

Lab note 0826-0830

0826

1. Preparation 100 mg/mL chloramphenicol solution

materials	volume
chloramphenicol	0.3 g
ethanol	3 mL

Dispensing reagent for 1.5 mL tube

Store in the freezer

2. Making culture medium (TGY broth/ TGY agar plate/ LB broth/ LB agar plate)

3. Pre-culture (*D.radiodurans*)

4. Culture(*D.radiodurans*)

0827

1. Colony PCR

Reagent

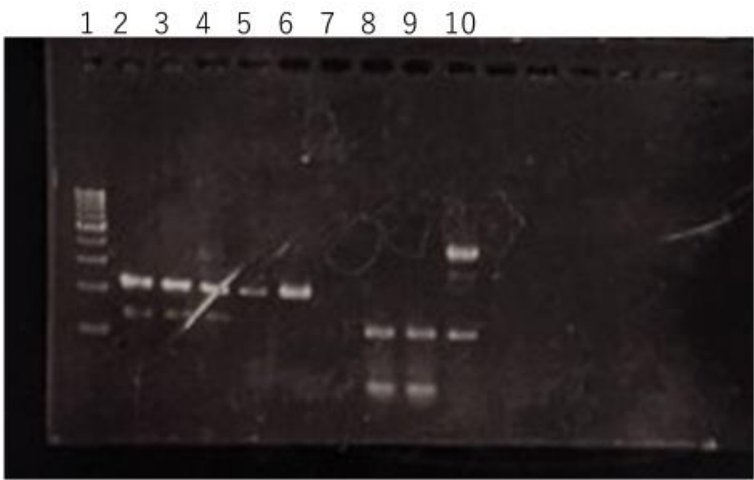
one sample

materials	concentration	volume(μ L)
2 \times PCR buffer	1X	25
dNTP	0.4mM	10
KOD Fx Pol	1U/50 μ L	1.0
forward primer(VII)	0.15~0.3 μ M	1.5
reverse primer(VI)	0.15~0.3 μ M	1.5
D.W	-	up to 50
total	-	50

The cycling conditions

reaction		temp.(°C)	time
cycle 1 (X1)	step 1	94	2:00
cycle 2 (X35)	step 1	98	0:10
	step 2	57.4	0:05
	step 3	68	0:08
cycle 3 (X1)	step 1	4	∞

Electrophoresis



- 1 OneSTEP Ladder Marker 500
- 2 recA
- 3 recA
- 4 recA
- 5 recA+histag
- 6 recA+histag
- 7 recA+histag
- 8 pSB1C3
- 9 pSB1C3
- 10 pSB1C3

2. Inverse PCR

Reagent

one sample

materials	concentration	volume(μL)
2 × PCR buffer	1X	25
dNTP	0.4 mM	10
KOD Fx DNA polymerase	1 U/50μL	1.0
Fw primer	0.15~0.3 μL	1.5
Rv primer	0.15~0.3 μL	1.5
pSB1C3		1.0
D.W.	-	11
total		51

The cycling conditions

reaction		temp.(°C)	time
cycle 1 (× 1)	step 1	94	2:00
cycle 2 (× 35)	step 1	98	0:10
	step 2	58	0:05
	step 3	68	1:30
cycle 3 (× 1)	step 1	4	∞

0829

1. Inverse PCR

Reagent for PCR tube

one sample

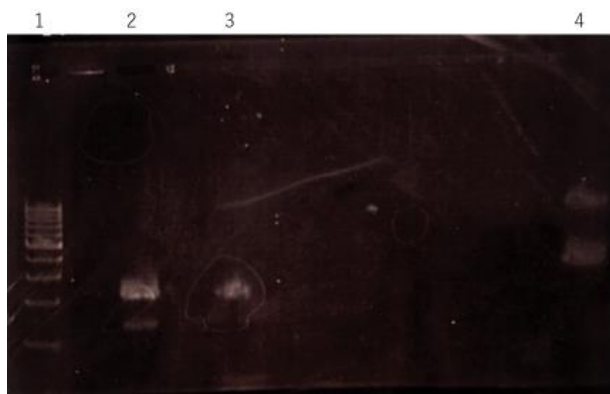
materials	concentration	volume(μL)
KOD One	0.5mM	25
Fw primer	0.15~0.3 μL	1.5
Rv primer	0.15~0.3 μL	1.5
D.W.	-	12
total		50

Add 10 μL template each tube

The cycling conditions

reaction		temp.(°C)	time
cycle 1 (× 1)	step 1	94	2:00
cycle 2 (× 35)	step 1	98	0:10
	step 2	58	0:05
	step 3	68	1:30
cycle 3 (× 1)	step 1	4	∞

Electrophoresis

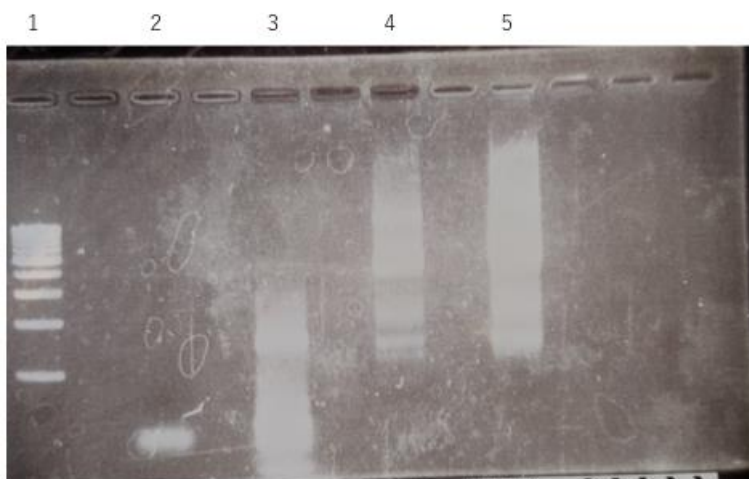


1 OneSTEP Ladder Marker 500

2 recA

3 recA+histag

4 pSB1C3-A



1 OneSTEP Ladder Marker 500

2 recA+histag

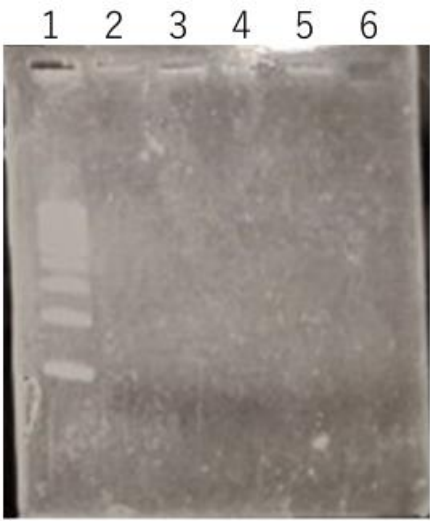
3 pSB1C3(8/27)

4 pSB1C3-B

5 pSB1C3-C

2. Gel extraction

Electrophoresis



- 1 OneSTEP Ladder Maeker 500
- 2 recA
- 3 recA+histag
- 4 pSB1C3-A
- 5 pSB1C3-B
- 6 pSB1C3-B

3. Pre-culture (*D.radiodurans*)

0830

1. Colony PCR

Reagent

one sample

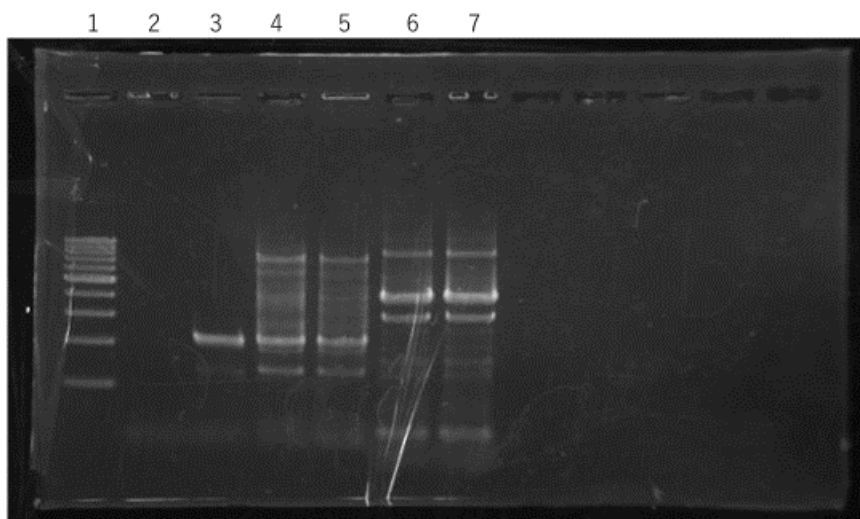
materials	concentration	volume(μ L)
2 \times PCR buffer	1X	25
dNTP	0.4mM	10
KOD Fx Pol	1U/50 μ L	10
forward primer	0.15~0.3 μ M	1.5
reverse primer	0.15~0.3 μ M	1.5
D.W	-	up to 50
total	-	50

sample	Forward primer	reverse primer
1	VII	VI
2	VII	VI
3	VII	I -Rec A
4	VII	I -Rec A

The cycling conditions

reaction		temp.(°C)	time
cycle 1 (X1)	step 1	94	2:00
cycle 2 (X35)	step 1	98	0:10
	step 2	58	0:05
	step 3	68	1:15
cycle 3 (X1)	step 1	4	∞

Electrophoresis



1 OneSTEP Ladder Marker 500

2 recA+histag

3 recA+histag

4 recA

5 recA

6 pSB1C3

7 pSB1C3

2. Inverse PCR

Reagent

one sample

materials	concentration	volume(μ L)
2 \times PCR buffer	1X	25
dNTP	0.4mM	10
KOD Fx Pol	1U/50 μ L	10
forward primer (h)	0.15~0.3 μ M	1.5
reverse primer (g)	0.15~0.3 μ M	1.5
D.W	-	up to 50
total	-	50

Add 10 μ L template

The cycling conditions

reaction		temp.(°C)	time
cycle 1 (X1)	step 1	94	2:00
cycle 2 (X35)	step 1	98	0:10
	step 2	58	0:05
	step 3	68	1:15
cycle 3 (X1)	step 1	4	∞