

Lab note 1014-1018

1014

## 1. Colony PCR (pSB1C3)

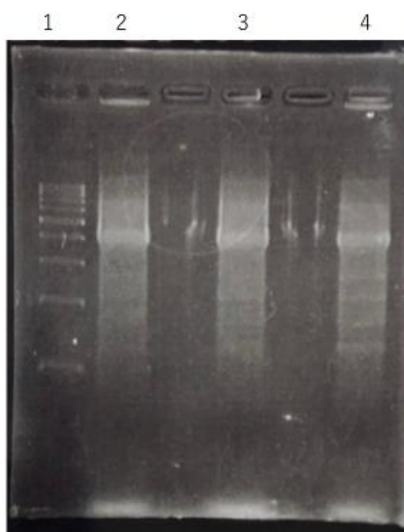
### Reagent

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

### Cycling condition

reaction		temp.(°C)	time
cycle 1 (X1)	step 1	94	2:00
cycle 2 (X35)	step 1	98	0:10
	step 2	55	0:05
	step 3	68	0:22
cycle 3 (X1)	step 1	4	∞

## 2. Electrophoresis



1 OneSTEP Ladder Marker 500

2 pSB1C3

3 pSB1C3

4 pSB1C3

### 3. Transformation

### 4. PCR

#### Reagent

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

#### Cycling condition

reaction		temp.(°C)	time
cycle 1	step 1	98	2:00
cycle 2	step 1	98	0:10
	step 2	60	0:05
	step 3	68	0:12
cycle 3	step 1	4	∞

1015

1. Electrophoresis



- 1 OneSTEP Ladder Marker 100
- 2 recA
- 3 recA
- 4 recA
- 5 recA
- 6 pSB1C3
- 7 pSB1C3
- 8 pSB1C3

2. Colony PCR (pSB1C3)

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
1	h	g
2	pSB1C3 for recA + pSB1C3	pSB1C3 for recA + pSB1C3
3	pSB1C3 for recA + pSB1C3	pSB1C3 for recA + pSB1C3
4	pSB1C3 for recA + pSB1C3	pSB1C3 for recA + pSB1C3

#### Cycling condition

reaction		temp.(°C)	time
cycle 1	step 1	94	2:00
cycle 2	step 1	98	0:10
	step 2	68	0:15
cycle 3	step 1	4	∞

### 3. Colony PCR (recA)

#### Reagent

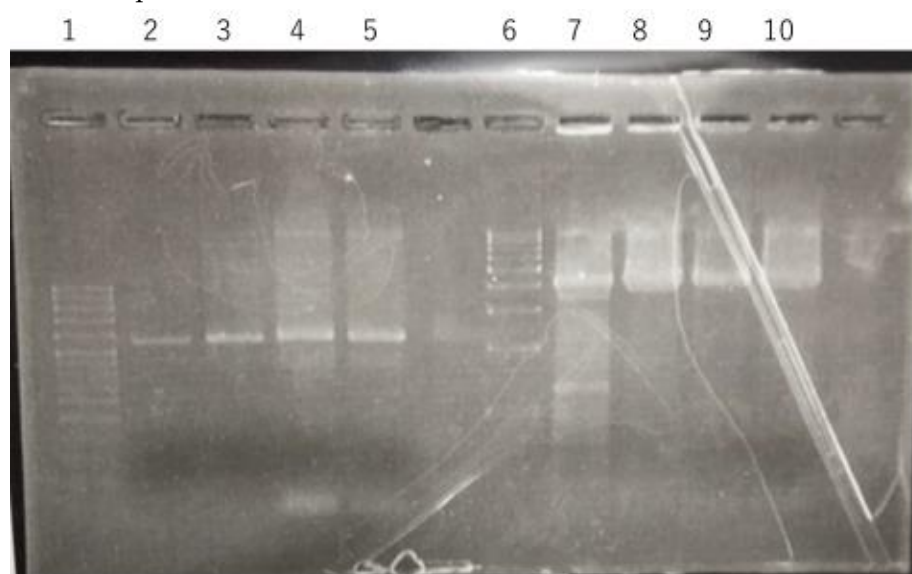
materials	volume (μL)
D.W.	22
KOD One	25
forward primer	1.5
reverse primer	1.5
total	50

Sample	forward primer	reverse primer
1	VII	X
2	pSB1C3 for recA + pSB1C3	pSB1C3 for recA + pSB1C3
3	pSB1C3 for recA + pSB1C3	pSB1C3 for recA + pSB1C3
4	pSB1C3 for recA + pSB1C3	pSB1C3 for recA + pSB1C3

#### Cycling condition

reaction		temp.(°C)	time
cycle 1	step 1	98	2:00
cycle 2	step 1	98	0:10
	step 2	60	0:05
	step 3	68	0:12
cycle 3	step 1	4	∞

#### 4. Electrophoresis



1 OneSTEP Ladder Marker 100

2 recA

3 recA

4 recA

5 recA

6 OneSTEP Ladder Marker 500

7 pSB1C3

8 pSB1C3

9 pSB1C3

10 pSB1C3

## 5. Colony PCR

### Reagent

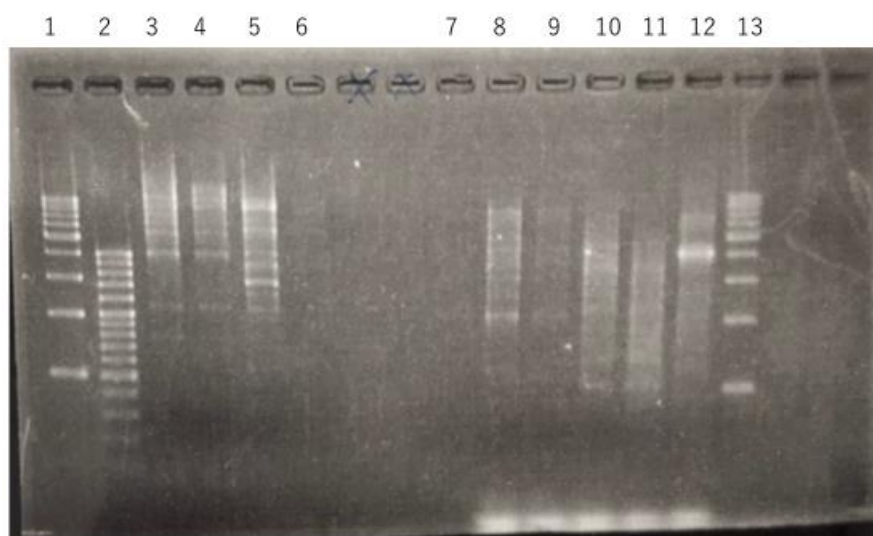
recA	
materials	volume (μL)
D.W.	22
KOD One	25
forward primer VII	1.5
reverse primer X	1.5
total	50

pSB1C3	
materials	volume (μL)
D.W.	22
KOD One	25
forward primer h	1.5
reverse primer g	1.5
total	50

### Cycling condition

reaction		temp.(°C)	time
cycle 1	step 1	94	2:00
cycle 2	step 1	98	0:10
	step 2	60	0:05
	step 3	68	0:12
cycle 3	step 1	4	∞

## Electrophoresis



1 OneSTEP Ladder Marker 500

2 OneSTEP Ladder Marker 100

3 recA

4 recA

5 recA

6 recA

7 recA(*D.radiodurans*)

8 pSB1C3

9 pSB1C3

10 pSB1C3

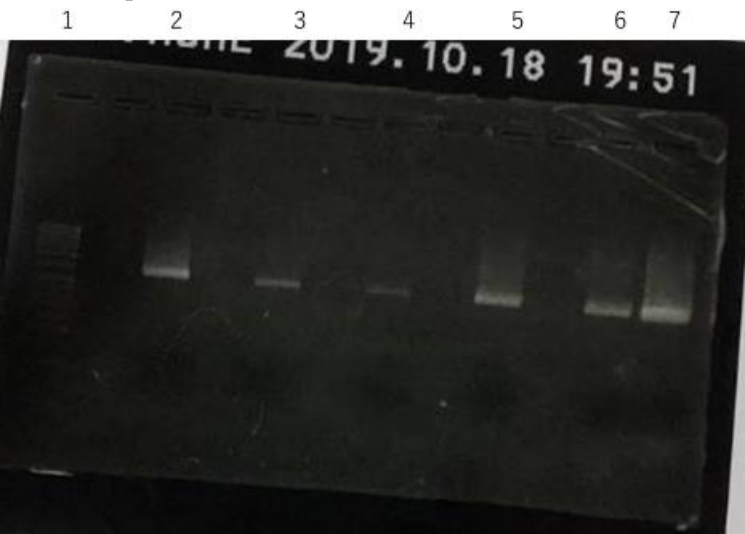
11 pSB1C3

12 pSB1C3(RFP)

13 OneSTEP Ladder Marker 500

1016

1. Electrophoresis



- 1 OneSTEP Ladder Marker 100
- 2 pqqE
- 3 pqqE
- 4 pqqE
- 5 pprM
- 6 pprM
- 7 pprM

2. Colony PCR

Reagent

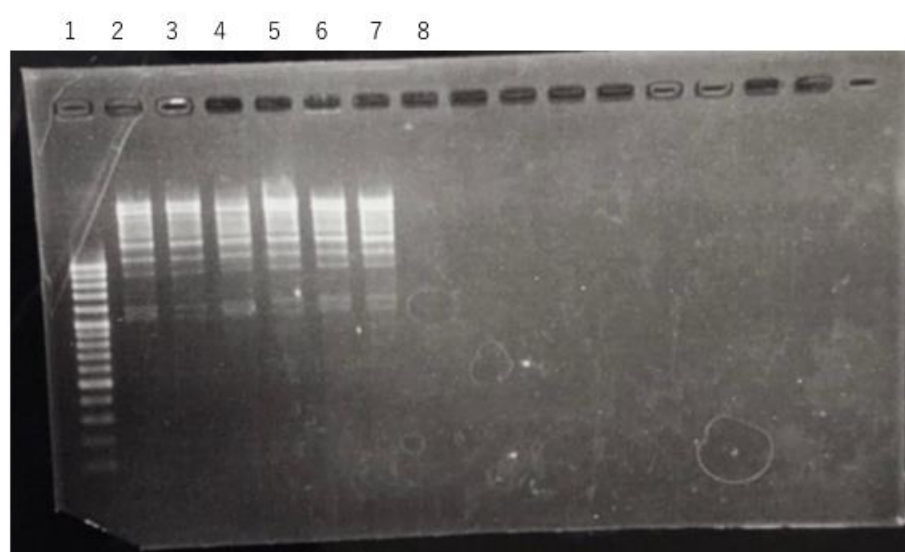
materials	volume (μL)
D.W.	22
KOD One	25
forward primer VII	1.5
reverse primer X	1.5
total	50



### Cycling condition

reaction		temp.(°C)	time
cycle 1 (X1)	step 1	98	2:00
cycle 2 (X30)	step 1	98	0:10
	step 2	60	0:05
	step 3	68	0:12
cycle 3 (X1)	step 1	4	∞

### 3. Electrophoresis



1 OneSTEP Ladder Marker

2 recA

3 recA

4 recA

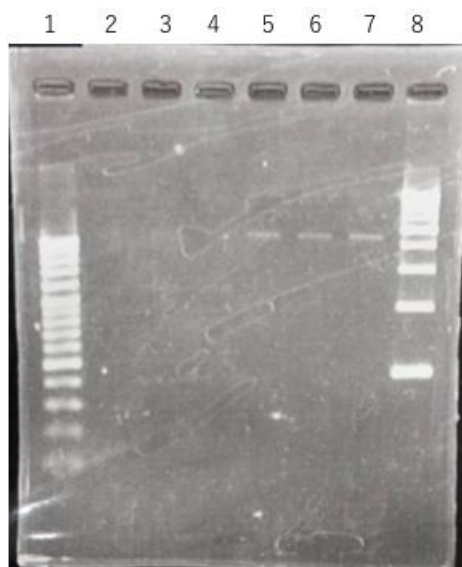
5 recA

6 recA

7 recA

8 positive control

#### 4. Gel extraction



1 OneSTEP Ladder Marker 100

2 recA

3 recA

4 recA

5 recA

6 recA

7 recA

8 OneSTEP Ladder Marker 500

#### 5. Infusion

#### 6. Transformation



1017

## 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
1	III	VIII
2	III	VIII
3	III	VIII
4	I	O
5	I	O
6	I	O

### Cycling condition

reaction		temp.(°C)	time
cycle 1	step 1	94	2:00
cycle 2	step 1	98	0:10
	step 2	68	0:12
cycle 3	step 1	4	∞

## 2. pre-culture

1018

## 1. Colony PCR

### Reagent

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

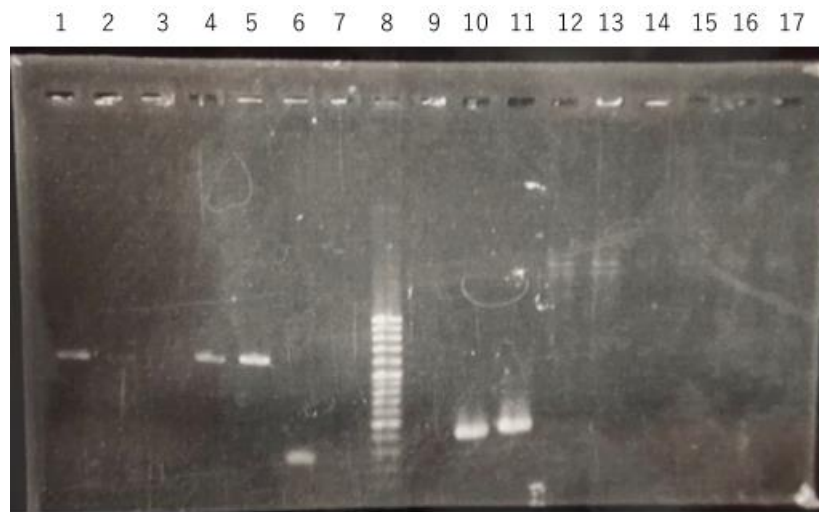
Sample	colony
1	<i>D. rad</i>
2	pprM (PCR product)
3	pprM (PCR product)
4	A
5	B
6	C
7	D
8	E
9	F

### Cycling condition

reaction		temp.(°C)	time
cycle 1	step 1	94	2:00
cycle 2	step 1	98	0:10
	step 2	59	0:05
	step 3	68	0:05
cycle 3	step 1	4	∞

1021

1. Electrophoresis



1 pqqE

2 pqqE

3 pqqE

4 pqqE

5 pqqE

6 Lac

7 Lac

8 OneSTEP Ladder Marker 100

9 positive control(*D. radiodurans*)

10 positive control(*D. radiodurans*)

11 pprM

12 pprM

13 pprM

14 pprM

15 pprM

16 pprM

17 pprM

## 2. PCR

### Reagent

materials	volume( $\mu$ L)
KOD One Pol	25
template DNA	1.0
forward primer	1.5
reverse primer	1.5
D.W	21
total	50

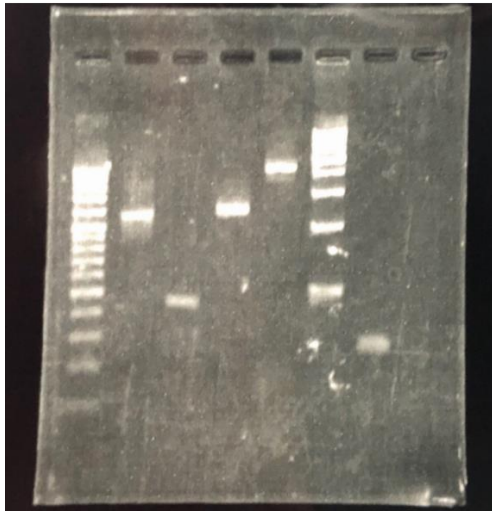
sample	forward primer	reverse primer
1	Ka	Ke
2	Ka	Ke
3	VII	XI
4	VII	XI

### Cycling condition

reaction		temp.(°C)	time
cycle 1 (× 1)	step 1	94	2:00
cycle 2 (× 30)	step 1	98	0:10
	step 2	34	0:05
	step 3	68	0:01
cycle 3 (× 1)	step 1	4	$\infty$

### 3. Electrophoresis

1 2 3 4 5 6 7



1 One STEP Ladder Marker 100

2 recA

3 pprM

4 pqqE

5 pSB1C3

6 One STEP Ladder Marker 500

7 Promoter

### 4. Gel extraction

Electrophoresis

1 2 3 4 5



1 OneSTEP Ladder Marker 100

2 pprM

3 pprM

4 Lac+pprM

5 Lac+pprM