FIG. 1A

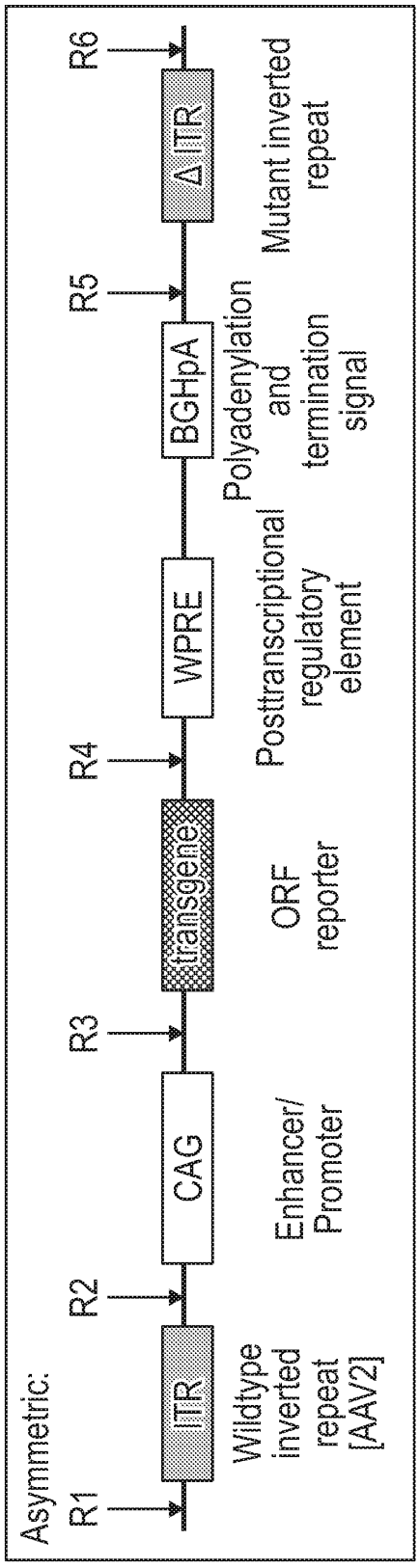

Asymmetric:
R1
ITR
R2
CAG
R3
transgene
R4
WPRE
R5
BGHpA
R6
Δ ITR
Wildtype inverted repeat [AAV2]
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
Mutant inverted repeat

FIG. 1B

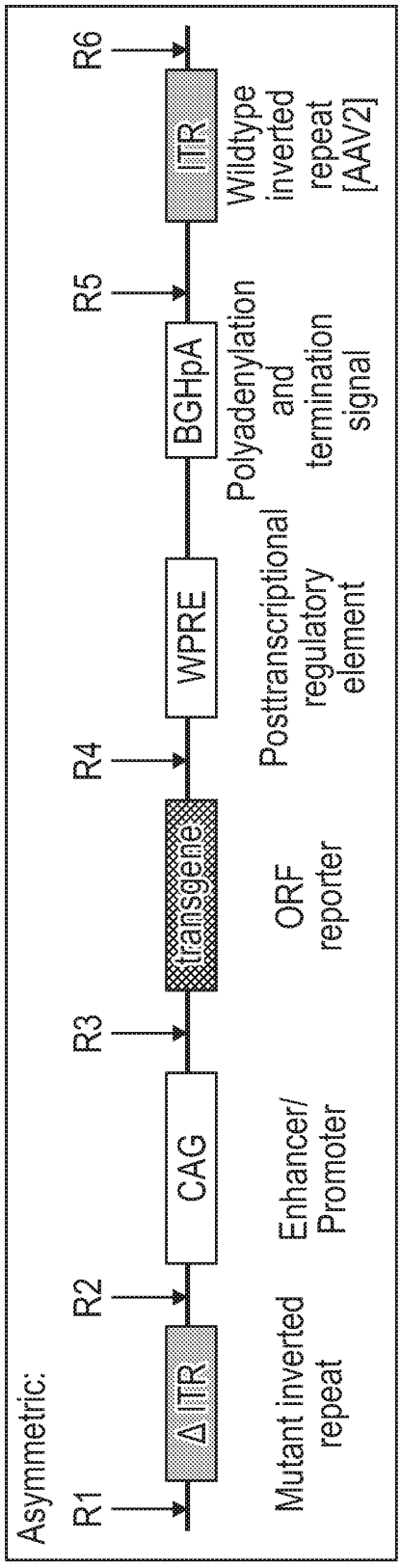

Asymmetric:
R1
Δ ITR
R2
CAG
R3
transgene
R4
WPRE
R5
BGHpA
R6
ITR
Mutant inverted repeat
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
Wildtype inverted repeat [AAV2]

FIG. 1C

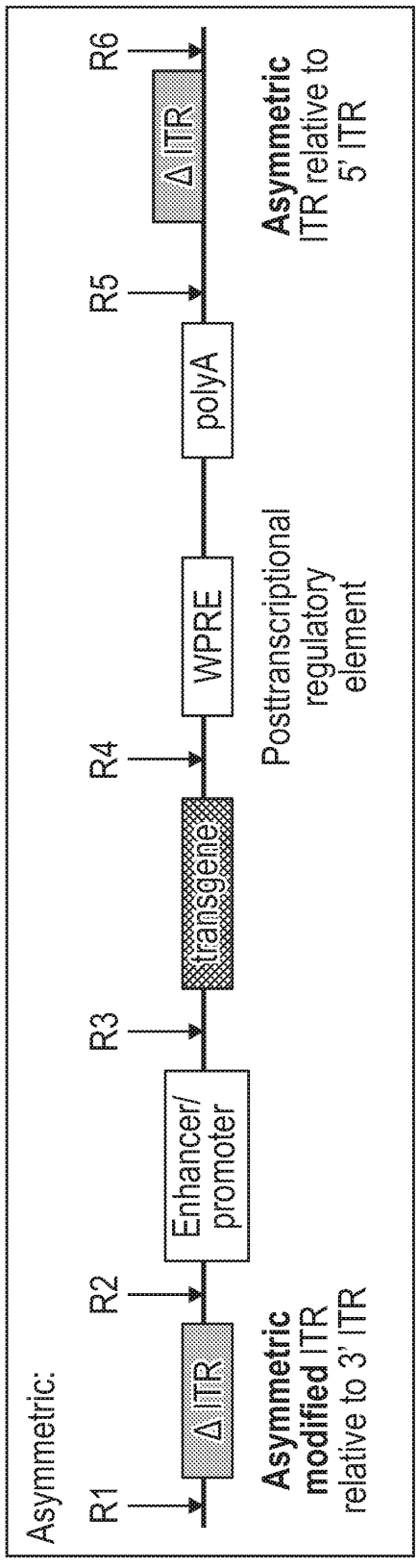

Asymmetric:
R1
R2
R3
R4
R5
R6
ΔITR
Enhancer/ promoter
transgene
WPRE
polyA
ΔITR
Asymmetric modified ITR relative to 3' ITR
Posttranscriptional regulatory element
Asymmetric ITR relative to 5' ITR

FIG. 1D

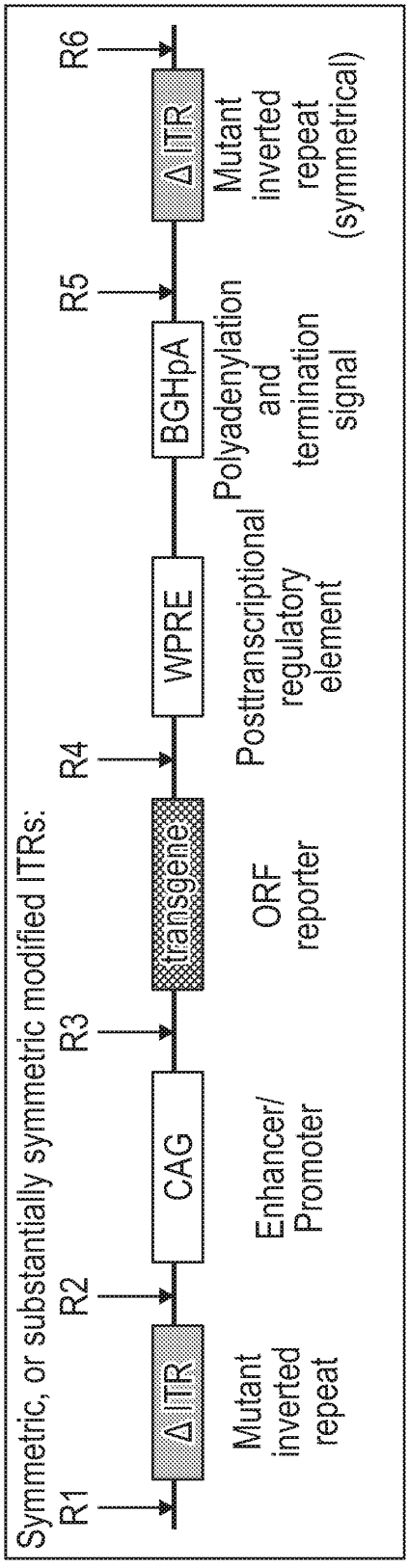

Symmetric, or substantially symmetric modified ITRs:
R1
R2
R3
R4
R5
R6
ΔITR
CAG
transgene
WPRE
BGHpA
ΔITR
Mutant inverted repeat
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
Mutant inverted repeat (symmetrical)

FIG. 1E

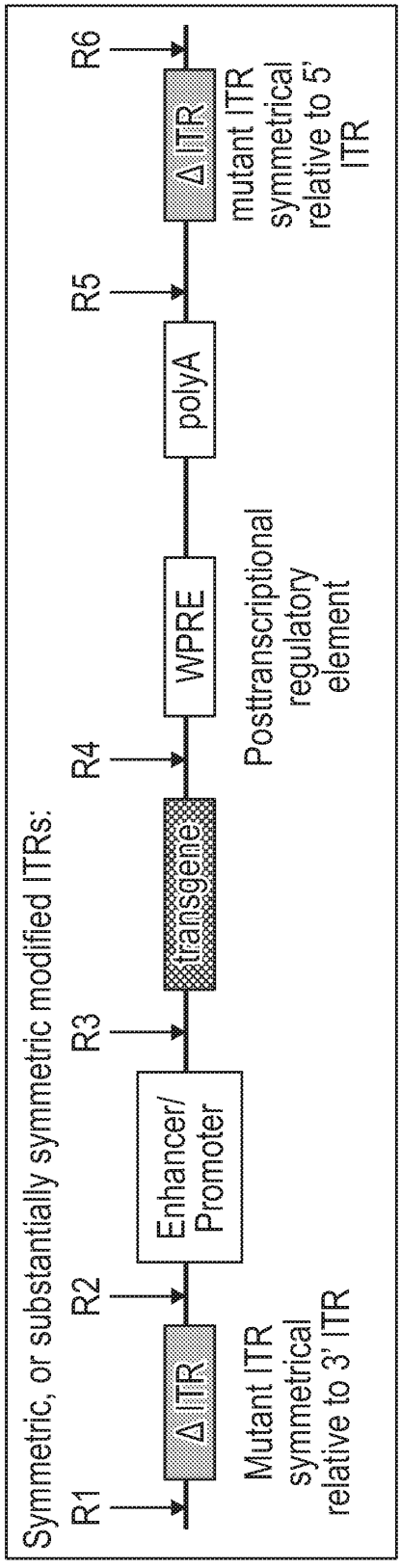

Symmetric, or substantially symmetric modified ITRs:
R1
R2
R3
R4
R5
R6
ΔITR
Enhancer/ Promoter
transgene
WPRE
polyA
ΔITR
Mutant ITR symmetrical relative to 3' ITR
Posttranscriptional regulatory element
mutant ITR symmetrical relative to 5' ITR

FIG. 1F

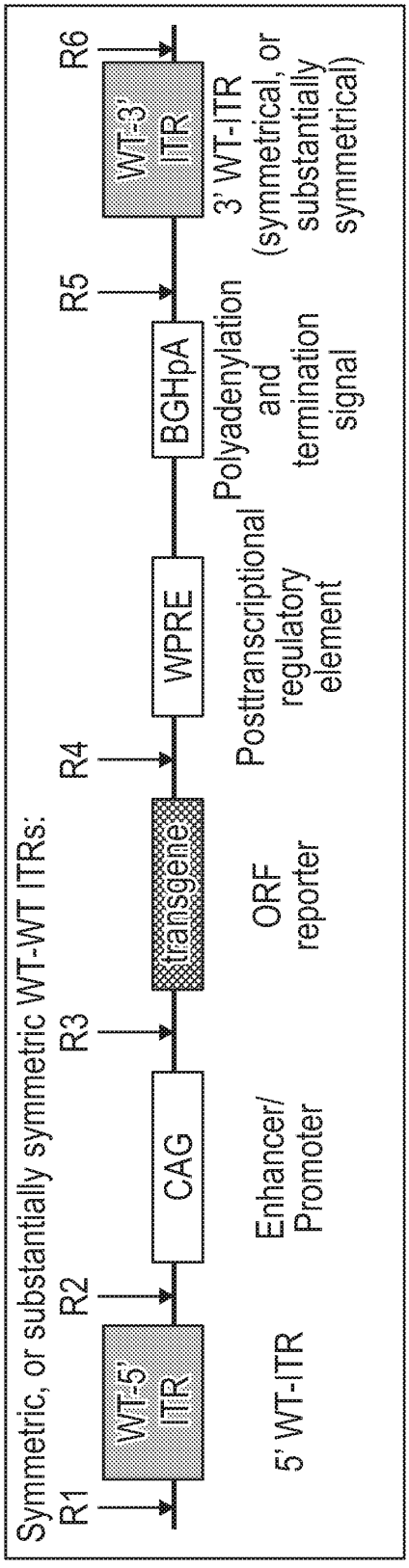
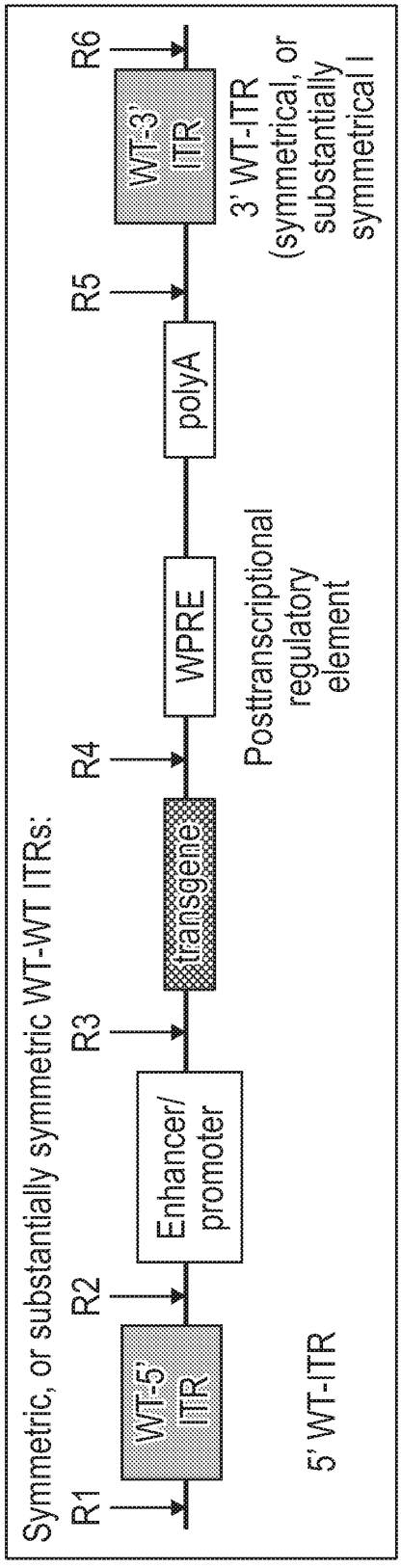

Symmetric, or substantially symmetric WT-WT ITRs:
R1
WT-5' ITR
R2
CAG
R3
transgene
R4
WPRE
R5
BGHpA
R6
WT-3' ITR
5' WT-ITR
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
3' WT-ITR (symmetrical, or substantially symmetrical)

FIG. 1G


Symmetric, or substantially symmetric WT-WT ITRs:
R1
WT-5' ITR
R2
Enhancer/ promoter
R3
transgene
R4
WPRE
R5
polyA
R6
WT-3' ITR
5' WT-ITR
Posttranscriptional regulatory element
3' WT-ITR (symmetrical, or substantially symmetrical I

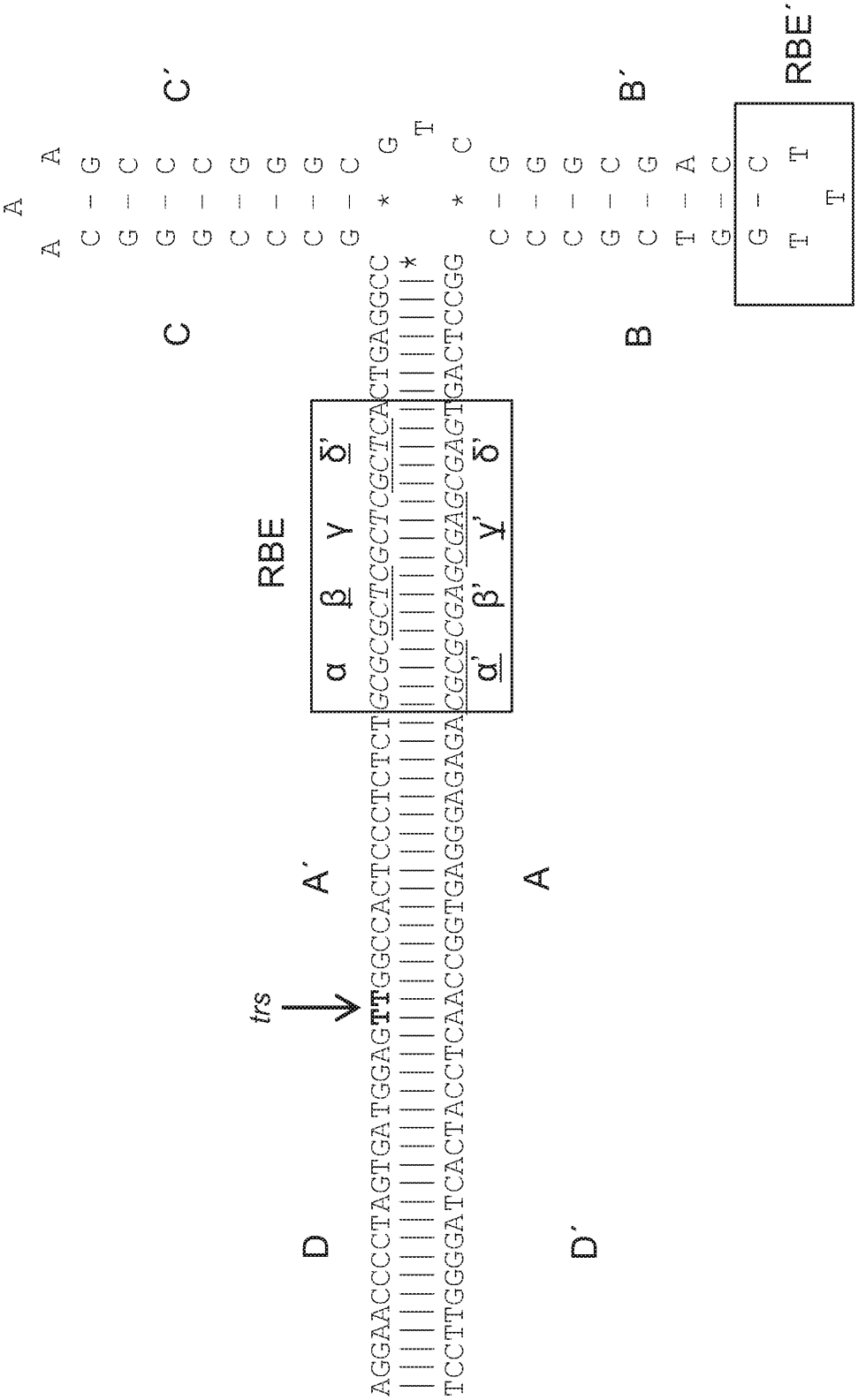


FIG. 2A

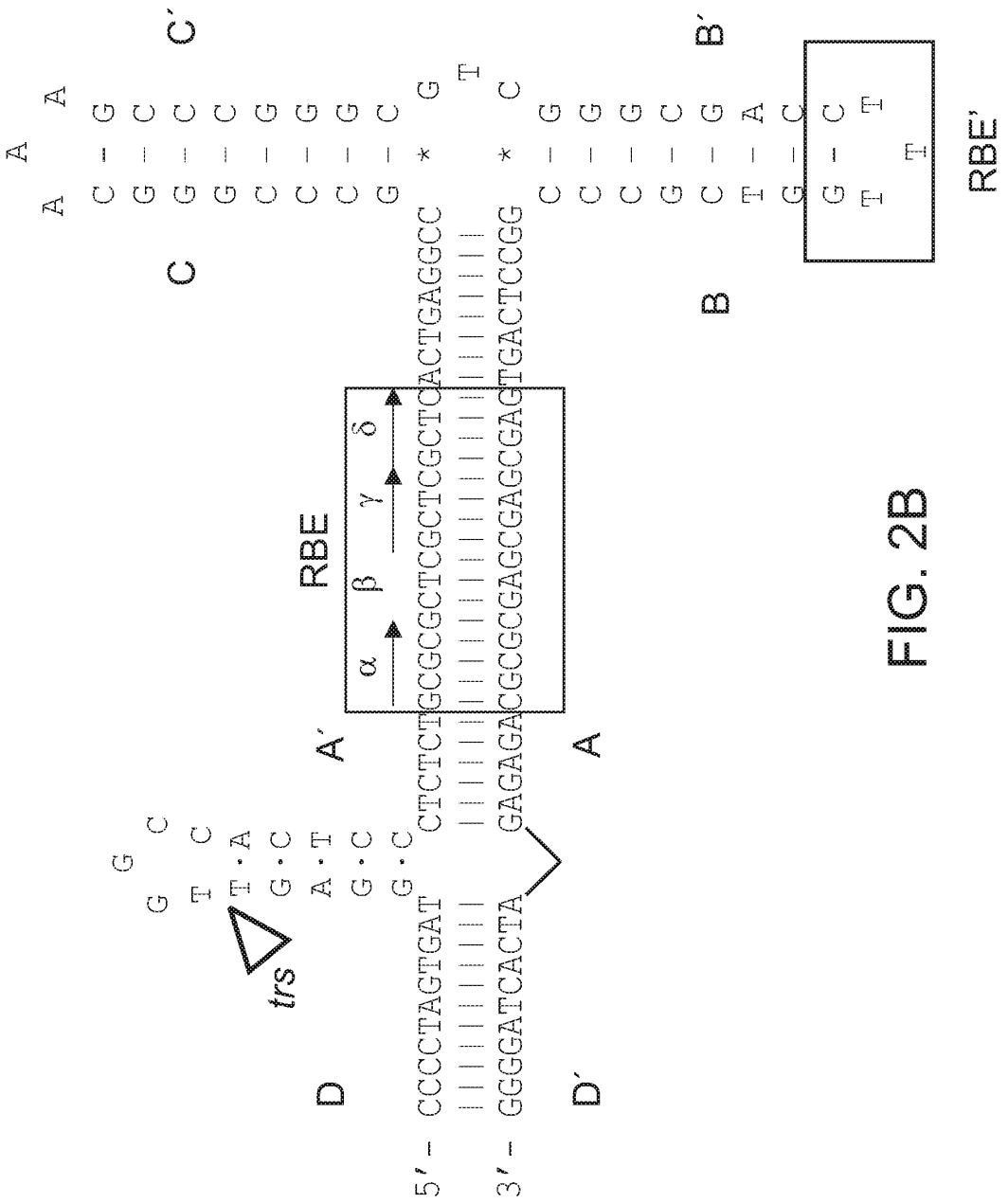
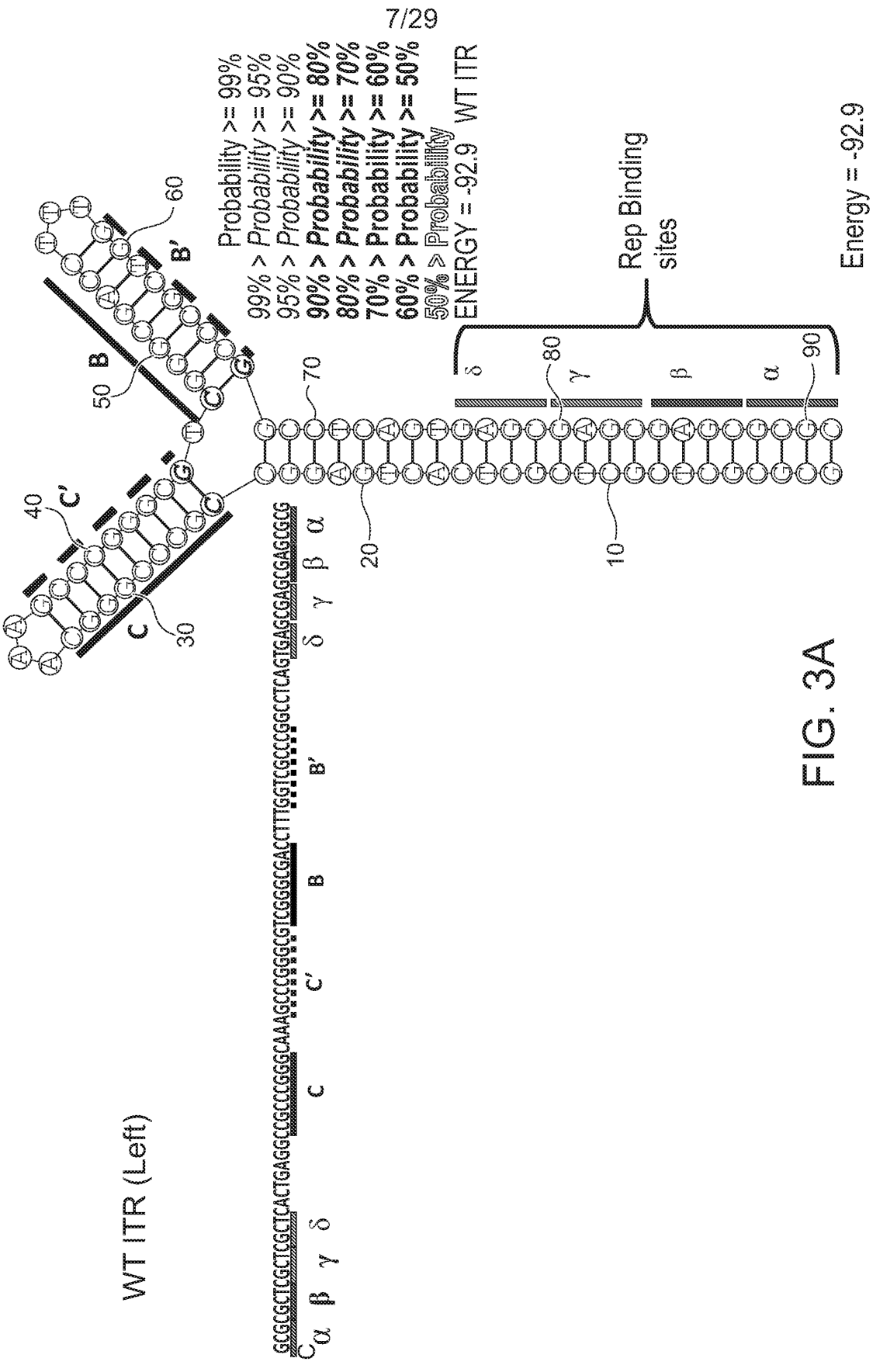


FIG. 2B


WT ITR (Left)
GCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCG
Cα β γ δ C C' B B' δ γ β α
Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability
ENERGY = -92.9 WT ITR
Rep Binding sites
Energy = -92.9
FIG. 3A

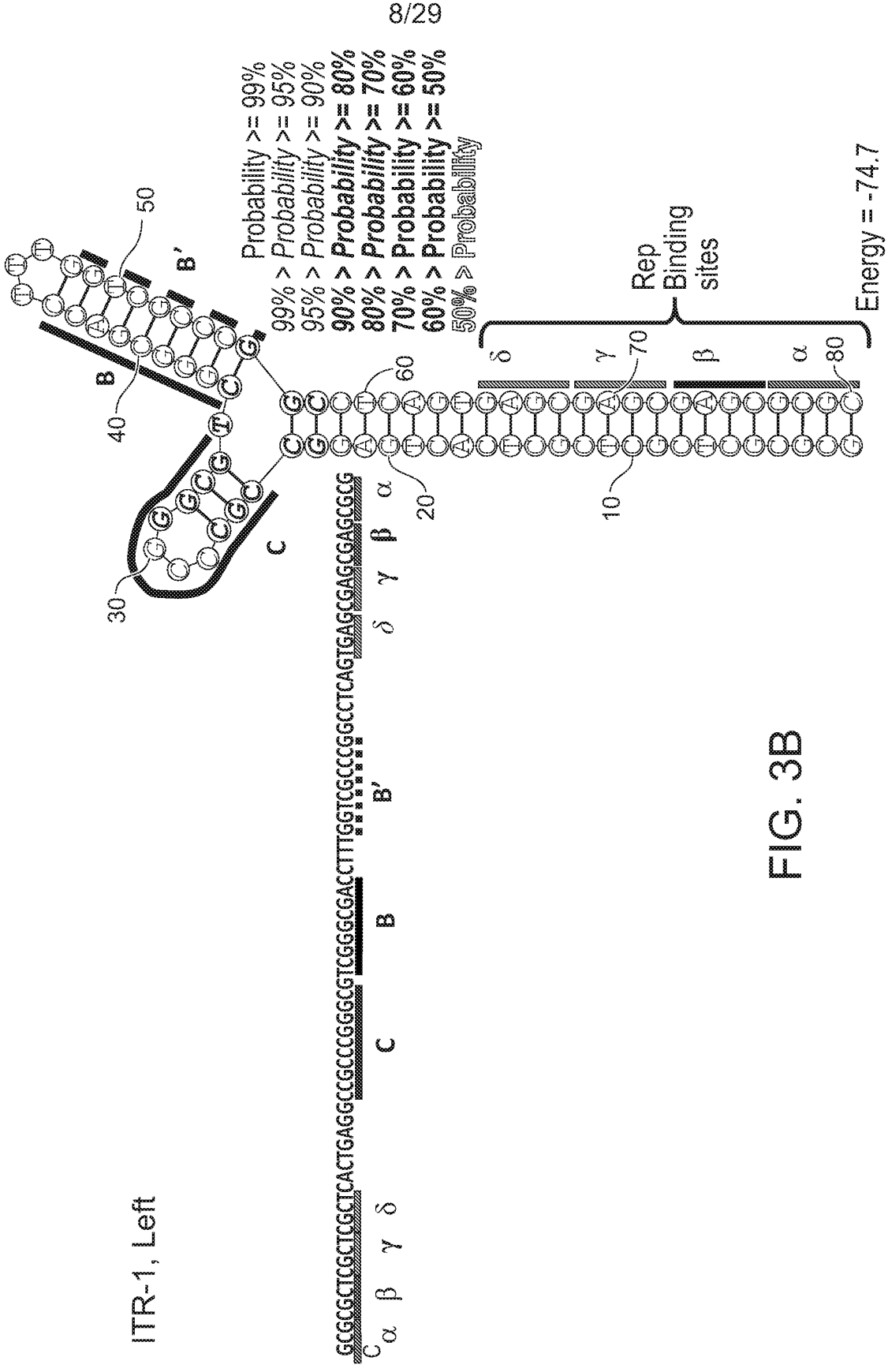

ITR-1, Left
Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability
Rep Binding sites
Energy = -74.7
α β γ δ
C
B
B'
δ γ β α
10
20
30
40
50
60
70
80

FIG. 3B

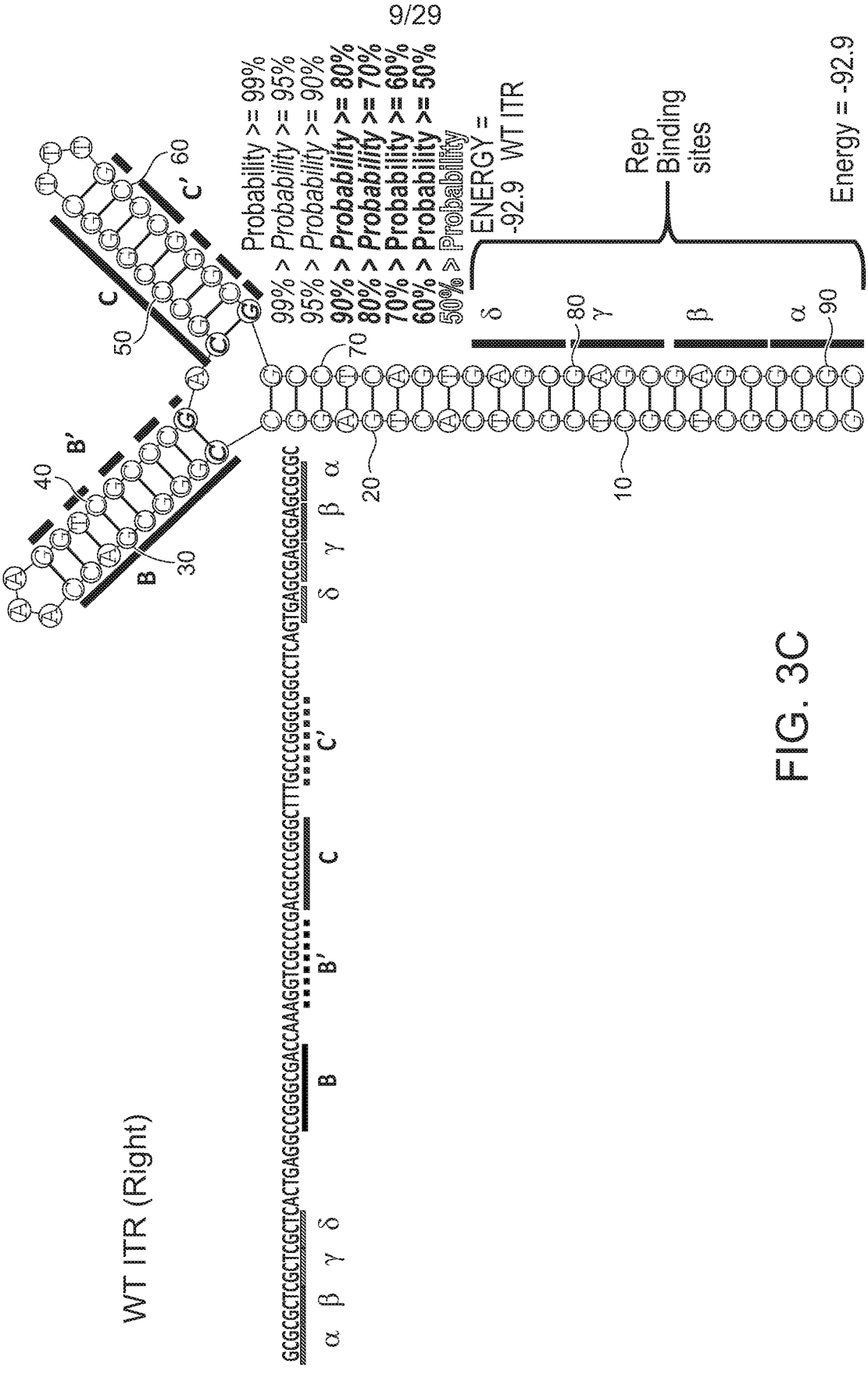

WT ITR (Right)
GCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGC
α β γ δ B B' C C'
δ γ β α
Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability
ENERGY = -92.9 WT ITR
Rep Binding sites
Energy = -92.9
δ γ β α
10 20 30 40 50 60 70 80 90
B B' C C'

FIG. 3C

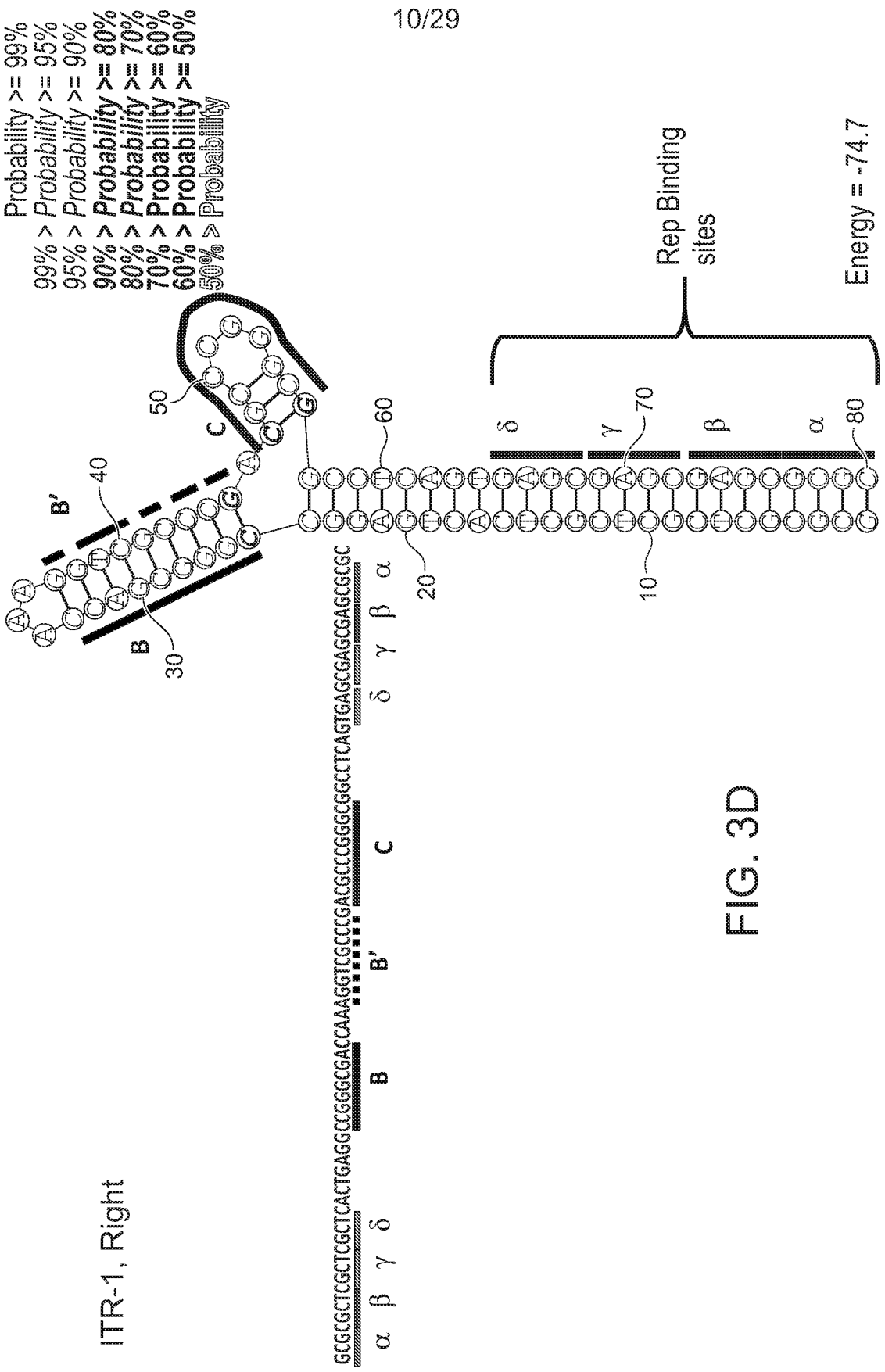

Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability
Rep Binding sites
Energy = -74.7
ITR-1, Right
GCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGC
α β γ δ
B
B'
C
δ γ β α
10
20
30
40
50
60
70
80

FIG. 3D

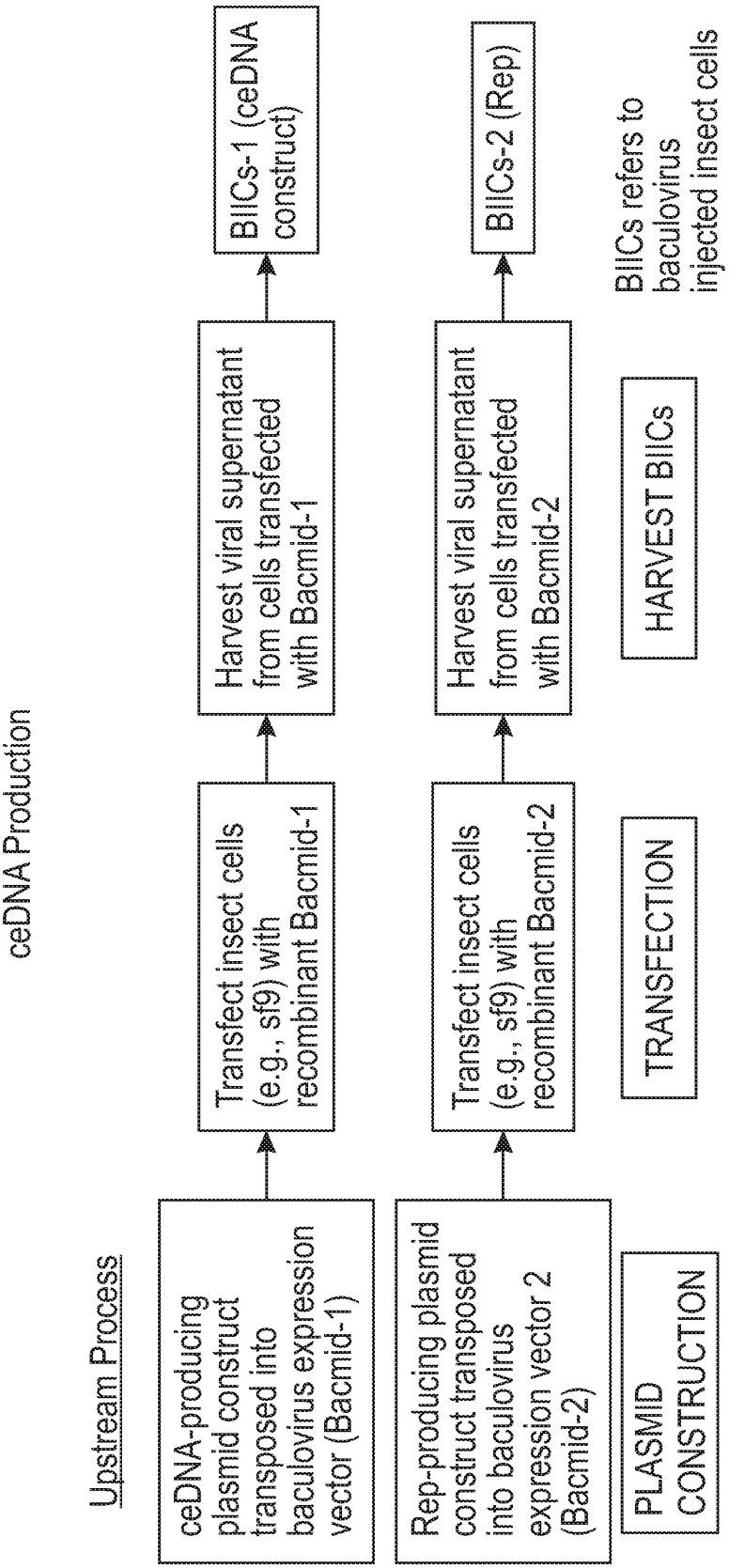

ceDNA Production
Upstream Process
ceDNA-producing plasmid construct transposed into baculovirus expression vector (Bacmid-1)
Rep-producing plasmid construct transposed into baculovirus expression vector 2 (Bacmid-2)
PLASMID CONSTRUCTION
Transfect insect cells (e.g., sf9) with recombinant Bacmid-1
Transfect insect cells (e.g., sf9) with recombinant Bacmid-2
TRANSFECTION
Harvest viral supernatant from cells transfected with Bacmid-1
Harvest viral supernatant from cells transfected with Bacmid-2
HARVEST BIICs
BIICs-1 (ceDNA construct)
BIICs-2 (Rep)
BIICs refers to baculovirus injected insect cells

FIG. 4A

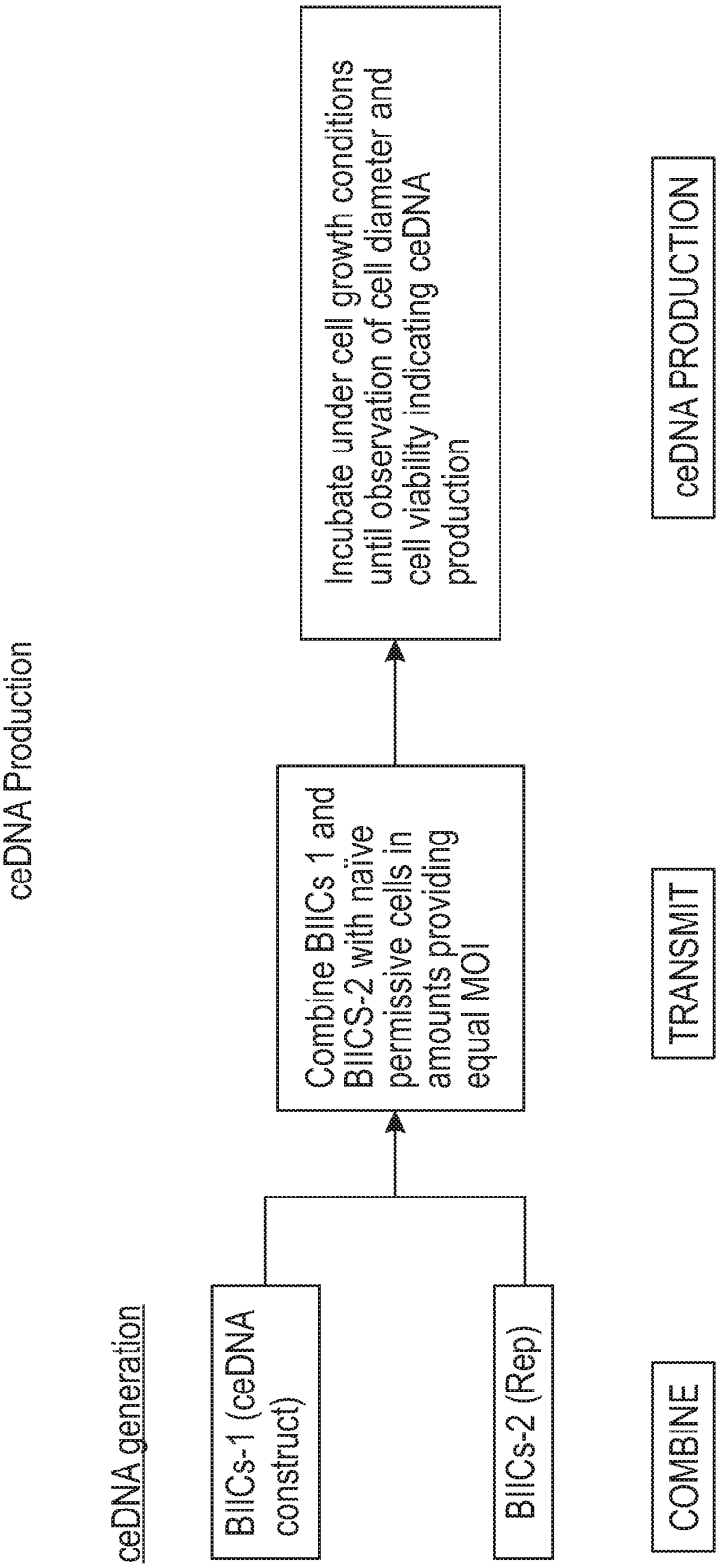

ceDNA Production
ceDNA generation
BIICs-1 (ceDNA construct)
BIICs-2 (Rep)
COMBINE
Combine BIICs 1 and BIICS-2 with naïve permissive cells in amounts providing equal MOI
TRANSMIT
Incubate under cell growth conditions until observation of cell diameter and cell viability indicating ceDNA production
ceDNA PRODUCTION

FIG. 4B

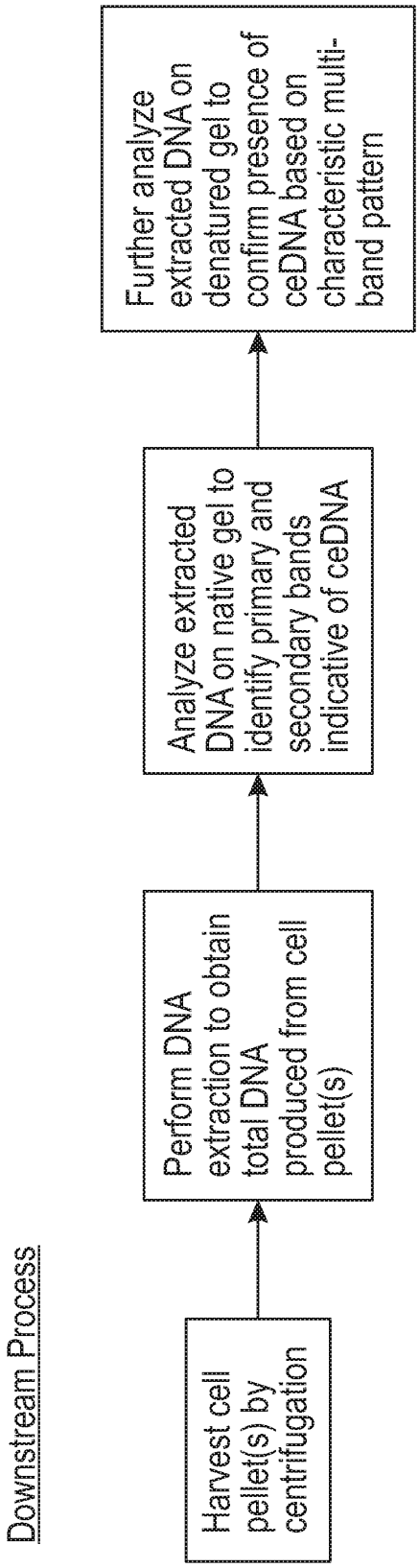

ceDNA Production
Downstream Process
Harvest cell pellet(s) by centrifugation
Perform DNA extraction to obtain total DNA produced from cell pellet(s)
Analyze extracted DNA on native gel to identify primary and secondary bands indicative of ceDNA
Further analyze extracted DNA on denatured gel to confirm presence of ceDNA based on characteristic multi-band pattern
HARVEST
EXTRACT
Confirm ceDNA Production

FIG. 4C

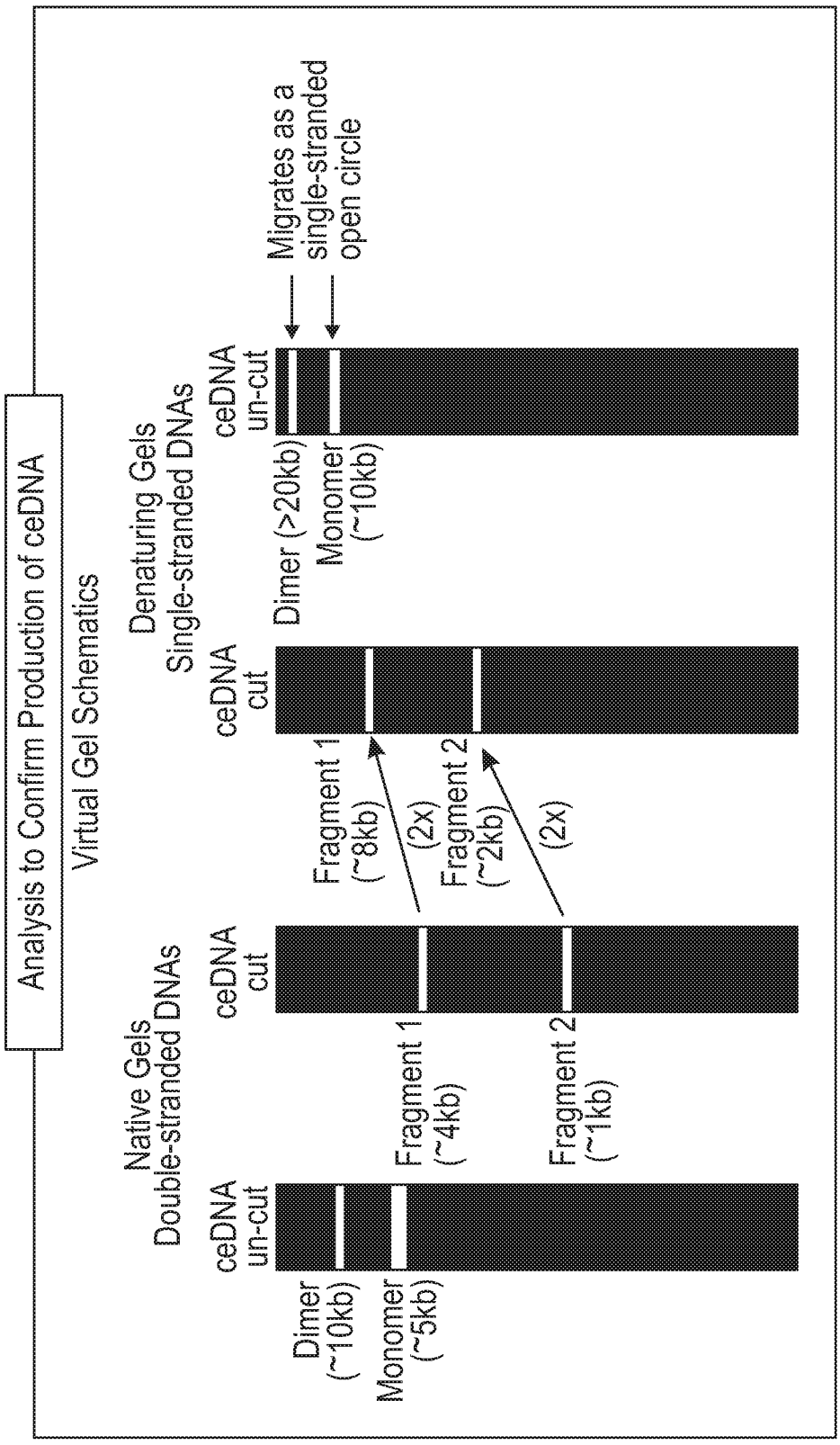

Analysis to Confirm Production of ceDNA
Virtual Gel Schematics
Native Gels
Double-stranded DNAs
ceDNA un-cut
Dimer (~10kb)
Monomer (~5kb)
ceDNA cut
Fragment 1 (~4kb)
Fragment 2 (~1kb)
Denaturing Gels
Single-stranded DNAs
ceDNA cut
Fragment 1 (~8kb)
(2x)
Fragment 2 (~2kb)
(2x)
ceDNA un-cut
Dimer (>20kb)
Monomer (~10kb)
Migrates as a single-stranded open circle

FIG. 4D

FIG. 4E

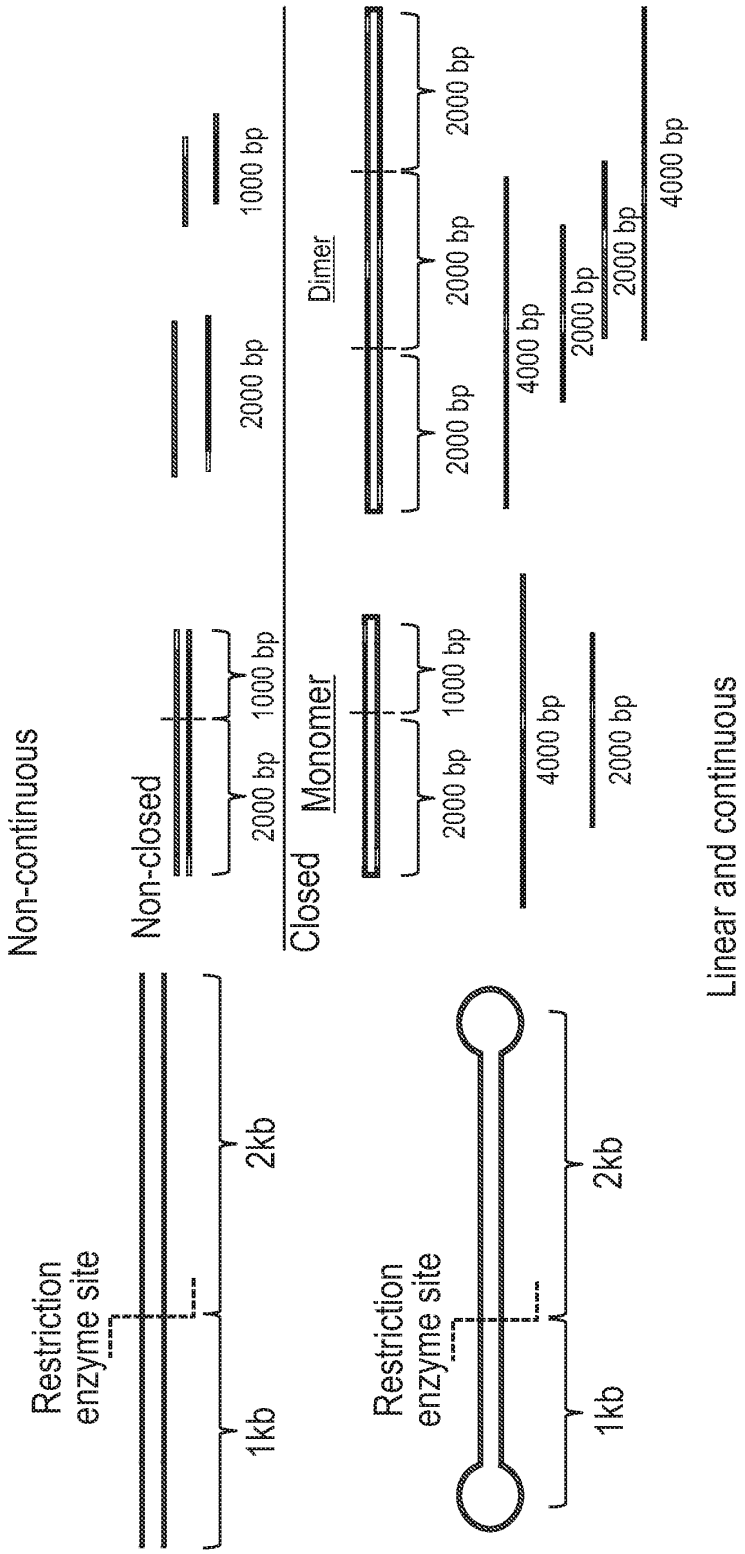

Non-continuous
Non-closed
Closed
Monomer
Dimer
Linear and continuous
Restriction enzyme site
1kb
2kb
2000 bp
1000 bp
4000 bp

FIG. 5

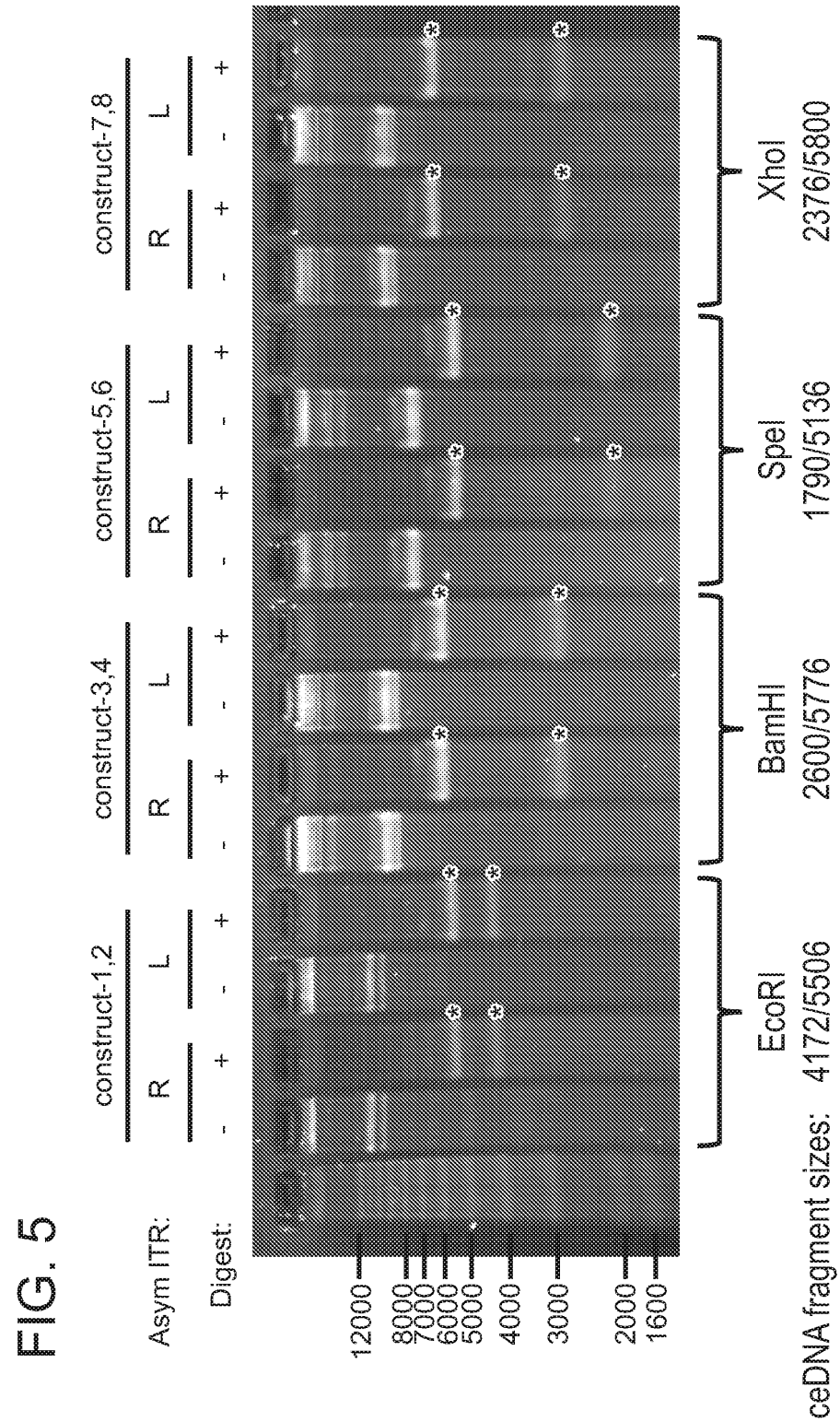

construct-1,2
construct-3,4
construct-5,6
construct-7,8
Asym ITR:
R L R L R L R L
Digest:
- + - + - + - + - + - + - + - +
12000
8000
7000
6000
5000
4000
3000
2000
1600
EcoRI
BamHI
SpeI
XhoI
ceDNA fragment sizes: 4172/5506 2600/5776 1790/5136 2376/5800

FIG. 6

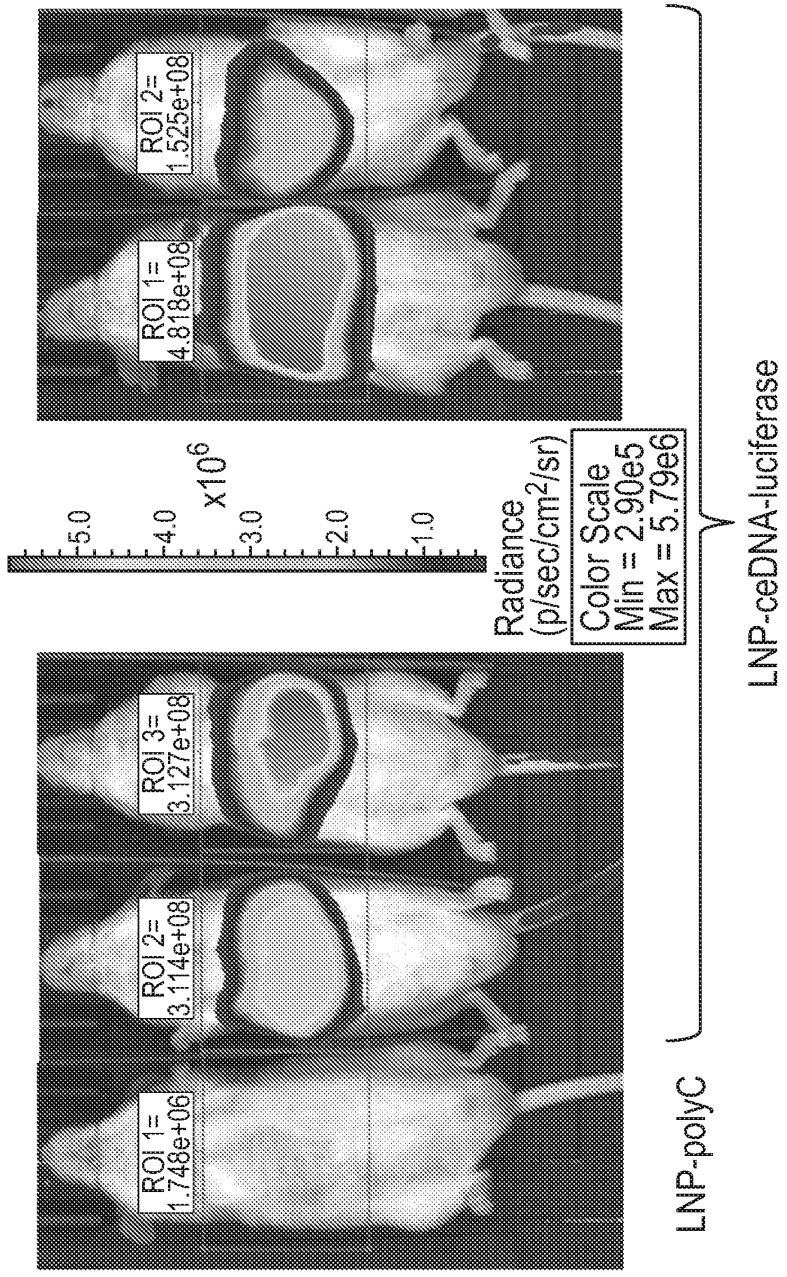
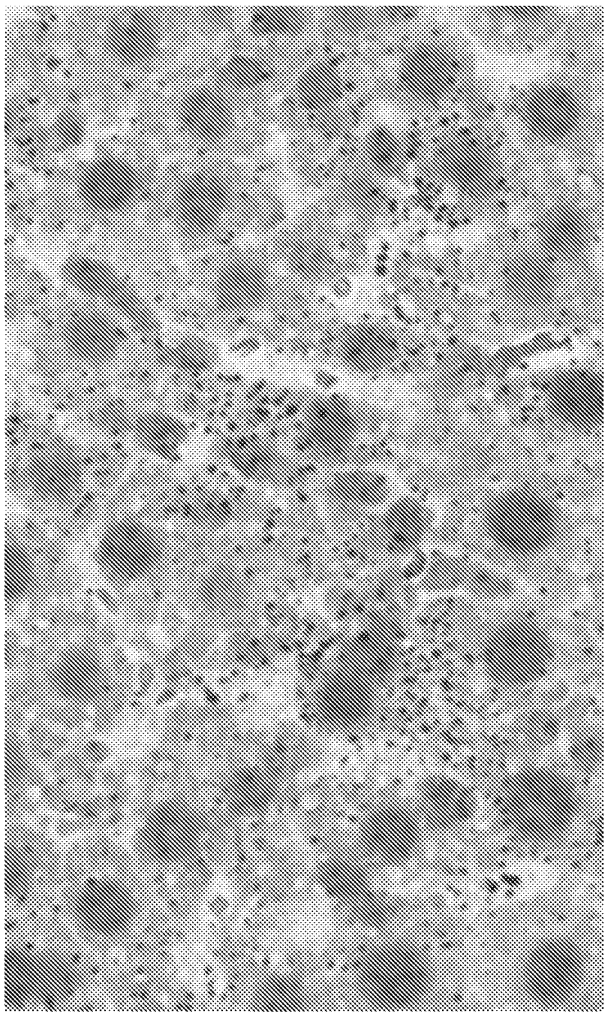

ROI 1= 1.748e+06
ROI 2= 3.114e+08
ROI 3= 3.127e+08
ROI 1= 4.818e+08
ROI 2= 1.525e+08
5.0
4.0
x10^6
3.0
2.0
1.0
Radiance (p/sec/cm²/sr)
Color Scale Min = 2.90e5 Max = 5.79e6
LNP-polyC
LNP-ceDNA-luciferase

FIG. 7



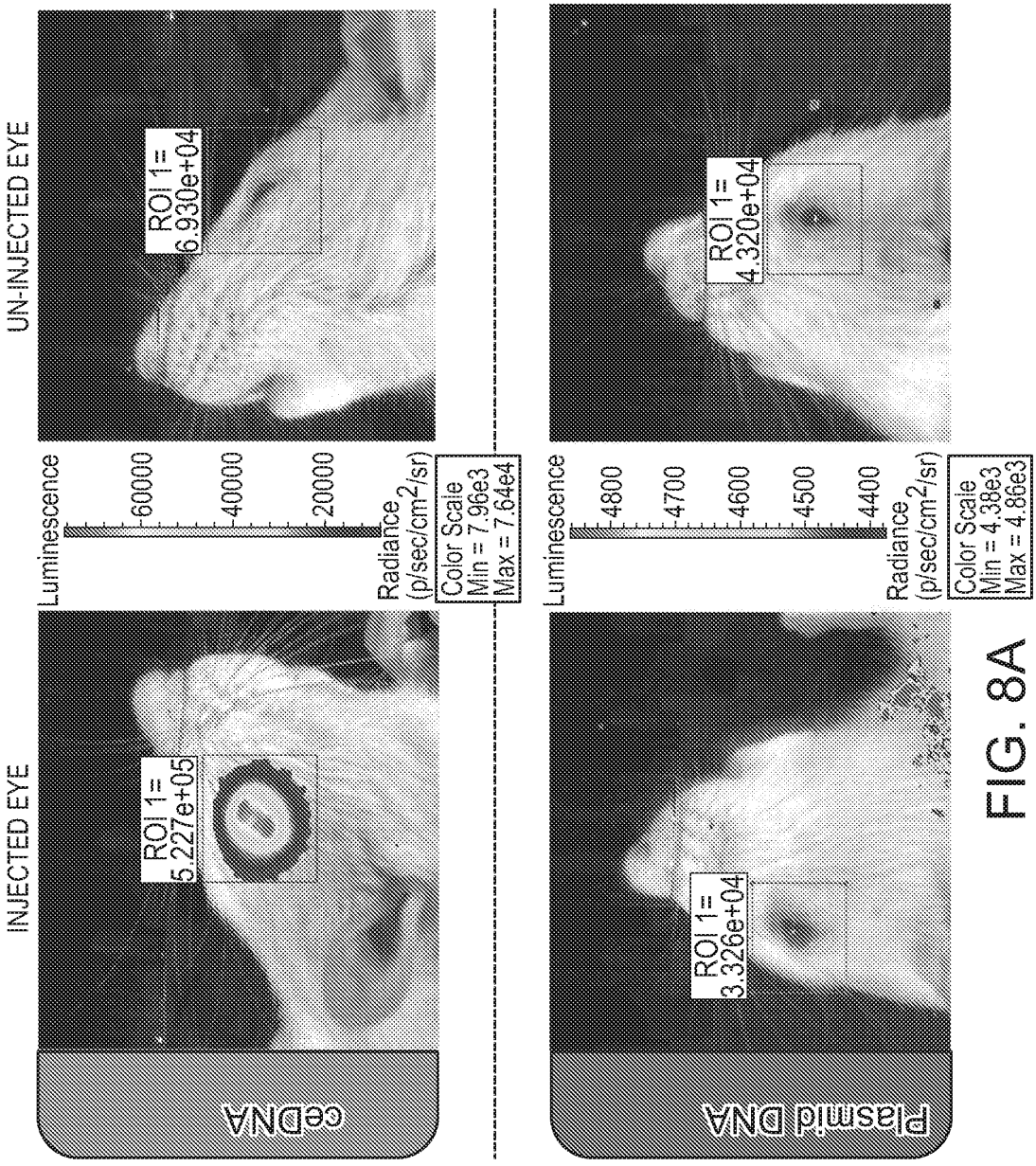

UN-INJECTED EYE
INJECTED EYE
ROI 1= 6.930e+04
ROI 1= 5.227e+05
ROI 1= 4.320e+04
ROI 1= 3.326e+04
Luminescence
60000
40000
20000
Radiance (p/sec/cm2/sr)
Color Scale Min = 7.96e3 Max = 7.64e4
Luminescence
4800
4700
4600
4500
4400
Radiance (p/sec/cm2/sr)
Color Scale Min = 4.38e3 Max = 4.86e3
ceDNA
Plasmid DNA

FIG. 8A

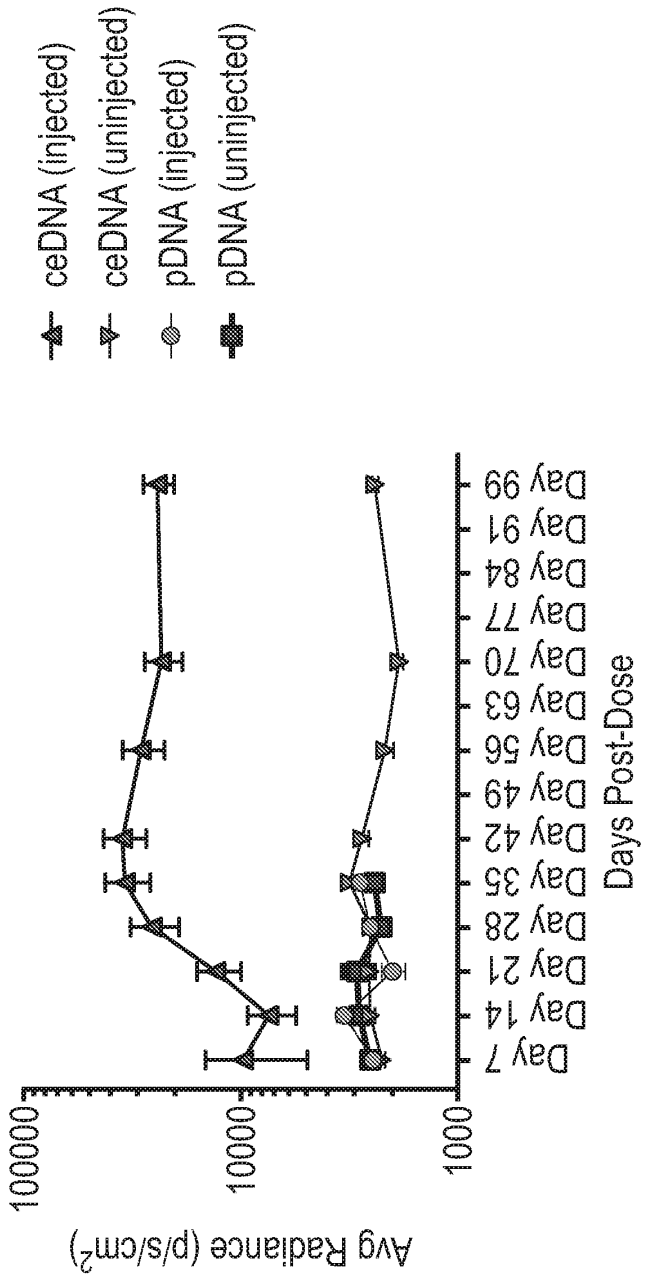

ceDNA (injected)
ceDNA (uninjected)
pDNA (injected)
pDNA (uninjected)
Avg Radiance (p/s/cm²)
100000
10000
1000
Day 7
Day 14
Day 21
Day 28
Day 35
Day 42
Day 49
Day 56
Day 63
Day 70
Day 77
Day 84
Day 91
Day 99
Days Post-Dose

FIG. 8B

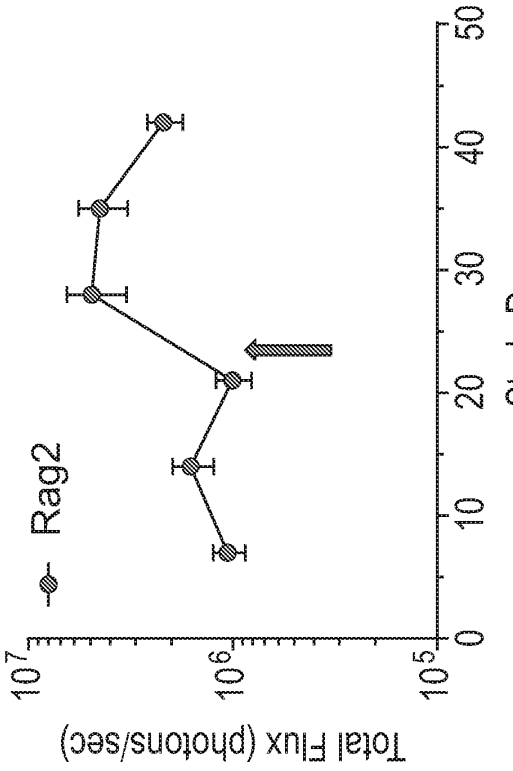

Rag2
Total Flux (photons/sec)
10^7
10^6
10^5
0
10
20
30
40
50
Study Day

FIG. 9B

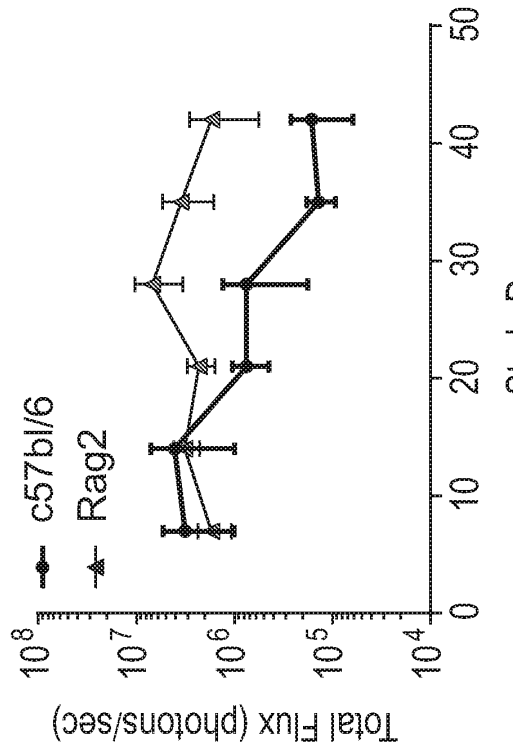

c57bl/6
Rag2
Total Flux (photons/sec)
10^8
10^7
10^6
10^5
10^4
0
10
20
30
40
50
Study Day

FIG. 9A

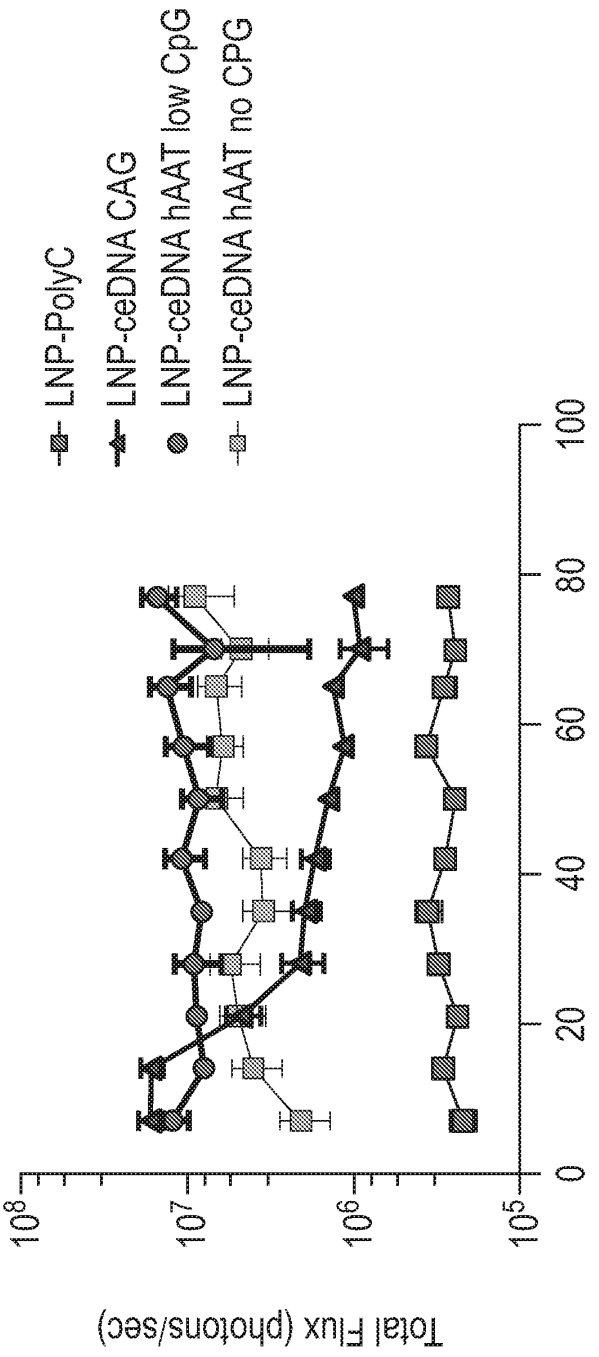

LNP-PolyC
LNP-ceDNA CAG
LNP-ceDNA hAAT low CpG
LNP-ceDNA hAAT no CPG
Total Flux (photons/sec)
10^8
10^7
10^6
10^5
0
20
40
60
80
100
Study Day

FIG. 10

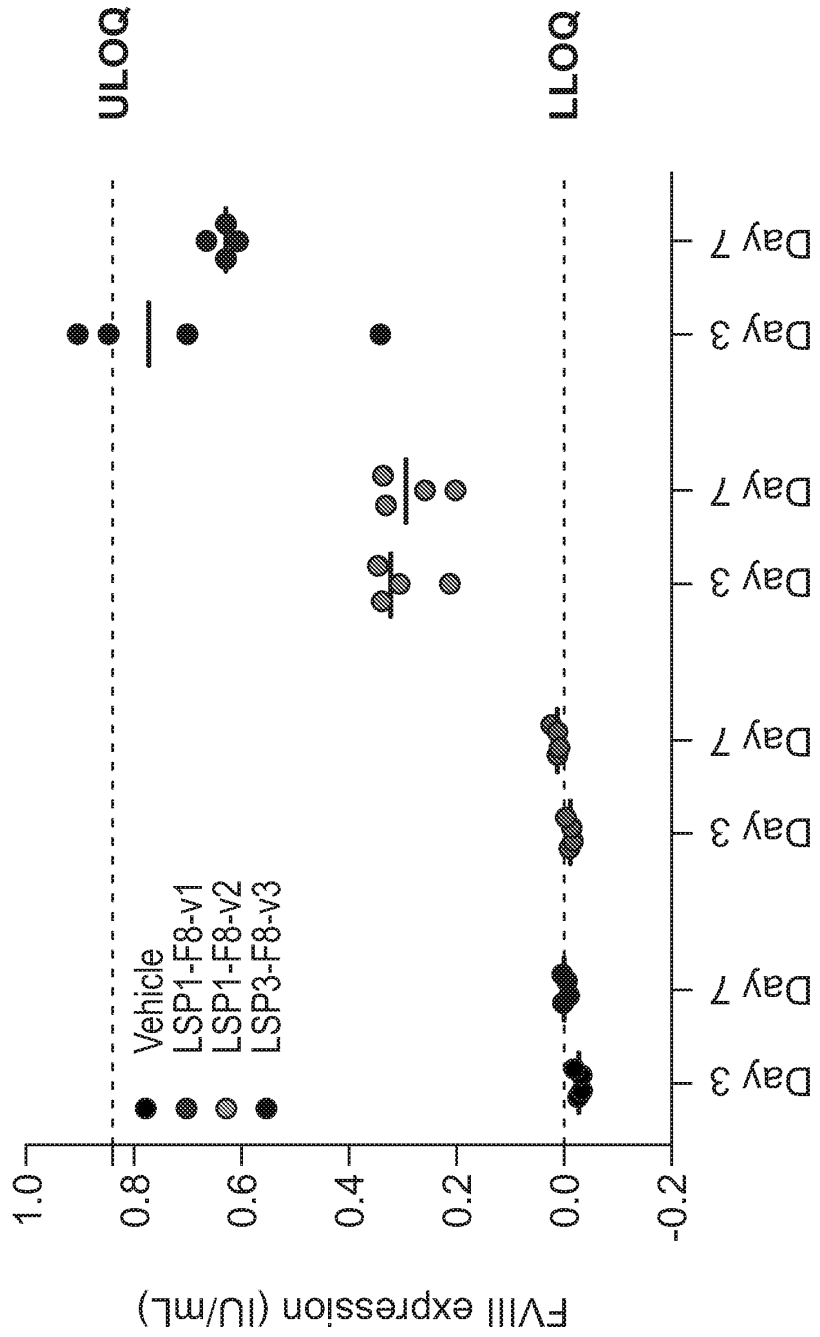

FVIII
ULOQ
LLOQ
Vehicle
LSP1-F8-v1
LSP1-F8-v2
LSP3-F8-v3
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
FVIII expression (IU/mL)
Day 3
Day 7
Day 3
Day 7
Day 3
Day 7
Day 3
Day 7

FIG. 11A

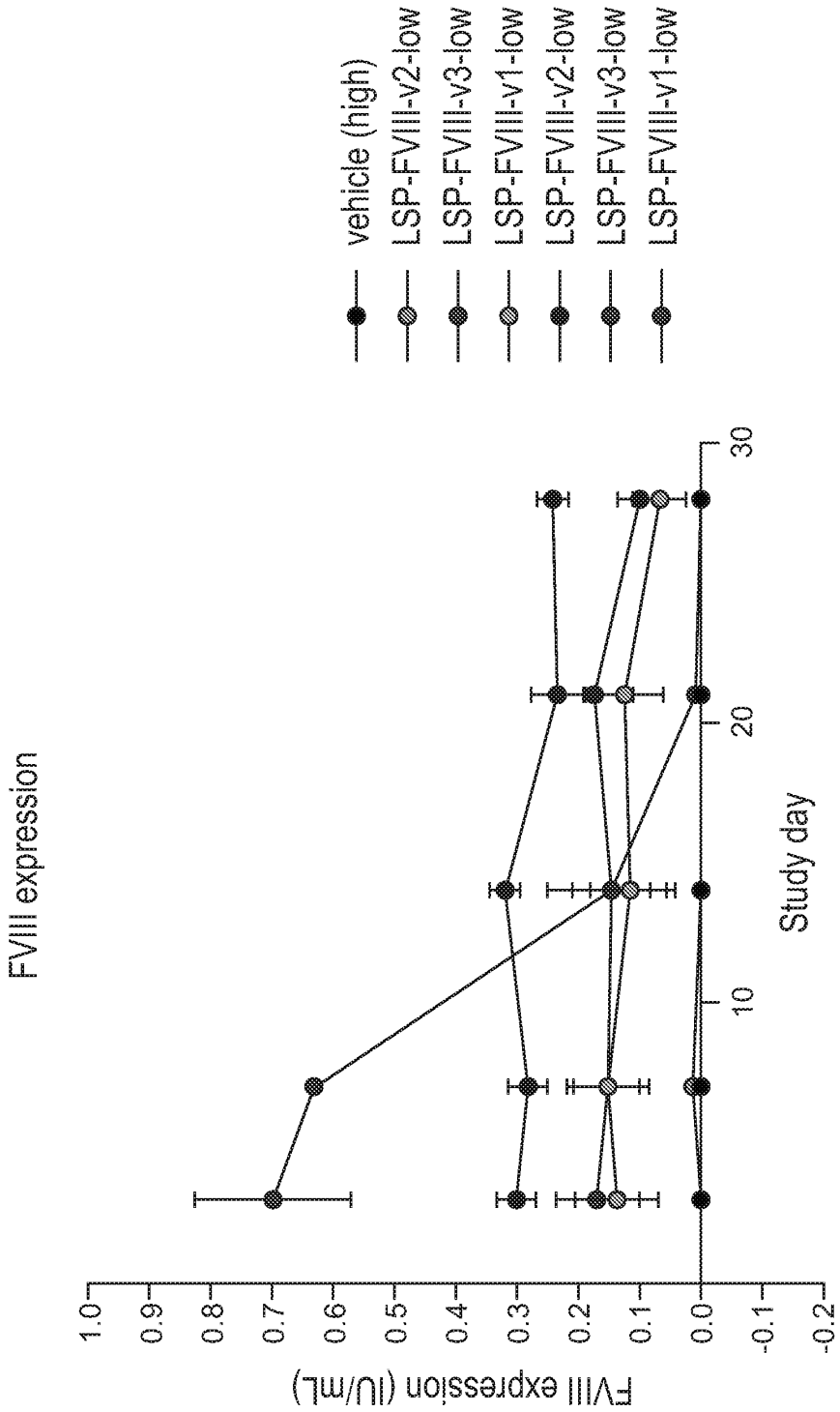

FVIII expression
FVIII expression (IU/mL)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
10
20
30
Study day
vehicle (high)
LSP-FVIII-v2-low
LSP-FVIII-v3-low
LSP-FVIII-v1-low
LSP-FVIII-v2-low
LSP-FVIII-v3-low
LSP-FVIII-v1-low

FIG. 11B

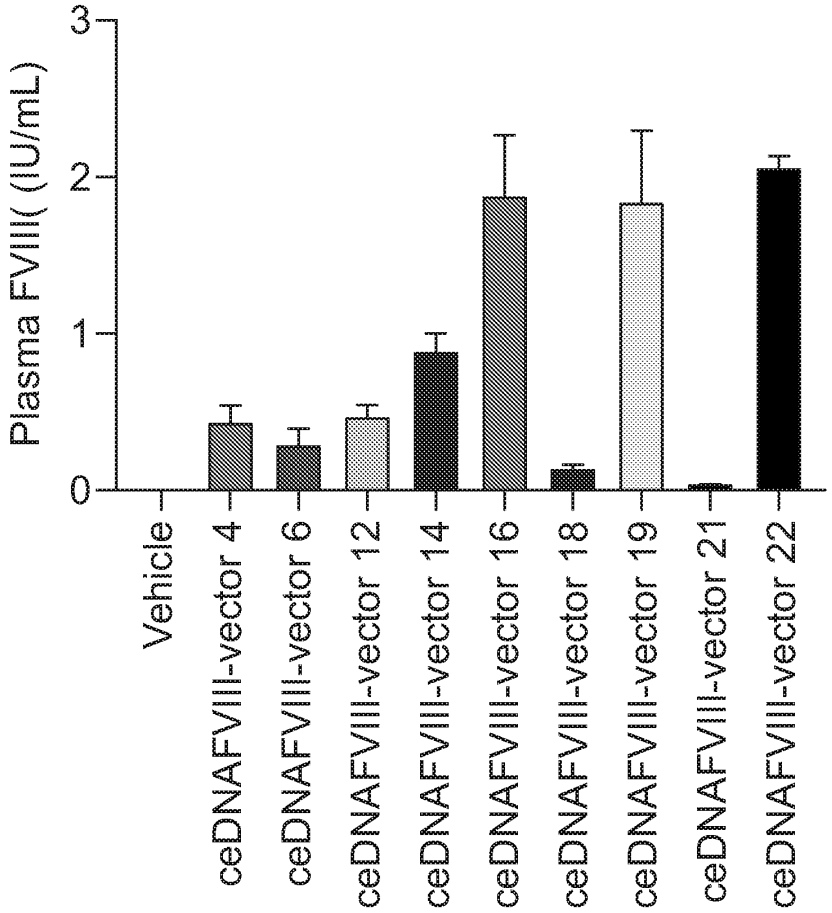

Plasma FVIII((IU/mL)
3
2
1
0
Vehicle
ceDNAFVIII-vector 4
ceDNAFVIII-vector 6
ceDNAFVIII-vector 12
ceDNAFVIII-vector 14
ceDNAFVIII-vector 16
ceDNAFVIII-vector 18
ceDNAFVIII-vector 19
ceDNAFVIII-vector 21
ceDNAFVIII-vector 22

FIG. 12

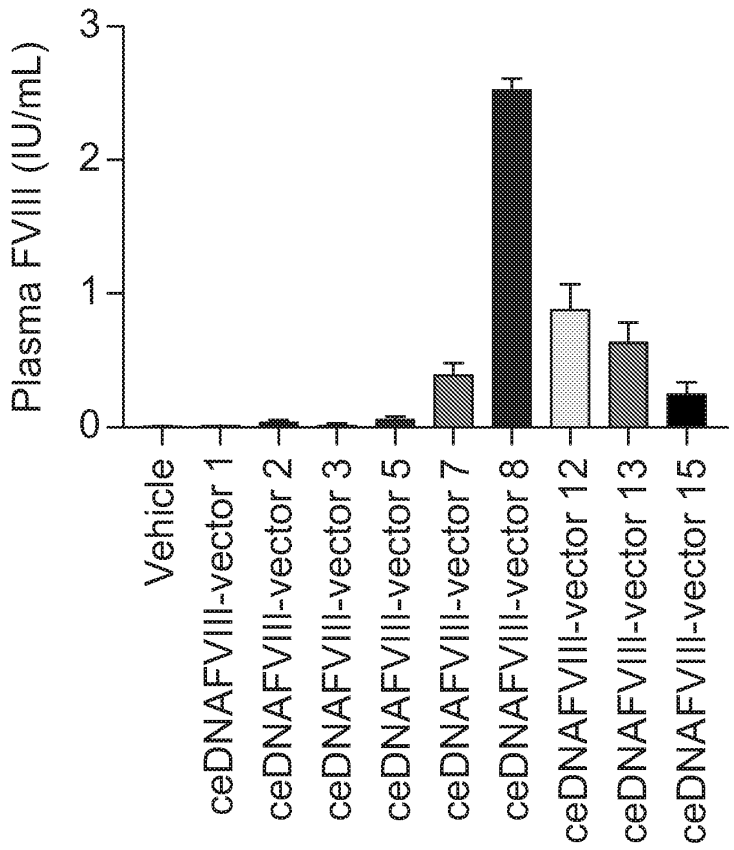

Plasma FVIII (IU/mL)
3
2
1
0
Vehicle
ceDNAFVIII-vector 1
ceDNAFVIII-vector 2
ceDNAFVIII-vector 3
ceDNAFVIII-vector 5
ceDNAFVIII-vector 7
ceDNAFVIII-vector 8
ceDNAFVIII-vector 12
ceDNAFVIII-vector 13
ceDNAFVIII-vector 15

FIG. 13

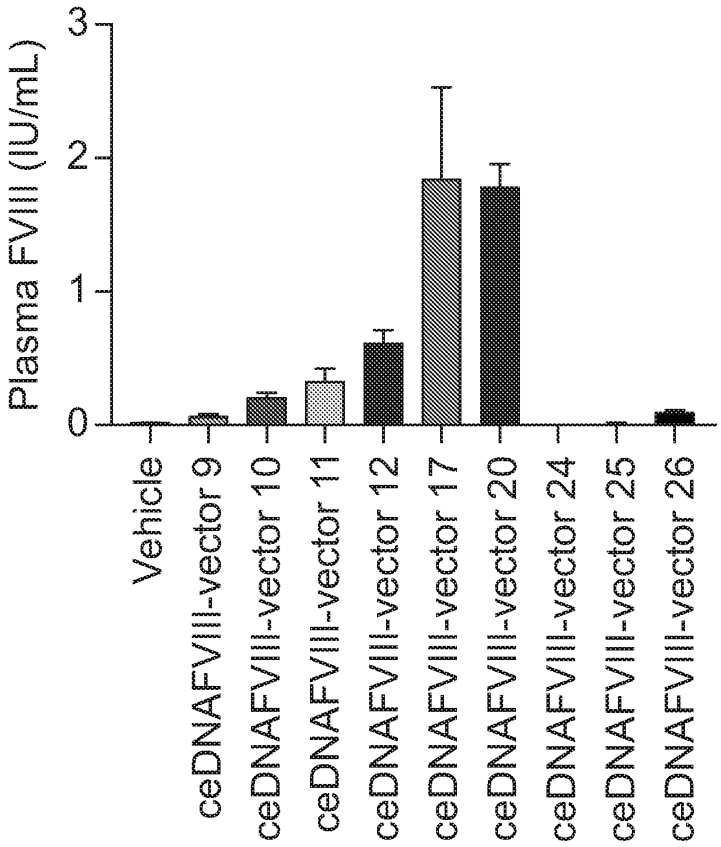

Plasma FVIII (IU/mL)
3
2
1
0
Vehicle
ceDNAFVIII-vector 9
ceDNAFVIII-vector 10
ceDNAFVIII-vector 11
ceDNAFVIII-vector 12
ceDNAFVIII-vector 17
ceDNAFVIII-vector 20
ceDNAFVIII-vector 24
ceDNAFVIII-vector 25
ceDNAFVIII-vector 26

FIG. 14

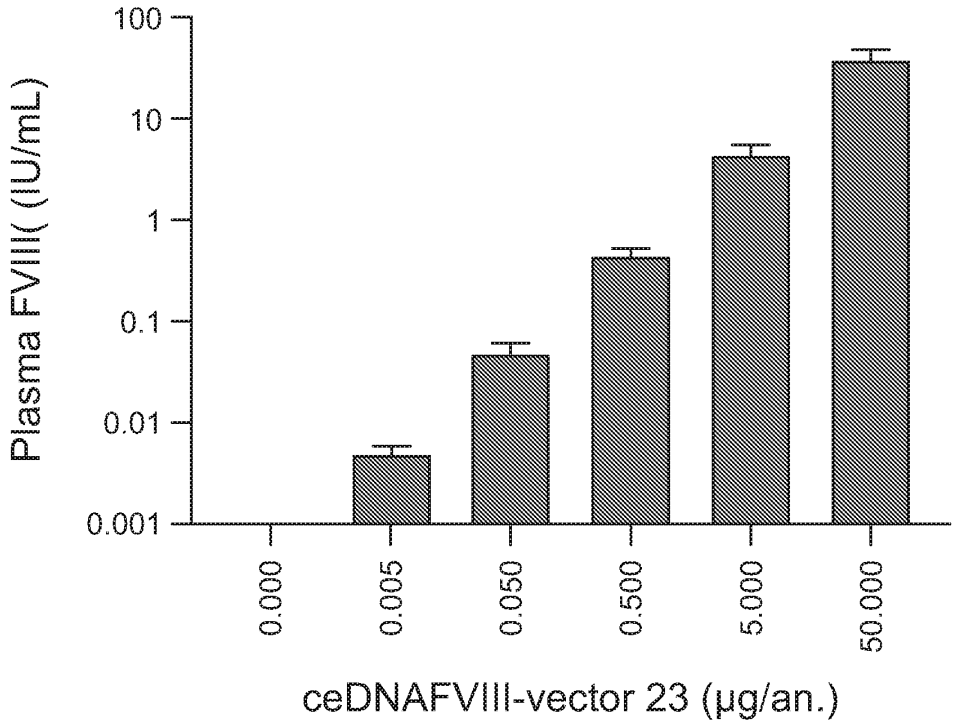

100
10
1
0.1
0.01
0.001
Plasma FVIII((IU/mL)
0.000
0.005
0.050
0.500
5.000
50.000
ceDNAFVIII-vector 23 (µg/an.)

FIG. 15

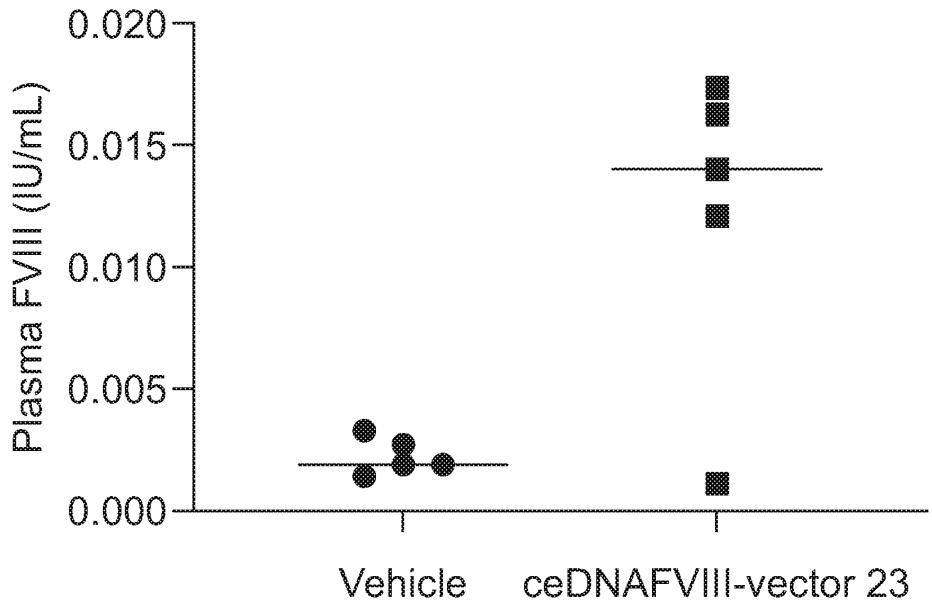

0.020
0.015
0.010
0.005
0.000
Plasma FVIII (IU/mL)
Vehicle
ceDNAFVIII-vector 23

FIG. 16