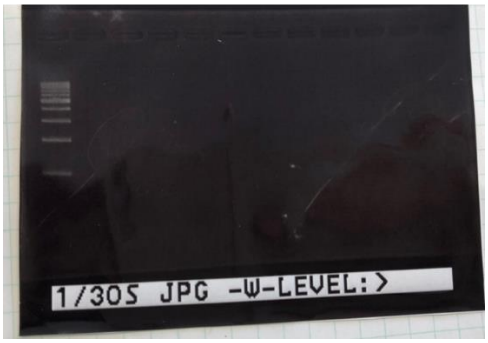
Lab note　　0923-0927

0923

1. Electrophoresis

1 2 3 4



1 One STEP Ladder Marker 500

2 pSB1C3

3 pSB1C3

4 pSB1C3

2. Pre-culture

3. PCR

Reagent

one sample

materials	volume(μL)
KOD One Pol	25
template DNA	1.0
forward primer	1.5
reverse primer	1.5
D.W	20
total	49

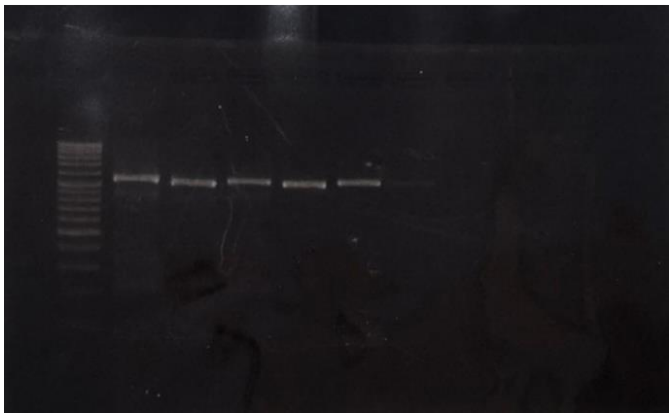
sample	forward primer	reverse primer	template
1	Ⅰ	tsu	10-fold dilution
2	Ⅶ	X	10-fold dilution
3	Ⅰ	tsu	100-fold dilution
4	Ⅶ	X	100-fold dilution
5	Ⅰ	tsu	dH_2O
6	Ⅶ	tsu	dH_2O

Cycling condition

reaction		temp.(℃)	time
cycle 1 (×1)	step 1	98	2:00
cycle 2 (×30)	step 1	98	0:10
	step 2	68	1:00
cycle 3 (×1)	step 1	4	∞

4. Electrophoresis

1 2 3 4 5 6 7



1 One STEP Ladder Maker 100

2 Homologous sequence addition

3 recA

4 Homologous sequence addition

5 recA

6 Negative control

7 Negative control

0924

1. Colony PCR

Reagent

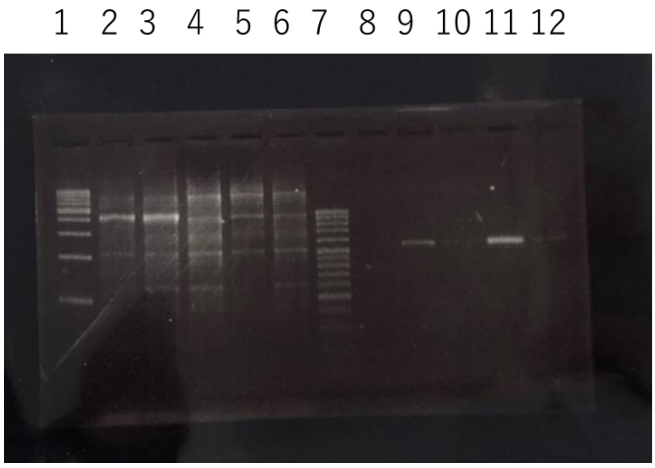
materials	volume (μL)
D.W.	22
KOD One	25
forward primer	1.5
reverse primer	1.5
total	50

Sample	forward primer	reverse primer	colony
1	h	g	vector RFP 1
2	h	g	vector RFP 2
3	Ⅶ	X	insert 9/24 Colony1
4	Ⅶ	X	insert 9/24 Colony2
5	Ⅶ	X	insert 9/24 Colony3
6	h	g	vector 9/24 Colony4
7	h	g	vector 9/24 Colony5
8	h	g	vector 9/23 Colony6

Cycling condition

reaction		temp.(°C)	time
cycle 1 (X1)	step 1	98	2:00
cycle 2 (X30)	step 1	98	0:10
	step 2	68	2:20
cycle 3 (X1)	step 1	4	∞

2. Electrophoresis



1 One STEP Ladder Marker 500

2 vector(RFP)

3 vector(RFP)

4 vector

5 vector

6 vector

7 One STEP Ladder Marker 100

8 recA

9 recA

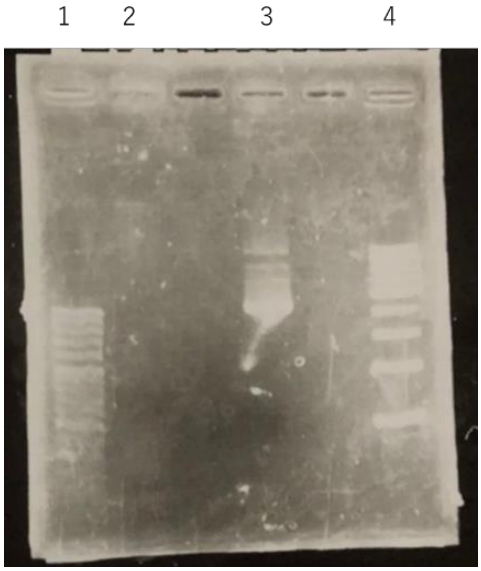
10 recA

11 recA

12 recA

0925

1. Plasmid extraction



1 One STEP Ladder Maker 100

2 recA

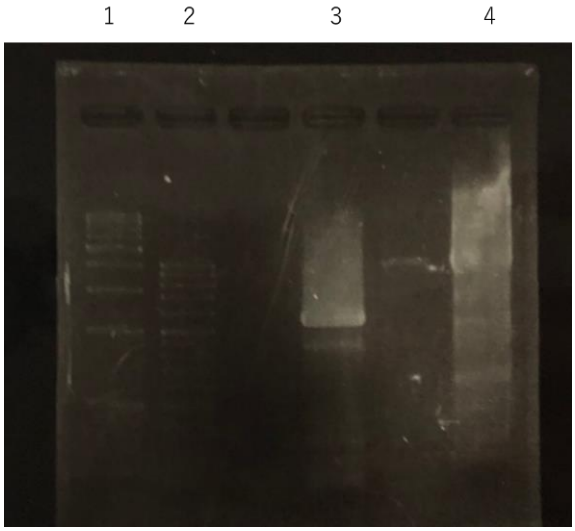
3 vector(RFP)

4 One STEP Ladder 500

2. Making culture medium LB agar plate

0926

1. Electrophoresis



1 One STEP Ladder Maker 100

2 One STEP Ladder Maker 500

3 recA

4 pSB1C3

2. Transformation

3. Colony PCR

Reagent

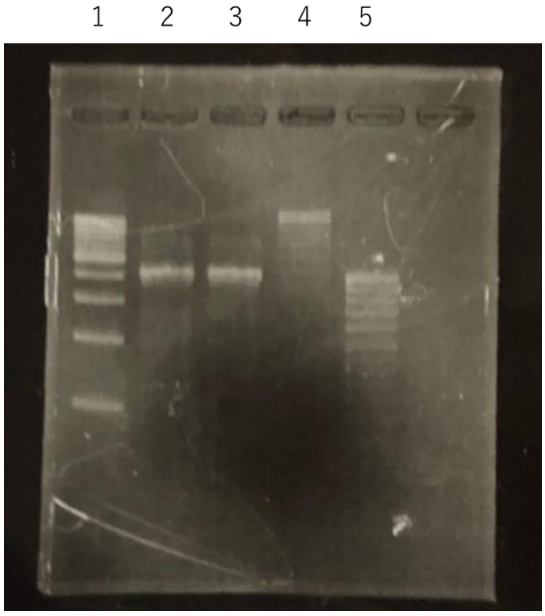
materials	volume (μL)
D.W.	22
KOD One	25
forward primer	1.5
reverse primer	1.5
total	50

Sample	forward primer	reverse primer	colony
1	h	g	RFP
2	h	g	RFP
3	Ⅶ	Ⅹ	RFP

Cycling condition

reaction		temp.(℃)	time
cycle 1 (X1)	step 1	94	2:00
cycle 2 (X30)	step 1	98	0:10
	step 2	58	0:05
	step 3	68	0:12
cycle 3 (X1)	step 1	4	∞

4. Electrophoresis



1 One STEP Ladder Maker 500

2 pSB1C3

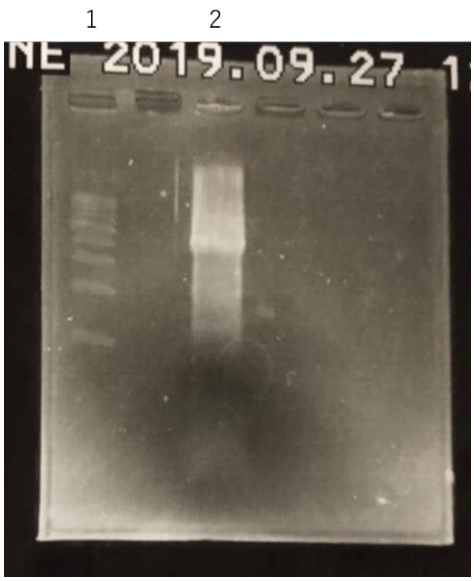
3 pSB1C3

4 recA

5 One STEP Ladder Maker 100

0927

1. Electrophoresis



1 One STEP Ladder Maker 500

2 pSB1C3

3. Colony PCR

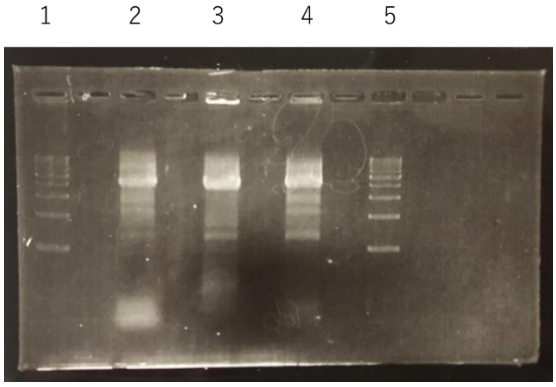
Reagent

materials	volume (μL)
D.W.	22
KOD One	25
forward primer	1.5
reverse primer	1.5
total	50

Cycling condition

reaction		temp.(℃)	time
cycle 1 (×1)	step 1	94	2:00
cycle 2 (×30)	step 1	98	0:10
	step 2	68	0:11
cycle 3 (×1)	step 1	4	∞

Electrophoresis



1 One STEP Ladder Maker 500

2 pSB1C3

3 pSB1C3(RFP)

4 pSB1C3(RFP)

5 One STEP Ladder Maker 500

4. PCR for homologous sequence addition

Reagent

materials	volume (μL)
D.W.	21
KOD One	25
forward primer	1.5
reverse primer	1.5
template	1
total	50

sample	forward primer	reverse primer	template
1	recA for pSB1C3+recA	recA for pSB1C3+recA	10-fold dilution
2	recA for pSB1C4+recA	recA for pSB1C4+recA	10-fold dilution
3	recA for pSB1C5+recA	recA for pSB1C5+recA	100-fold dilution
4	recA for pSB1C6+recA	recA for pSB1C6+recA	100-fold dilution
5	recA for pSB1C7+recA	recA for pSB1C7+recA	dH_2O
6	recA for pSB1C8+recA	recA for pSB1C8+recA	dH_2O

Cycling condition

reaction		temp.(℃)	time
cycle 1 (×1)	step 1	98	2:00
cycle 2 (×30)	step 1	98	0:10
	step 2	68	0:16
cycle 3 (×1)	step 1	4	∞