
Primer 1 SP029
Primer 2 SP028
Template SM008
H2O 30+2(MgSO4)
DNA polymerase FastPfu mix15
All 50

Product length 5870 bp
Process
1 95 1min
2 95 20s
3 55 20s
4 72 210s
Go to 2 32 cycle
5 72 2min
6 12 ∞

PCR
Primer 1 SP036
Primer 2 SP037
Template SM010
H2O 32
DNA polymerase FastPfu mix15
All 50

Product length 256 bp
Process
1 95 1min
2 95 20s
3 55 20s
4 72 30s
Go to 2 32 cycle
5 72 2min
6 12 ∞

PCR
Primer 1 SP038
Primer 2 SP039
Template SM010
H2O 32
DNA polymerase FastPfu mix15

All 50

Product length 250 bp
Process
1 95 1min
2 95 20s
3 55 20s
4 72 30s
Go to 2 32 cycle
5 72 2min
6 12 ∞

PCR
Primer 1 SP036
Primer 2 SP042
Template SM004
H2O 32
DNA polymerase FastPfu mix15
All 50

Product length 256 bp
Process
1 95 1min
2 95 20s
3 55 20s
4 72 30s
Go to 2 32 cycle
5 72 2min
6 12 ∞

PCR
Primer 1 SP030
Primer 2 SP031
Template SM008
H2O 32
DNA polymerase FastPfu mix15
All 50

Product length 166 bp
Process
1 95 1min
2 95 20s
3 55 20s
4 72 30s
Go to 2 32 cycle
5 72 2min
6 12 ∞

PCR

Primer 1 SP030
Primer 2 SP031
Template SM008
H2O 32
DNA polymerase FastPfu mix15
All 50

Product length 166 bp
Process
1 95 1min
2 95 20s
3 55 20s
4 72 30s
Go to 2 32 cycle
5 72 2min
6 12 ∞

PCR
Primer 1 SP032
Primer 2 SP033
Template SM008
H2O 32
DNA polymerase FastPfu mix15
All 50

Product length 160 bp
Process
1 95 1min
2 95 20s
3 55 20s
4 72 30s
Go to 2 32 cycle
5 72 2min
6 12 ∞

8.1(Thur)

Constuction of SM004 006 011

Gel extraction (operator: Uranium)

√Gel extraction
□Liquid extraction

Elution buffer
☒EB
□H2O

Elution volume：30 μL
Name：SM005fr1，SM006fr1，SM005fr2，SM005fr3，SM006fr2，SM007fr2，SM011fr2，SM011fr3
Concentration(ng/ul)：10，40，20，35，20，10，20，15

PCR Template (operator: Uranium)
□Purified genome
√Purified plasmid
□Single colony
□Bacteria liquid
□Boiled Bacteria cell lysate

PCR
Primer 1 SP028
Primer 2 SP029
Template SM008
H2O　30+2MgSO44
DNA polymerase Fastpfu Mix 15
All　50x2

Product length 5870 bp
Process
1 95 1min
2 95 20s
3 55 20s
4 72 30s
Go to 2 7 cycle
5 95 20s
6 58 20s
7 72 30s
Go to 5 25 cycle
5 72 5min
6 12 ∞

Colony selection (operator:)
From
√ Agar plates
□Glycerol stock
□Liquid starter

Strain SM015
Antibiotics resistance Cm
Medium LB
Volume Inoculating single colonies on plates
Temperature 37
Number 8

8.1

Gel extraction (operator: Uranium)

Gel extraction

□Liquid extraction

Elution buffer

☒EB

□H2O

Elution volume：30 μL

Name：SM005fr1，SM006fr1，SM005fr2，SM005fr3，SM006fr2，SM007fr2，SM011fr2，SM011fr3

Concentration(ng/ul)：10，40，20，35，20，10，20，15

PCR Template (operator: Uranium)

□Purified genome

√Purified plasmid

□Single colony

□Bacteria liquid

□Boiled Bacteria cell lysate

PCR

Primer 1 SP028

Primer 2 SP029

Template SM008

H2O 30+2MgSO44

DNA polymerase Fastpfu Mix 15

All 50x2

Product length 5870 bp

Process

1 95 1min

2 95 20s

3 55 20s

4 72 30s

Go to 2 7 cycle

5 95 20s

6 58 20s

7 72 30s

Go to 5 25 cycle

5 72 5min

6 12 ∞

Colony selection (operator:)

From

√ Agar plates

□Glycerol stock

□Liquid starter

Strain SM015

Antibiotics resistance Cm

Medium LB

Volume Inoculating single colonies on plates

Temperature 37

Number 8

8.2

Golden Gate for SM005,SM006

Backbone p Pet28b

Fragment 1 MFP5(both)

Fragment 2 MFP5(SM005)

H2O 1

T4 Ligase Buffer NEB 2

Restriction enzyme BbsI （NEB） 1

T4 Ligase NEB 1

All 20

Process

1 37 5min

2 16 10min

Go to 2 10 cycle

3 37 10min

4 50 5min

5 80 5min

6 12 ∞

Transformation (operator: Uranium)

□Electroporation 1 μL

√Chemical transformation 10μL

Host:DH5a,50ul each

Plasmid to construct：sm005,sm006

Recovery medium：LB，300 μL

Volume of bacterial liquid to spread：150 μL

Agar plate and antibiotic to use：LB，Kan

Transformation conditions：heat shock at 42°C for 90s

PCR Template (operator: Cherie)

□Purified genome

√Purified plasmid

□Single colony

□Bacteria liquid

□Boiled Bacteria cell lysate

PCR		
Primer 1	SP028	0.6

Primer 2	SP029	0.6
Template	SM008	0.6
H2O		18
DNA polymerase	fastpfu mix	9
All		30
MgSO4		1.2
Product length	5870bp	

PCR		
Primer 1	SP034	0.6
Primer 2	SP035	0.6
Template	SM010	0.6
H2O		18
DNA polymerase	fastpfu mix	9
All		30
MgSO4		1.2
Product length	5960bp	

8.3

PCR Template (operator: Uranium)

□Purified genome

□Purified plasmid

√Single colony

□Bacteria liquid

□Boiled Bacteria cell lysate

PCR

Primer 1 SP004 F 1.5

Primer 2 SP004 R 1.5

Template single colony of SM006

H2O 12

DNA polymerase Q5 Mix 13

25/tube×2

Product length 1681bp

Process

1 94 10min

2 94 30s

3 55 30s

4 72 30s

Go to 2 35cycle

5 72 2min

6 12 ∞

8.6

Preparing bacterial glycerol stocks (operator: Adonie)

Strain name： S027

Number in stocks: 2

8.7

PCR Template (operator: Adonie)

□Purified genome

□Purified plasmid

 Single colony

□Bacteria liquid

□Boiled Bacteria cell lysate

PCR	Name	Volume
Primer 1	SP004	1.5
Primer 2	SP004	1.5
Template	SM005 & SM011 colony	/
H2O		12
DNA polymerase	2x Starmix	15
All		30
Product length	1617bp	

Process	Temperature	Time
1	95	1min
2	95	30s
3	55	30s
4	72	1min 30s
Go to	32 cycle	
5	72	2 min
6	12	∞

Gel Picture

8.8

Preparing bacterial glycerol stocks (operator: Ginny Amy)

Strain name：S028,S029,S030,S031,S032,S033

Number in stocks：2 for each\

PCR Template (operator: Ginny Amy)

□Purified genome

□Purified plasmid

√Single colony

□Bacteria liquid

□Boiled Bacteria cell lysate

PCR x8u

Primer 1 sp004 F 1.5

Primer 2 sp004 R 1.5

Template SM014

H2O 12

DNA polymerase R-taq 15

All 30

Product length 1400 bp

8.9

Sequencing Results Analysis of SM005, SM006 and SM014

Primers: SP004 F/R

SM005:

- Wrong insertion of mfp3 appears in sequencing results
- 8 mismatches

Conclusion: PCR template may be faulty

SM006:

- 4 gaps/insertions

- pET28b isn't loaded（空载）

Conclusion: ???

SM014

- 1 gap in cp19k that could lead to frame shifting（移码）
- Otherwise ok

Conclusion: Specimen number 19 should be sent for sequencing again

8.12

Inoculation (operator:Uranium)

From

□ Agar plates

√Glycerol stock

□Liquid starter

Strain S006,S010

Antibiotics Kan

Medium LB

Volume 3mL in 15mL tube, single colony

Temperature 37

Number 1

8.13

Preparing bacterial glycerol stocks (operator: Adonie)

Strain name：S034

Number in stocks：2

Plasmid extraction (operator: Adonie)

strain	pET28b-rBalcp19k-linker-mfp5-7*His (SM014)

plasmid	SM014

strain	pET28b-CsgA-linker-mfp3-7*his (SM008)
plasmid	SM008

strain	pET28b-csgA-linker-mfp5-7*His (SM010)
plasmid	SM010

Elution buffer

TB

H2O

Elution volume: 50μl

Concentration(A260/280):

SM014(1): 80.7 ng/ul

SM014(2): 96.15 ng/ul

SM010: 132.2 ng/ul

SM008: 122 ng/ul

8.14

Sequencing results analysis (operator: XYZ)

Sample	sequencing result	next plan
SM006-4/8	empty vector	stop
SM011-8(csgA-mfp3-mfp3-mfp3)	the sequence is the same as SM010 (csgA-mfp5) resulting from contaminated PCR templates	stop

SM014-4/6/8/11/13/15	all correct, therefore the SM014-19/20 have been sent to sequence several days ago probably be right.	preparing glycerol stocks

PCR Template (operator: Adonie)

□Purified genome

√Purified plasmid

□Single colony

□Bacteria liquid

□Boiled Bacteria cell lysate

SM005

Fr1

PCR	Name	Volume
Primer 1	SP035	1.5
Primer 2	SP034	1.5
Template	SM010	1
H2O		16
DNA polymerase	FASTPFU	9
MgSO4		1
All		30
Product length	5460bp	

Fr2

PCR	Name	Volume
Primer 1	SP036	1.5
Primer 2	SP037	1.5

Template	SM010	1
H2O		17
DNA polymerase	FASTPFU	9
All		30
Product length	256bp	

Fr3

PCR	Name	Volume
Primer 1	SP038	1.5
Primer 2	SP039	1.5
Template	SM010	1
H2O		17
DNA polymerase	FASTPFU	9
All		30
Product length	250bp	

SM016

Fr1

PCR	Name	Volume
Primer 1	SP034	1.5
Primer 2	SP035	1.5
Template	SM010	1
H2O		16

DNA polymerase	FASTPFU	9
MgSO4		1
All		30
Product length	5960	

Fr2

PCR	Name	Volume
Primer 1	SP034	1.5
Primer 2	SP035	1.5
Template	SM010	1
H2O		16
DNA polymerase	FASTPFU	9
All		30
Product length	256bp	

SM017

PCR	Name	Volume
Primer 1	SP049	1.5
Primer 2	SP003_R	1.5
Template	SM010	1
H2O		16
DNA polymerase	FASTPFU	9
MgSO4		1
All		30

Product length	5521bp	

SM018

PCR	Name	Volume
Primer 1	SP050	1.5
Primer 2	SP003_R	1.5
Template	SM010	1
H2O		16
DNA polymerase	FASTPFU	9
MgSO4	u	1
All		30
Product length	5431bp	

Process

Process	Temperature	Time
1	98	1min
2	98	30sec
3	62	30sec, -0.5°C/cyc
4	72	3min
Go to 2	12 cycle	
5	98	30sec
6	55	30sec
7	72	3min
Go to 6	22 cycle	
7	72	7min

8	12	forever

Process		
1	98	10min
2	98	30s
3	55	30s
4	72	30s
Go to 2	32 cycle	
5	72	2min
6	12	∞

Gel Picture:

SM005 Fr1 will not undergo gel extraction because it's band did not display the right results. Replace this sample with SM016 Fr1.

Gel Extraction (operator: Diana, Adonie)

√Gel extraction

□Liquid extraction

Elution buffer

☒EB

□H2O

Elution volume: 30ul

Name：SM005 Fr2

Concentration(A260/280nm)：7.9ng/ul

Name：SM005 Fr3

Concentration(A260/280nm)：8ng/ul

Name：SM016 Fr1
Concentration(A260/280nm): 15.1ng/ul

Name：SM016 Fr2
Concentration(A260/280nm)：8.45ng/ul

Name：SM017 Fr1
Concentration(A260/280nm)：27.15ng/ul

Name：SM018 Fr1
Concentration(A260/280nm)：8.25ng/ul

The concentrations of gel extraction are too low for GoldenGate Assembly. Another round of PCR and gel extraction will be undertaken.

Transformation (operator: Diana)
□Electroporation 1μL
√Chemical transformation 0.5μL
1. Host: BL21(DE3)
Plasmid to construct：SM014, SM015

2. Host: BL21(DE3) Rosetta
Plasmid to construct：SM014
Recovery medium：LB
Volume of bacterial liquid to spread：200 μL
Agar plate and antibiotic to use：LB，Cm + Kan
Transformation conditions：heat shock at 42°C for 90s

8.15

PCR Template (operator: Adonie)

□Purified genome
√Purified plasmid
□Single colony
□Bacteria liquid
□Boiled Bacteria cell lysate

SM005
Fr1

PCR	Name	Volume
Primer 1	SP035	1.5
Primer 2	SP034	1.5
Template	SM010	1
H2O		16
DNA polymerase	FASTPFU	9
MgSO4		1
All		30
Product length	5460bp	

Fr2

PCR	Name	Volume
Primer 1	SP036	1.5
Primer 2	SP037	1.5
Template	SM010	1
H2O		17
DNA polymerase	FASTPFU	9

All		30
Product length	256bp	

Fr3

PCR	Name	Volume
Primer 1	SP038	1.5
Primer 2	SP039	1.5
Template	SM010	1
H2O		17
DNA polymerase	FASTPFU	9
All		30
Product length	250bp	

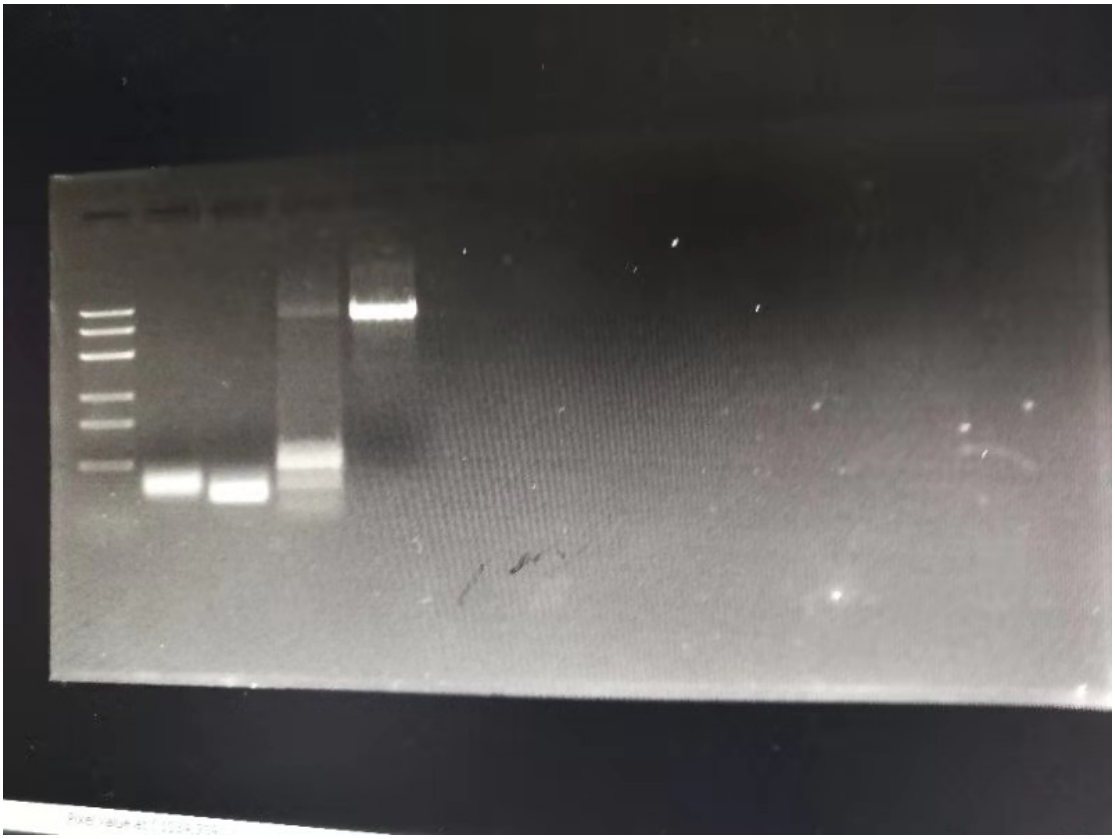
SM018

PCR	Name	Volume
Primer 1	SP050	1.5
Primer 2	SP003_R	1.5
Template	SM010	1
H2O		16
DNA polymerase	FASTPFU	9
MgSO4		1
All		30
Product length	5431bp	

Process	Temperature	Time
1	98	1min
2	98	30sec
3	62	30sec, -0.5°C/cyc
4	72	3min
Go to 2	12 cycle	
5	98	30sec
6	55	30sec
7	72	3min
Go to 6	22 cycle	
7	72	7min
8	12	forever

Process		
1	98	10min
2	98	30s
3	55	30s
4	72	30s
Go to 2	32 cycle	
5	72	2min
6	12	∞

Gel Picture:



Gel extraction (operator: Uranium)

√Gel extraction

□Liquid extraction

Elution buffer

☒EB

□H2O

Elution volume：30 μL

Name：SM005Fr2,Fr3,Fr1,SM018 Fr1

Concentration(A260/280nm)：14.2 ng/μL

8.17

PCR of all constructed vector (Operator: Adonie)

PCR of Sy003 and SM015 again

□Purified genome

√Purified plasmid

□Single colony

□Bacteria liquid

□Boiled Bacteria cell lysate

Sy003

PCR	Name	Volume
Primer 1	SP004-F	1.5
Primer 2	SP004-R	1.5
Template	Sy003	1
H2O		10
DNA polymerase	Starmix 2x	10
All		20
Product length	1140bp	

SM015

PCR	Name	Volume
Primer 1	SP024	1.5
Primer 2	SP025	1.5
Template	Sy003	1
H2O		10

DNA polymerase	Starmix 2x	10
All		20
Product length	425bp	

Process	Temperature	Time
1	95	1min
2	95	30s
3	58	45s
4	72	45s
Go to	32 cycle	
5	72	5 min
6	12	∞

Construction of SM005/016/017/018

Clony PCR

□Purified genome

□Purified plasmid

√Single colony

□Bacteria liquid

□Boiled Bacteria cell lysate

PCR	Name	Volume
Primer 1	SP004-F	1.5
Primer 2	SP004-R	1.5
Template	SM005/016/017/018 colonies	/
H2O		12
DNA polymerase	Starmix 2x	15
All		30
Product length	SM005 - 1617 bp SM016 - 1329 bp SM017 - 738 bp SM018 - 648 bp	

Number of colonies:

SM005 - 6

SM016 - 3

SM017 - 7

SM018 - 8

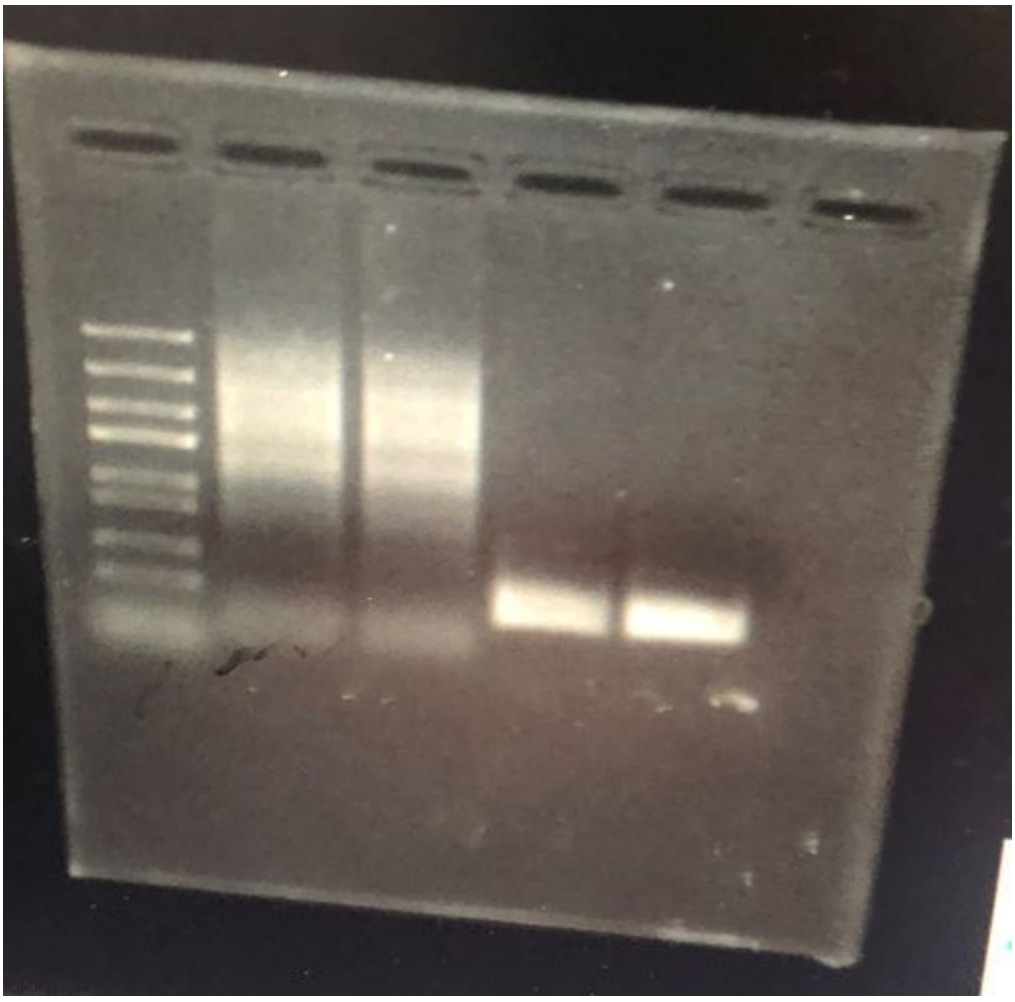
Process	Temperature	Time
1	95	1min
2	95	30s
3	55	30s
4	72	1min 30s
Go to	32 cycle	
5	72	2 min
6	12	∞

Gel picture

Construction of SM019

DnpI digestion and gel electropherosis

Gel Picture:



M SM019 Fr2 SM019 Fr2 SM019 Fr1 SM019 Fr1

Analysis:

SM019 Fr2： smear bands. PCR again.

SM019 Fr2: Correct size. Cut and store in -20 in E&A box

8.18

Construction of SM005/016/017/018

Gel electrophoresis

Results analysis
Bands of SM016/017/018 are right while SM005 are smaller than predicted.

Lane	samples	predicted size	band size	send for sequencing
1	Trans 5K			
2-7	SM005	1617	1200	1
8-10	SM016	1329	1300	1, 2
11-17	SM017	738	700	3, 5
18-25	SM018	648	600	7, 8

To be Sent for sequencing

in 4 refrigerator

Colony inoculation on agar plates

Colonies sent for sequencing were inoculated on Kan plates.

PCR of all constructed vector

Inoculation of DH5α/Sy003

From

□ Agar plates

√Glycerol stock

□Liquid starter

Strain	S021 DH5α/Sy003
Antibiotics	Kan
Medium	LB
Temperature	37
Number	1

8.19

PCR of all constructed vector

Colony PCR of sy003(S021)

PCR Template

□Purified genome

□Purified plasmid

√Single colony

□Bacteria liquid

□Boiled Bacteria cell lysate

PCR		
Primer 1	SP004-F	1
Primer 2	SP004-R	1
Template	S021 colonies	/
H2O		8
DNA polymerase	2XStarmix	10
All		20
		20/tube×3
Product length	1140 bp	
Process		
1	95	10min
2	95	30s
3	55	30s
4	72	45s
Go to 2	32 cycle	
5	72	5min
6	12	∞

pET28b empty vector was set as control.

Gel electrophoresis

Analysis: Nonspecific bands(~600bp) still present.

8.21

Construction of SM005/016/017/018

Sequencing

Sample	Primer	Requirements
□Bacteria liquid □plasmid □purified PCR √unpurified PCR SM005 No.1 SM017 No.1&2 SM018 No.3&5 SM016 No.7&8	SP004-F SP004-R	√ Forward □ Reverse √ Both

Results analysis:

SM005: empty vectors. Next plan: PCR by using gel extracted samples as templates.

SM017: No.5 is correct while No.3 has 3 insertions. Next plan: preparing glycerol stocks of No.5.

SM018: Both No.7 and No.8 are correct. Next plan: preparing glycerol stocks of No.7 and No.8.

SM016: empty vectors. Next plan: PCR by using gel extracted samples as templates.

8.24

PCR Template (operator: Adonie)

□Purified genome

√Purified plasmid

□Single colony

□Bacteria liquid

□Boiled Bacteria cell lysate

SM005/SM016

Fr1

PCR	Name	Volume
Primer 1	SP035	1.5
Primer 2	SP034	1.5
Template	SM016 Fr1 (Gel extraction)	1
H2O		30
DNA polymerase	FASTPFU	15
MgSO4		1
All		50
Product length	5460bp	

SM005 Fr2

PCR	Name	Volume
Primer 1	SP036	1.5
Primer 2	SP037	1.5
Template	SM005 Fr2 (Gel extraction)	1
H2O		31
DNA polymerase	FASTPFU	15
All		50
Product length	256bp	

SM005 Fr3

PCR	Name	Volume
Primer 1	SP038	1.5
Primer 2	SP039	1.5
Template	SM005 Fr3 (Gel extraction)	1
H2O		30
DNA polymerase	FASTPFU	15
All		50
Product length	250bp	

SM016 Fr2

PCR	Name	Volume
Primer 1	SP044	1.5
Primer 2	SP043	1.5
Template	SM016 Fr2 (Gel extraction)	1
H2O		30
DNA polymerase	FASTPFU	15
All		50
Product length	198bp	

SM019 Fr1

PCR	Name	Volume
Primer 1	SP047	1.5

Primer 2	SP048	1.5
Template	SM008	1
H2O		30
DNA polymerase	FASTPFU	15
MgSO4		1
All		50
Product length	5483bp	

Process:

Process	Temperature	Time
1	98	1min
2	98	30sec
3	62	30sec, -0.5°C/cyc
4	72	3min
Go to 2	12 cycle	
5	98	30sec
6	55	30sec
7	72	3min
Go to 6	22 cycle	
7	72	7min
8	12	forever

Process		
1	98	10min

2	98	30s
3	55	30s
4	72	30s
Go to 2	32 cycle	
5	72	2min
6	12	∞

Gel extraction (operator: Uranium)

√Gel extraction

□Liquid extraction

Elution buffer

⊠EB

□H2O

Elution volume：30 μL

Name：SM005 fr2，SM005fr3，SM016fr2，SM018fr1

Concentration(A260/280nm)：20 ng/μL，9ng/ul，8ng/ul，12.5ng/ul