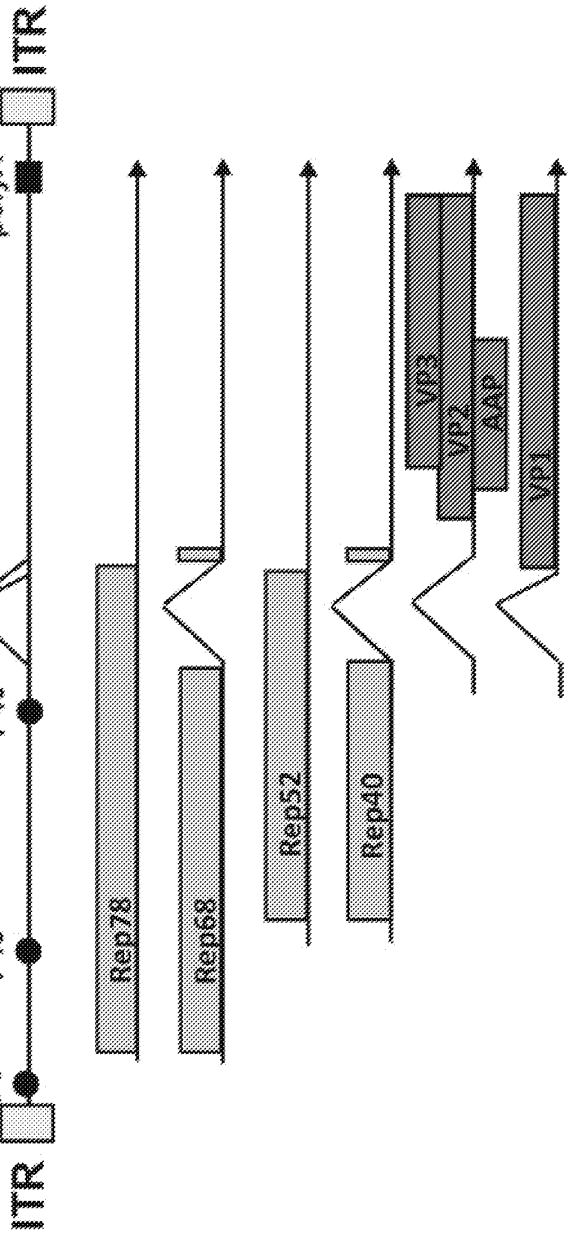

ITR
polyA
ITR
P5
P19
P40
Rep78
Rep68
Rep52
Rep40
VP3
VP2
AAP
VP1

Fig. 1

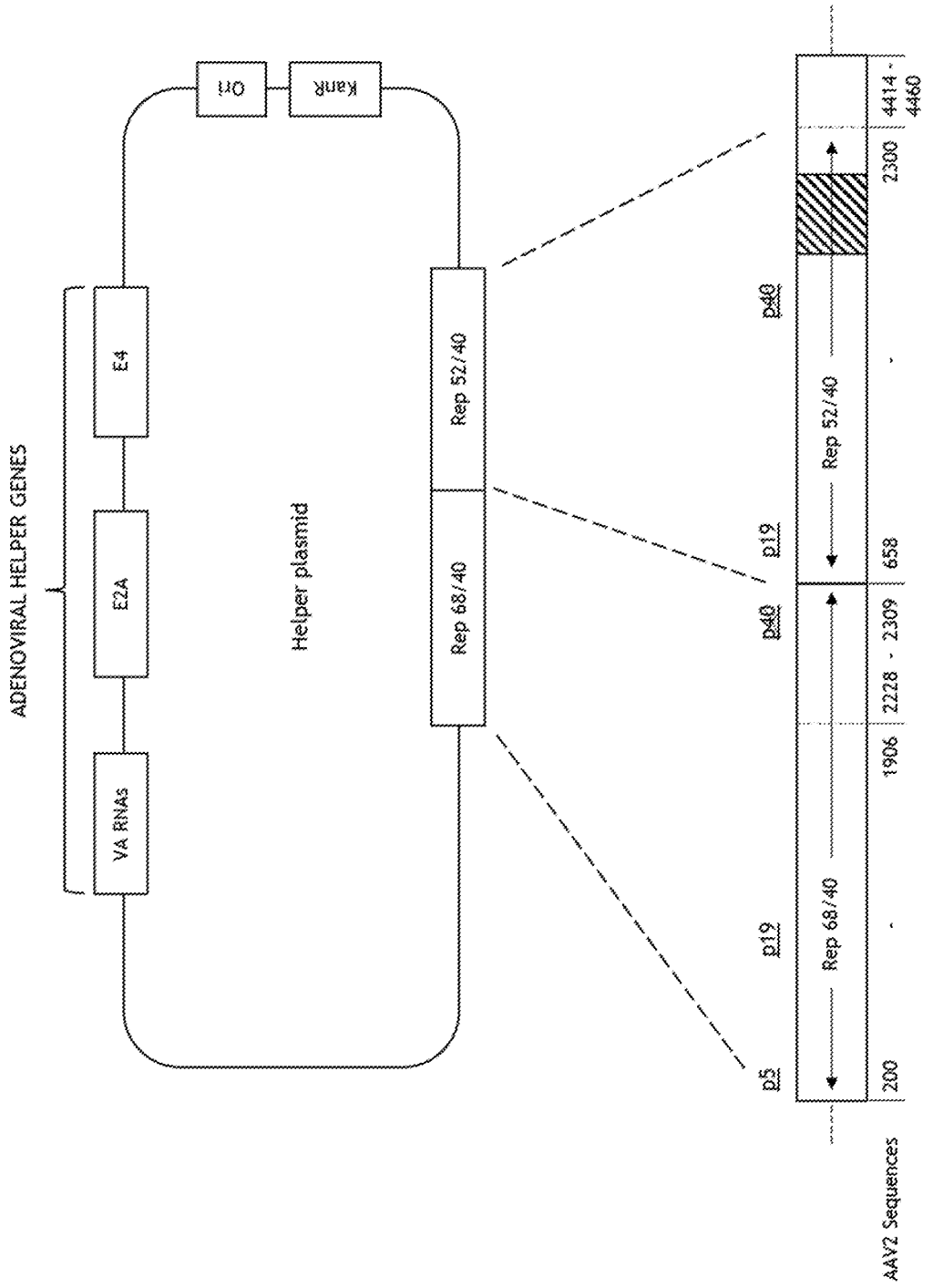

ADENOVIRAL HELPER GENES
VA RNAs
E2A
E4
Helper plasmid
KanR
Ori
Rep 68/40
Rep 52/40
p5
p19
p40
Rep 68/40
Rep 52/40
200
1906
2228 - 2309
658
2300
4414 - 4460
AAV2 Sequences

Fig. 2

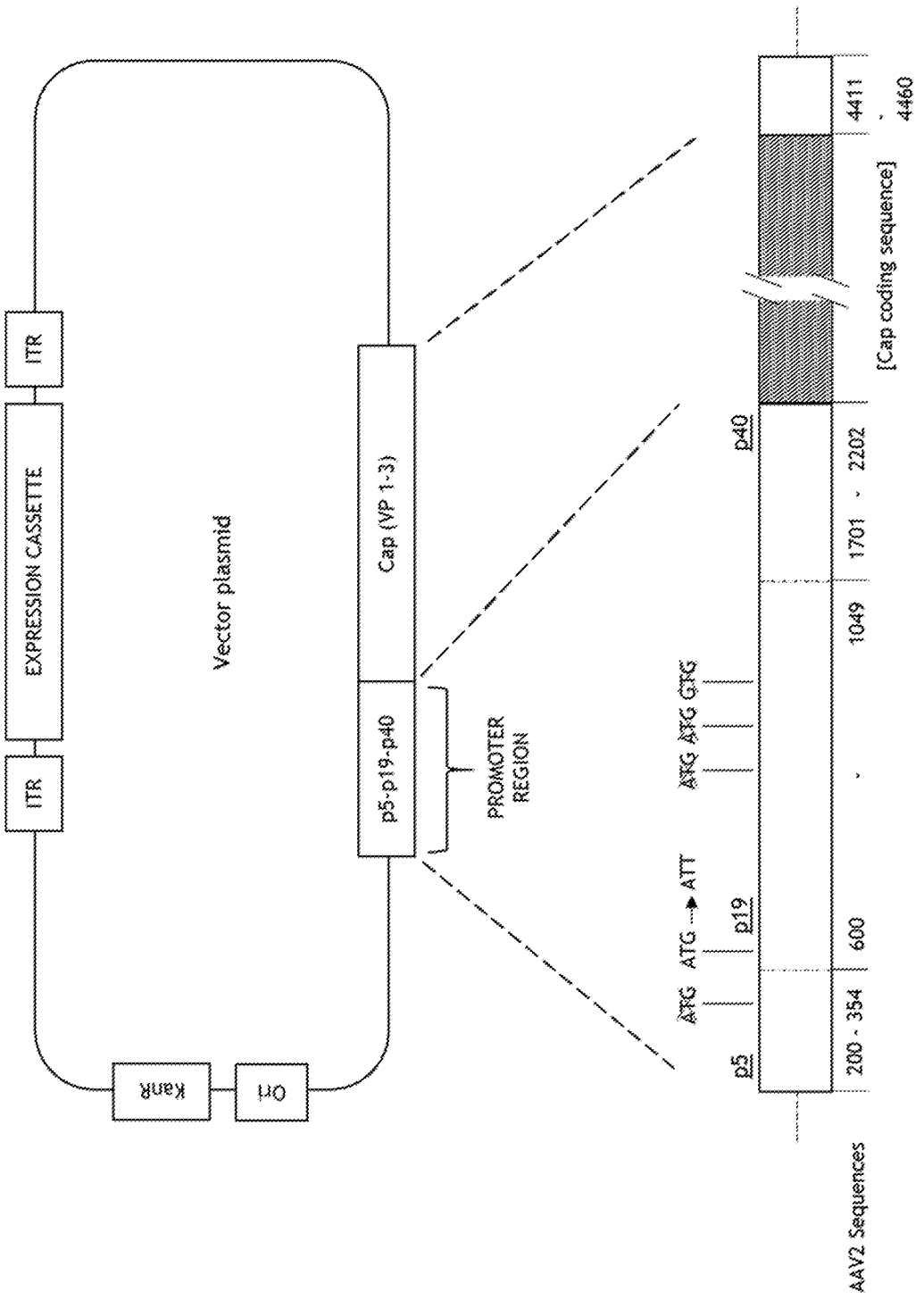

ITR
EXPRESSION CASSETTE
ITR
Vector plasmid
kanR
Ori
p5-p19-p40
Cap (VP 1-3)
PROMOTER REGION
AAV2 Sequences
p5
ATG
ATG → ATT
p19
ATG ATG GTG
p40
200 - 354
600
1049
1701 - 2202
4411 - 4460
[Cap coding sequence]

Fig. 3

Fig. 4

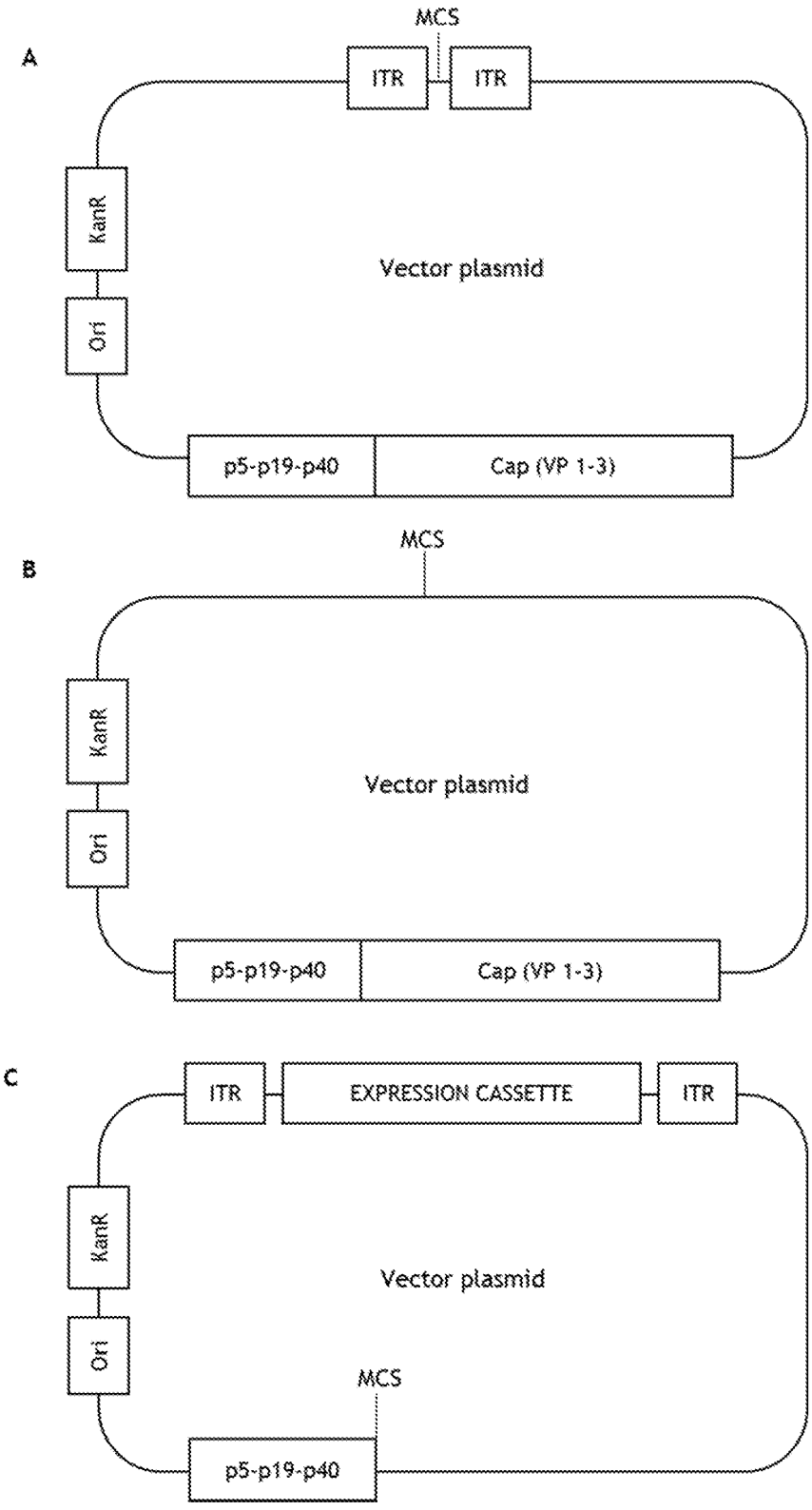

A
MCS
ITR
ITR
KanR
Ori
Vector plasmid
p5-p19-p40
Cap (VP 1-3)
B
MCS
KanR
Ori
Vector plasmid
p5-p19-p40
Cap (VP 1-3)
C
ITR
EXPRESSION CASSETTE
ITR
KanR
Ori
Vector plasmid
MCS
p5-p19-p40

Fig. 4 (cont.)

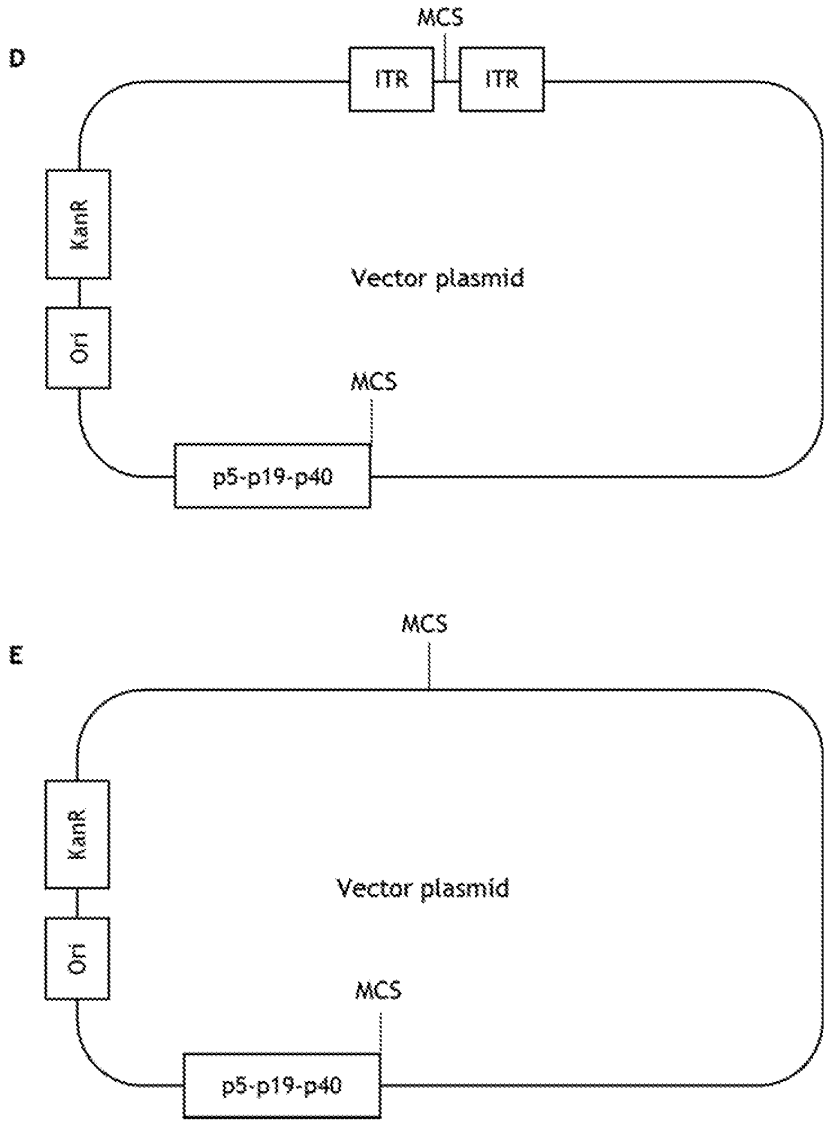
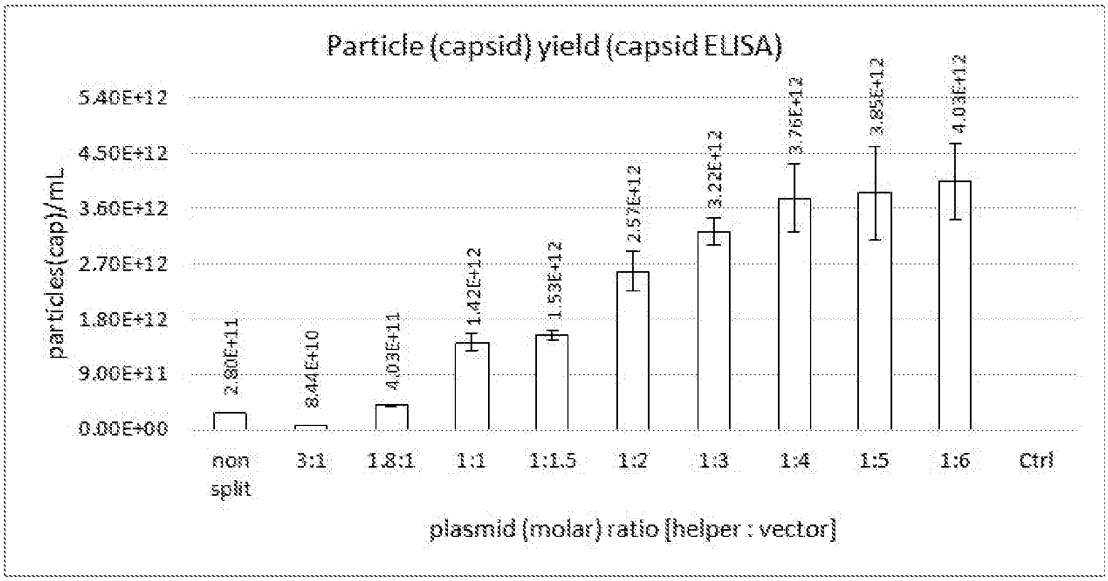

D
MCS
ITR
ITR
KanR
Vector plasmid
Ori
MCS
p5-p19-p40
E
MCS
KanR
Vector plasmid
Ori
MCS
p5-p19-p40

Fig. 5

A


Particle (capsid) yield (capsid ELISA)
particles(cap)/mL
5.40E+12
4.50E+12
3.60E+12
2.70E+12
1.80E+12
9.00E+11
0.00E+00
2.80E+11
8.44E+10
4.03E+11
1.42E+12
1.53E+12
2.57E+12
3.22E+12
3.76E+12
3.85E+12
4.03E+12
non split
3:1
1.8:1
1:1
1:1.5
1:2
1:3
1:4
1:5
1:6
Ctrl
plasmid (molar) ratio [helper : vector]

B

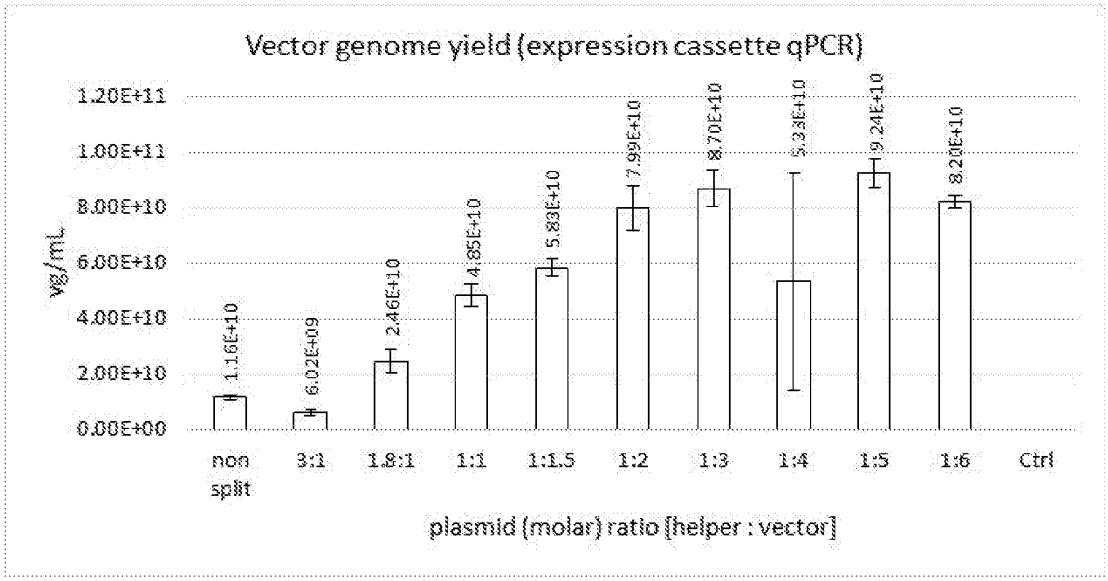

Vector genome yield (expression cassette qPCR)
vg/mL
1.20E+11
1.00E+11
8.00E+10
6.00E+10
4.00E+10
2.00E+10
0.00E+00
1.16E+10
6.02E+09
2.46E+10
4.85E+10
5.83E+10
7.99E+10
8.70E+10
5.33E+10
9.24E+10
8.20E+10
non split
3:1
1.8:1
1:1
1:1.5
1:2
1:3
1:4
1:5
1:6
Ctrl
plasmid (molar) ratio [helper : vector]

Fig. 5 (cont.)

C

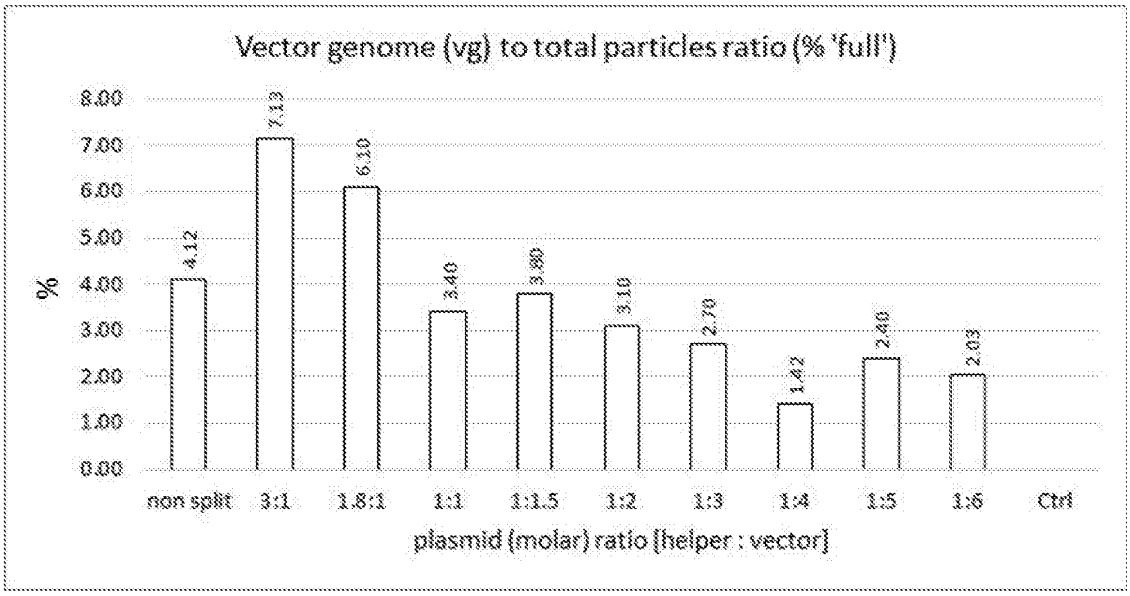
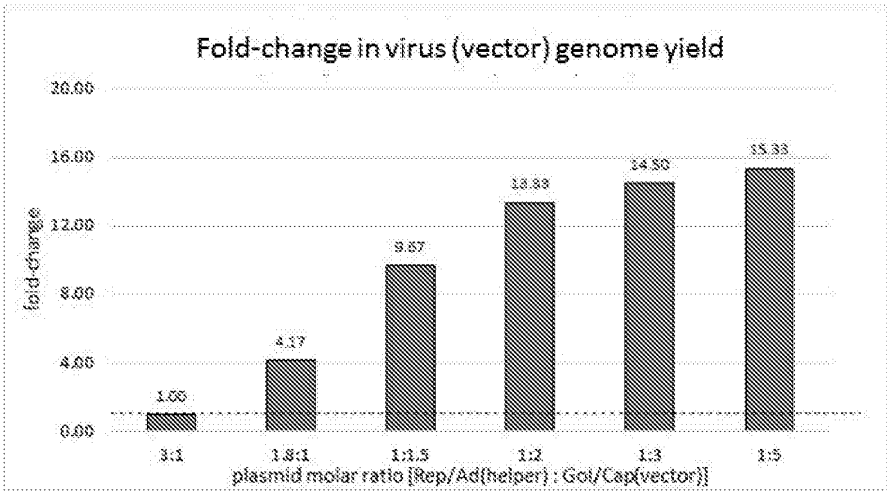

Vector genome (vg) to total particles ratio (% 'full')
%
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
4.12
7.13
6.10
3.40
3.80
3.10
2.70
1.42
2.40
2.03
non split
3:1
1.8:1
1:1
1:1.5
1:2
1:3
1:4
1:5
1:6
Ctrl
plasmid (molar) ratio [helper : vector]

Fig. 6

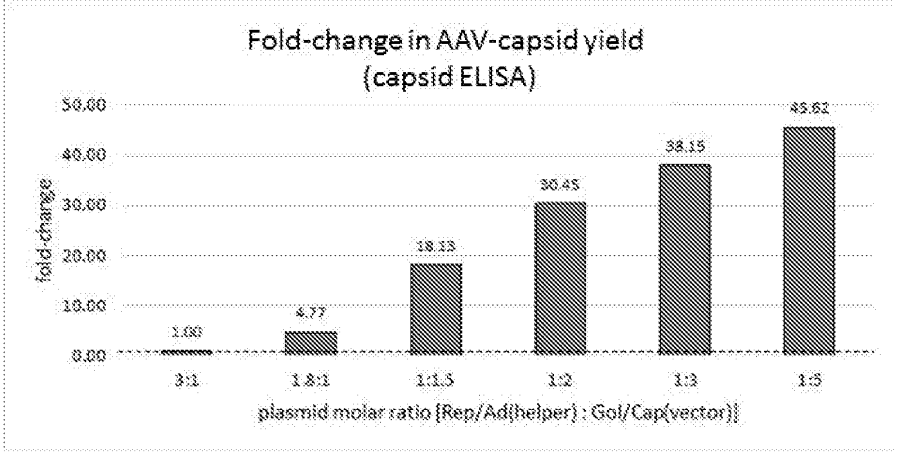
	Result	Detection
Batch 1	No replication	<10 rcAAV per 1E+10 vg
Batch 2	No replication	<10 rcAAV per 1E+10 vg
Batch 3	No replication	<10 rcAAV per 1E+10 vg

Fig. 7

A


Fold-change in virus (vector) genome yield
fold-change
20.00
16.00
12.00
8.00
4.00
0.00
1.00
4.17
9.67
13.33
14.50
15.33
3:1
1.8:1
1:1.5
1:2
1:3
1:5
plasmid molar ratio [Rep/Ad(helper) : GoI/Cap(vector)]

B


Fold-change in AAV-capsid yield
(capsid ELISA)
fold-change
50.00
40.00
30.00
20.00
10.00
0.00
1.00
4.77
18.13
30.45
38.15
45.62
3:1
1.8:1
1:1.5
1:2
1:3
1:5
plasmid molar ratio [Rep/Ad(helper) : GoI/Cap(vector)]

C

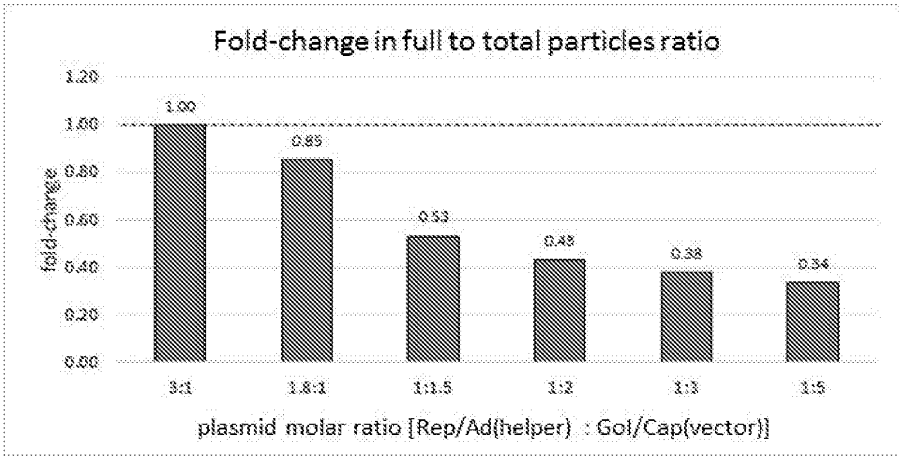

Fold-change in full to total particles ratio
fold-change
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.00
0.85
0.53
0.43
0.38
0.34
3:1
1.8:1
1:1.5
1:2
1:3
1:5
plasmid molar ratio [Rep/Ad(helper) : GoI/Cap(vector)]

Fig. 8

A

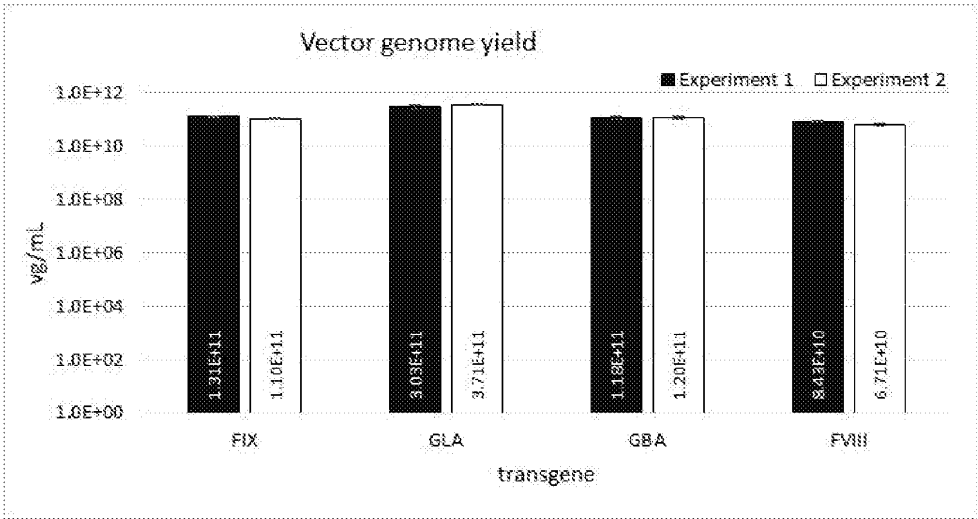
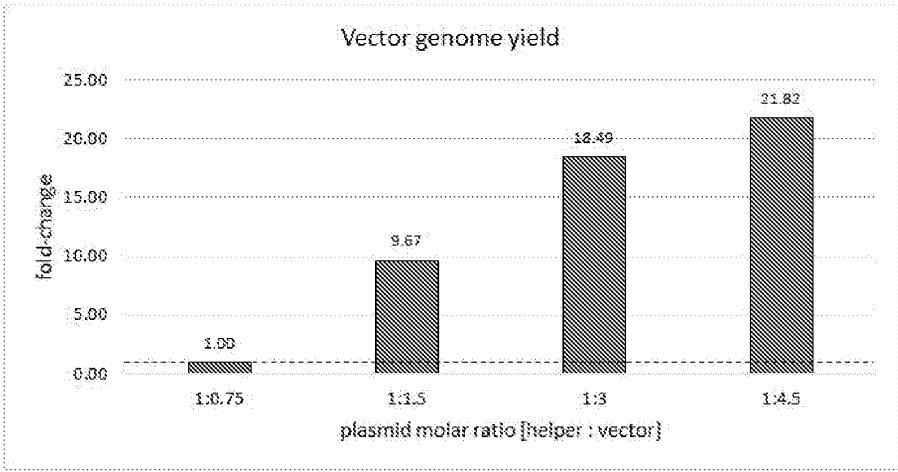
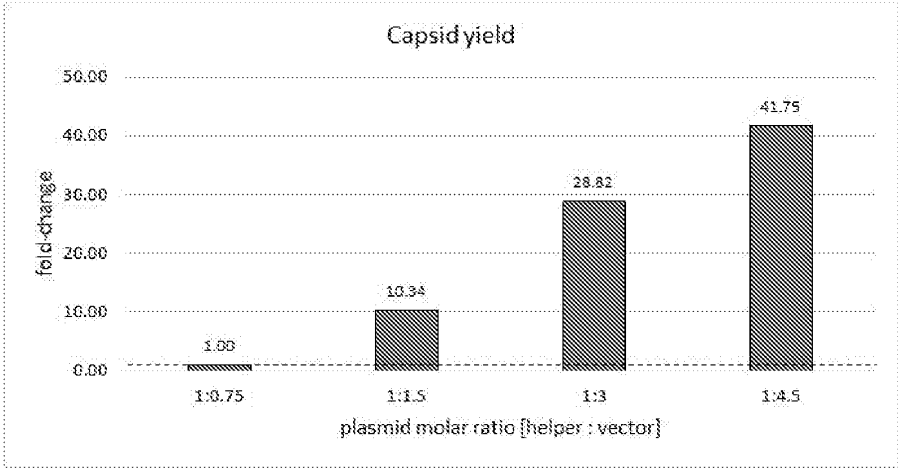

Vector genome yield
Experiment 1
Experiment 2
1.0E+12
1.0E+10
1.0E+08
1.0E+06
1.0E+04
1.0E+02
1.0E+00
vg/mL
1.31E+11
1.10E+11
3.03E+11
3.71E+11
1.13E+11
1.20E+11
8.43E+10
6.71E+10
FIX
GLA
GBA
FVIII
transgene

Fig. 8 (cont.)

B



C



D

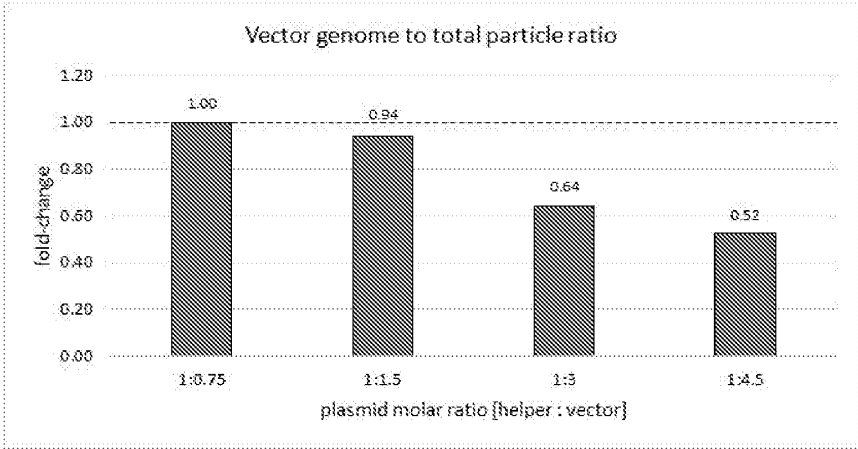
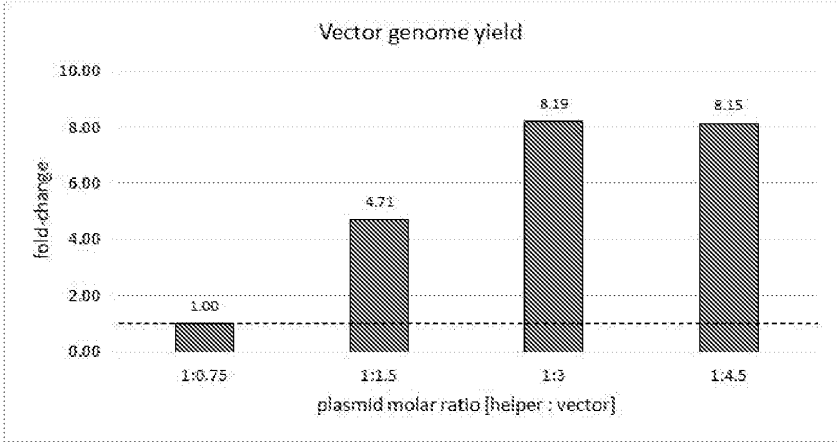
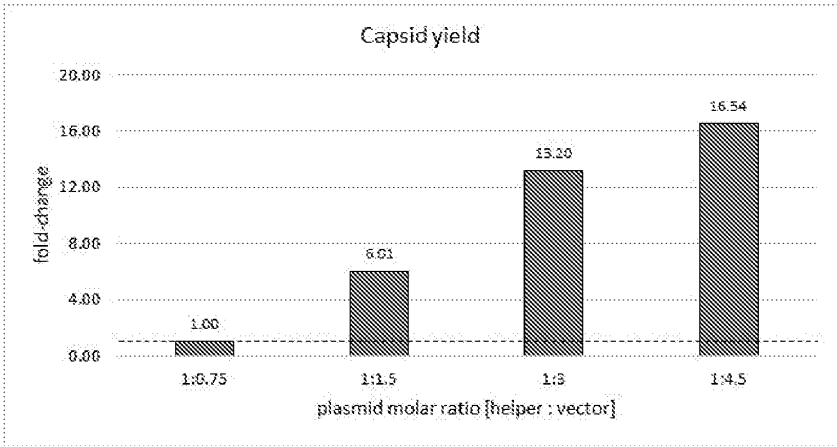


Fig. 8 (cont.)

E



F



G

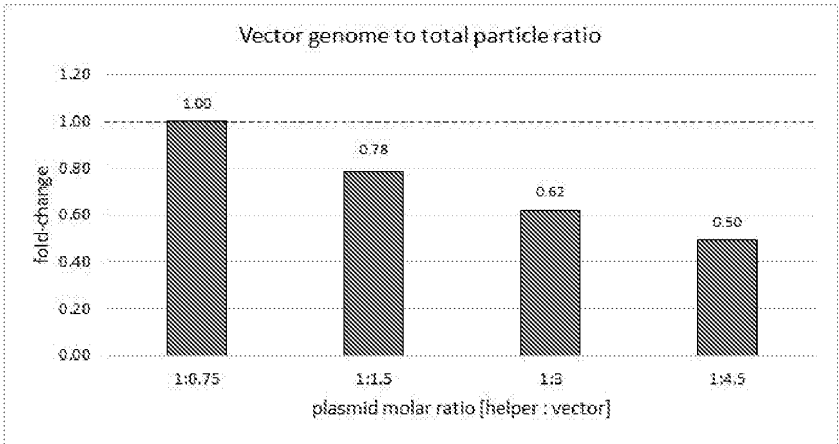


Fig. 8 (cont.)

H

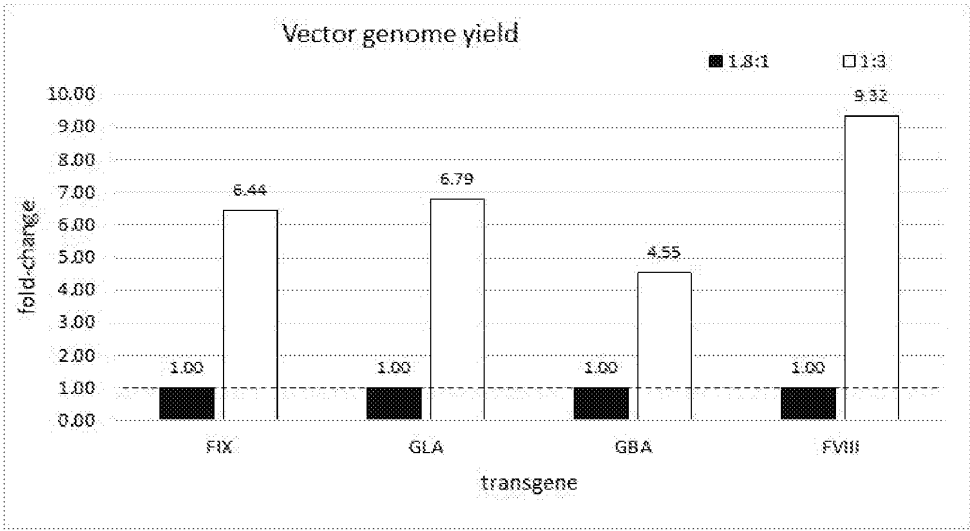

Vector genome yield
1.8:1
1:3
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
fold-change
6.44
6.79
4.55
9.32
1.00
1.00
1.00
1.00
FIX
GLA
GBA
FVIII
transgene

Fig. 9

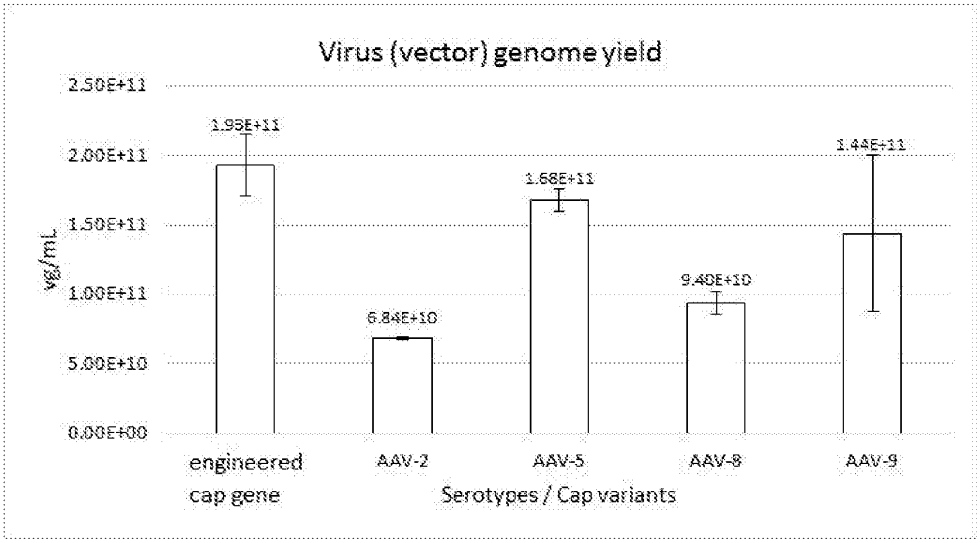

Virus (vector) genome yield
2.50E+11
2.00E+11
1.50E+11
1.00E+11
5.00E+10
0.00E+00
vg/mL
1.93E+11
6.84E+10
1.68E+11
9.40E+10
1.44E+11
engineered cap gene
AAV-2
AAV-5
AAV-8
AAV-9
Serotypes / Cap variants

Fig. 10

SEQ ID NO: 1

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SEQ ID NO: 2

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SEQ ID NO: 4

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SEQ ID NO: 7

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SEQ ID NO: 8

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SEQ ID NO: 9

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Fig. 11

A

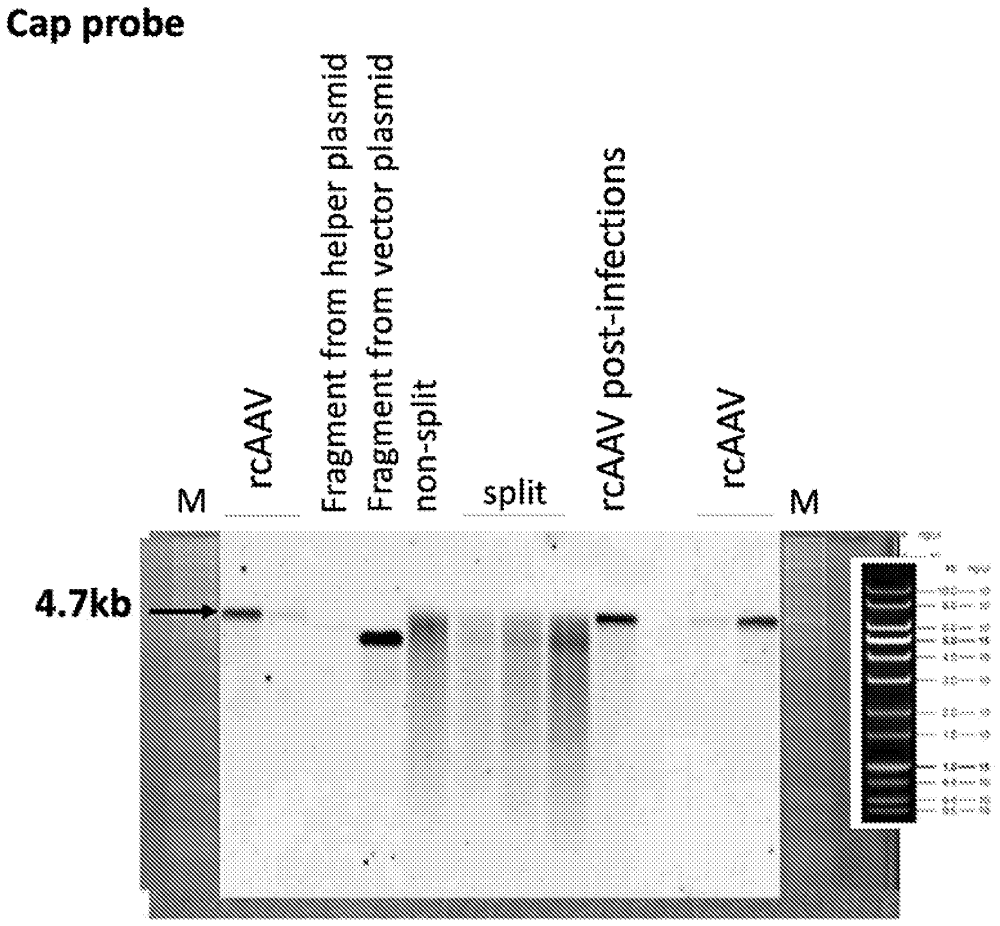

Cap probe
M
rcAAV
Fragment from helper plasmid
Fragment from vector plasmid
non-split
split
rcAAV post-infections
rcAAV
M
4.7kb

Fig. 11 (cont.)

B

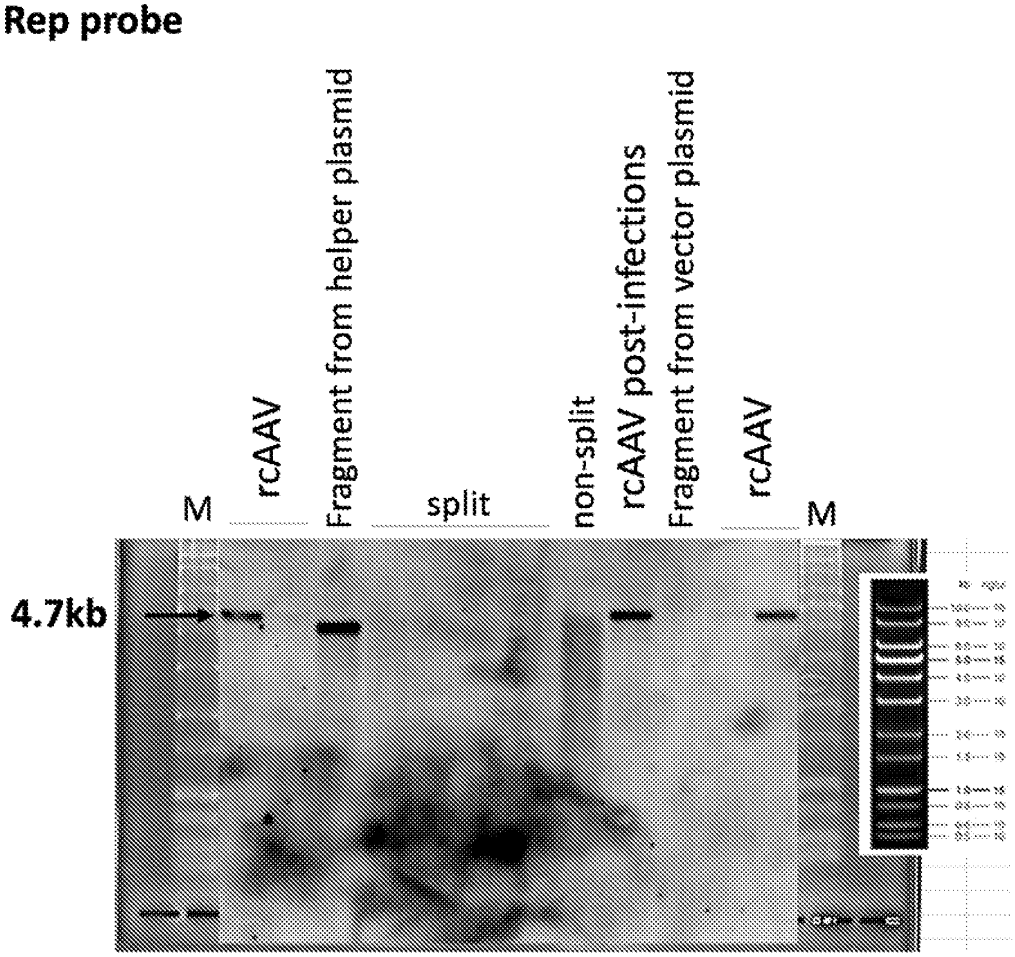

Rep probe
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rcAAV
Fragment from helper plasmid
split
non-split
rcAAV post-infections
Fragment from vector plasmid
rcAAV
M
4.7kb

Fig. 12

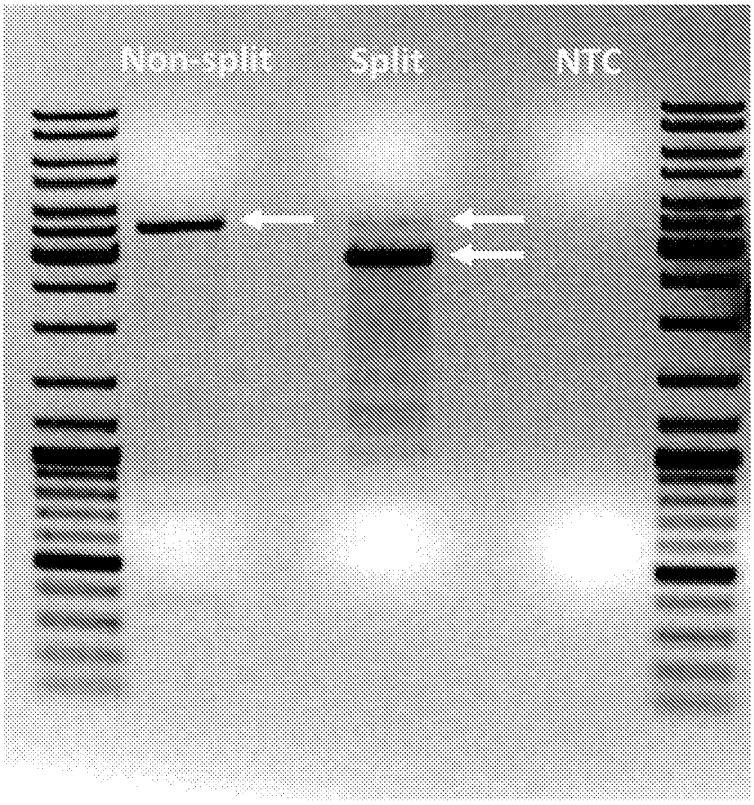
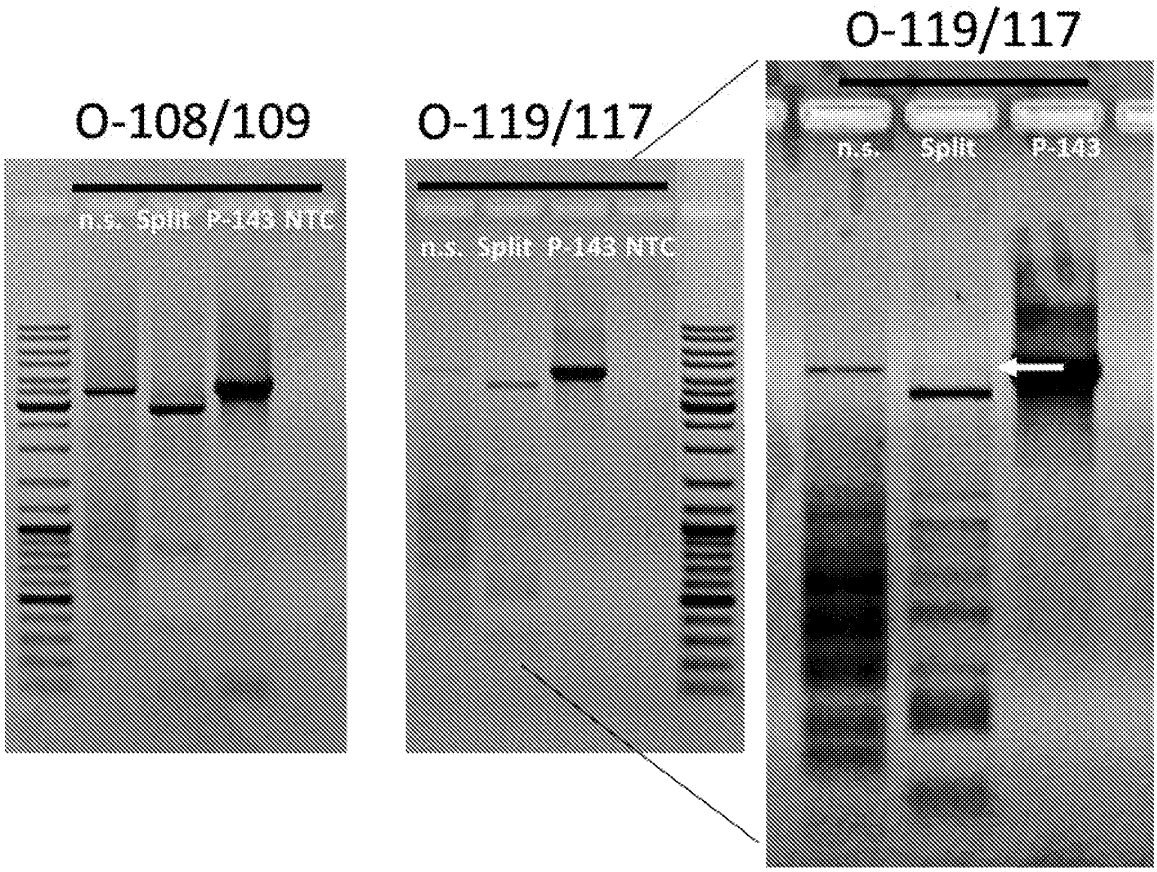


Fig. 13


O-108/109
n.s. Split P-143 NTC
O-119/117
n.s. Split P-143 NTC
O-119/117
n.s. Split P-143

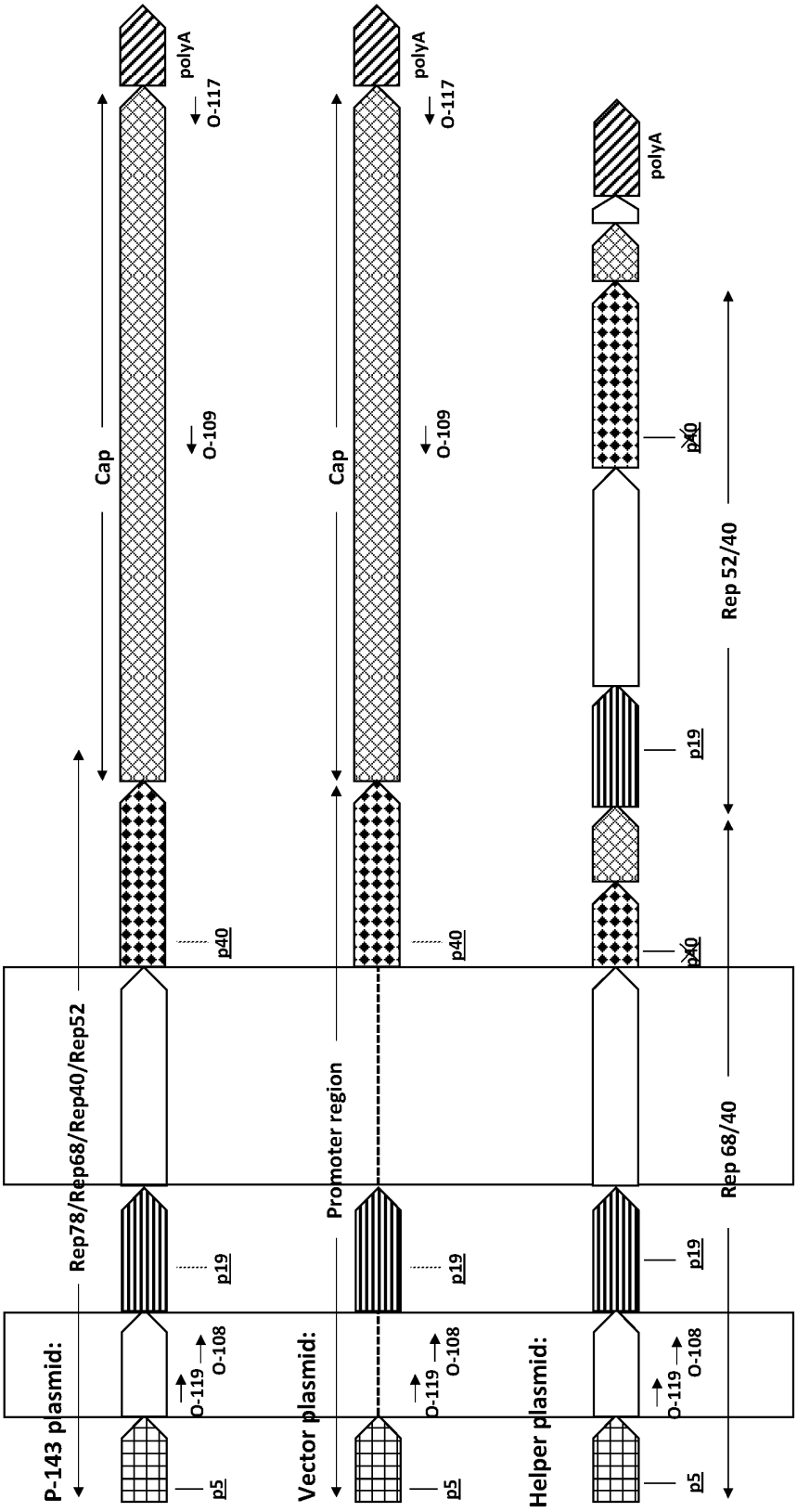

P-143 plasmid:
Vector plasmid:
Helper plasmid:
Rep78/Rep68/Rep40/Rep52
Promoter region
Rep 68/40
Rep 52/40
Cap
polyA
O-117
O-109
O-119
O-108
p5
p19
p40

Fig. 14

Fig. 15

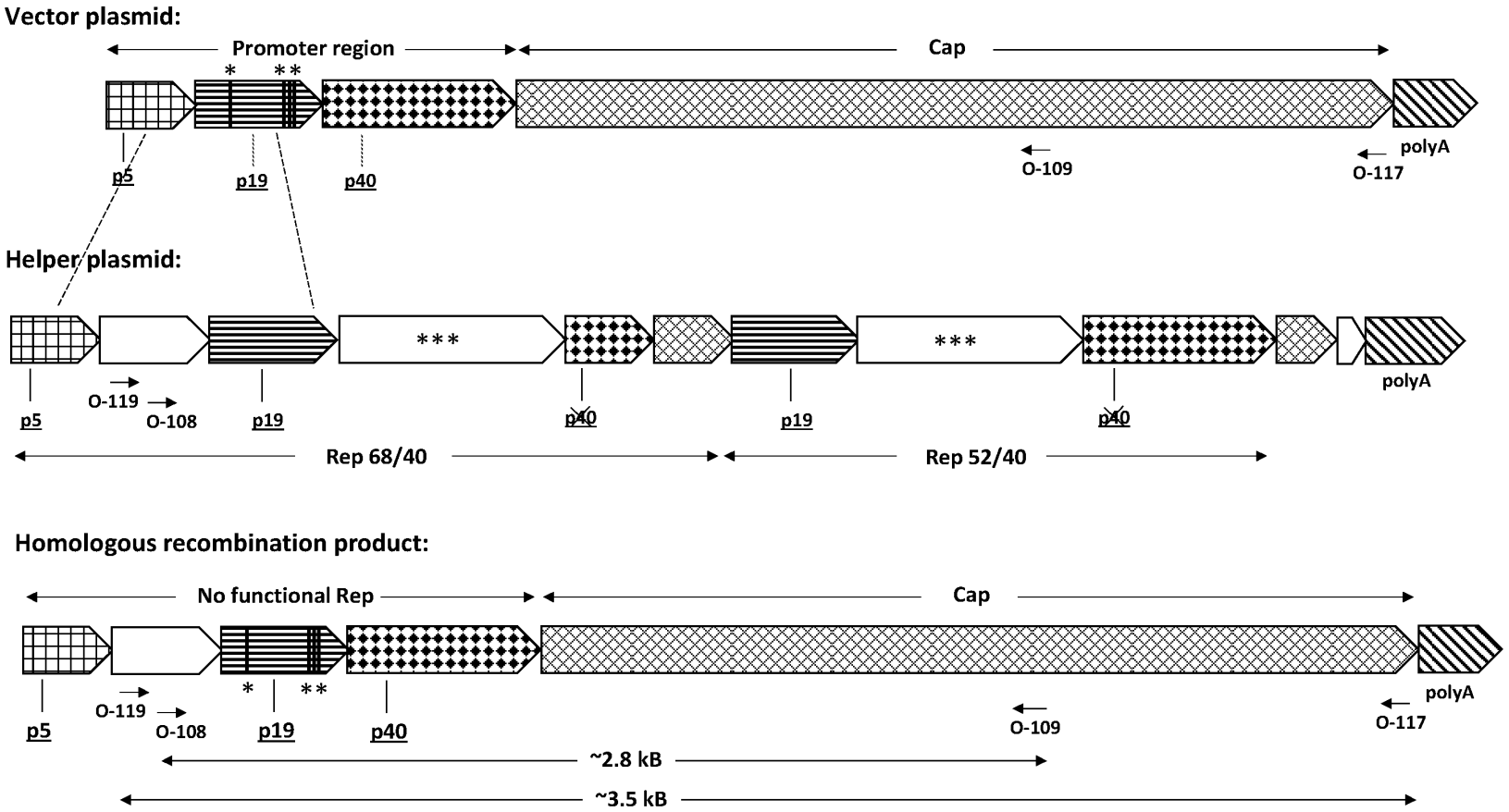
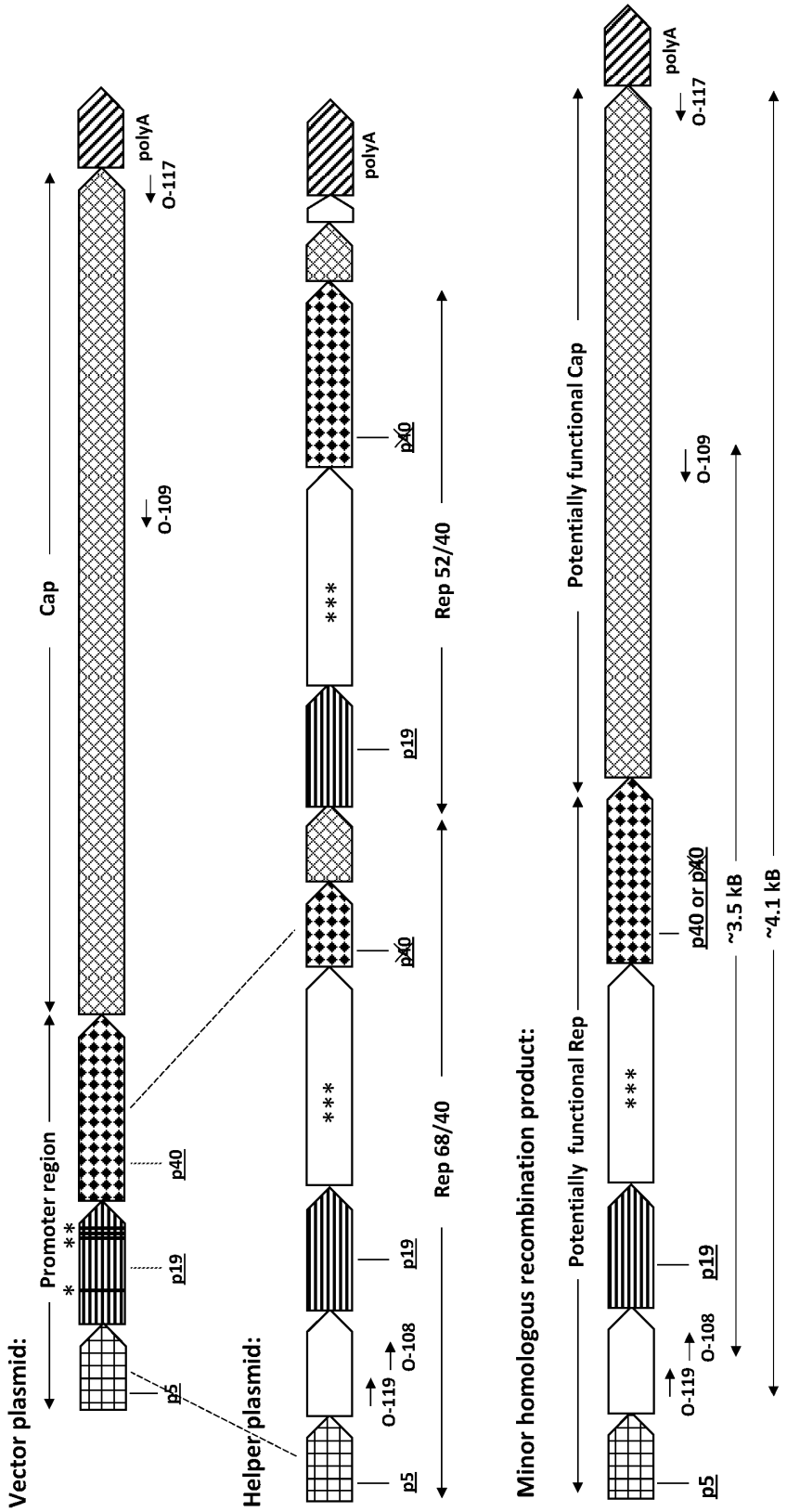

Vector plasmid:
Promoter region
Cap
*
**
p5
p19
p40
O-109
polyA
O-117
Helper plasmid:
***
***
O-119
O-108
p5
p19
p40
p19
p40
polyA
Rep 68/40
Rep 52/40
Homologous recombination product:
No functional Rep
Cap
*
**
O-119
O-108
p5
p19
p40
O-109
polyA
O-117
~2.8 kB
~3.5 kB

Fig. 16


Vector plasmid:
Promoter region
Cap
polyA
O-117
O-109
p5
p19
p40
Helper plasmid:
O-119
O-108
Rep 68/40
Rep 52/40
***
Minor homologous recombination product:
Potentially functional Rep
Potentially functional Cap
p40 or p40
~3.5 kB
~4.1 kB