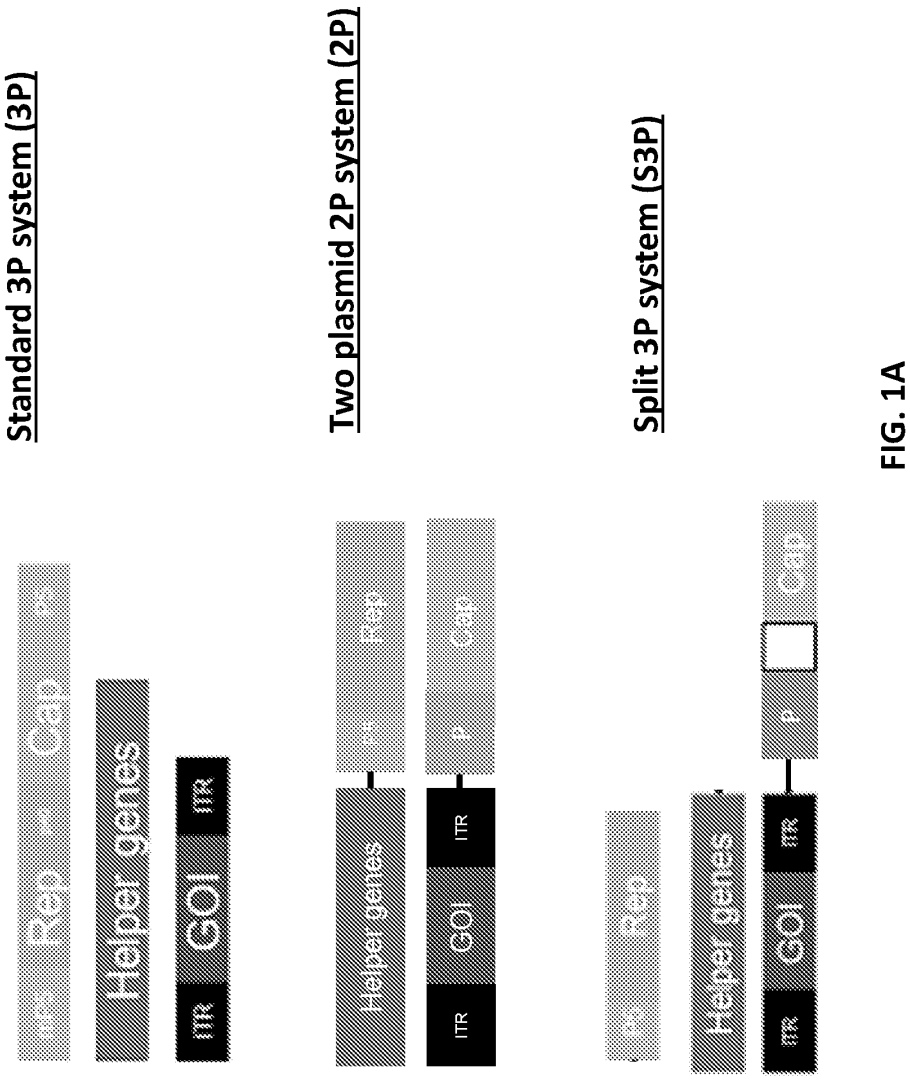

Standard 3P system (3P)
ITR
Rep
Cap
Helper genes
ITR
GOI
ITR
Two plasmid 2P system (2P)
Rep
Cap
Helper genes
ITR
GOI
ITR
Split 3P system (S3P)
Rep
Cap
Helper genes
P
ITR
GOI
ITR

FIG. 1A

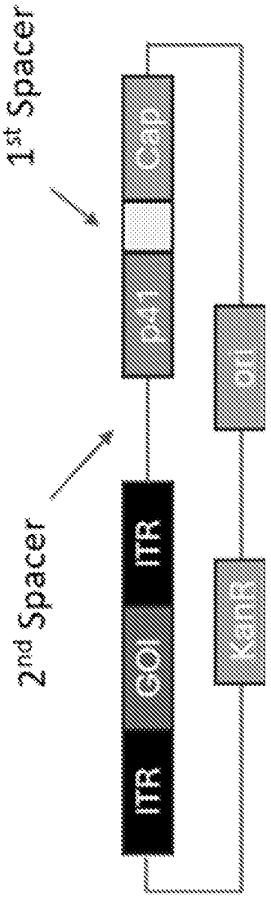


FIG. 1B

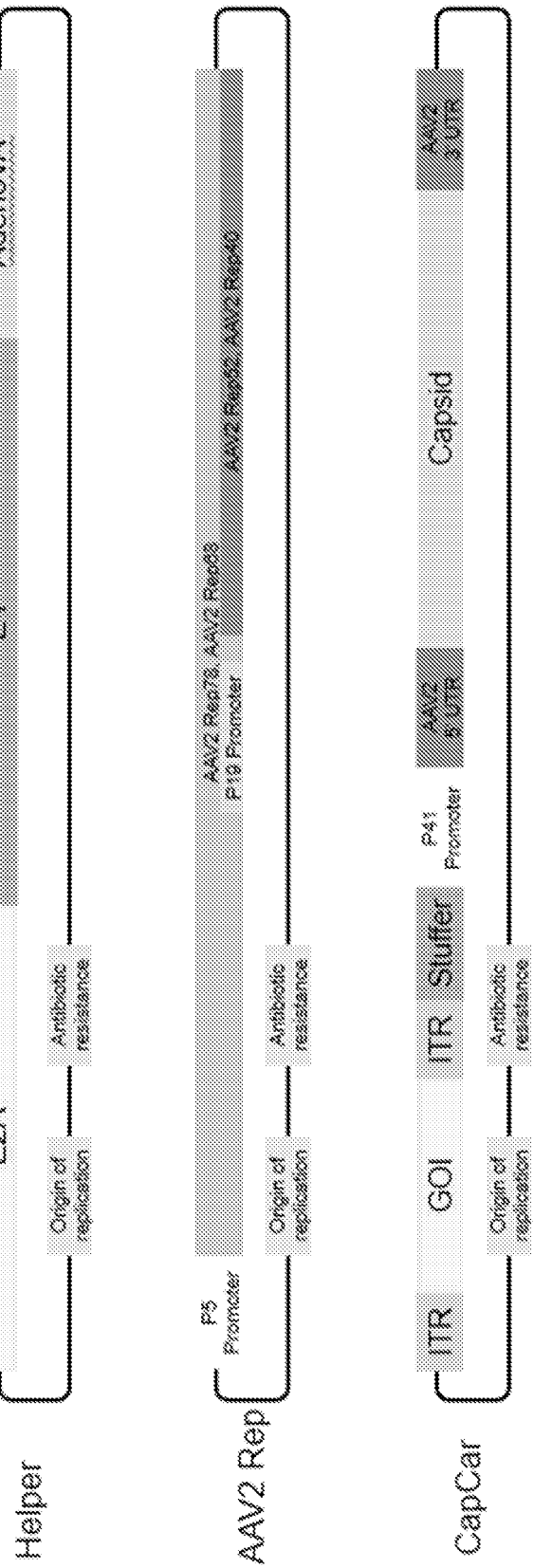

Helper
E2A
E4
AdenoVA
Origin of replication
Antibiotic resistance
AAV2 Rep
P5 Promoter
AAV2 Rep78, AAV2 Rep68
P19 Promoter
AAV2 Rep52, AAV2 Rep40
Origin of replication
Antibiotic resistance
CapCar
ITR
GOI
Origin of replication
ITR
Stuffer
P41 Promoter
AAV2 5'UTR
Capsid
AAV2 3'UTR
Antibiotic resistance

FIG. 2

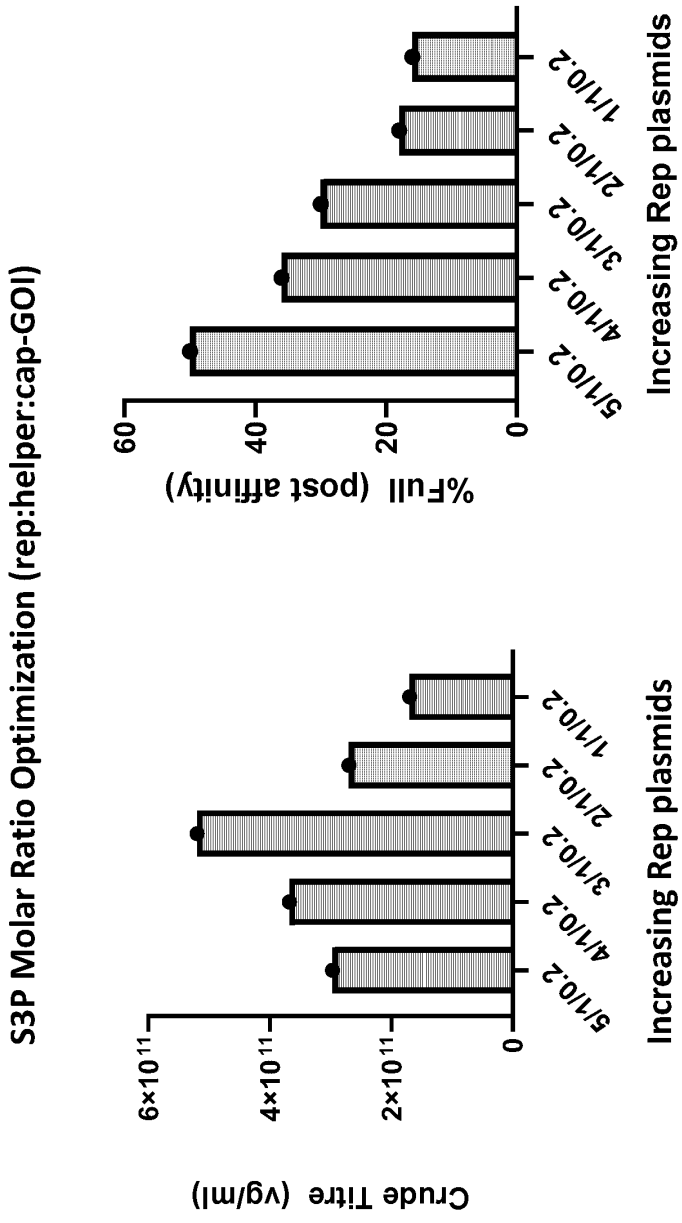

S3P Molar Ratio Optimization (rep:helper:cap-GOI)
Crude Titre (vg/ml)
6×10^11
4×10^11
2×10^11
0
5/1/0.2
4/1/0.2
3/1/0.2
2/1/0.2
1/1/0.2
Increasing Rep plasmids
%Full (post affinity)
60
40
20
0
5/1/0.2
4/1/0.2
3/1/0.2
2/1/0.2
1/1/0.2
Increasing Rep plasmids

FIG. 3

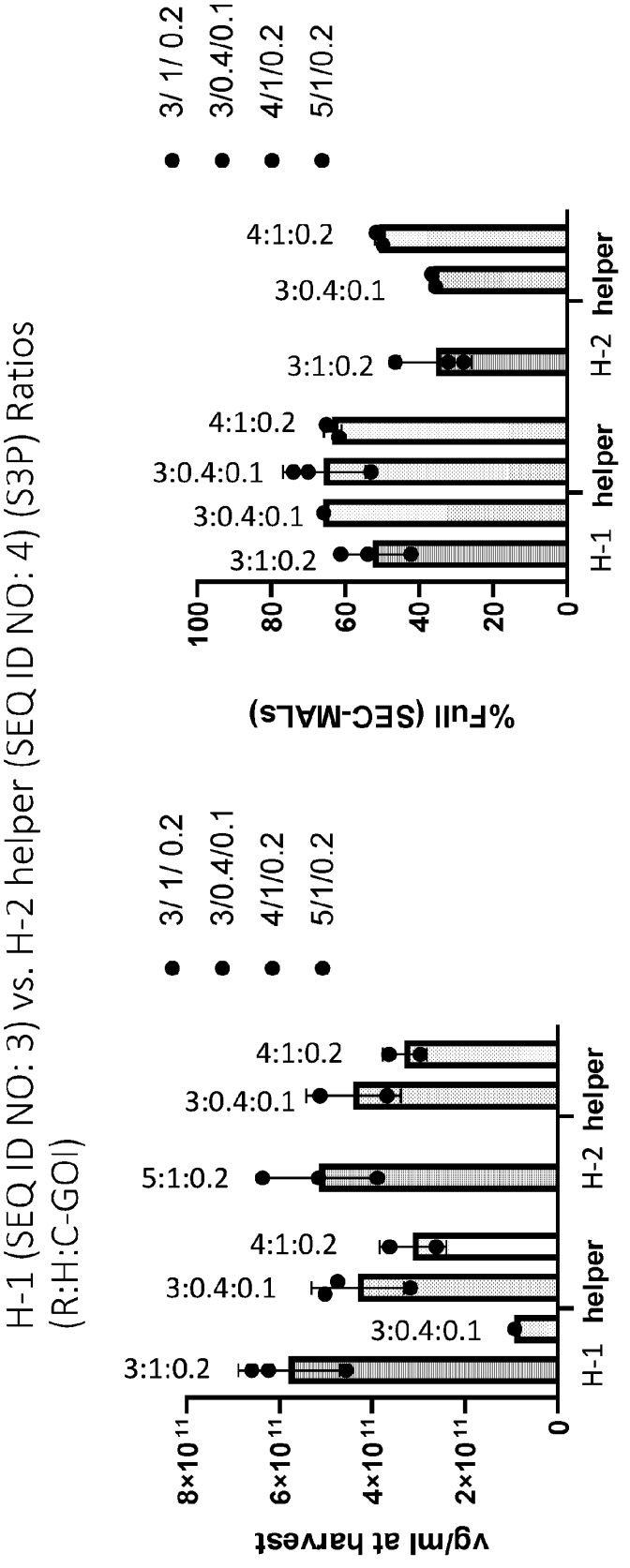

H-1 (SEQ ID NO: 3) vs. H-2 helper (SEQ ID NO: 4) (S3P) Ratios
(R:H:C-GOI)
3/ 1/ 0.2
3/0.4/0.1
4/1/0.2
5/1/0.2
vg/ml at harvest
8×10^11
6×10^11
4×10^11
2×10^11
0
3:1:0.2
3:0.4:0.1
4:1:0.2
3:0.4:0.1
4:1:0.2
5:1:0.2
3:0.4:0.1
4:1:0.2
H-1 helper
H-2 helper
%Full (SEC-MALS)
100
80
60
40
20
0
3:1:0.2
3:0.4:0.1
4:1:0.2
3:0.4:0.1
3:1:0.2
4:1:0.2
H-1 helper
H-2 helper

FIG. 4

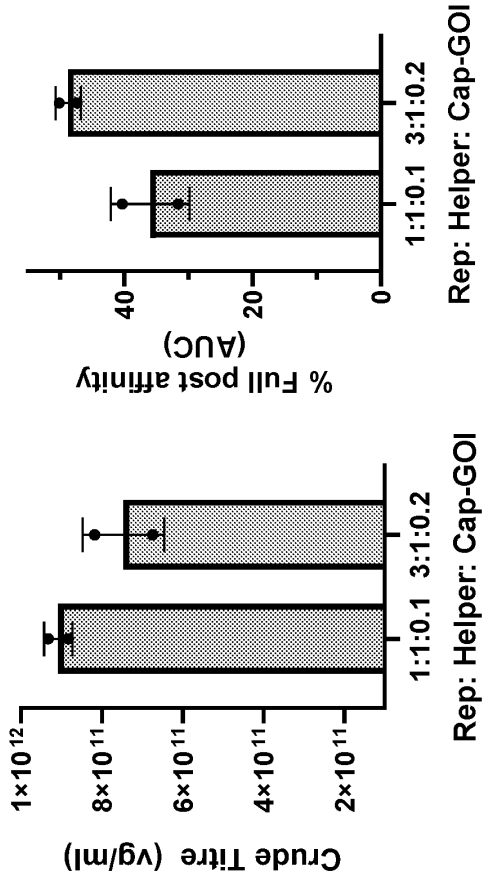

Crude Titre (vg/ml)
1×10^12
8×10^11
6×10^11
4×10^11
2×10^11
1:1:0.1
3:1:0.2
Rep: Helper: Cap-GOI
% Full post affinity (AUC)
40
20
0
1:1:0.1
3:1:0.2
Rep: Helper: Cap-GOI

FIG. 5

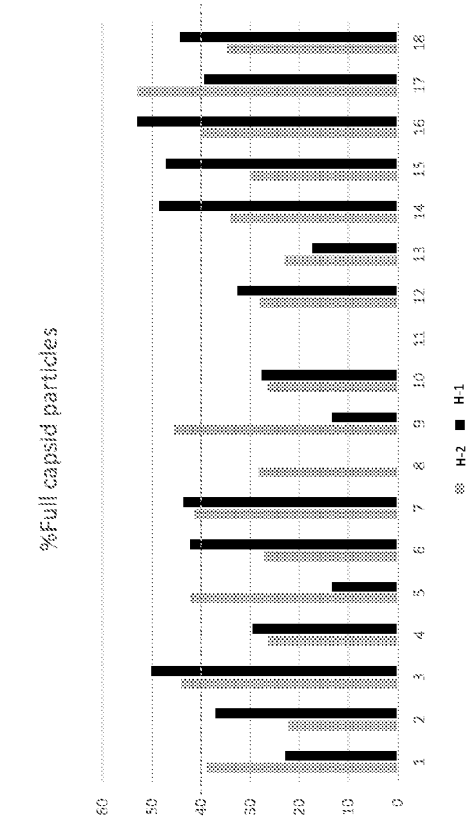
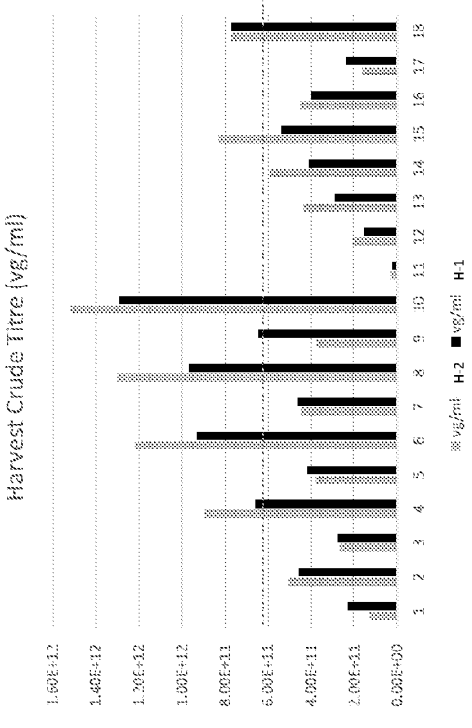
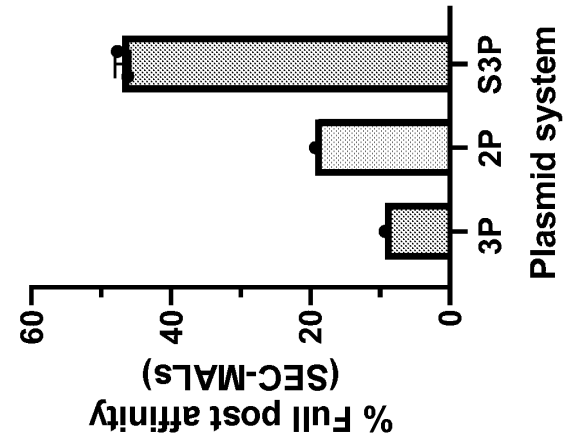

%Full capsid particles
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
H-2
H-1

FIG. 6


Harvest Crude Titre (vg/ml)
1.60E+12
1.40E+12
1.20E+12
1.00E+12
8.00E+11
6.00E+11
4.00E+11
2.00E+11
0.00E+00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
vg/ml H-2 vg/ml H-1


% Full post affinity
(SEC-MALs)
60
40
20
0
3P
2P
S3P
Plasmid system

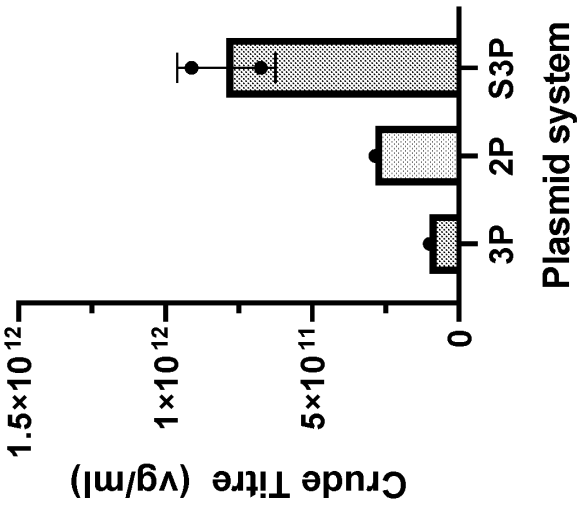

Crude Titre (vg/ml)
1.5×10^12
1×10^12
5×10^11
0
3P
2P
S3P
Plasmid system

FIG. 7

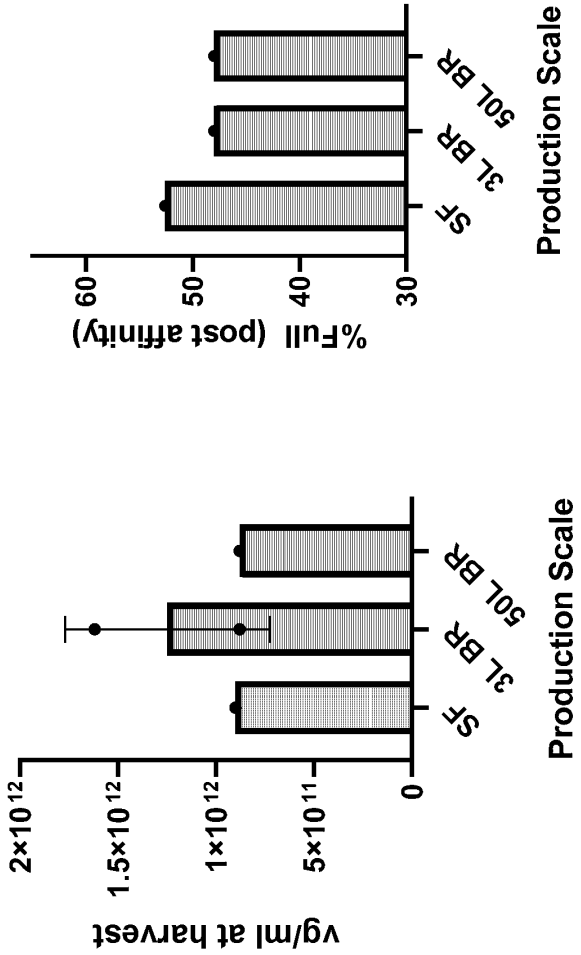

%Full (post affinity)
60
50
40
30
SF
3L BR
50L BR
Production Scale
vg/ml at harvest
2×10^12
1.5×10^12
1×10^12
5×10^11
0
SF
3L BR
50L BR
Production Scale

FIG. 8

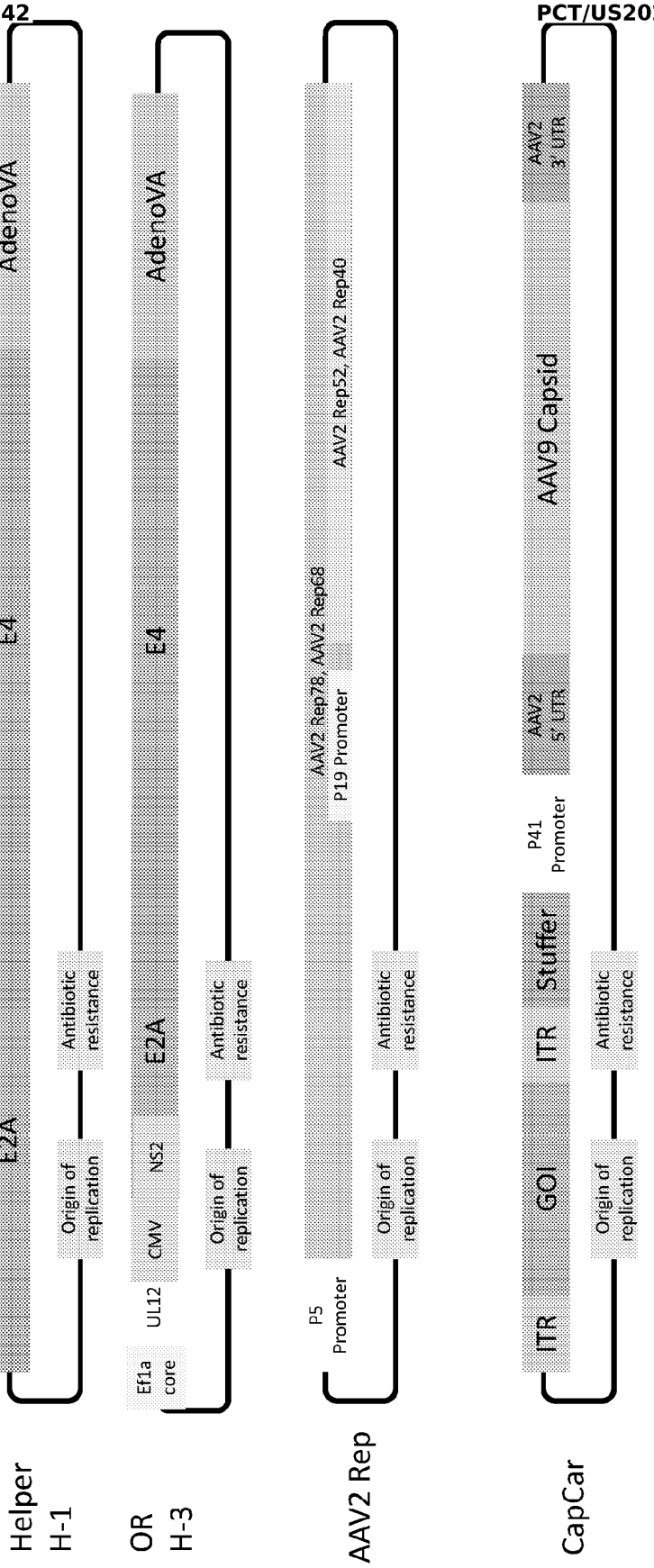

Helper
H-1
E2A
E4
AdenoVA
Origin of replication
Antibiotic resistance
OR
H-3
Ef1a core
UL12
CMV
NS2
E2A
E4
AdenoVA
Origin of replication
Antibiotic resistance
AAV2 Rep
P5 Promoter
AAV2 Rep78, AAV2 Rep68
P19 Promoter
AAV2 Rep52, AAV2 Rep40
Origin of replication
Antibiotic resistance
CapCar
ITR
GOI
ITR
Stuffer
Origin of replication
Antibiotic resistance
P41 Promoter
AAV2 5' UTR
AAV9 Capsid
AAV2 3' UTR

FIG. 9

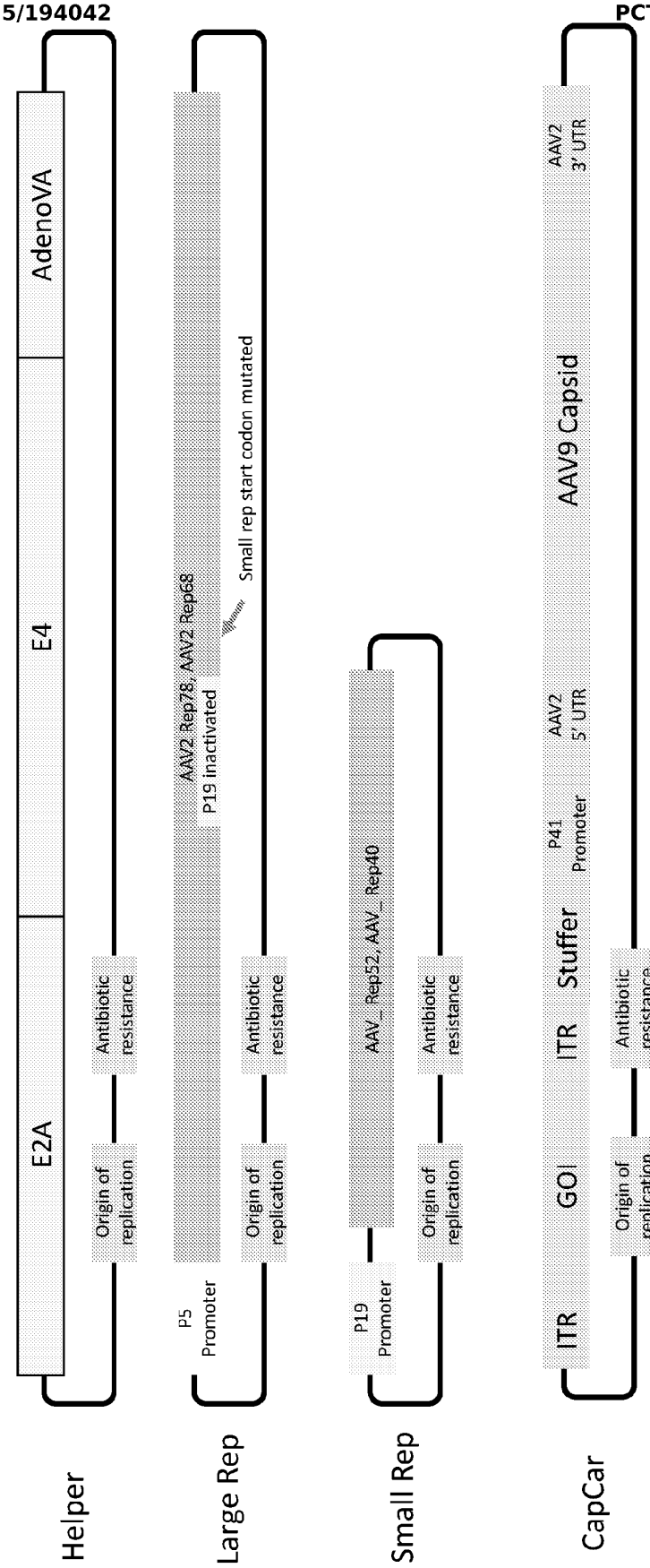

Helper
E2A
E4
AdenoVA
Origin of replication
Antibiotic resistance
Large Rep
P5 Promoter
AAV2 Rep78, AAV2 Rep68
P19 inactivated
Small rep start codon mutated
Origin of replication
Antibiotic resistance
Small Rep
P19 Promoter
AAV_ Rep52, AAV_ Rep40
Origin of replication
Antibiotic resistance
CapCar
ITR
GOI
ITR
Stuffer
P41 Promoter
AAV2 5' UTR
AAV9 Capsid
AAV2 3' UTR
Origin of replication
Antibiotic resistance

FIG. 10

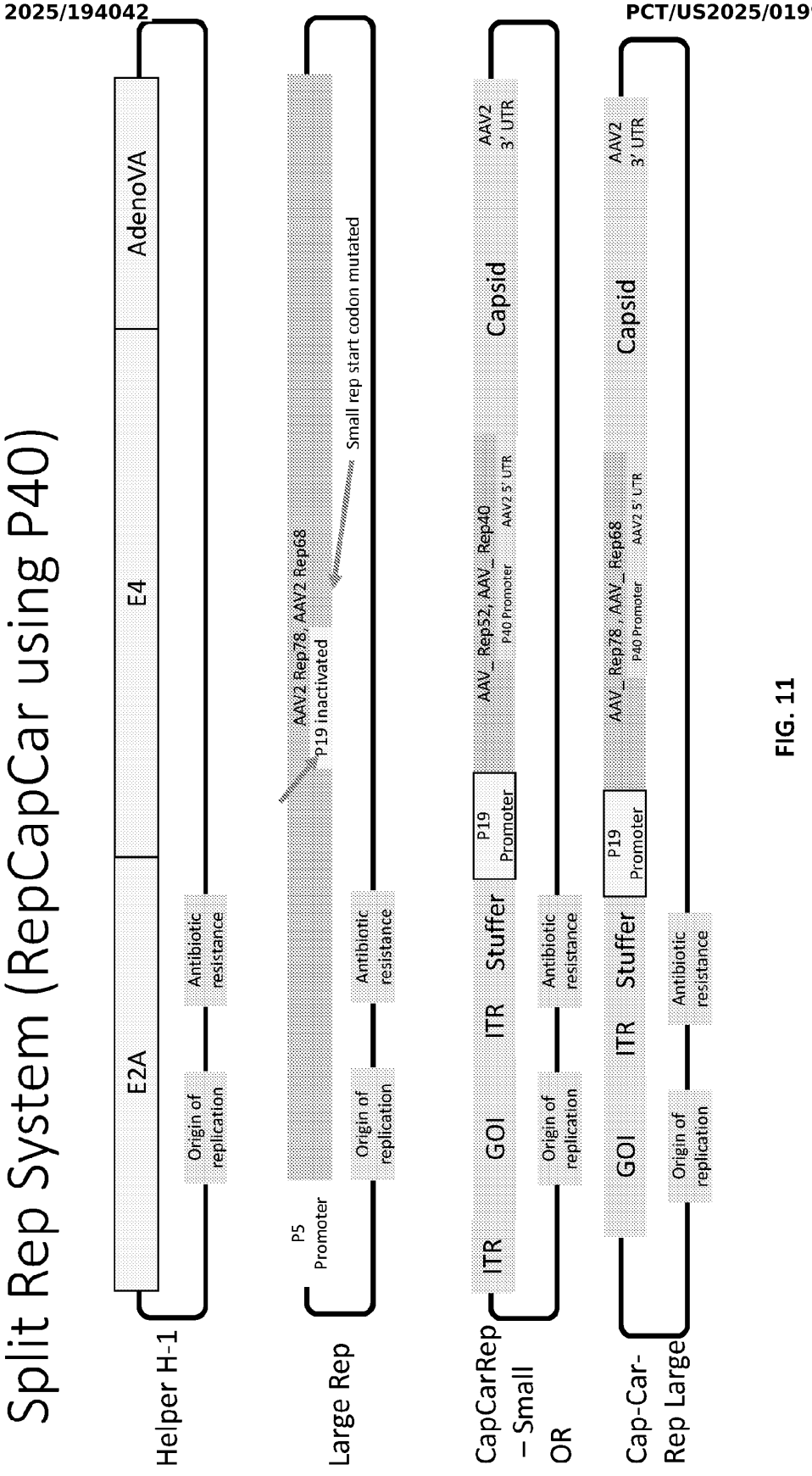

Split Rep System (RepCapCar using P40)
Helper H-1
E2A
E4
AdenoVA
Origin of replication
Antibiotic resistance
Large Rep
P5 Promoter
AAV2 Rep78, AAV2 Rep68
P19 inactivated
Small rep start codon mutated
Origin of replication
Antibiotic resistance
CapCarRep – Small OR
ITR
GOI
ITR
Stuffer
P19 Promoter
AAV_ Rep52, AAV_ Rep40
P40 Promoter
AAV2 5' UTR
Capsid
AAV2 3' UTR
Origin of replication
Antibiotic resistance
Cap-Car-Rep Large
GOI
ITR
Stuffer
P19 Promoter
AAV_ Rep78 , AAV_ Rep68
P40 Promoter
AAV2 5' UTR
Capsid
AAV2 3' UTR
Origin of replication
Antibiotic resistance

FIG. 11

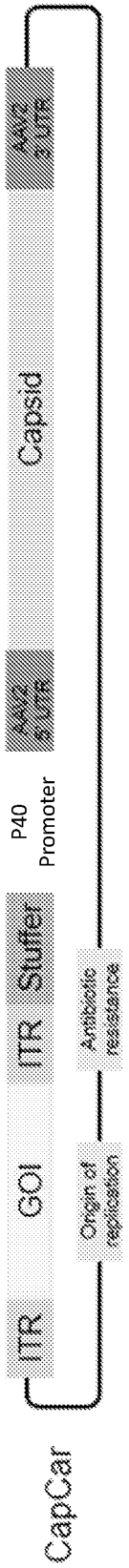

CapCar
ITR
GOI
ITR
Stuffer
P40
Promoter
AAV2
5'UTR
Capsid
AAV2
3'UTR
Origin of
replication
Antibiotic
resistance

FIG. 12

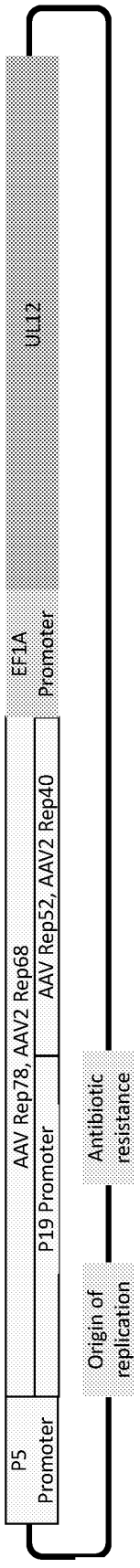

UL12
EF1A Promoter
AAV Rep78, AAV2 Rep68
AAV Rep52, AAV2 Rep40
P19 Promoter
P5 Promoter
Antibiotic resistance
Origin of replication

FIG. 13

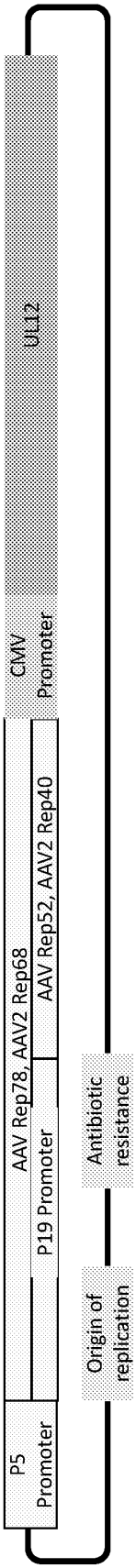

UL12
CMV Promoter
AAV Rep78, AAV2 Rep68
AAV Rep52, AAV2 Rep40
P19 Promoter
Antibiotic resistance
Origin of replication
P5 Promoter

FIG. 14

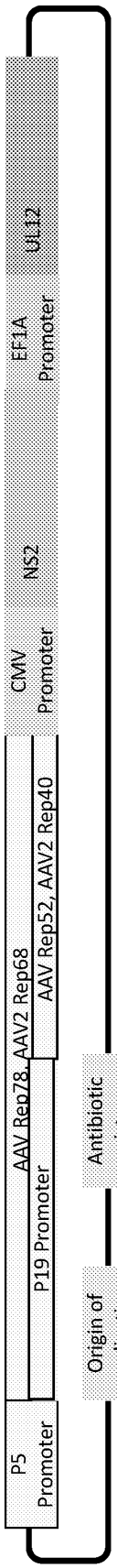

P5 Promoter
AAV Rep78, AAV2 Rep68
P19 Promoter
AAV Rep52, AAV2 Rep40
CMV Promoter
NS2
EF1A Promoter
UL12
Antibiotic resistance
Origin of replication

FIG. 15

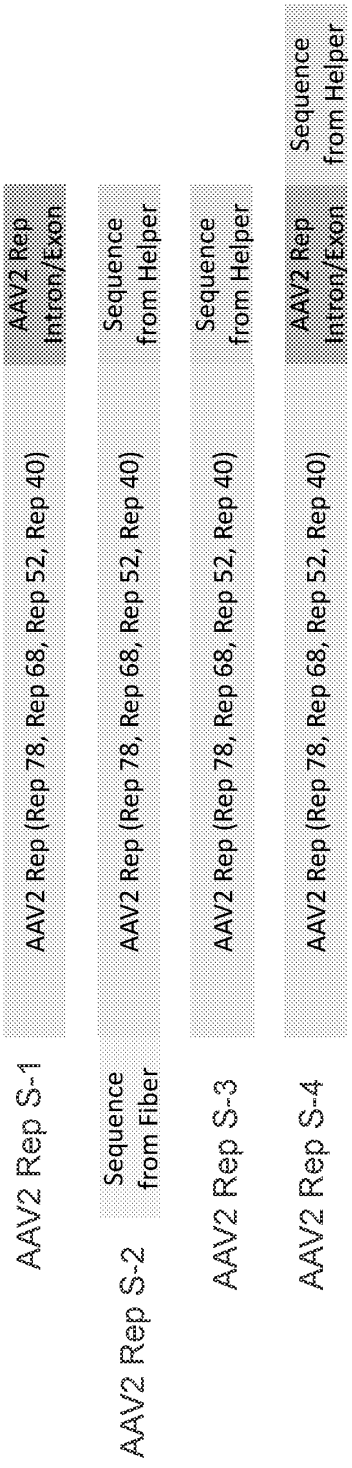

AAV2 Rep S-1
AAV2 Rep (Rep 78, Rep 68, Rep 52, Rep 40)
AAV2 Rep Intron/Exon
AAV2 Rep S-2
Sequence from Fiber
AAV2 Rep (Rep 78, Rep 68, Rep 52, Rep 40)
Sequence from Helper
AAV2 Rep S-3
AAV2 Rep (Rep 78, Rep 68, Rep 52, Rep 40)
Sequence from Helper
AAV2 Rep S-4
AAV2 Rep (Rep 78, Rep 68, Rep 52, Rep 40)
AAV2 Rep Intron/Exon
Sequence from Helper

FIG. 16

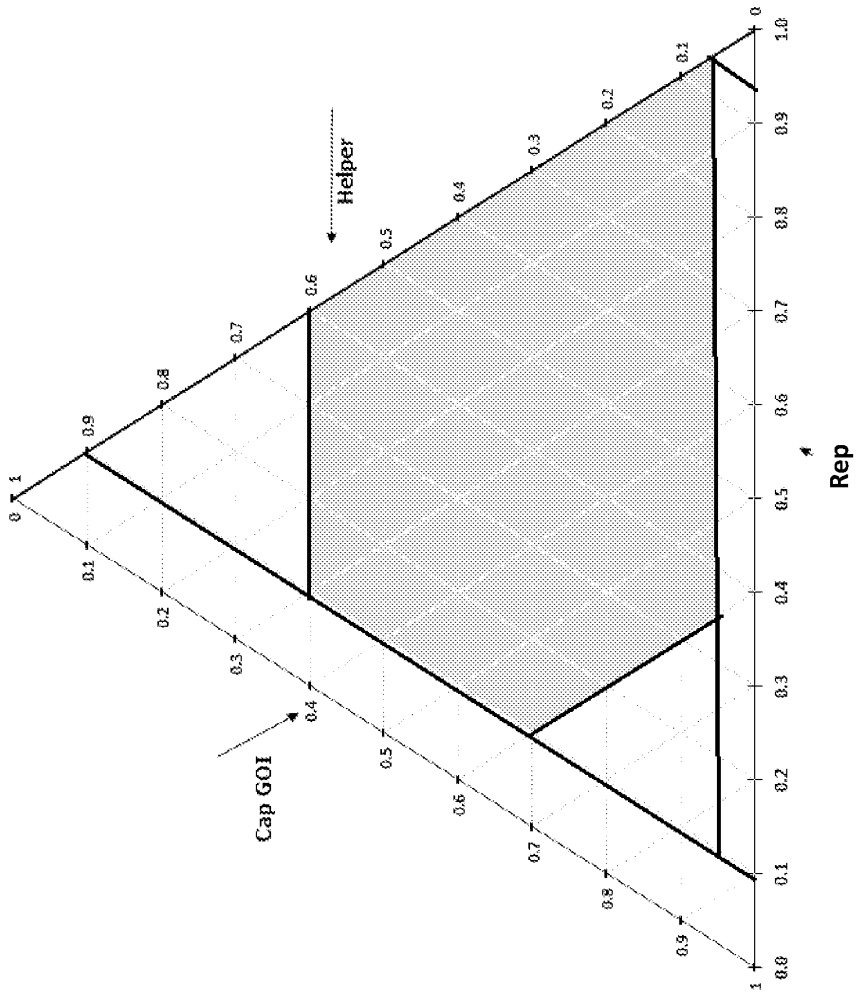


FIG. 17

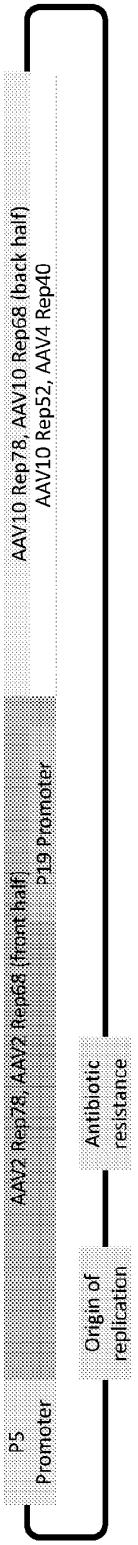

AAV2/4
Hybrid Rep
P5
Promoter
AAV2 Rep78, AAV2 Rep68 (front half)
P19 Promoter
AAV10 Rep78, AAV10 Rep68 (back half)
AAV10 Rep52, AAV4 Rep40
Origin of
replication
Antibiotic
resistance

FIG. 18

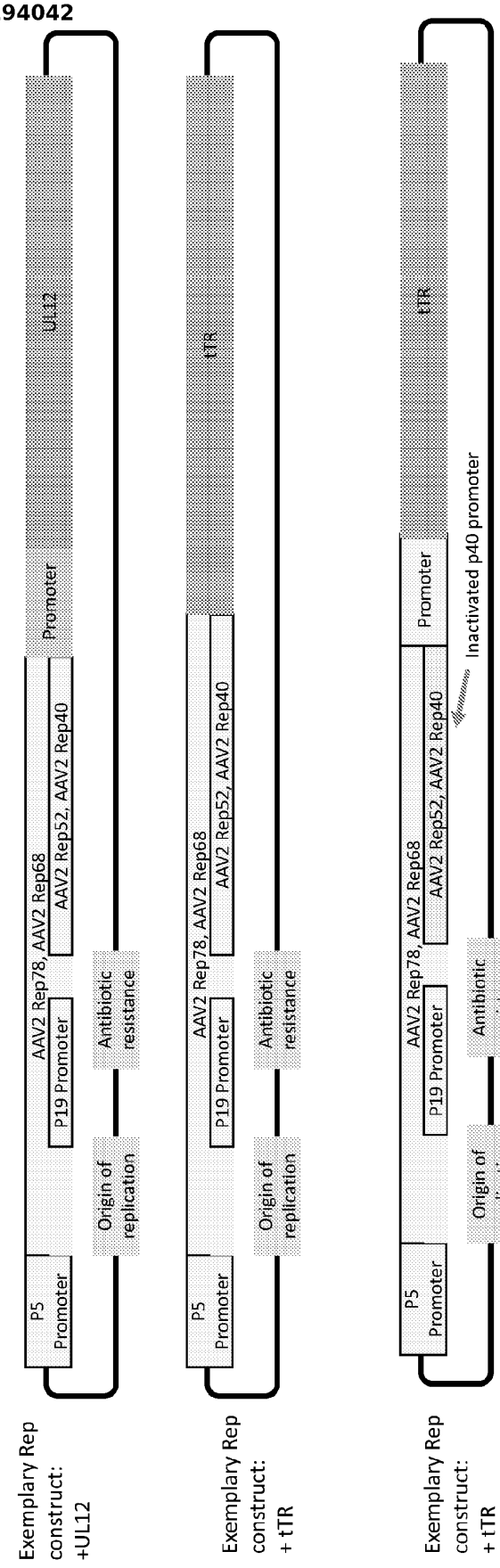

Exemplary Rep construct: +UL12
P5 Promoter
AAV2 Rep78, AAV2 Rep68
P19 Promoter
AAV2 Rep52, AAV2 Rep40
Promoter
UL12
Origin of replication
Antibiotic resistance
Exemplary Rep construct: + tTR
P5 Promoter
AAV2 Rep78, AAV2 Rep68
P19 Promoter
AAV2 Rep52, AAV2 Rep40
tTR
Origin of replication
Antibiotic resistance
Exemplary Rep construct: + tTR
P5 Promoter
AAV2 Rep78, AAV2 Rep68
P19 Promoter
AAV2 Rep52, AAV2 Rep40
Inactivated p40 promoter
Promoter
tTR
Origin of replication
Antibiotic resistance

FIG. 19

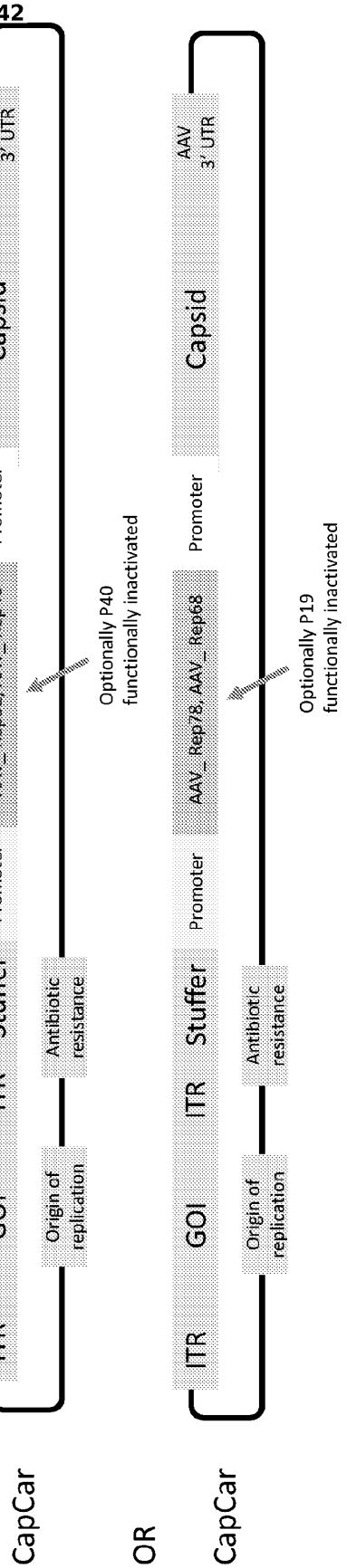

CapCar
ITR
GOI
ITR
Stuffer
Promoter
AAV_Rep52, AAV_Rep40
Promoter
Capsid
AAV 3' UTR
Origin of replication
Antibiotic resistance
Optionally P40 functionally inactivated
OR
CapCar
ITR
GOI
ITR
Stuffer
Promoter
AAV_Rep78, AAV_Rep68
Promoter
Capsid
AAV 3' UTR
Origin of replication
Antibiotic resistance
Optionally P19 functionally inactivated

FIG. 20