FIG. 1A

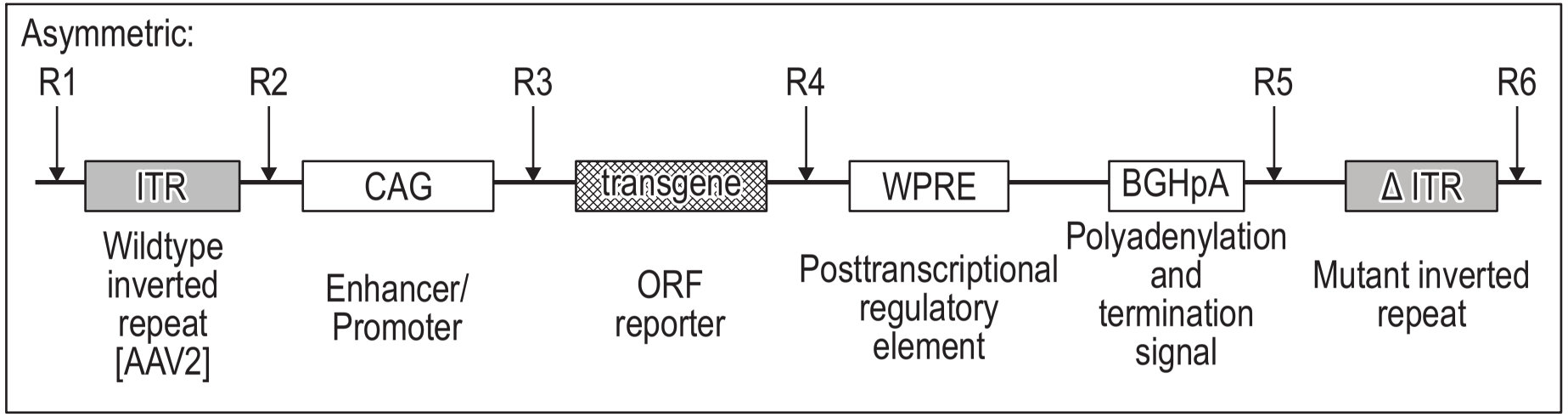

Asymmetric:
R1
R2
R3
R4
R5
R6
ITR
CAG
transgene
WPRE
BGHpA
Δ ITR
Wildtype inverted repeat [AAV2]
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
Mutant inverted repeat

FIG. 1B

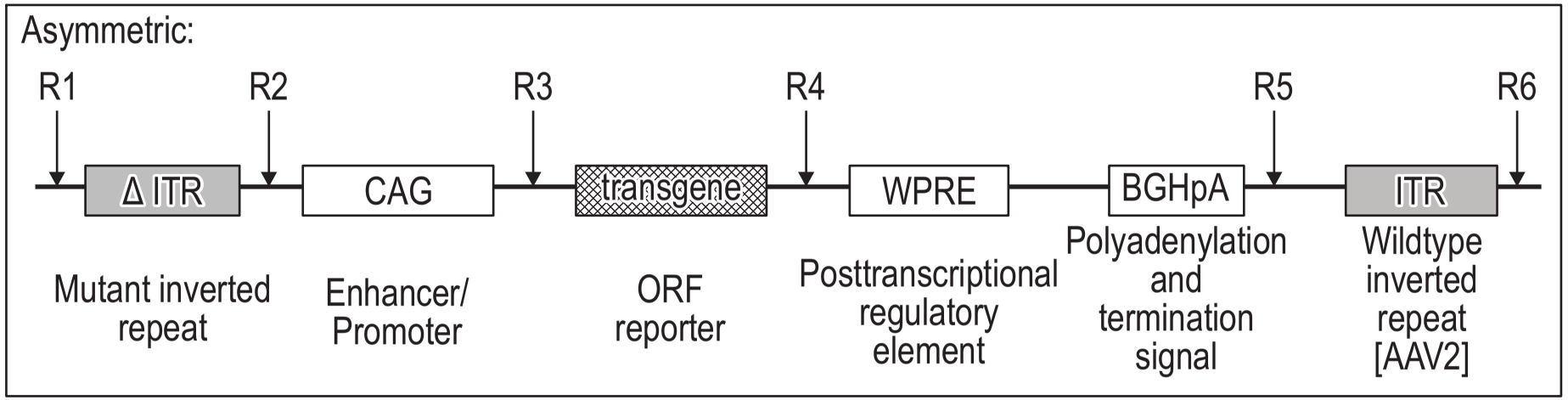

Asymmetric:
R1
R2
R3
R4
R5
R6
Δ ITR
CAG
transgene
WPRE
BGHpA
ITR
Mutant inverted repeat
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
Wildtype inverted repeat [AAV2]

FIG. 1C

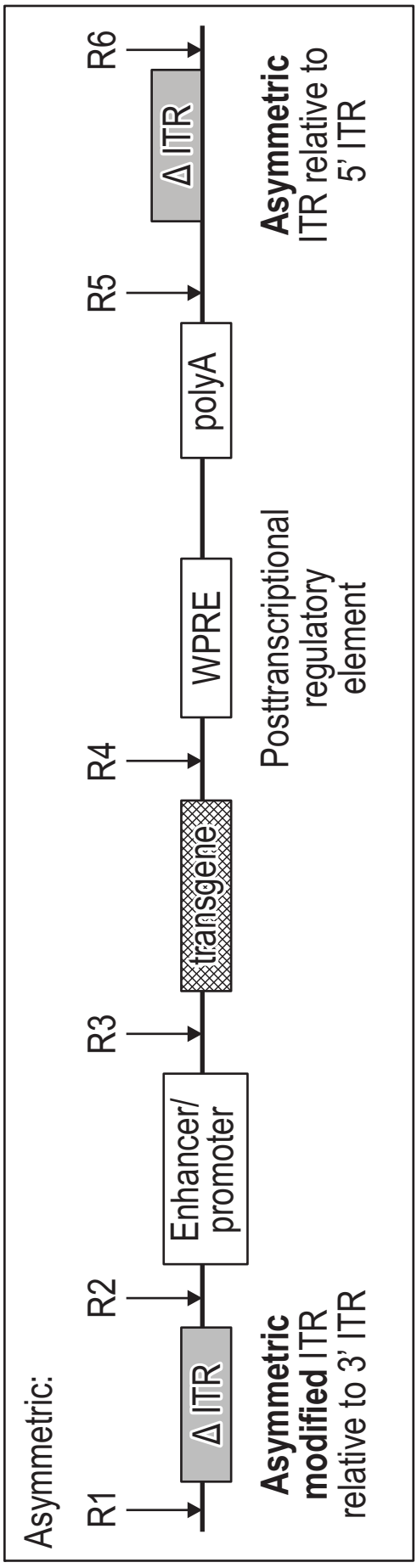

Asymmetric:
R1
R2
R3
R4
R5
R6
Δ ITR
Enhancer/ promoter
transgene
WPRE
polyA
Δ ITR
Asymmetric modified ITR relative to 3' ITR
Posttranscriptional regulatory element
Asymmetric ITR relative to 5' ITR

FIG. 1D

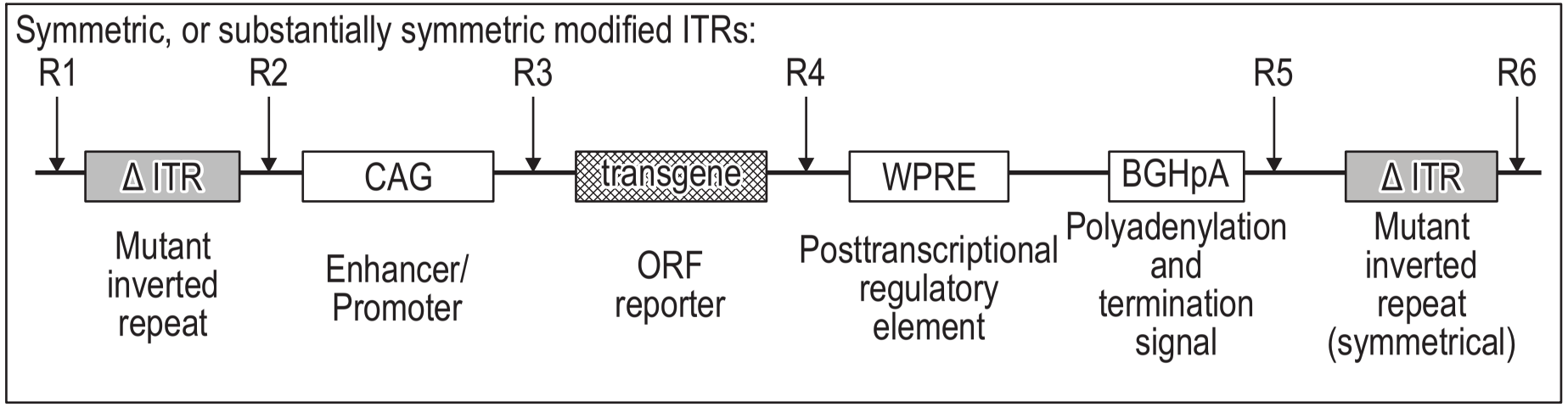

Symmetric, or substantially symmetric modified ITRs:
R1
R2
R3
R4
R5
R6
Δ ITR
CAG
transgene
WPRE
BGHpA
Δ ITR
Mutant inverted repeat
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
Mutant inverted repeat (symmetrical)

FIG. 1E

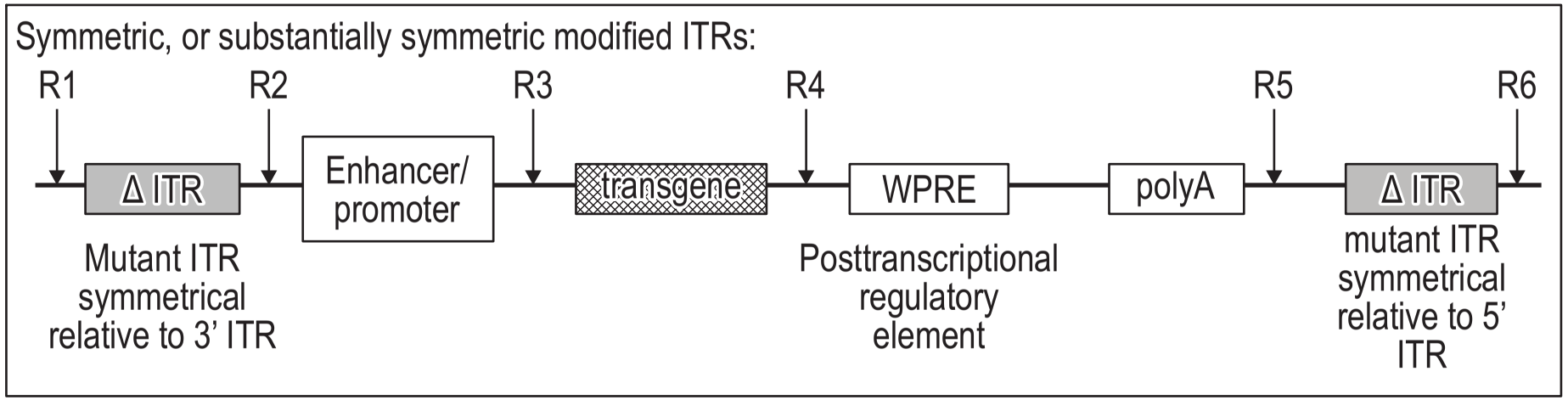

Symmetric, or substantially symmetric modified ITRs:
R1
R2
R3
R4
R5
R6
Δ ITR
Enhancer/ promoter
transgene
WPRE
polyA
Δ ITR
Mutant ITR symmetrical relative to 3' ITR
Posttranscriptional regulatory element
mutant ITR symmetrical relative to 5' ITR

FIG. 1F

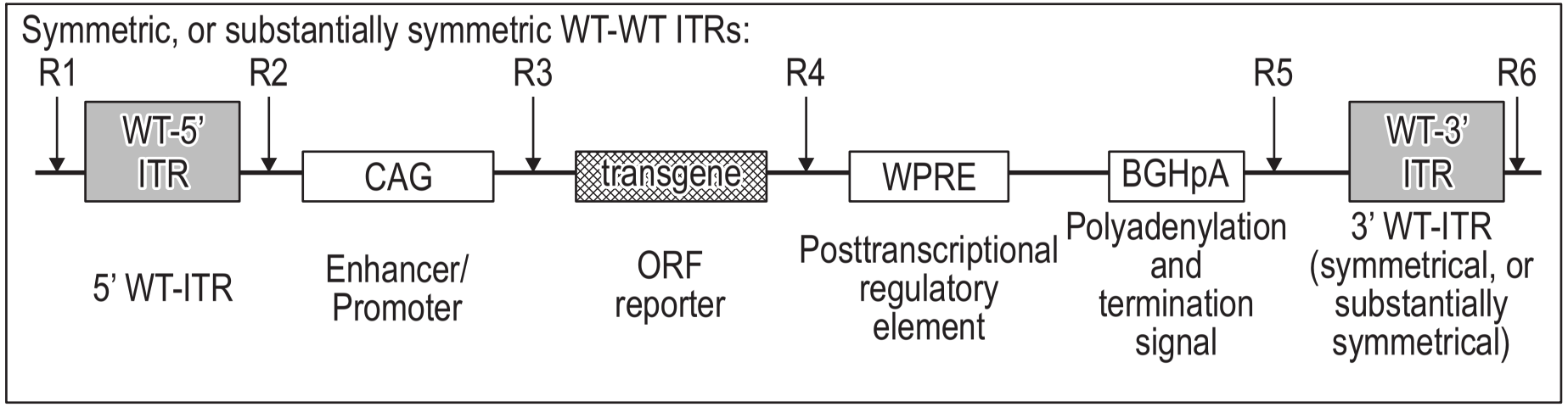
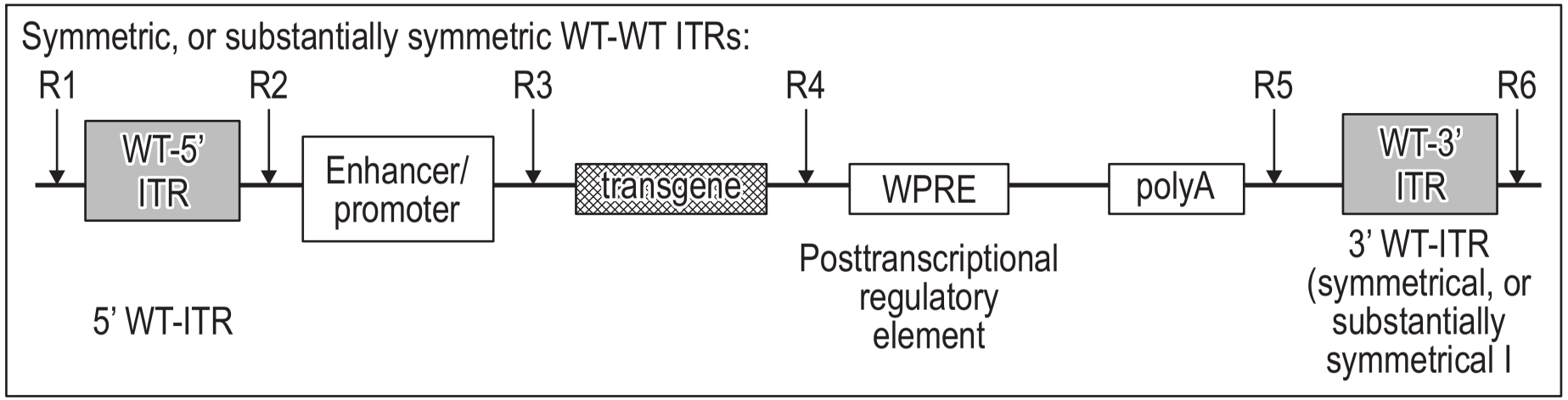

Symmetric, or substantially symmetric WT-WT ITRs:
R1
R2
R3
R4
R5
R6
WT-5' ITR
CAG
transgene
WPRE
BGHpA
WT-3' ITR
5' WT-ITR
Enhancer/ Promoter
ORF reporter
Posttranscriptional regulatory element
Polyadenylation and termination signal
3' WT-ITR (symmetrical, or substantially symmetrical)

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

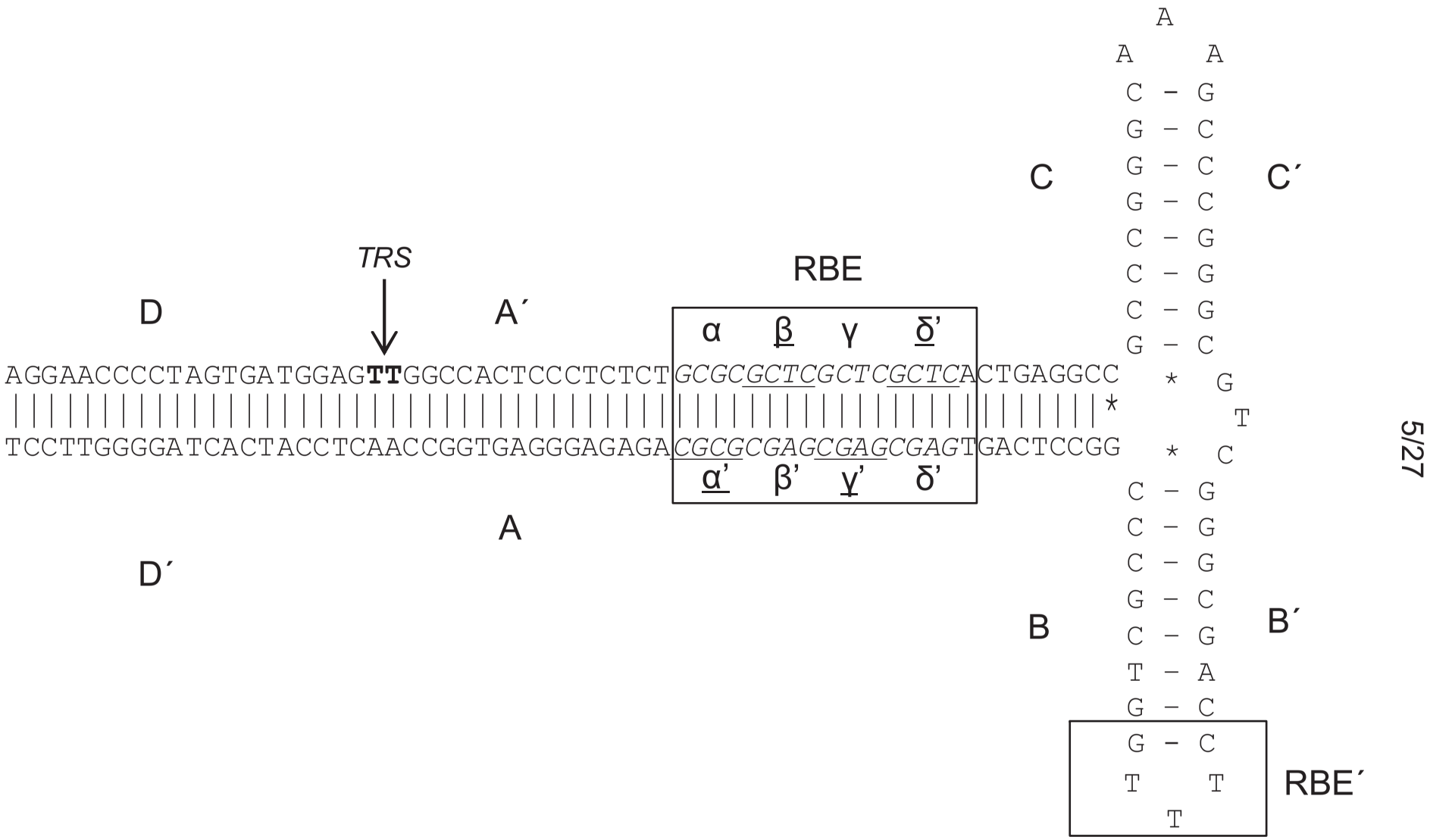

TRS
RBE
D
A´
α β γ δ'
AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCC
TCCTTGGGGATCACTACCTCAACCGGTGAGGGAGAGACGCGCGAGCGAGCGAGTGACTCCGG
α' β' γ' δ'
A
D´
C
C´
B
B´
RBE´
5/27

FIG. 2A

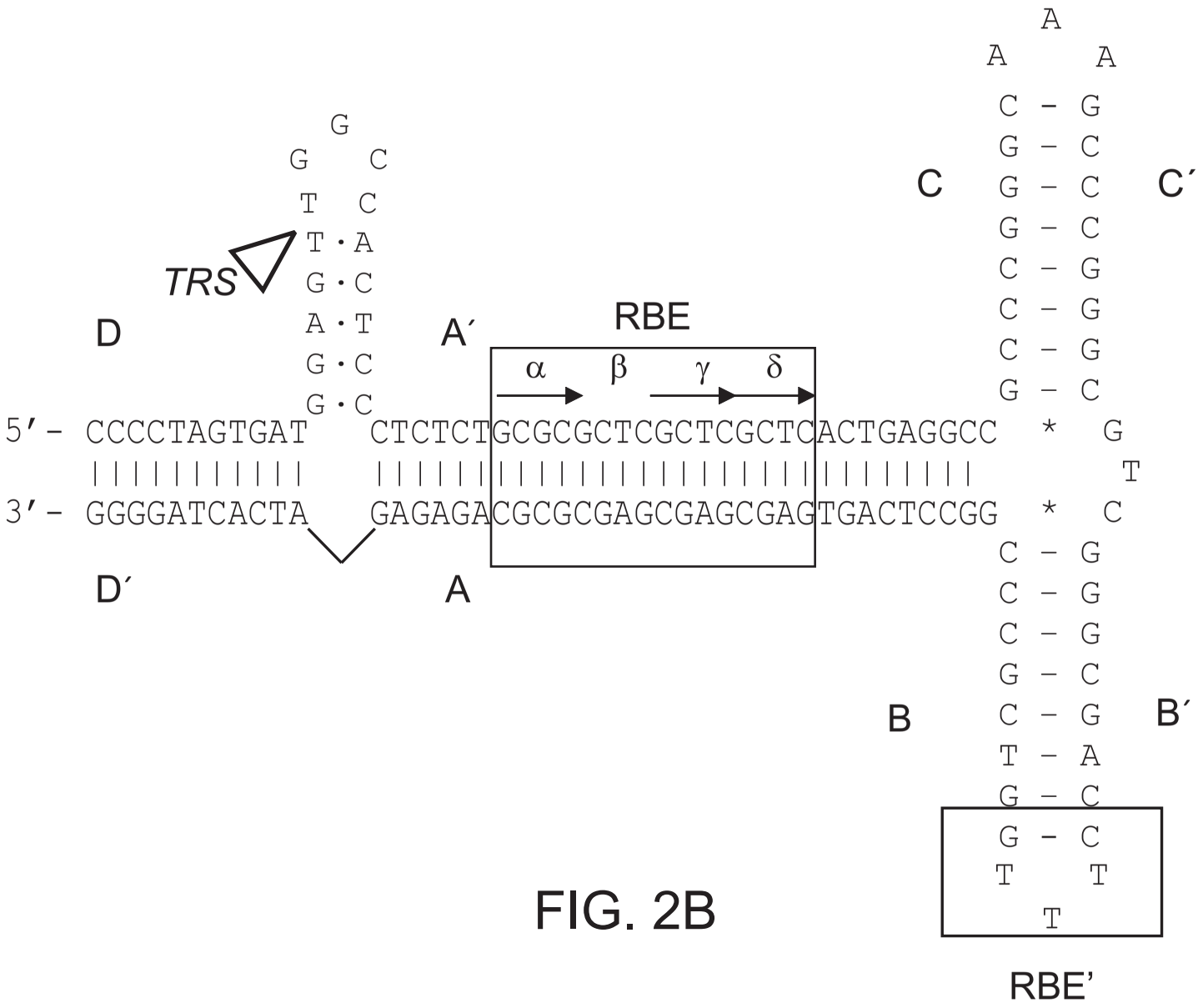

TRS
D
A´
RBE
α
β
γ
δ
5′- CCCCTAGTGAT CTCTCTGCGCGCTCGCTCGCTCACTGAGGCC
3′- GGGGATCACTA GAGAGACGCGCGAGCGAGCGAGTGACTCCGG
D´
A
C
C´
B
B´
RBE'

FIG. 2B

WT ITR (Left)

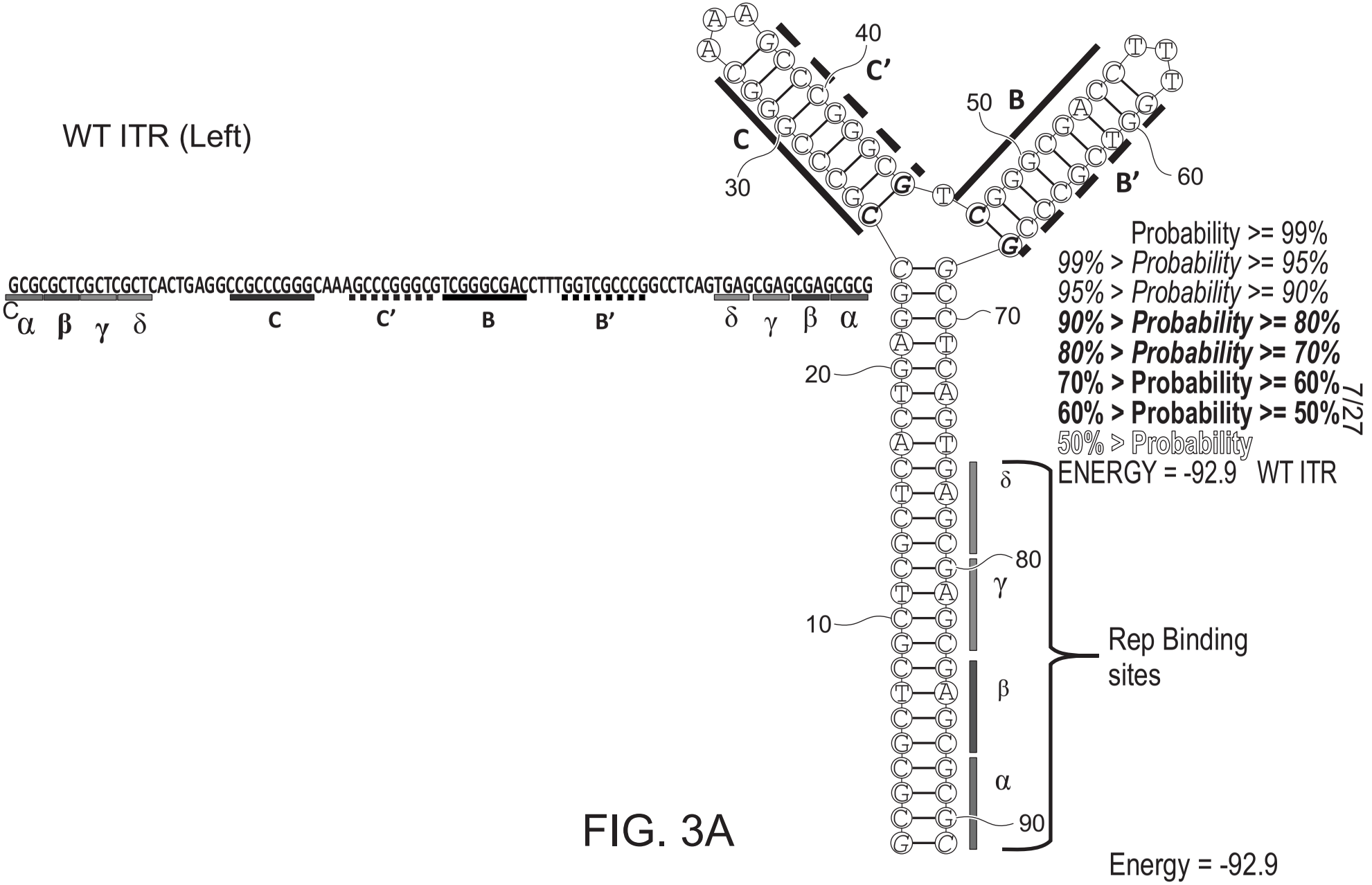


FIG. 3A

ITR-1, Left

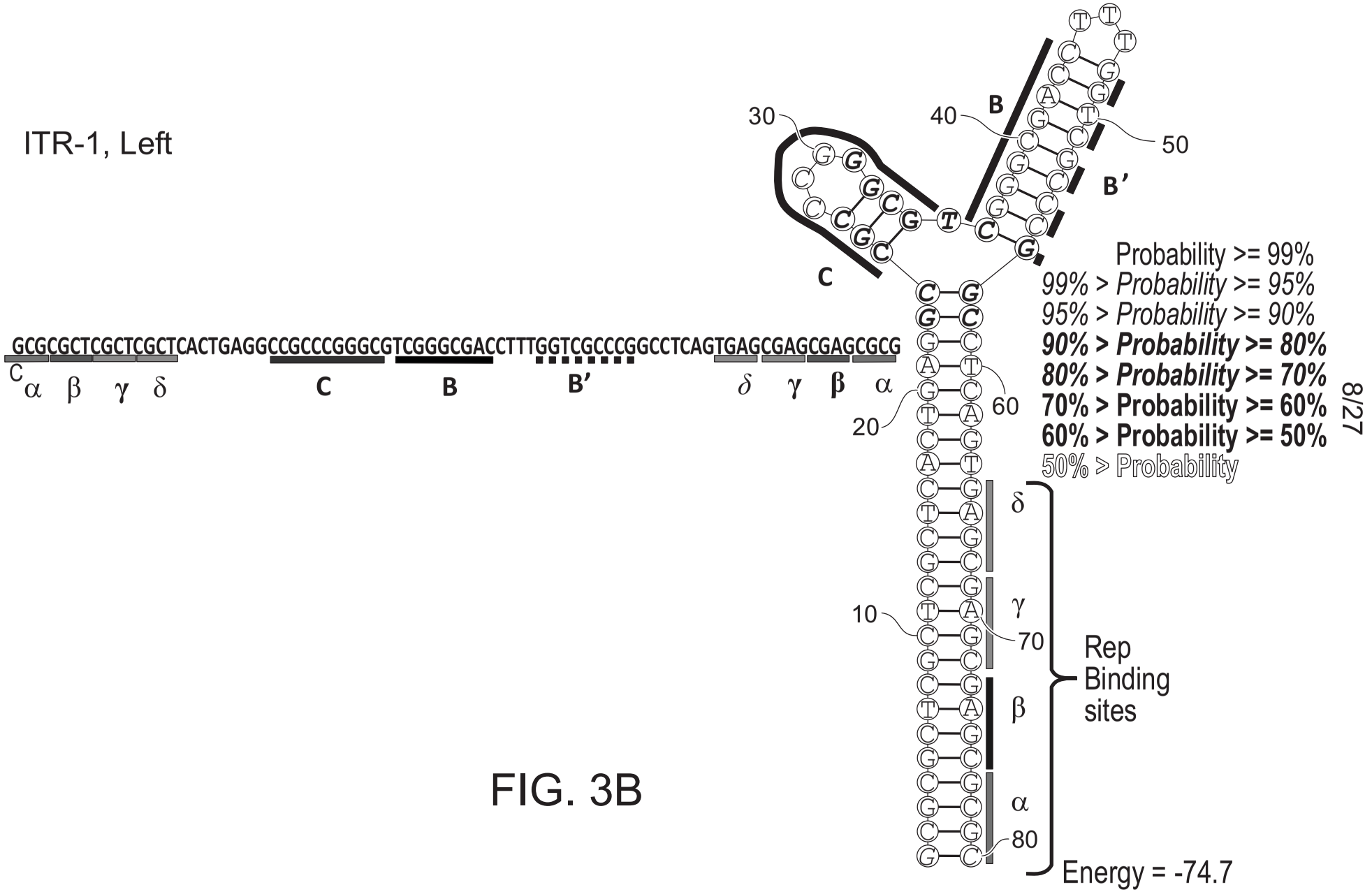
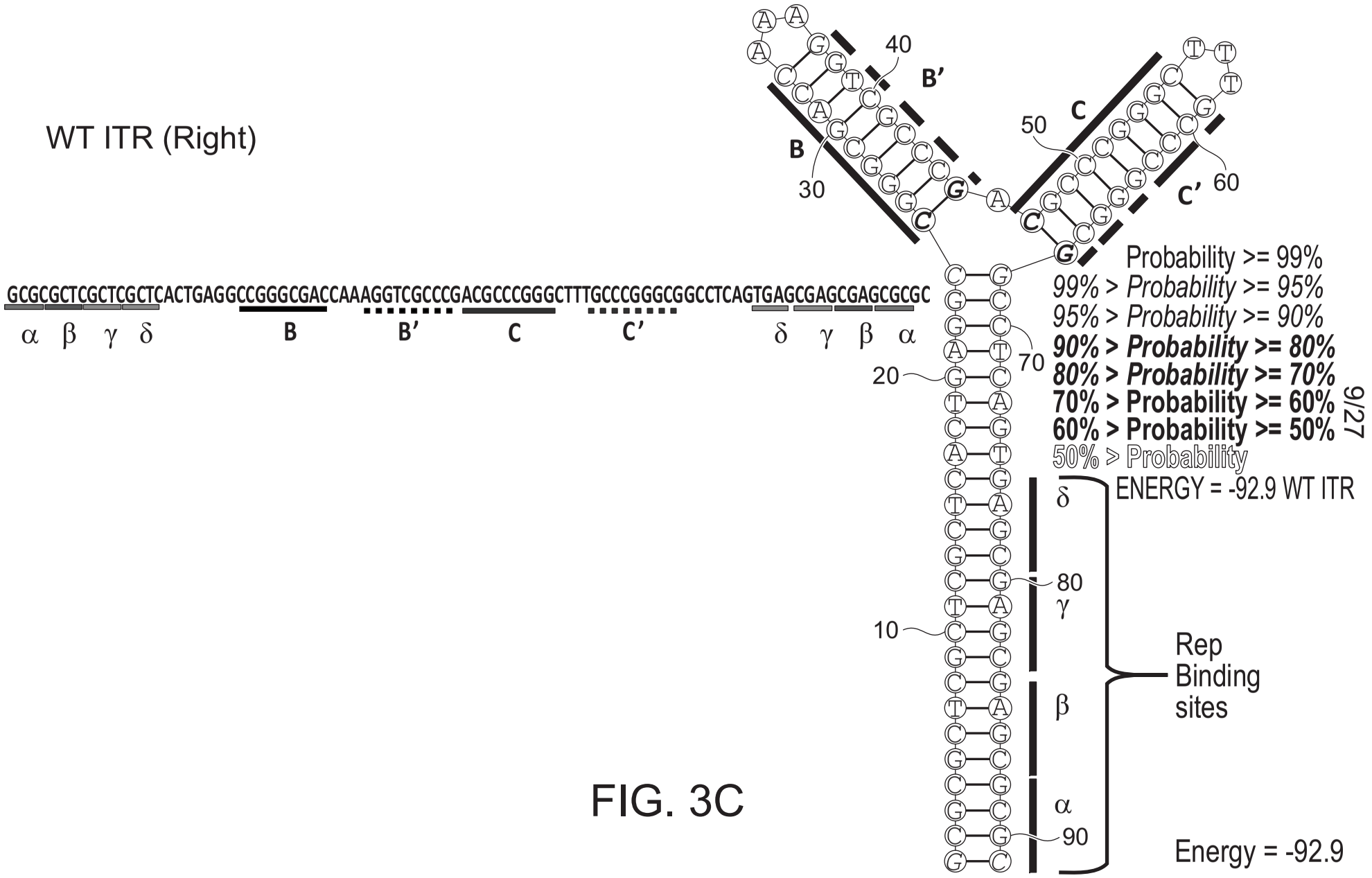


FIG. 3B

WT ITR (Right)



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FIG. 3C

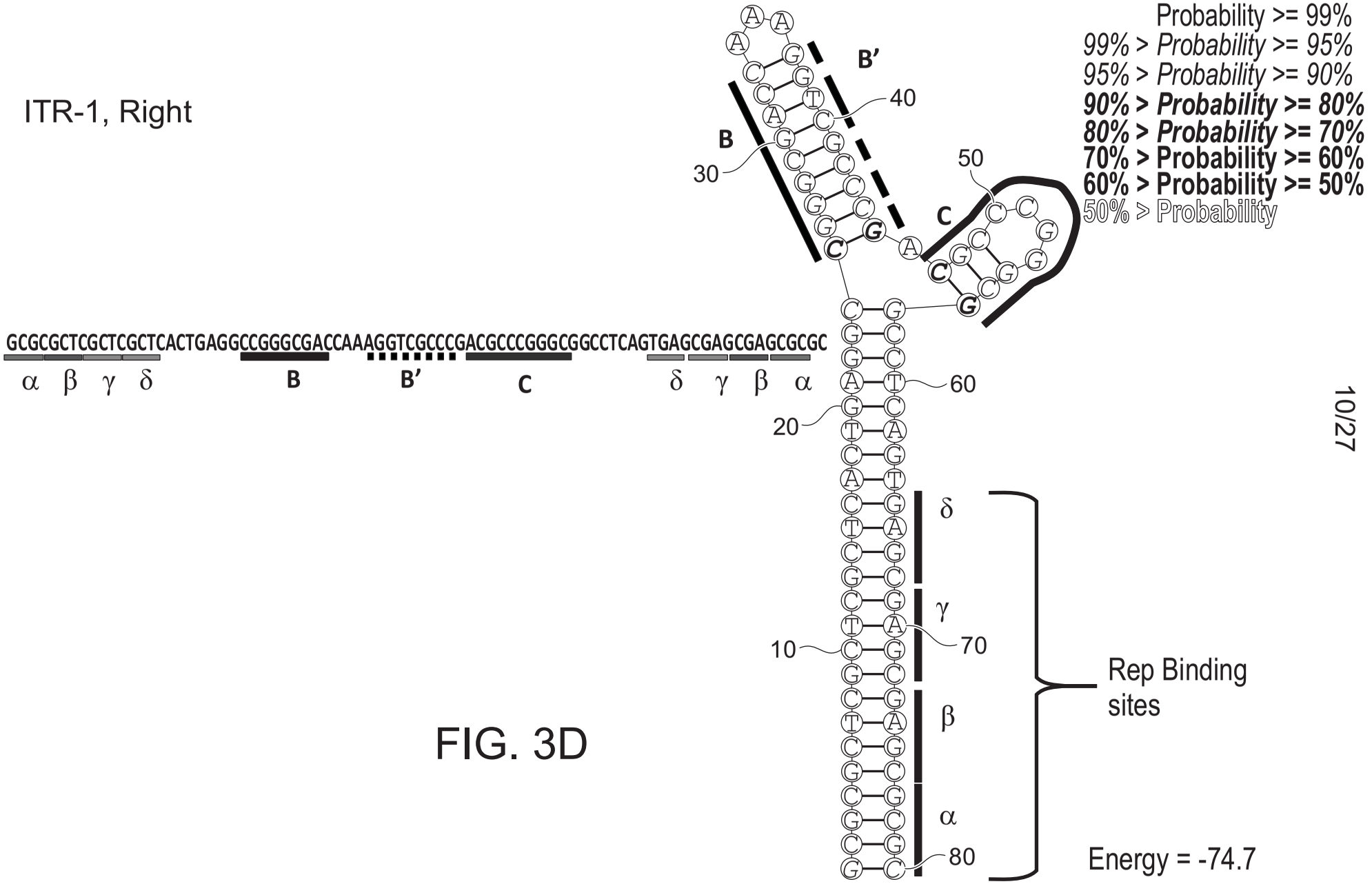

ITR-1, Right
Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability
GCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGC
α β γ δ
B
B'
C
δ γ β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

FIG. 3D

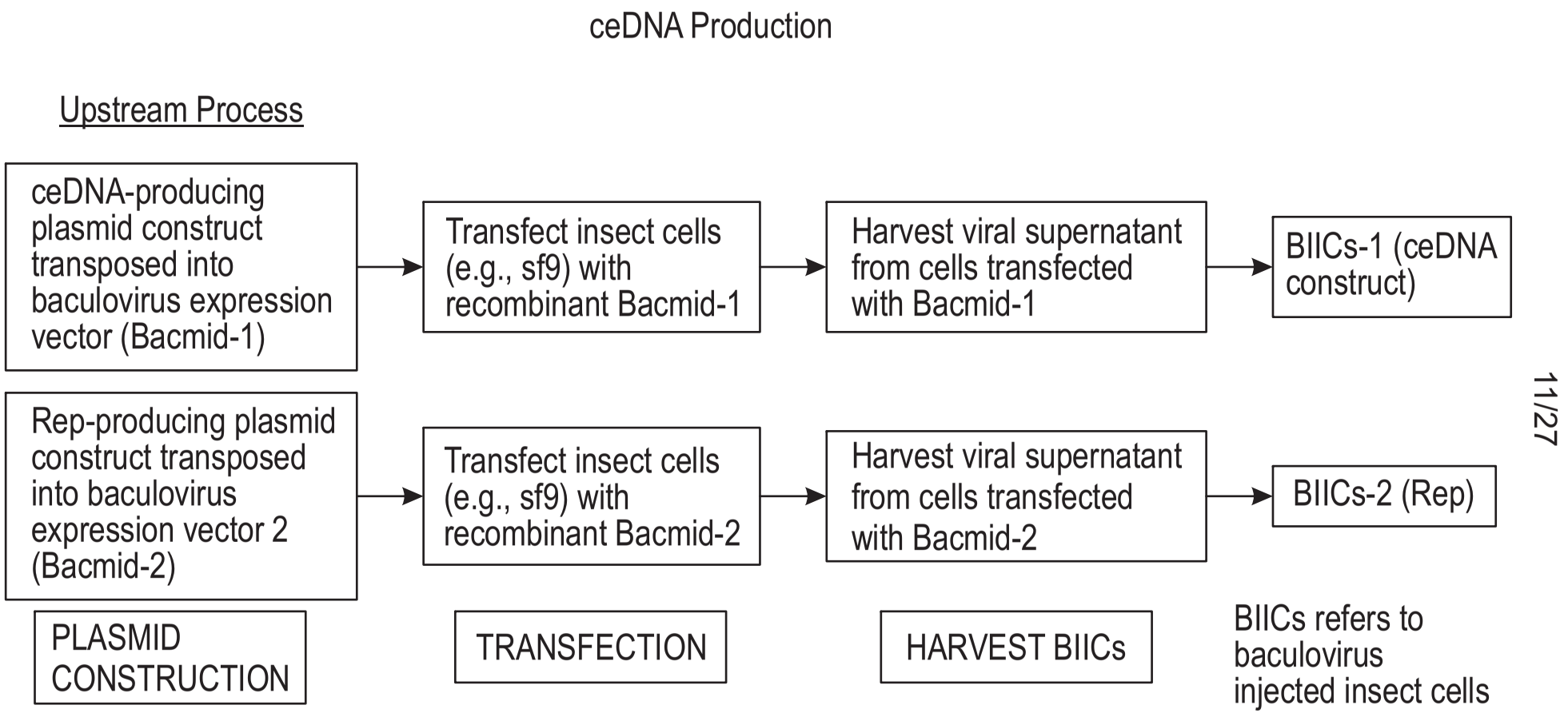

ceDNA Production
Upstream Process
ceDNA-producing plasmid construct transposed into baculovirus expression vector (Bacmid-1)
Transfect insect cells (e.g., sf9) with recombinant Bacmid-1
Harvest viral supernatant from cells transfected with Bacmid-1
BIICs-1 (ceDNA construct)
Rep-producing plasmid construct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

FIG. 4A

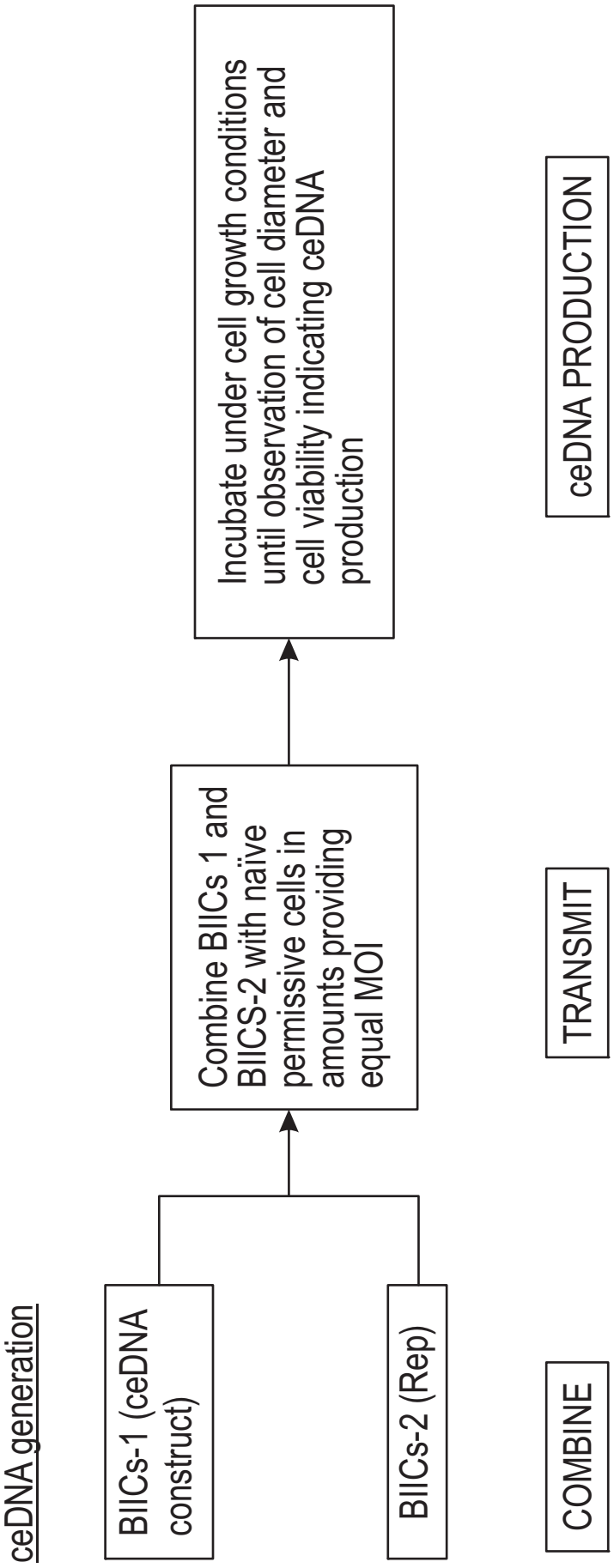

ceDNA Production
ceDNA generation
BIICs-1 (ceDNA construct)
BIICs-2 (Rep)
COMBINE
Combine BIICs 1 and BIICS-2 with naïve permissive cells in amounts providing equal MOI
TRANSMIT
Incubate under cell growth conditions until observation of cell diameter and cell viability indicating ceDNA production
ceDNA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

FIG. 4B

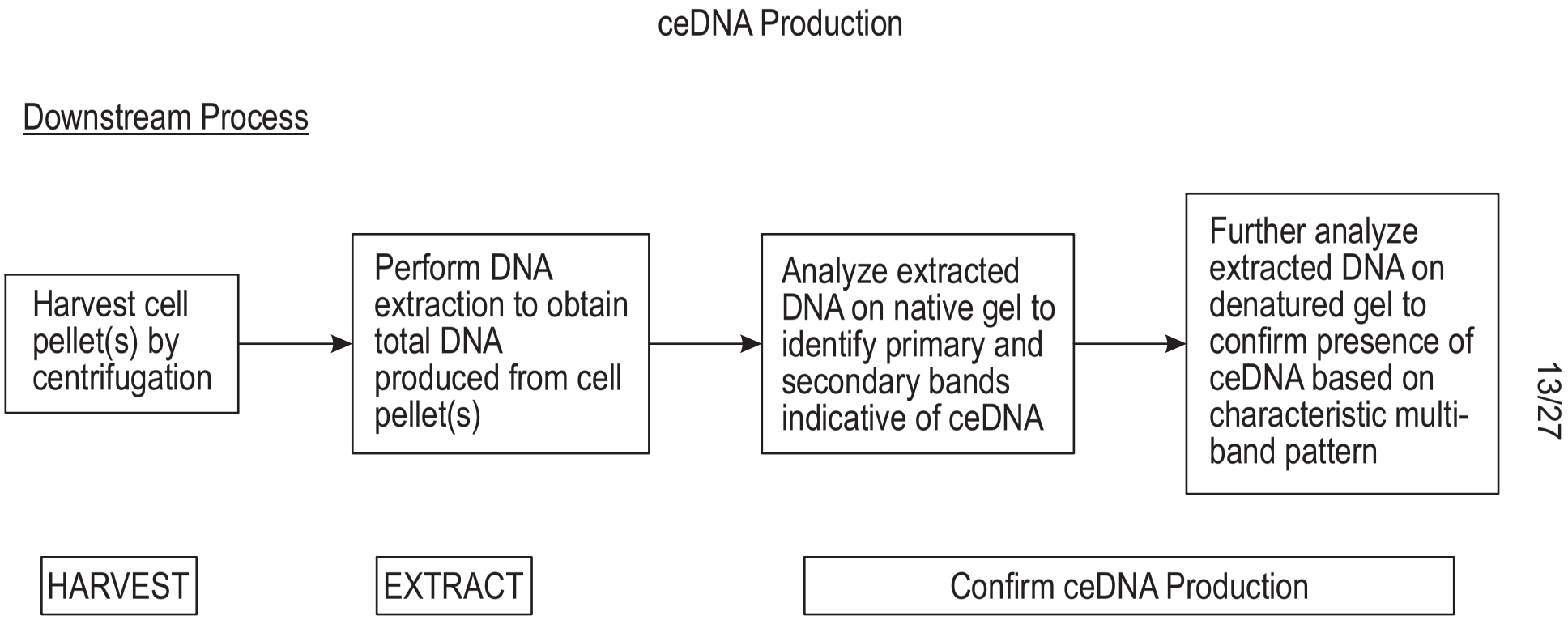

ceDNA Production
Downstream Process
Harvest cell pellet(s) by centrifugation
Perform DNA extraction to obtain total DNA produced from cell pellet(s)
Analyze extracted DNA on native gel to identify primary and secondary bands indicative of ceDNA
Further analyze extracted DNA on denatured gel to confirm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

FIG. 4C

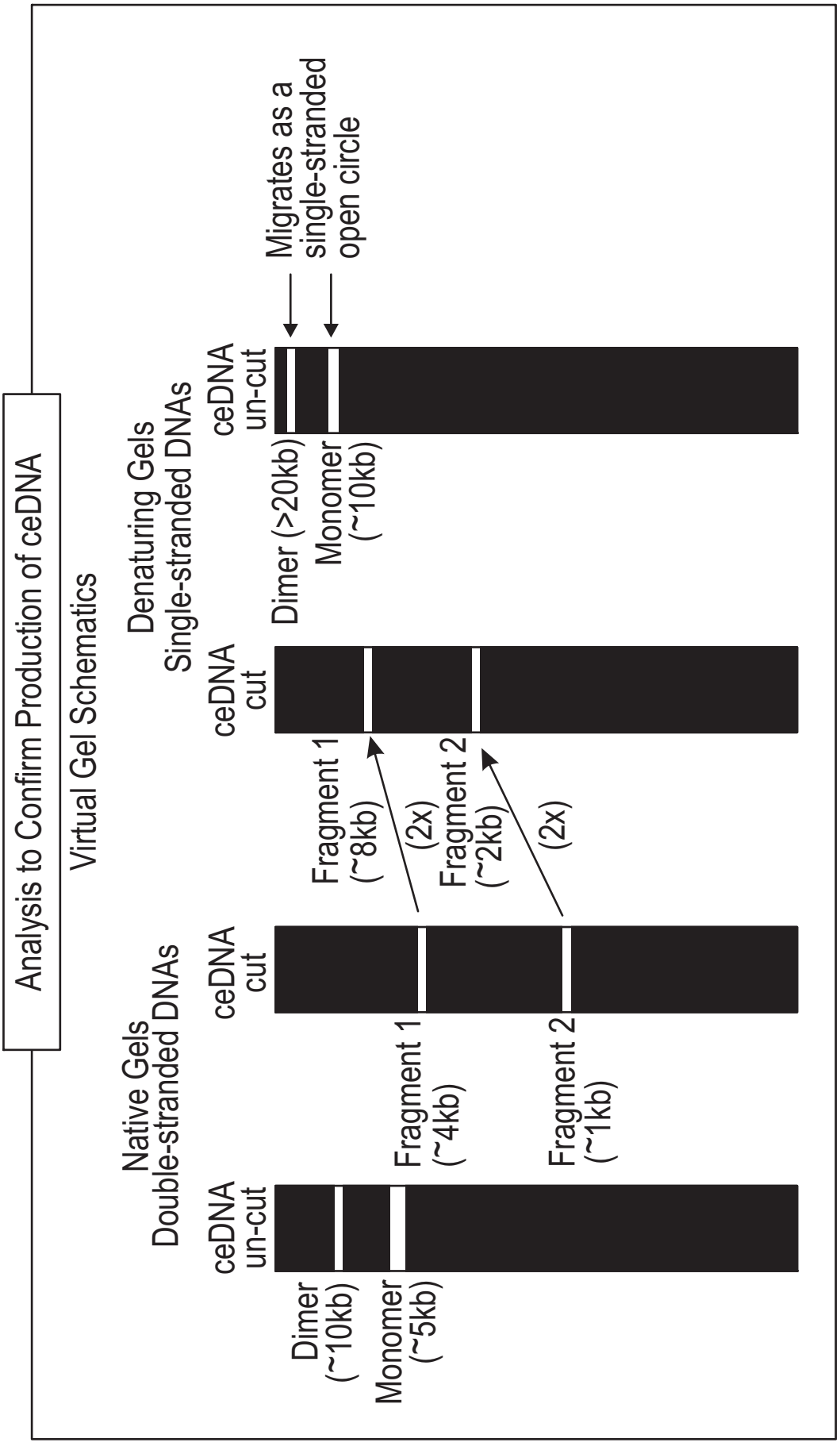

Analysis to Confirm Production of ceDNA
Virtual Gel Schematics
Native Gels
Double-stranded DNAs
ceDNA
un-cut
Dimer
(~10kb)
Monomer
(~5kb)
ceDNA
cut
Fragment 1
(~4kb)
Fragment 2
(~1kb)
Denaturing Gels
Single-stranded DNAs
ceDNA
cut
Fragment 1
(~8kb)
(2x)
Fragment 2
(~2kb)
(2x)
ceDNA
un-cut
Dimer (>20kb)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

FIG. 4D

FIG. 4E

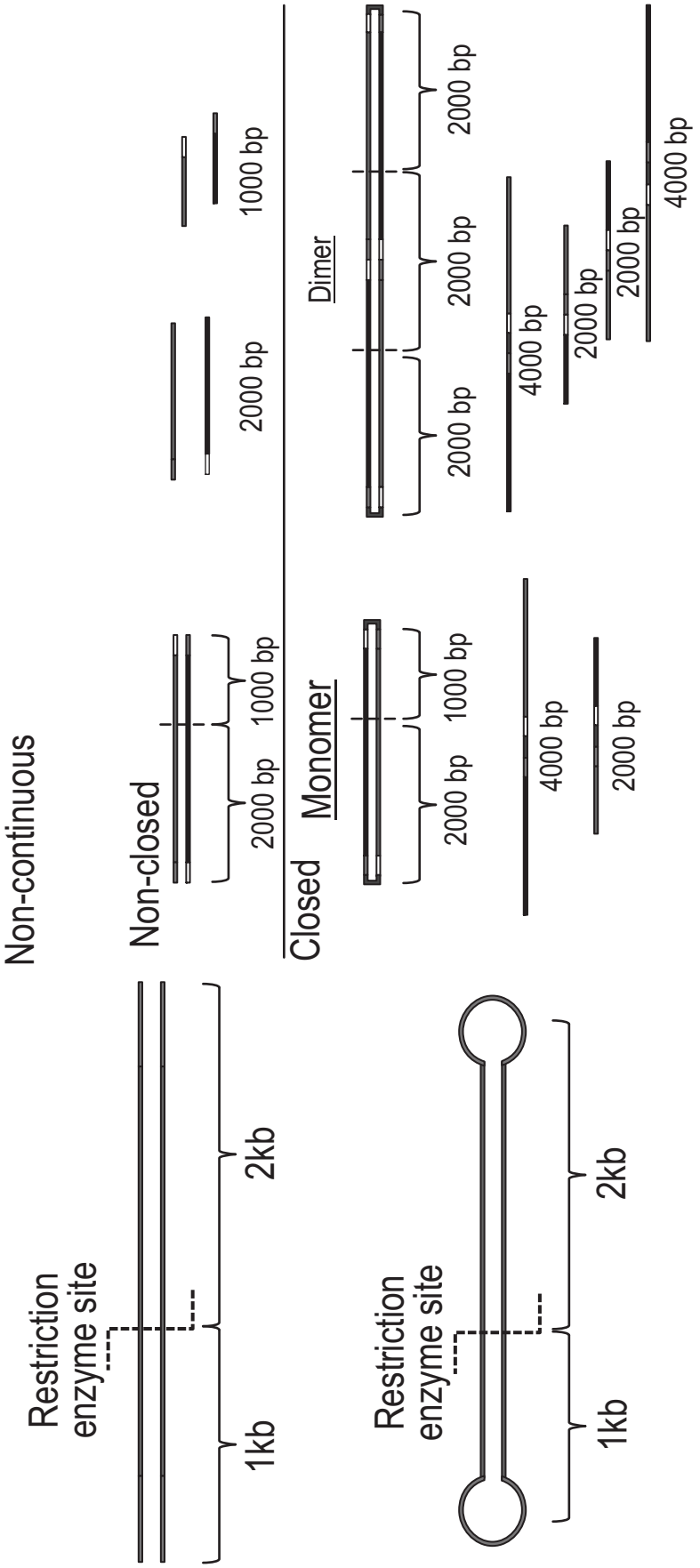

Non-continuous
Non-closed
Closed
Monomer
Dimer
Restriction enzyme site
1kb
2kb
2000 bp
1000 bp
4000 bp
Linear and continuous

FIG. 5

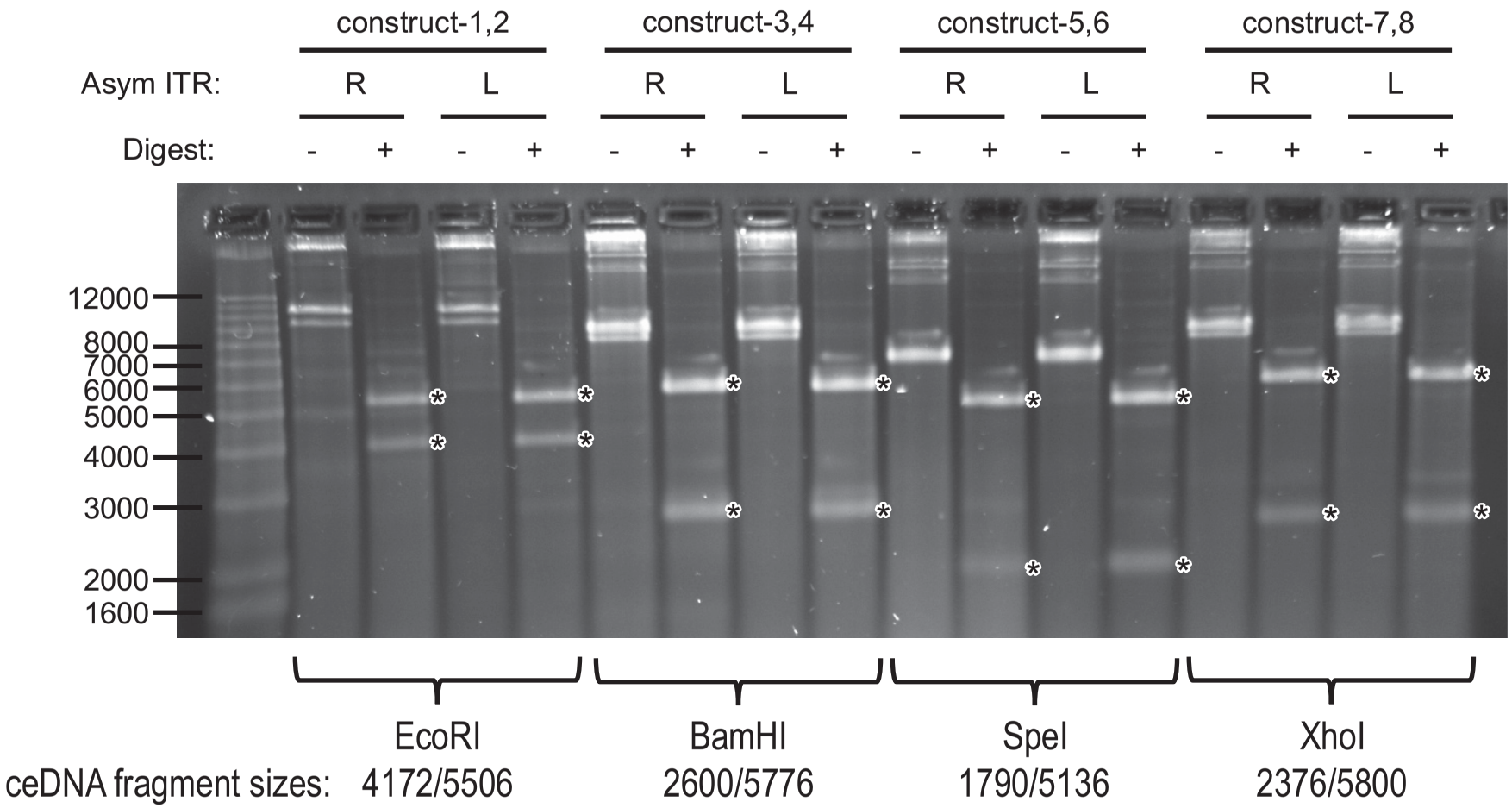

construct-1,2
construct-3,4
construct-5,6
construct-7,8
Asym ITR:
R
L
Digest:
-
+
12000
8000
7000
6000
5000
4000
3000
2000
1600
EcoRI
BamHI
SpeI
XhoI
ceDNA fragment sizes:
4172/5506
2600/5776
1790/5136
2376/5800

FIG. 6

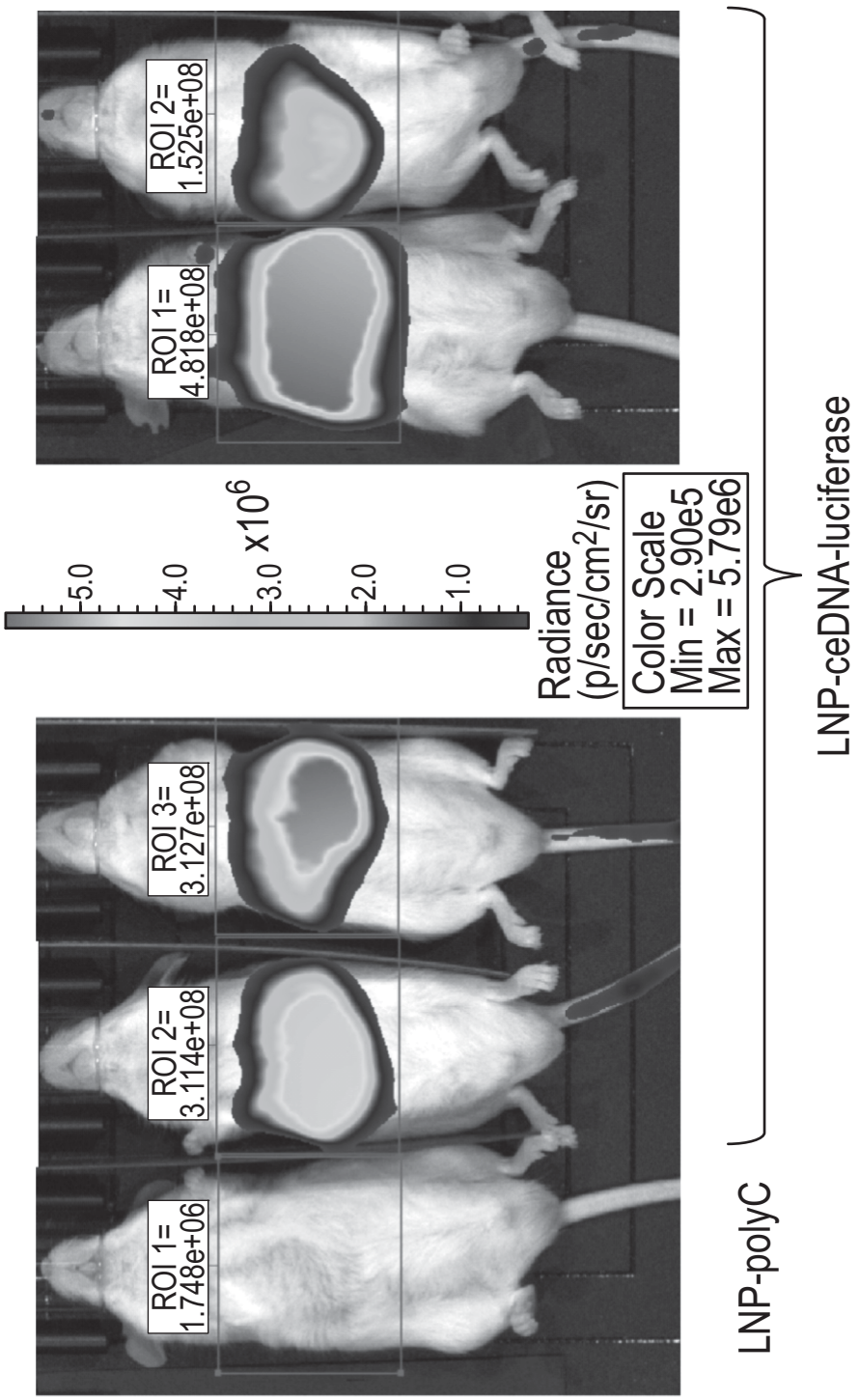
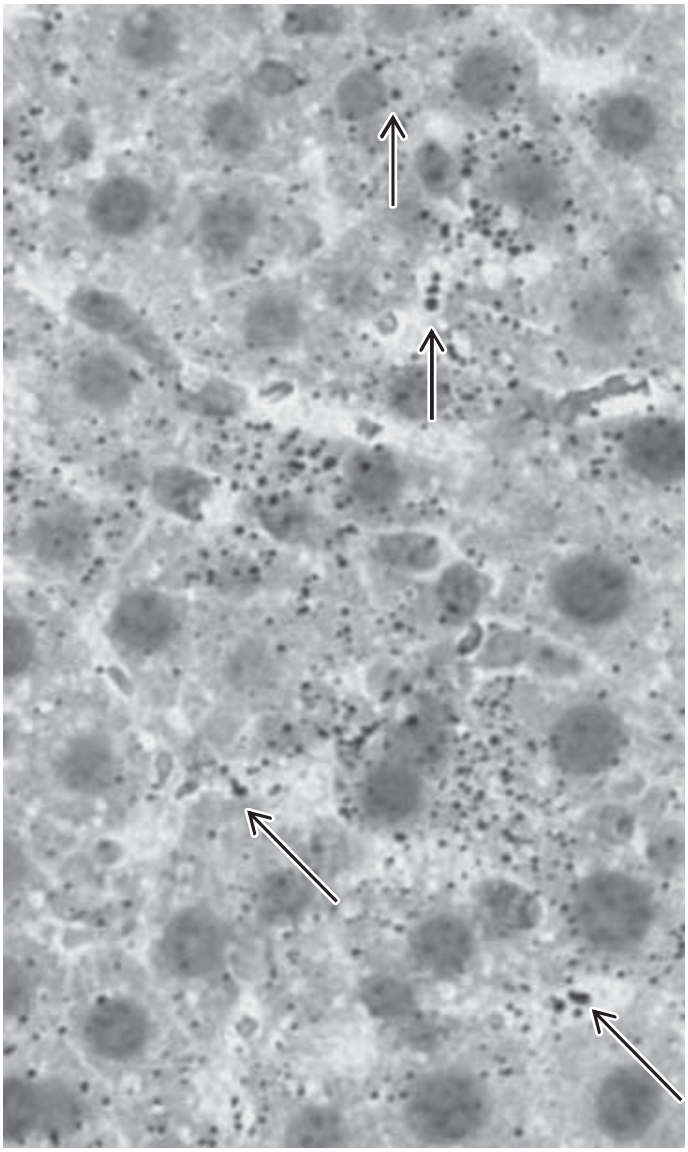

ROI 1= 1.748e+06
ROI 2= 3.114e+08
ROI 3= 3.127e+08
ROI 1= 4.818e+08
ROI 2= 1.525e+08
5.0
4.0
3.0
2.0
1.0
x10^6
Radiance (p/sec/cm²/sr)
Color Scale
Min = 2.90e5
Max = 5.79e6
LNP-polyC
LNP-ceDNA-luciferase

FIG. 7



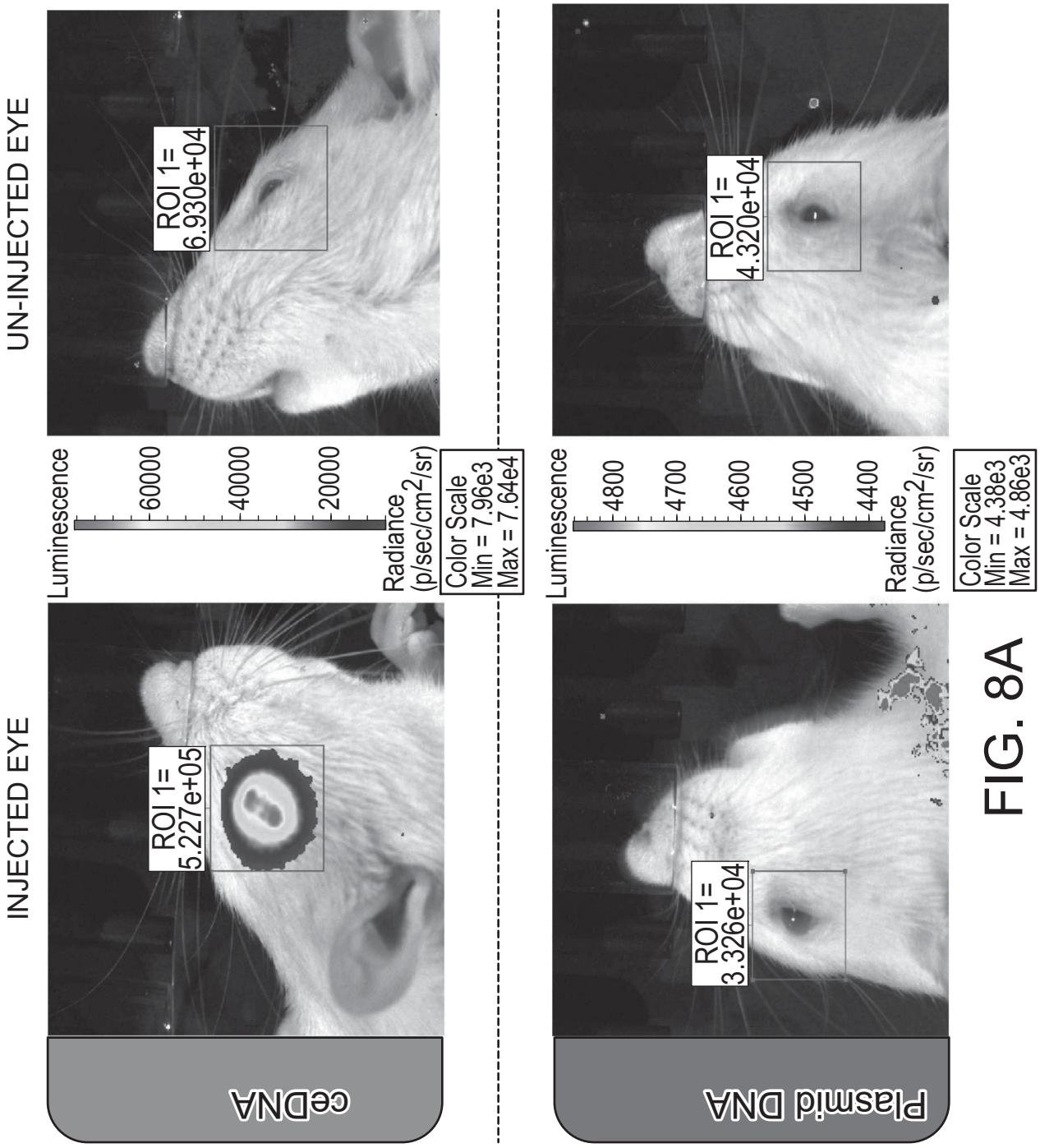

UN-INJECTED EYE
INJECTED EYE
ROI 1= 6.930e+04
ROI 1= 5.227e+05
ROI 1= 4.320e+04
ROI 1= 3.326e+04
Luminescence
60000
40000
20000
Radiance (p/sec/cm2/sr)
Color Scale Min = 7.96e3 Max = 7.64e4
Luminescence
4800
4700
4600
4500
4400
Radiance (p/sec/cm2/sr)
Color Scale Min = 4.38e3 Max = 4.86e3
ceDNA
Plasmid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

FIG. 8A

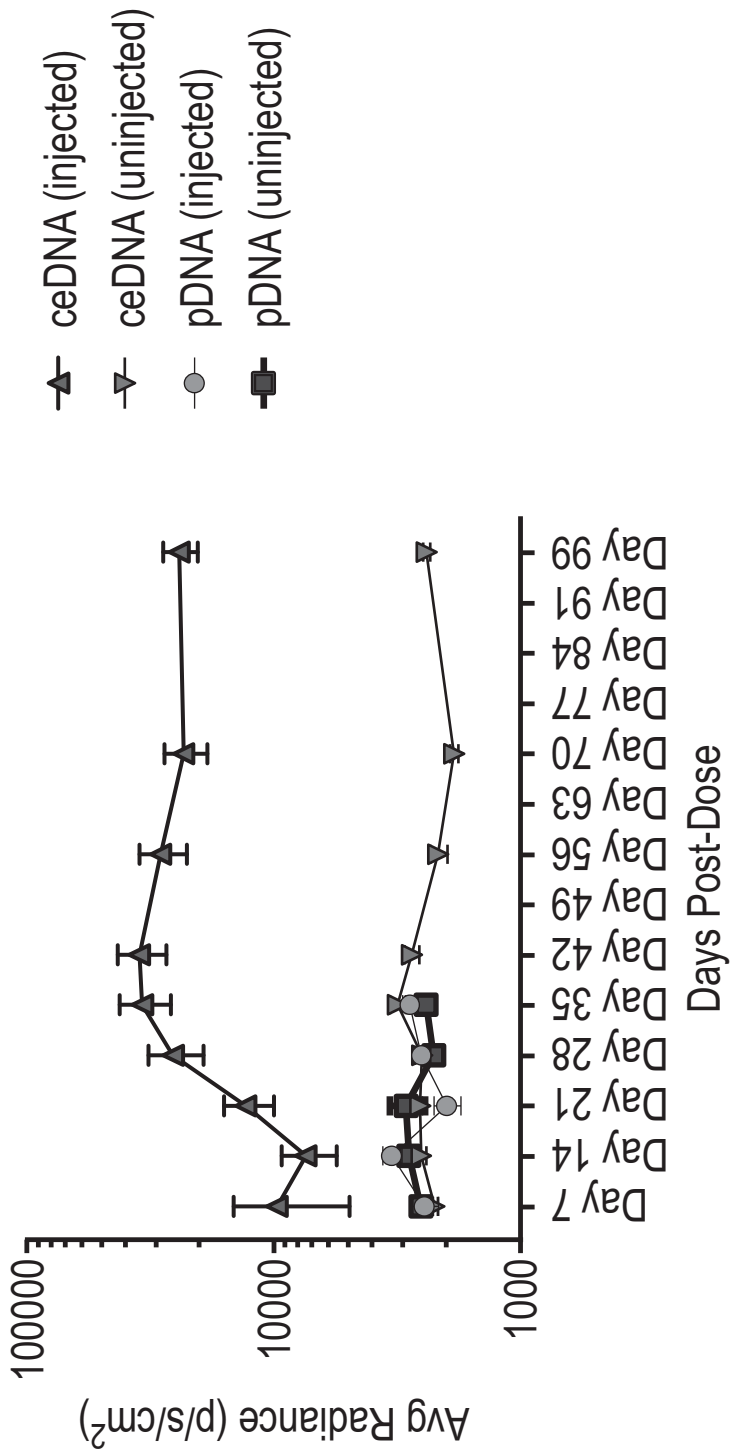

ceDNA (injected)
ceDNA (uninjected)
pDNA (injected)
pDNA (uninjected)
Avg Radiance (p/s/cm²)
100000
10000
1000
Day 7
Day 14
Day 21
Day 28
Day 35
Day 42
Day 49
Day 56
Day 63
Day 70
Day 77
Day 84
Day 91
Day 99
Days Post-Dose

FIG. 8B

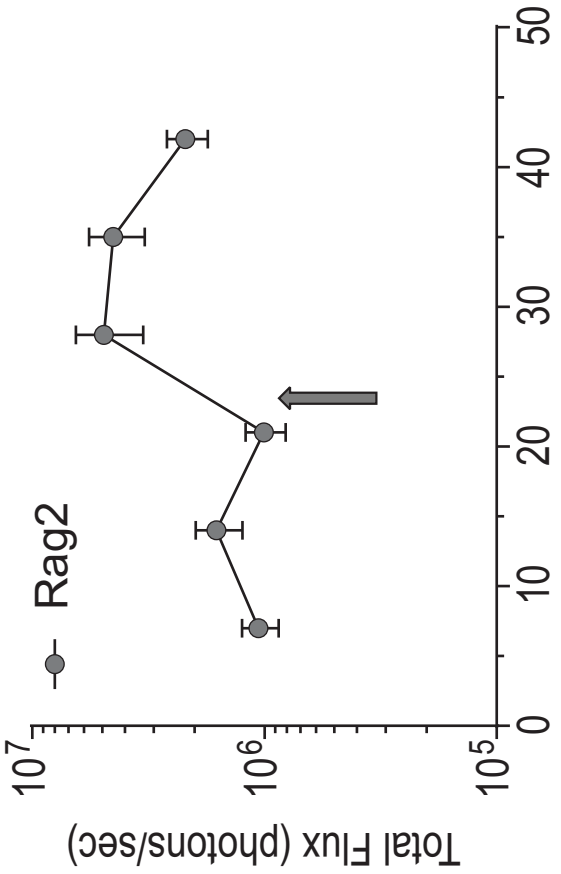

Rag2
Total Flux (photons/sec)
10^7
10^6
10^5
0
10
20
30
40
50
Study Day

FIG. 9B

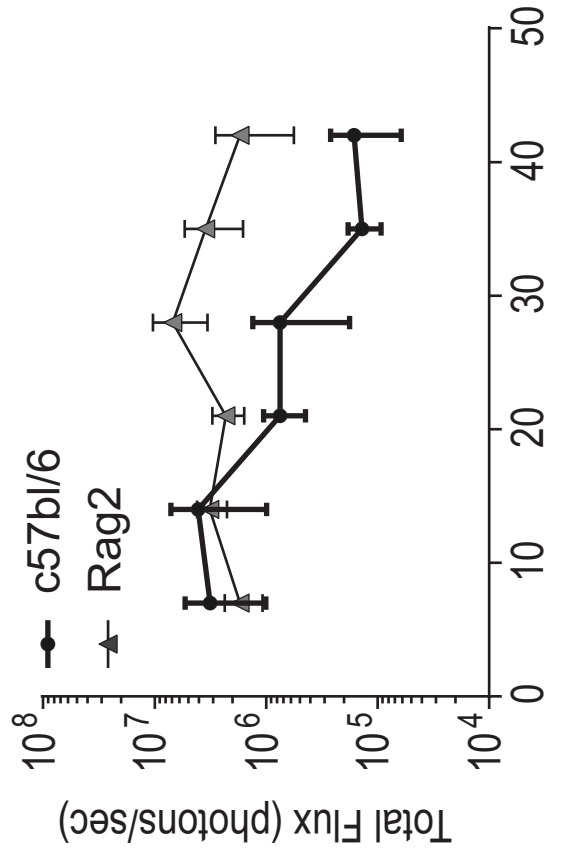

c57bl/6
Rag2
Total Flux (photons/sec)
10^8
10^7
10^6
10^5
10^4
0
10
20
30
40
50
Study Day

FIG. 9A

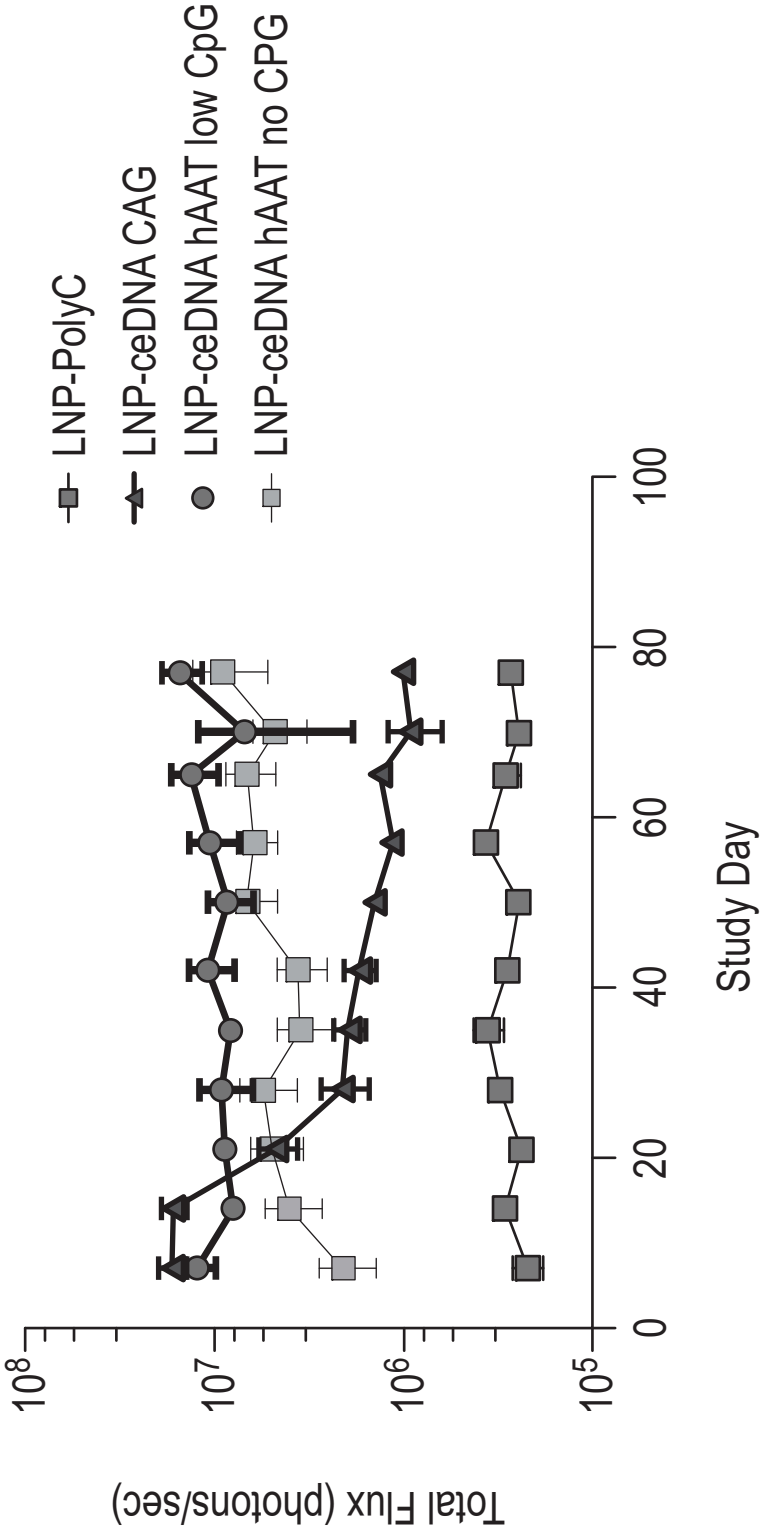

LNP-PolyC
LNP-ceDNA CAG
LNP-ceDNA hAAT low CpG
LNP-ceDNA hAAT no CPG
Total Flux (photons/sec)
10^8
10^7
10^6
10^5
0
20
40
60
80
100
Study Day

FIG. 10

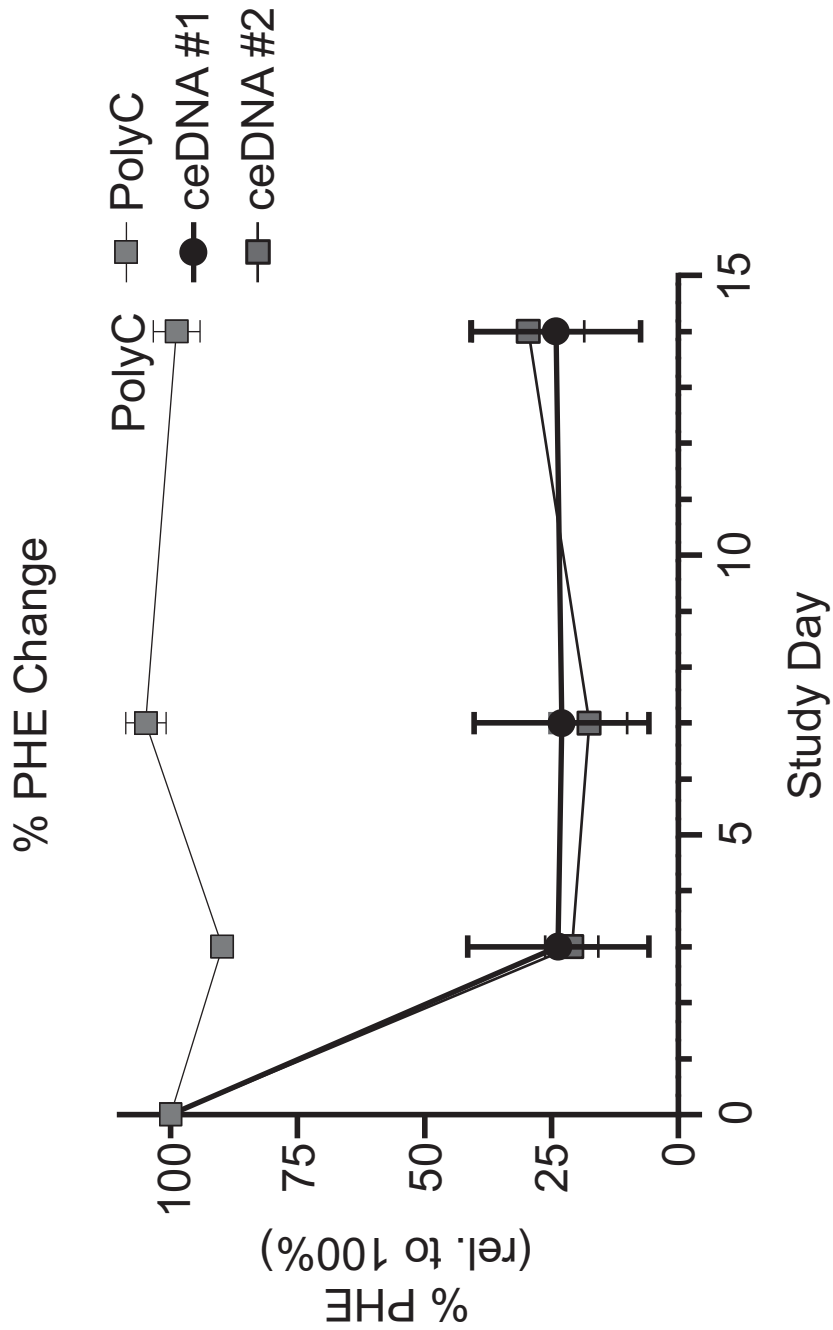

% PHE Change
PolyC
ceDNA #1
ceDNA #2
PolyC
% PHE
(rel. to 100%)
100
75
50
25
0
0
5
10
15
Study Day

FIG. 11

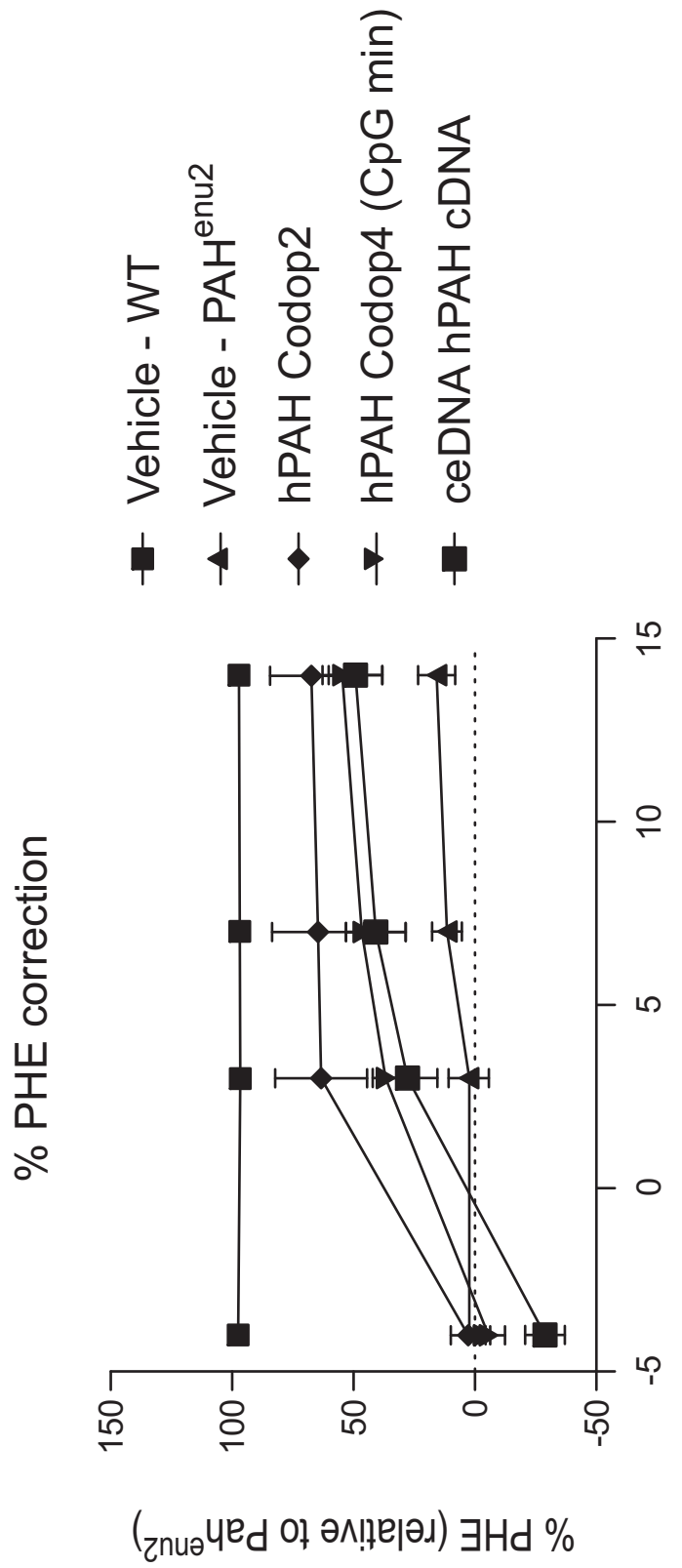
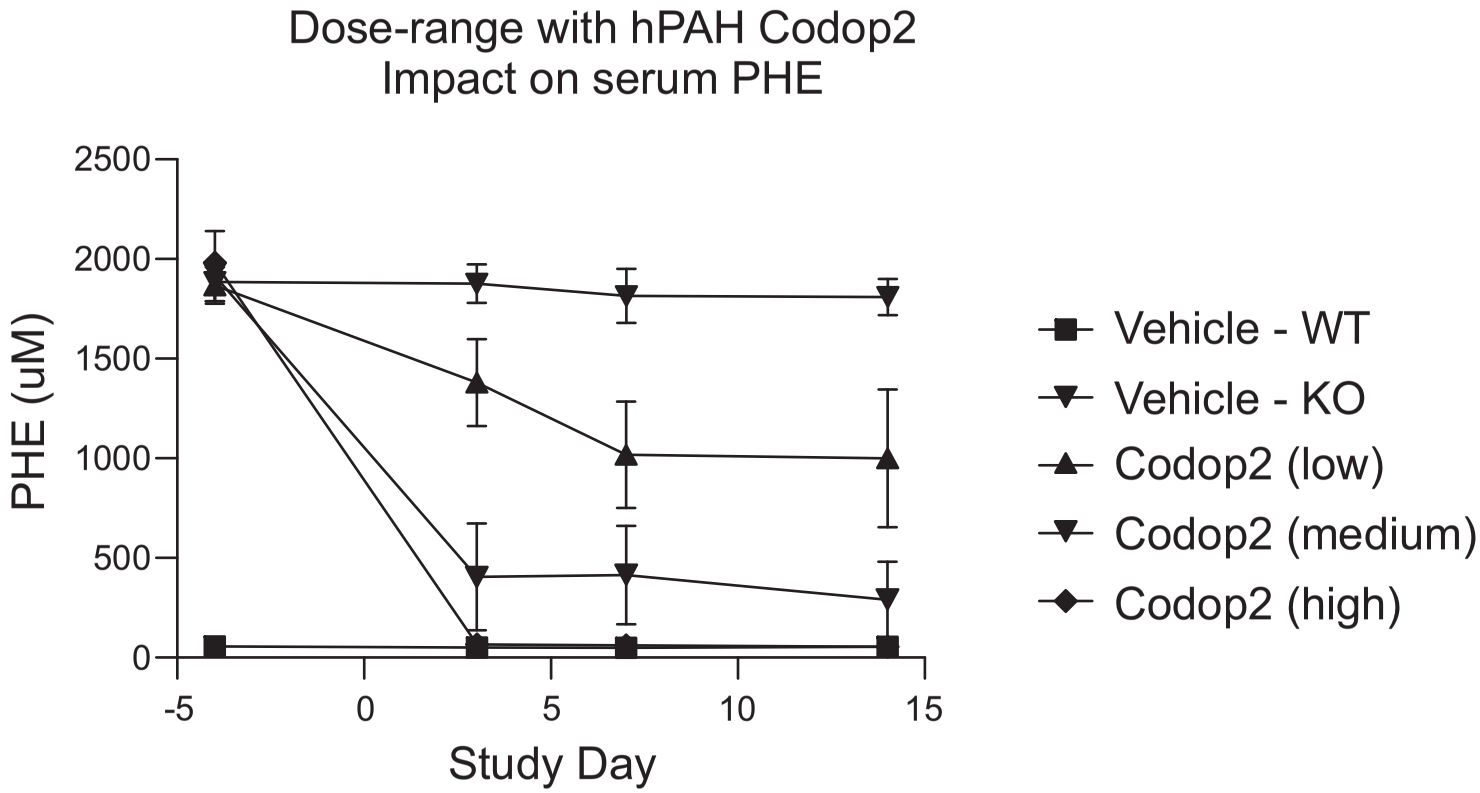

% PHE correction
Vehicle - WT
Vehicle - PAH^{enu2}
hPAH Codop2
hPAH Codop4 (CpG min)
ceDNA hPAH cDNA
% PHE (relative to Pah^{enu2})
150
100
50
0
-50
-5
0
5
10
15
Study day

FIG. 12



Codop2: hPAH Codop2 (ceDNA human PAH codon optimized ORF)

FIG. 13

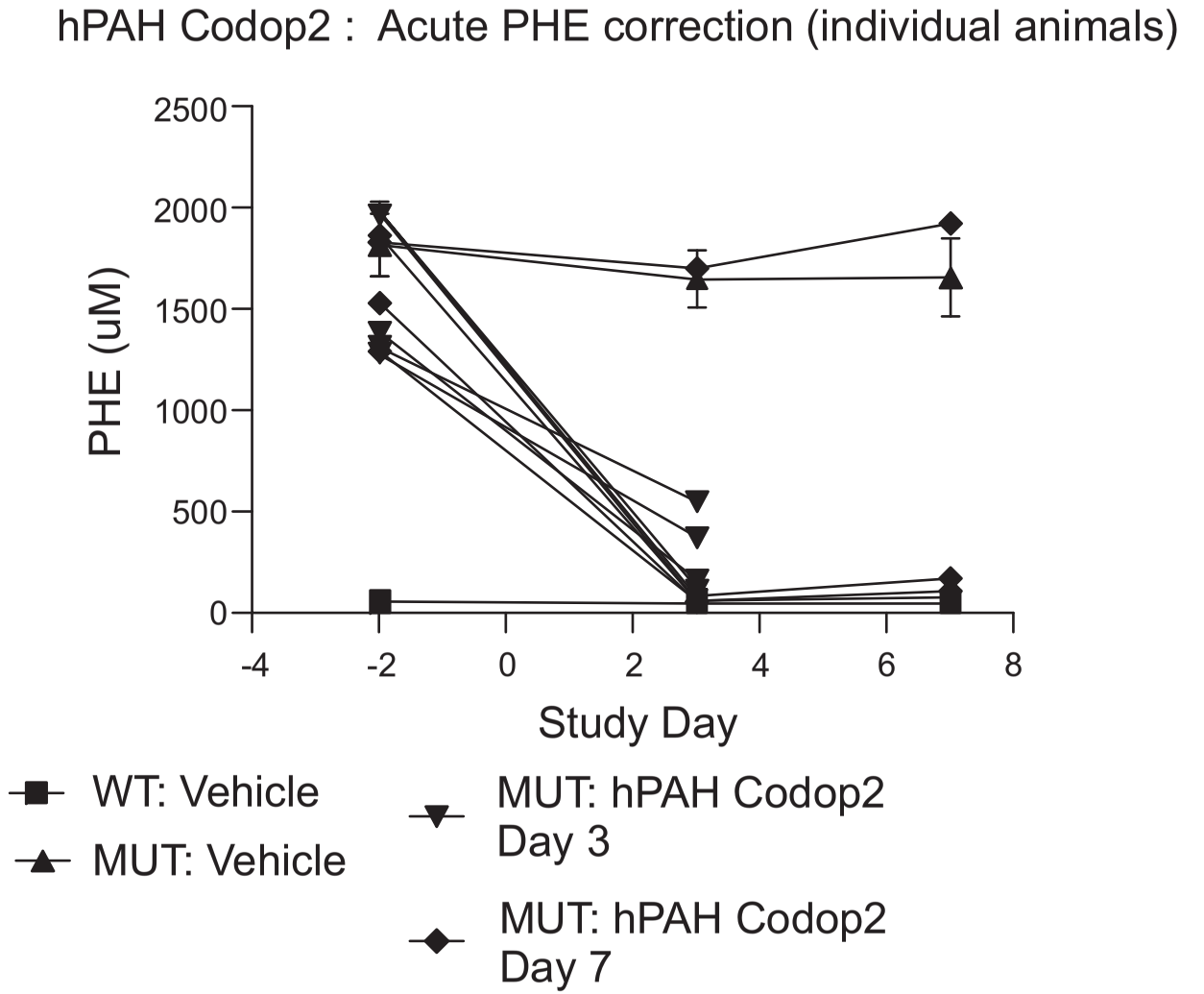

hPAH Codop2 : Acute PHE correction (individual animals)
PHE (uM)
2500
2000
1500
1000
500
0
-4
-2
0
2
4
6
8
Study Day
WT: Vehicle
MUT: Vehicle
MUT: hPAH Codop2 Day 3
MUT: hPAH Codop2 Day 7

FIG. 14A

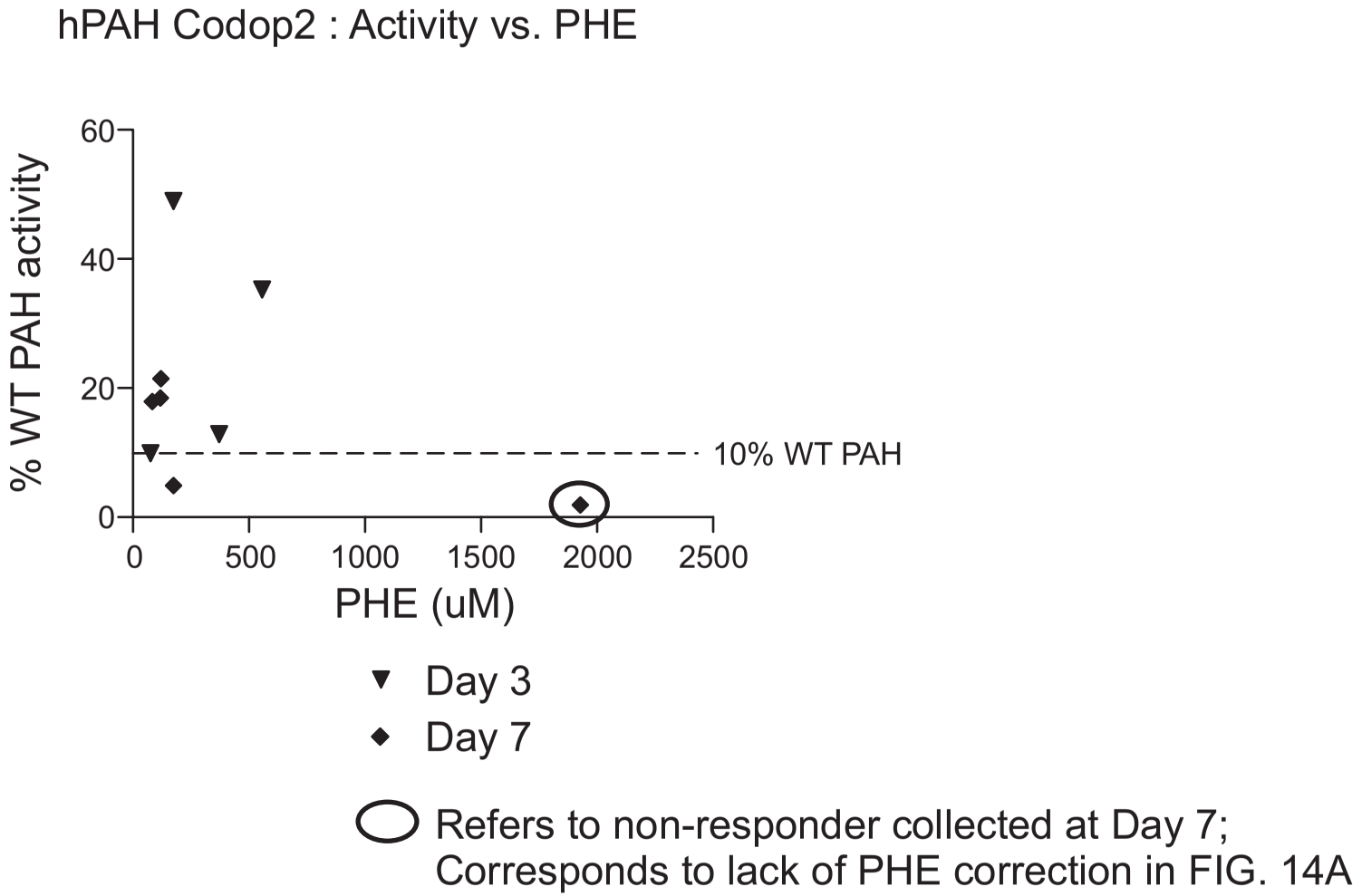

hPAH Codop2 : Activity vs. PHE
% WT PAH activity
60
40
20
0
10% WT PAH
0
500
1000
1500
2000
2500
PHE (uM)
Day 3
Day 7
Refers to non-responder collected at Day 7;
Corresponds to lack of PHE correction in FIG. 14A

FIG. 14B