

## Lab note 0909-0913

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### 1. colony PCR

#### Reagent

one sample

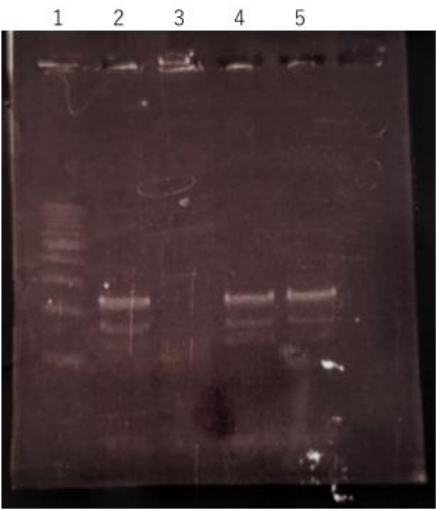
materials	concentration	volume( $\mu$ L)
2 $\times$ PCR buffer	1X	25
dNTP	0.4mM	10
KOD Fx Pol	1U/50 $\mu$ L	1.0
forward primer(VII)	0.15~0.3 $\mu$ M	1.5
reverse primer(VI)	0.15~0.3 $\mu$ M	1.5
D.W	-	up to 50
total	-	50

#### 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	$\infty$

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1. Electrophoresis



- 1 OneSTEP Ladder Marker 500
- 2 recA
- 3 recA
- 4 recA
- 5 recA

2. Pre-culture

3. PCR

Reagent

one sample (lac)

materials	concentration	volume(μL)
PCR buffer	1X	25
dNTP	0.4 mM	10
D.W	-	8.5
Template DNA	-	5
Primer Ko	0.15~0.3 μL	0.25
Primer Ka	0.15~0.3 μL	0.25
KOD Pol	1 U/50μL	1
total		50

one sample (colony PCR product)

materials	concentration	volume( $\mu$ L)
PCR buffer	1X	25
dNTP	0.4 mM	10
D.W	-	13.5
Primer Ko	0.15~0.3 $\mu$ L	0.25
Primer Ka	0.15~0.3 $\mu$ L	0.25
KOD Pol	1 U/50 $\mu$ L	1
total		50

#### Cycling condition

reaction		temp.( $^{\circ}$ C)	time
cycle 1 ( $\times$ 1)	step 1	94	2:00
cycle 2 ( $\times$ 35)	step 1	98	0:10
	step 2	58	0:05
	step 3	68	1:40
cycle 3 ( $\times$ 1)	step 1	4	$\infty$

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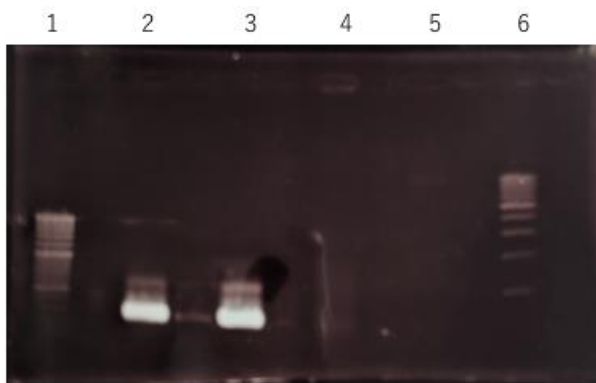
#### 1. Electrophoresis



- 1 OneSTEP Ladder Marker 100
- 2 Lac
- 3 Lac
- 4 recA
- 5 vector
- 6 OneSTEP Ladder Marker 500

## 2. Gel extraction

### Electrophoresis



- 1 OneSTEP Ladder Marker 100
- 2 Lac
- 3 Lac
- 4 vector
- 5 vector
- 6 OneSTEP Ladder Marker 500

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1. Colony PCR

Reagent

one sample

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

Cycling condition

reaction		temp.( $^{\circ}$ C)	time
cycle 1 (X1)	step 1	94	2:00
cycle 2 (X35)	step 1	98	0:10
	step 2	65	0:05
	step 3	68	1:00
cycle 3 (X1)	step 1	4	$\infty$

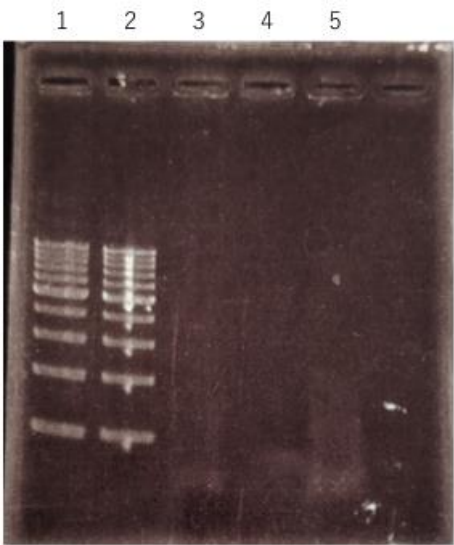
Electrophoresis



- 1 OneSTEP Ladder Marker 100
- 2 Lac
- 3 Lac

0913

1. Electrophoresis



- 1 OneSTEP Ladder Marker 500
- 2 mistake
- 3 vector
- 4 vector
- 5 vector

2. Colony PCR

Reagent

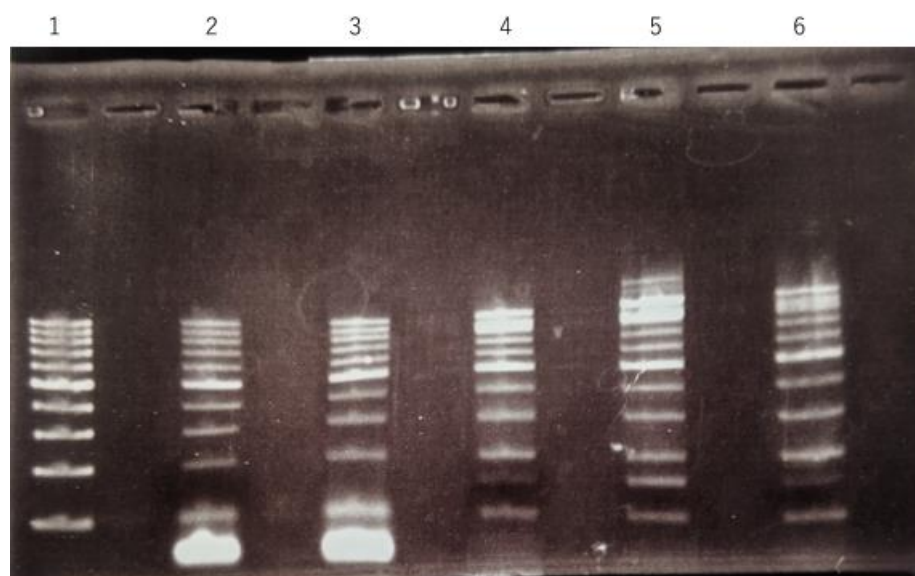
one sample		
materials	concentration	volume( $\mu$ L)
KOD One Pol	1U/50 $\mu$ L	22
forward primer	10 $\mu$ M	1.5
reverse primer	10 $\mu$ M	1.5
D.W	-	up to 50
total	-	50

### 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	0:15
cycle 3 (X1)	step 1	4	∞

### 3. Gel extraction

#### Electrophoresis



1 OneSTEP Ladder Marker 500

2 Lac

3 Lac

4 vector

5 vector

6 vector