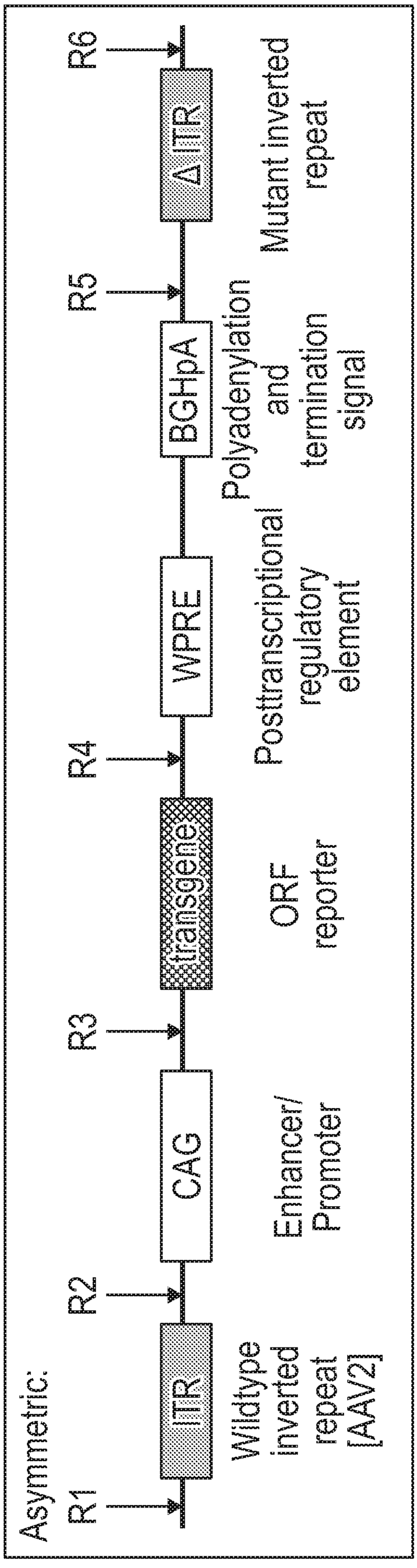

Asymmetric:
R1
ITR
Wildtype inverted repeat [AAV2]
R2
CAG
Enhancer/ Promoter
R3
transgene
ORF reporter
R4
WPRE
Posttranscriptional regulatory element
R5
BGHpA
Polyadenylation and termination signal
R6
ΔITR
Mutant inverted repeat

FIG. 1A

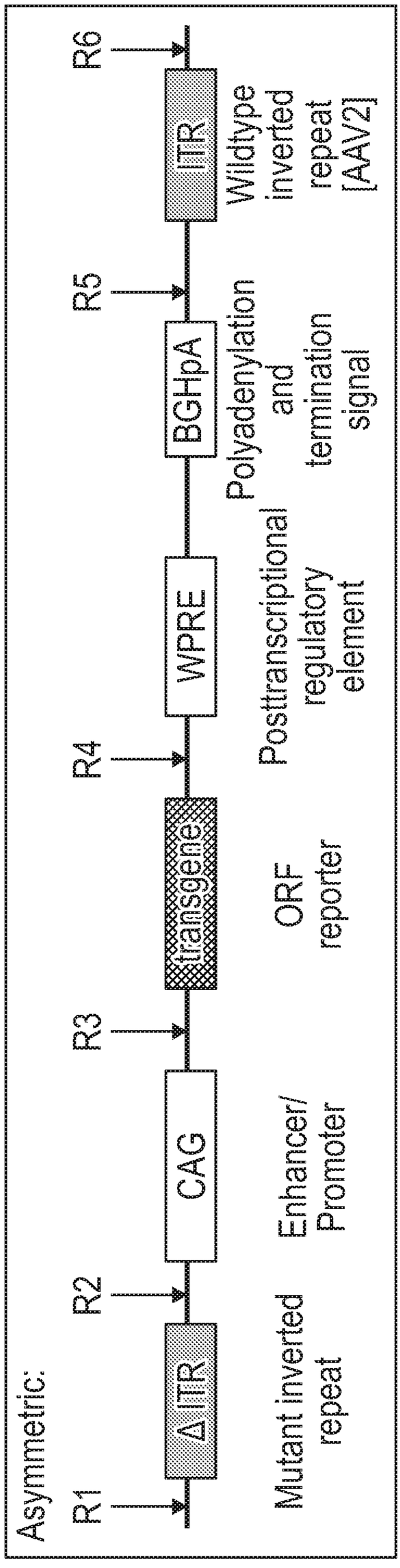

Asymmetric:
R1
ΔITR
Mutant inverted repeat
R2
CAG
Enhancer/ Promoter
R3
transgene
ORF reporter
R4
WPRE
Posttranscriptional regulatory element
R5
BGHpA
Polyadenylation and termination signal
R6
ITR
Wildtype inverted repeat [AAV2]

FIG. 1B

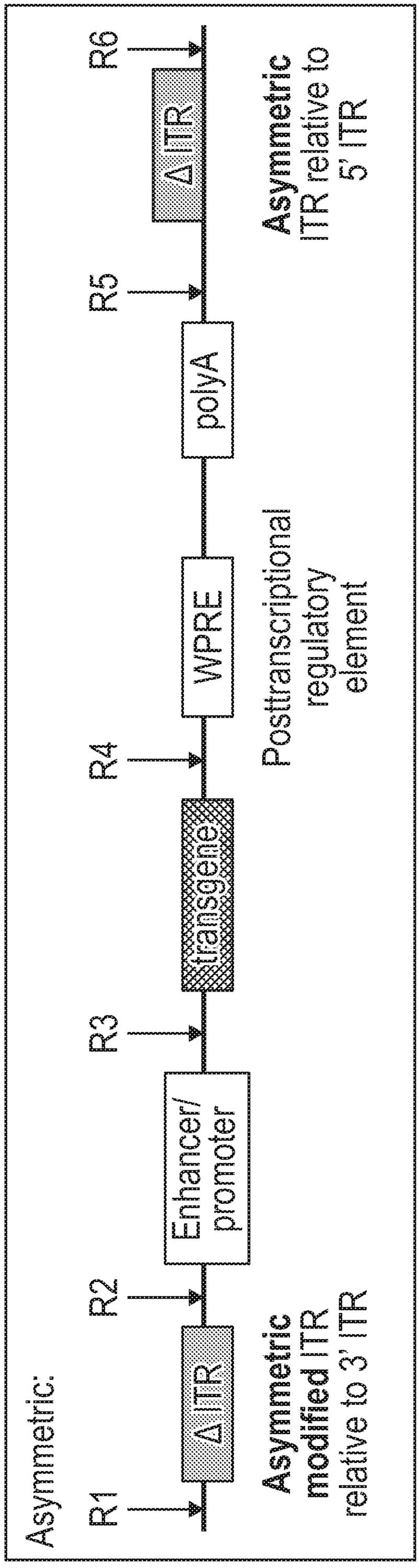

Asymmetric:
R1
ΔITR
Asymmetric
modified ITR
relative to 3' ITR
R2
Enhancer/
promoter
R3
transgene
R4
WPRE
Posttranscriptional
regulatory
element
R5
polyA
R6
ΔITR
Asymmetric
ITR relative to
5' ITR

FIG. 1C

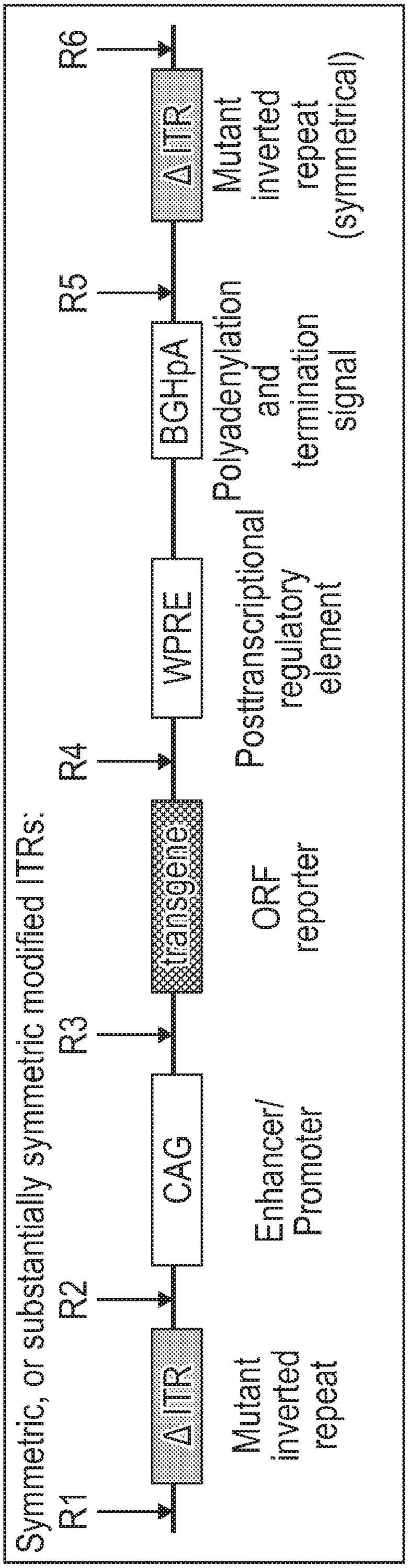

Symmetric, or substantially symmetric modified ITRs:
R1
R2
Δ ITR
Mutant inverted repeat
R3
CAG
Enhancer/ Promoter
R4
transgene
ORF reporter
Posttranscriptional regulatory element
R5
WPRE
R6
BGHpA
Polyadenylation and termination signal
Δ ITR
Mutant inverted repeat (symmetrical)

FIG. 1D

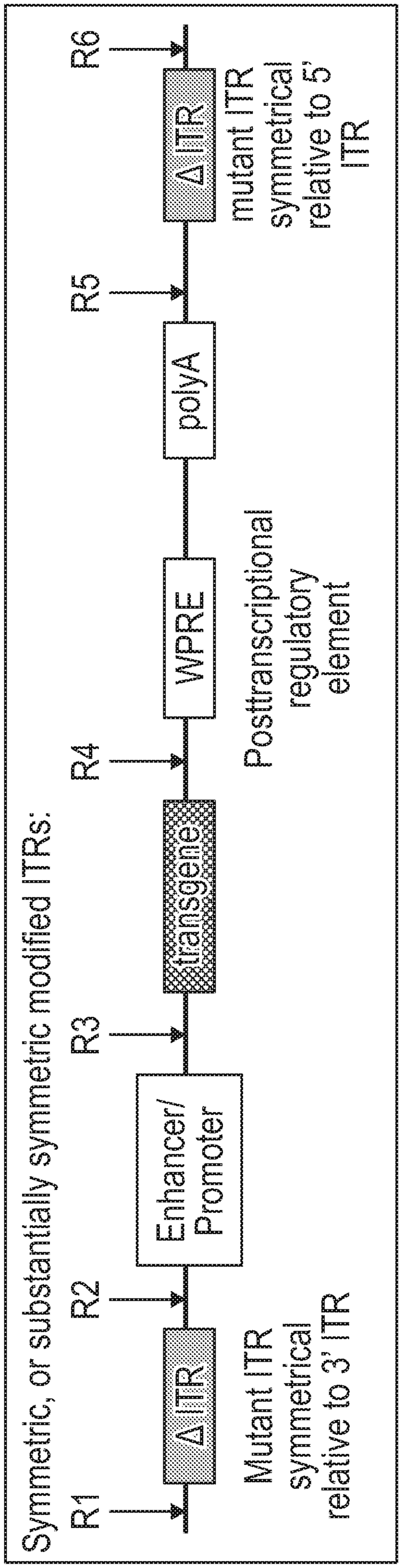

Symmetric, or substantially symmetric modified ITRs:
R1
R2
Δ ITR
Mutant ITR symmetrical relative to 3' ITR
R3
Enhancer/ Promoter
R4
transgene
ORF reporter
Posttranscriptional regulatory element
R5
WPRE
R6
polyA
Δ ITR
mutant ITR symmetrical relative to 5' ITR

FIG. 1E

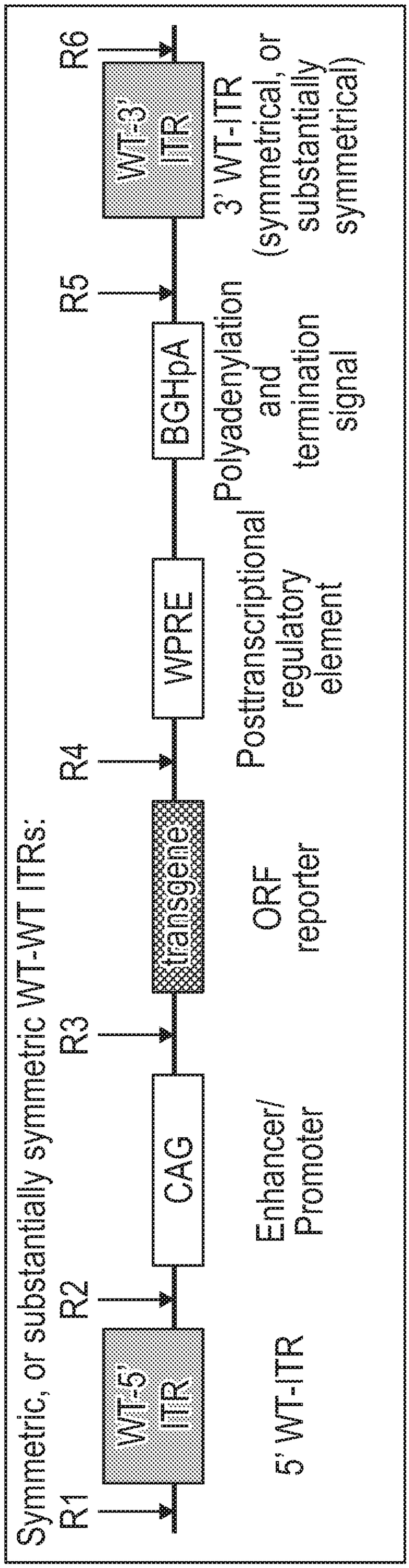

Symmetric, or substantially symmetric WT-WT ITRs:
R1
WT-5' ITR
R2
5' WT-ITR
R3
CAG
Enhancer/ Promoter
R4
transgene
ORF reporter
R5
WPRE
Posttranscriptional regulatory element
R6
BGHpA
Polyadenylation and termination signal
3' WT-ITR (symmetrical, or substantially symmetrical)

FIG. 1F

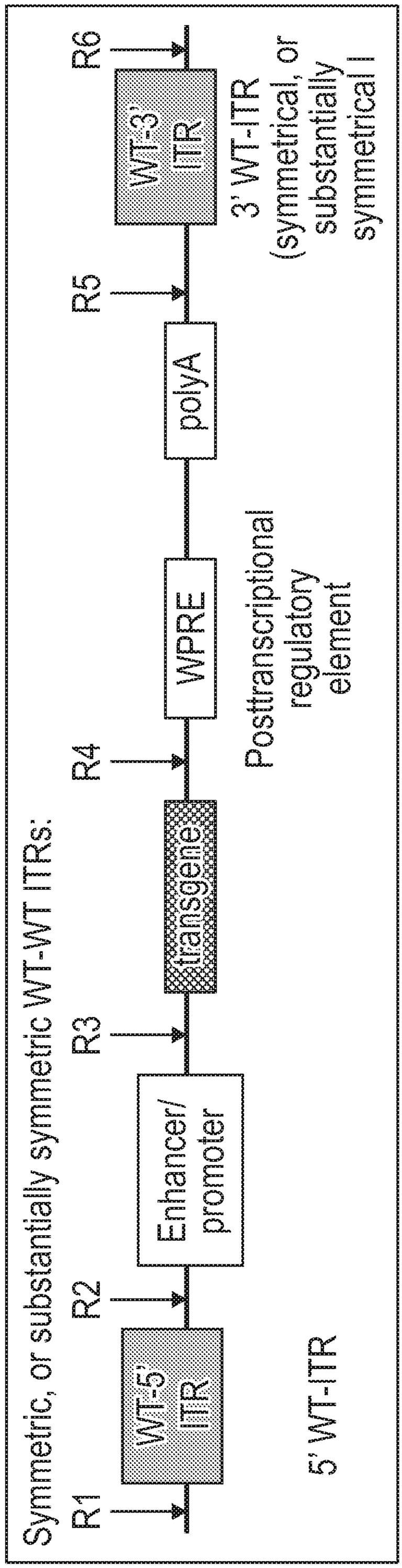

Symmetric, or substantially symmetric WT-WT ITRs:
R1
WT-5' ITR
R2
5' WT-ITR
R3
Enhancer/ promoter
R4
transgene
ORF reporter
R5
WPRE
Posttranscriptional regulatory element
R6
polyA
3' WT-ITR (symmetrical, or substantially symmetrical I

FIG. 1G

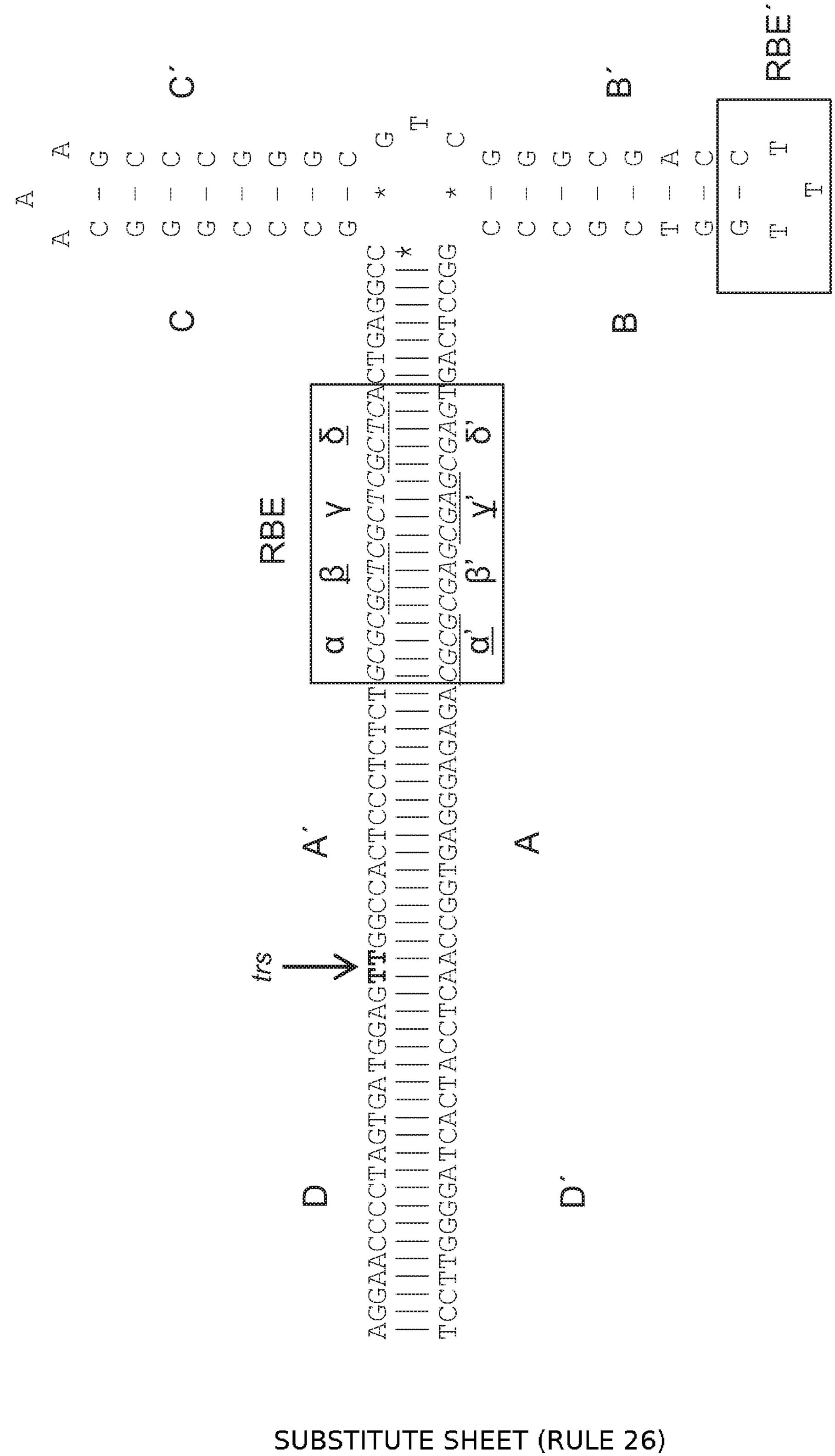
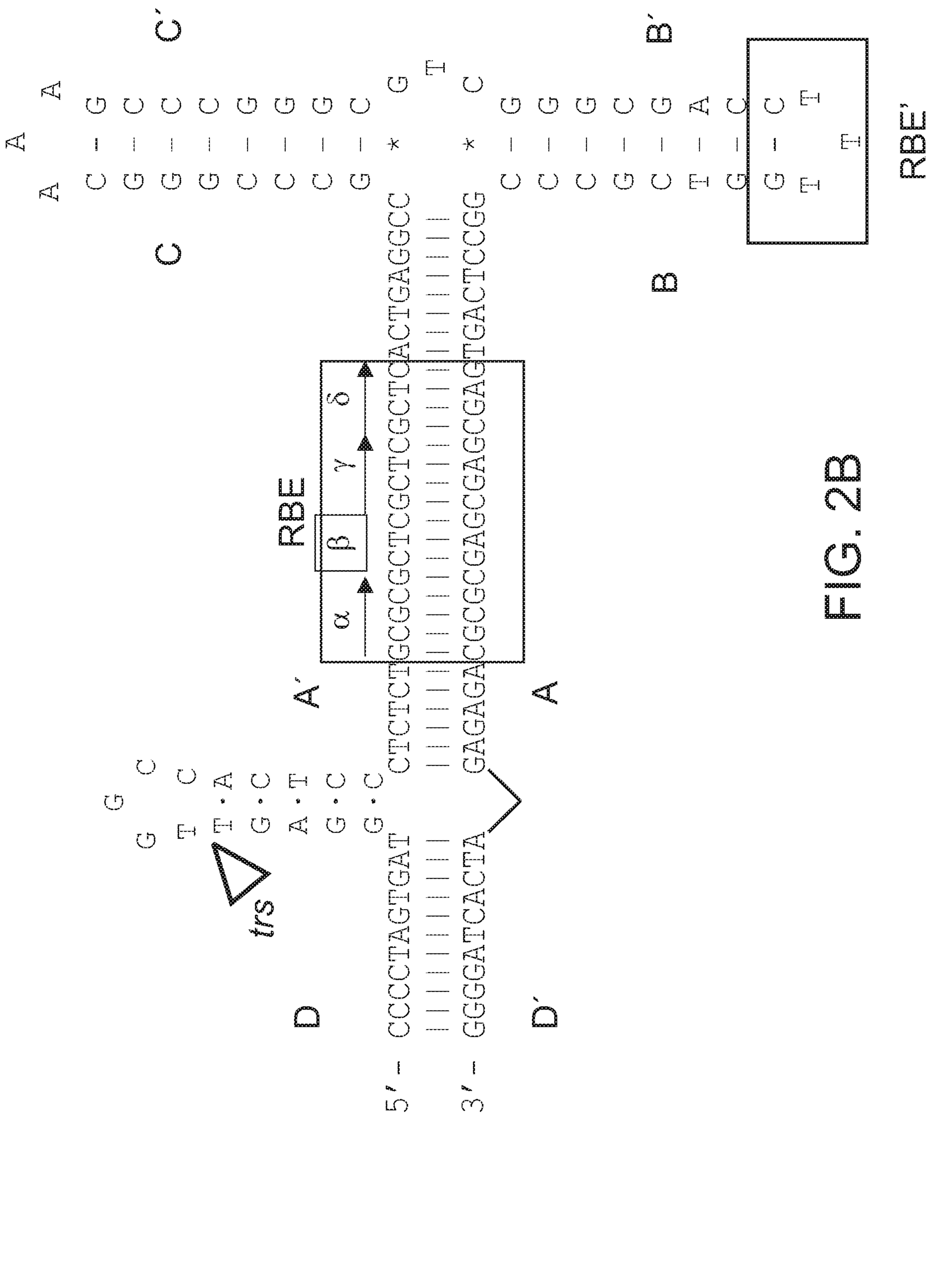

A
C
G
G
G
C
C
C
G
A
G
C
C
C
C
G
G
G
C
G
A
C
C
T
T
G
C
C
C
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T
C
G
T
C
C
C
G
G
G
C
G
A
C
C
T
T
G
G
T
T
RBE
α β γ δ
α' β' γ' δ'
trs
A
A'
B
B'
C
C'
D
D'
RBE'

FIG. 2A


D
5′ – CCCCTTT

WT ITR (Left)

GCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCG

α β γ δ C C' B B' δ γ β α

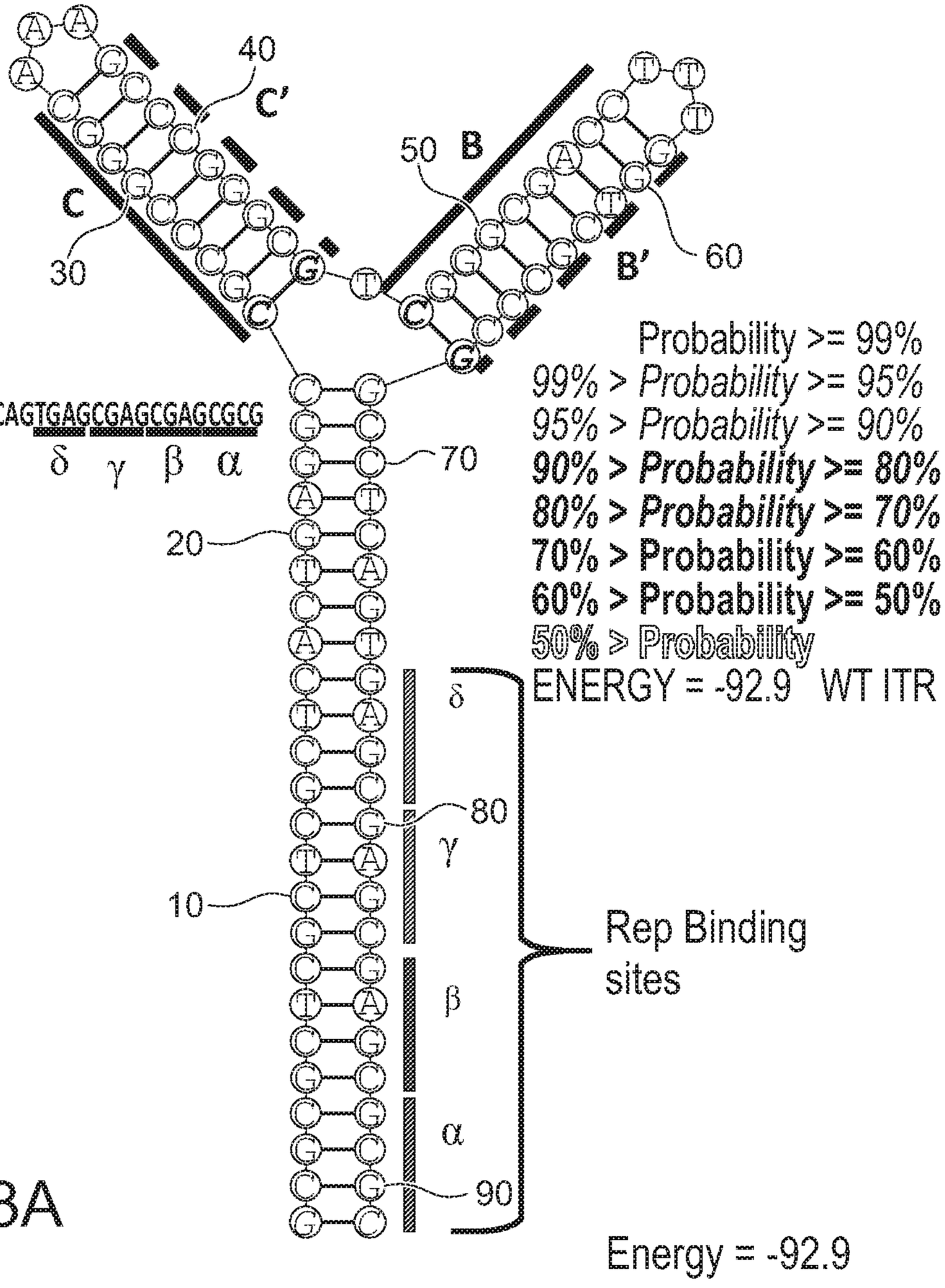


FIG. 3A

SUBSTITUTE SHEET (RULE 26)

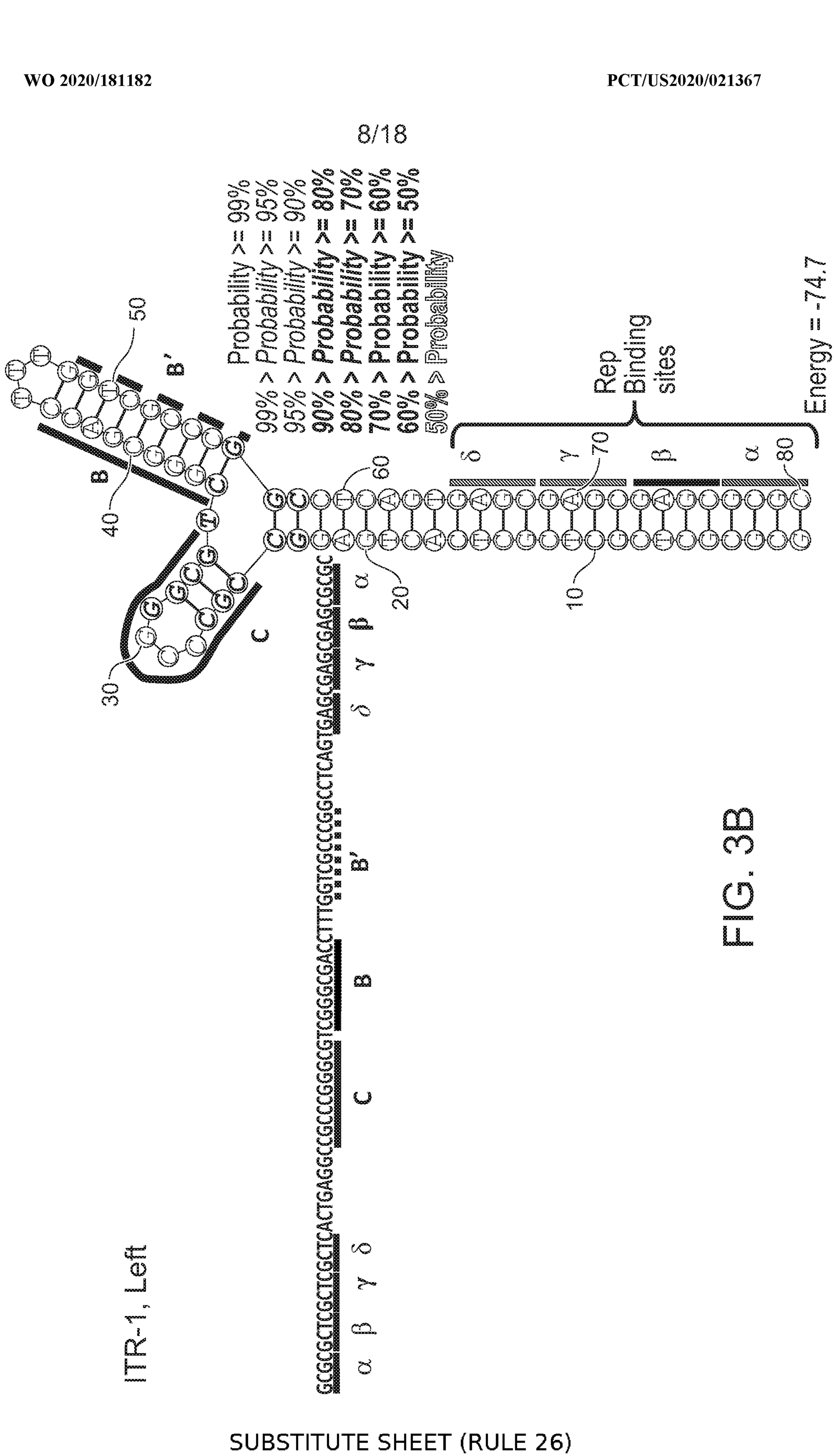

Probability >= 99%
Probability >= 95%
Probability >= 90%
Probability >= 80%
Probability >= 70%
Probability >= 60%
Probability >= 50%
99% > Probability
95% > Probability
90% > Probability
80% > Probability
70% > Probability
60% > Probability
50% > Probability
Rep Binding sites
Energy = -74.7
δ γ β α
80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

FIG. 3B

WT ITR (Right)

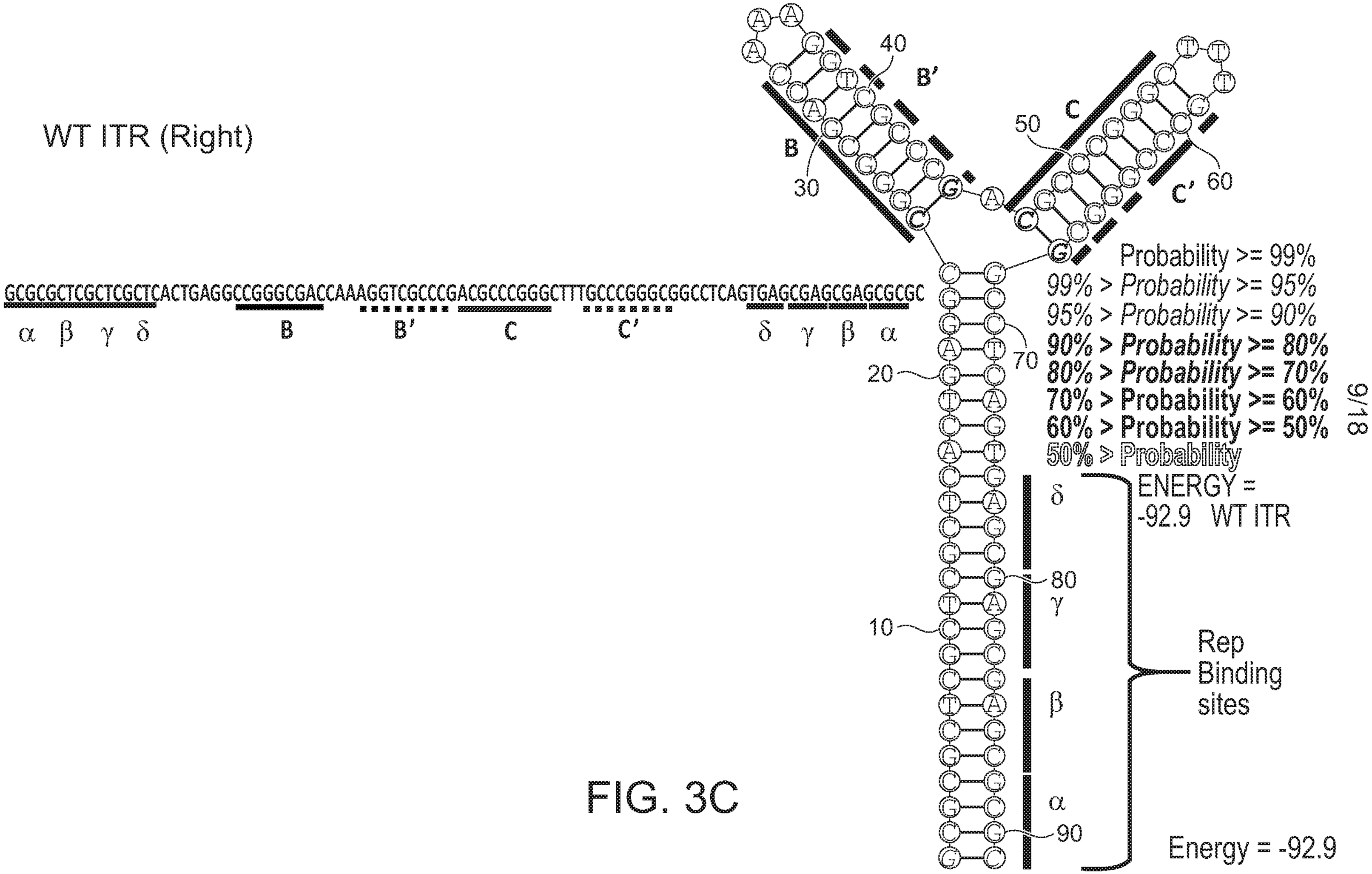


FIG. 3C

ITR-1, Right

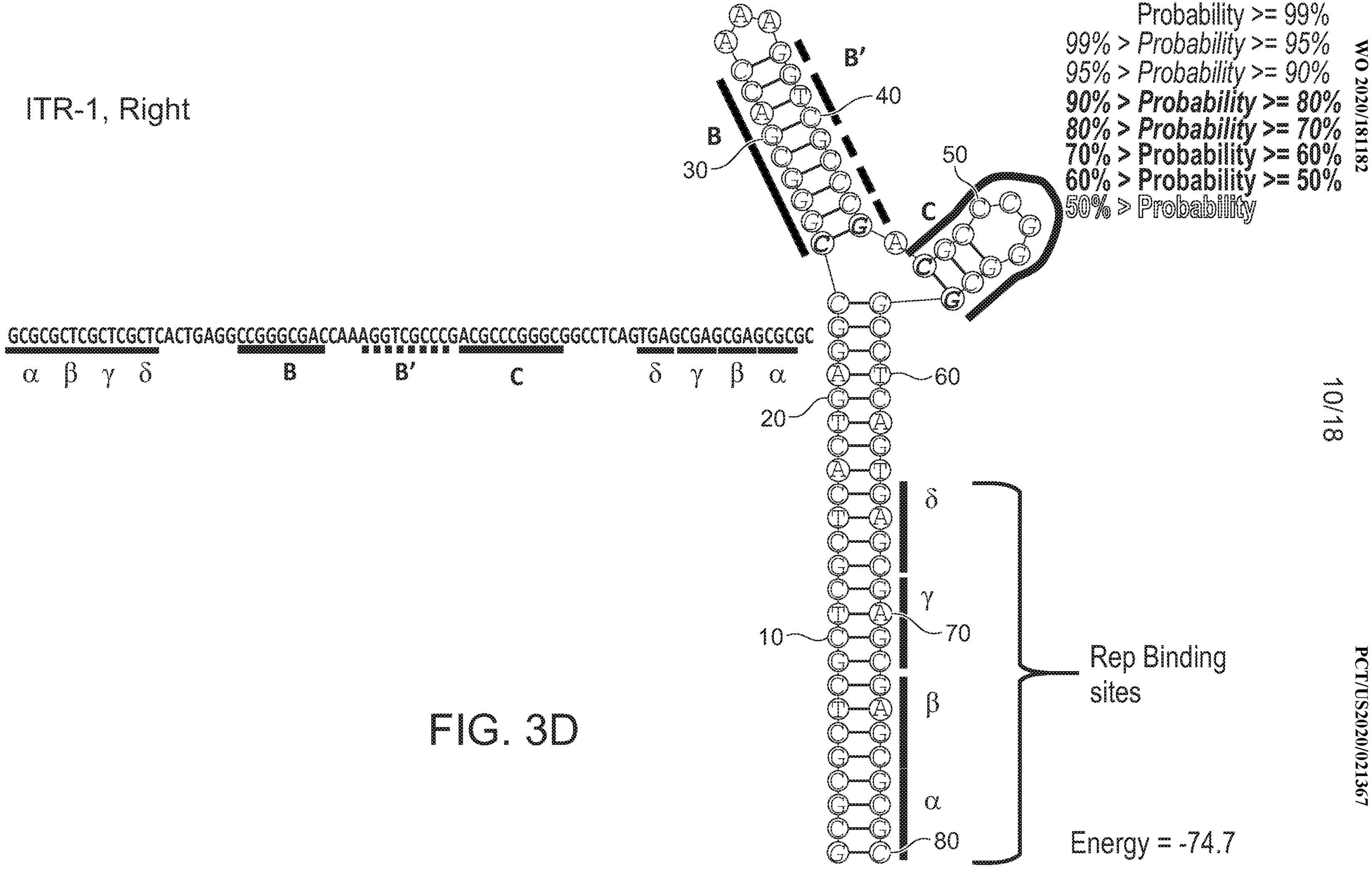


FIG. 3D

SUBSTITUTE SHEET (RULE 26)

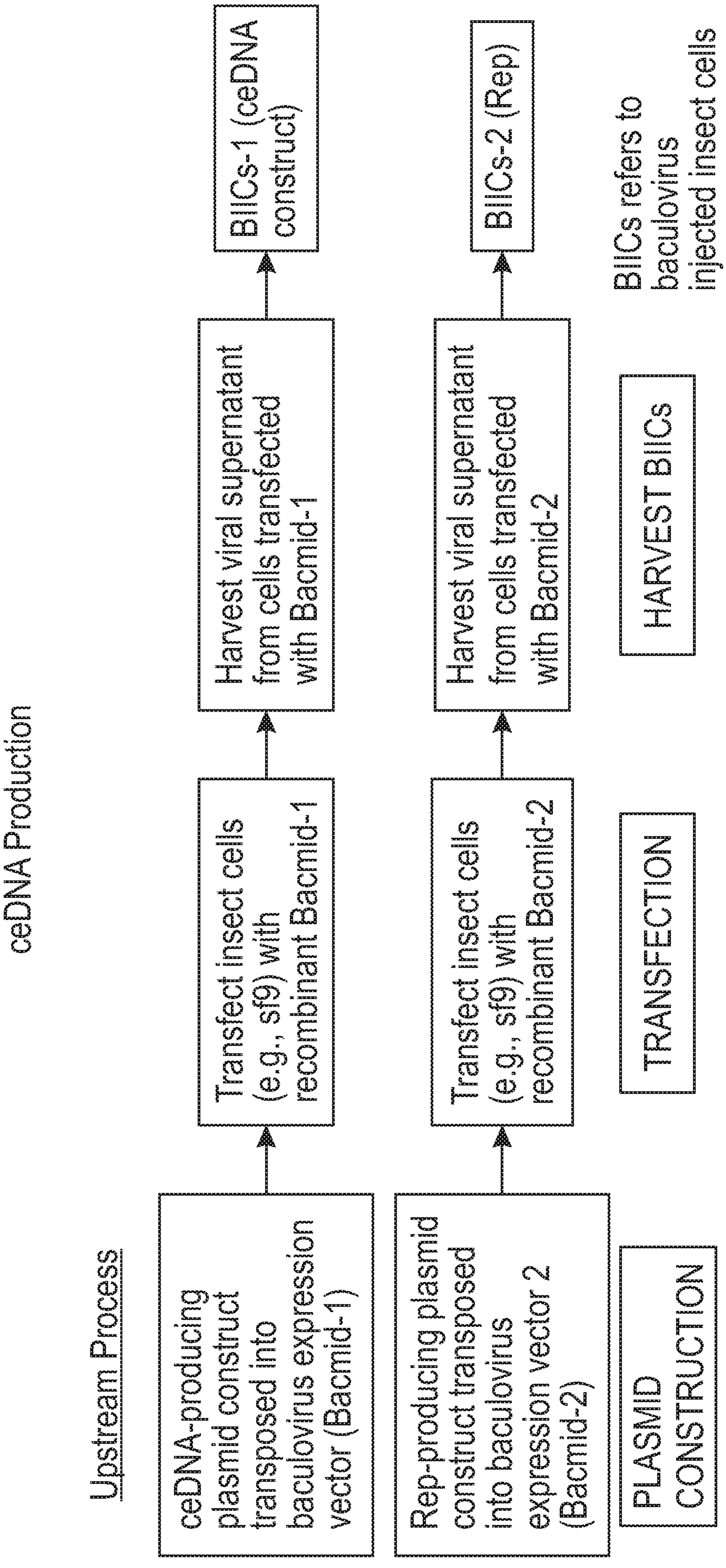

Upstream Process
ceDNA Production
ceDNA-producing plasmid construct transposed into baculovirus expression vector (Bacmid-1)
Rep-producing plasmid construct transposed into baculovirus expression vector 2 (Bacmid-2)
PLASMID CONSTRUCTION
Transfect insect cells (e.g., sf9) with recombinant Bacmid-1
Transfect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

FIG. 4A

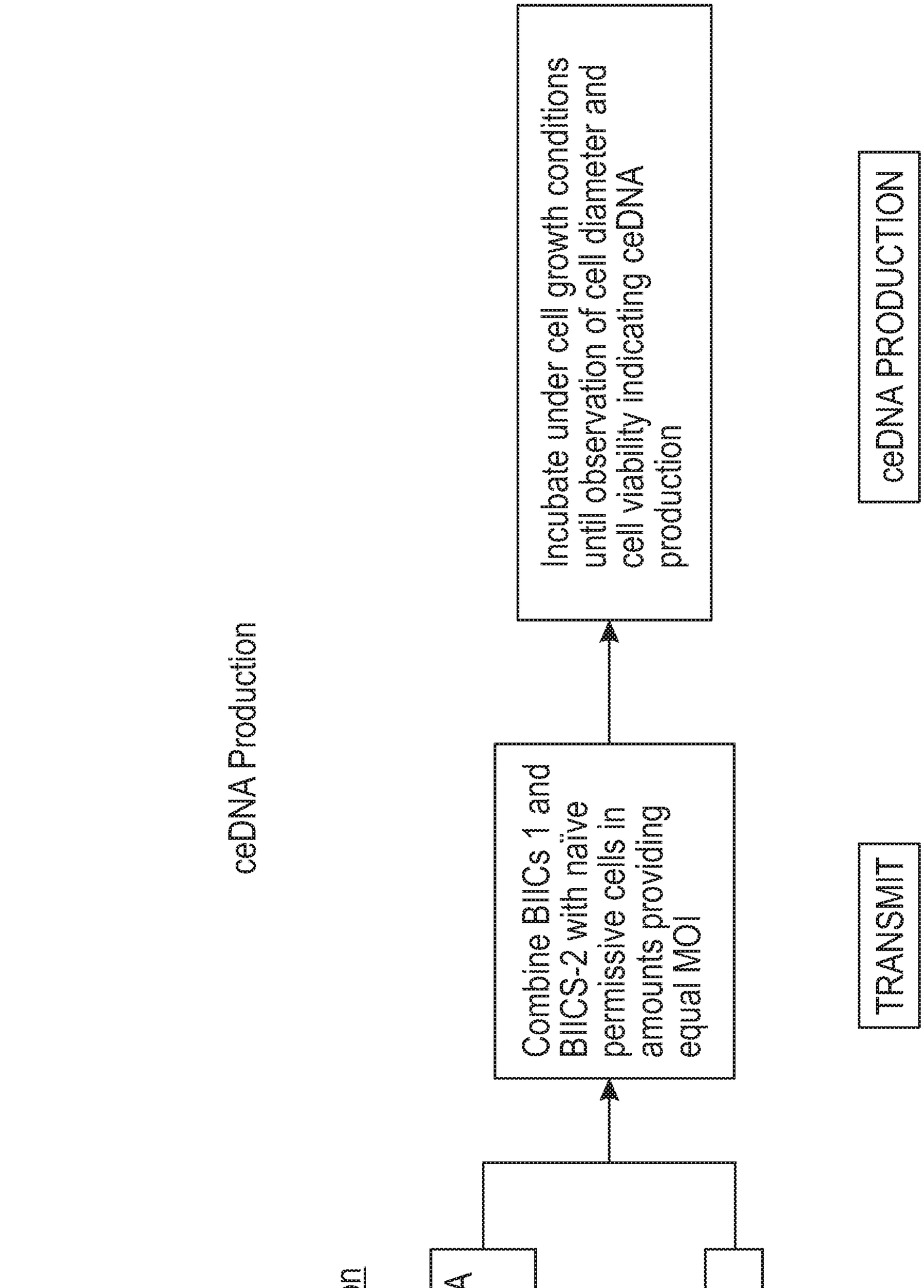

ceDNA generation
BICs-1 (ceDNA construct)
BICs-2 (Rep)
COMBINE
TRANSMIT
Combine BICs 1 and BICS-2 with naïve permissive cells in amounts providing equal MOI
Incubate under cell growth conditions until observation of cell diameter and cell viability indicating ceDNA production
ceDNA PRODUCTION
ceDNA Production

FIG. 4B

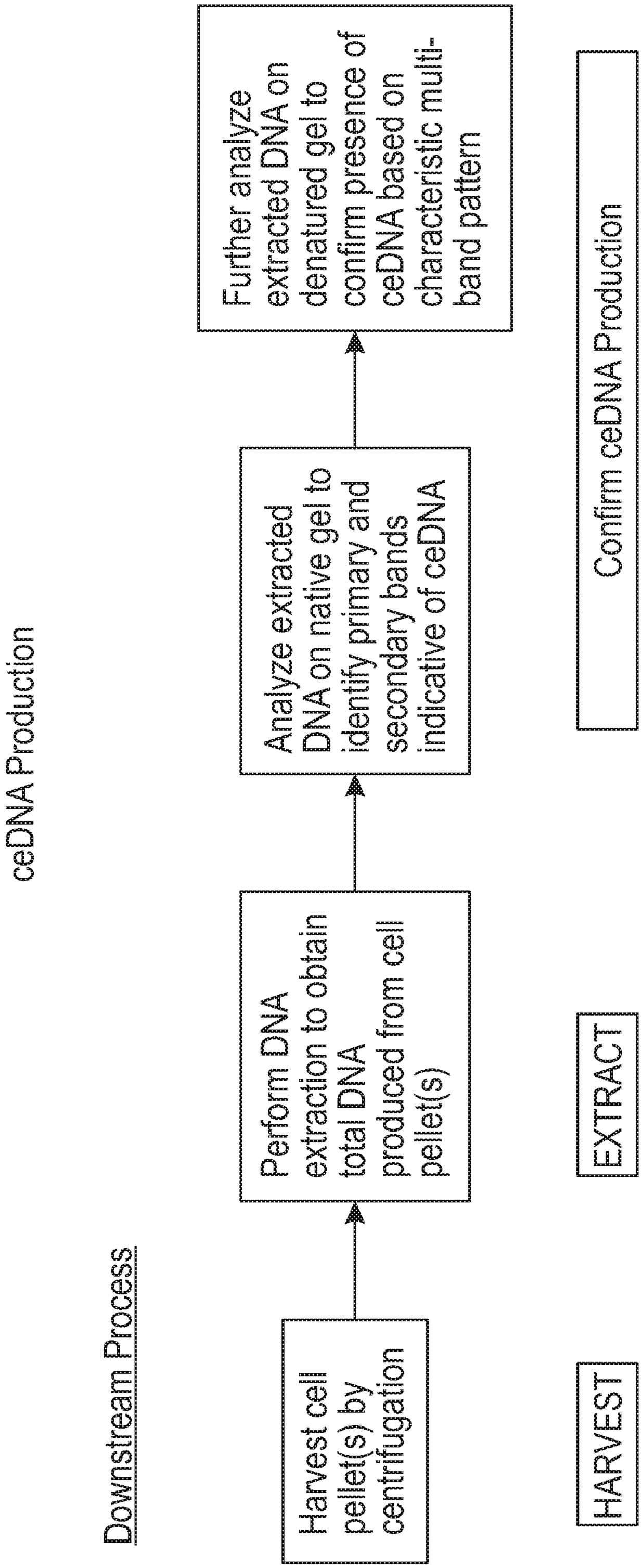

Downstream Process
Harvest cell pellet(s) by centrifugation
HARVEST
Perform DNA extraction to obtain total DNA produced from cell pellet(s)
EXTRACT
Analyze extracted DNA on native gel to identify primary and secondary bands indicative of ceDNA
Confirm ceDNA Production
Further analyze extracted DNA on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

FIG. 4C

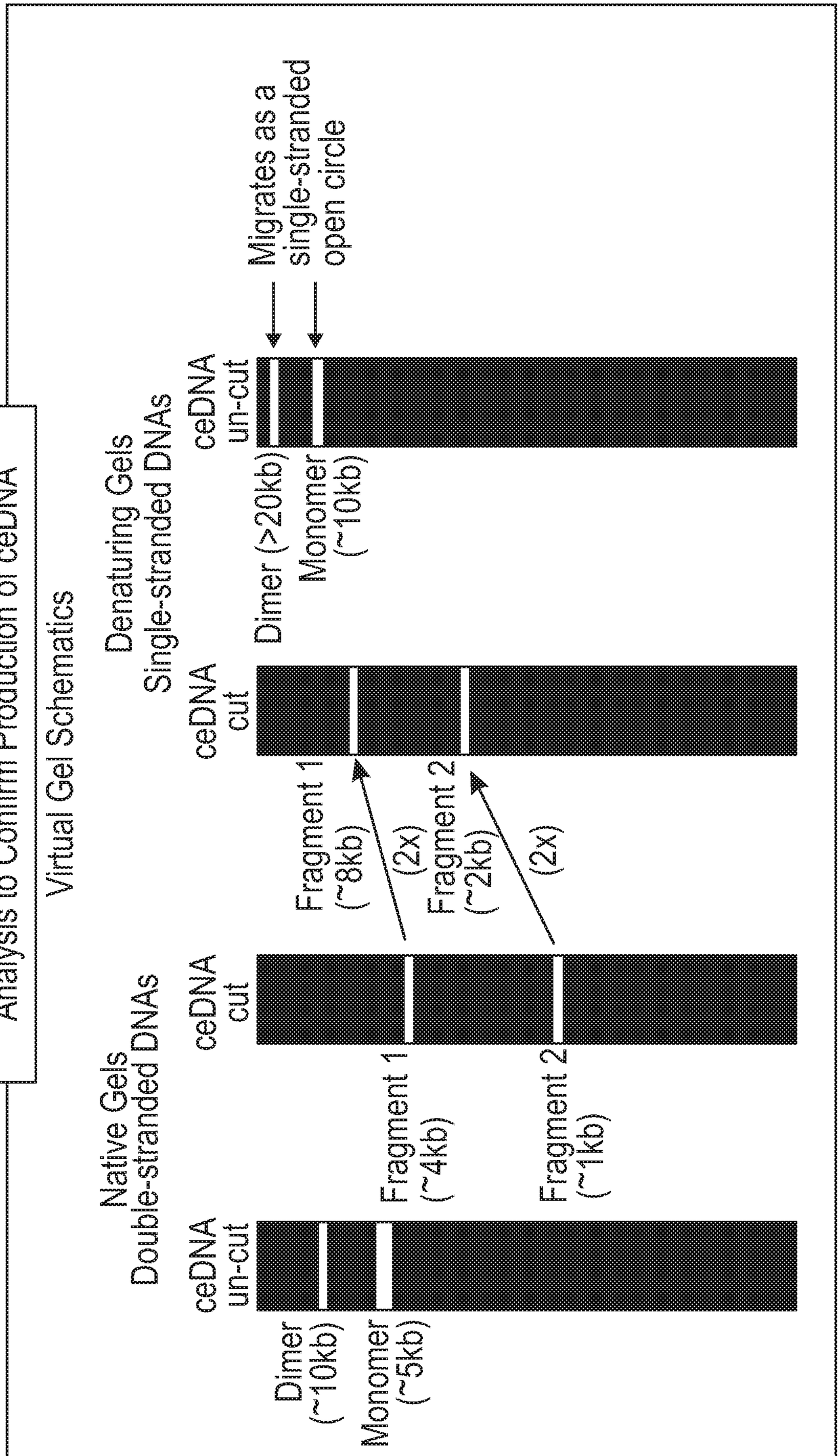

Analysis to Confirm Production of ceDNA
Virtual Gel Schematics
Denaturing Gels
Single-stranded DNAs
ceDNA cut
ceDNA un-cut
Dimer (>20kb)
Monomer (~10kb)
Migrates as a single-stranded open circle
Fragment 1 (~8kb)
(2x)
Fragment 2 (~2kb)
(2x)
Fragment 1 (~4kb)
Fragment 2 (~1kb)
Native Gels
Double-stranded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

FIG. 4D

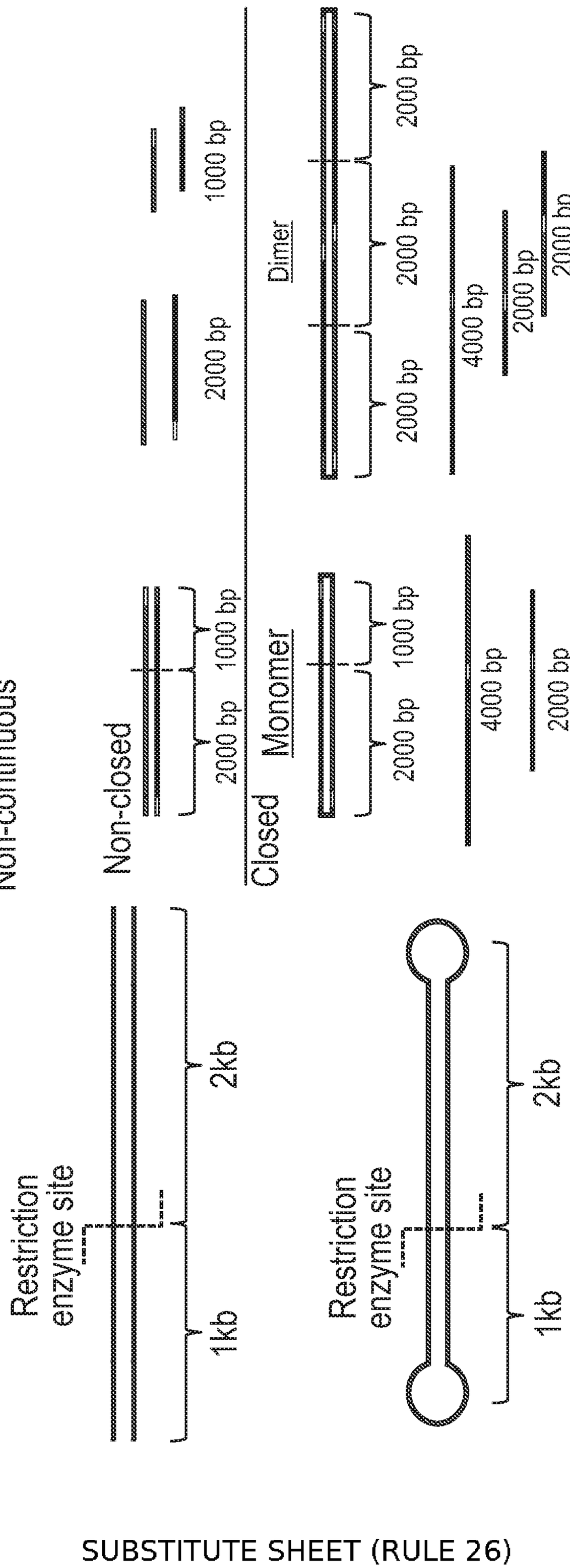

Non-continuous
2000 bp
1000 bp
Dimer
2000 bp
2000 bp
2000 bp
4000 bp
2000 bp
2000 bp
4000 bp
Non-closed
2000 bp
1000 bp
Closed
Monomer
2000 bp
1000 bp
4000 bp
2000 bp
Linear and continuous
Restriction enzyme site
2kb
1kb
Restriction enzyme site
2kb
1kb

FIG. 4E

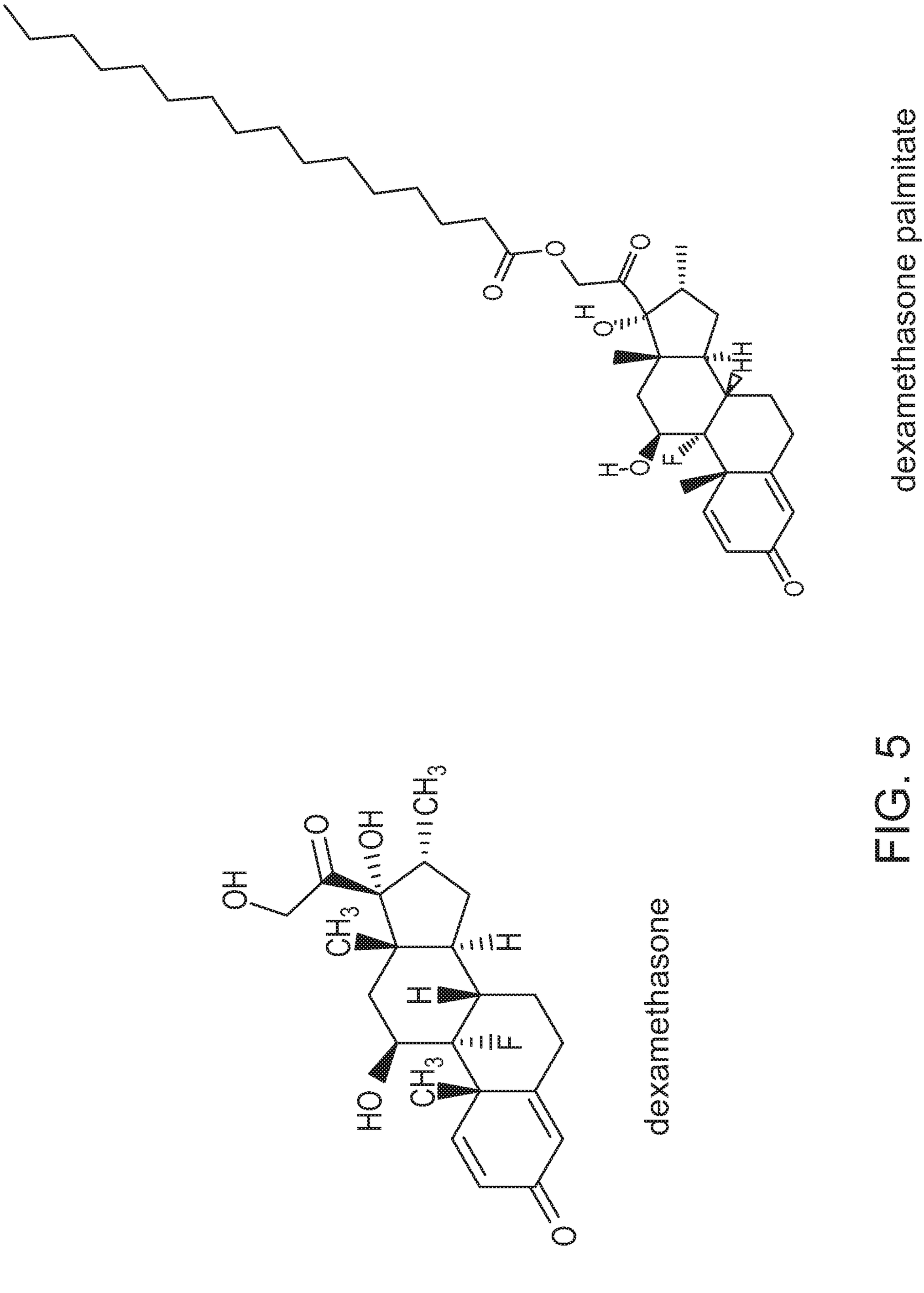


FIG. 5

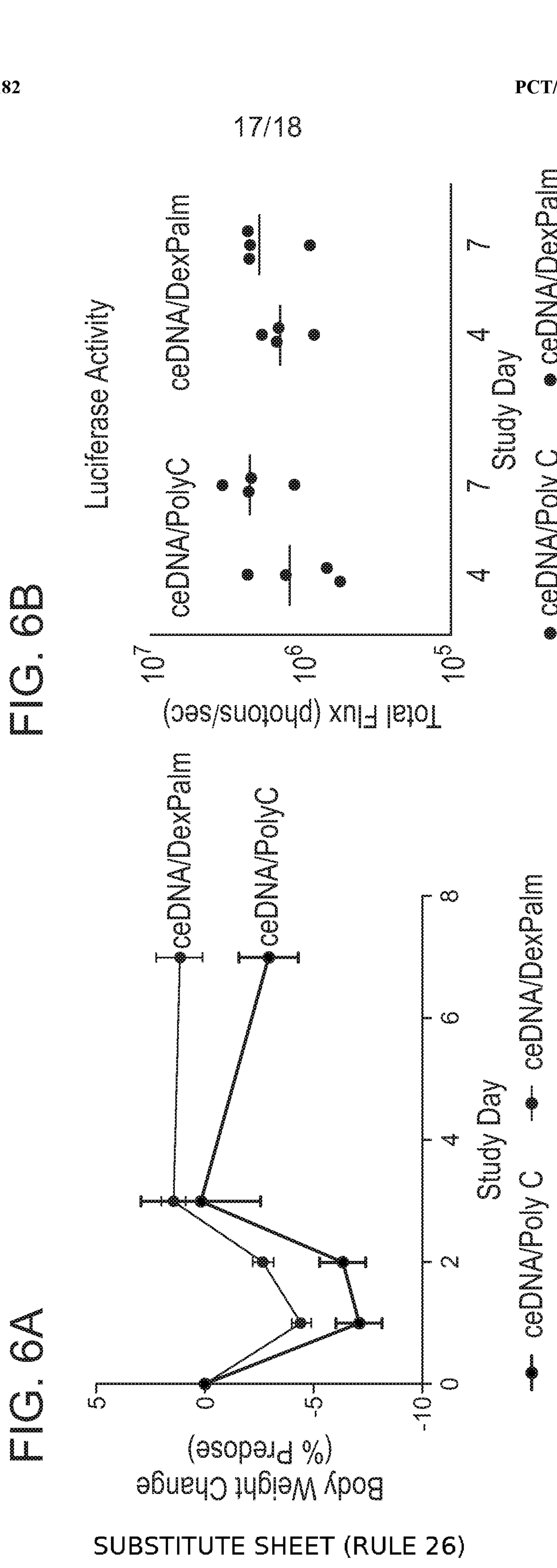

Luciferase Activity
ceDNA/DexPalm
Total Flux (photons/sec)
10^7
10^6
10^5
4
7
Study Day
ceDNA/DexPalm
ceDNA/PolyC
ceDNA/PolyC
4
7
Study Day
ceDNA/DexPalm
ceDNA/DexPalm
ceDNA/PolyC
Body Weight Change
(% Predose)
5
0
-5
-10
0
2
4
6
8
Study Day
ceDNA/DexPalm
ceDNA/Poly C

FIG. 6B

FIG. 6A

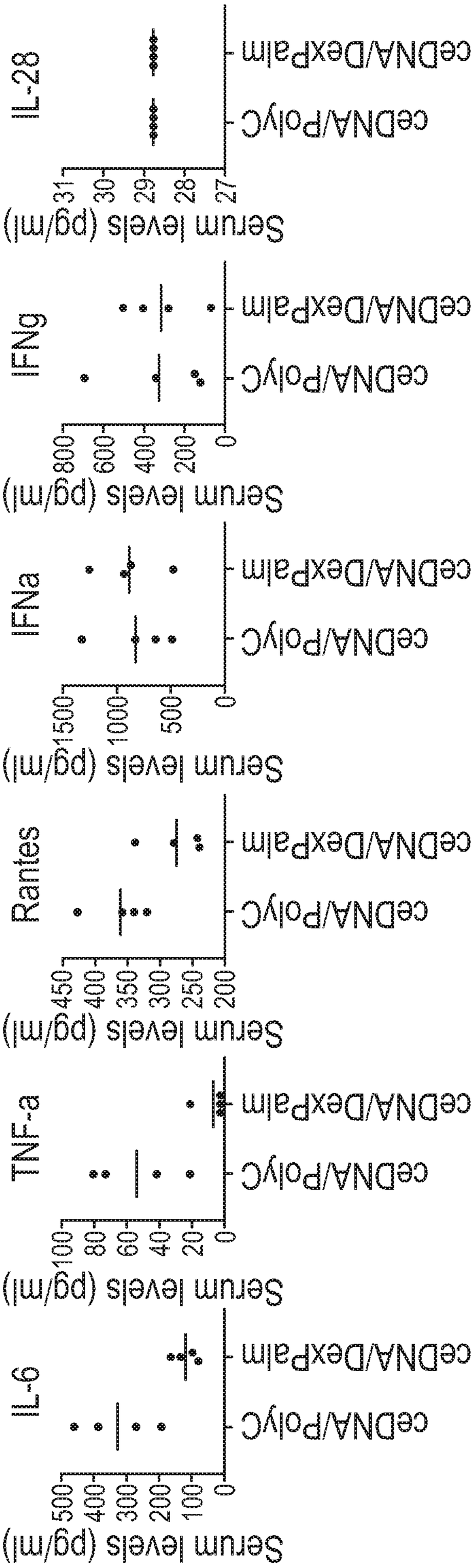

IL-28
IFNg
IFNα
Rantes
TNF-a
IL-6
IP-10
MIP-1b
MIP-1α
MCP-1
IL-18
IL-1b
ceDNA/PolyC
ceDNA/DexPalm
Serum levels (pg/ml)

FIG. 6C