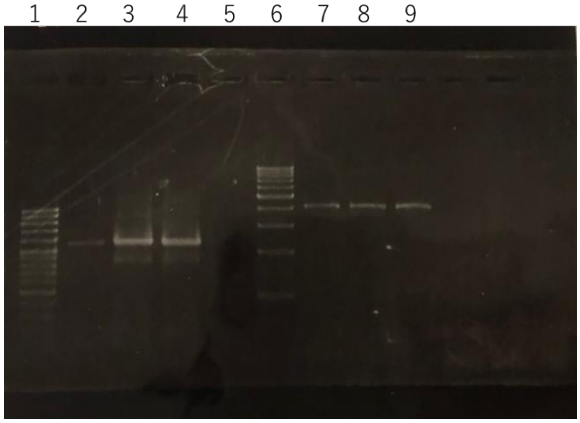
Lab note　　0930-1004

0930

1. Electrophoresis



1 One STEP Ladder Marker 100

2 recA

3 recA

4 recA

5 recA

6 One STEP Ladder Marker 500

7 pSB1C3

8 pSB1C3

9 pSB1C3

2. Infusion

Reagent

one sample

materials	volume(μL)
insert DNA ①A	12
vector DNA B-1	2
HD Enzyme	4
dH_2O	up to 20
total	20

3. Transformation

4. PCR for homologous addition

Reagent

materials	volume (μL)
D.W.	21
KOD One	25
forward primer pSB1C3 for pSB1C3+recA	1.5
reverse primer pSB1C3 for pSB1C3+reaA	1.5
template	1
total	50

sample	temple
1	10-fold dilution
2	100-fold dilution
4	D.W.

Cycling condition

reaction		temp.(°C)	time
cycle 1 (×1)	step 1	94	2:00
cycle 2 (×30)	step 1	98	0:10
	step 2	58	0:05
	step 3	68	0:12
cycle 3 (×1)	step 1	4	∞

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1. Colony PCR

Reagent

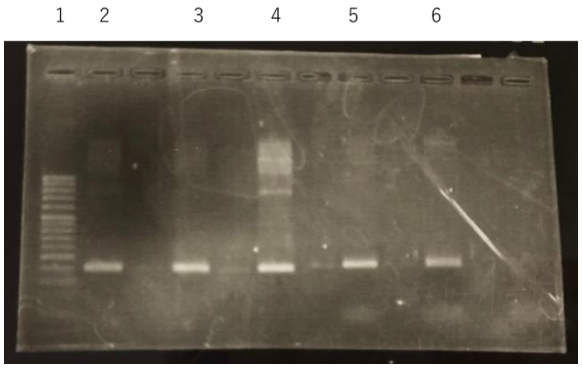
materials	volume (μL)
D.W.	22
KOD One	25
forward primer	1.5
reverse primer	1.5
total	50

Sample	forward primer	reverse primer
1	V	IX
2	V	IX
3	U	Chi
4	U	Chi

Cycling condition

reaction		temp.(°C)	time
cycle 1 (X30)	step 1	98	0:10
	step 2	56	0:05
	step 3	68	0:01

Electrophoresis



1 One STEP Ladder Marker 100

2 pprM

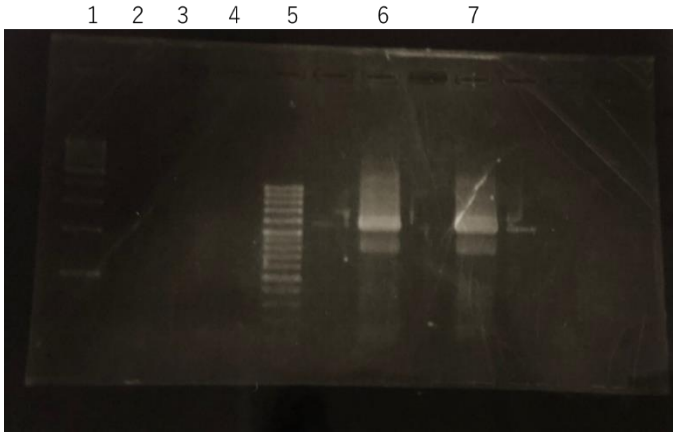
3 pprM

4 pprM

5 pprM

6 pprM

2. Electrophoresis



1 One STEP Ladder Marker 500

2 pSB1C3

3 pSB1C3

4 pSB1C3

5 One STEP Ladder Maker 100

6 recA

7 recA

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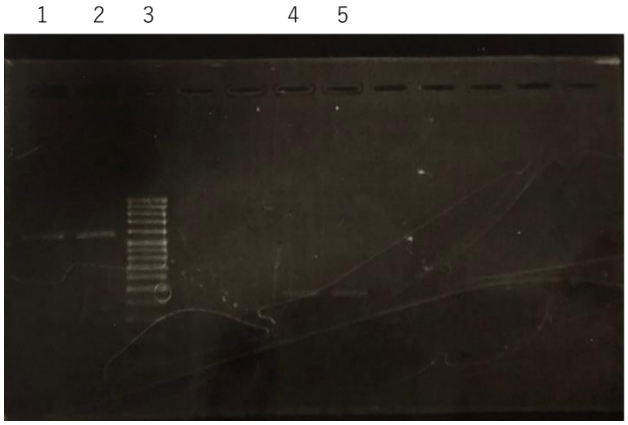
1. Gel extraction

Electrophoresis



1 recA

2 recA

3 One STEP Ladder Marker

4 pprM

5 pprM

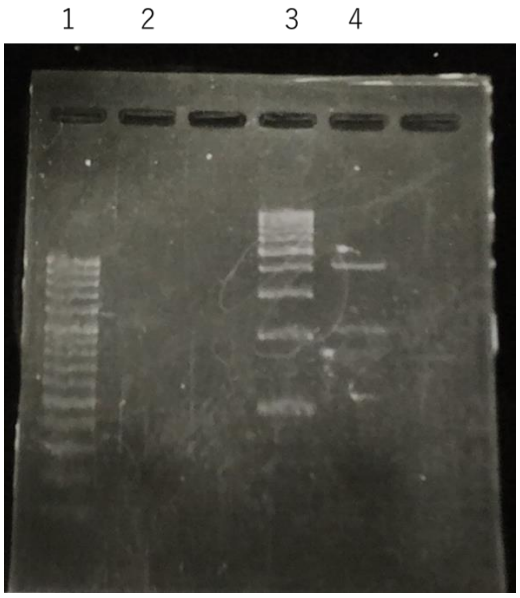
2. Infusion

3. Transformation

4. Restriction enzyme digestion

materials	volume(μL)
D.W.	15
Buffer	2
DNA	1
Restriction enzyme Spe I	1
Restriction enzyme Xba I	1
total	20

5. Electrophoresis



1 One STEP Ladder marker 100

2 recA

3 One STEP Ladder Marker 500

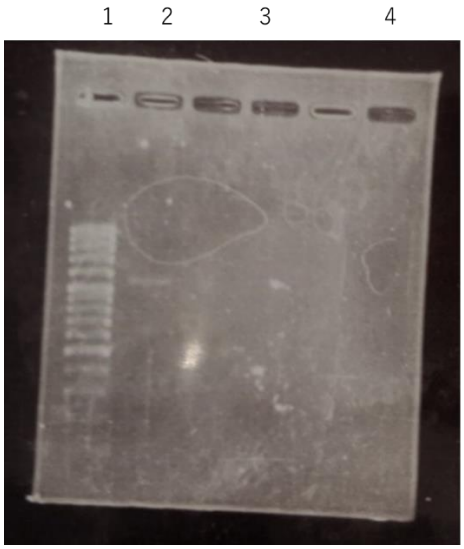
4 pSB1C3

6. Gel extraction

7. Restriction enzyme

materials	volume(μL)
D.W.	15
10XpprM	2
DNA	1
Restriction enzyme Spe I	1
Restriction enzyme Xba I	1
total	20

Electrophoresis



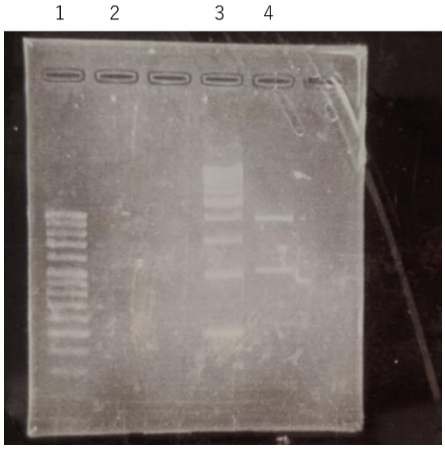
1 One STEP Ladder Marker 100

2 recA

3 recA

4 pprM

8. Electrophoresis



1 One STEP Ladder Marker 100

2 recA

3 One STEP Ladder Marker 500

4 pSB1C3

1003

1. Gel extraction

2. Ligation

materials	volume(μL)
vector P_5	2
insert(recA or pprM)	8
Ligation Mix	10
total	20

3. Transformation

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1. PCR

Reagent

materials	volume(μL)
2×PCR buffer	25
dNTP	10
KOD Fx Pol	1
forward primer	1.5
reverse primer	1.5
template	1
D.W.	10
total	50

Cycling condition

reaction		temp.(°C)	time
cycle 1 (×1)	step 1	94	2:00
cycle 2 (×10)	step 1	98	0:10
	step 2	68	0:13
cycle 3 (×1)	step 1	4	∞