Figure 1



Figure 2

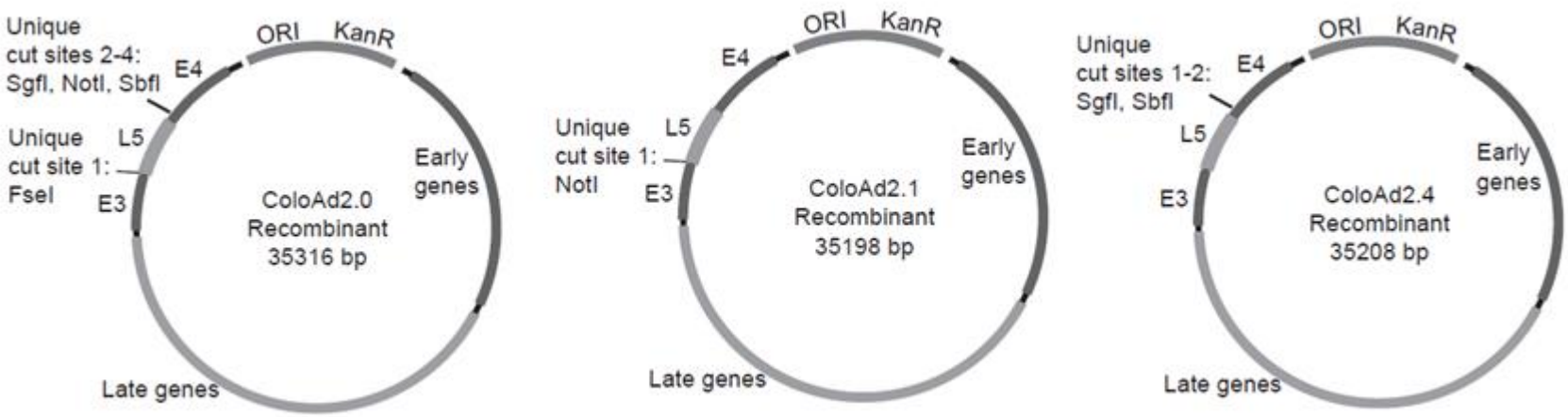


Figure 3

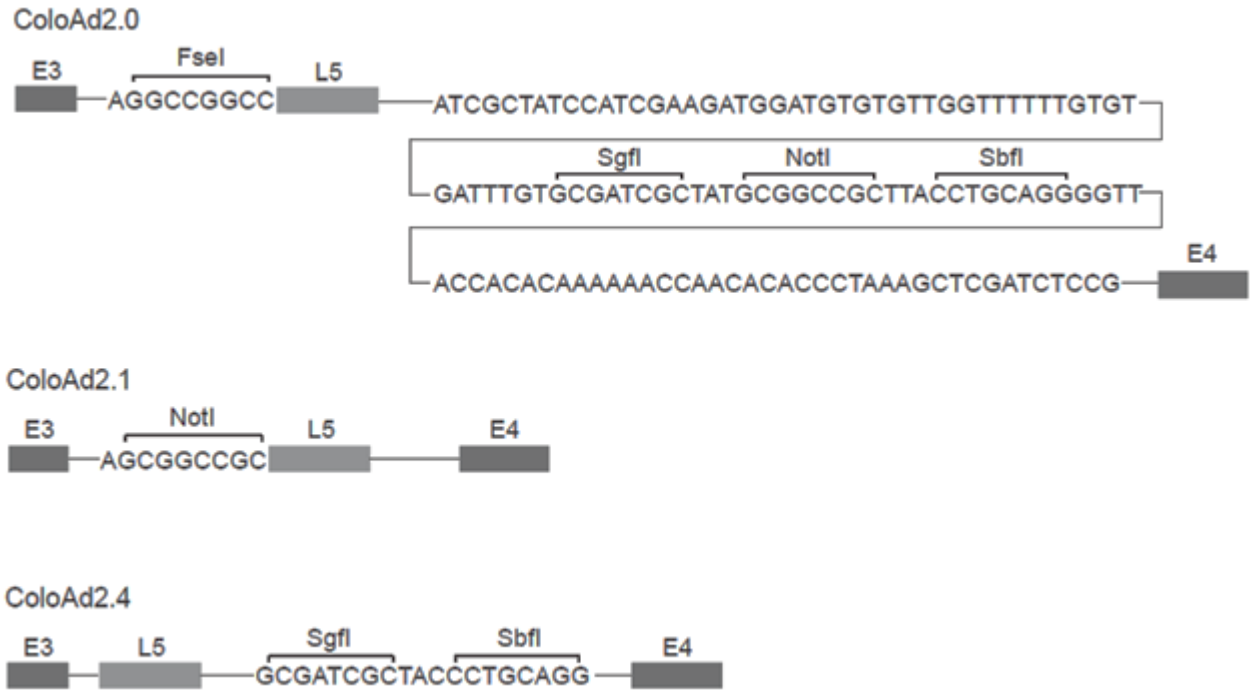


Figure 4



Figure 5

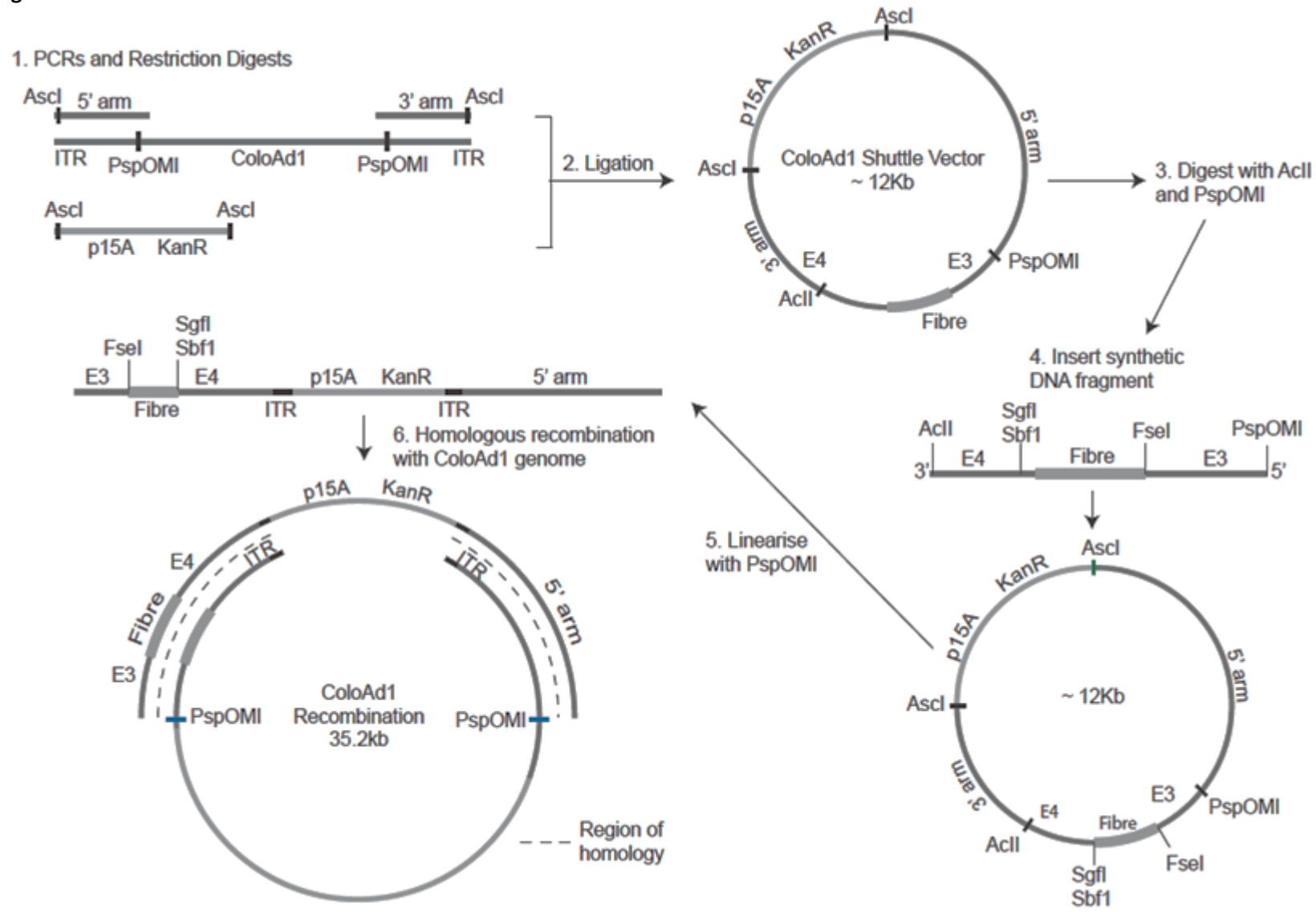

1. PCRs and Restriction Digests
AscI
5' arm
3' arm
AscI
ITR
PspOMI
ColoAd1
PspOMI
ITR
AscI
AscI
p15A
KanR
2. Ligation
AscI
KanR
p15A
5' arm
ColoAd1 Shuttle Vector
~ 12Kb
AscI
3' arm
E4
E3
PspOMI
AclI
Fibre
3. Digest with AclI
and PspOMI
4. Insert synthetic
DNA fragment
AclI
SgfI
Sbf1
FseI
PspOMI
3'
E4
Fibre
E3
5'
5. Linearise
with PspOMI
AscI
KanR
p15A
5' arm
~ 12Kb
AscI
3' arm
E4
E3
PspOMI
AclI
Fibre
FseI
SgfI
Sbf1
FseI
SgfI
Sbf1
E3
E4
p15A
KanR
5' arm
Fibre
ITR
ITR
6. Homologous recombination
with ColoAd1 genome
p15A
KanR
E4
ITR
ITR
5' arm
Fibre
E3
PspOMI
ColoAd1
Recombination
35.2kb
PspOMI
Region of
homology

Figure 6

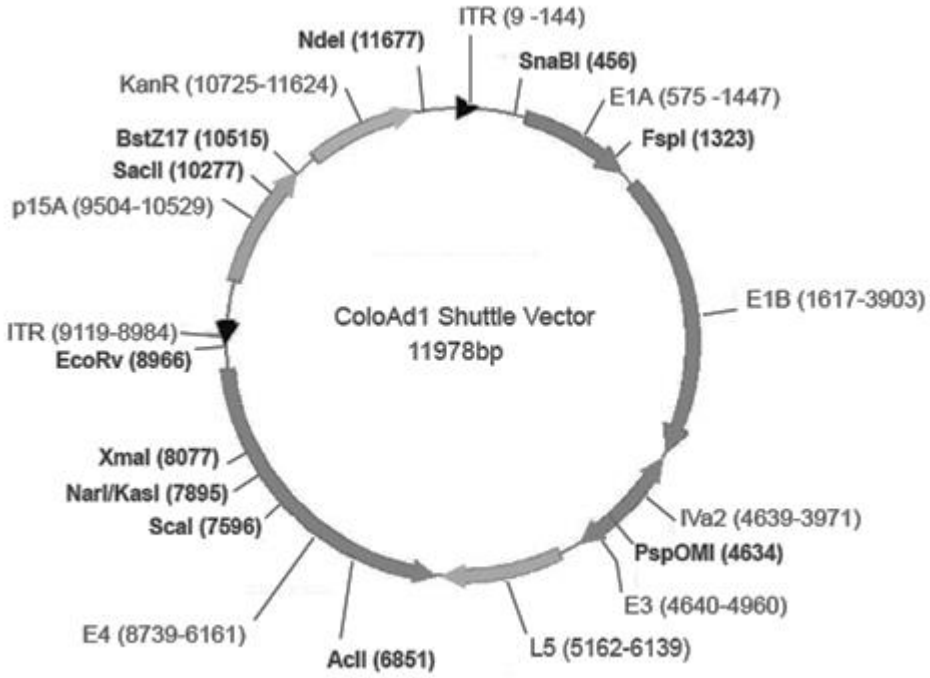


Figure 7

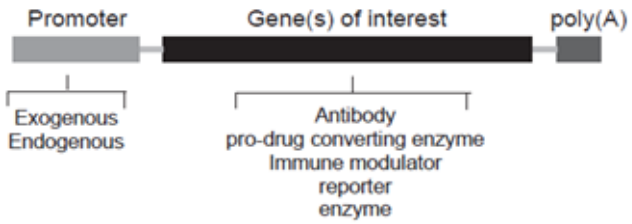


Figure 8

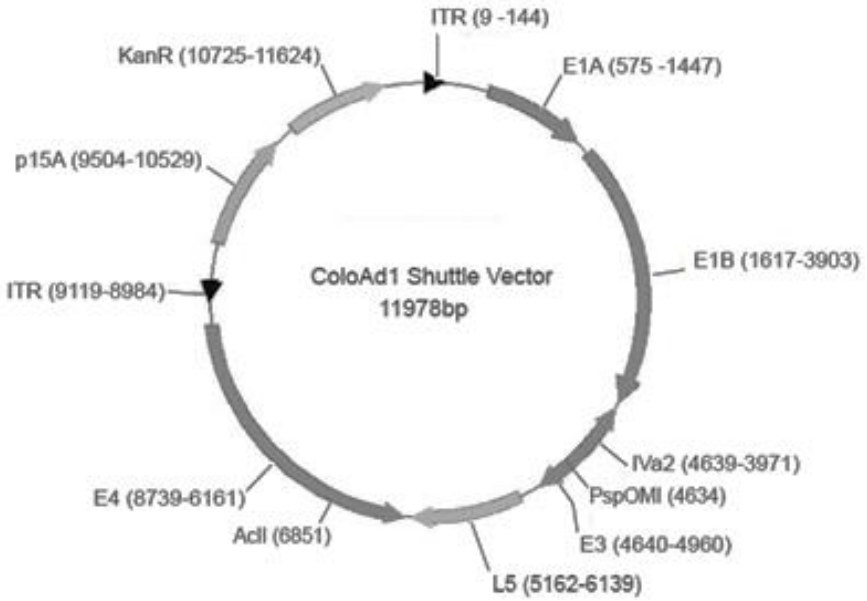


Figure 9

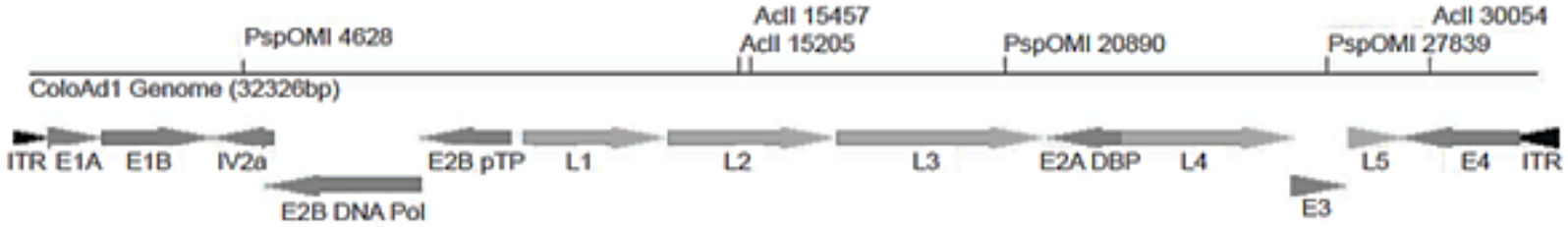


Figure 10

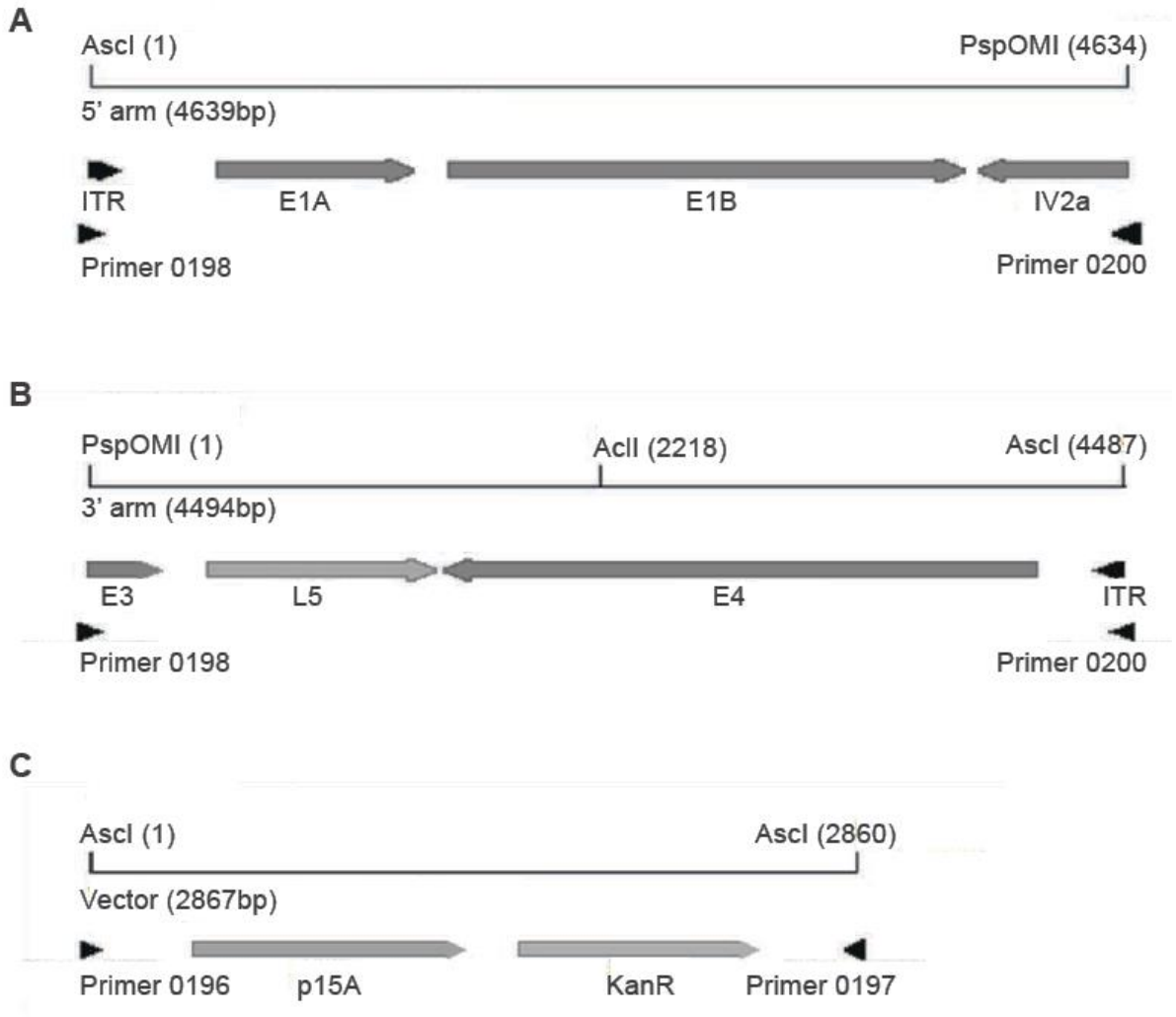

A
AscI (1)
PspOMI (4634)
5' arm (4639bp)
ITR
E1A
E1B
IV2a
Primer 0198
Primer 0200
B
PspOMI (1)
AclI (2218)
AscI (4487)
3' arm (4494bp)
E3
L5
E4
ITR
Primer 0198
Primer 0200
C
AscI (1)
AscI (2860)
Vector (2867bp)
Primer 0196
p15A
KanR
Primer 0197

Figure 11

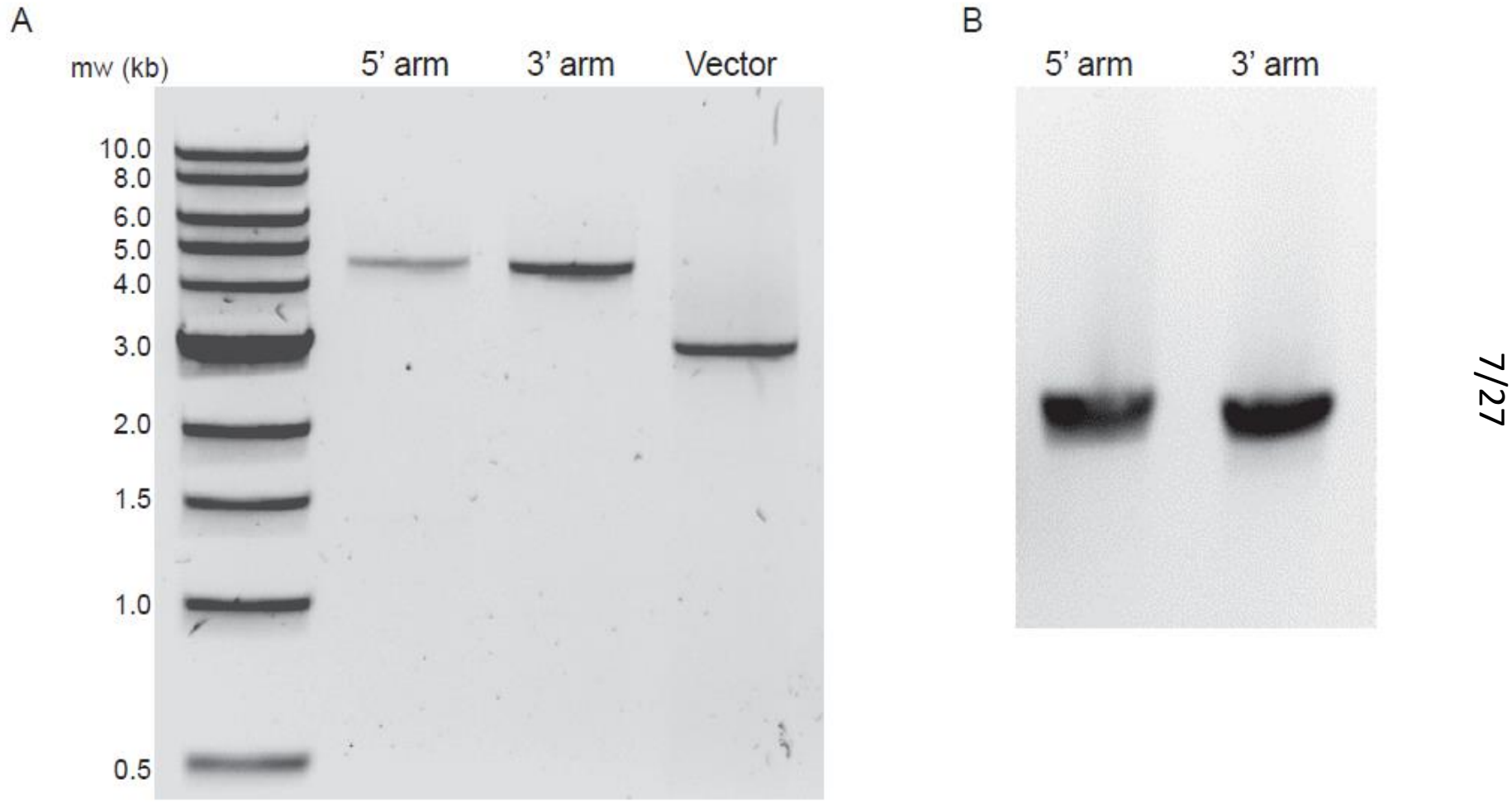


Figure 12

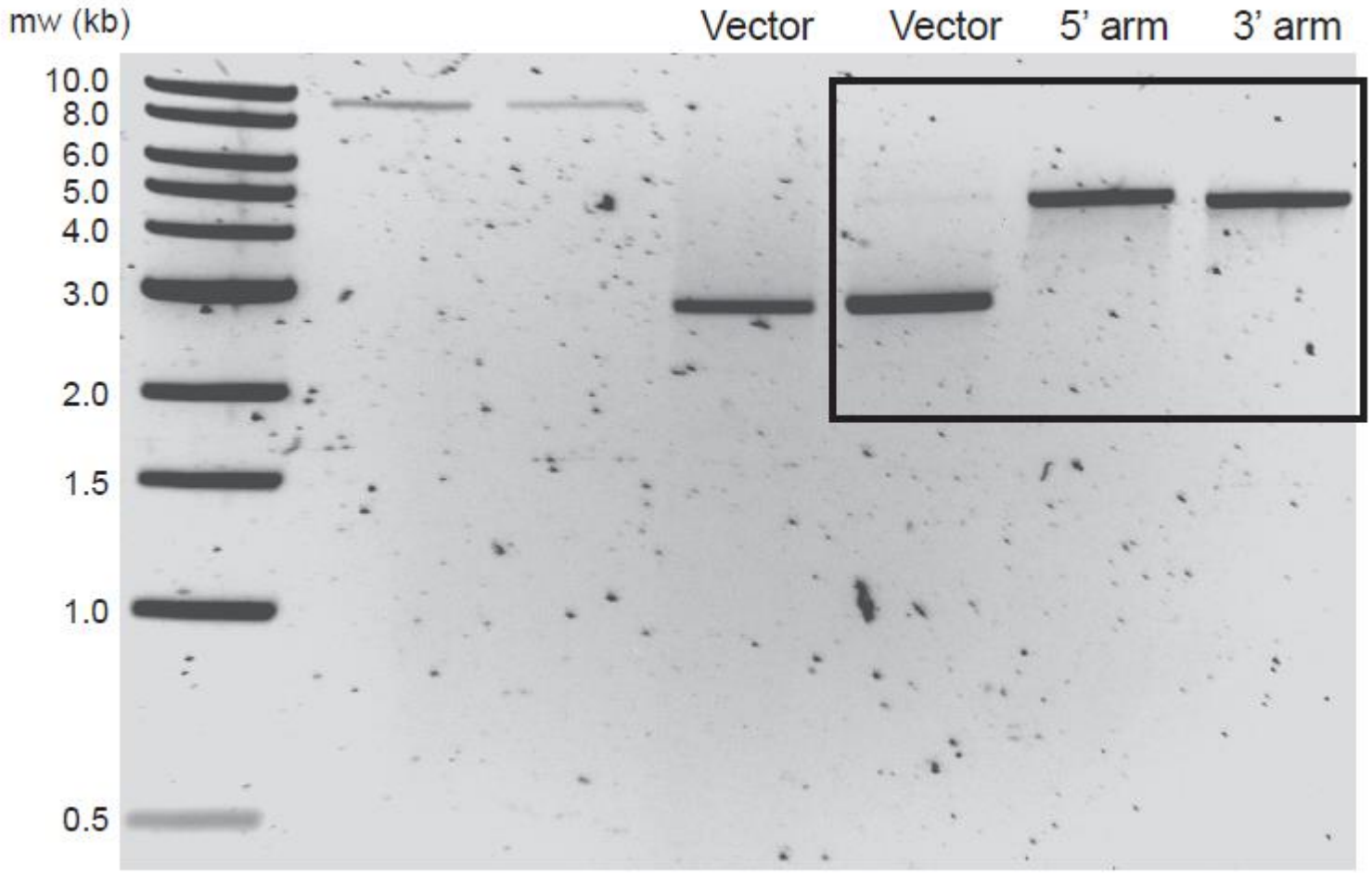


Figure 13

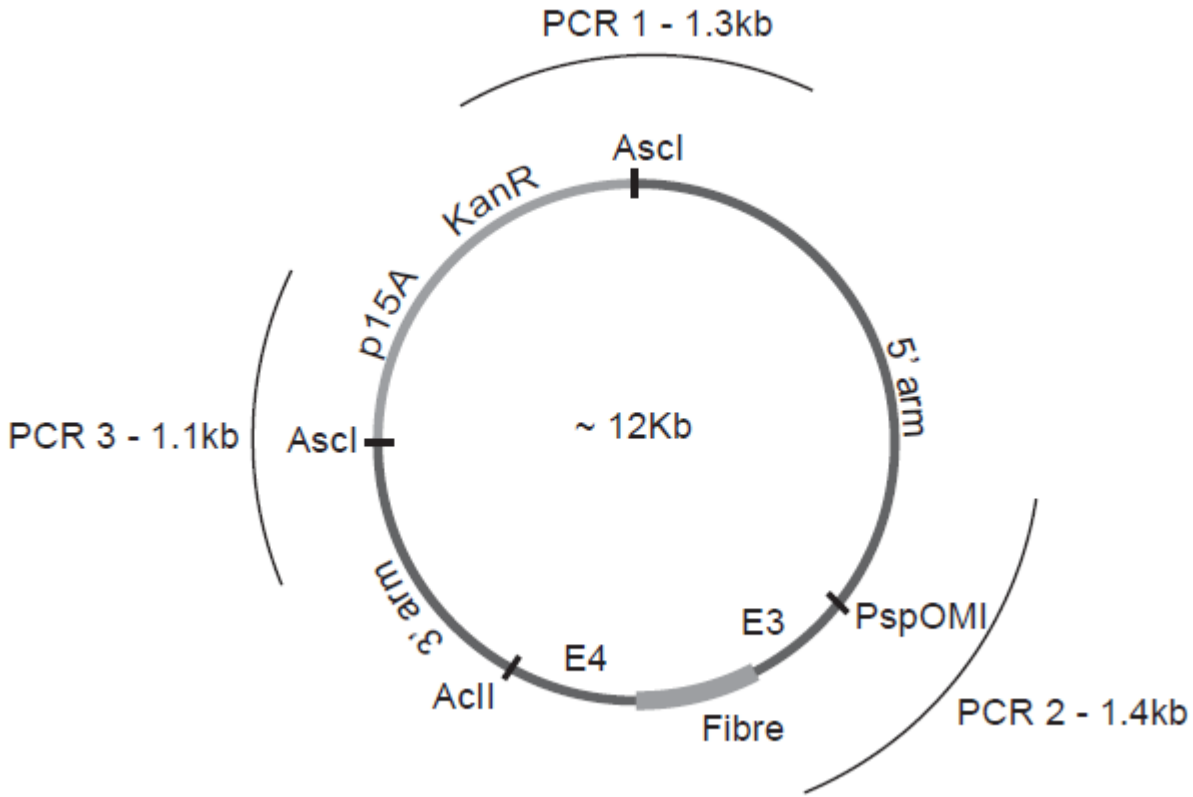

PCR 1 - 1.3kb
AscI
KanR
p15A
5' arm
PCR 3 - 1.1kb
AscI
~ 12Kb
3' arm
E3
PspOMI
E4
AclI
Fibre
PCR 2 - 1.4kb

Figure 14

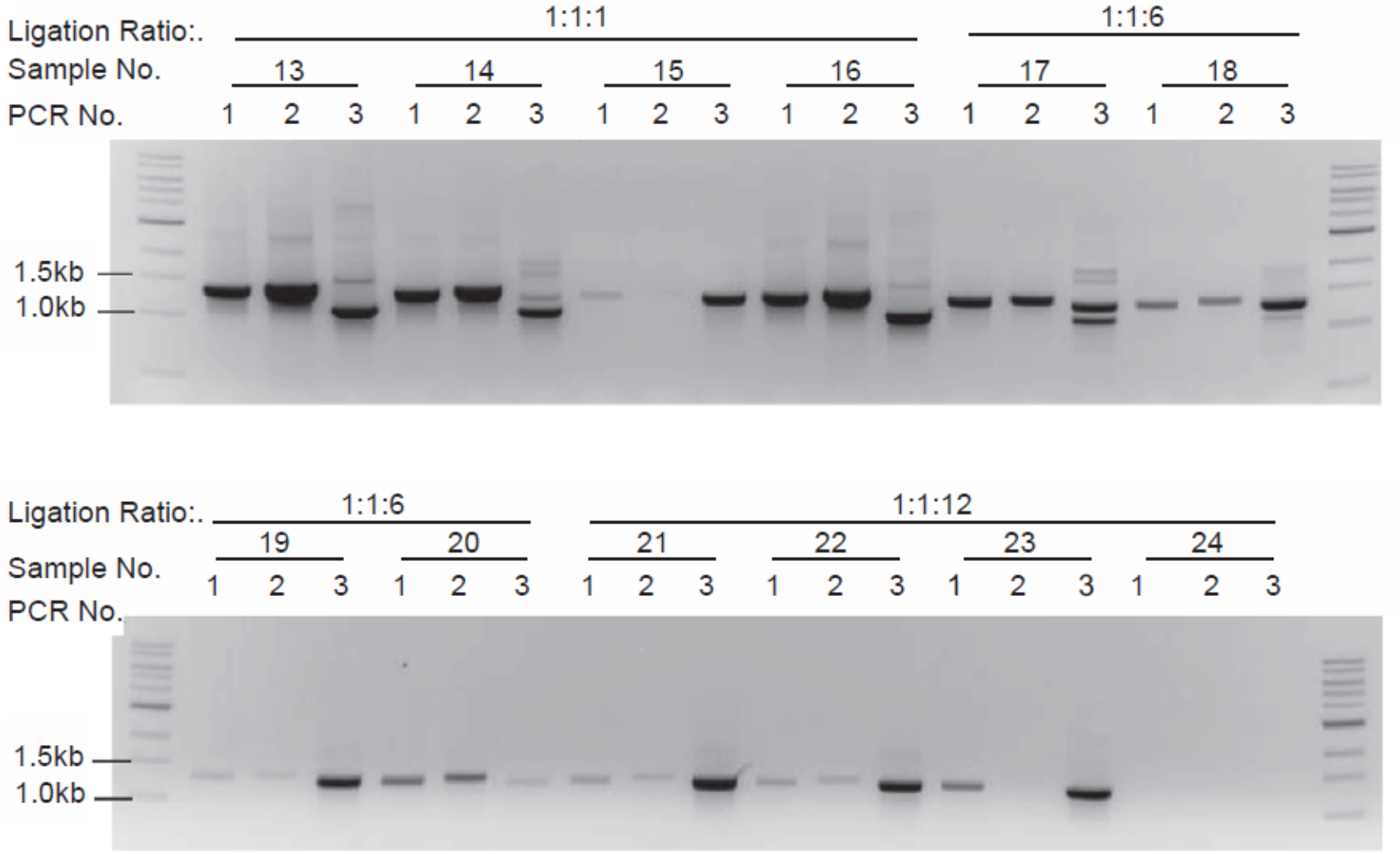

Ligation Ratio:.
1:1:1
1:1:6
Sample No.
13
14
15
16
17
18
PCR No.
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
1.5kb
1.0kb
Ligation Ratio:.
1:1:6
1:1:12
Sample No.
19
20
21
22
23
24
PCR No.
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
1.5kb
1.0kb

Figure 15

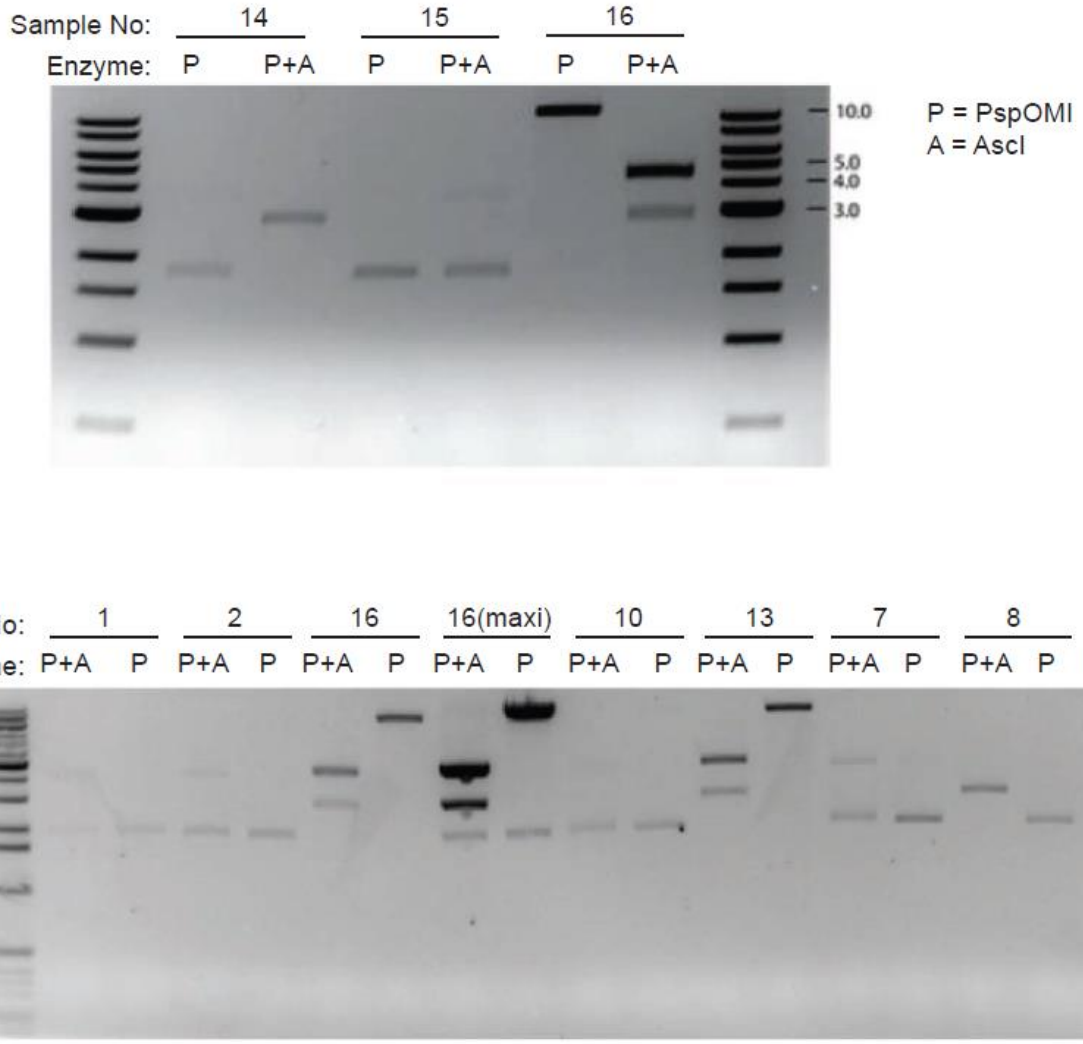

Sample No: 14 15 16
Enzyme: P P+A P P+A P P+A
10.0
5.0
4.0
3.0
P = PspOMI
A = AscI
Sample No: 1 2 16 16(maxi) 10 13 7 8 9
Enzyme: P+A P P+A P P+A P P+A P P+A P P+A P P+A P P+A P P+A P

Figure 16

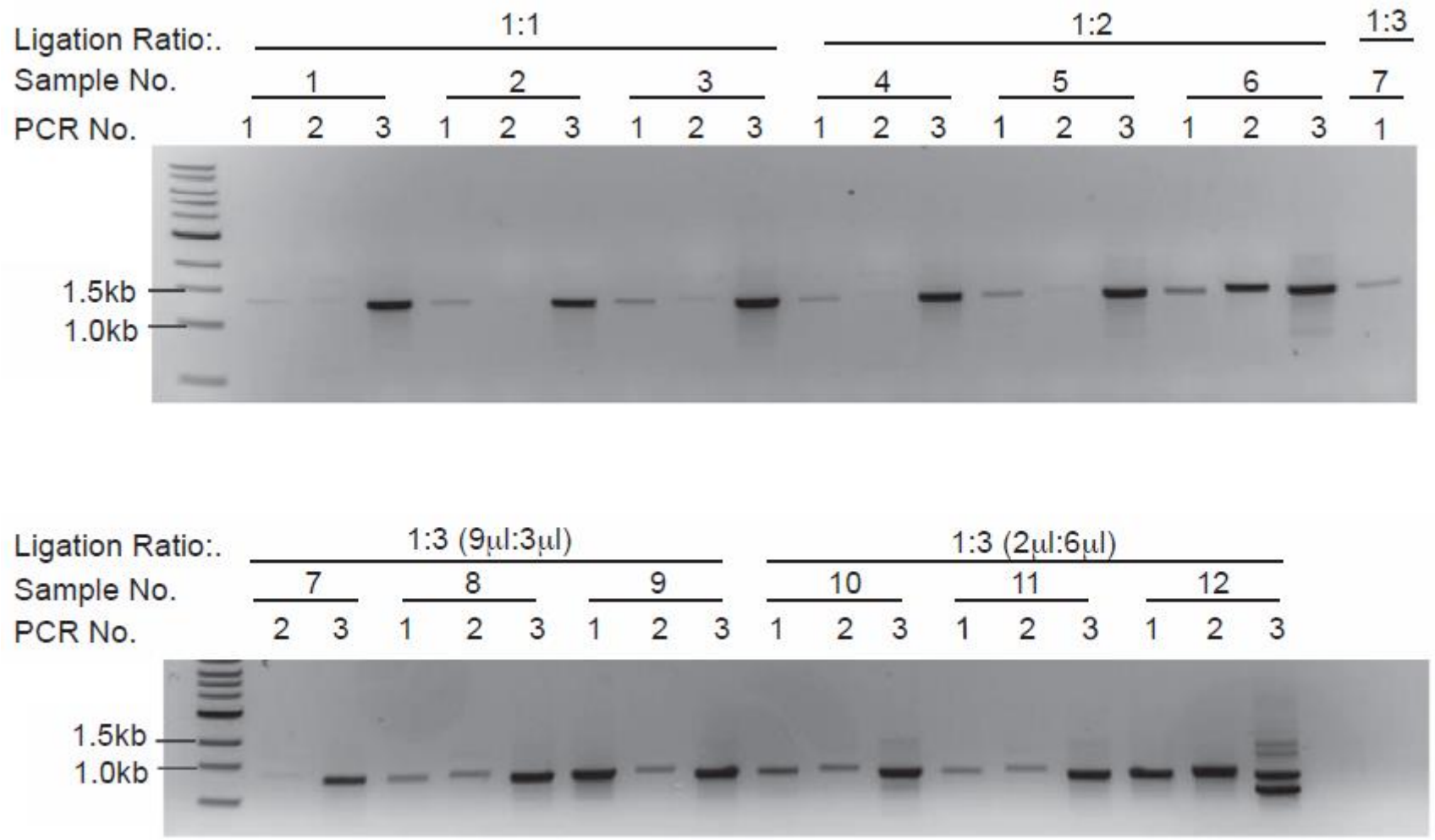

Ligation Ratio:.
1:1
1:2
1:3
Sample No.
1
2
3
4
5
6
7
PCR No.
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1
1.5kb
1.0kb
Ligation Ratio:.
1:3 (9μl:3μl)
1:3 (2μl:6μl)
Sample No.
7
8
9
10
11
12
PCR No.
2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
1.5kb
1.0kb

Figure 17

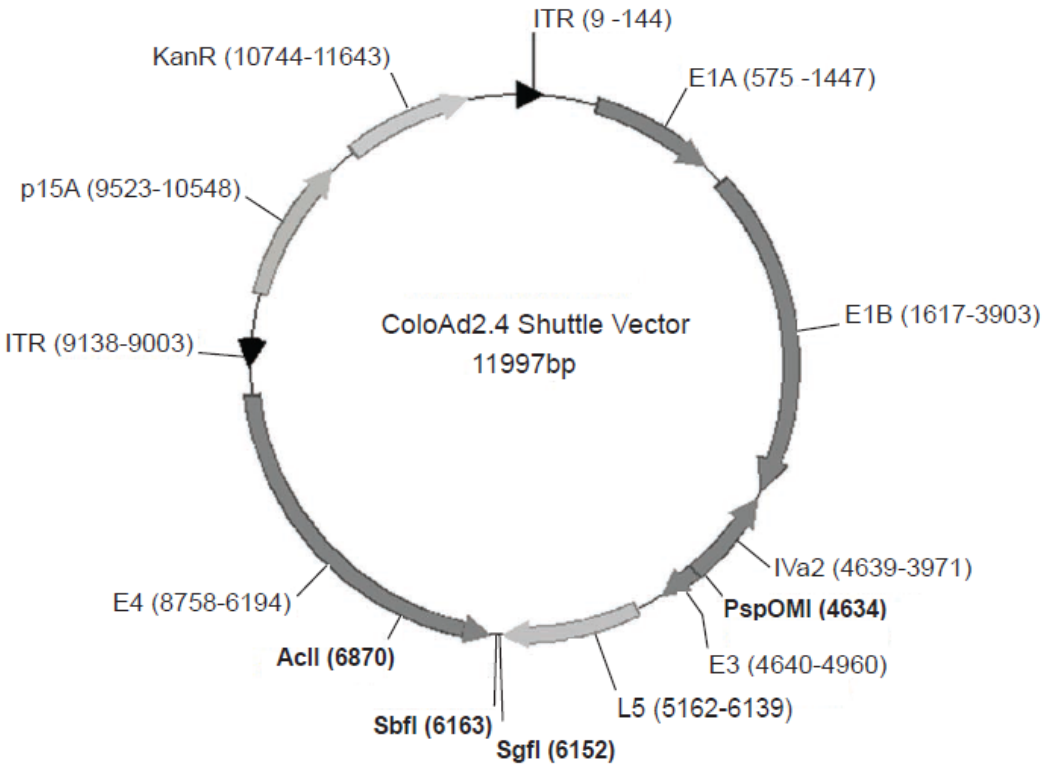


Figure 18



Figure 19

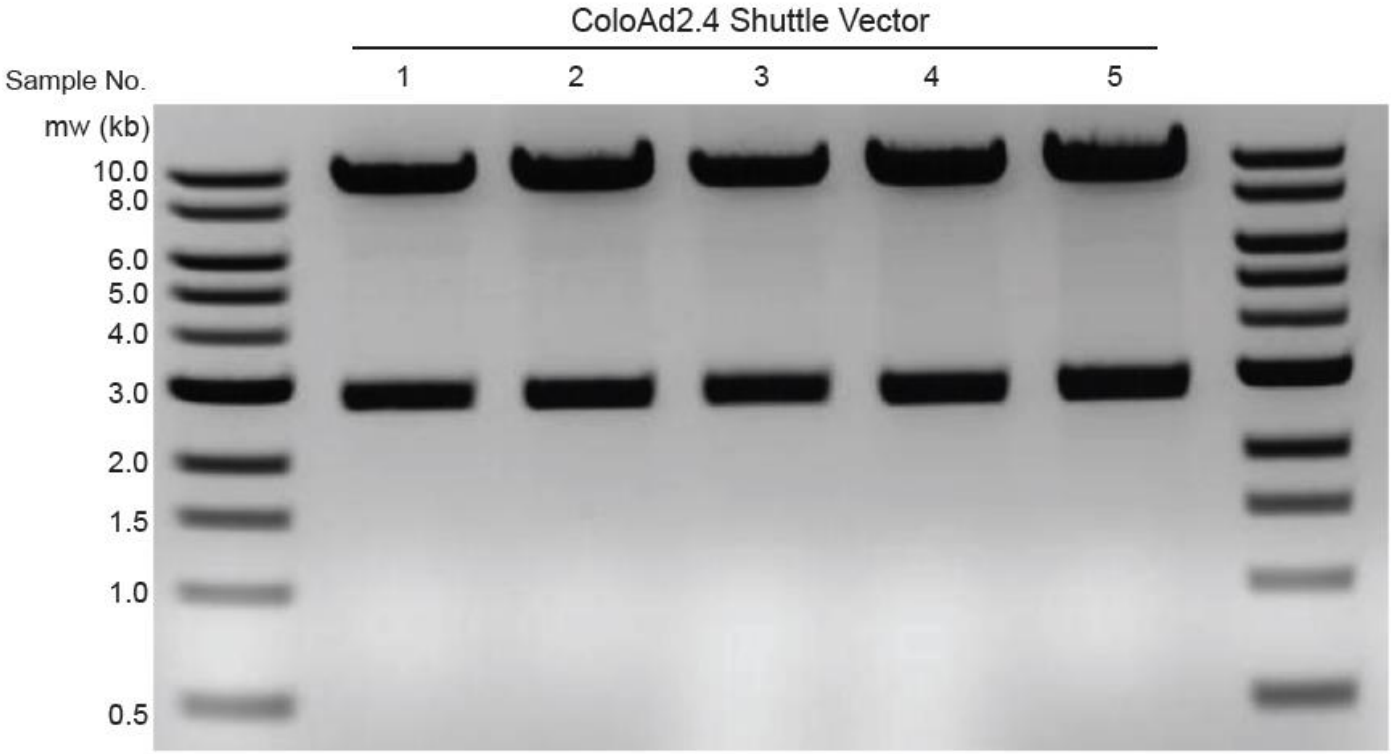

ColoAd2.4 Shuttle Vector
Sample No.
1
2
3
4
5
mw (kb)
10.0
8.0
6.0
5.0
4.0
3.0
2.0
1.5
1.0
0.5

Figure 20

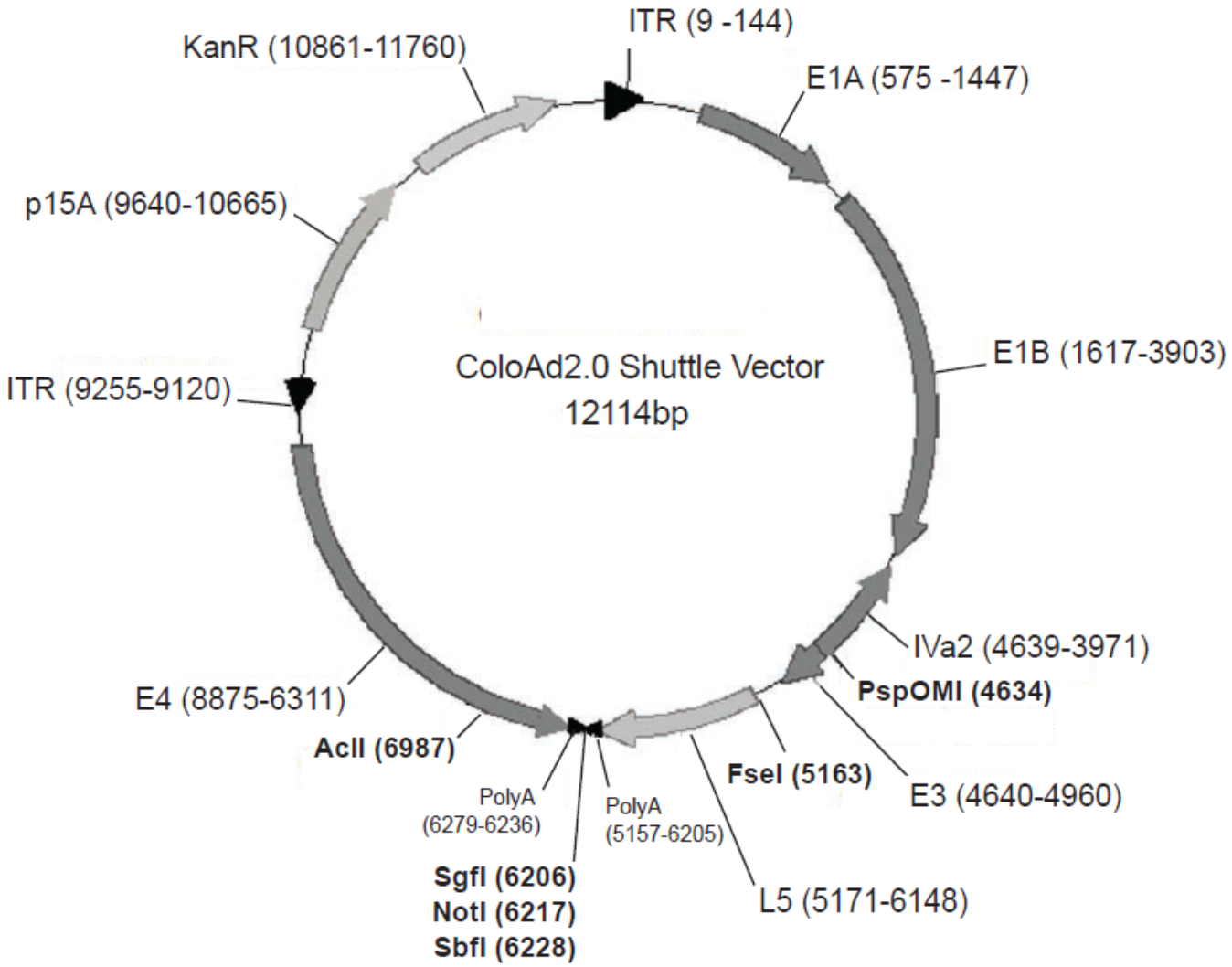

ITR (9 -144)
KanR (10861-11760)
E1A (575 -1447)
p15A (9640-10665)
ColoAd2.0 Shuttle Vector
12114bp
E1B (1617-3903)
ITR (9255-9120)
IVa2 (4639-3971)
PspOMI (4634)
E4 (8875-6311)
AclI (6987)
FseI (5163)
E3 (4640-4960)
PolyA
(6279-6236)
PolyA
(5157-6205)
SgfI (6206)
NotI (6217)
SbfI (6228)
L5 (5171-6148)

Figure 21

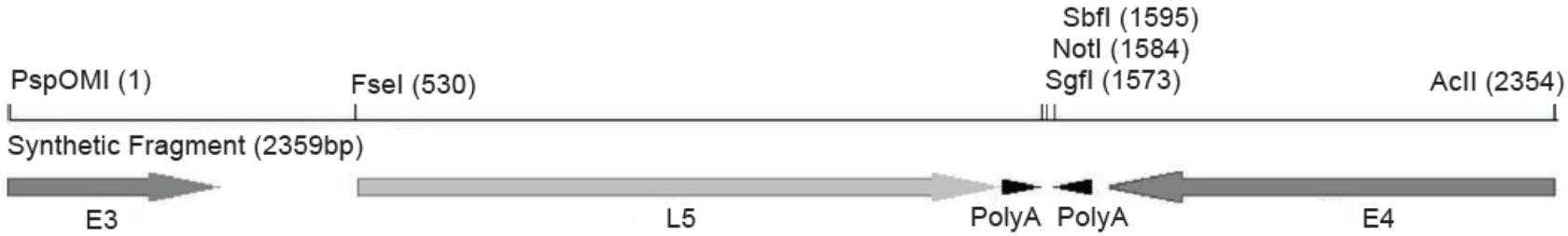


Figure 22

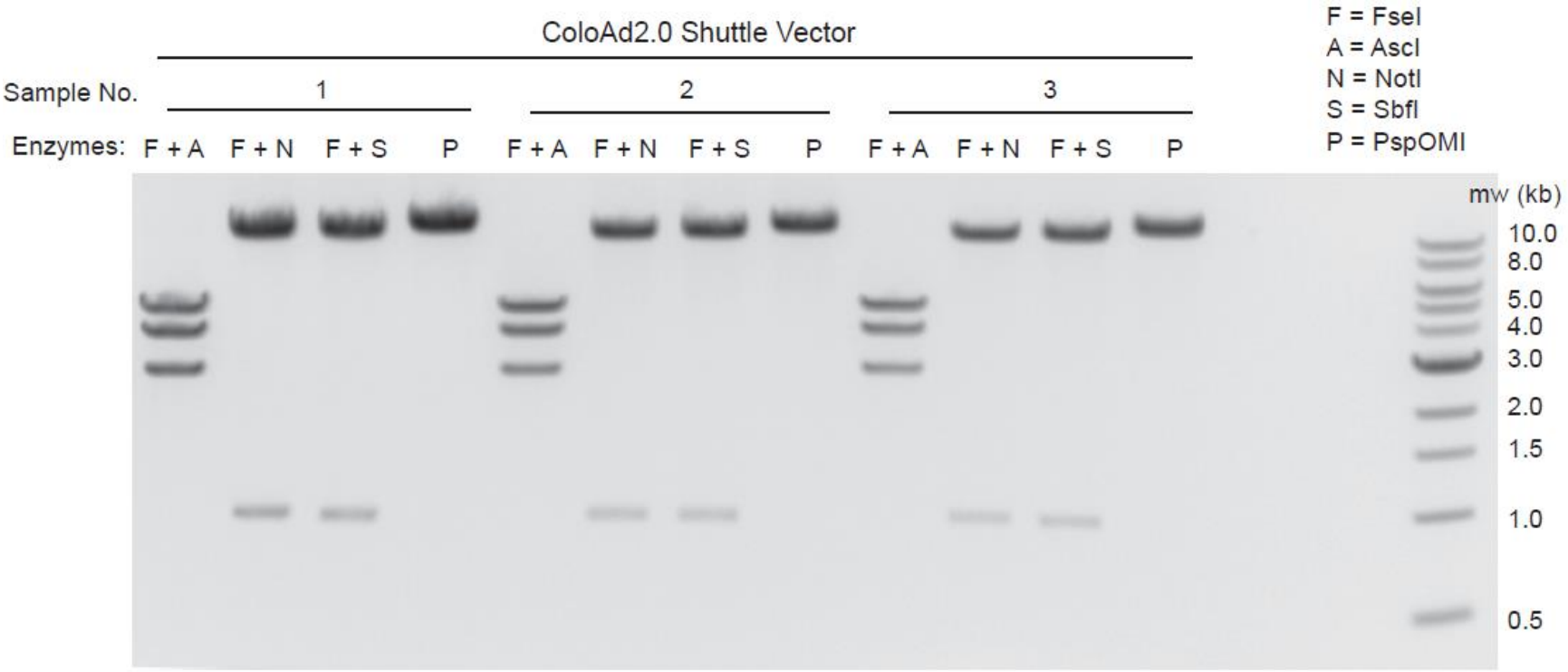


Figure 23

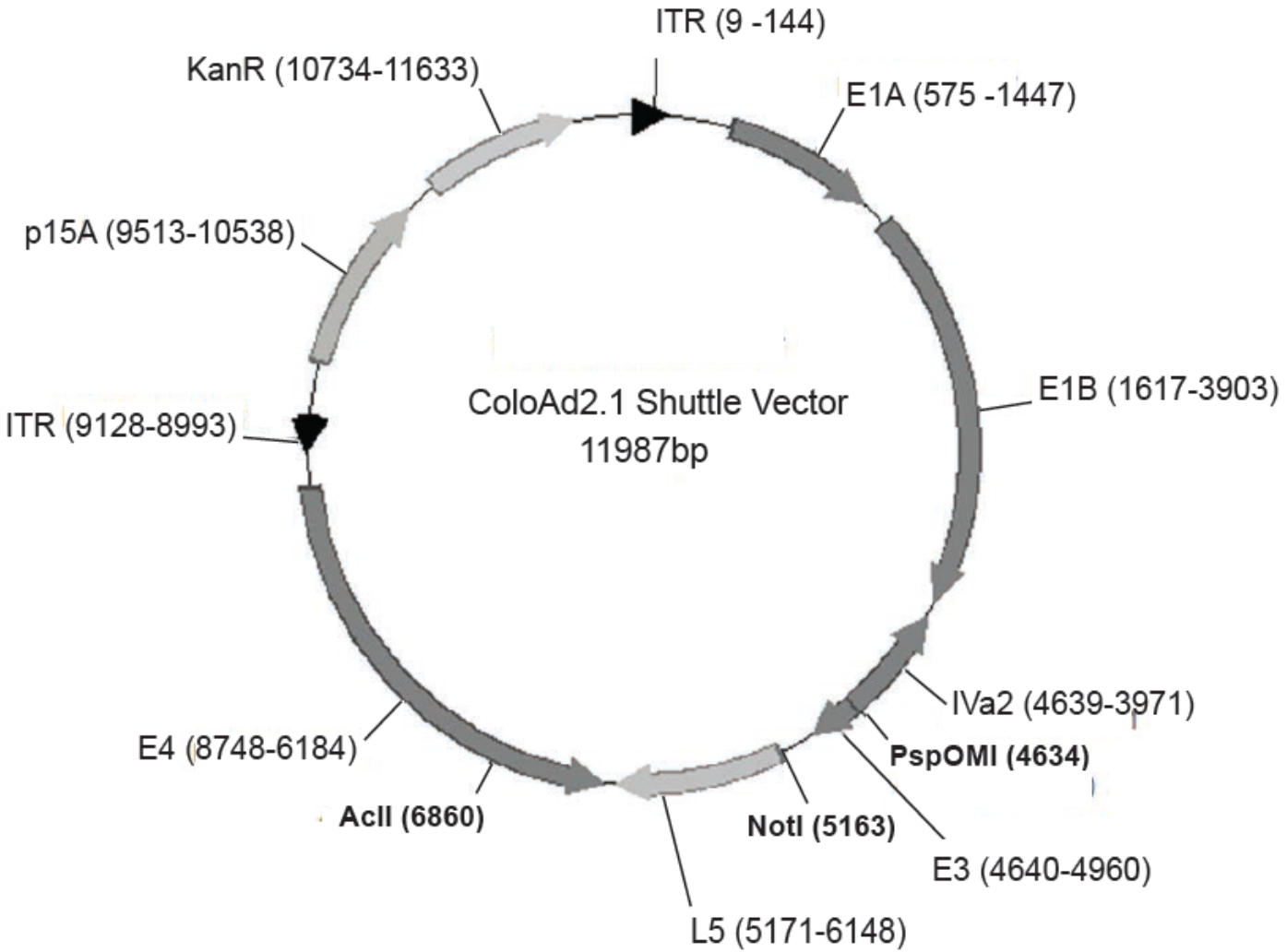

ITR (9 -144)
KanR (10734-11633)
E1A (575 -1447)
p15A (9513-10538)
ColoAd2.1 Shuttle Vector
11987bp
E1B (1617-3903)
ITR (9128-8993)
IVa2 (4639-3971)
PspOMI (4634)
E4 (8748-6184)
AclI (6860)
NotI (5163)
E3 (4640-4960)
L5 (5171-6148)

Figure 24

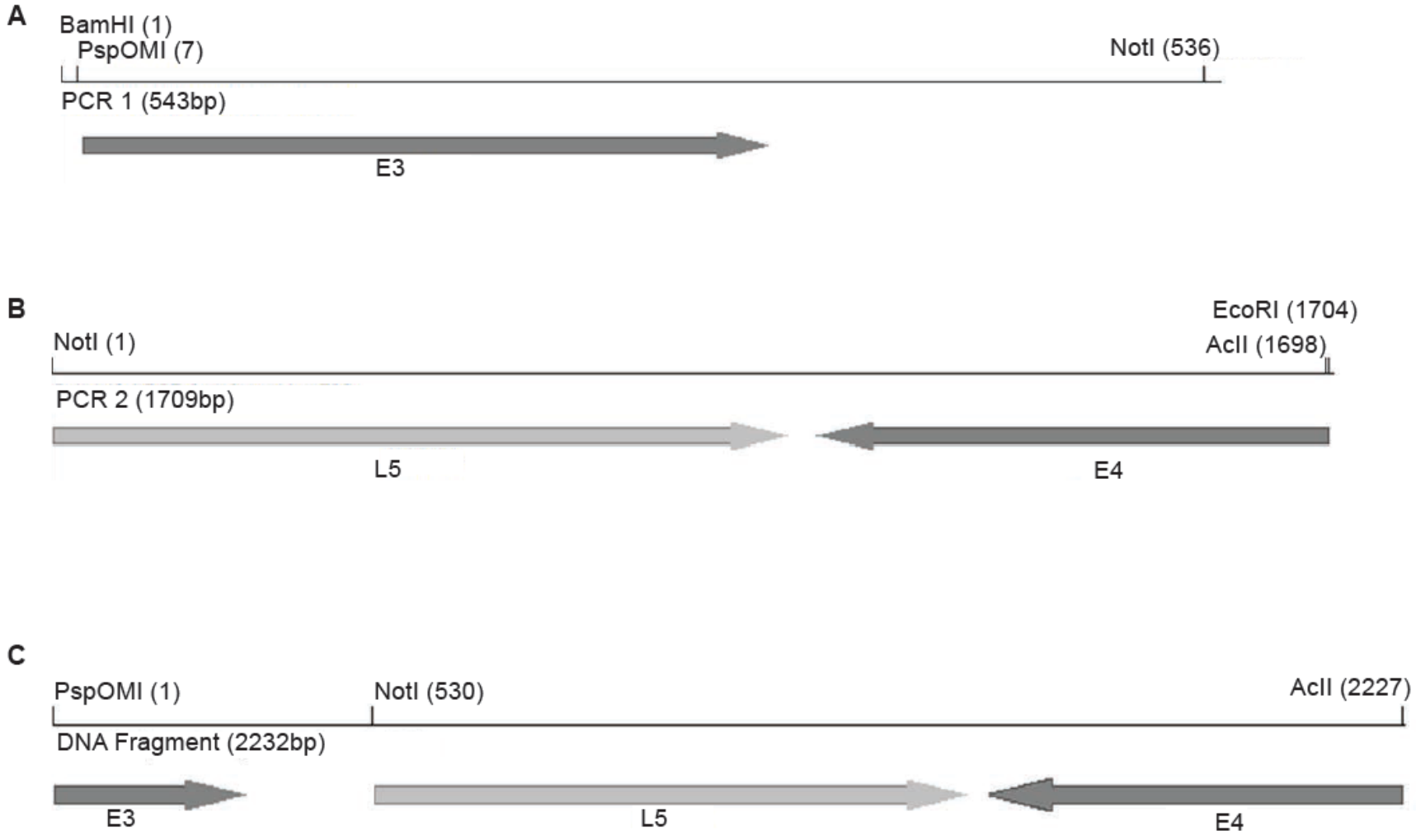

A
BamHI (1)
PspOMI (7)
NotI (536)
PCR 1 (543bp)
E3
B
EcoRI (1704)
NotI (1)
AclI (1698)
PCR 2 (1709bp)
L5
E4
C
PspOMI (1)
NotI (530)
AclI (2227)
DNA Fragment (2232bp)
E3
L5
E4

Figure 25

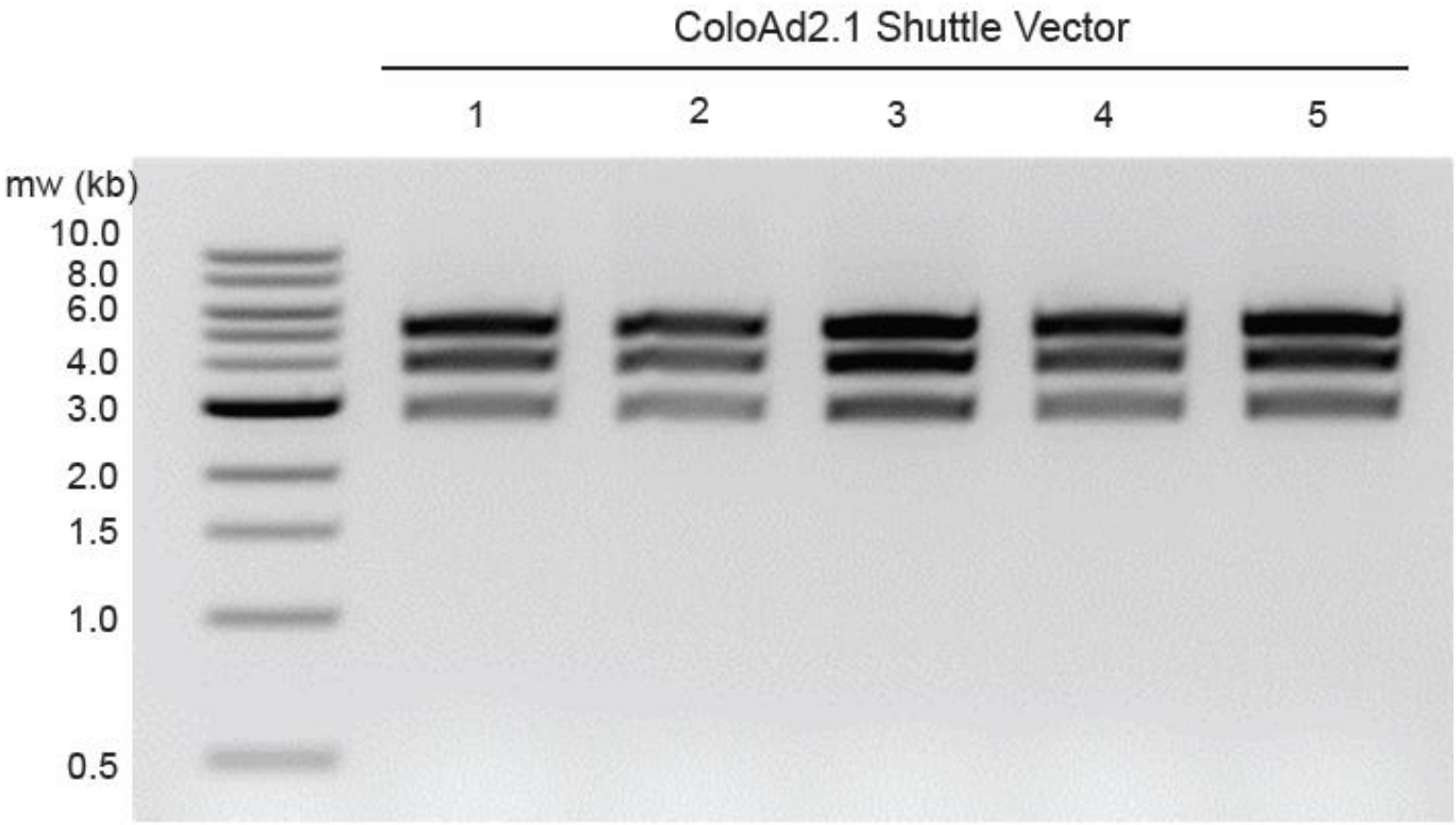


Figure 26

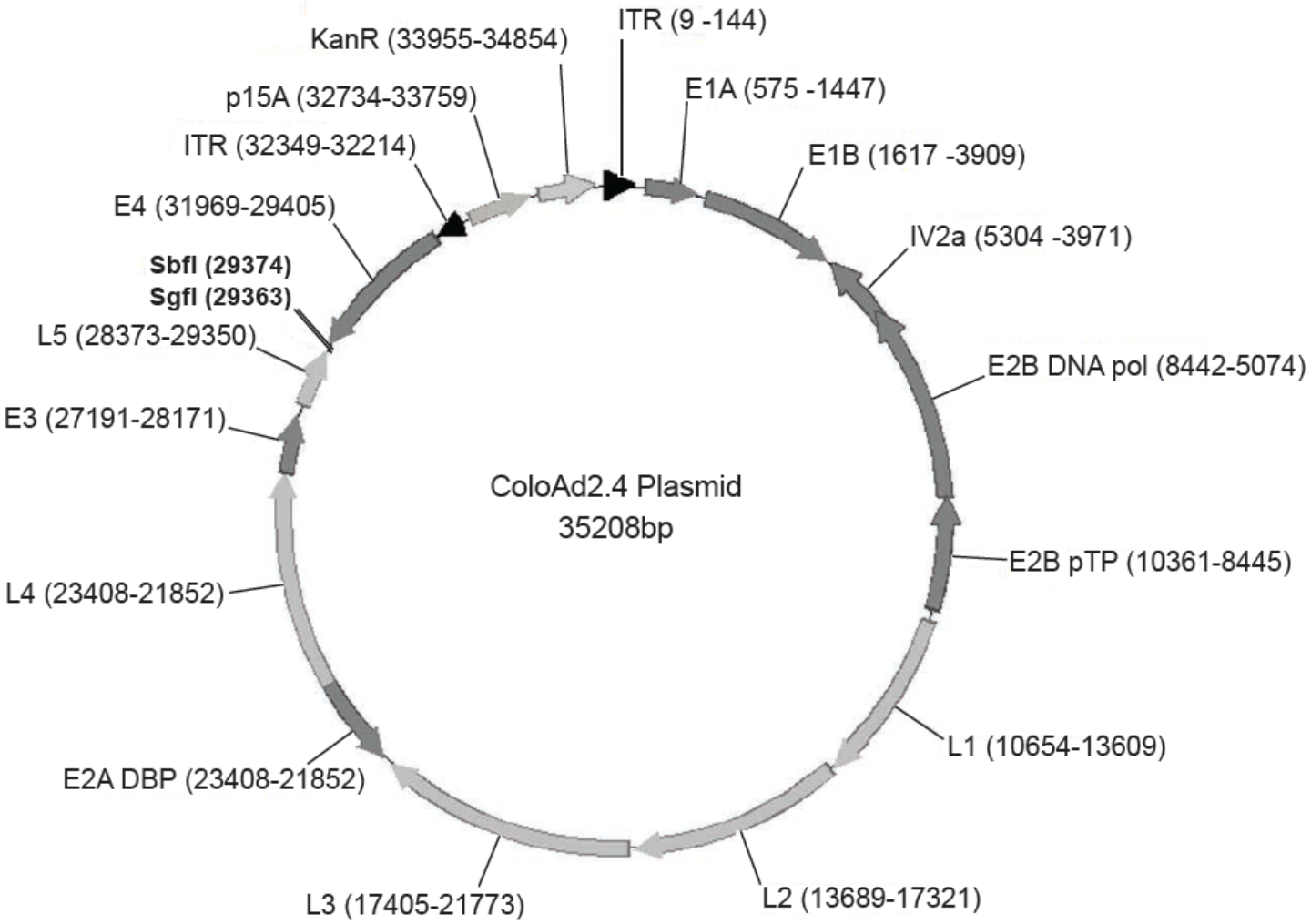

ITR (9 -144)
KanR (33955-34854)
E1A (575 -1447)
p15A (32734-33759)
E1B (1617 -3909)
ITR (32349-32214)
IV2a (5304 -3971)
E4 (31969-29405)
SbfI (29374)
SgfI (29363)
L5 (28373-29350)
E2B DNA pol (8442-5074)
E3 (27191-28171)
ColoAd2.4 Plasmid
35208bp
E2B pTP (10361-8445)
L4 (23408-21852)
L1 (10654-13609)
E2A DBP (23408-21852)
L3 (17405-21773)
L2 (13689-17321)

Figure 27

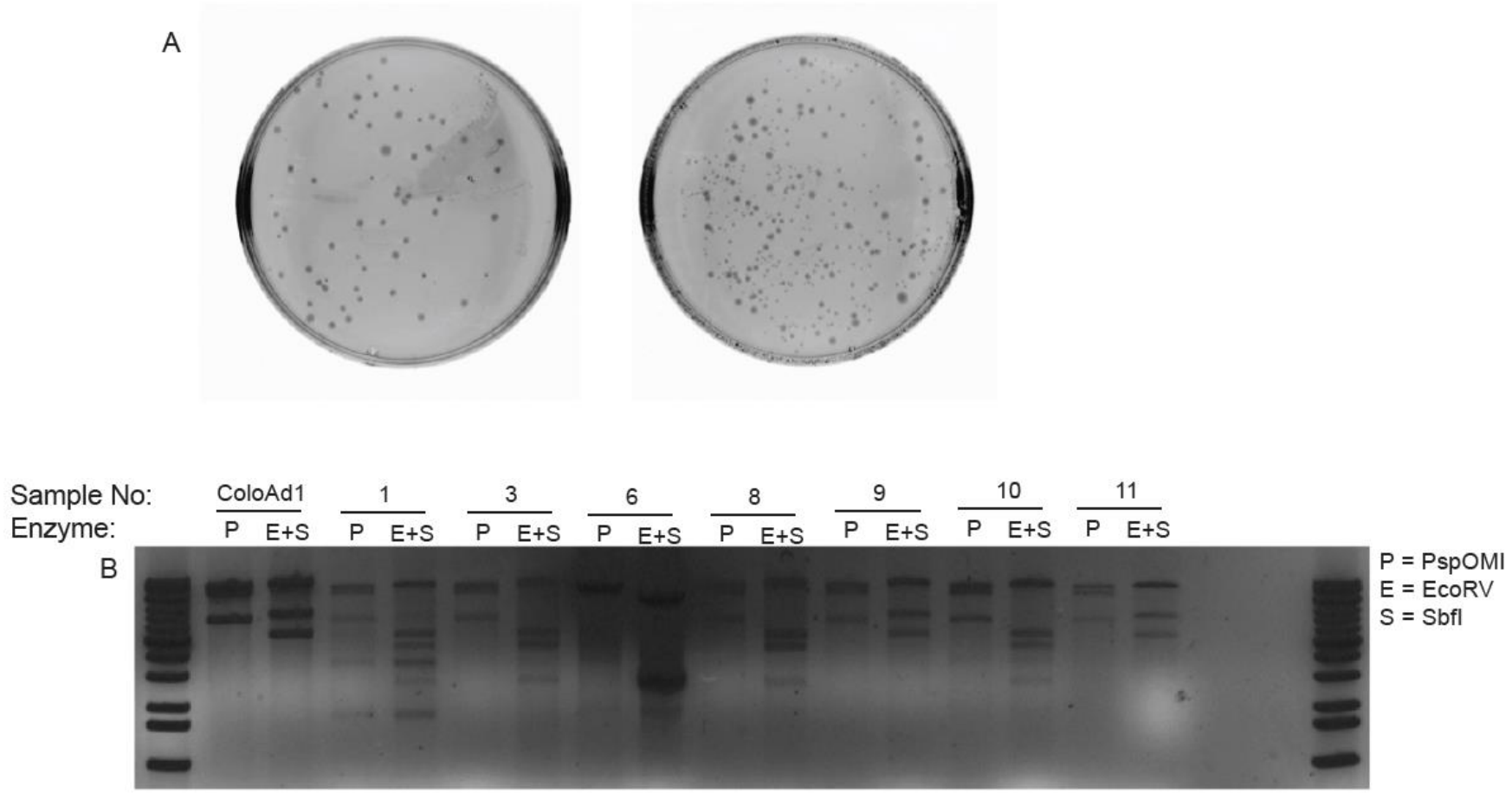

A
Sample No:
ColoAd1
1
3
6
8
9
10
11
Enzyme:
P
E+S
B
P = PspOMI
E = EcoRV
S = SbfI

Figure 28

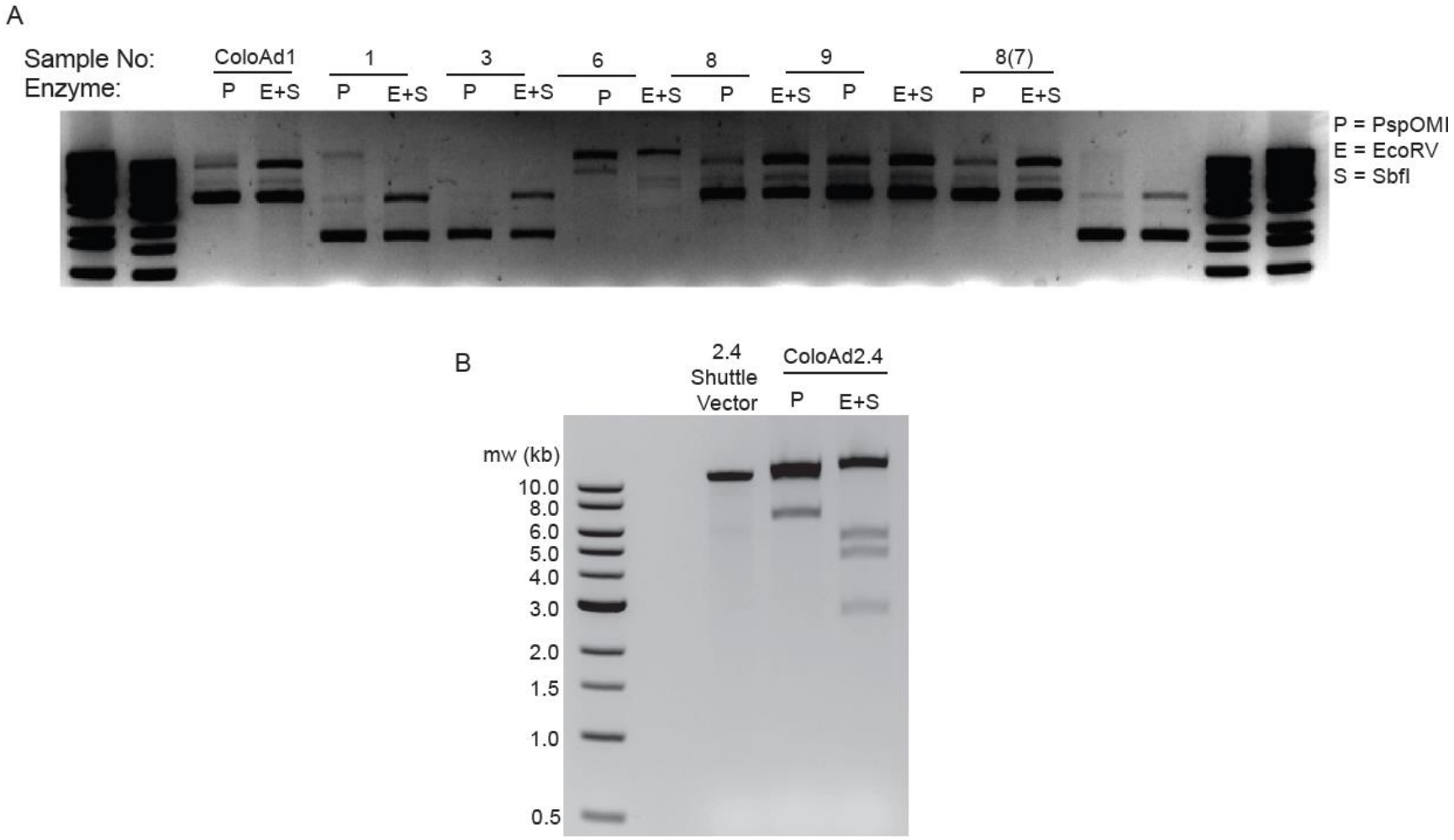

A
Sample No:
Enzyme:
ColoAd1
P
E+S
1
3
6
8
9
8(7)
P = PspOMI
E = EcoRV
S = SbfI
B
2.4 Shuttle Vector
ColoAd2.4
mw (kb)
10.0
8.0
6.0
5.0
4.0
3.0
2.0
1.5
1.0
0.5

Figure 29

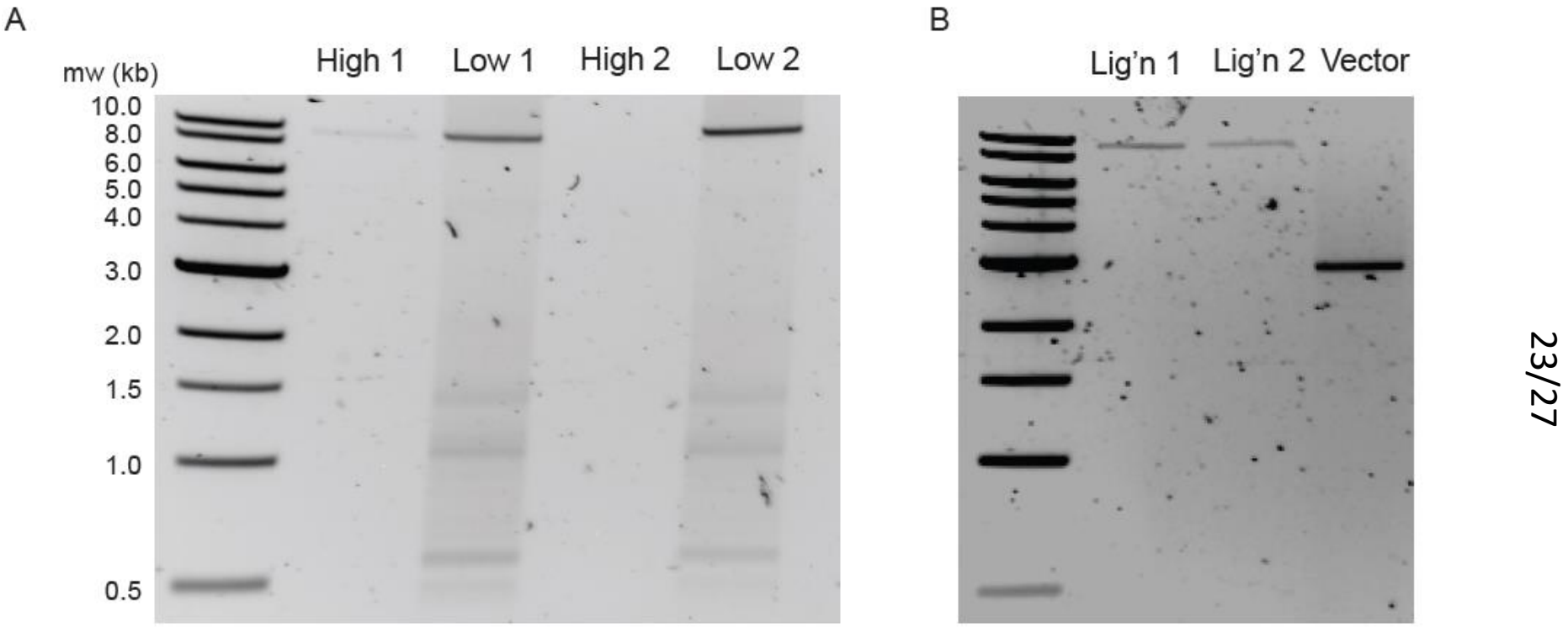

A
mw (kb)
10.0
8.0
6.0
5.0
4.0
3.0
2.0
1.5
1.0
0.5
High 1
Low 1
High 2
Low 2
B
Lig'n 1
Lig'n 2
Vector

Figure 30

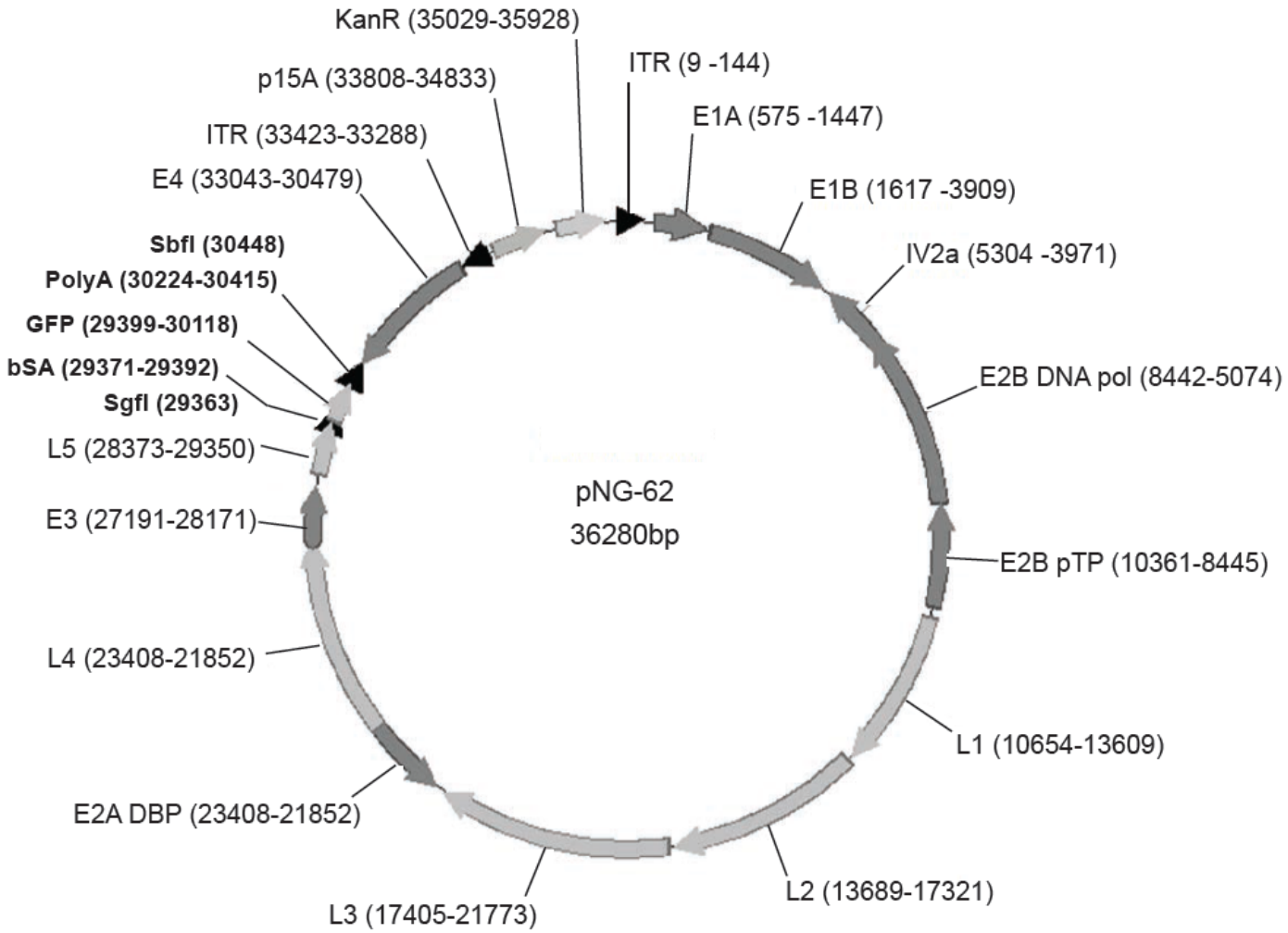

KanR (35029-35928)
ITR (9 -144)
p15A (33808-34833)
E1A (575 -1447)
ITR (33423-33288)
E1B (1617 -3909)
E4 (33043-30479)
IV2a (5304 -3971)
SbfI (30448)
PolyA (30224-30415)
GFP (29399-30118)
bSA (29371-29392)
SgfI (29363)
E2B DNA pol (8442-5074)
L5 (28373-29350)
pNG-62
36280bp
E3 (27191-28171)
E2B pTP (10361-8445)
L4 (23408-21852)
L1 (10654-13609)
E2A DBP (23408-21852)
L2 (13689-17321)
L3 (17405-21773)

Figure 31

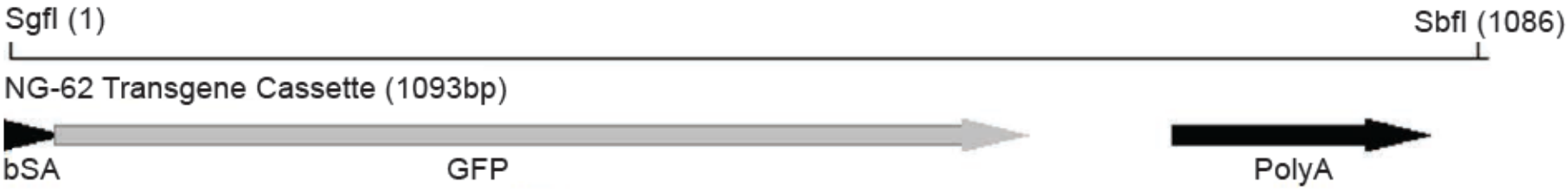


Figure 32

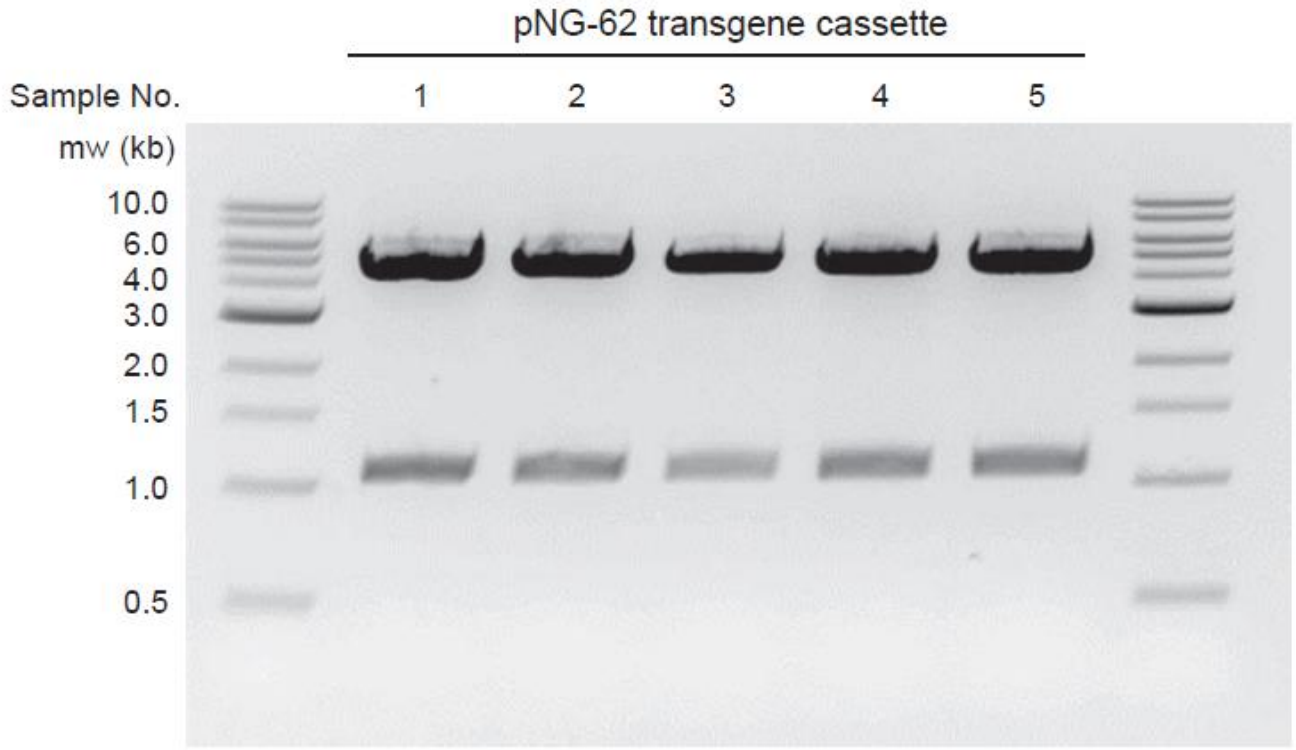


Figure 33

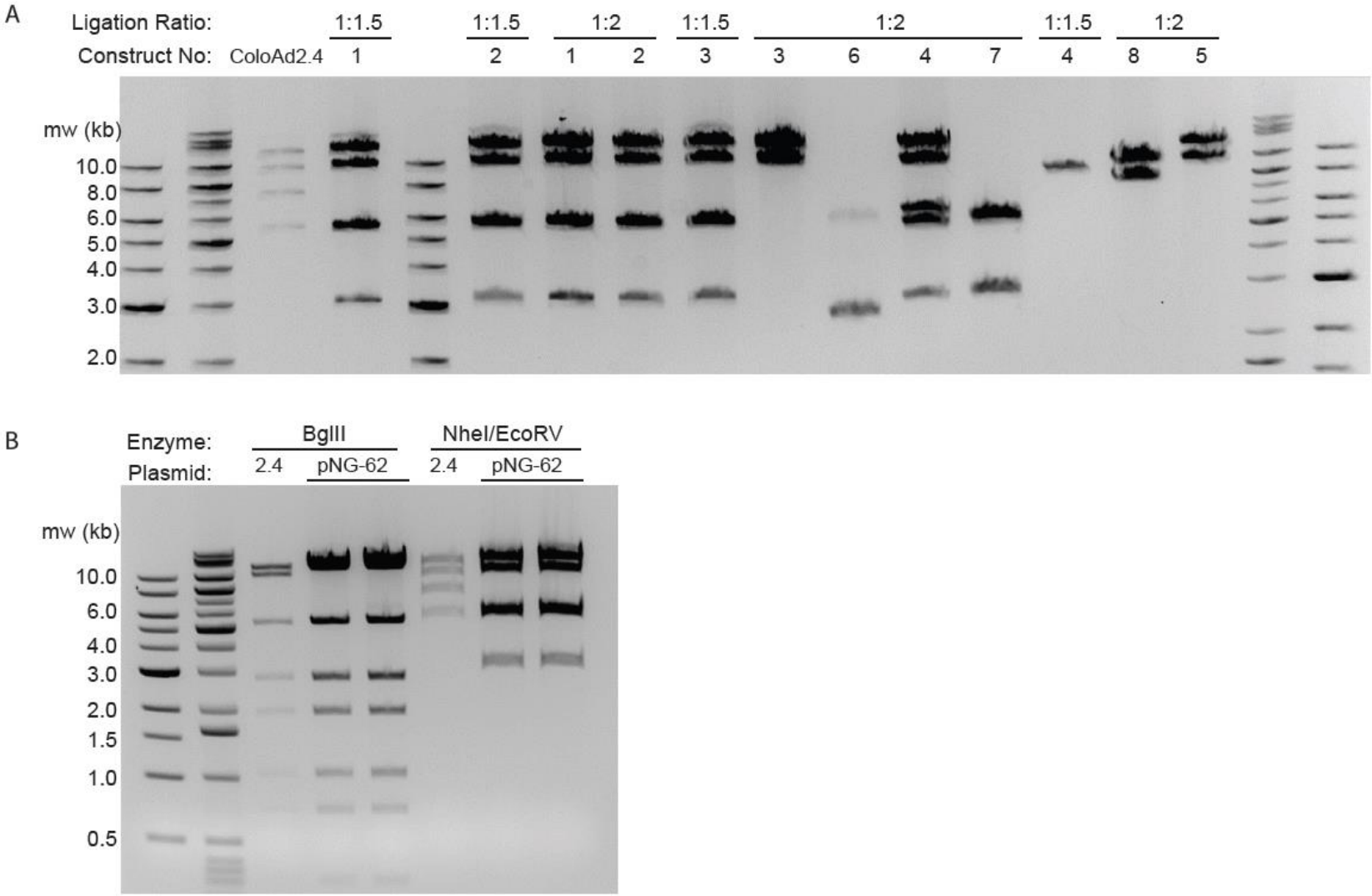
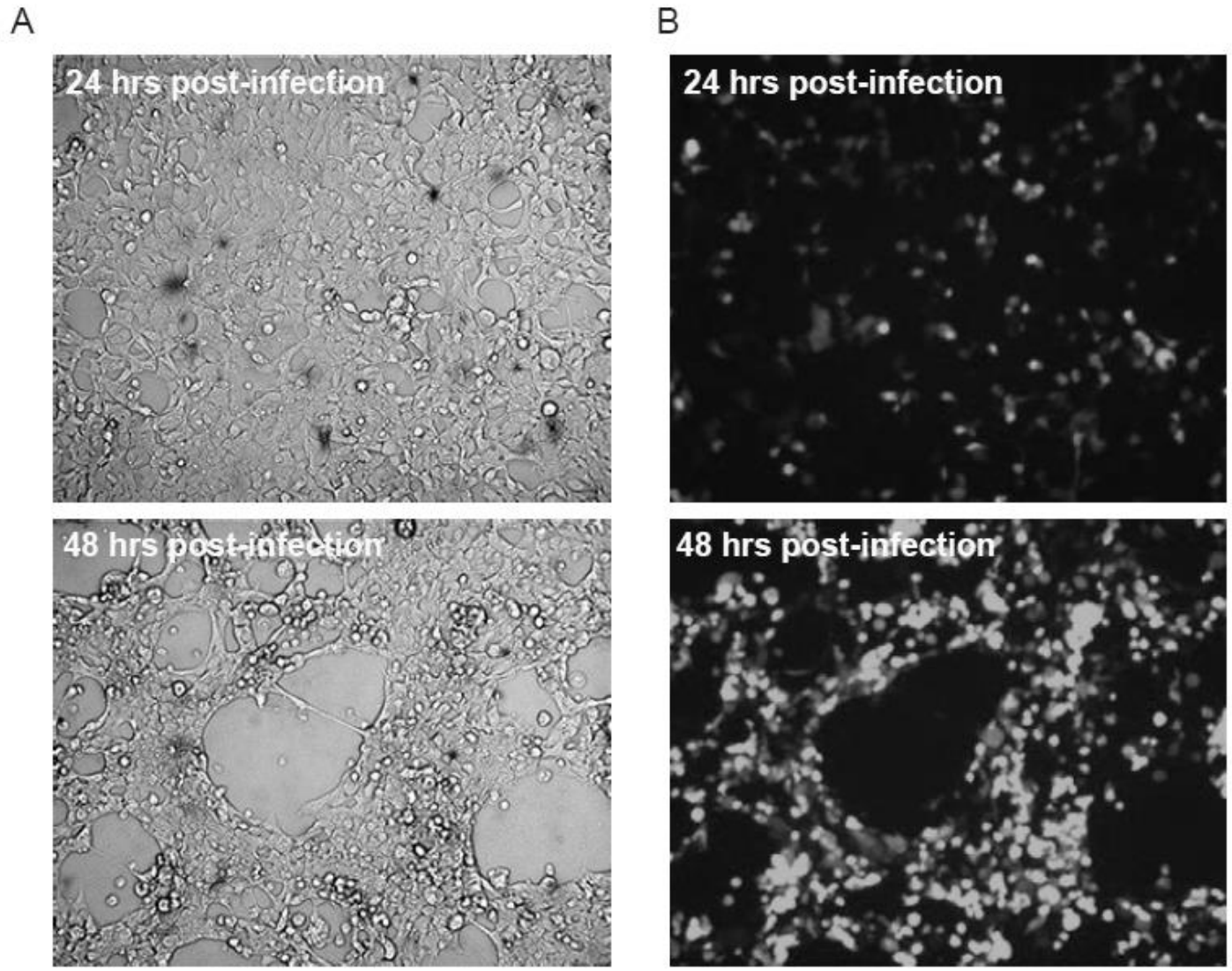

A
Ligation Ratio:
1:1.5
1:1.5
1:2
1:1.5
1:2
1:1.5
1:2
Construct No:
ColoAd2.4
1
2
1
2
3
3
6
4
7
4
8
5
mw (kb)
10.0
8.0
6.0
5.0
4.0
3.0
2.0
B
Enzyme:
BglII
NheI/EcoRV
Plasmid:
2.4
pNG-62
2.4
pNG-62
mw (kb)
10.0
6.0
4.0
3.0
2.0
1.5
1.0
0.5

Figure 34


A
B
24 hrs post-infection
24 hrs post-infection
48 hrs post-infection
48 hrs post-infection