

Lab note 1007-1011

1007

1. Colony PCR

Reagent

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 V	0.15~0.3 μ M	1.5
reverse primer IX	0.15~0.3 μ M	1.5
D.W	-	up to 50
total	-	50

Sample	colony
1	10/2 so
2	10/2 so
3	<i>D. rad</i>

Cycling condition

reaction		temp.(°C)	time
cycle 1	step 1	94	2:00
cycle 2	step 1	98	0:10
	step 2	56	0:30
	step 3	68	0:30
cycle 3	step 1	4	∞

2. Restriction enzyme treatment

materials	volume(μL)
10 X pprM	2
DNA (pprM or recA)	1
Spe I	1
Xba I	1
D.W.	15
total	20

3. Electrophoresis



- 1 OneSTEP Ladder Marker 500
- 2 recA
- 3 recA
- 4 recA
- 5 recA
- 6 pprM
- 7 pprM
- 8 pprM
- 9 pprM(*D. radiodurans*)

4. Gel extraction



1 Lac

2 Lac

3 Lac

5. Electrophoresis



1 OneSTEP Ladder Marker 500

2 Template

3 pprM

4 pprM

5 pprM

6. PCR

Reagent

materials	volume(μ L)
2 \times PCR buffer	25
dNTP	10
KOD Fx Pol	1
forward primer lac	1.5
reverse primer lac	1.5
template	1
D.W.	10
total	50

Cycling condition

reaction		temp.($^{\circ}$ C)	time
cycle 1	step 1	94	2:00
cycle 2	step 1	98	0:10
	step 2	68	1:15
cycle 3	step 1	4	∞

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1. Electrophoresis



1 OneSTEP Ladder Marker 100

2 pSB1C3

3 pSB1C3

4 recA

5 rppM

2. Infusion

3. Electrophoresis



1 OneSTEP Ladder Marker 500

2 recA

1009

1. Infusion

2. Transformation

3. Colony PCR (pprM)

Reagent

materials	concentration	volume(μ L)
2 \times PCR buffer	1X	25
dNTP	0.4mM	10
KOD Fx Pol	1U/50 μ L	5.0
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	colony
1	V	IX	A
2	U	Chi	B
3	U	Chi	C
4	U	Chi	D
5	U	Chi	E

Cycling condition

reaction		temp.(°C)	time
cycle 1	step 1	94	2:00
cycle 2	step 1	98	0:10
	step 2	56	0:30
	step 3	68	0:30
cycle 3	step 1	4	∞

1010

1. 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 (×1)	step 1	98	2:00
cycle 2 (×30)	step 1	98	0:10
	step 2	60	0:05
	step 3	68	0:12
cycle 3 (×1)	step 1	4	∞

2. Electrophoresis



1 OneSTEP Ladder Marker 500

2 recA

3 pqqE

4 recA

5 pprM

6 pprM

7 pprM

8 pprM

1011

1. Gel extraction

1 2 3 4



1 OneSTEP Ladder Marker 100

2 pqqE

3 pqqE

4 pSB1C3