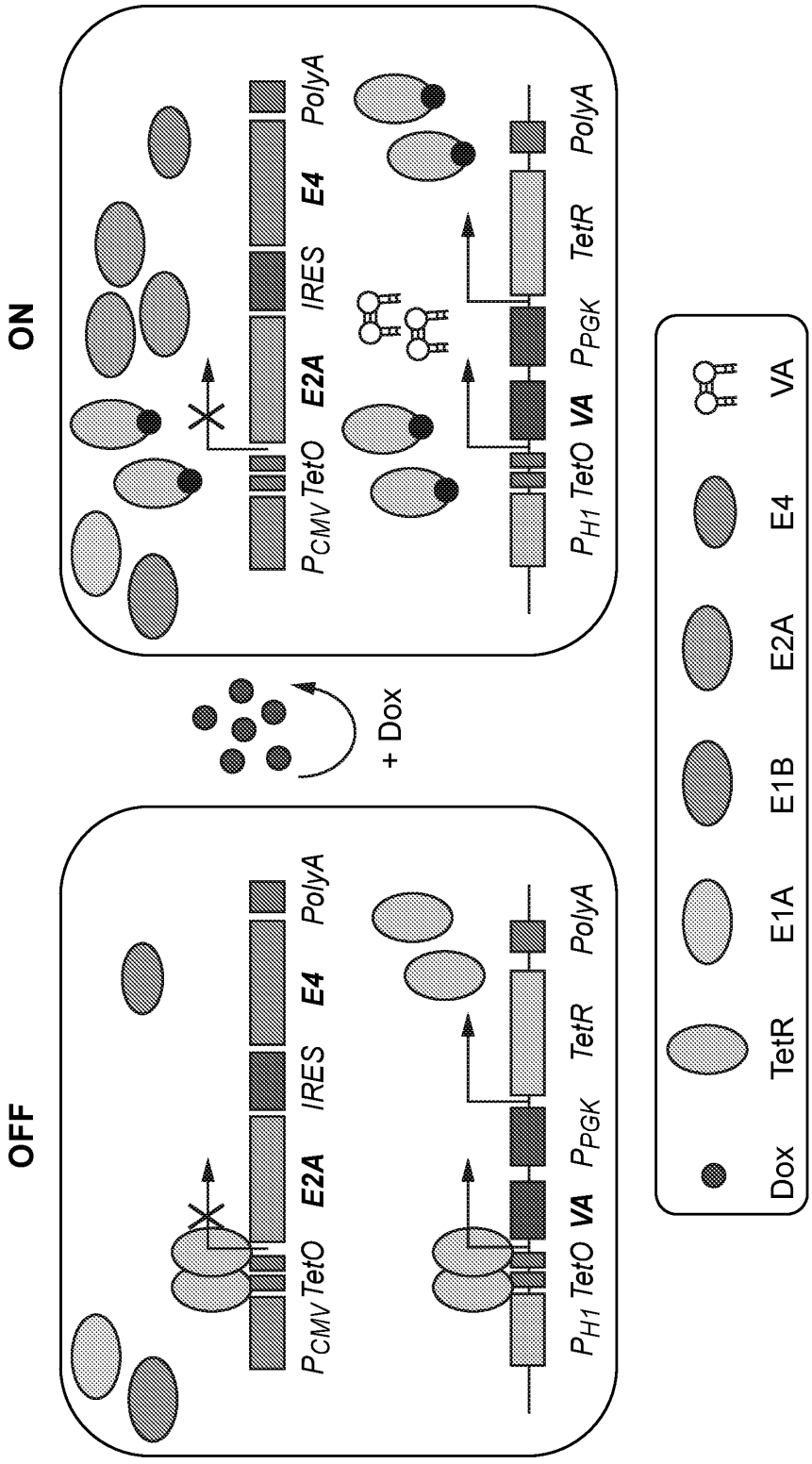
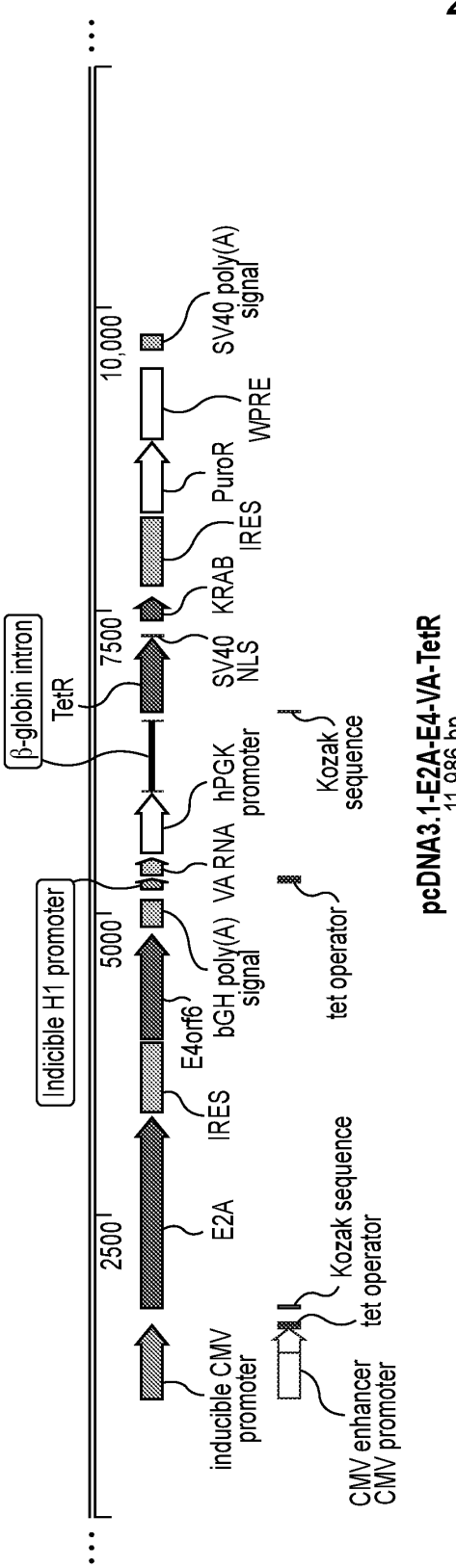
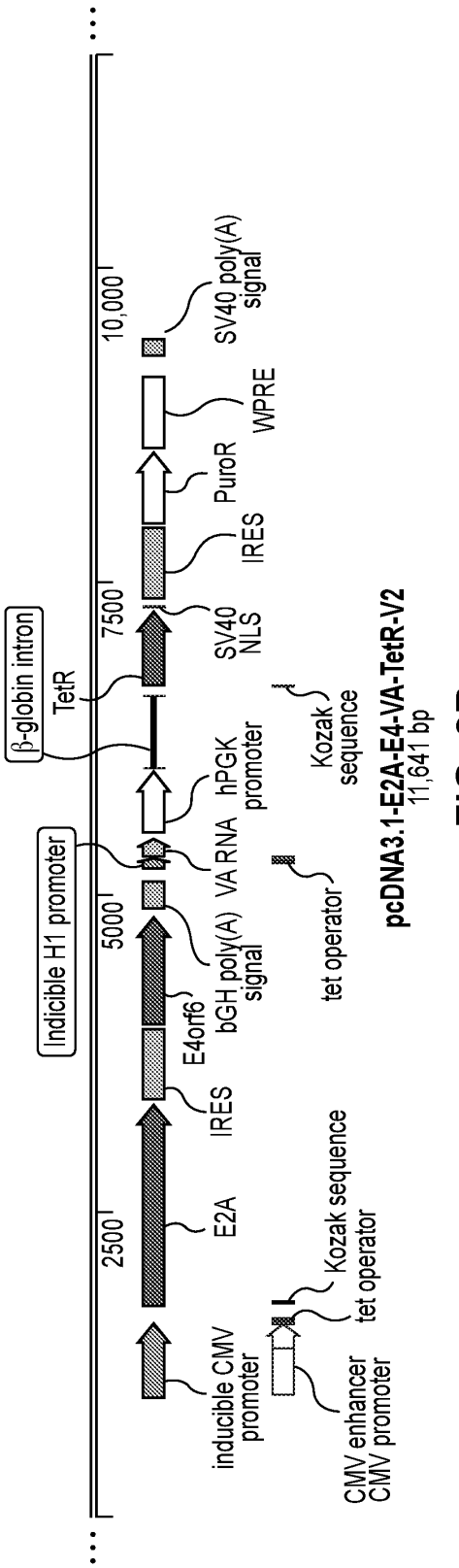

ON
OFF
+ Dox
P_{CMV} TetO E2A IRES E4 PolyA
P_{H1} TetO VA P_{PGK} TetR PolyA
Dox
TetR
E1A
E1B
E2A
E4
VA

FIG. 1



pcDNA3.1-E2A-E4-VA-TetR
11,986 bp

FIG. 2A



pcDNA3.1-E2A-E4-VA-TetR-V2
11,641 bp

FIG. 2B

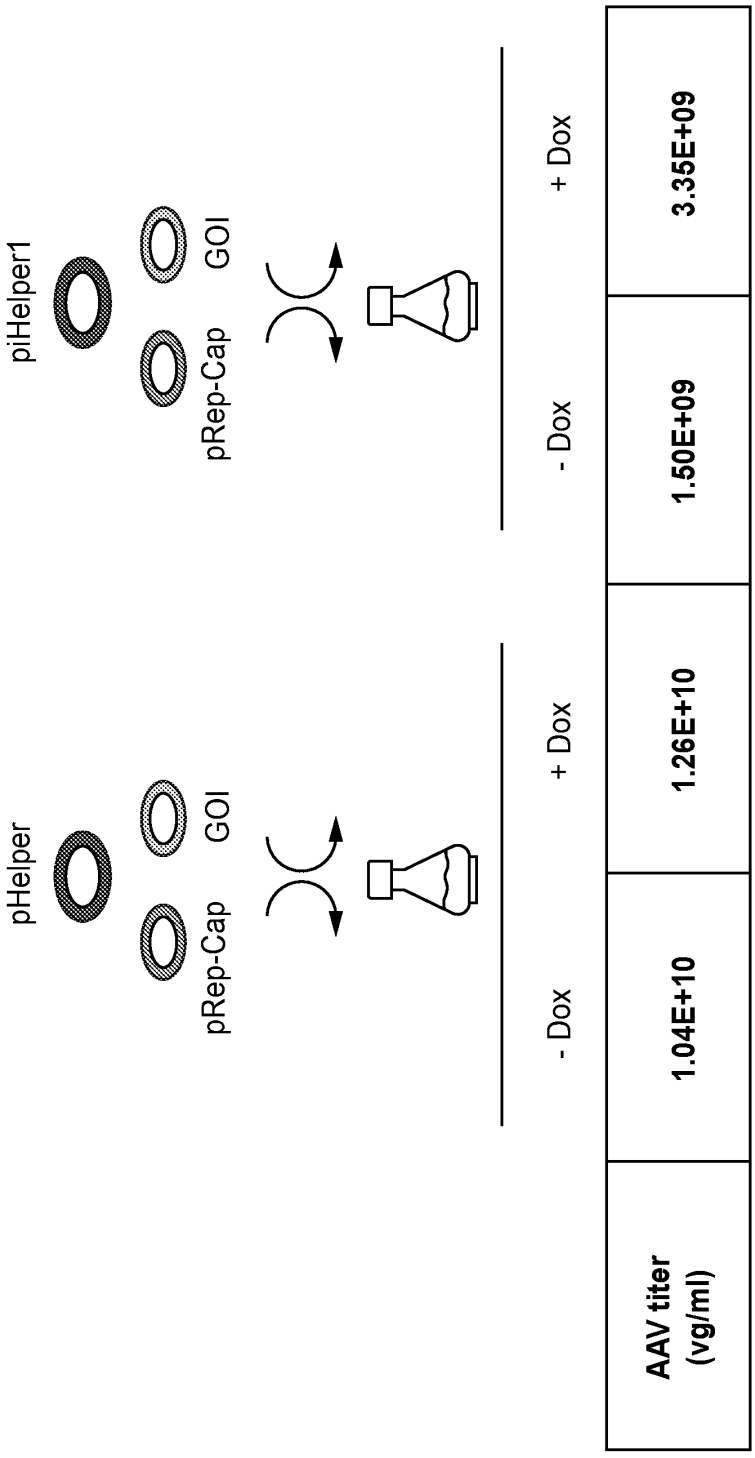

piHelper1
pRep-Cap
GOI
pHelper
pRep-Cap
GOI
- Dox
+ Dox
- Dox
+ Dox
AAV titer (vg/ml)
1.04E+10
1.26E+10
1.50E+09
3.35E+09

FIG. 3

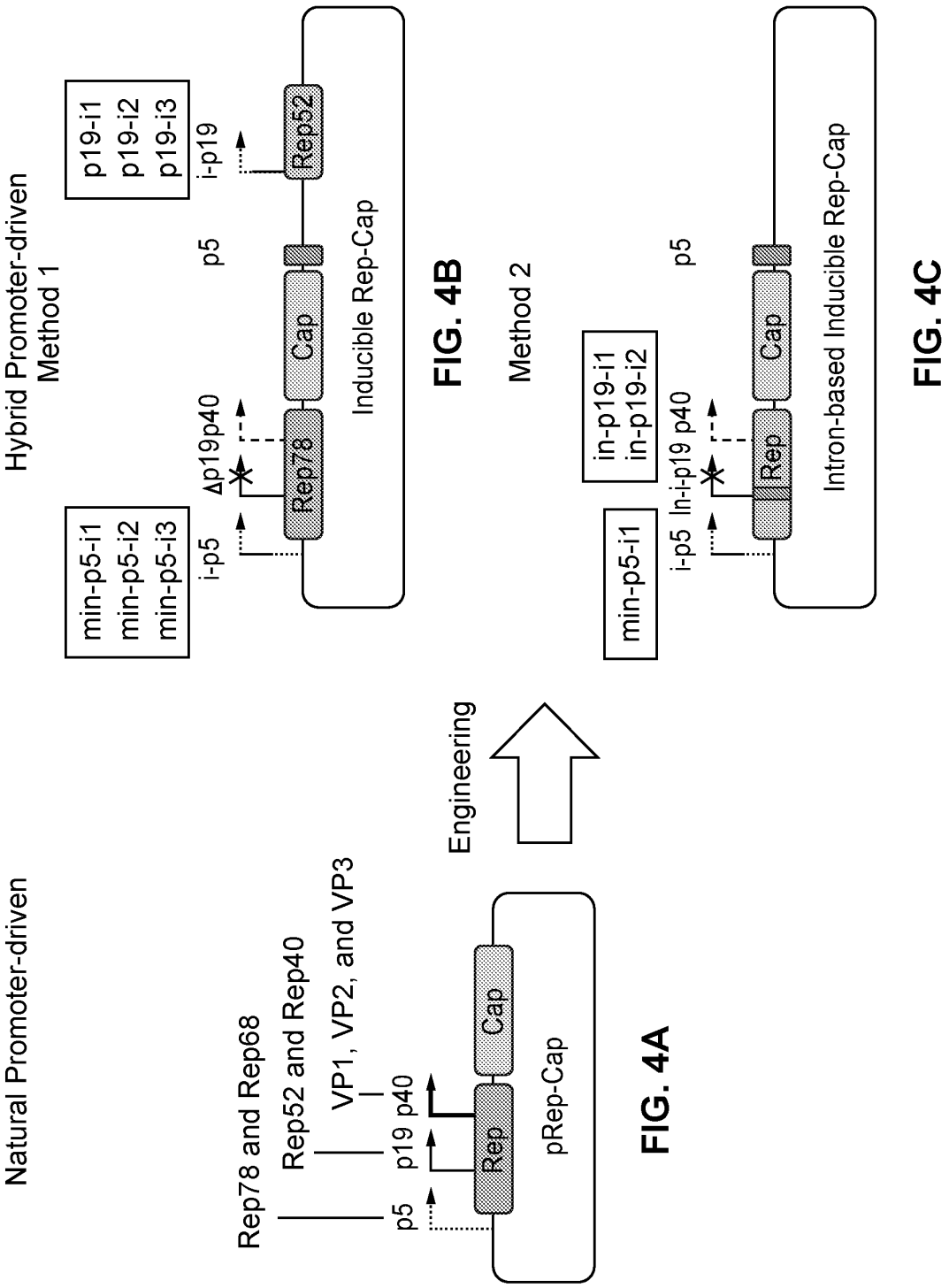


FIG. 4A

FIG. 4B

FIG. 4C

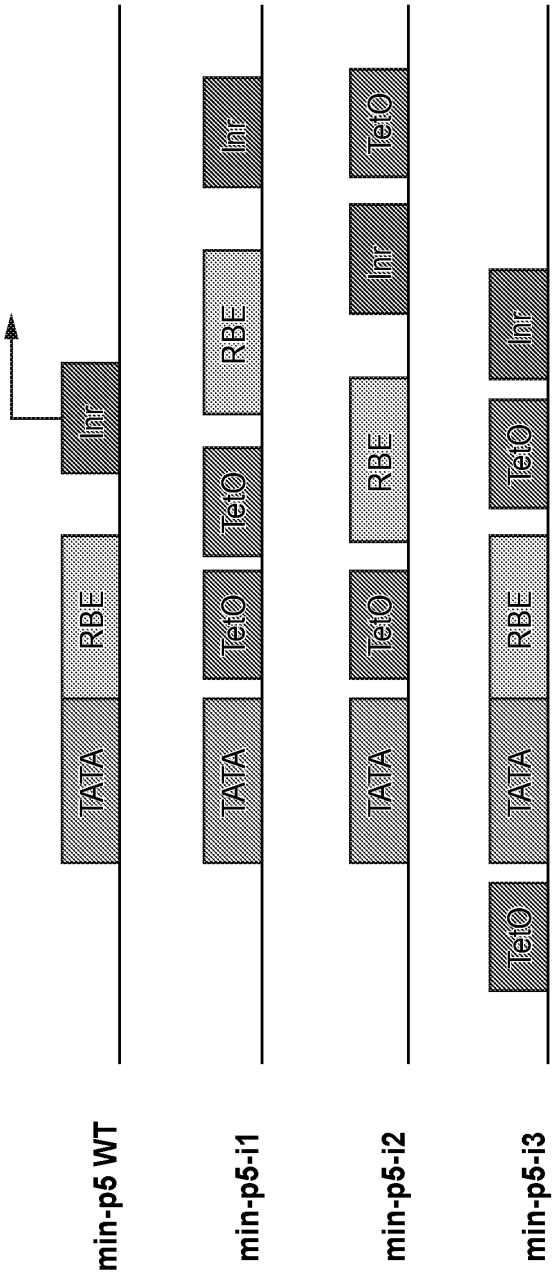

TATA
RBE
Inr
TATA
TetO
TetO
RBE
Inr
TATA
TetO
RBE
TetO
Inr
TetO
TATA
RBE
TetO
Inr
min-p5 WT
min-p5-i1
min-p5-i2
min-p5-i3

FIG. 5

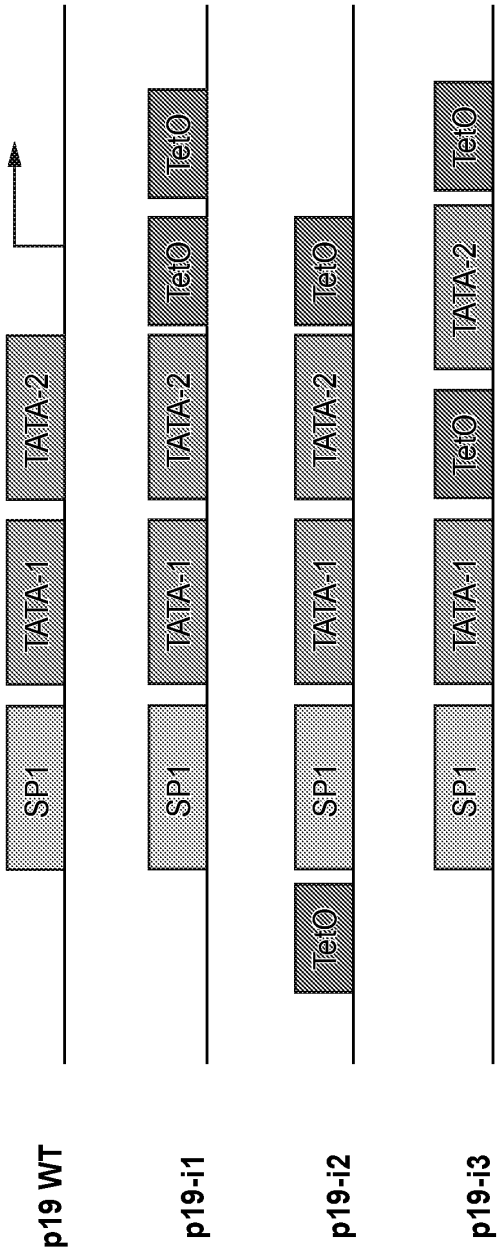


FIG. 6

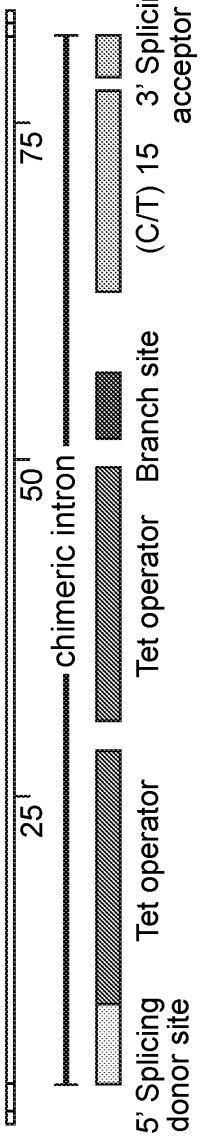

25
50
75
chimeric intron
5' Splicing donor site
Tet operator
Tet operator
Branch site
(C/T) 15
3' Splicing acceptor site
chimeric TetO intron
83 bp

FIG. 7A

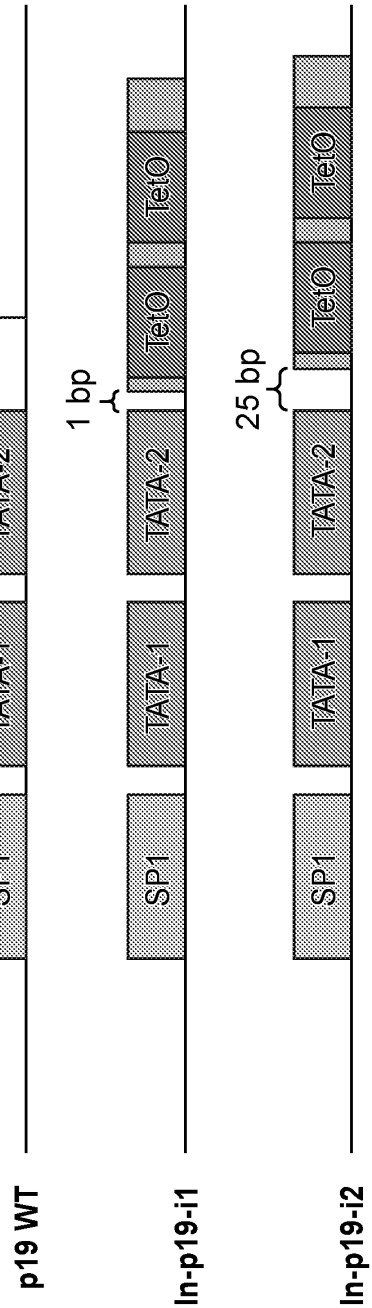

p19 WT
SP1
TATA-1
TATA-2
ln-p19-i1
1 bp
SP1
TATA-1
TATA-2
TetO
TetO
ln-p19-i2
25 bp
SP1
TATA-1
TATA-2
TetO
TetO

FIG. 7B

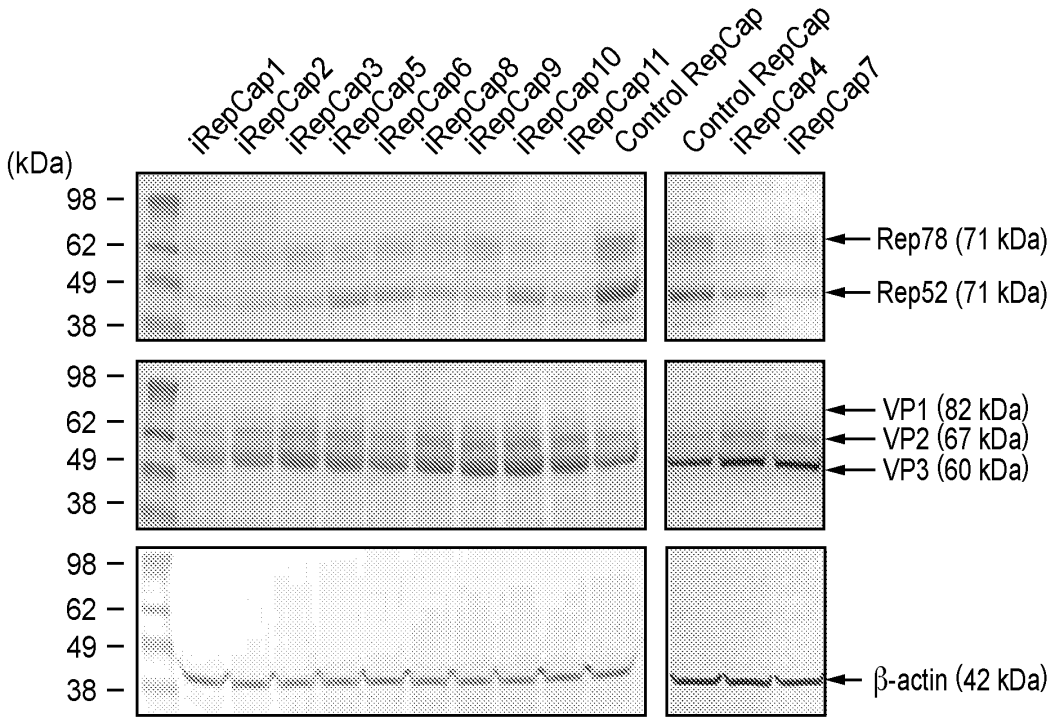

iRepCap1
iRepCap2
iRepCap3
iRepCap5
iRepCap6
iRepCap8
iRepCap9
iRepCap10
iRepCap11
Control RepCap
Control RepCap
iRepCap4
iRepCap7
(kDa)
98
62
49
38
Rep78 (71 kDa)
Rep52 (71 kDa)
VP1 (82 kDa)
VP2 (67 kDa)
VP3 (60 kDa)
β-actin (42 kDa)

FIG. 8A

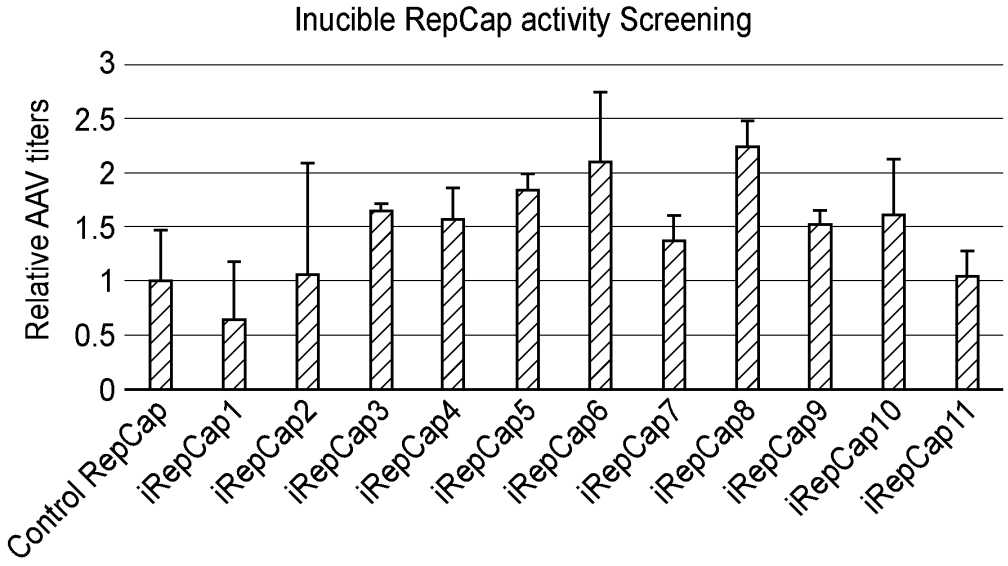

Inucible RepCap activity Screening
Relative AAV titers
3
2.5
2
1.5
1
0.5
0
Control RepCap
iRepCap1
iRepCap2
iRepCap3
iRepCap4
iRepCap5
iRepCap6
iRepCap7
iRepCap8
iRepCap9
iRepCap10
iRepCap11

FIG. 8B

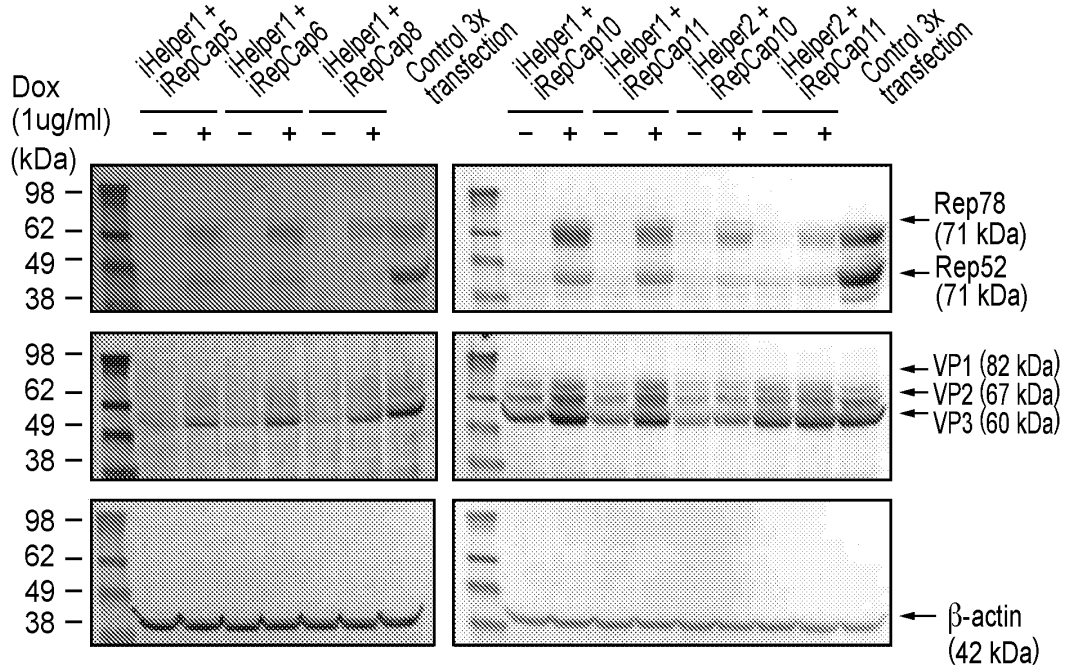

iHelper1 + iRepCap5
iHelper1 + iRepCap6
iHelper1 + iRepCap8
Control 3x transfection
iHelper1 + iRepCap10
iHelper1 + iRepCap11
iHelper2 + iRepCap10
iHelper2 + iRepCap11
Control 3x transfection
Dox (1ug/ml)
– + – + – + – + – + – + – +
(kDa)
98
62
49
38
Rep78 (71 kDa)
Rep52 (71 kDa)
VP1 (82 kDa)
VP2 (67 kDa)
VP3 (60 kDa)
β-actin (42 kDa)

FIG. 9A

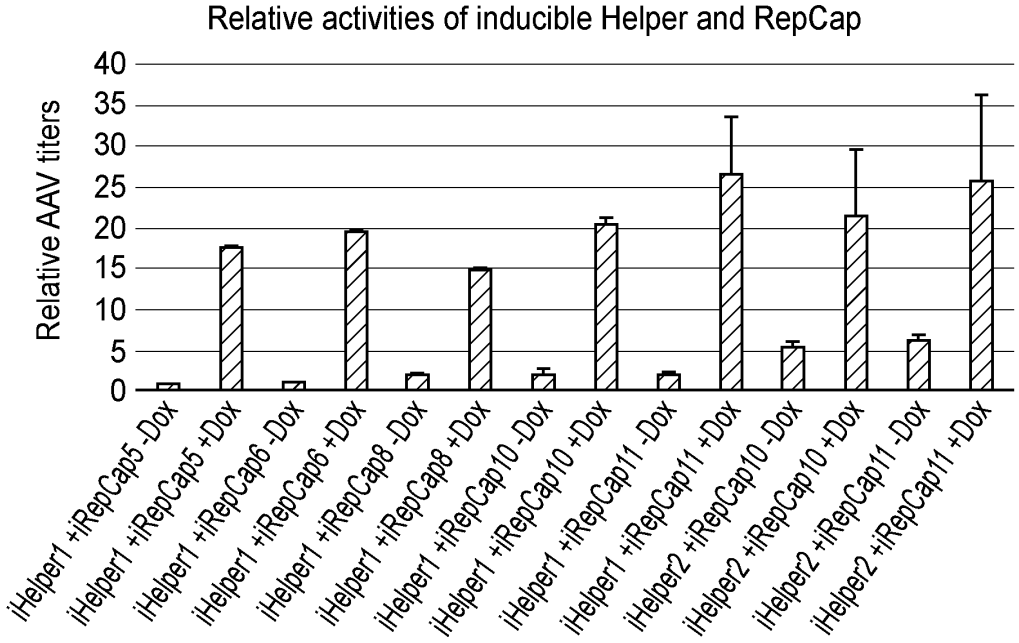

Relative activities of inducible Helper and RepCap
Relative AAV titers
40
35
30
25
20
15
10
5
0
iHelper1 +iRepCap5 -Dox
iHelper1 +iRepCap5 +Dox
iHelper1 +iRepCap6 -Dox
iHelper1 +iRepCap6 +Dox
iHelper1 +iRepCap8 -Dox
iHelper1 +iRepCap8 +Dox
iHelper1 +iRepCap10 -Dox
iHelper1 +iRepCap10 +Dox
iHelper1 +iRepCap11 -Dox
iHelper1 +iRepCap11 +Dox
iHelper2 +iRepCap10 -Dox
iHelper2 +iRepCap10 +Dox
iHelper2 +iRepCap11 -Dox
iHelper2 +iRepCap11 +Dox

FIG. 9B

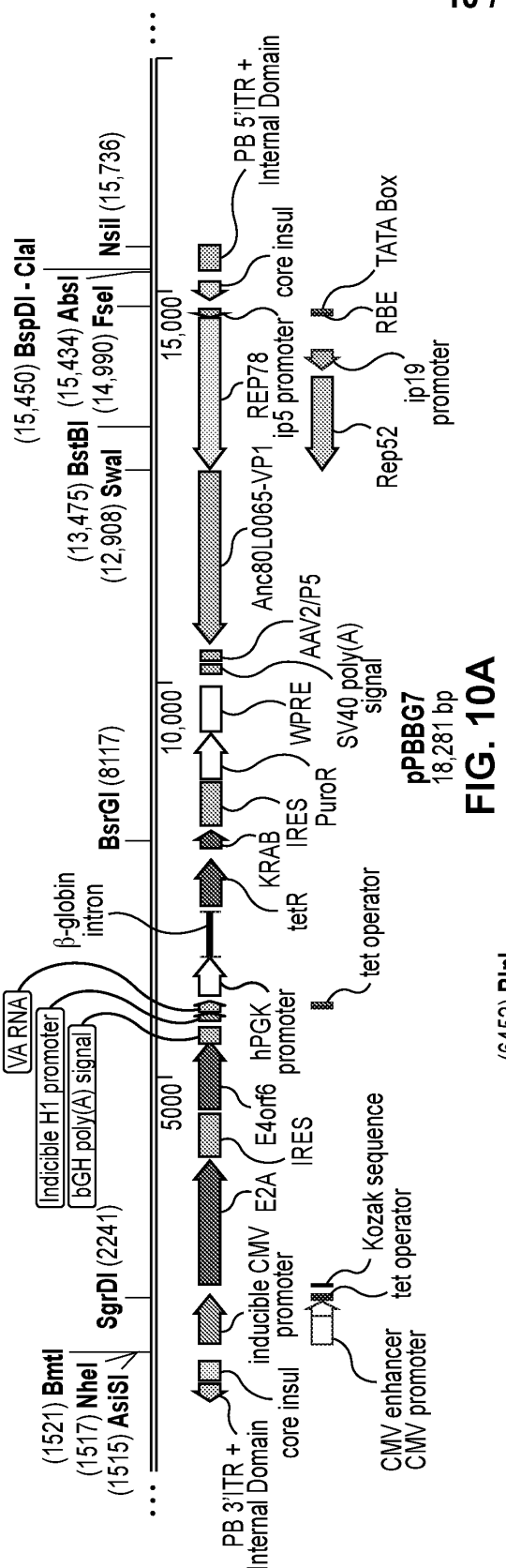


FIG. 10A

FIG. 10B

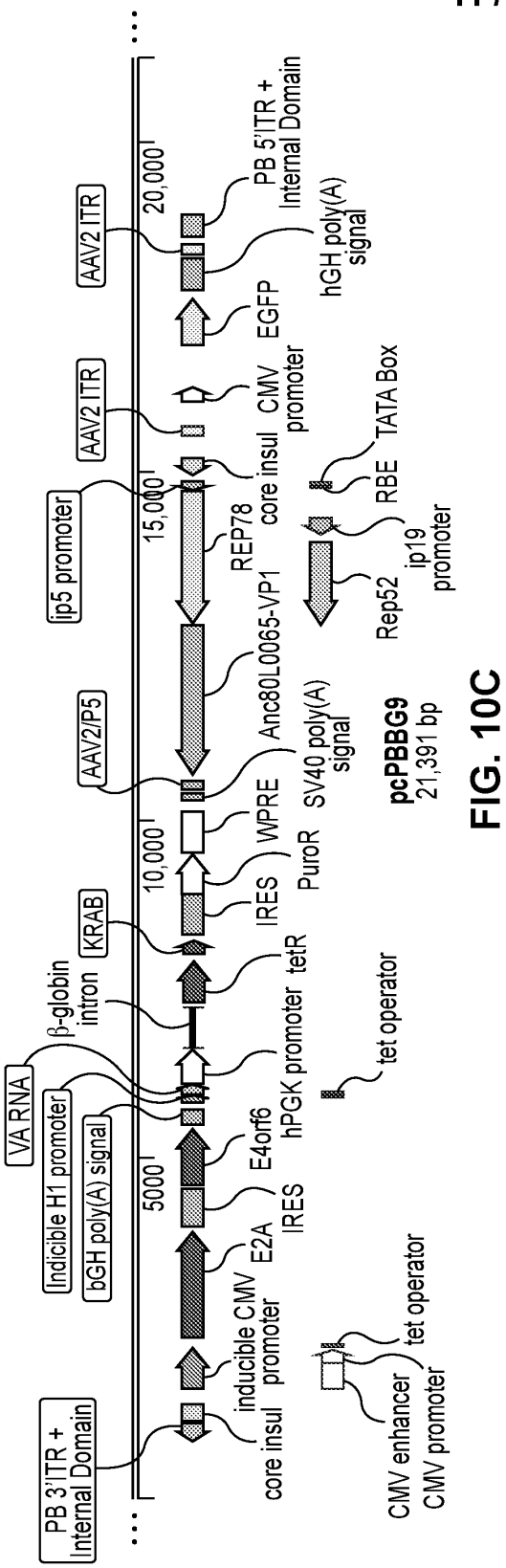


FIG. 10C

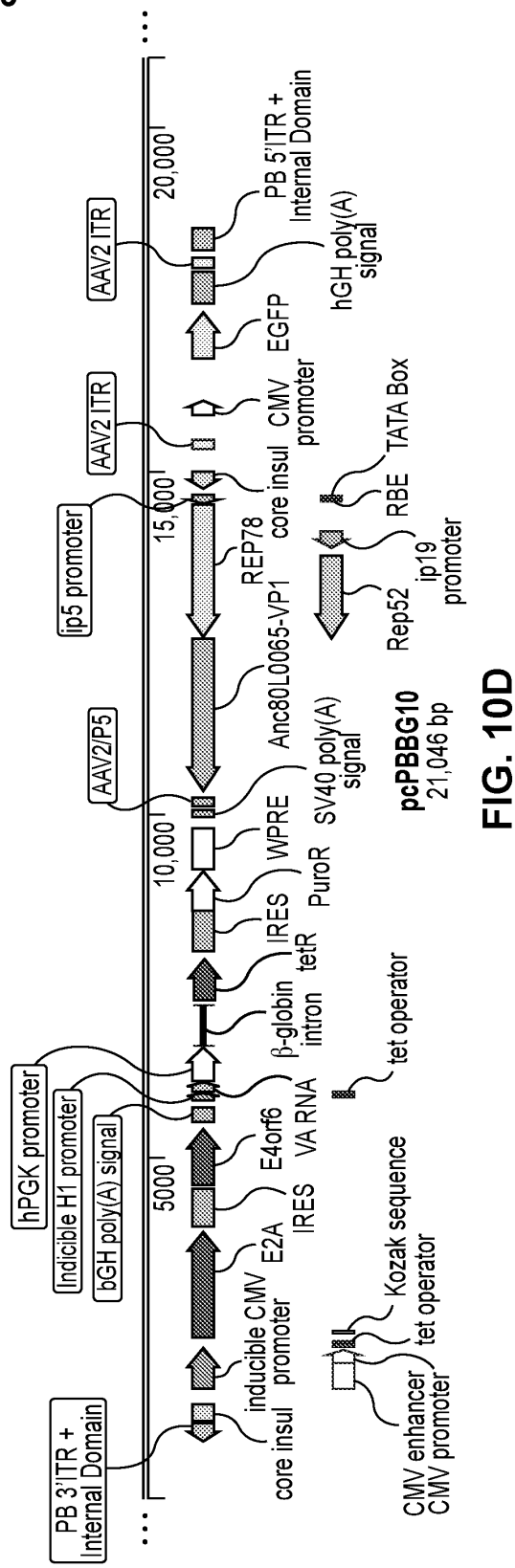
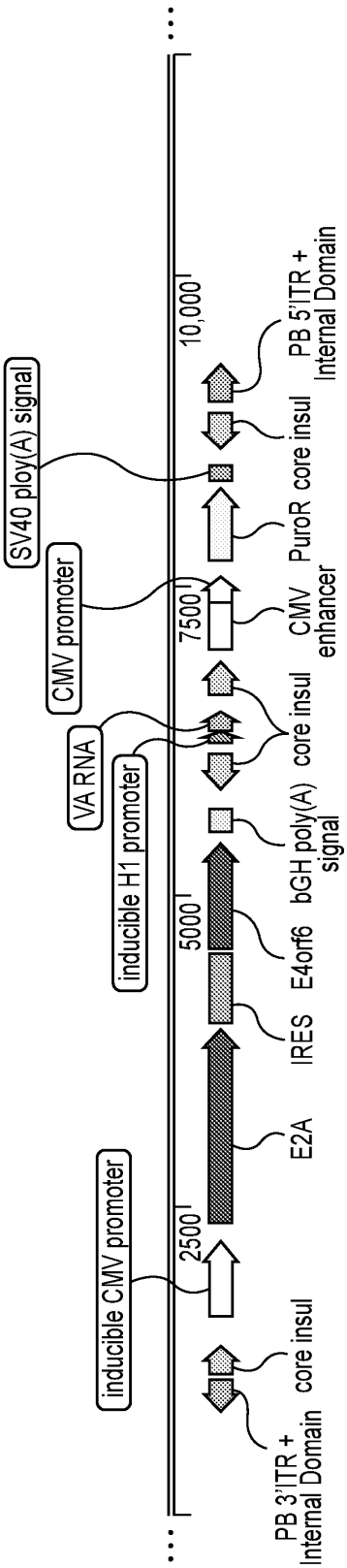
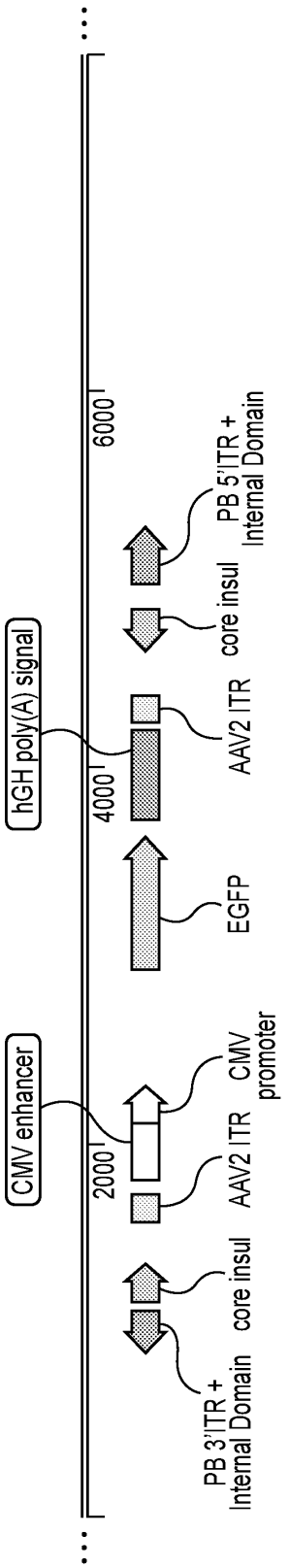


FIG. 10D



PBBG-iHelper-Puro
11,801 bp

FIG. 11A



PBBG-ITRGFP
7798 bp

FIG. 11B

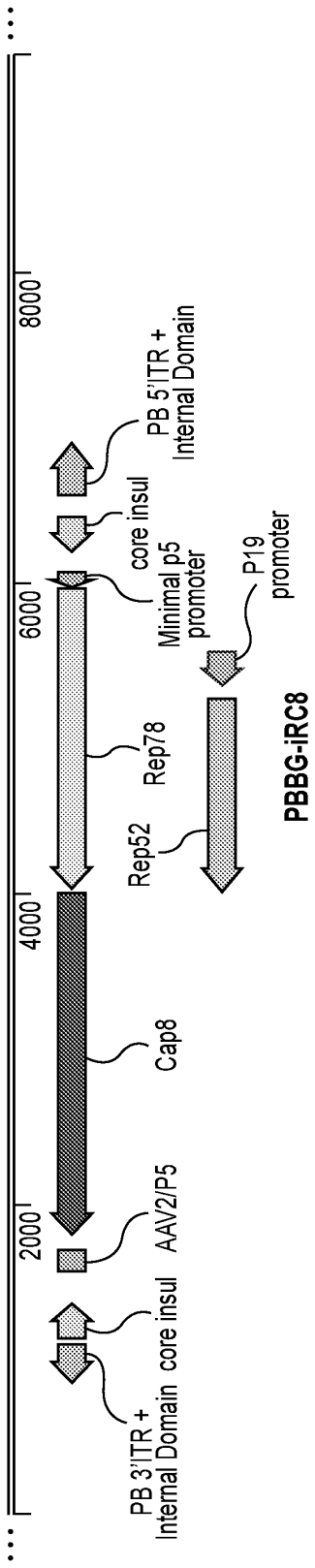


FIG. 11C

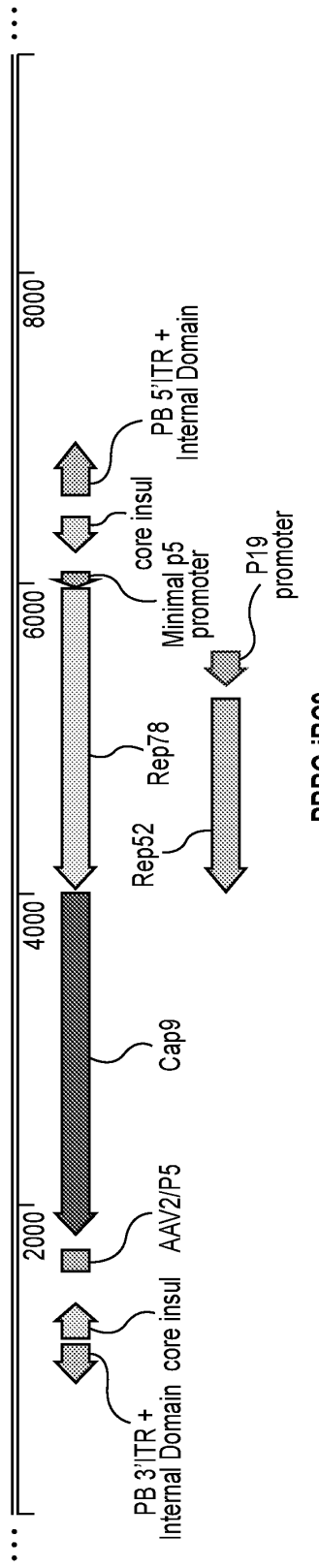


FIG. 11D

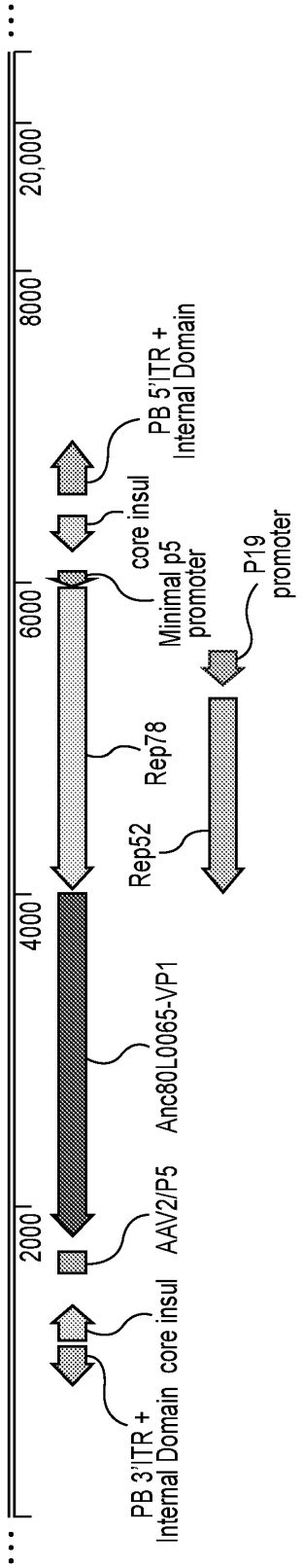

2000
4000
6000
8000
20,000
PB 3'ITR + Internal Domain
core insul
AAV2/P5
Anc80L0065-VP1
Rep52
Rep78
Minimal p5 promoter
core insul
PB 5'ITR + Internal Domain
P19 promoter
PBBG-Anc80iRC
9393 bp

FIG. 11E

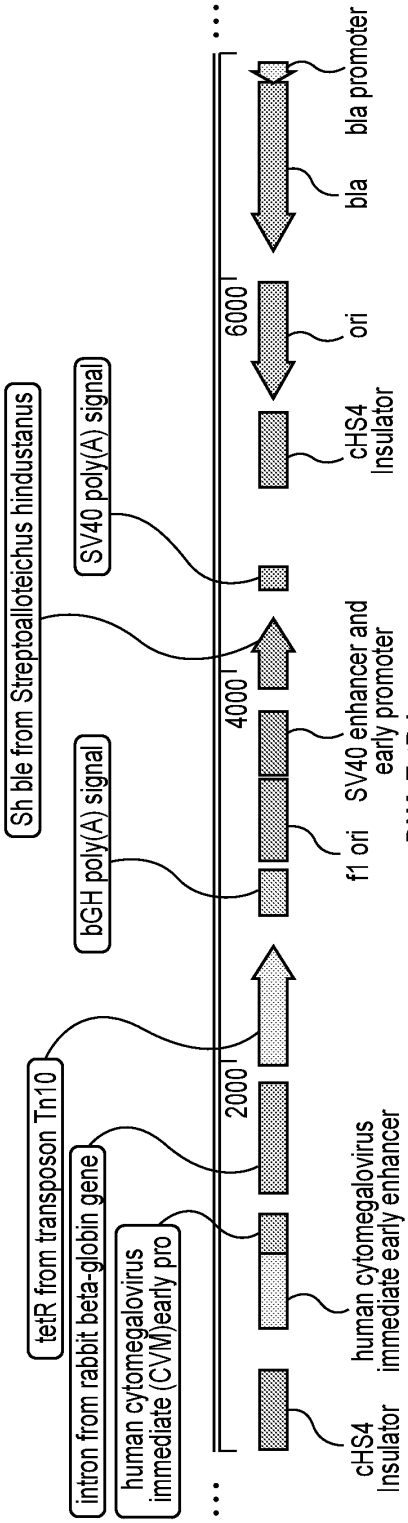


FIG. 12A

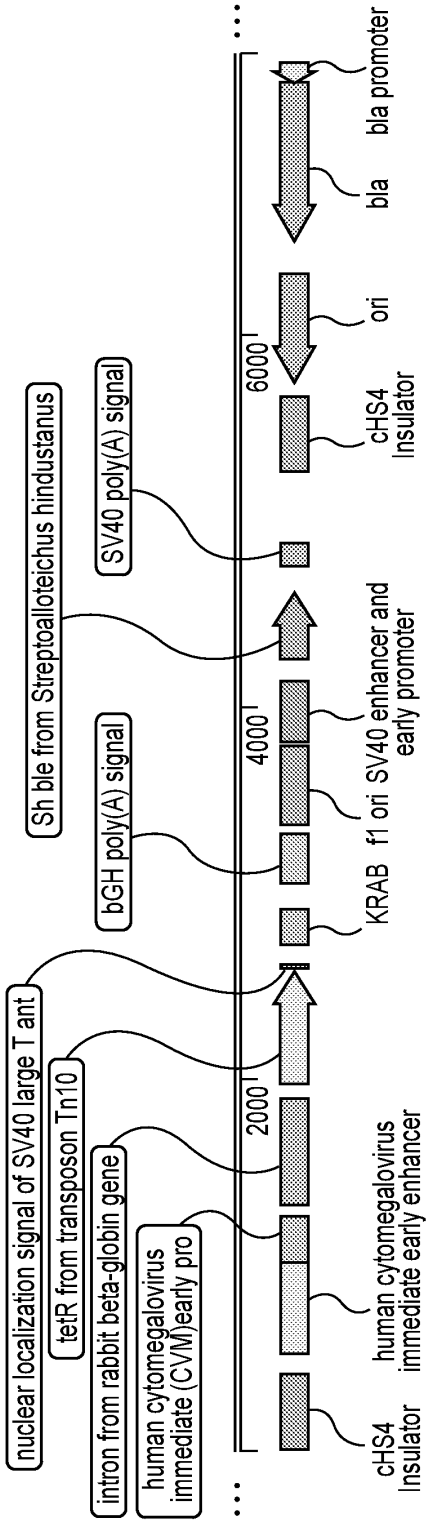


FIG. 12B