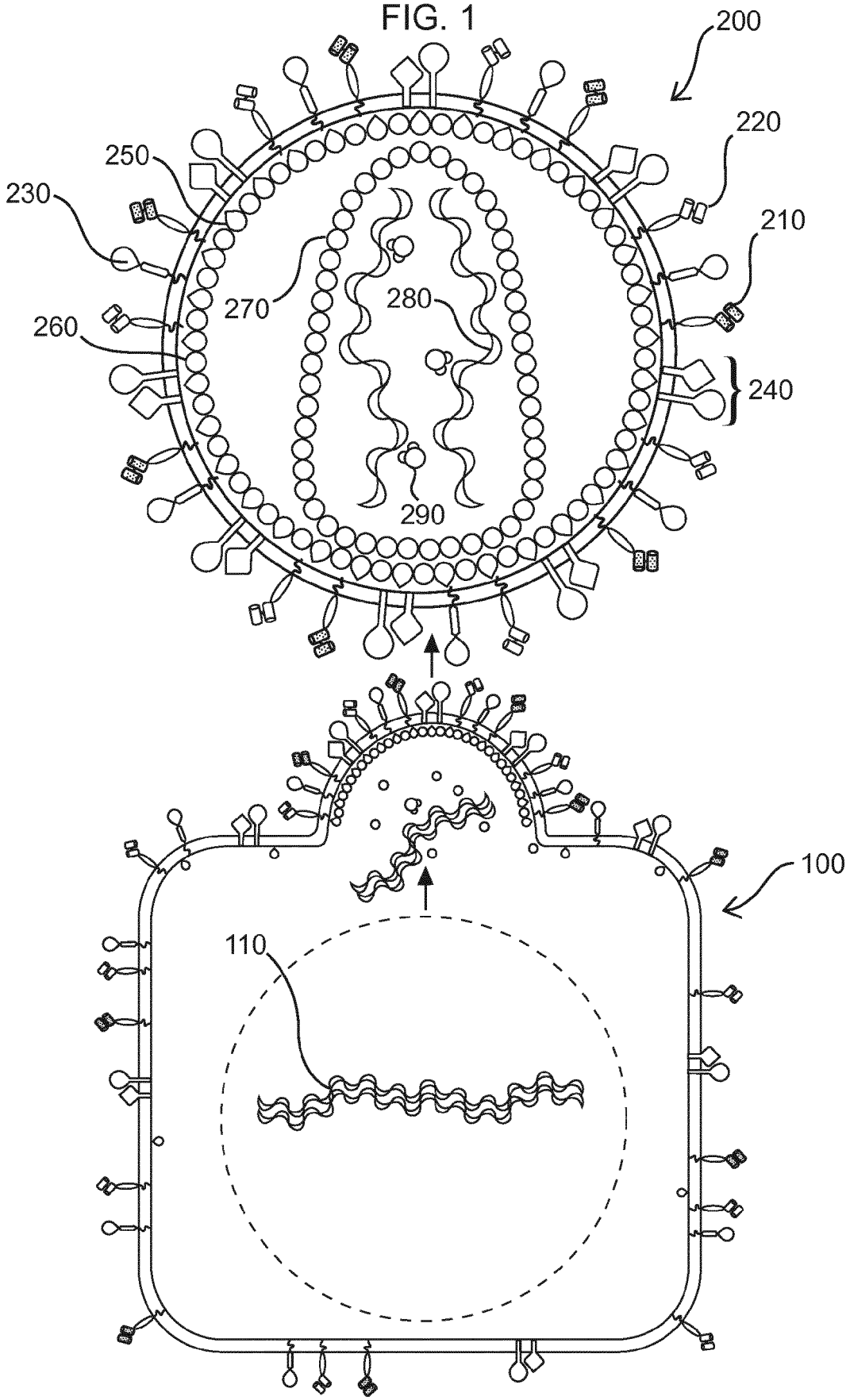
FIG. 1



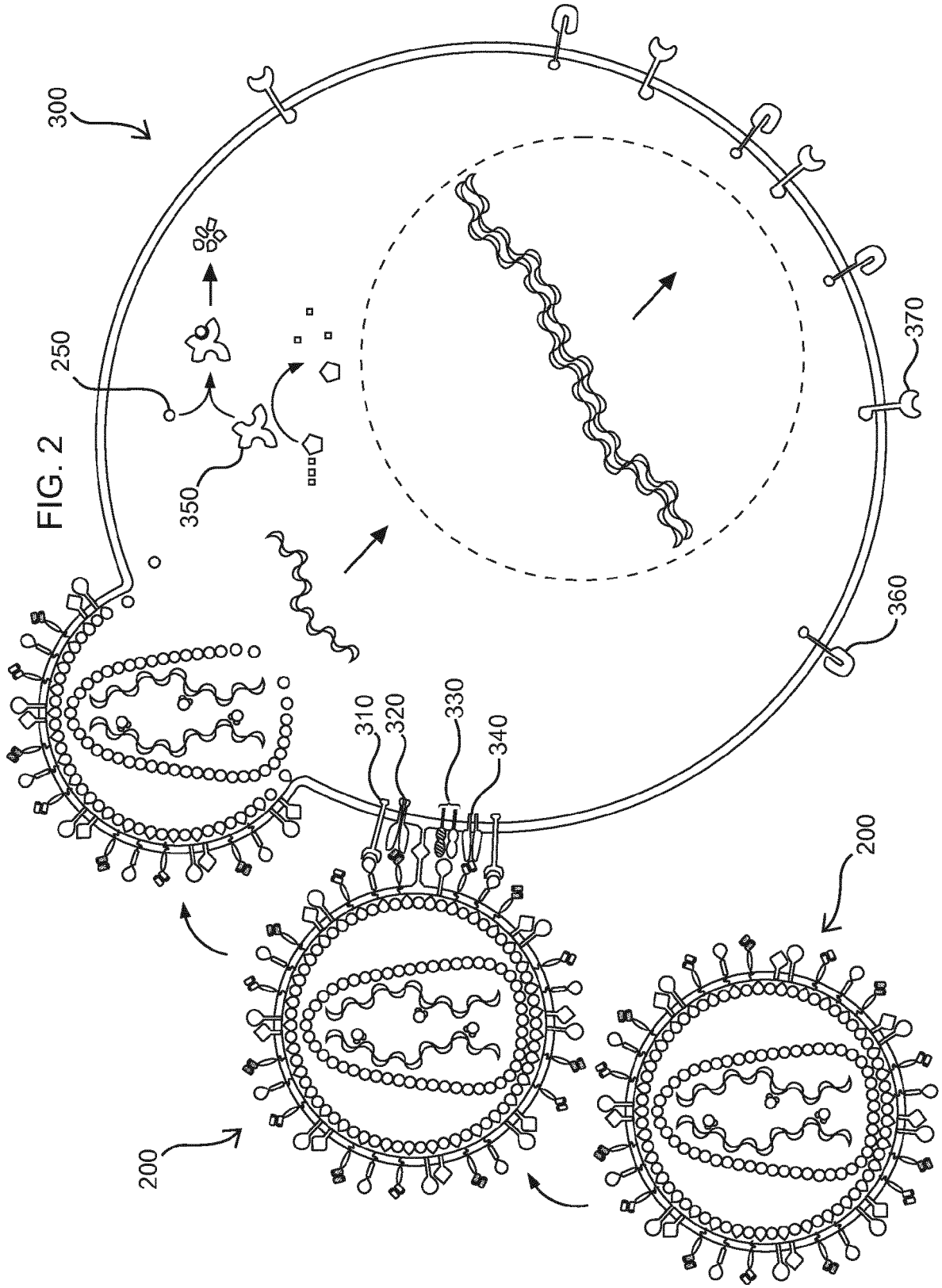


FIG. 2

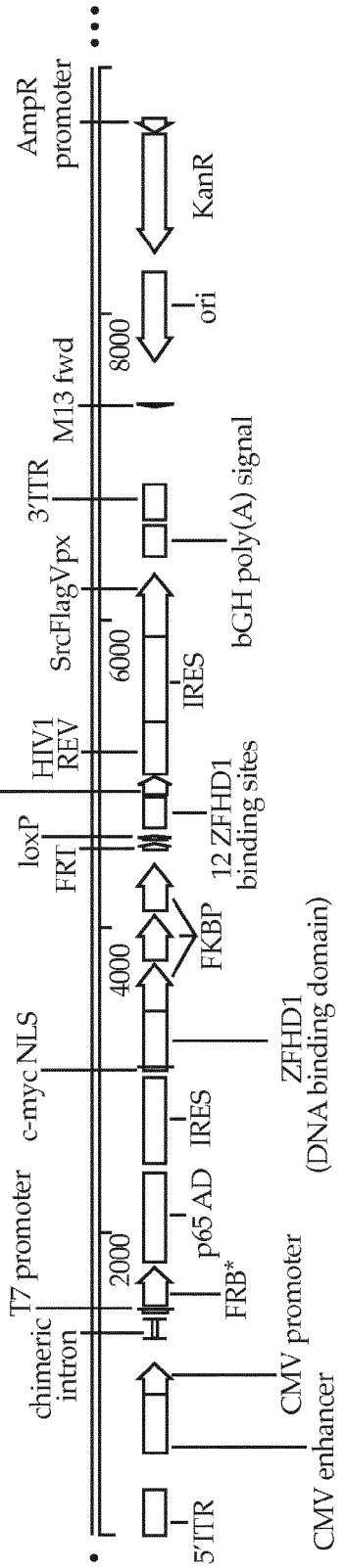

5'ITR
CMV enhancer
CMV promoter
FRB*
p65 AD
T7 promoter
chimeric intron
2000
IRES
c-myc NLS
ZFHD1
(DNA binding domain)
4000
FKBP
FRT
loxP
minimal IL-2 promoter
12 ZFHD1 binding sites
HIV1 REV
IRES
6000
SrcFlagVpx
3'ITR
bGH poly(A) signal
M13 fwd
8000
ori
KanR
AmpR promoter

FIG. 3A

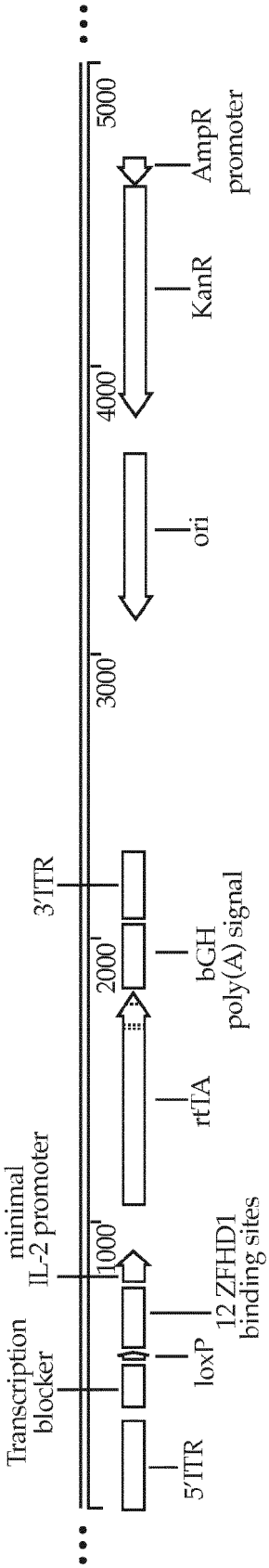

5'ITR
loxP
12 ZFHD1 binding sites
Transcription blocker
minimal IL-2 promoter
1000
rtTA
2000
bGH poly(A) signal
3'ITR
3000
ori
4000
KanR
5000
AmpR promoter

FIG. 3B

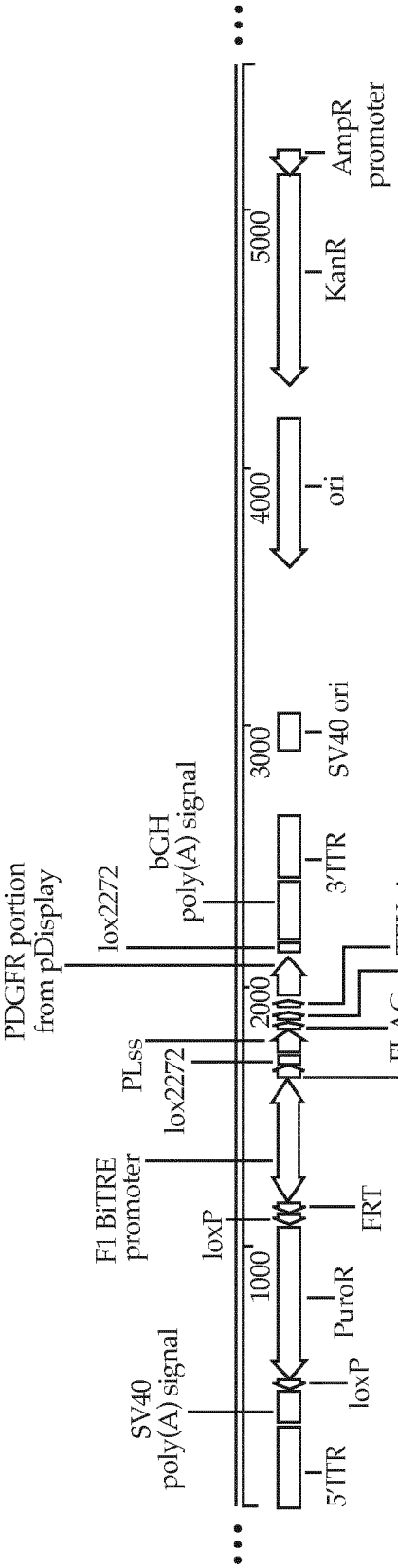

PDGFR portion
from pDisplay
lox2272
bGH
poly(A) signal
3'TR
SV40 ori
TEV site
Myc
FLAG
FRT (mutant)
PLss
lox2272
F1 BiTRE
promoter
loxP
FRT
SV40
poly(A) signal
1000
2000
3000
4000
5000
5'TR
loxP
PuroR
KanR
ori
AmpR
promoter

FIG. 3C

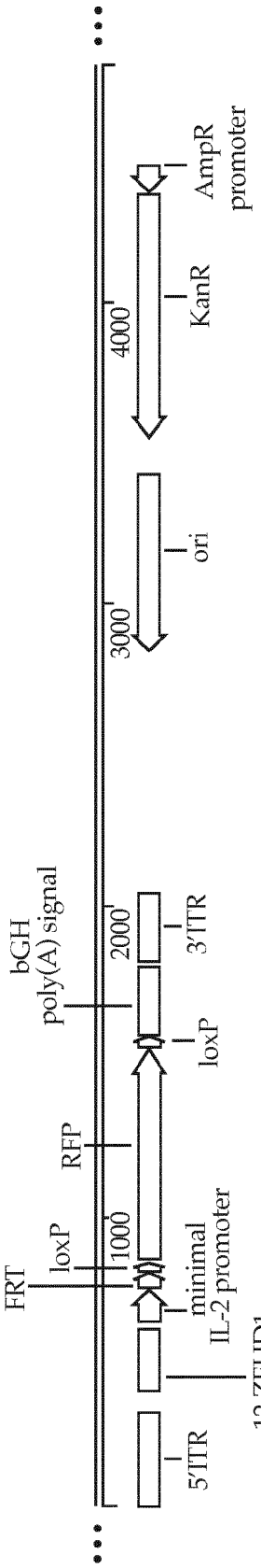

bGH
poly(A) signal
3'TR
FRT
loxP
RFP
loxP
1000
2000
3000
4000
5'TR
minimal
IL-2 promoter
12 ZFHD1
binding sites
ori
KanR
AmpR
promoter

FIG. 3D

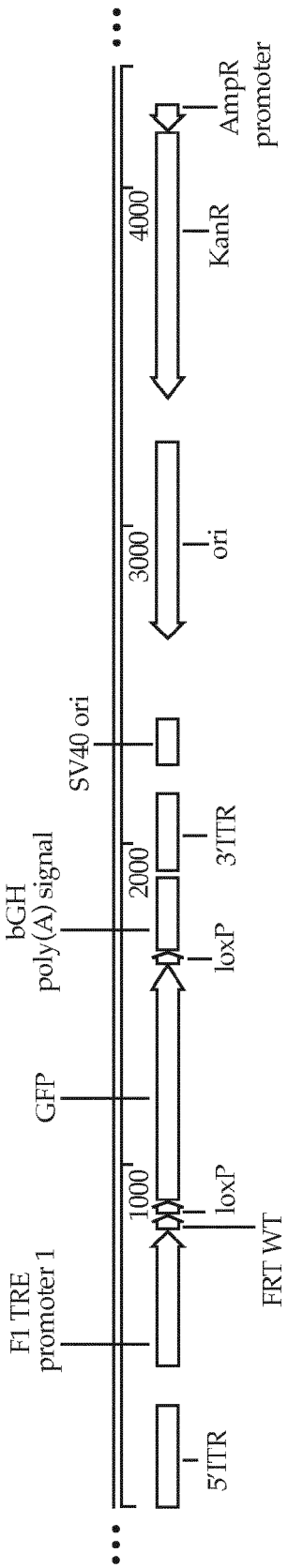

5'ITR
F1 TRE
promoter 1
1000
loxP
FRT WT
GFP
bGH
poly(A) signal
2000
loxP
3'ITR
SV40 ori
3000
ori
4000
KanR
AmpR
promoter

FIG. 3E

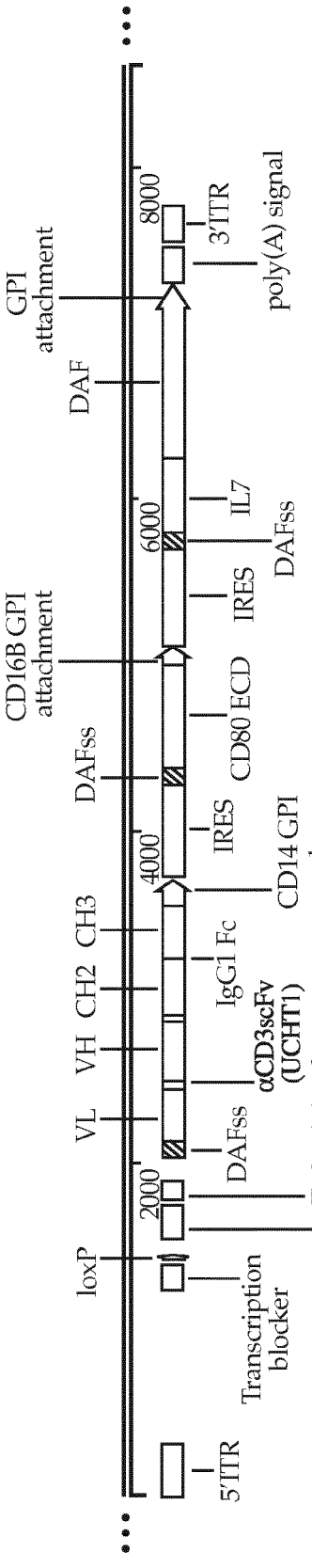

loxP
VL
VH
CH2
CH3
DAFss
CD16B GPI attachment
DAF
GPI attachment
2000
4000
6000
8000
5'TTR
Transcription blocker
DAFss
αCD3scFv (UCHT1)
IgG1 Fc
IRES
CD80 ECD
CD14 GPI attachment
IRES
IL7
DAFss
3'TTR
poly(A) signal
IL-2 minimal promoter
12 ZFHD1 binding sites

FIG. 4A

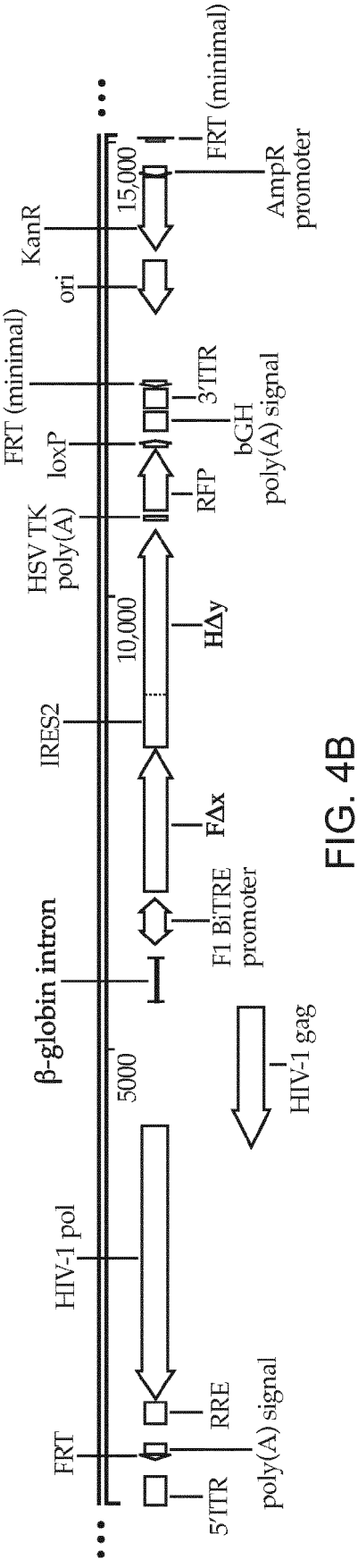

FRT
HIV-1 pol
β-globin intron
IRES2
HSV TK poly(A)
FRT (minimal)
loxP
KanR
ori
FRT (minimal)
AmpR promoter
5000
10,000
15,000
5'TTR
RRE
poly(A) signal
F1 BiTRE promoter
FΔx
HΔy
RFP
3'TTR
bGH poly(A) signal
HIV-1 gag

FIG. 4B

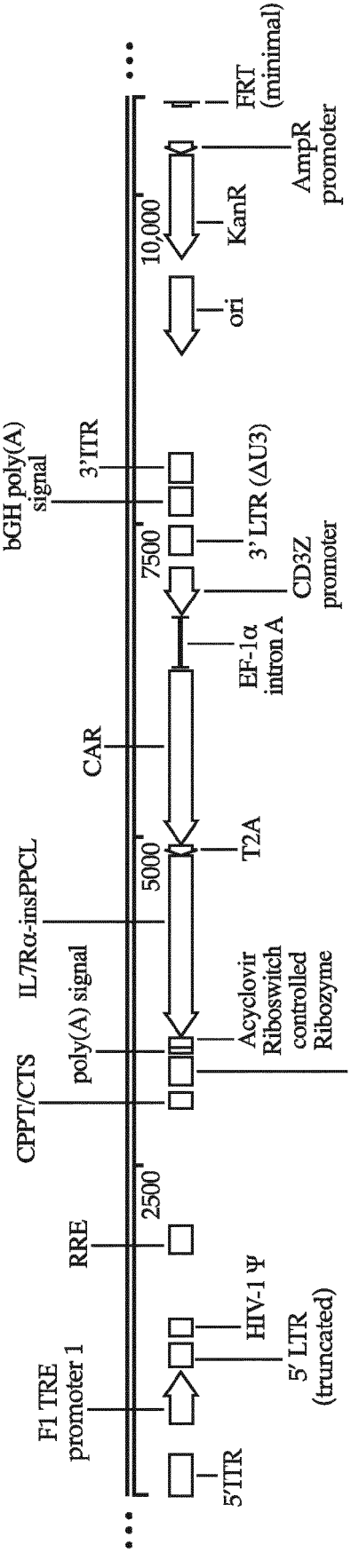
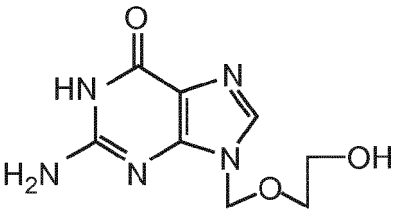
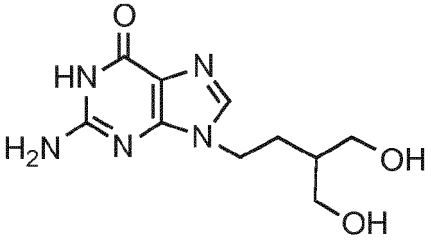

5'TTR
F1 TRE promoter 1
HIV-1 Ψ
5' LTR (truncated)
RRE
2500'
CPPT/CTS
IL7Rα-insPPCL
poly(A) signal
5000'
CAR
T2A
Acyclovir Riboswitch controlled Ribozyme
bGH poly(A) signal
EF-1α intron A
7500'
CD3Z promoter
3' LTR (ΔU3)
bGH poly(A) signal
3'TTR
10,000'
ori
KanR
AmpR promoter
FRT (minimal)

FIG. 4C



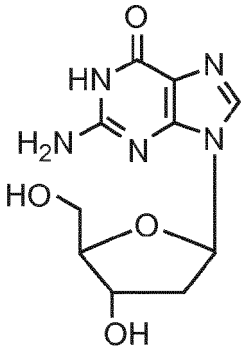
Acyclovir

FIG. 5A



Penciclovir

FIG. 5B



2′-Deoxyguanosine

FIG. 5C

aaaaaaaataaaatcaatagcaaatattaagatttttaagaaataaaaaattaatattaatttacaactgaatataaaagaa**actta**
tacagggtagcataatgggctactgaccccgccttcaaacctatttggagactataagtgaaaaaccactctttaattatta
aagtttctttttatgtccaaaagacaagaagaaacttttttatttagttgaatttataataagagaaaaagaaaggatattatATGGC
AAAAATAAAAAACCAATATTACAACGAGTCTGTTTCGCCAATTGAATATGCGCAACAAGGATTTA
AAGGAAAAATGCGTTCAGTAAACTGAAACGTAGTAAATGATGAAAAAGATTTAGAGGTATGAAAT
AGAATTACACAAAACTTCTGATTGCCTGAAAAAATTCCAGTTTCAAATGATTTAACTTCATGAAGA
ACTTTGACACCAGAATGACAAGAATTAATTACAAGAACTTTTACAGGATTAACATTGTTAGATACA
ATTCAAGCTACTGTTGGTGATGTGGCTCAAGTTCCTAACTCATTAACTGACCATGAACAAGTAATT
TACACAAACTTTGCATTTATGGTTGCAGTTCACGCTAGATCATATGGTTCAATCTTTTCAACTTTAT
GTTCAAGTGAACAAATTGAAGAGGCTCATGAATGAGTTATCAATACAGAAACATTACAAGAAAGA
GCTAAAGCATTAATTCCTTATTATGTGAATGATGACCCTTTAAAGTCAAAAGTTGCAGCTGCTTTA
ATGCCAGGCTTCTTATTATATGGAGGCTTCTATTTACCATTTTACCTATCAGCTAGAGGTAAATTACC
AAACACTTCAGATATTATTAGATTAATATTAAGAGATAAAGTTATACATAACTACTATAGTGGTTATA
AATATCAAAAGAAAGTTGCTAAACTTTCTCCAGAAAAACAAGCTGAAATGAAAGAATTTGTTTTT
AAATTATTATATGAATTAATAGATTTAGAAAAAGCTTATTTGAAAGAATTGTATGAGGATTTTGGATT
AGCTGATGATGCTATTAGATTTAGTGTTTACAACGCAGGTAAATTTTTACAAAATTTAGGTTATGAT
TCACCGTTTACAGAAGAAGAAACAAGAATTGAGCCAGAAATATTCACACAATTATCAGCTAGAGC
TGATGAAAACCATGATTTCTTTTCAGGGAATGGCTCATCATATATTATGGGAGTTTCAGAAGAAAC
TGAAGATGACGATTGGGAGTTTtaa (SEQ ID NO:236)

FIG. 6

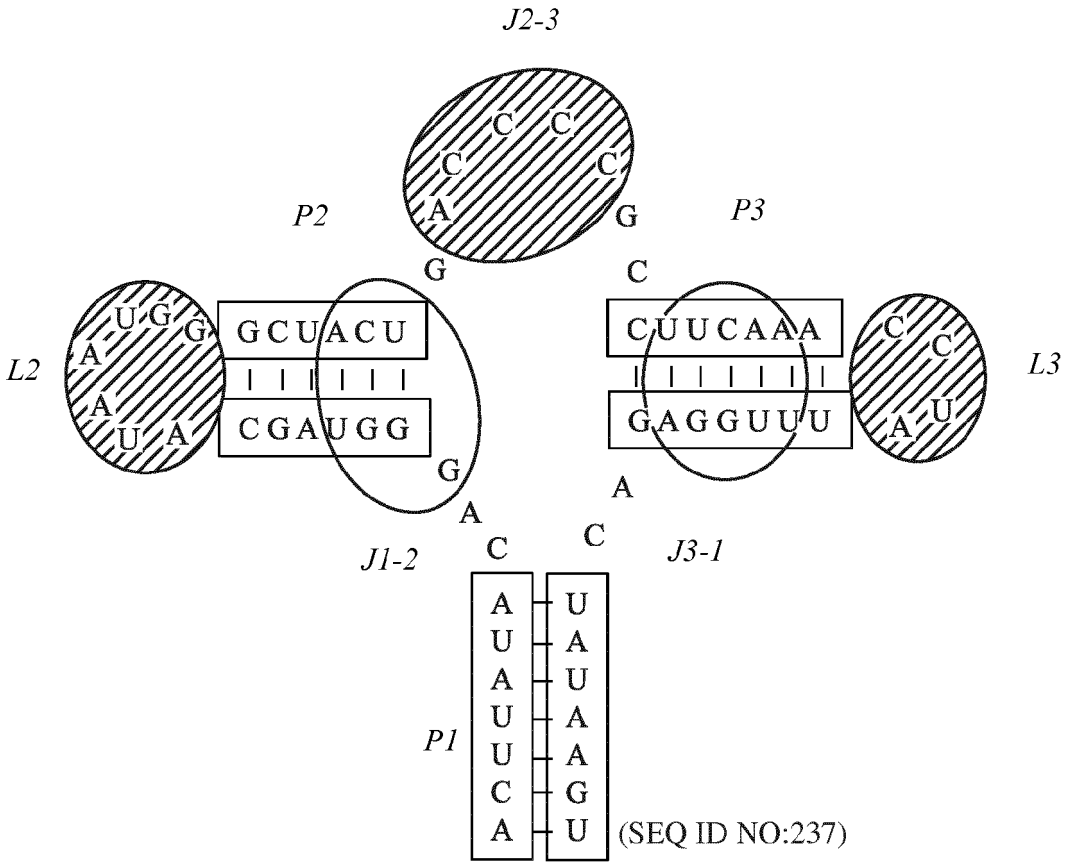
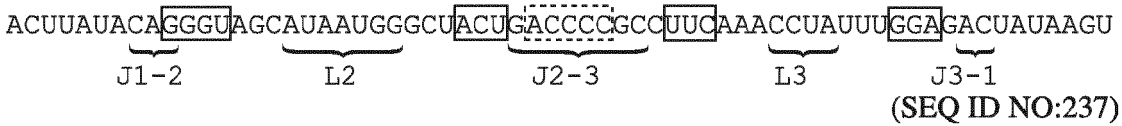
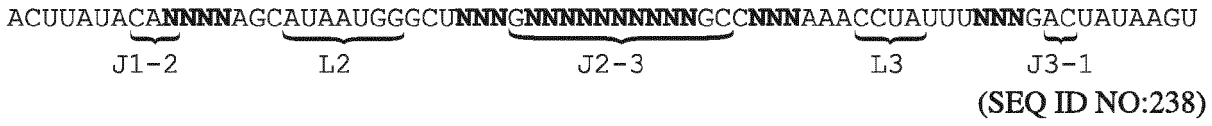

J2-3
C C
C C
A G
P2
P3
G
C
U G G
A
A
U A
L2
G C U A C U
C G A U G G
G
A
C U U C A A A
G A G G U U U
C C
A U
L3
A
J1-2
C
C
J3-1
A U
U A
A U
U A
U A
C G
A U
P1
(SEQ ID NO:237)

FIG. 7



(SEQ ID NO:237)

FIG. 8A



(SEQ ID NO:238)

FIG. 8B

FIG. 9

Oligo library for screen

CGCGCGACACTTATAGTCNNNAAATAGGTTTNNNGGCNNNNNNNNNNCNNNAGCCCATTATGCTNNNNTGTATAAGTGCCGCCC (SEQ ID NO:239)

T7 promoter amplification primer

TAATACGACTCACTATAGGGCGGCACTTATACA (SEQ ID NO:240)

Reverse amplification primer

CGCGCGACACTTATAGTC (SEQ ID NO:241)

tcaaaagcctggcggcgcggtcgtcagactcttttatatcgaatccccttgaaatacgaatgatatctaaaaaaac
aaaattaaagttcgggaatttttattttcagcctatgcaagagattagaatcttgatataatttattacaatataatagg
aa**cactcatataatcgcgtggatatggcacgcaagtttctaccgggcaccgtaaatgtccgactatgggtg**
agcaatggaaccgcacgtgtacggttttttgtgatatcagcattgcttgctctttatttgagcgggcaatgctttttta
ttctcataacggaggtagacaggATGGAAGCACTGAAACGGAAAATAGAGGAAGAAGGCGTC
GTATTATCTGATCAGGTATTGAAAGTGGATTCTTTTTTGAATCACCAAATTGATCCGCTG
CTTATGCAGAGAATTGGTGATGAATTTGCGTCTAGGTTTGCAAAAGACGGTATTACCAA
AATTGTGACAATCGAATCATCAGGTATCGCTCCCGCTGTAATGACGGGCTTGAAGCTG
GGTGTGCCAGTTGTCTTCGCGAGAAAGCATAAATCGTTAACACTCACCGACAACTTGC
TGACAGCGTCTGTTTATTCCTTTACGAAGCAAACAGAAAGCCAAATCGCAGTGTCTGG
GACCCACCTGTCGGATCAGGATCATGTGCTGATTATCGATGATTTTTTGGCAAATGGAC
AGGCAGCGCACGGGCTTGTGTCGATTGTGAAGCAAGCGGGAGCTTCTATTGCGGGAA
TCGGCATTGTTATTGAAAAGTCATTTCAGCCGGGAAGAGATGAACTTGTAAAACTGGG
CTACCGAGTGGAATCTTTGGCAAGAATTCAGTCTTTAGAAGAAGGAAAAGTGTCCTTC
GTACAGGAGGTTCATTCA**tga** (SEQ ID NO:242)

FIG. 10

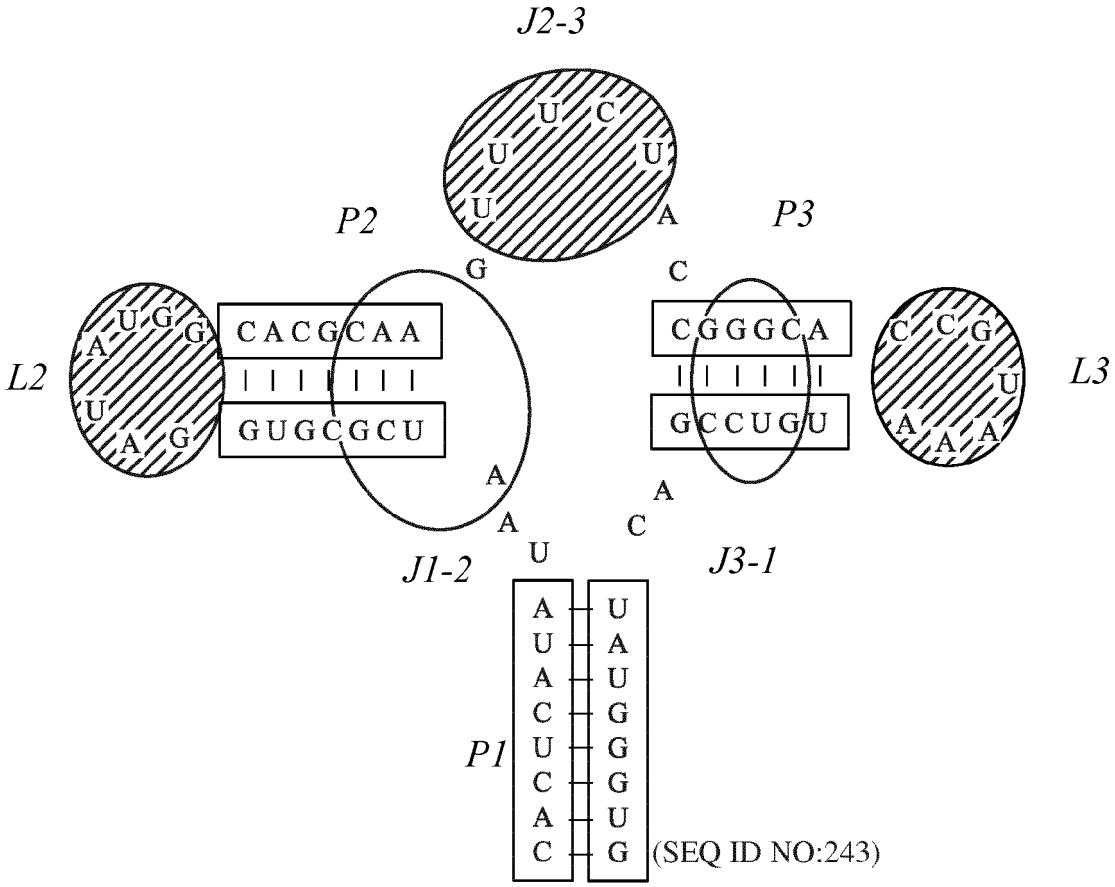

J2-3
U C
U U
U A
P2
P3
G
C
L2
U G G
A
U
A G
C A C G C A A
G U G C G C U
C G G G C A
G C C U G U
C C G
U
A A A
L3
A
A
A
C
U
J1-2
J3-1
P1
A U
U A
A U
C G
U G
C G
A U
C G
(SEQ ID NO:243)

FIG. 11



(SEQ ID NO: 243)

FIG. 12A

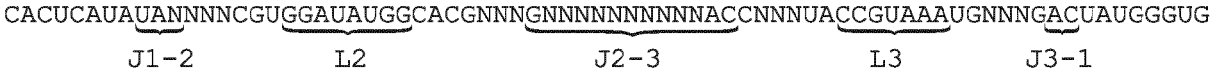


FIG. 12B

(SEQ ID NO: 244)

FIG. 13

Oligo library for screen

CGCGCGACCACCCATAGTC**NNN**CATTTACGGTG**NNN**GGT**NNNNNNNNNNN**C**NNN**CGTGCCATATCCACG
NNNNTATATGAGTGGCCGCCC (SEQ ID NO:245)

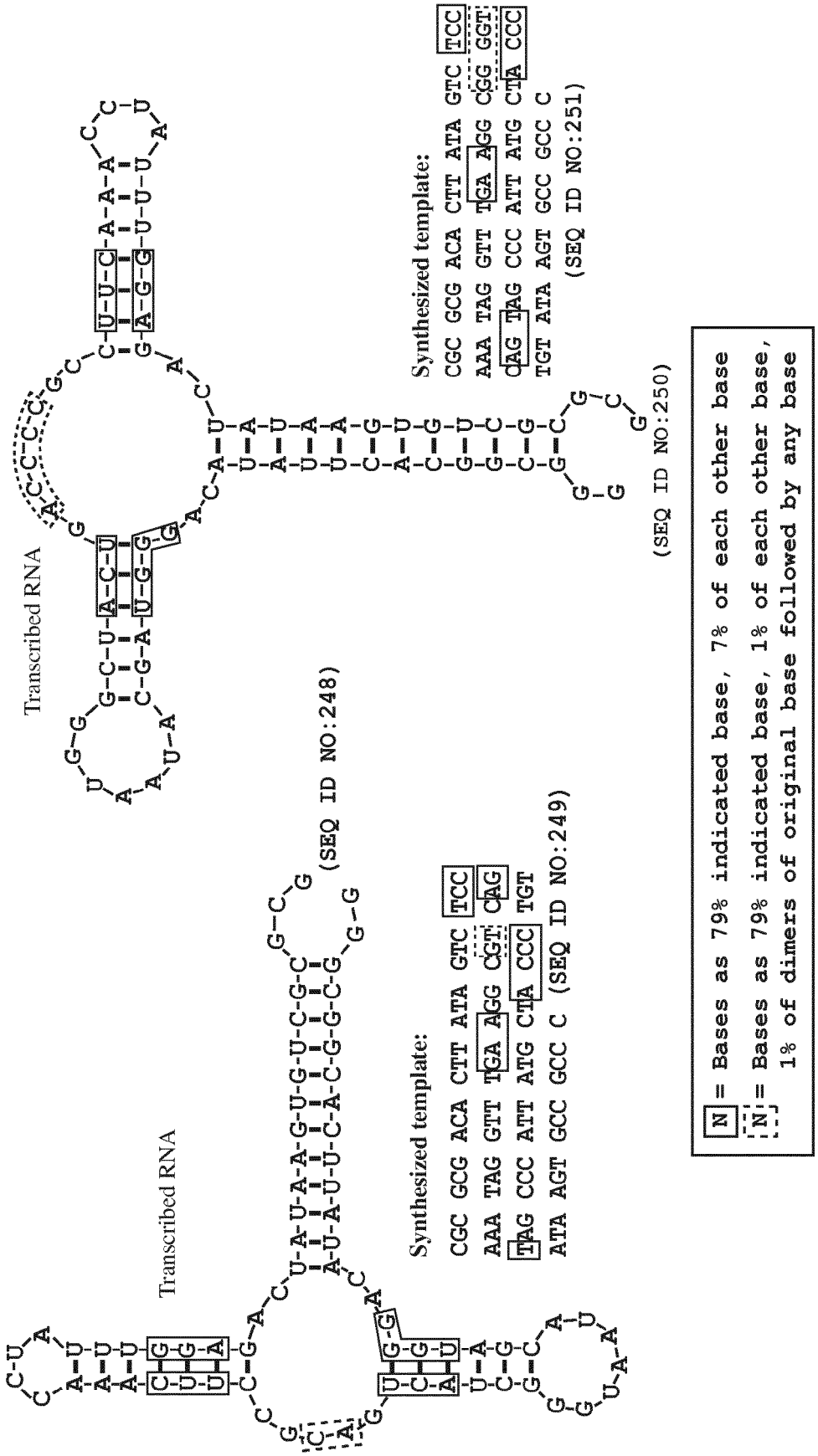
T7 promoter amplification primer

TAATACGACTCACTATAGGGCGGCCACTCATATA (SEQ ID NO:246)

Reverse amplification primer

CGCGCGACCACCCATAGTC (SEQ ID NO:247)

FIG. 14


Transcribed RNA
Synthesized template:
CGC GCG ACA CTT ATA GTC TCC
AAA TAG GTT TGA AGG CGT CAG
TAG CCC ATT ATG CTA CCC TGT
ATA AGT GCC GCC C (SEQ ID NO:249)
(SEQ ID NO:248)
Transcribed RNA
Synthesized template:
CGC GCG ACA CTT ATA GTC TCC
AAA TAG GTT TGA AGG CGT CAG
TAG CCC ATT ATG CTA CCC TGT
TGT ATA AGT GCC GCC C
(SEQ ID NO:251)
(SEQ ID NO:250)
N = Bases as 79% indicated base, 7% of each other base
N = Bases as 79% indicated base, 1% of each other base,
1% of dimers of original base followed by any base

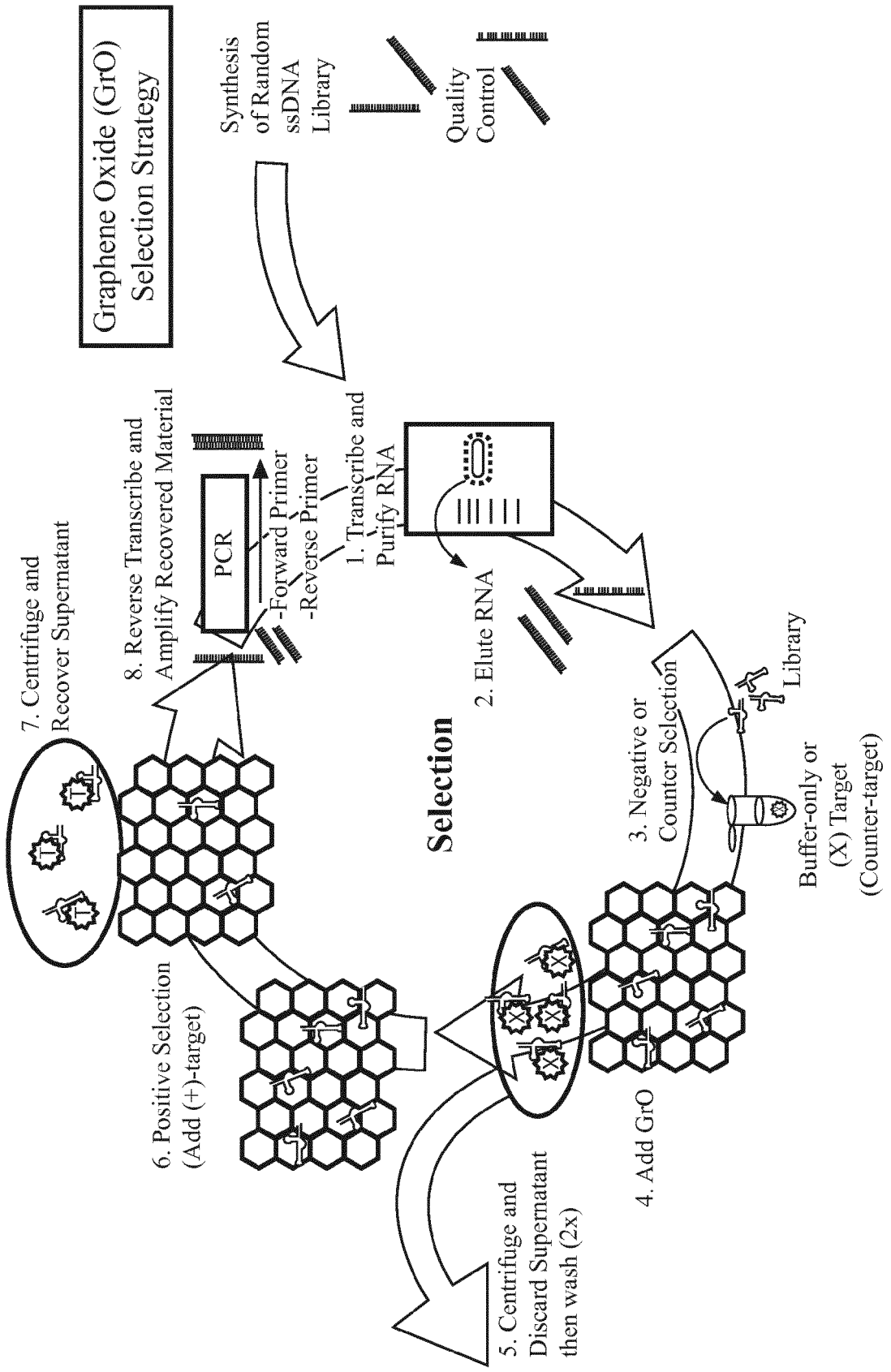

Graphene Oxide (GrO)
Selection Strategy
Synthesis
of Random
ssDNA
Library
Quality
Control
1. Transcribe and
Purify RNA
-Forward Primer
-Reverse Primer
PCR
8. Reverse Transcribe and
Amplify Recovered Material
7. Centrifuge and
Recover Supernatant
2. Elute RNA
Selection
3. Negative or
Counter Selection
Library
Buffer-only or
(X) Target
(Counter-target)
4. Add GrO
5. Centrifuge and
Discard Supernatant
then wash (2x)
6. Positive Selection
(Add (+)-target)

FIG. 15

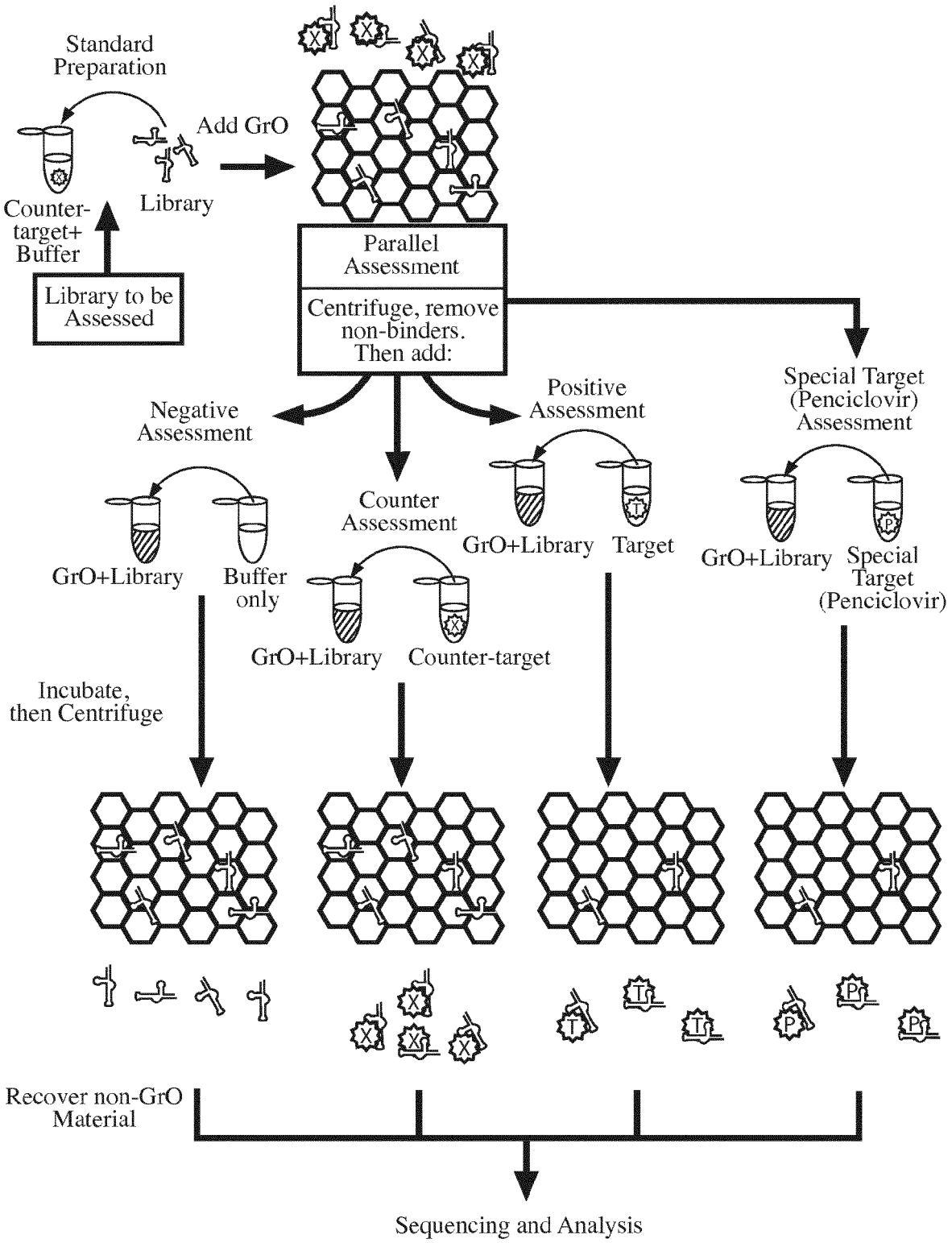

Standard Preparation
Add GrO
Library
Counter-target+ Buffer
Library to be Assessed
Parallel Assessment
Centrifuge, remove non-binders. Then add:
Negative Assessment
Positive Assessment
Special Target (Penciclovir) Assessment
Counter Assessment
GrO+Library
Buffer only
GrO+Library
Target
GrO+Library
Special Target (Penciclovir)
GrO+Library
Counter-target
Incubate, then Centrifuge
Recover non-GrO Material
Sequencing and Analysis

FIG. 16

F1A-795	**F1A-996**	**F1A-935**	**F1A-946**	**F1A-961**	**F1A-769**	**F1A-582**
ΔG = -23.30 kcal/mol	ΔG = -25.50 kcal/mol	ΔG = -25.40 kcal/mol	ΔG = -25.10 kcal/mol	ΔG = -27.40 kcal/mol	ΔG = -23.70 kcal/mol	ΔG = -23.10 kcal/mol
Sequence (5′->3′): GGG CGG CAC UUA UAC AGC GAA GCA UAA UGG CUA CUG ACG CCC UCA AAC CCU AUU UGC AGA CUA UAA GUG UCG CGC G	Sequence (5′->3′): GGG CGG CAC UUA UAC AGG GUA GCA UAA UGG CUU AGG ACG CCU UCA AAC CUA UCA AGA CUA UAA GUG UCG CGC G	Sequence (5′->3′): GGG CGG CAC UUA UAC AGG GUA GCA UAA UGG GCU ACU UGA CGC CUU CAC CUA UUU GUA GAC UAU AAG UGU CGC GCG	Sequence (5′->3′): GGG CGG CAC UUA UAC AGC GUA GCA UAA UGG GCU GCA GAC GCC GUC AAA CCU AUU UGC AGA CUA UAA GUG UCG CGC G	Sequence (5′->3′): GGG CGG CAC UUA UAC ACC GUA GCA UAA UGG GCU ACU GCC GCC GUC GAC CUU UUG GAG ACU AUA AGU GUC GCG CG	Sequence (5′->3′): GGG CGG CAC UUA UAC AGG UCA GCA UAA UGU GCU AGU GCG CCU UCA AAC CUA UUU AGA GAC UAU AAG UGU CGC GCG	Sequence (5′->3′): GGG CGG CAC UUA UAC AGC UUA GCG UAA UGG CUA CUG ACG CCG UCC AAA CCU AUU UAC AGA CUA UAA GUG UCG CGC G
(SEQ ID NO:87)	(SEQ ID NO:88)	(SEQ ID NO:89)	(SEQ ID NO:90)	(SEQ ID NO:91)	(SEQ ID NO:92)	(SEQ ID NO:93)

FIG. 17

F1P-584	**F1P-710**	**F1P-923**	**F1P-991**	**F1P-837**	**F1P-718**	**F1P-932**
ΔG = -24.10 kcal/mol	ΔG = -28.90 kcal/mol	ΔG = -23.00 kcal/mol	ΔG = -25.00 kcal/mol	ΔG = -23.70 kcal/mol	ΔG = -25.00 kcal/mol	ΔG = -27.70 kcal/mol
Sequence (5′->3′): GGG CGG CAC UUA UAC AGG GGA GCA UAA UGG GCU ACU GAC GCC UUU AAA CCU AUU UGA GGA CUA UAA GUG UCG CGC G	Sequence (5′->3′): GGG CGG CAC UUA UAC AUG GAA GCA UAA UGG GCU GCC GAC GGC CCU UAA CCU UUG GAG ACU AUA AGU GUC GCG CG	Sequence (5′->3′): GGG CGG CAC UUA UAC AGA UUA GCA UAA UGG GCU ACU GAC CCC GCC GGC AAA CCU AUU UGA AGA CUA UAA GUG UCG CGC G	Sequence (5′->3′): GGG CGG CAC UUA UAC AGU GUA GCA UAA UGG GCU ACU GUC GCA UCA AAC CUA UUU GGA GAC UAU AAG UGU CGC GCG	Sequence (5′->3′): GGG CGG CAC UUA UAC AGU GAA GCA UAA UGG GCU ACU GAC ACC CUU AAA CCU AUU UGC AGA CUA UAA GUG UCG CGC G	Sequence (5′->3′): GGG CGG CAC UUA UAC AGA GUA GCA UAA UGG GCU ACA GAC GCC GUC AAA CCU AUU UAC CGA CUA UAA GUG UCG CGC G	Sequence (5′->3′): GGG CGG CAC UUA UAC AGG GUG CAU AAU GGG CUA GUG ACG CCU UCA AAC CUA UUU GUA GAC UAU AAG UGU CGC GCG
(SEQ ID NO:94)	(SEQ ID NO:95)	(SEQ ID NO:96)	(SEQ ID NO:97)	(SEQ ID NO:98)	(SEQ ID NO:99)	(SEQ ID NO:100)

FIG. 18

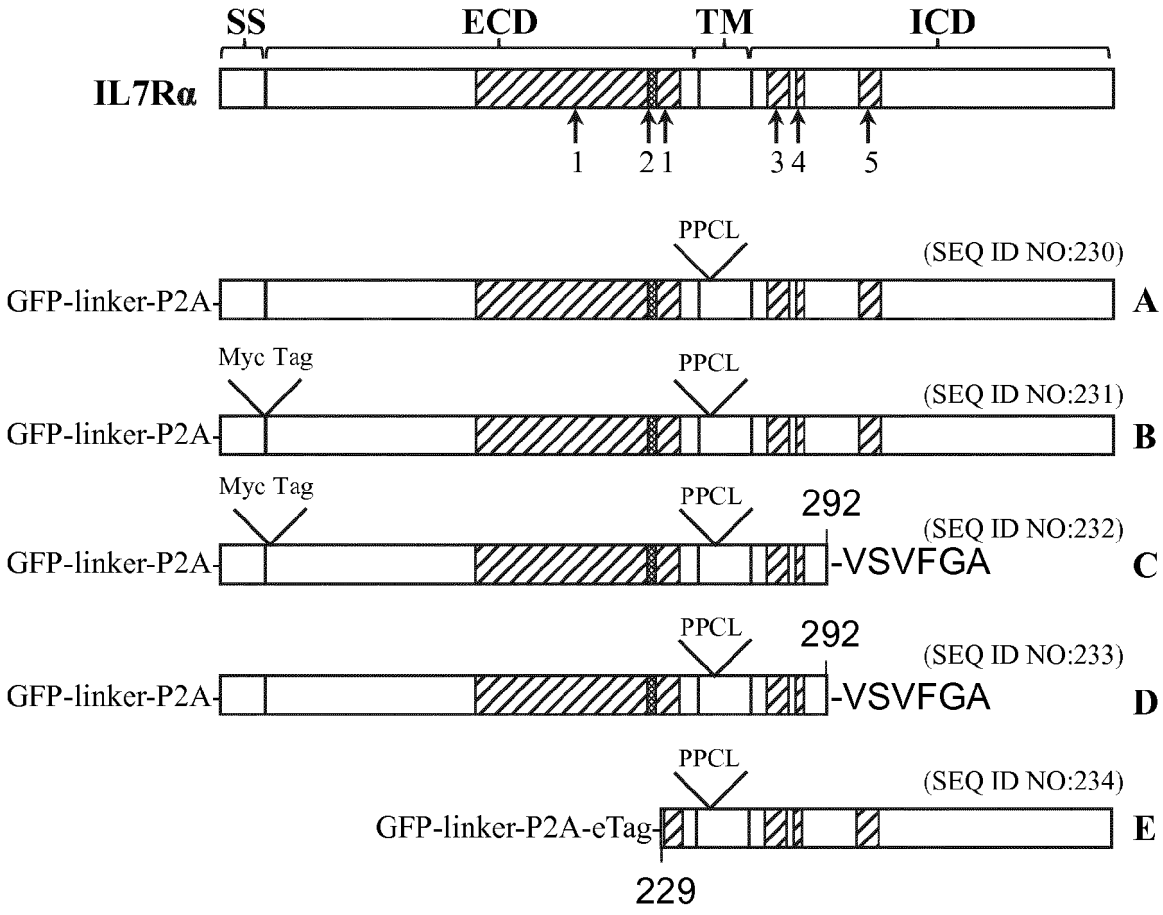


FIG. 19A

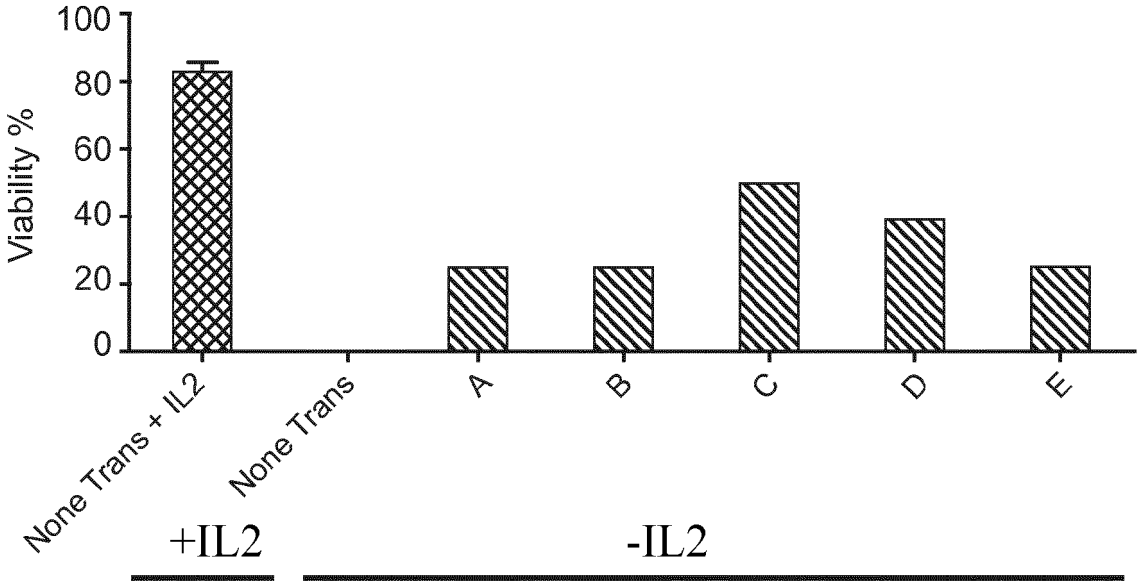


FIG. 19B

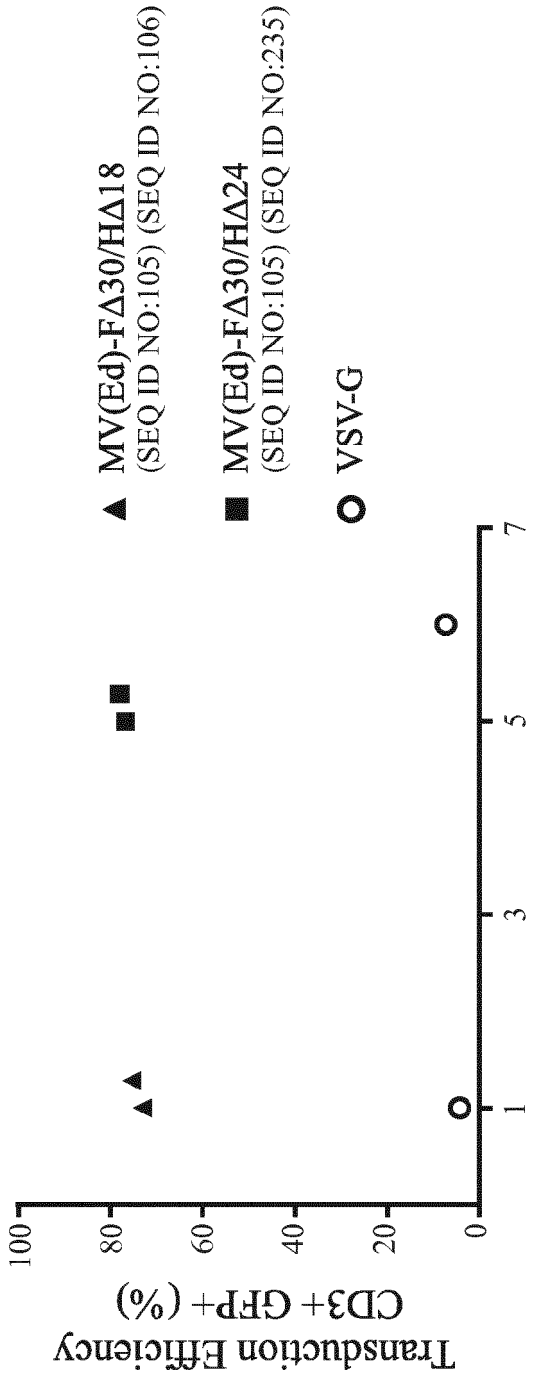

Transduction Efficiency
CD3+ GFP+ (%)
100
80
60
40
20
0
1
3
5
7
Multiplicity of Infection (MOI)
MV(Ed)-FΔ30/HΔ18
(SEQ ID NO:105) (SEQ ID NO:106)
MV(Ed)-FΔ30/HΔ24
(SEQ ID NO:105) (SEQ ID NO:235)
VSV-G

FIG. 20

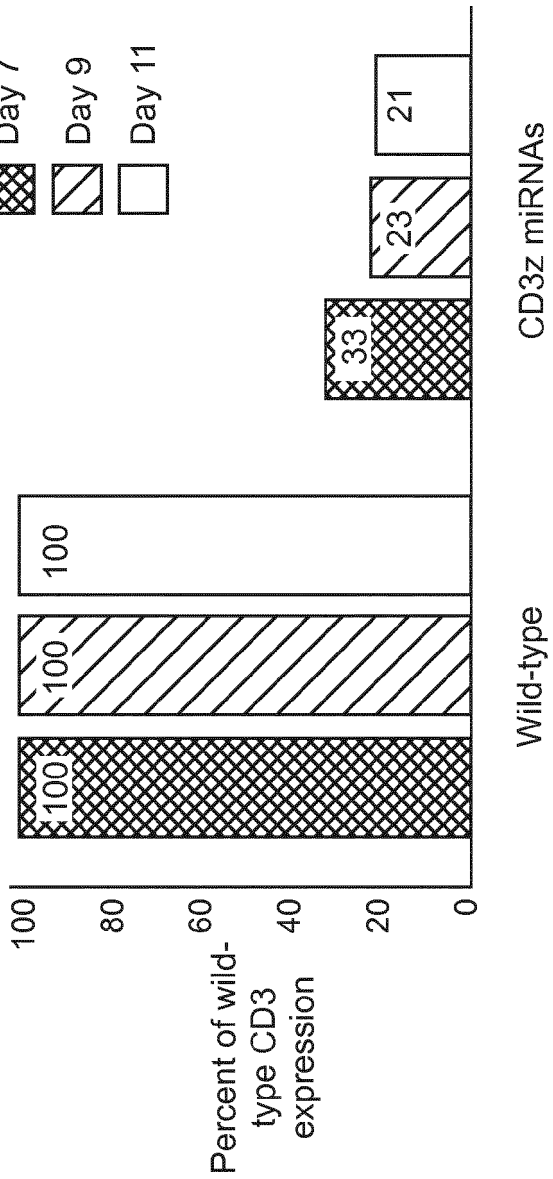

Day 7
Day 9
Day 11
Percent of wild-type CD3 expression
100
80
60
40
20
0
100
100
100
33
23
21
Wild-type
CD3z miRNAs

FIG. 21