FIG. 1A

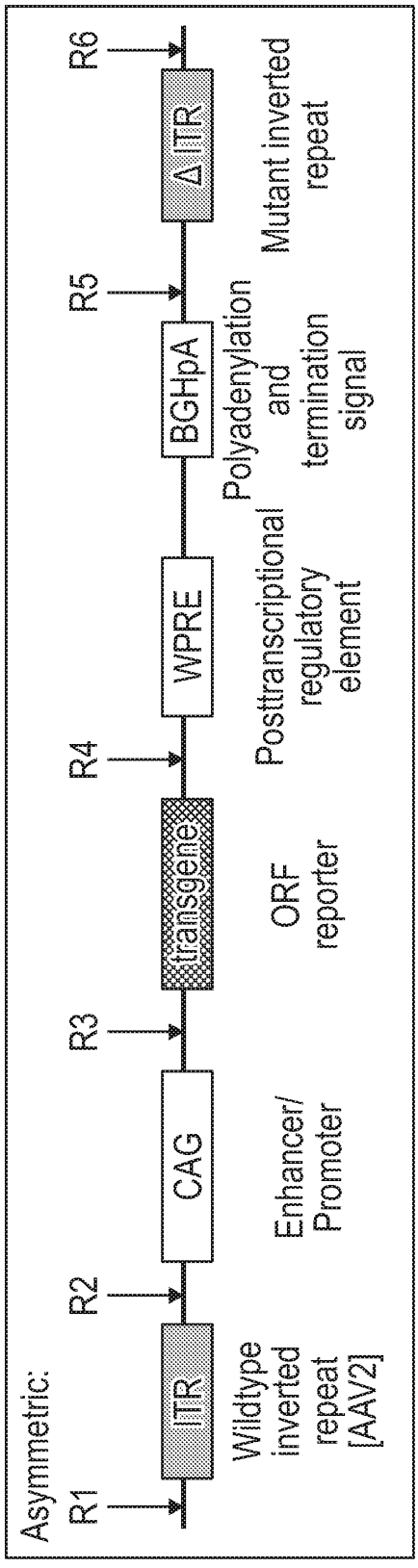

Asymmetric:
R1
R2
R3
R4
R5
R6
ITR
CAG
transgene
WPRE
BGHpA
Δ ITR
Wildtype inverted repeat [AAV2]
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
Mutant inverted repeat

FIG. 1B

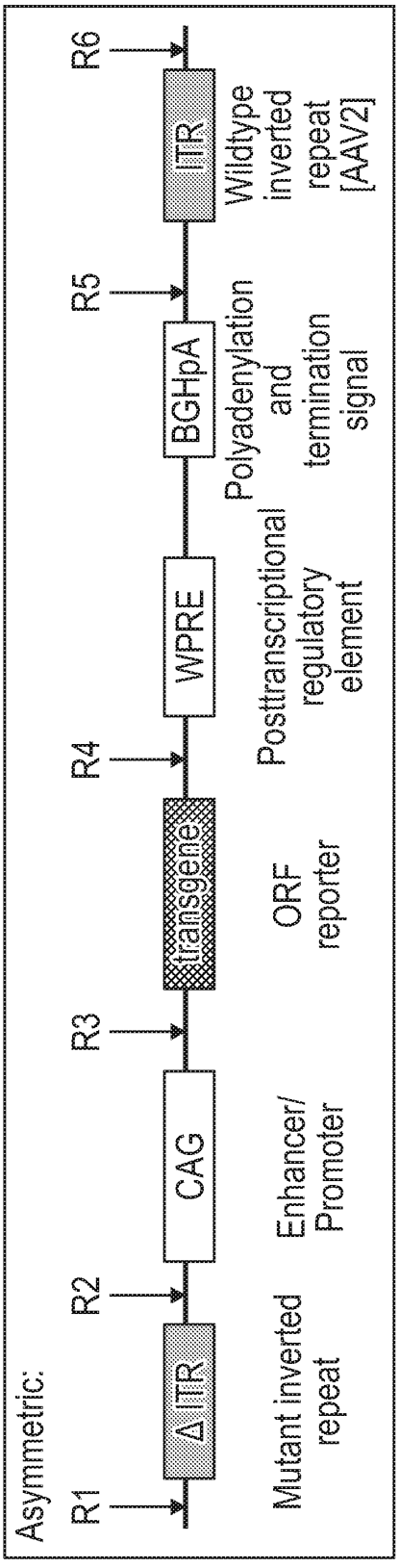

Asymmetric:
R1
R2
R3
R4
R5
R6
Δ ITR
CAG
transgene
WPRE
BGHpA
ITR
Mutant inverted repeat
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
Wildtype inverted repeat [AAV2]

FIG. 1C

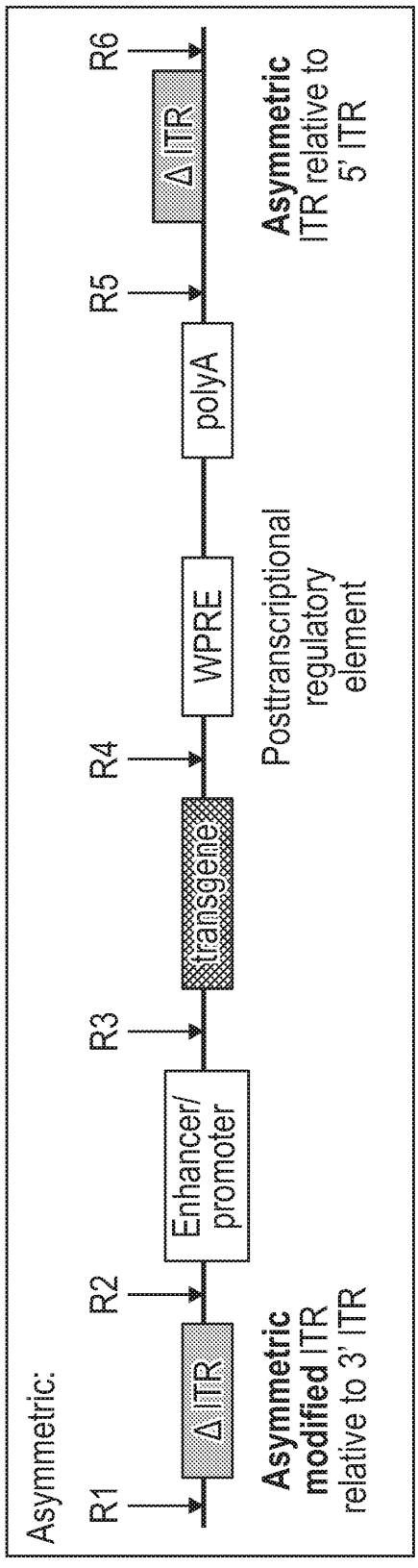

Asymmetric:
R1
ΔITR
R2
Enhancer/ promoter
R3
transgene
R4
WPRE
R5
polyA
R6
ΔITR
Asymmetric modified ITR relative to 3' ITR
Posttranscriptional regulatory element
Asymmetric ITR relative to 5' ITR

FIG. 1D

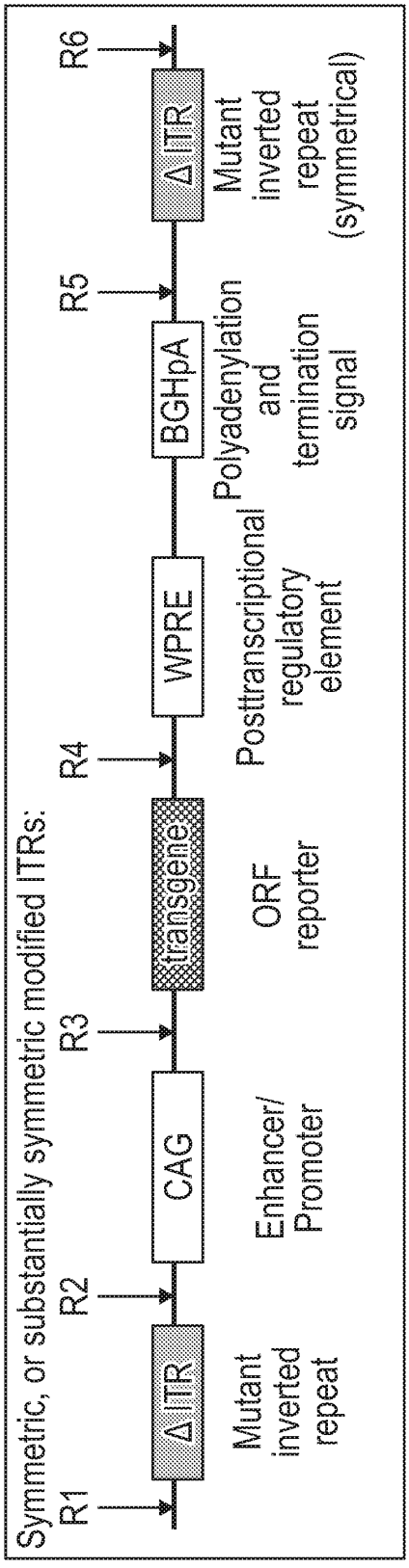

Symmetric, or substantially symmetric modified ITRs:
R1
R2
R3
R4
R5
R6
ΔITR
CAG
transgene
WPRE
BGHpA
ΔITR
Mutant inverted repeat
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
Mutant inverted repeat (symmetrical)

FIG. 1E

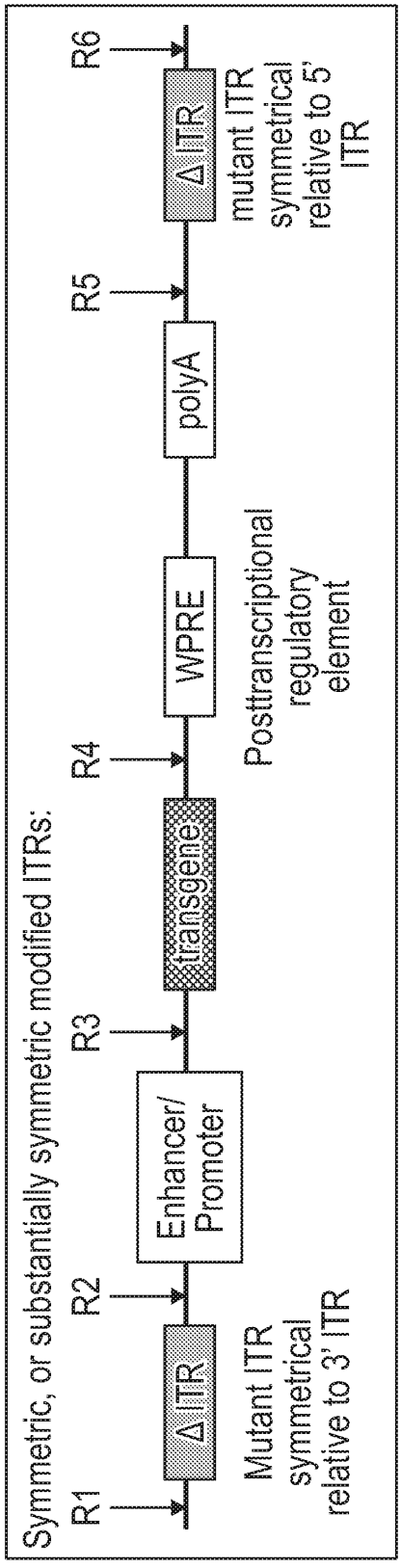

Symmetric, or substantially symmetric modified ITRs:
R1
R2
R3
R4
R5
R6
ΔITR
Enhancer/ Promoter
transgene
WPRE
polyA
ΔITR
Mutant ITR symmetrical relative to 3' ITR
Posttranscriptional regulatory element
mutant ITR symmetrical relative to 5' ITR

FIG. 1F

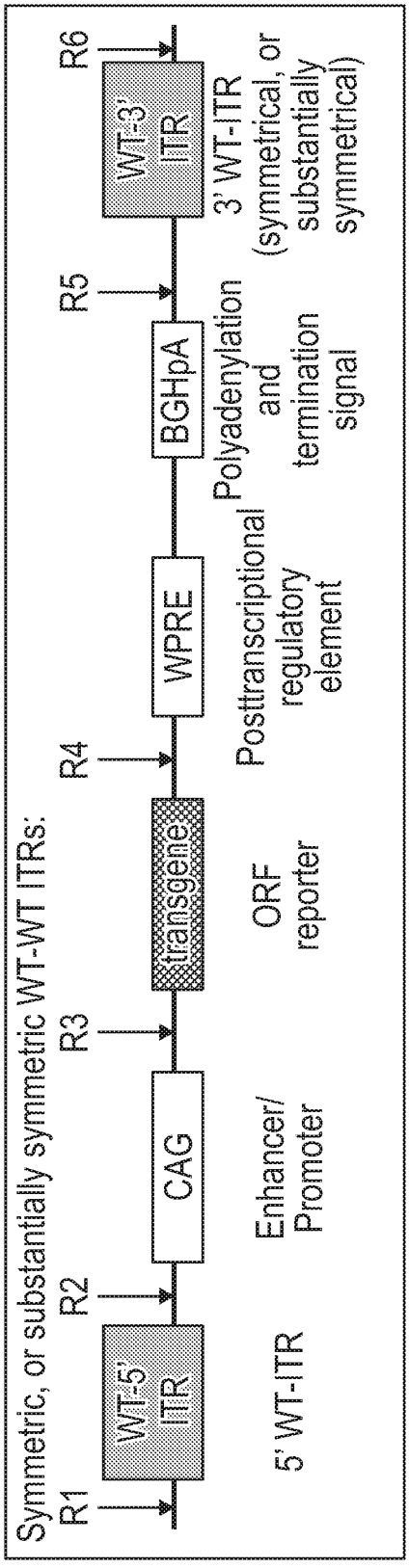
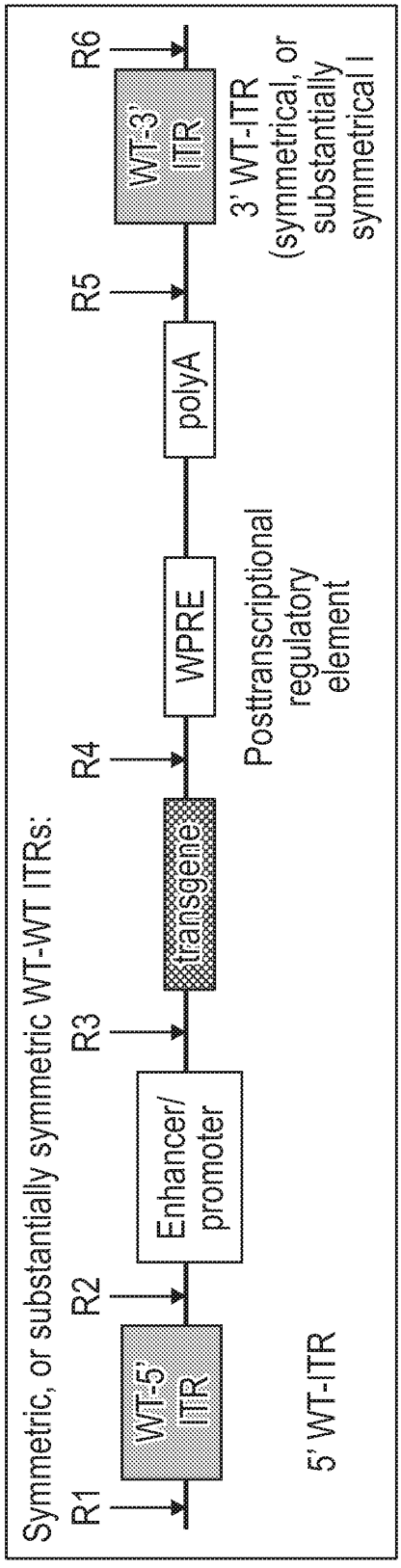

Symmetric, or substantially symmetric WT-WT ITRs:
R1
R2
R3
R4
R5
R6
WT-5' ITR
CAG
transgene
WPRE
BGHpA
WT-3' ITR
5' WT-ITR
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
3' WT-ITR (symmetrical, or substantially symmetrical)

FIG. 1G


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

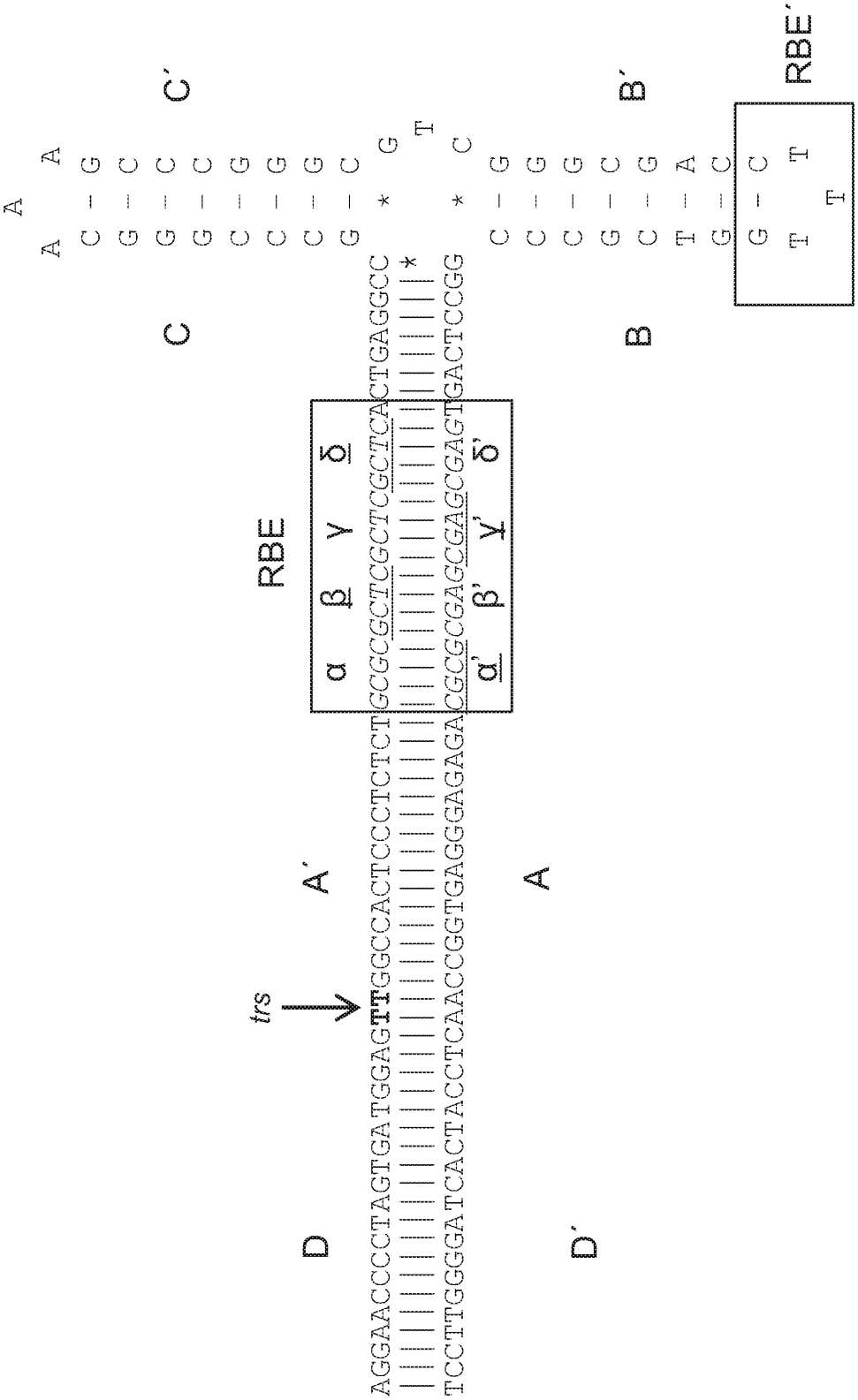

C´
C
B´
B
RBE´
RBE
α β γ δ
α' β' γ' δ'
trs
A´
A
D
D´
AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCC
TCCTTGGGGATCACTACCTCAACCGGTGAGGGAGAGACGCGCGAGCGAGCGAGTGACTCCGG

FIG. 2A

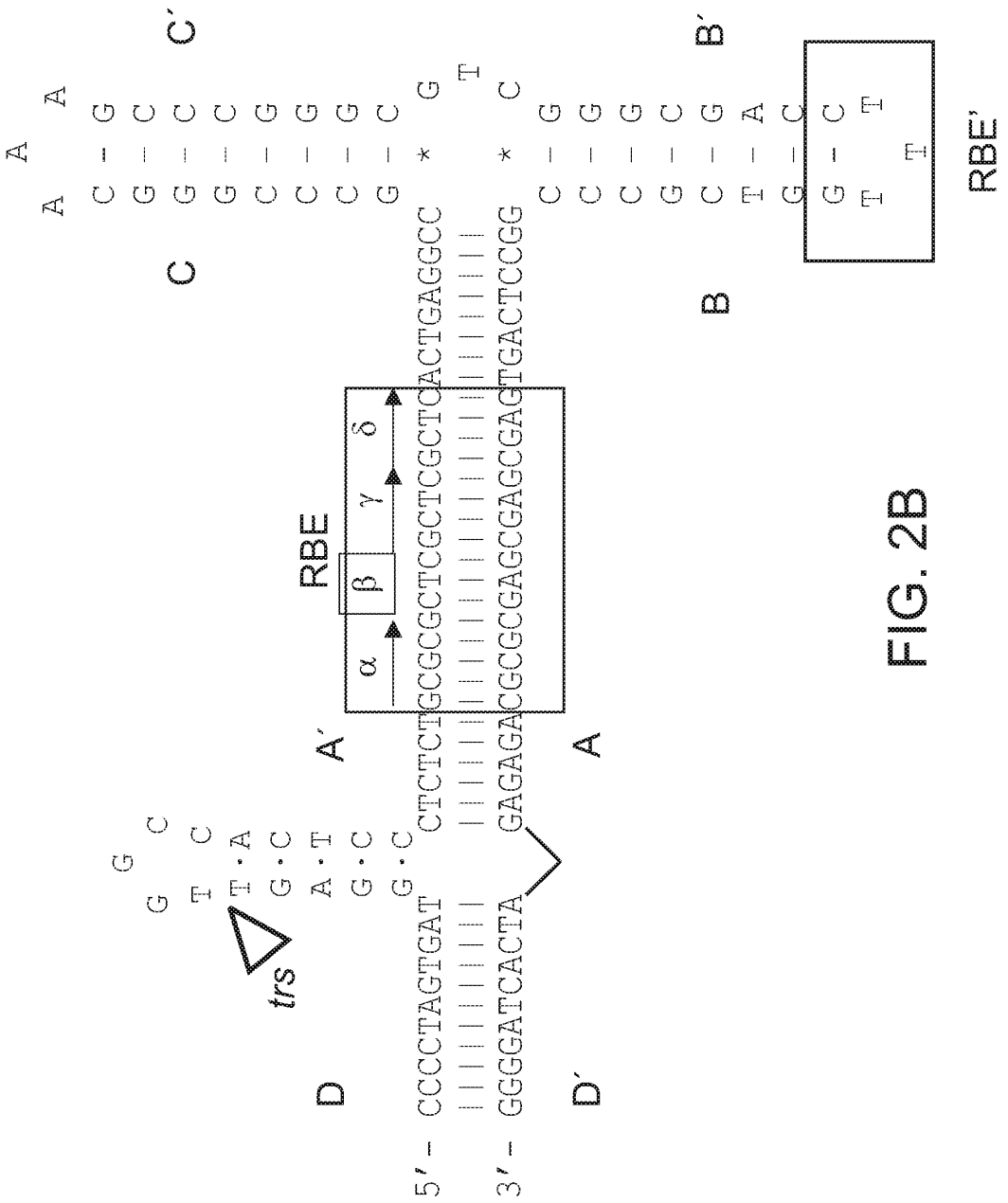


FIG. 2B

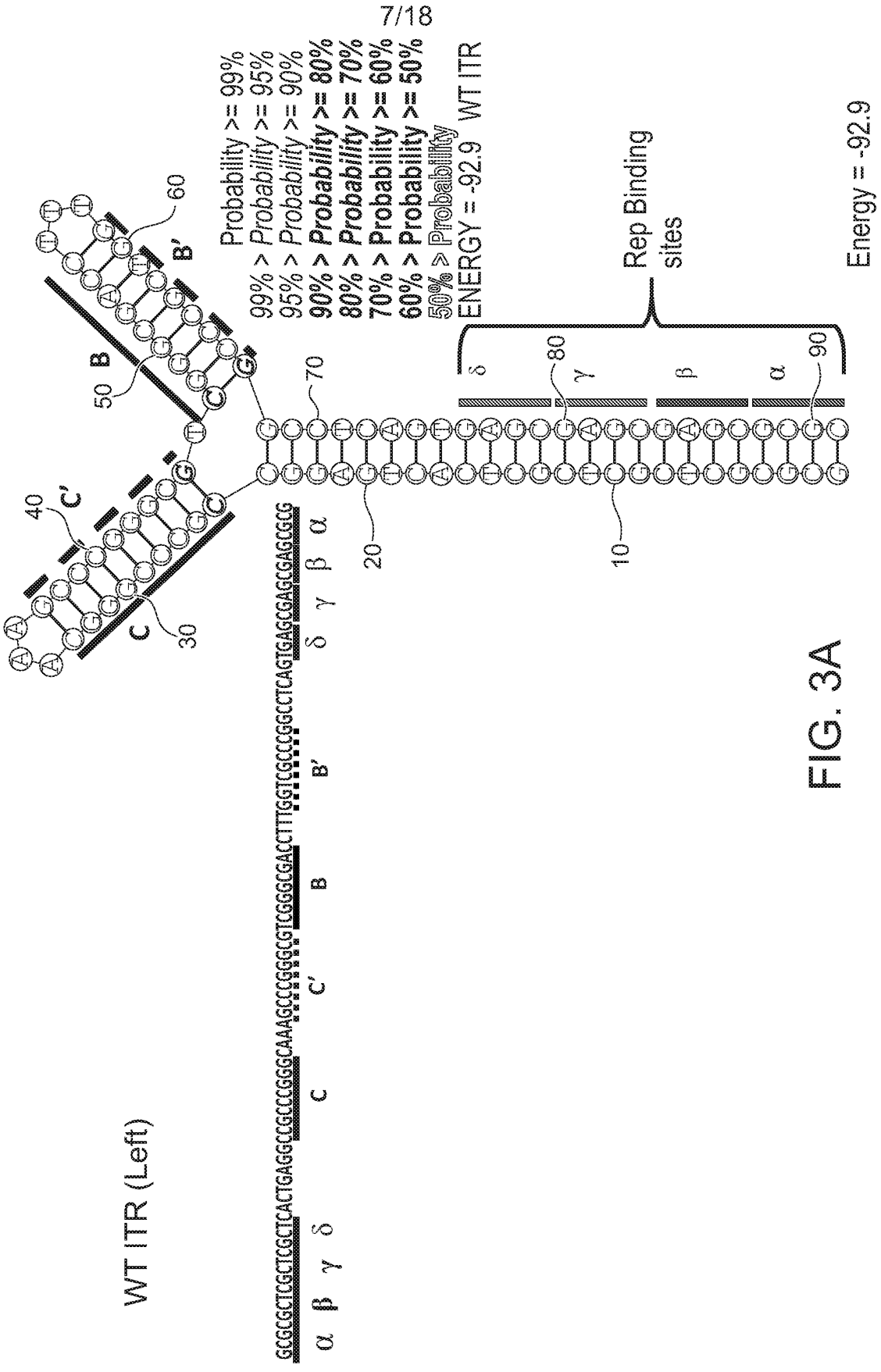

WT ITR (Left)
GCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCG
α β γ δ C C' B B' δ γ β α
Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50%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

FIG. 3A

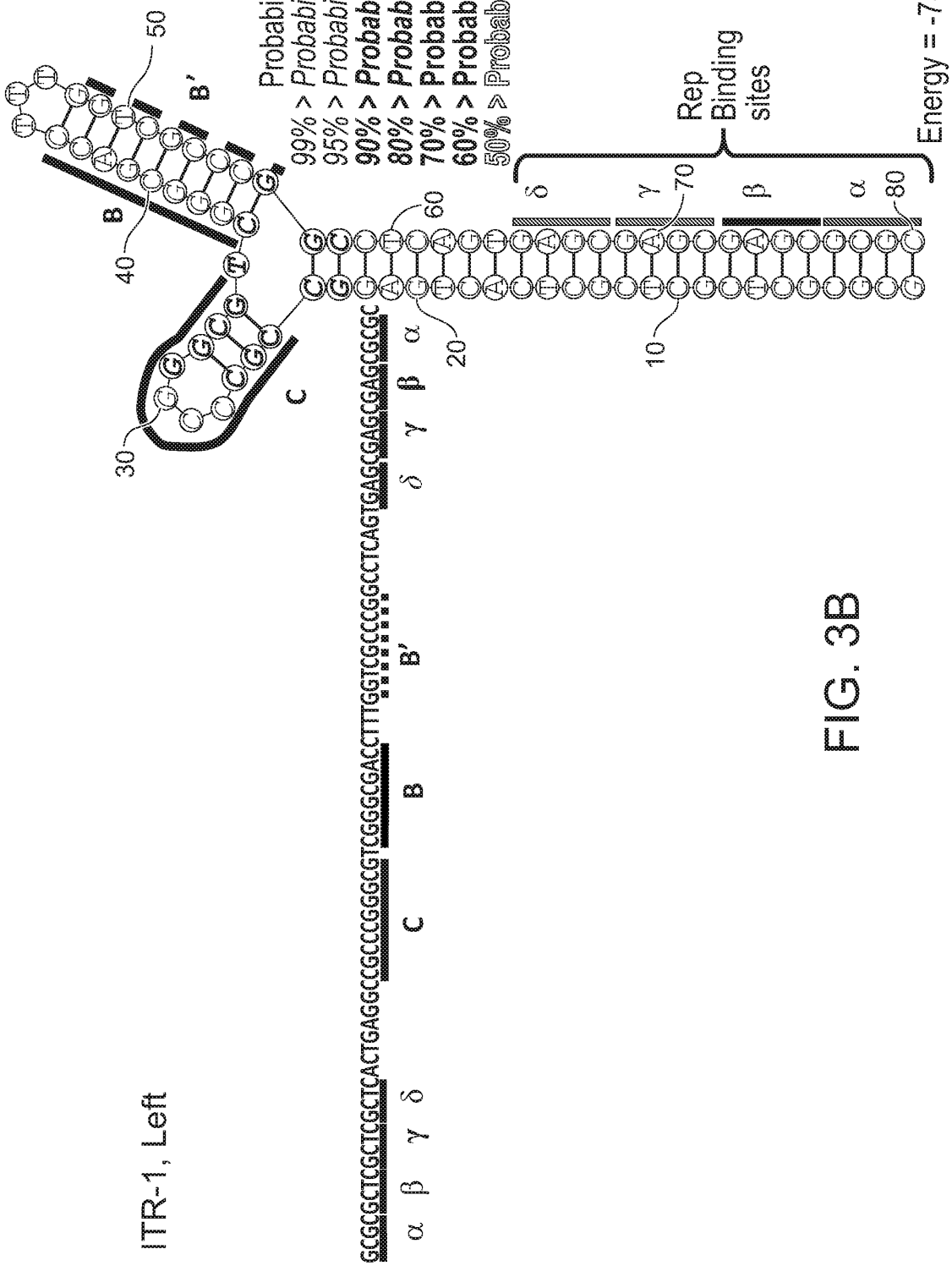

ITR-1, Left
Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability
Rep Binding sites
Energy = -74.7
α β γ δ
C
B
B'
δ γ β α
10
20
30
40
50
60
70
80

FIG. 3B

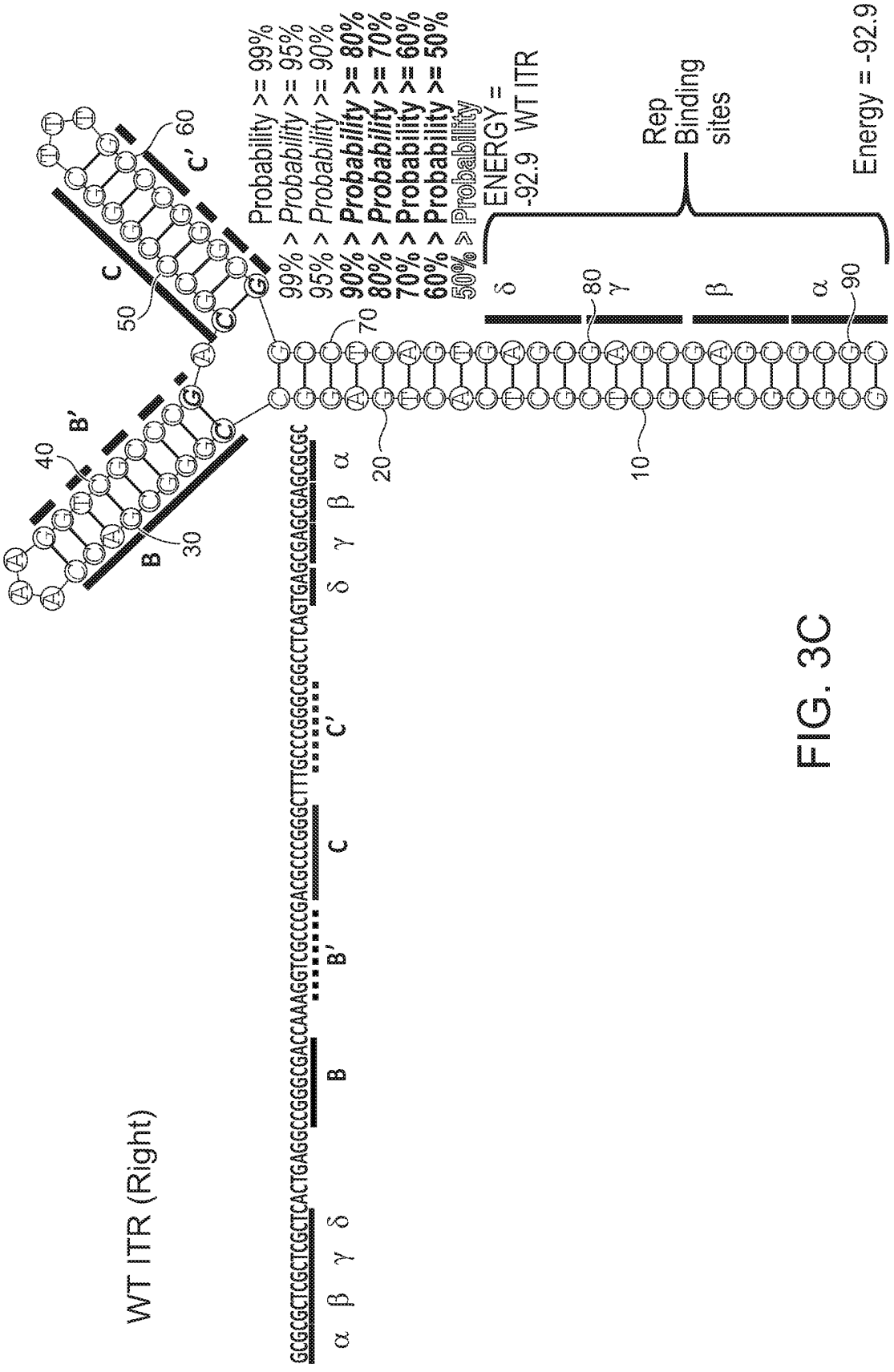

WT ITR (Right)
GCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGC
α β γ δ
B
B'
C
C'
δ γ β α
Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
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

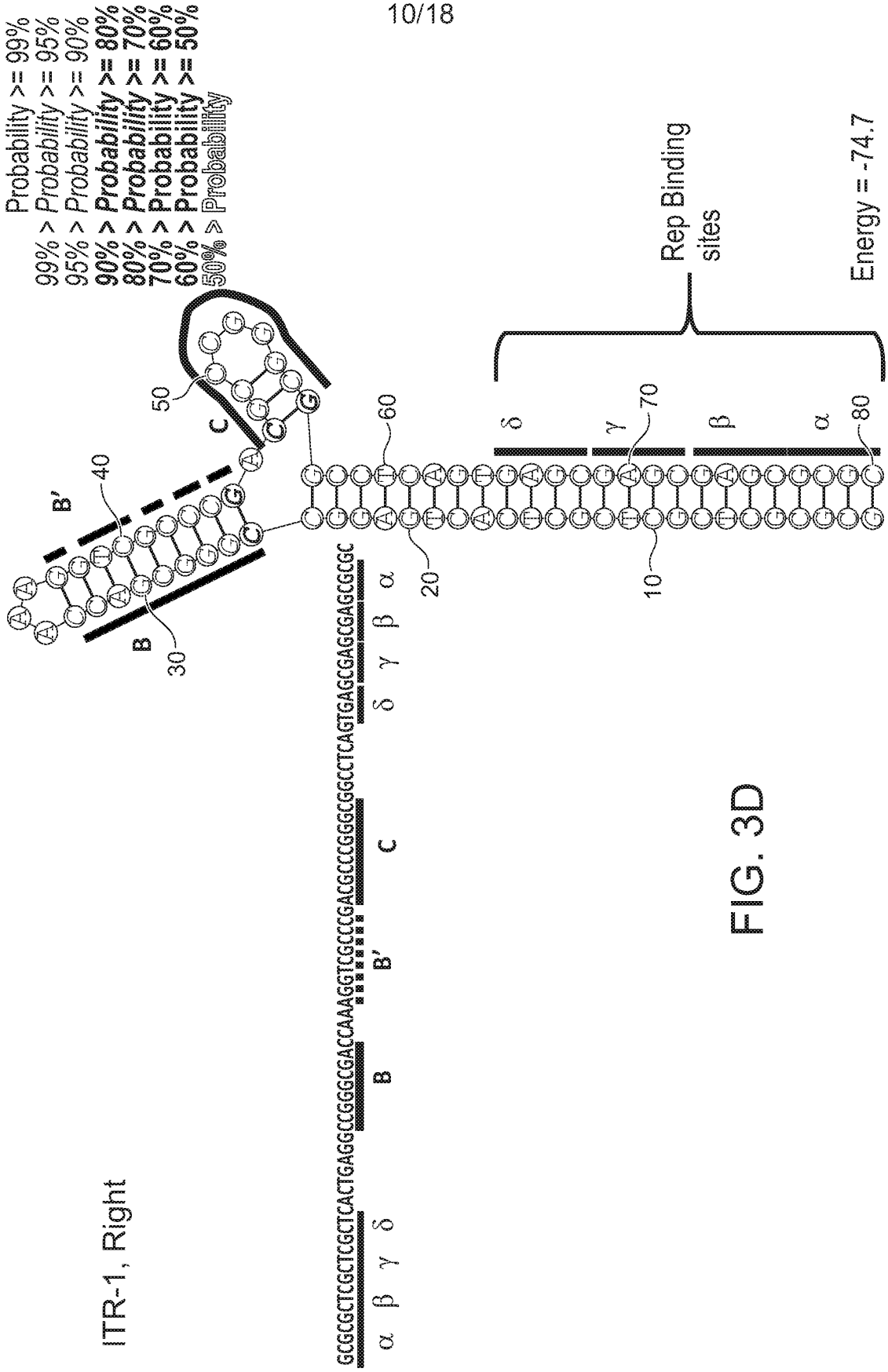

Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability
ITR-1, Right
GCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGC
α β γ δ
B
B'
C
δ γ β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

FIG. 3D

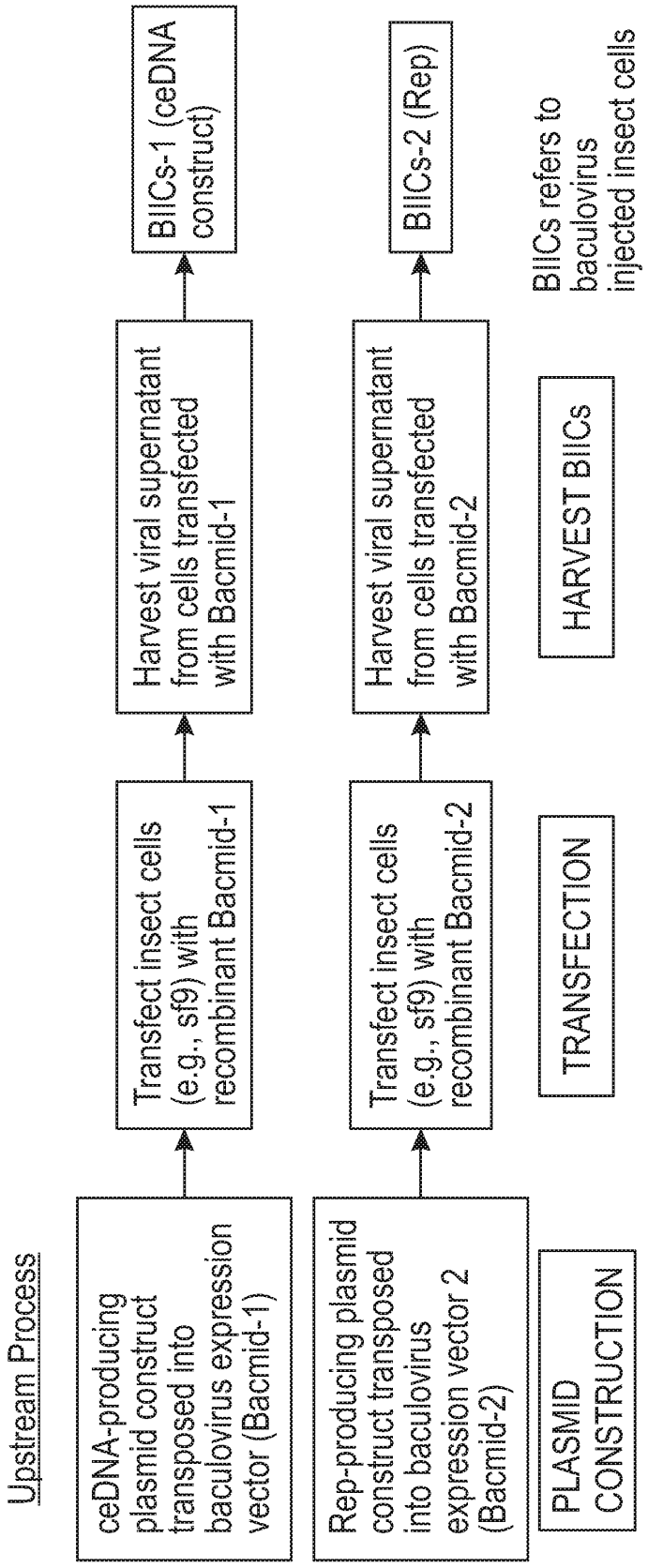

ceDNA Production
Upstream Process
ceDNA-producing plasmid construct transposed into baculovirus expression vector (Bacmid-1)
Rep-producing plasmid construct transposed into baculovirus expression vector 2 (Bacmid-2)
PLASMID CONSTRUCTION
Transfect insect cells (e.g., sf9) with recombinant Bacmid-1
Transfect 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

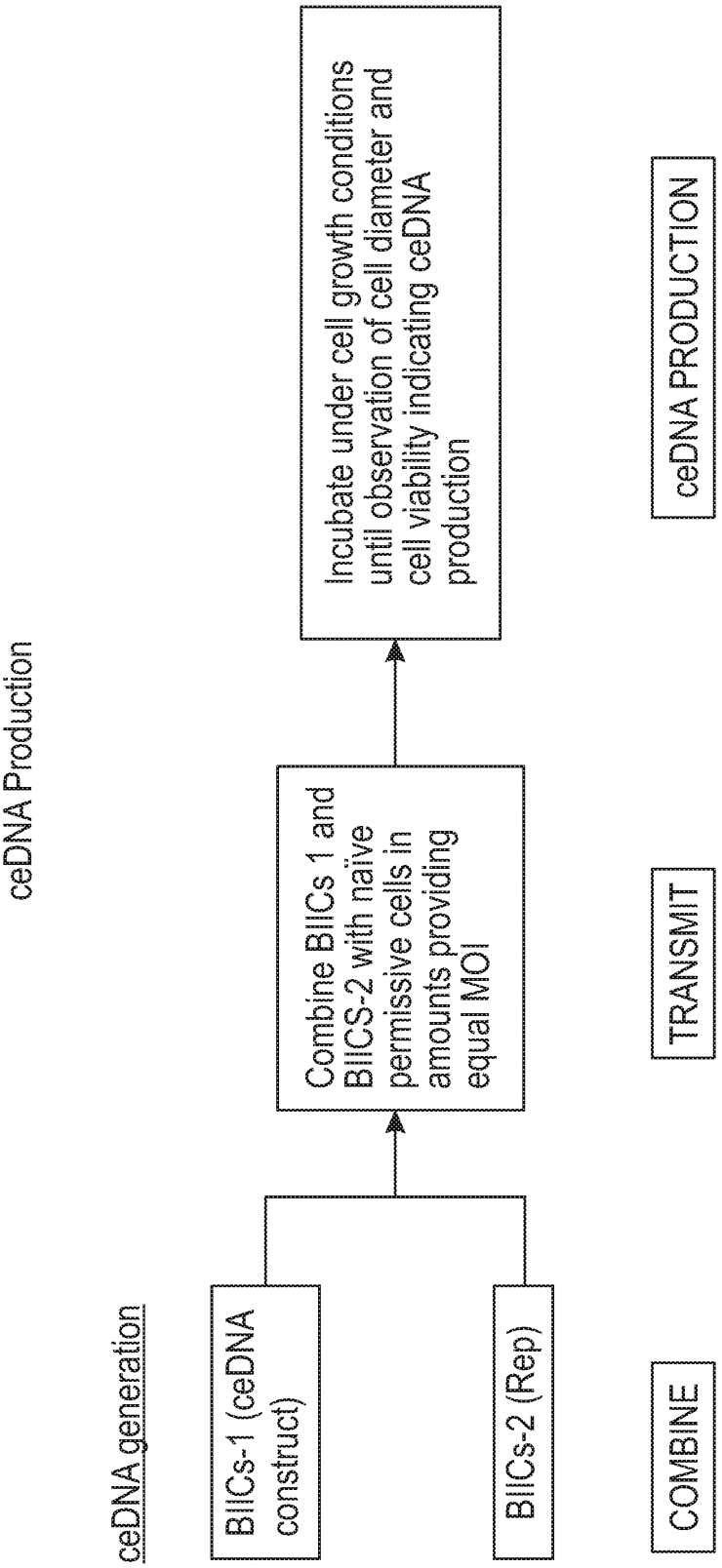

ceDNA Production
ceDNA generation
BIICs-1 (ceDNA construct)
BIICs-2 (Rep)
COMBINE
Combine BIICs 1 and BIICS-2 with naïve permissive cells in amounts providing equal MOI
TRANSMIT
Incubate under cell growth conditions until observation of cell diameter and cell viability indicating ceDNA production
ceDNA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

FIG. 4B

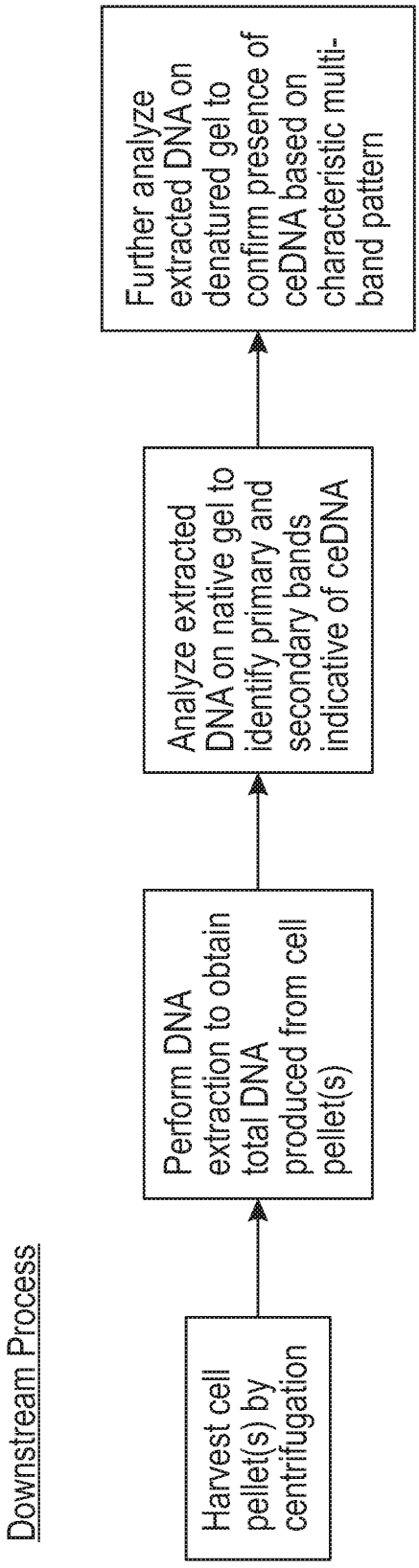

ceDNA Production
Downstream Process
Harvest cell pellet(s) by centrifugation
Perform DNA extraction to obtain total DNA produced from cell pellet(s)
Analyze extracted DNA on native gel to identify primary and secondary bands indicative of ceDNA
Further analyze extracted DNA on denatured gel to confirm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

FIG. 4C

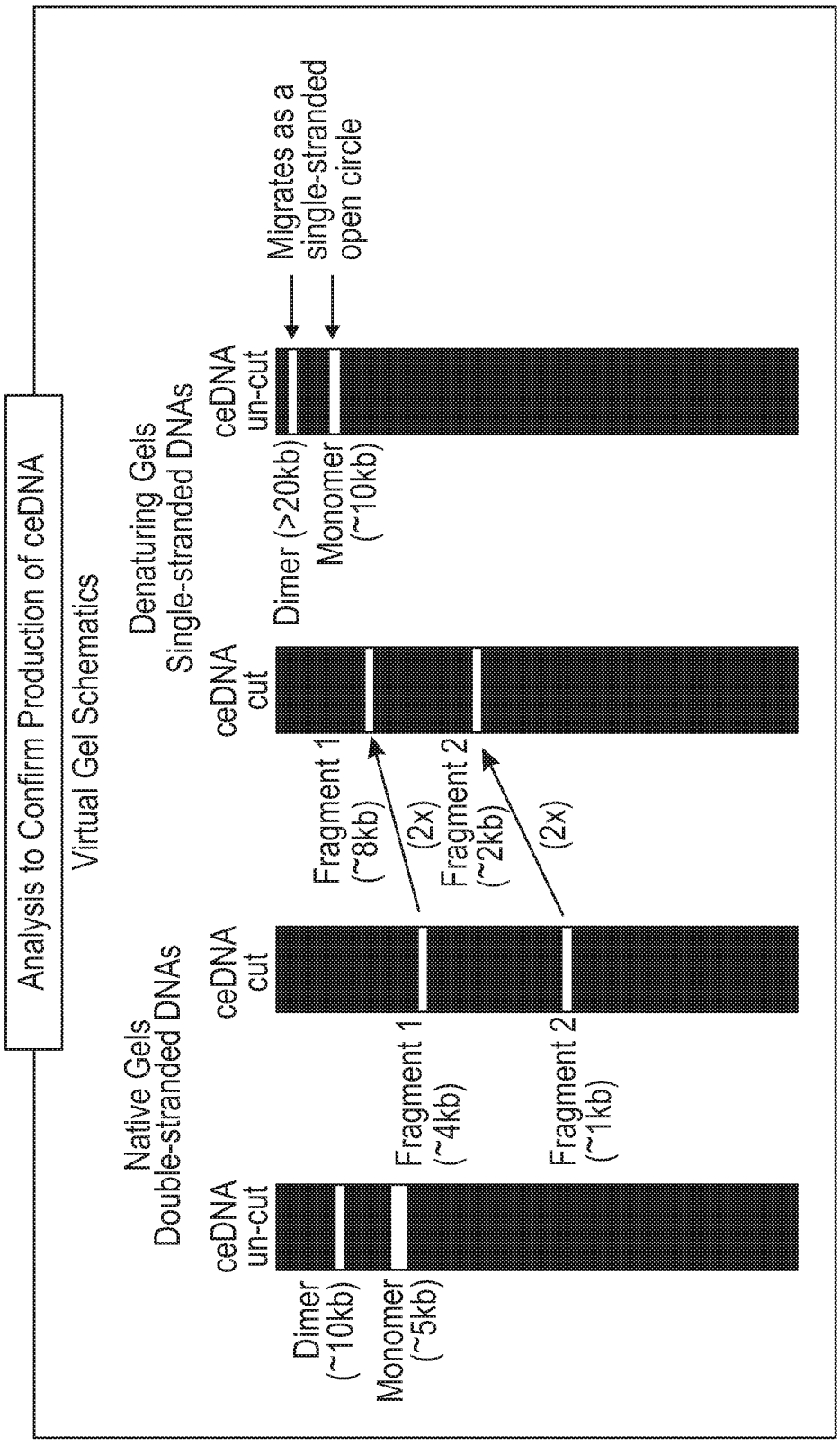
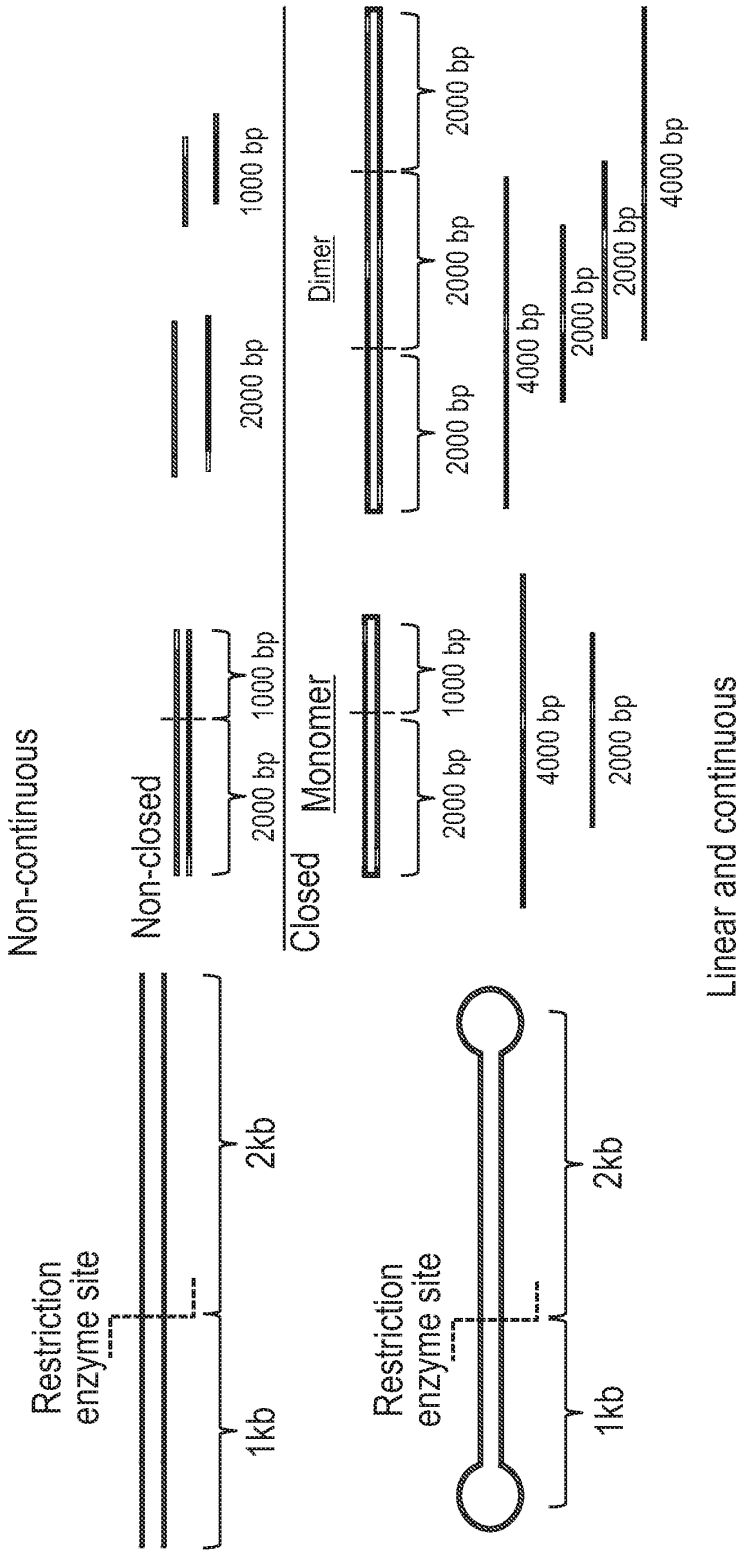
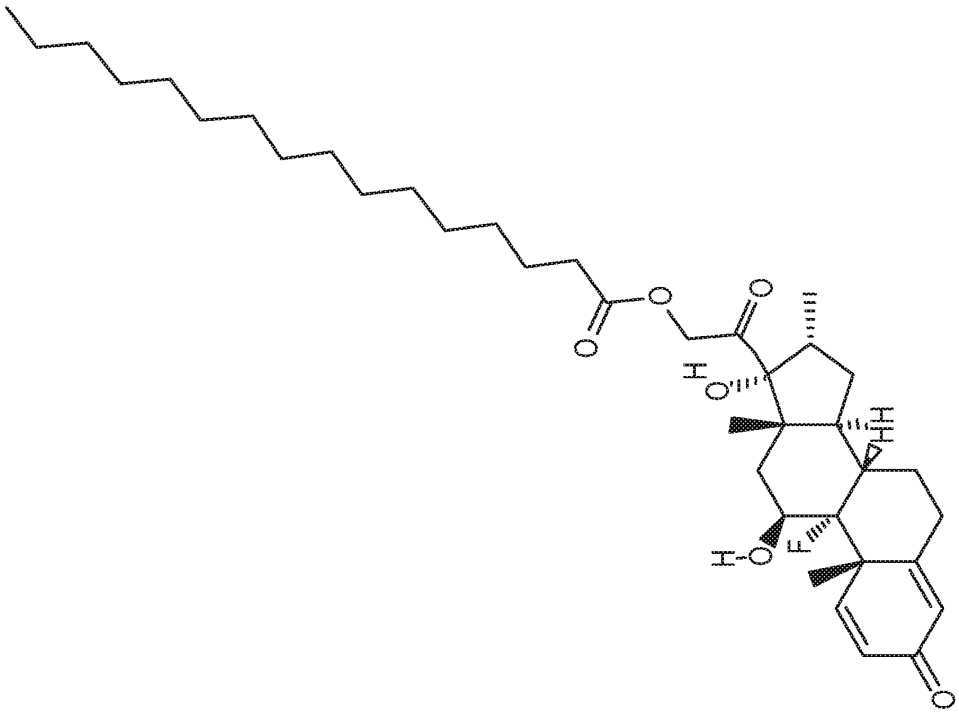

Analysis to Confirm Production of ceDNA
Virtual Gel Schematics
Native Gels
Double-stranded DNAs
ceDNA
un-cut
Dimer
(~10kb)
Monomer
(~5kb)
ceDNA
cut
Fragment 1
(~4kb)
Fragment 2
(~1kb)
Denaturing Gels
Single-stranded DNAs
ceDNA
cut
Fragment 1
(~8kb)
(2x)
Fragment 2
(~2kb)
(2x)
ceDNA
un-cut
Dimer (>20kb)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

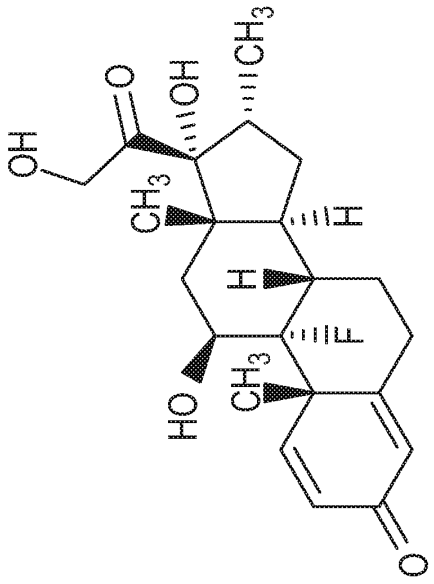
FIG. 4D

FIG. 4E


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



dexamethasone palmitate



dexamethasone

FIG. 5

FIG. 6A

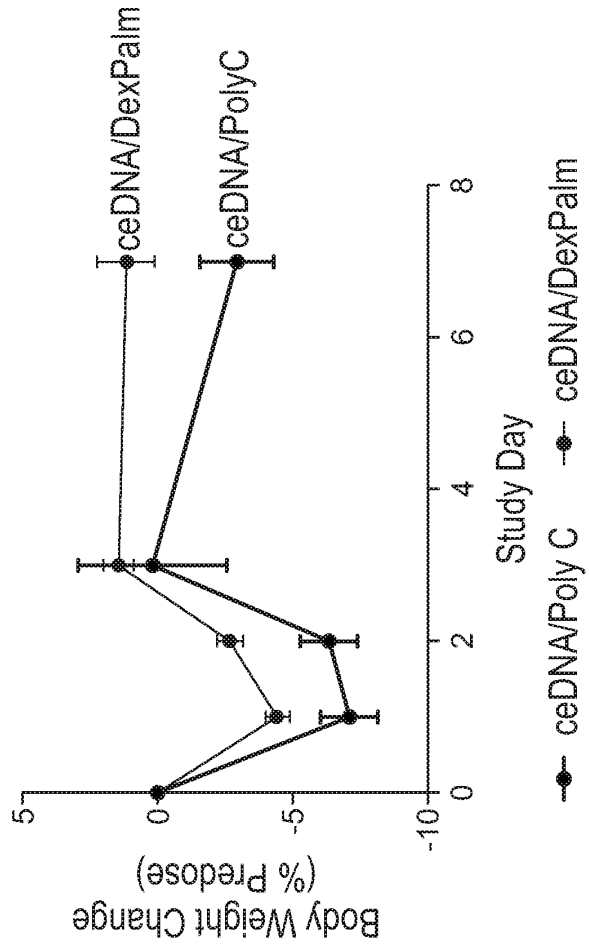

Body Weight Change
(% Predose)
5
0
-5
-10
0
2
4
6
8
Study Day
ceDNA/DexPalm
ceDNA/PolyC
ceDNA/Poly C
ceDNA/DexPalm

FIG. 6B

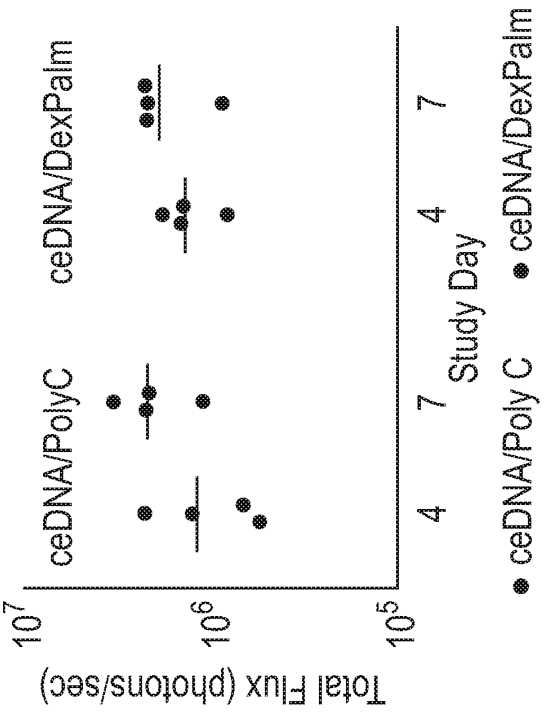
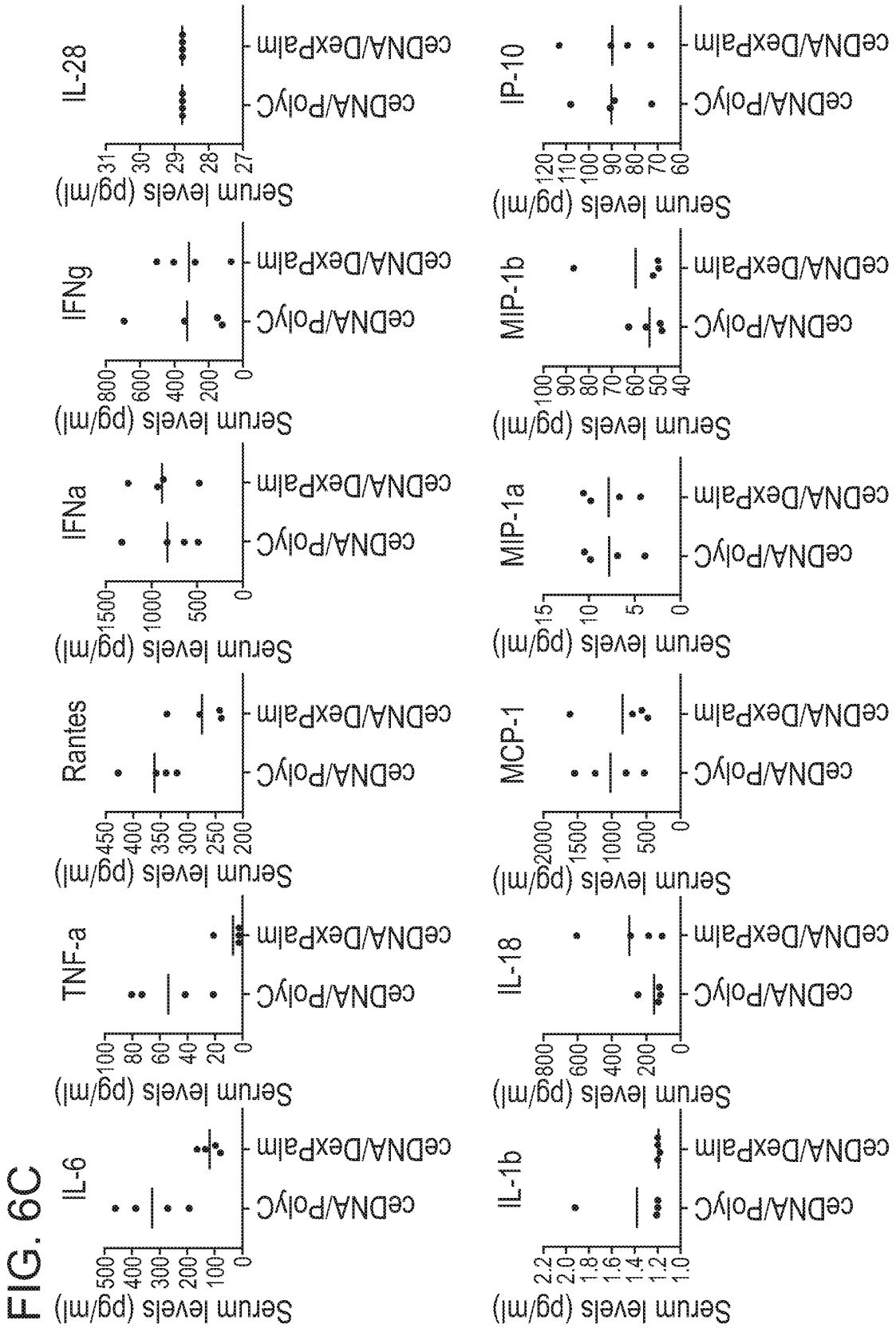

Luciferase Activity
Total Flux (photons/sec)
10^7
10^6
10^5
ceDNA/PolyC
ceDNA/DexPalm
4
7
Study Day
4
7
ceDNA/Poly C
ceDNA/DexPalm

FIG. 6C


IL-6
TNF-a
Rantes
IFNa
IFNg
IL-28
IL-1b
IL-18
MCP-1
MIP-1a
MIP-1b
IP-10
Serum levels (pg/ml)
ceDNA/PolyC
ceDNA/DexPalm