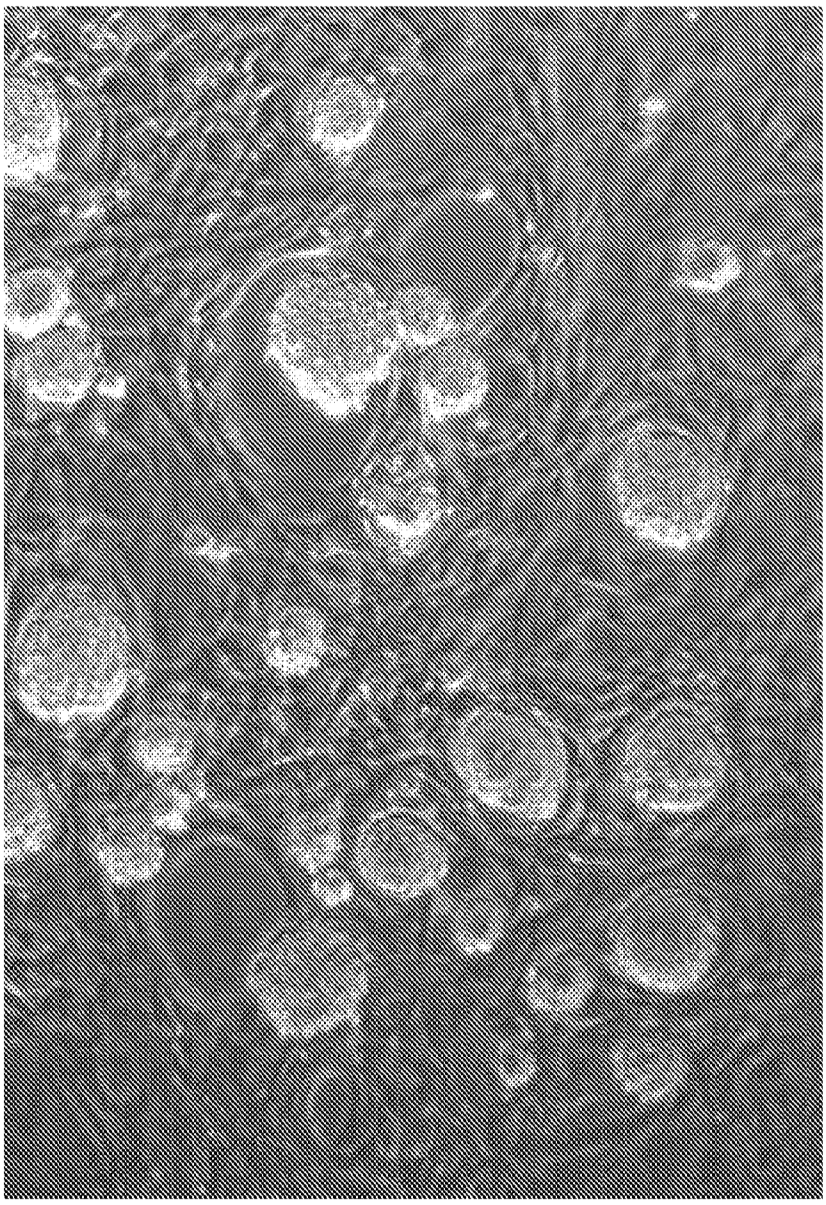


FIG. 1

FIG. 2A

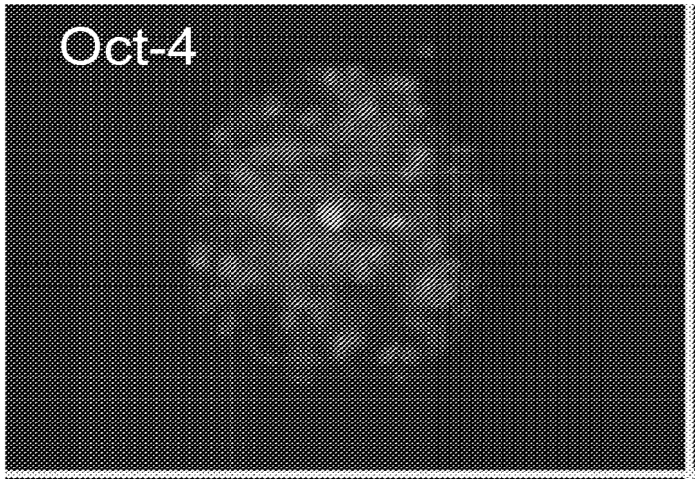


FIG. 2B

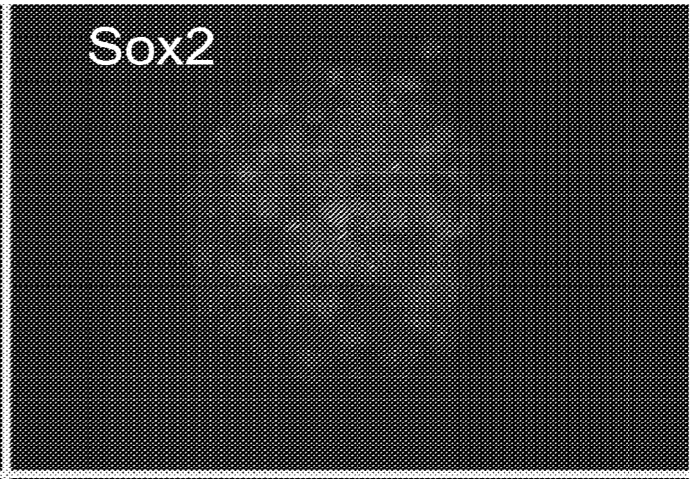


FIG. 2C

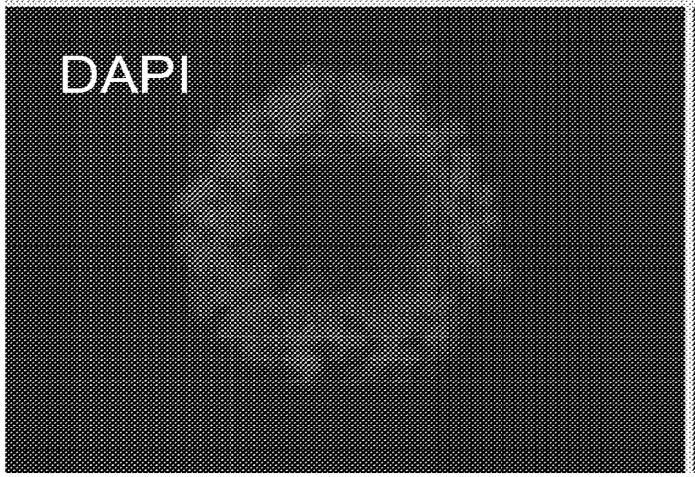
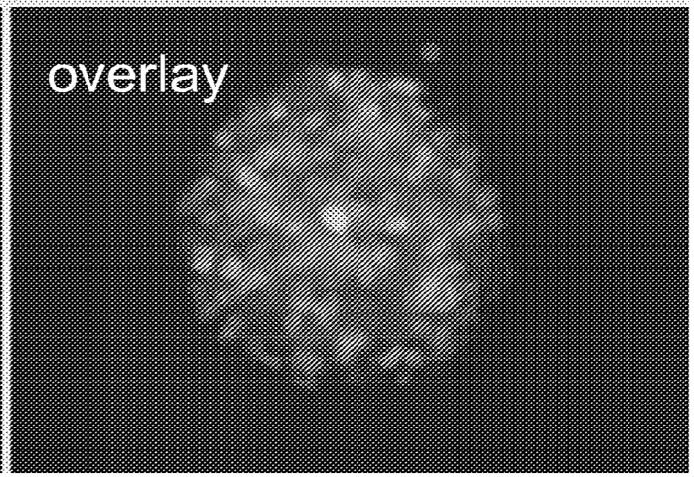


FIG. 2D



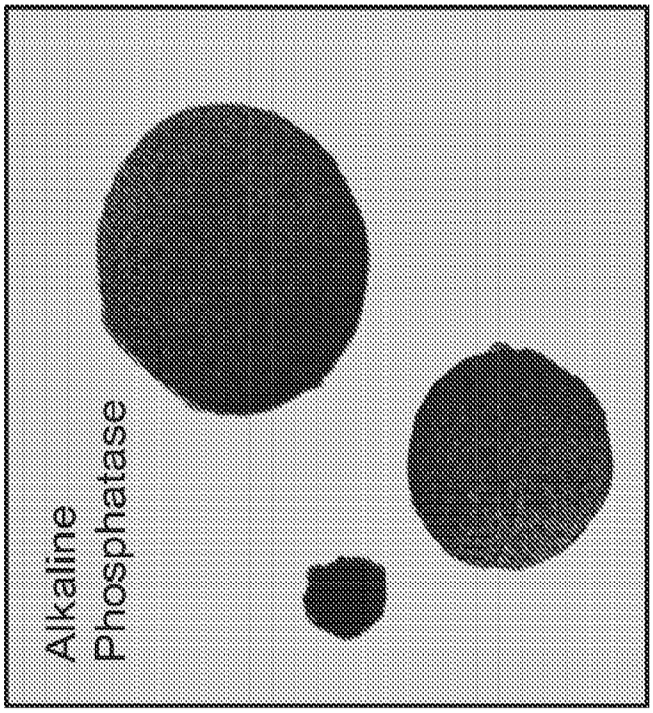


FIG. 3

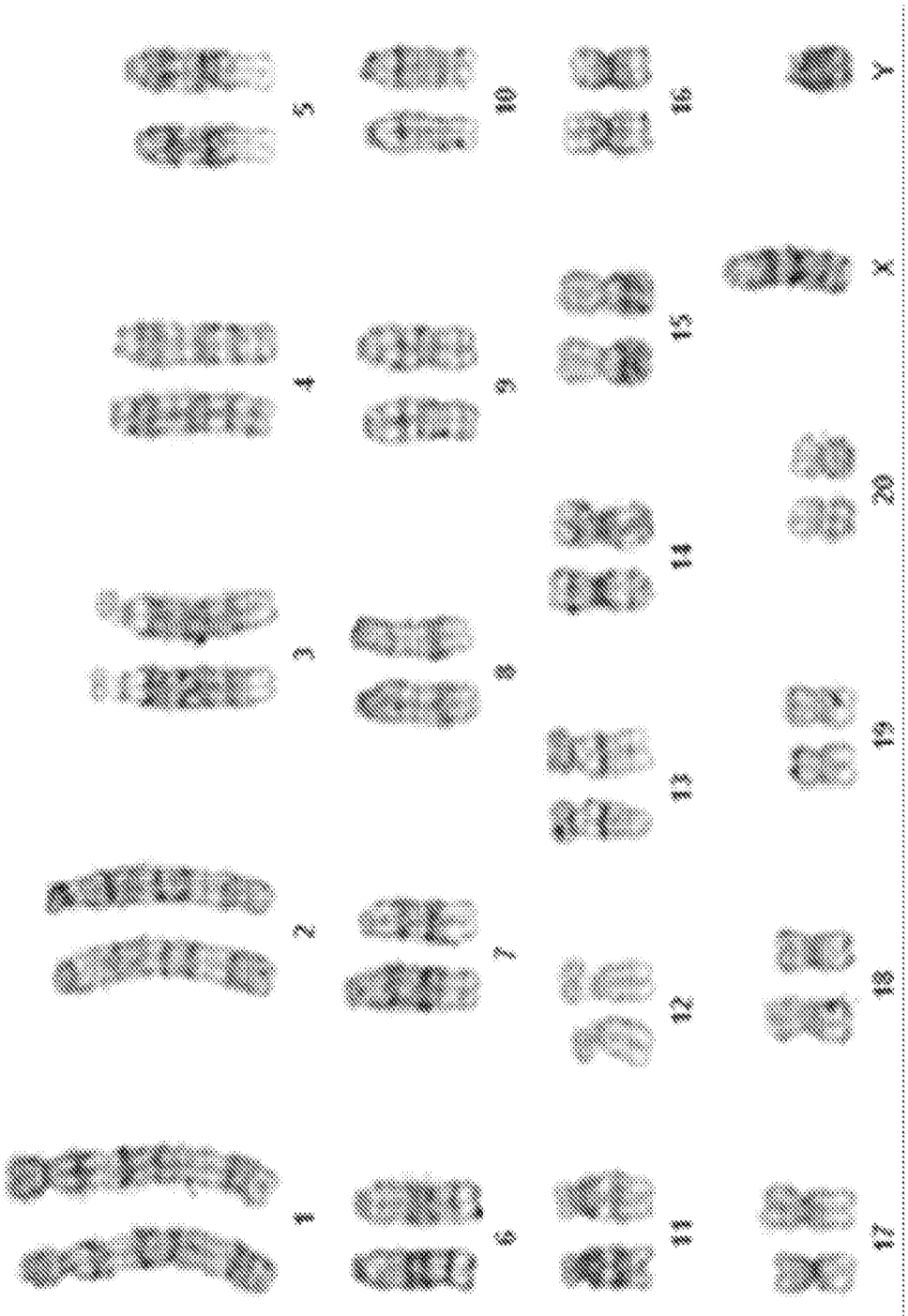
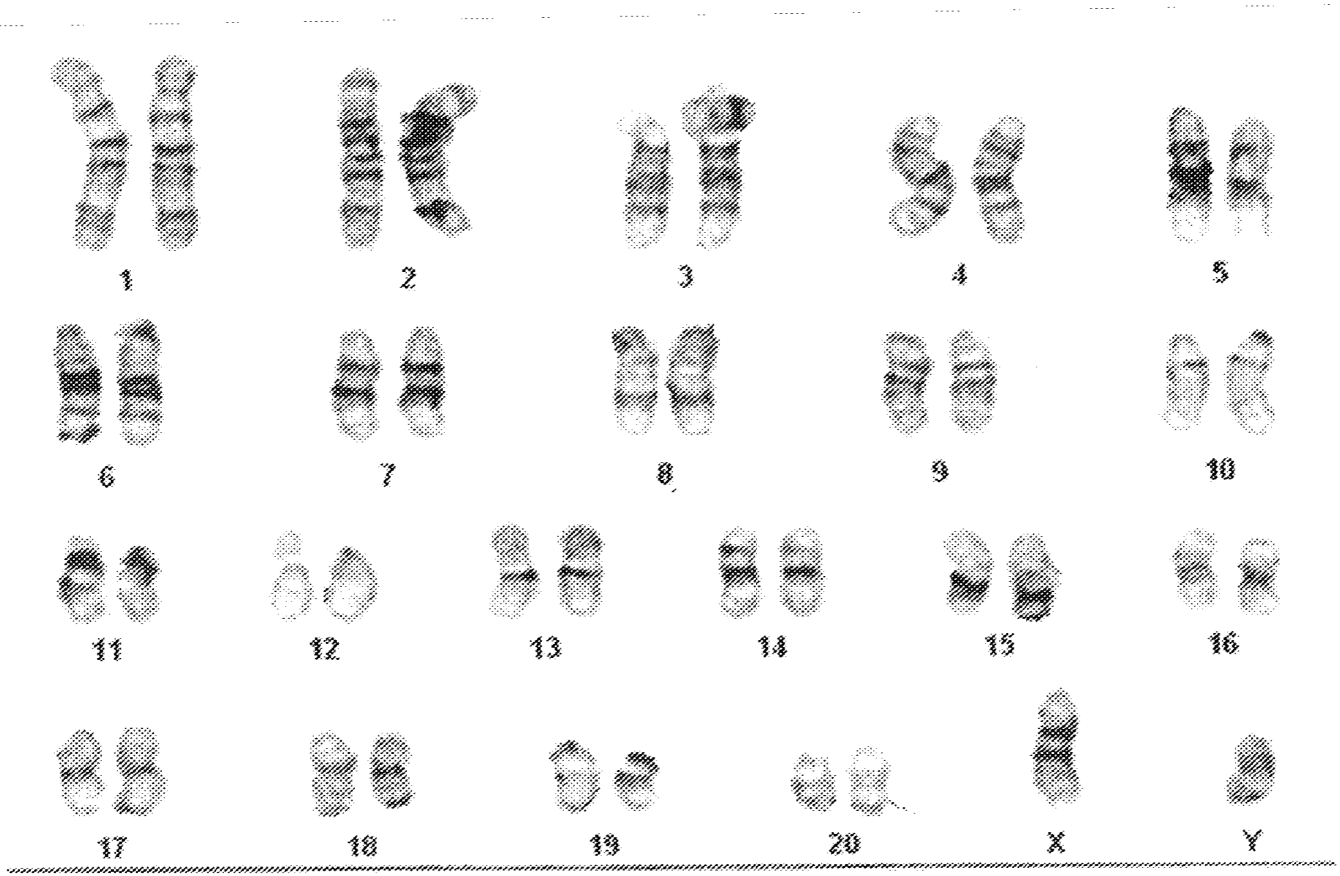


FIG. 4



Cell Results: 42, XY

FIG. 5A



Cell Results: 42, XY

FIG. 5B



Cell Results: 42, XY

FIG. 6A

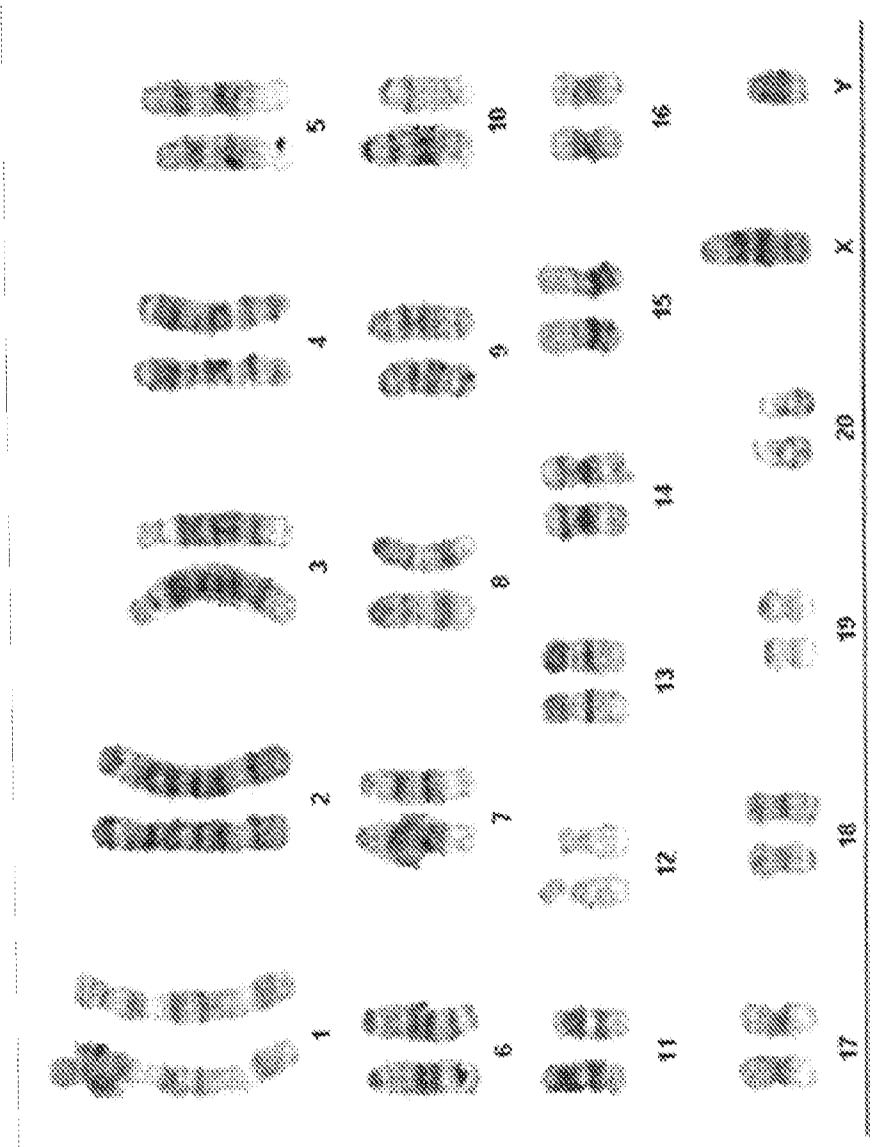
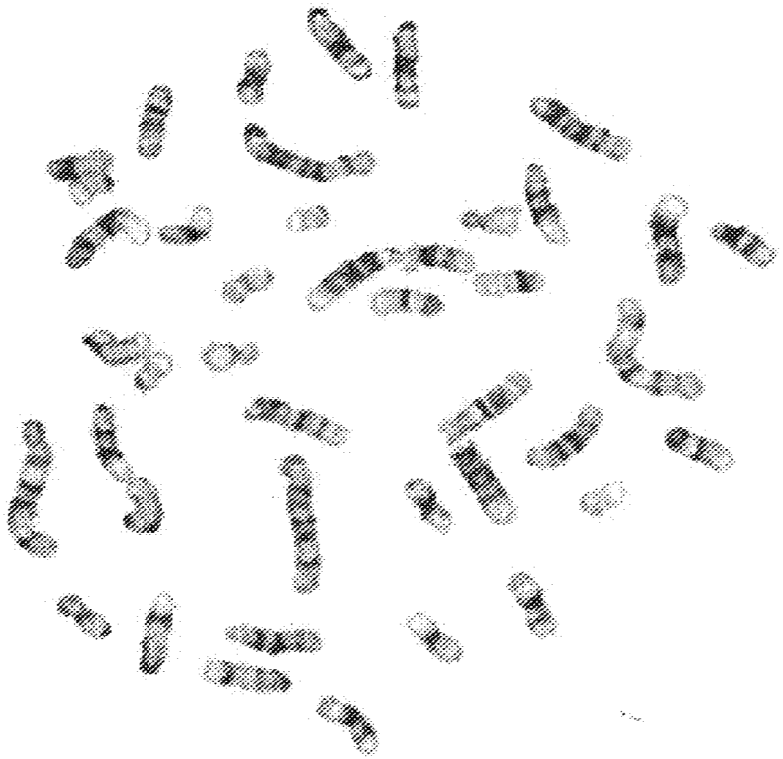
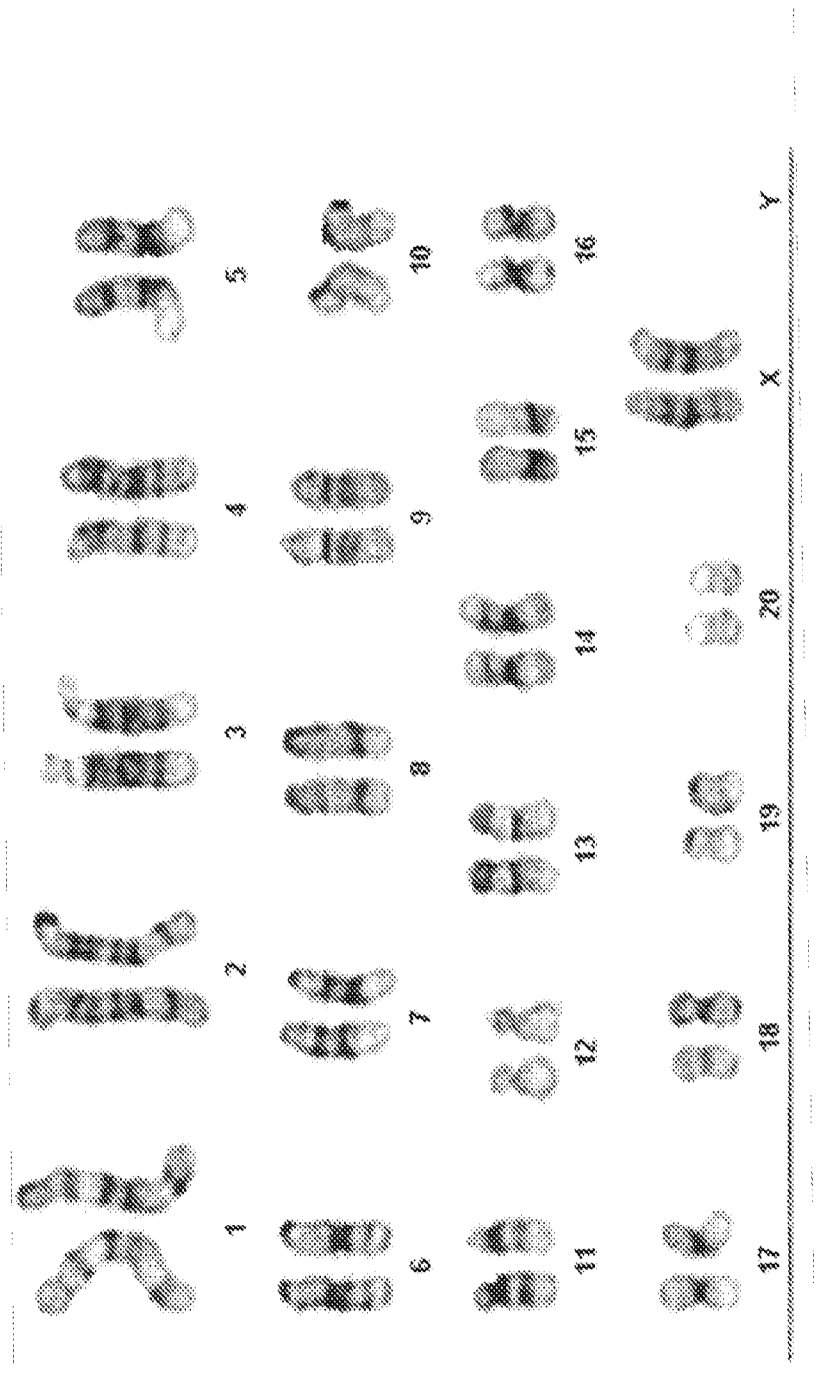


FIG. 6B



Cell Results: 42, XX

FIG. 7A



Cell Results: 42, XX

FIG. 7B

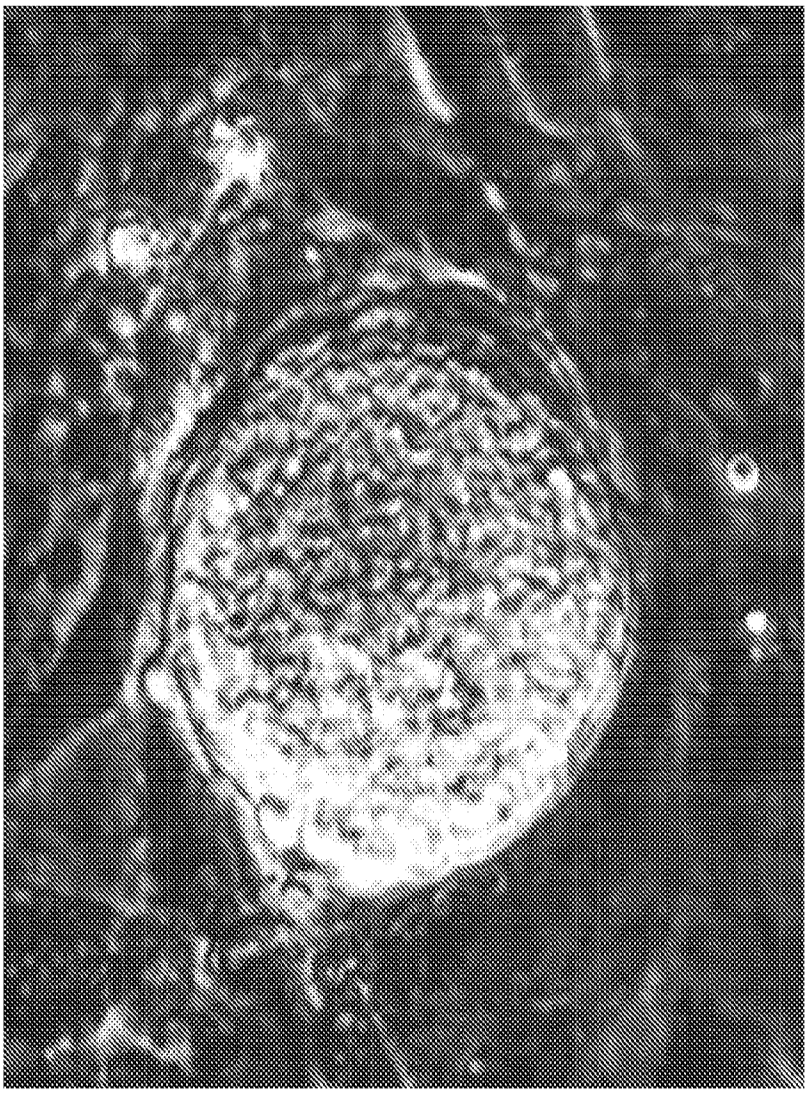


FIG. 8

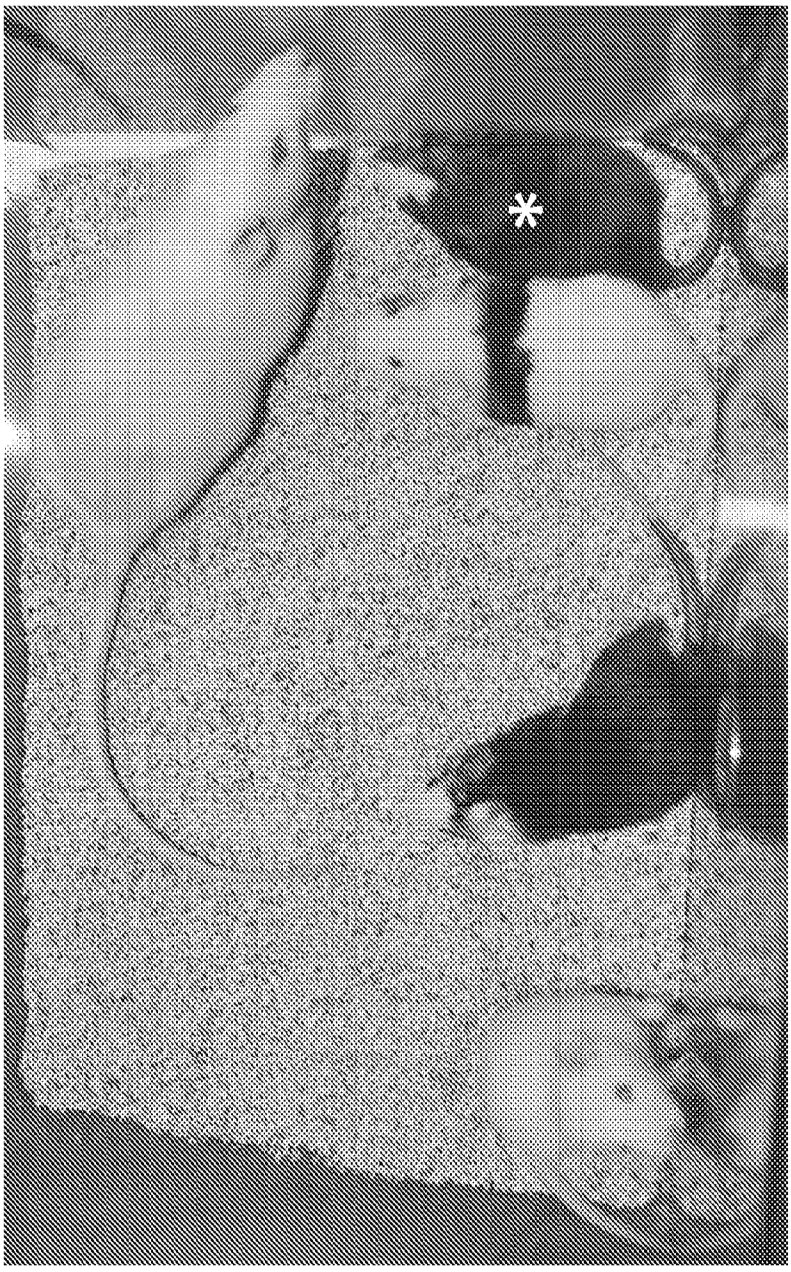


FIG. 9

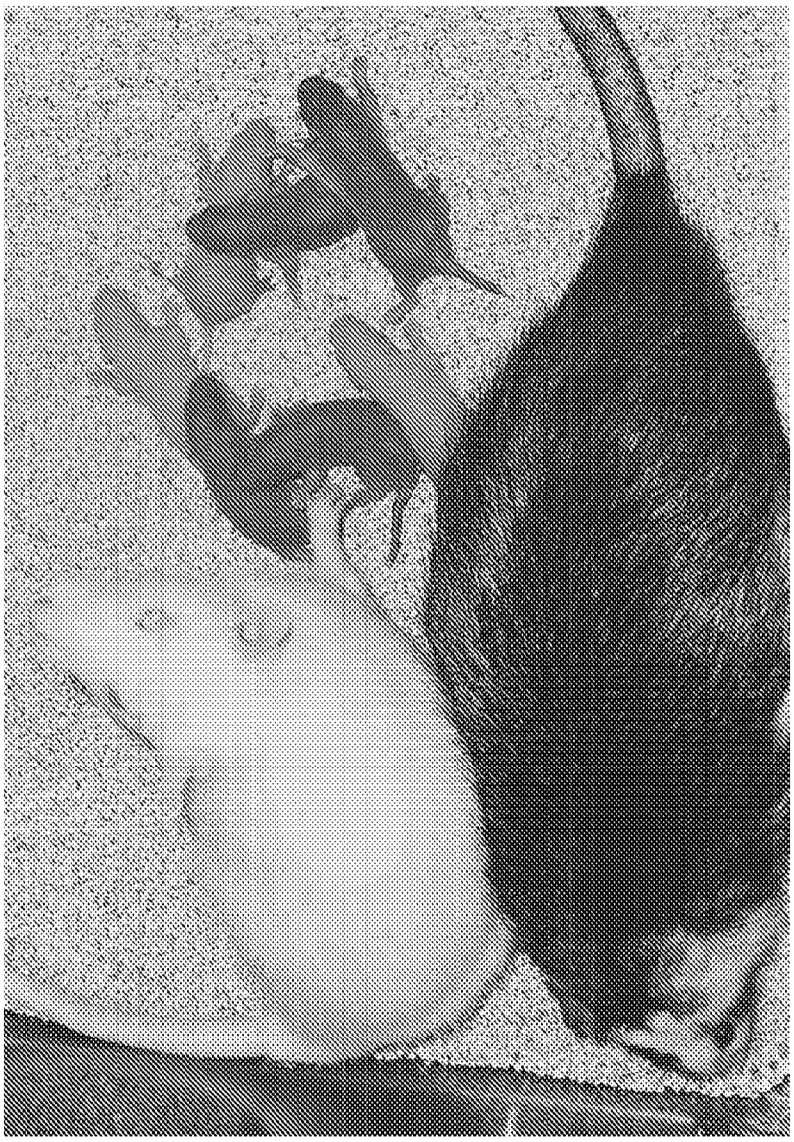


FIG. 10

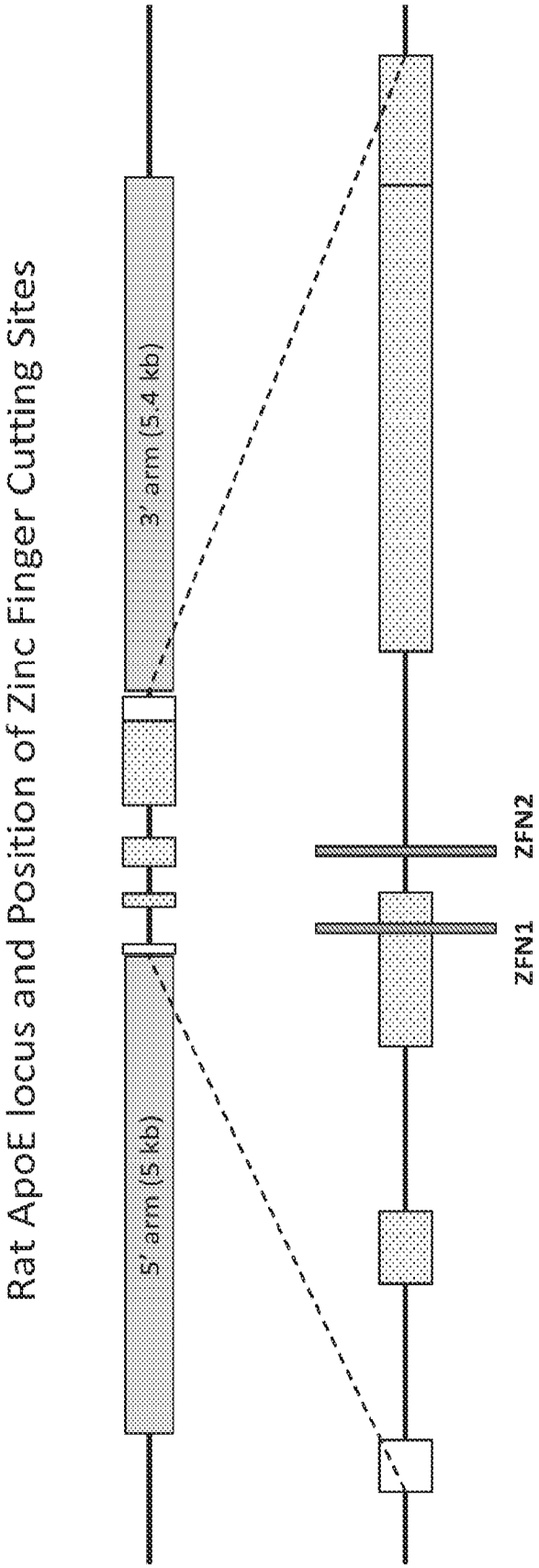

Rat ApoE locus and Position of Zinc Finger Cutting Sites
5' arm (5 kb)
3' arm (5.4 kb)
ZFN1
ZFN2

FIG. 11

FIG. 12A

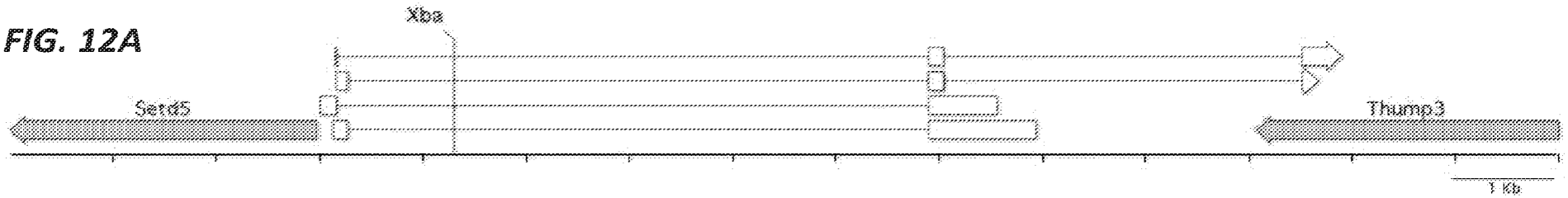


FIG. 12B

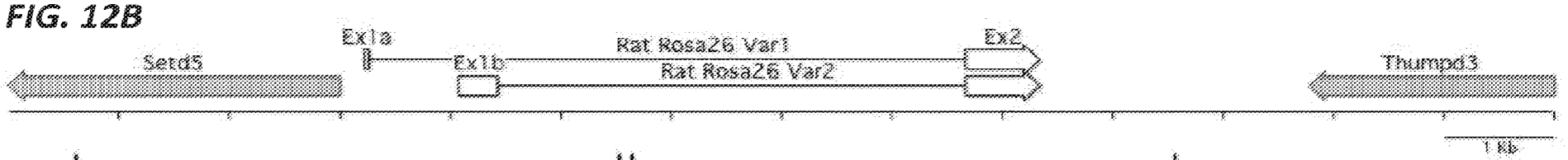
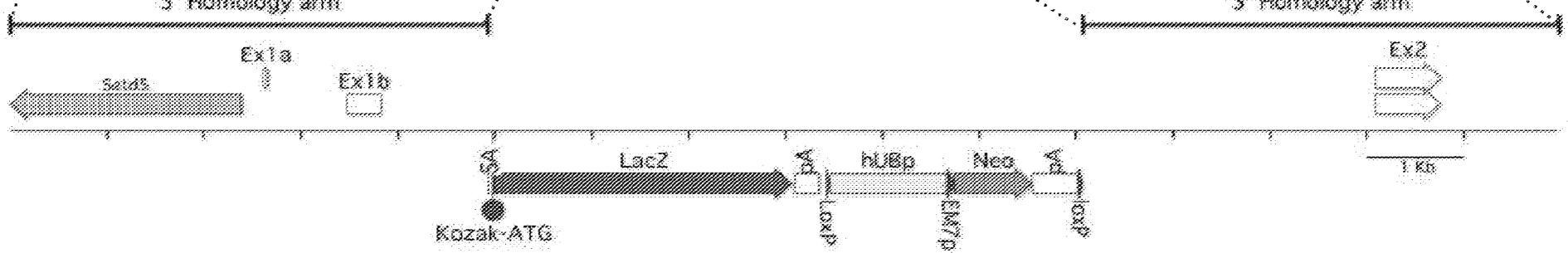


FIG. 12C



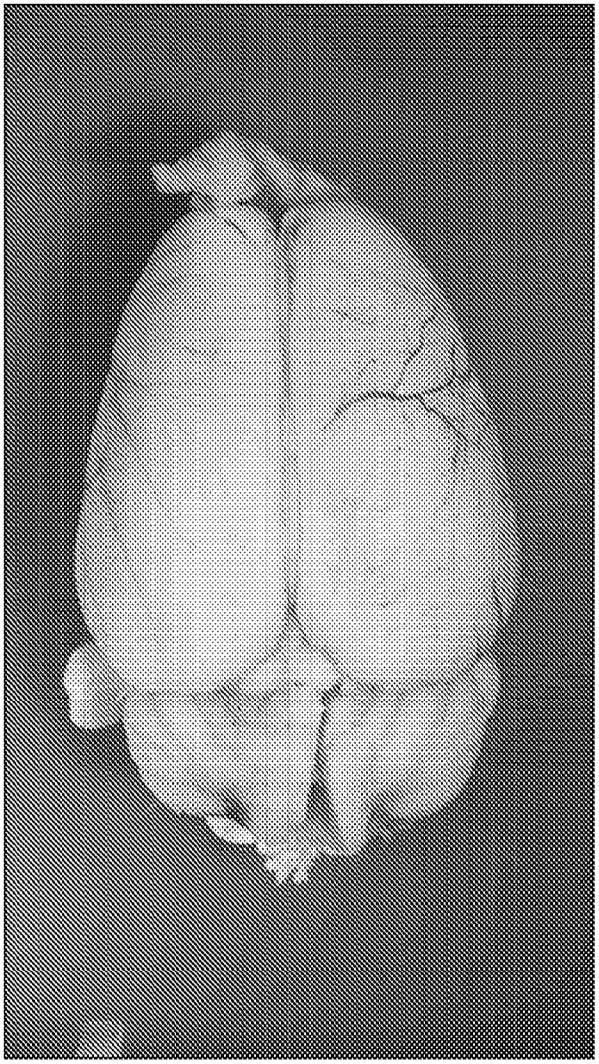


FIG. 13A

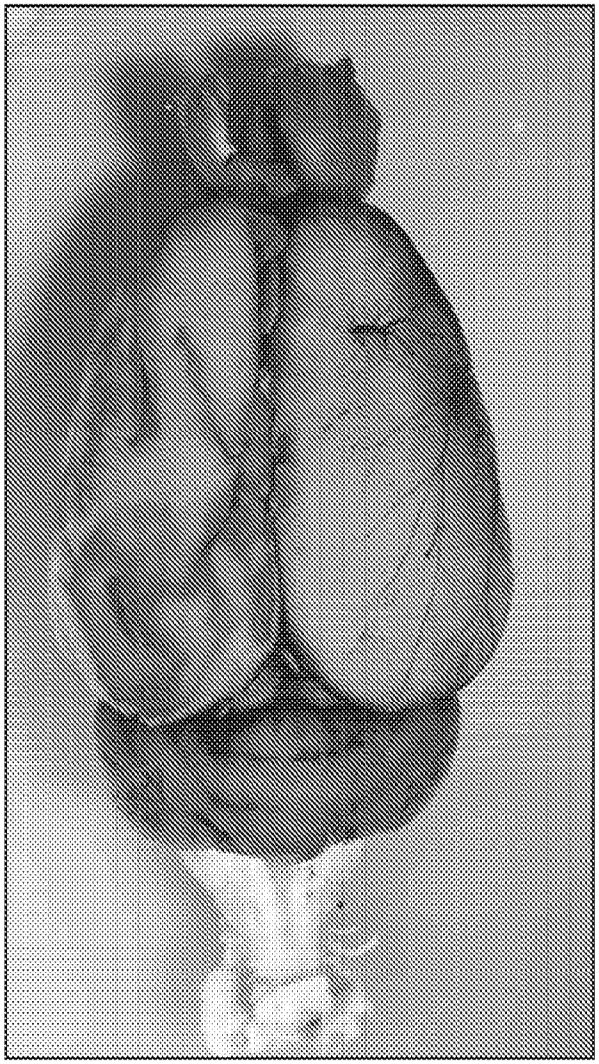


FIG. 13B

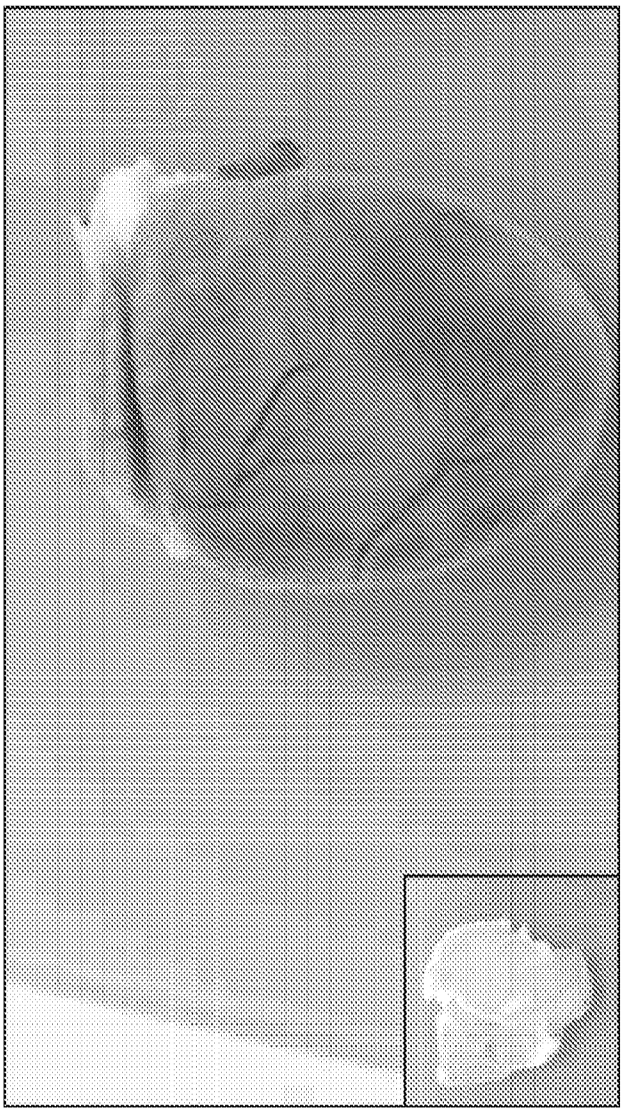


FIG. 13C

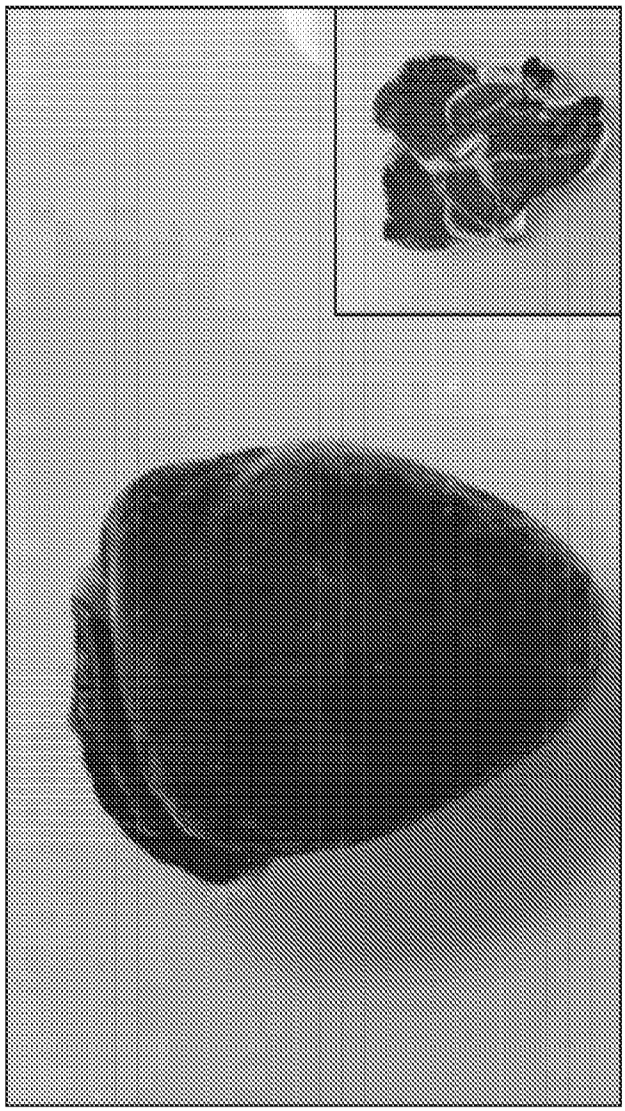


FIG. 13D



FIG. 13E

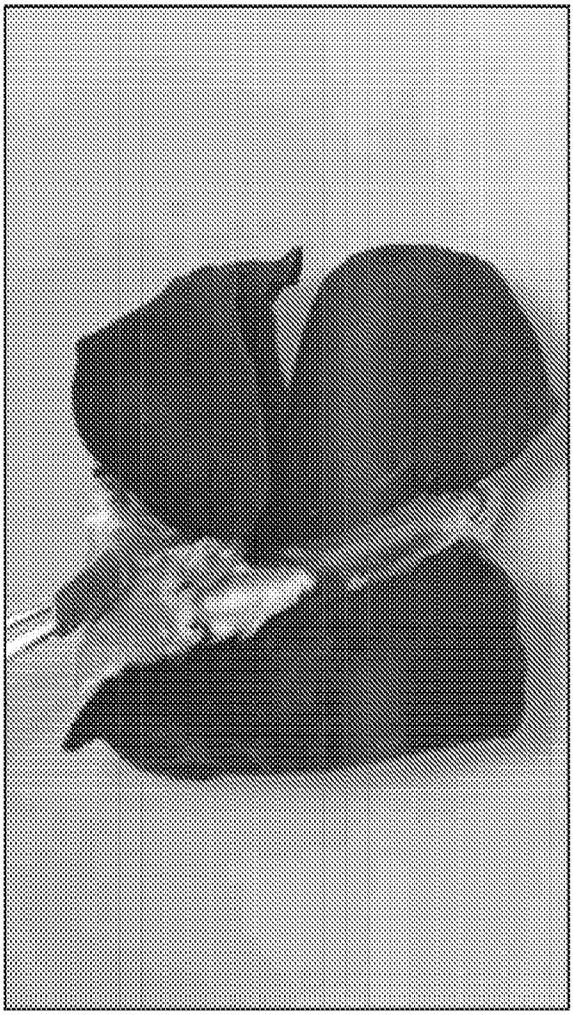


FIG. 13F

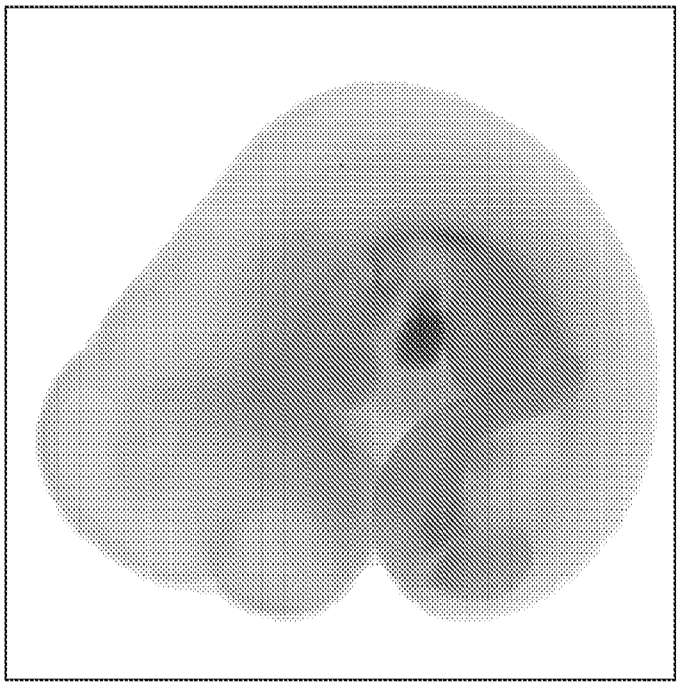


FIG. 13H

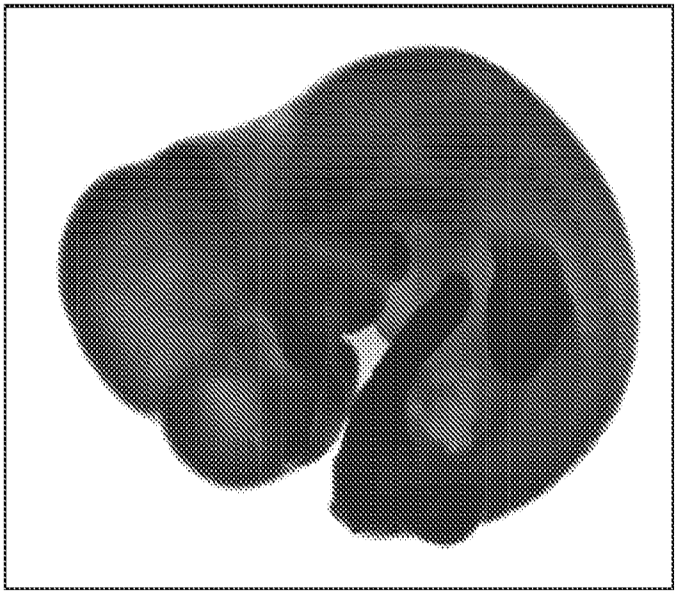


FIG. 13G

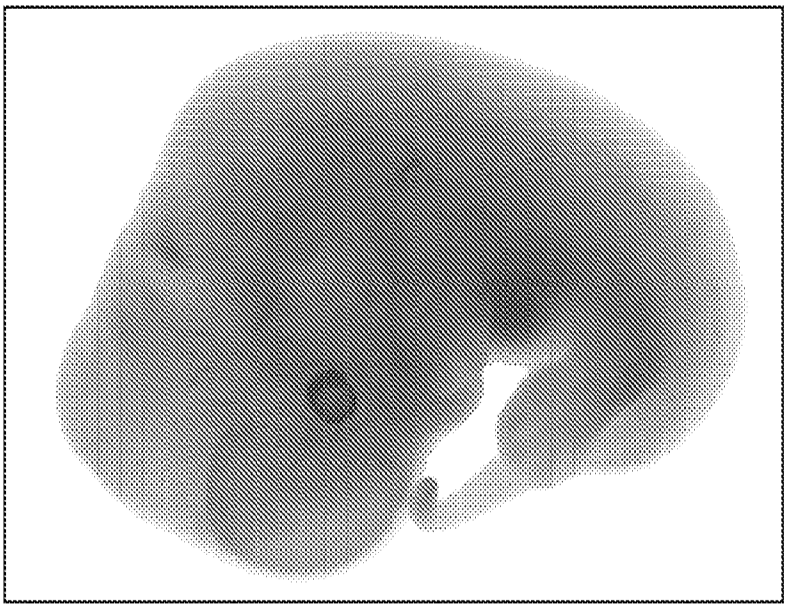


FIG. 13J

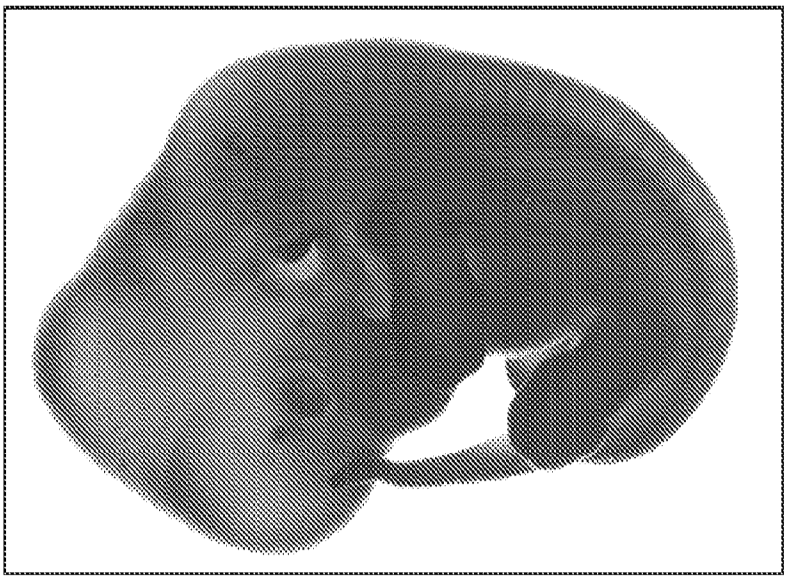


FIG. 13I

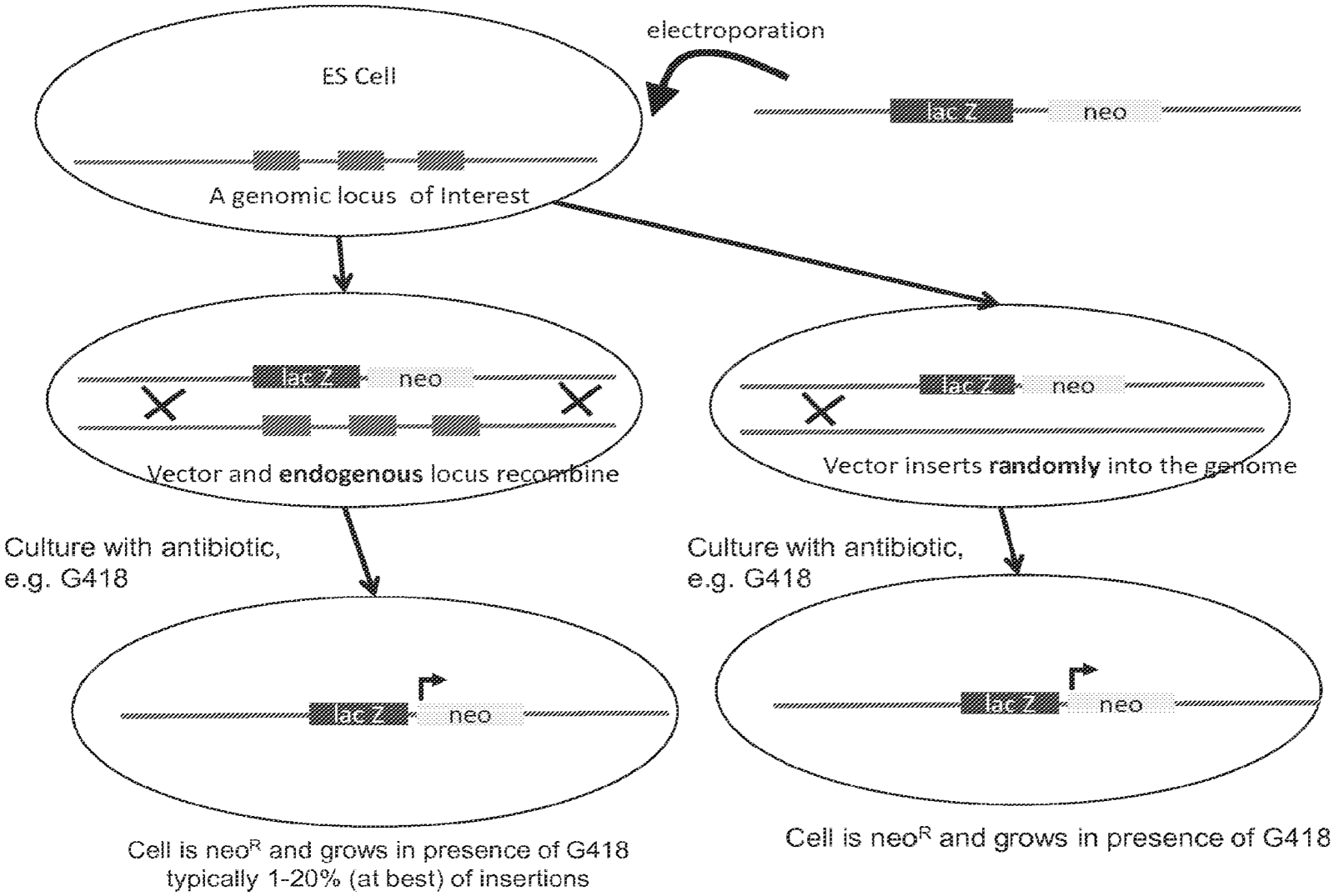

electroporation
ES Cell
A genomic locus of Interest
lac Z
neo
Vector and endogenous locus recombine
Vector inserts randomly into the genome
Culture with antibiotic,
e.g. G418
Culture with antibiotic,
e.g. G418
Cell is neoR and grows in presence of G418
typically 1-20% (at best) of insertions
Cell is neoR and grows in presence of G418

FIG. 14

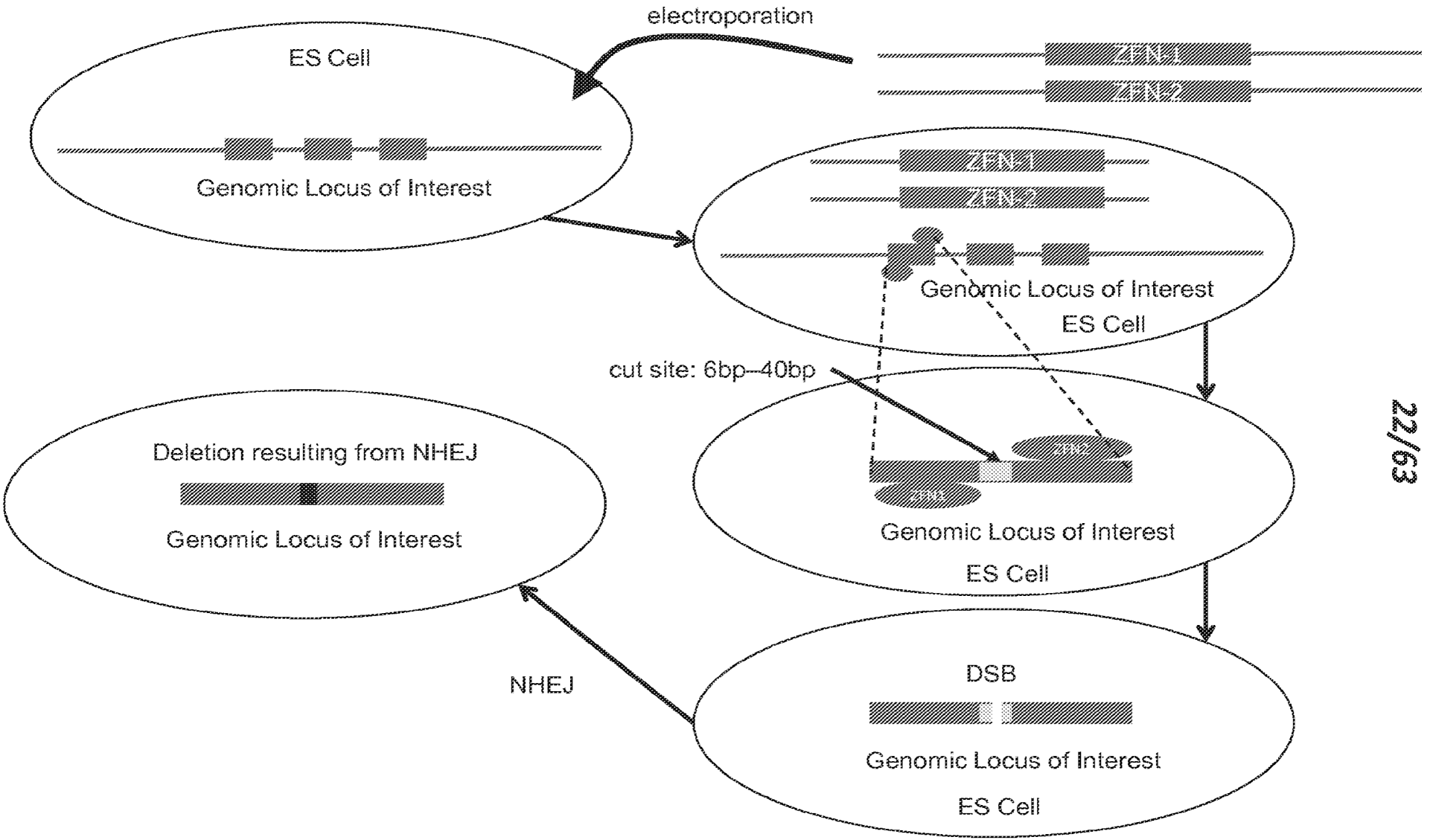

electroporation
ES Cell
Genomic Locus of Interest
ZFN-1
ZFN-2
Genomic Locus of Interest
ES Cell
cut site: 6bp–40bp
Deletion resulting from NHEJ
Genomic Locus of Interest
Genomic Locus of Interest
ES Cell
NHEJ
DSB
Genomic Locus of Interest
ES Cell

FIG. 15

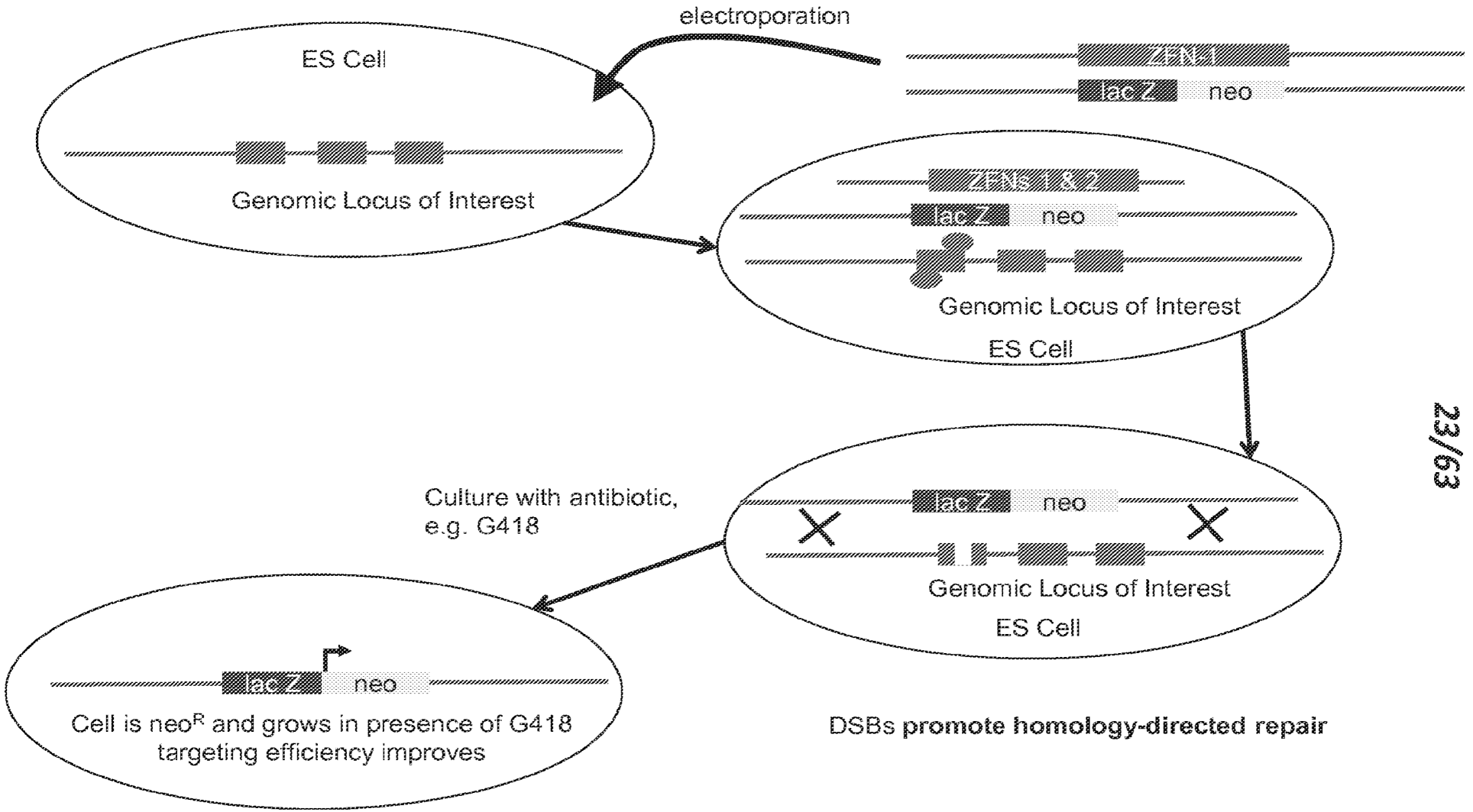
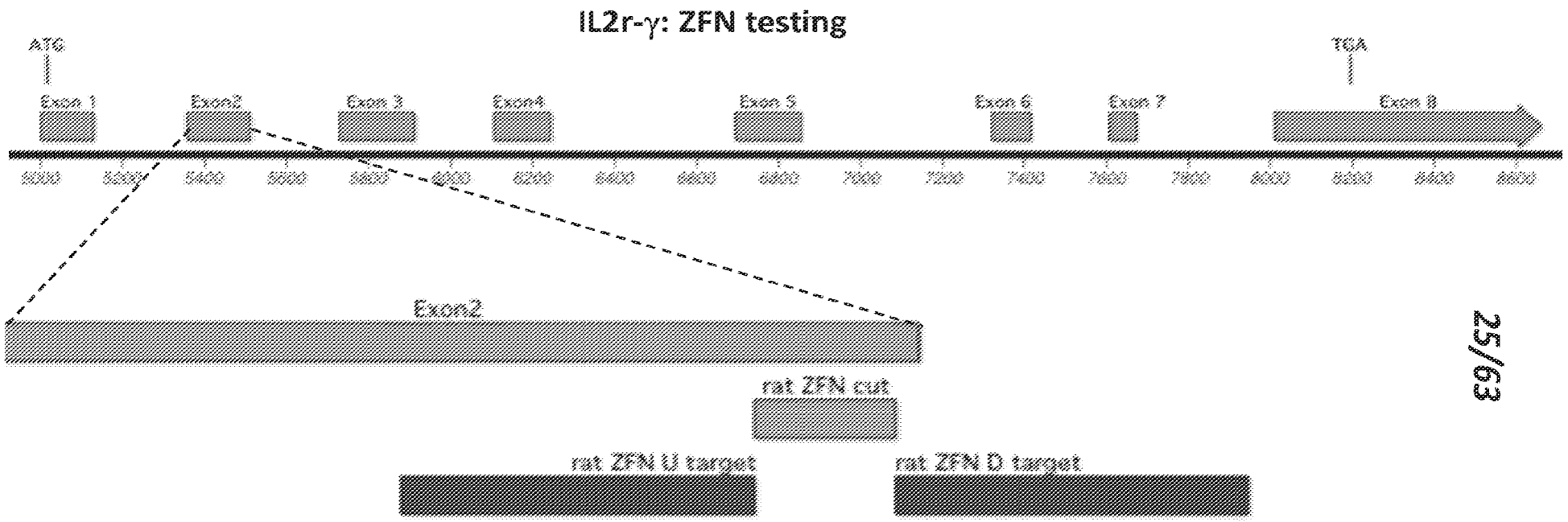

electroporation
ES Cell
Genomic Locus of Interest
ZFNs 1 & 2
lac Z
neo
Genomic Locus of Interest
ES Cell
Culture with antibiotic,
e.g. G418
lac Z
neo
Genomic Locus of Interest
ES Cell
lac Z
neo
Cell is neo^R and grows in presence of G418
targeting efficiency improves
DSBs promote homology-directed repair

FIG. 16



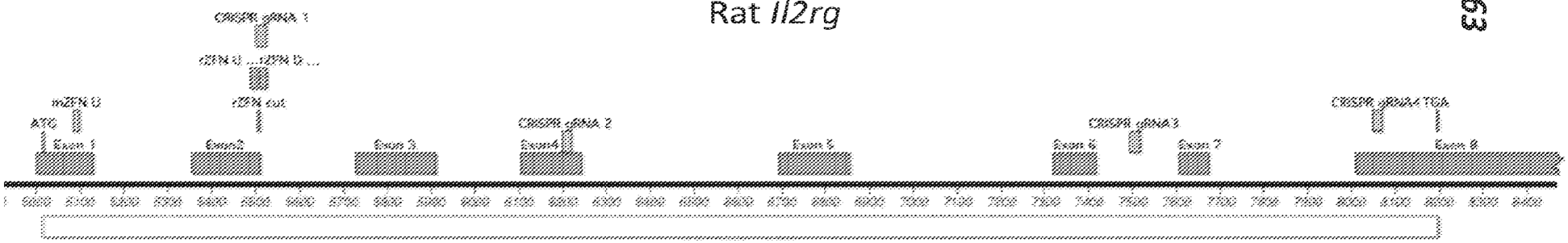
FIG. 17



TTCTGAGCCTCAGCCGACCAACCTCACTATGCACTATAGGTATGAGAAGGGGGAGG (SEQ ID NO: 93)
AAGACTCGGAGTCGGCTGGTTGGAGTGATACGTGATATCCATACTCTTCCCCCTCC

FIG. 18

FIG. 19


Rat *Il2rg*
ATG
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Exon 6
Exon 7
Exon 8
Il2rg ATG to TGA

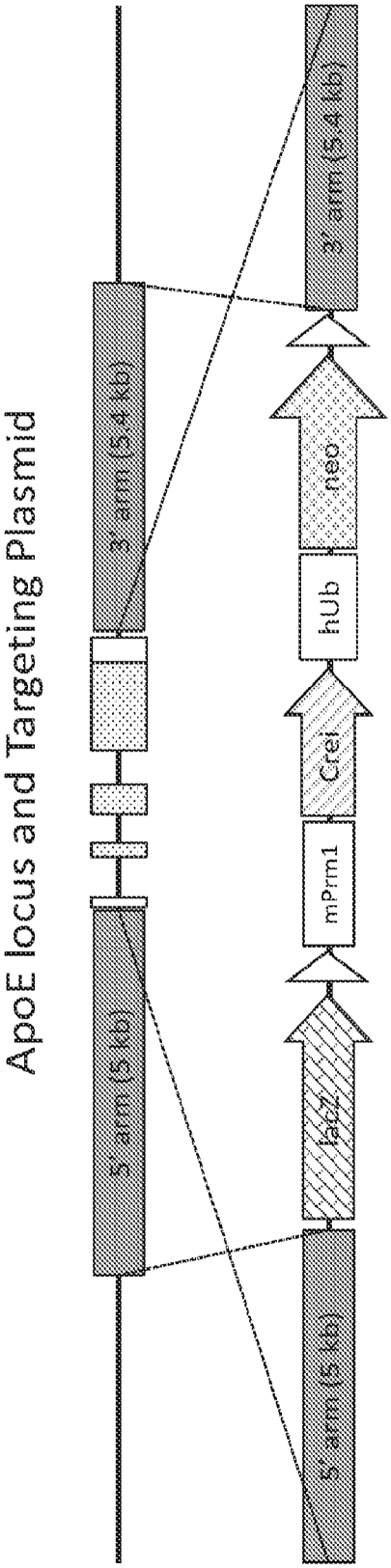

ApoE locus and Targeting Plasmid
5' arm (5 kb)
3' arm (5.4 kb)
fatC
mPrm1
Crei
hUb
neo
5' arm (5 kb)
3' arm (5.4 kb)

FIG. 20

FIG. 21A

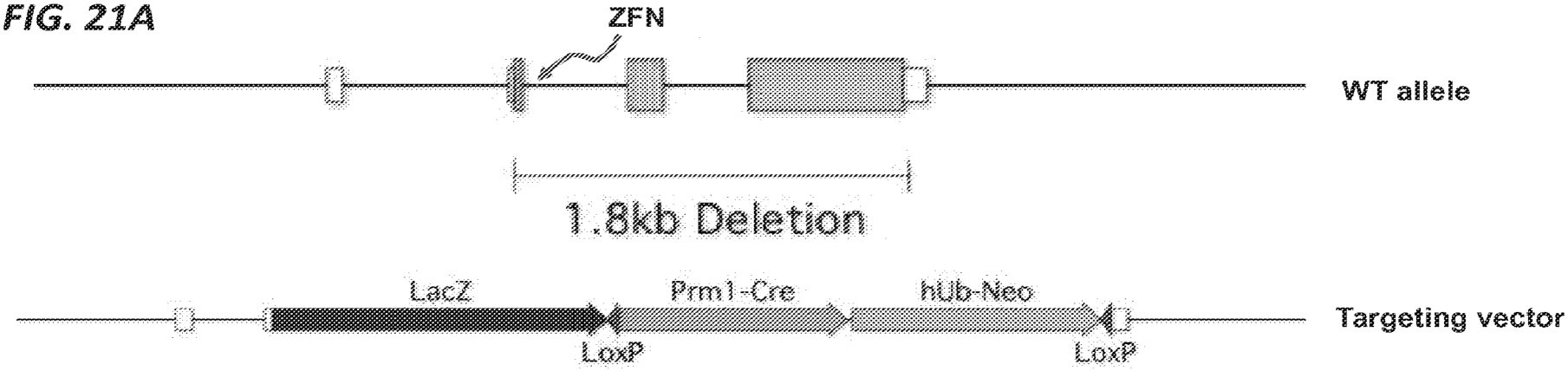
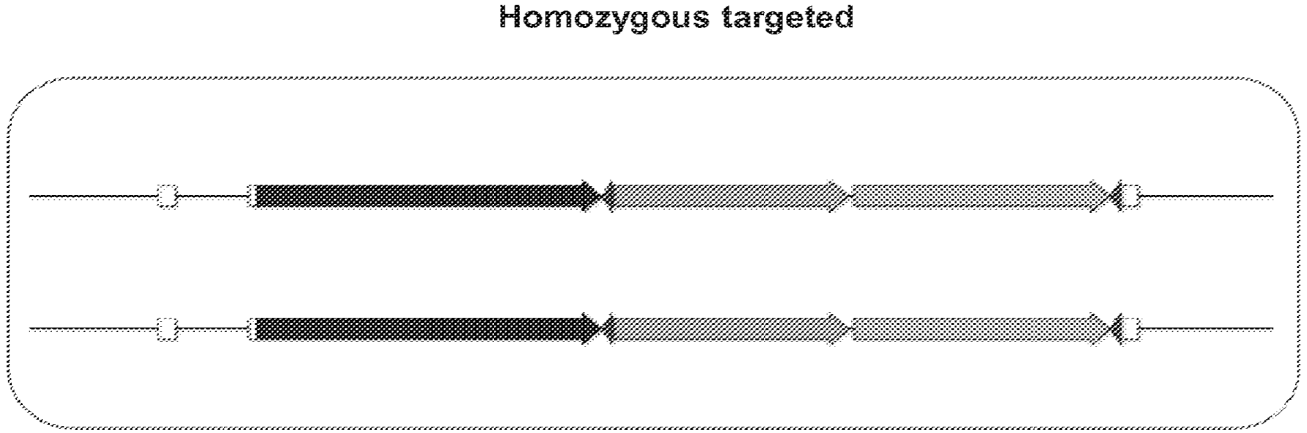


FIG. 21B



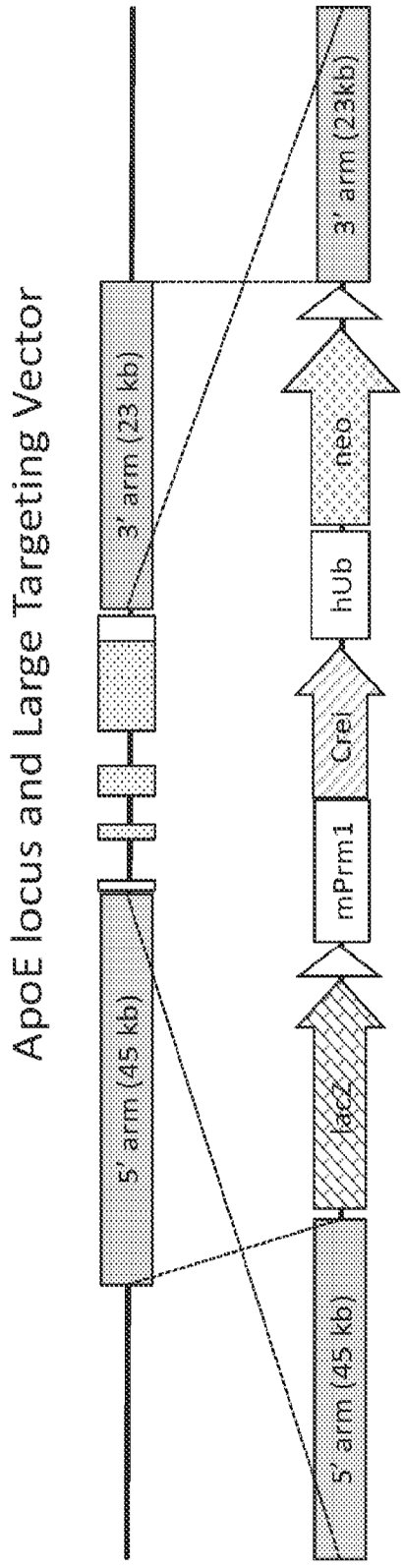

ApoE locus and Large Targeting Vector
5' arm (45 kb)
3' arm (23 kb)
lacZ
mPrm1
Cre
hUb
neo
5' arm (45 kb)
3' arm (23kb)

FIG. 22

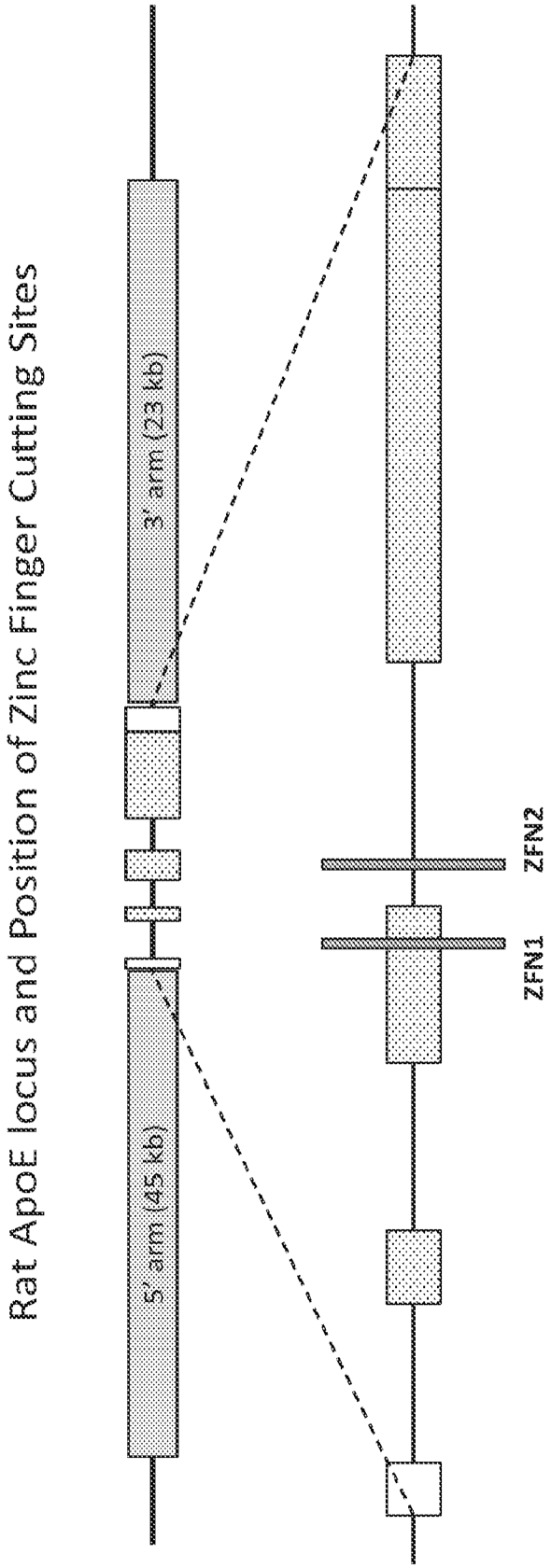

Rat ApoE locus and Position of Zinc Finger Cutting Sites
5' arm (45 kb)
3' arm (23 kb)
ZFN1
ZFN2

FIG. 23

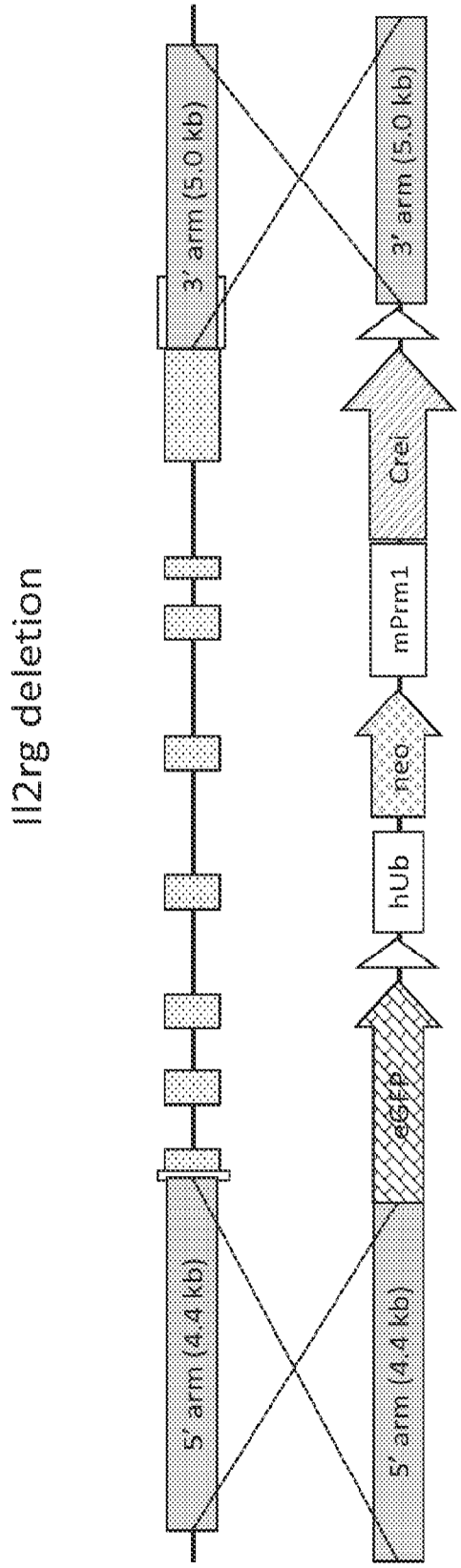

Il2rg deletion
5' arm (4.4 kb)
eGFP
hUb
neo
mPrm1
Cre
3' arm (5.0 kb)
5' arm (4.4 kb)
3' arm (5.0 kb)

FIG. 24

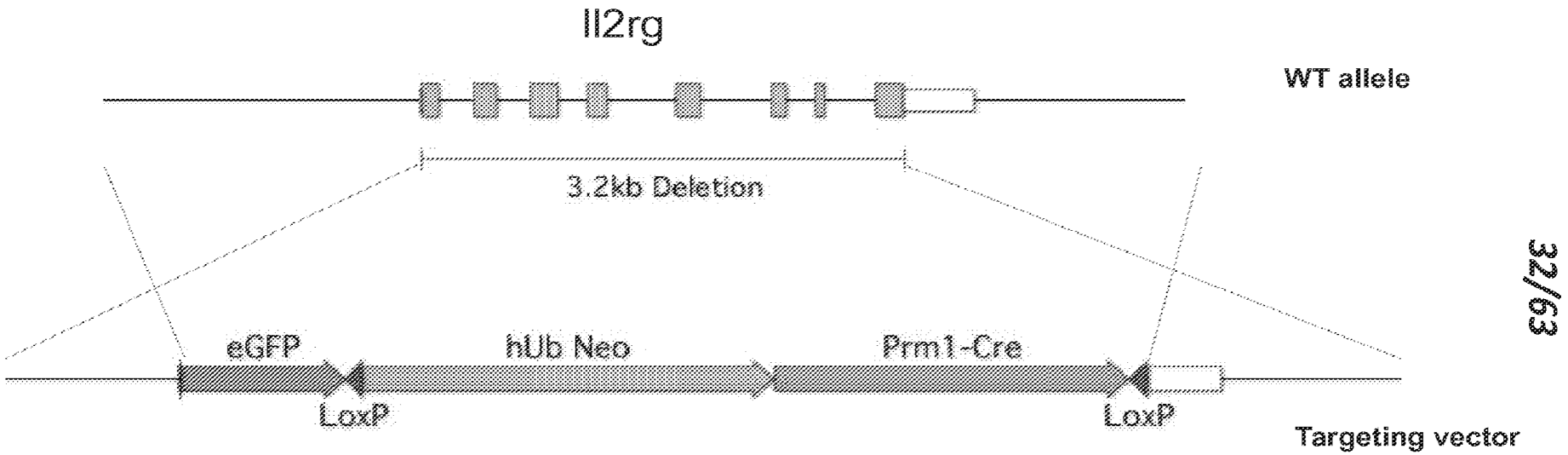

Il2rg
WT allele
3.2kb Deletion
eGFP
hUb Neo
Prm1-Cre
LoxP
LoxP
Targeting vector

FIG. 25

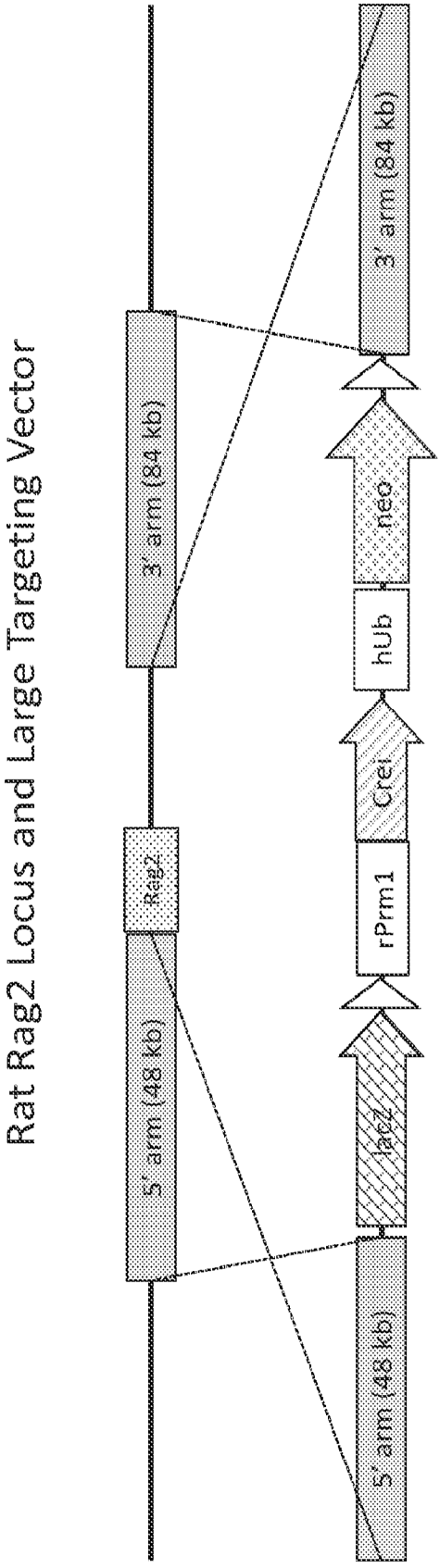

Rat Rag2 Locus and Large Targeting Vector
3' arm (84 kb)
Rag2
5' arm (48 kb)
3' arm (84 kb)
neo
hUb
Cre1
rPrm1
lacZ
5' arm (48 kb)

FIG. 26

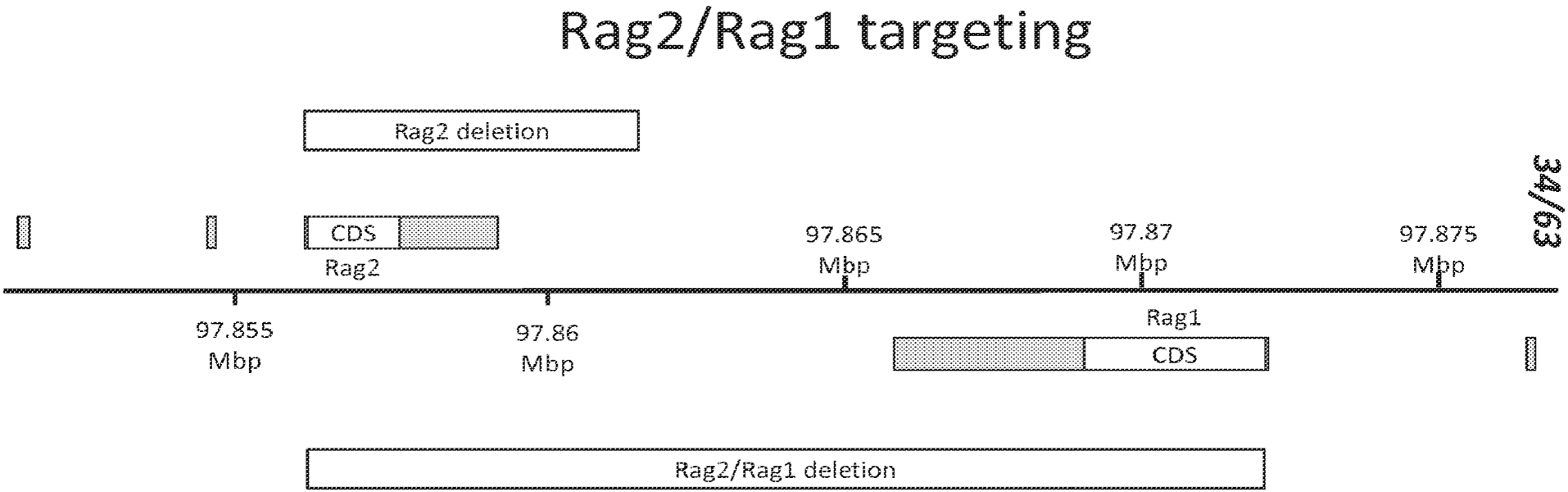

Rag2/Rag1 targeting
Rag2 deletion
CDS
Rag2
97.855
Mbp
97.86
Mbp
97.865
Mbp
97.87
Mbp
97.875
Mbp
Rag1
CDS
Rag2/Rag1 deletion

FIG. 27

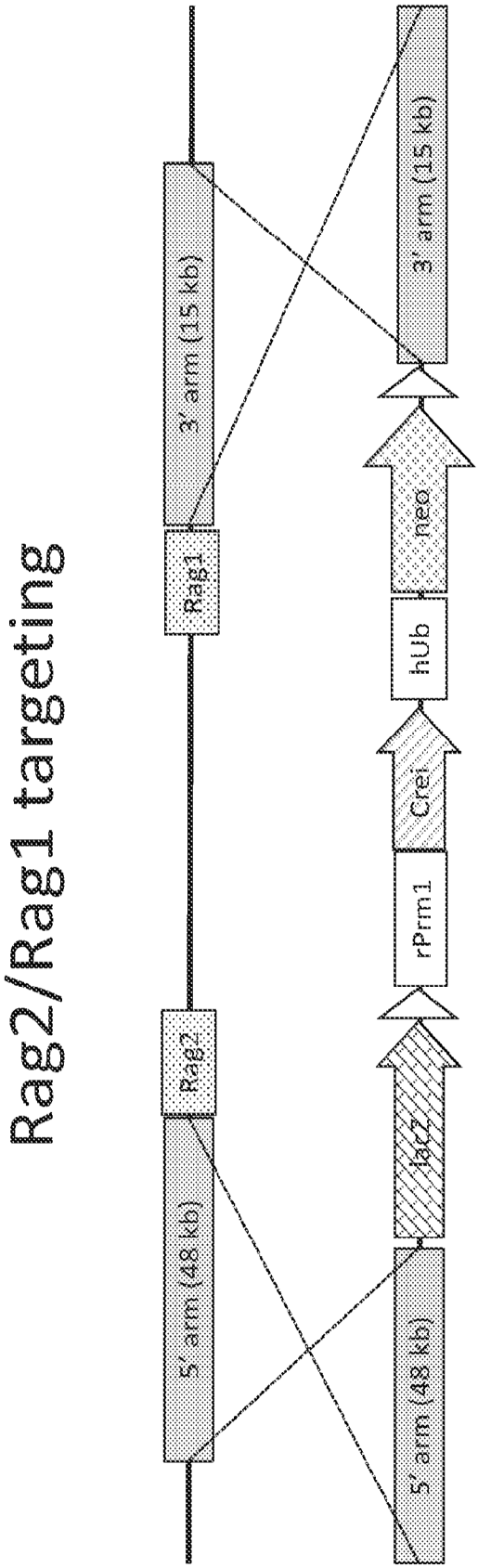

Rag2/Rag1 targeting
5' arm (48 kb)
Rag2
3' arm (15 kb)
Rag1
5' arm (48 kb)
lacZ
rPrm1
Crei
hUb
neo
3' arm (15 kb)

FIG. 28

FIG. 29A

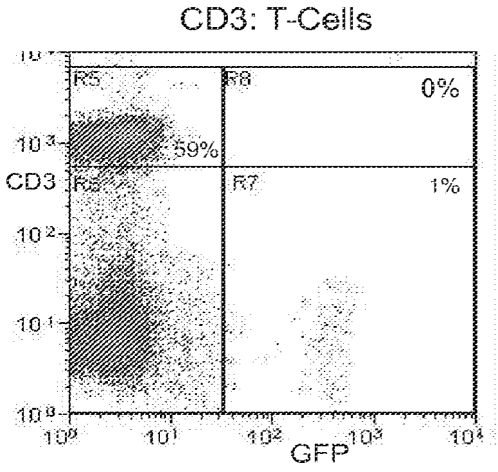


FIG. 29B

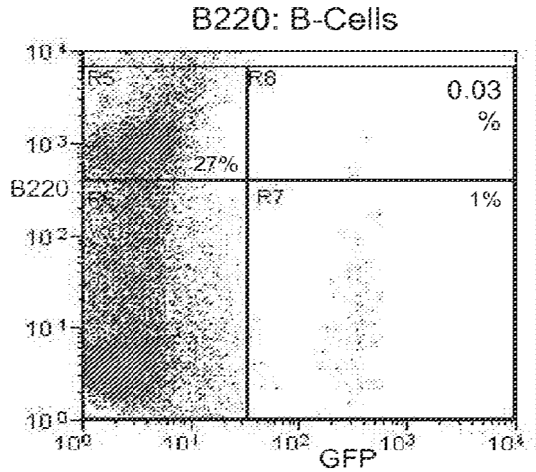
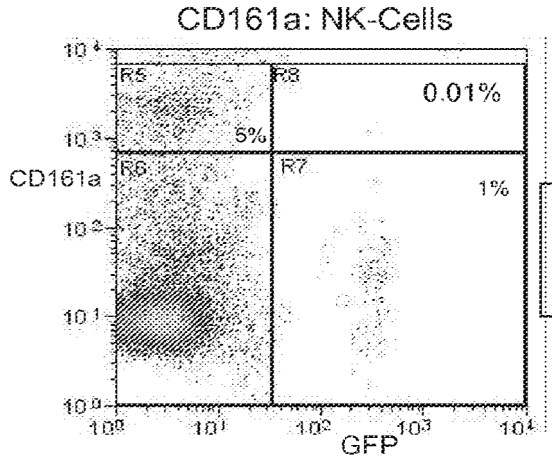


FIG. 29C



Il2rg$^{-/Y}$ chimera #3

36/63

FIG. 29D

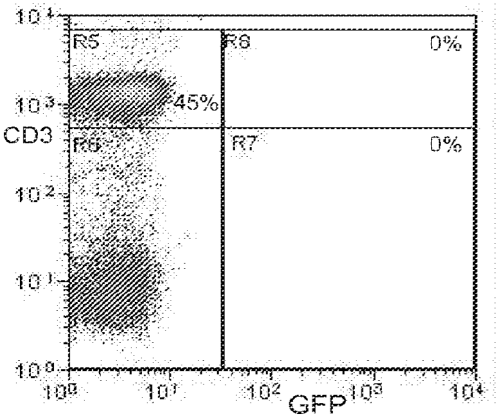


FIG. 29E

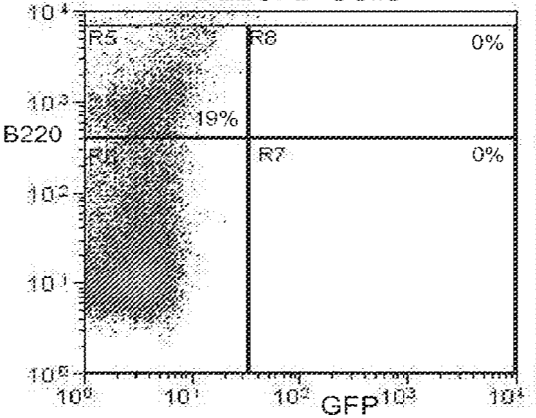
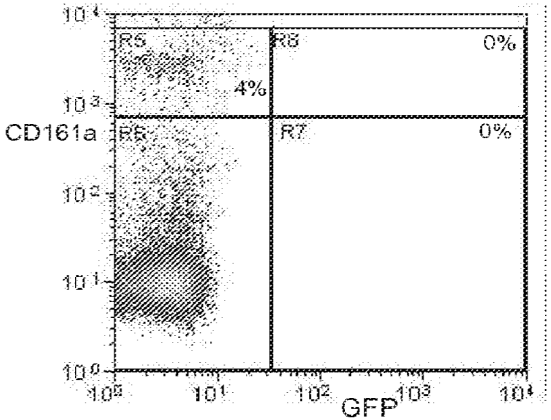


FIG. 29F



WT DA rat

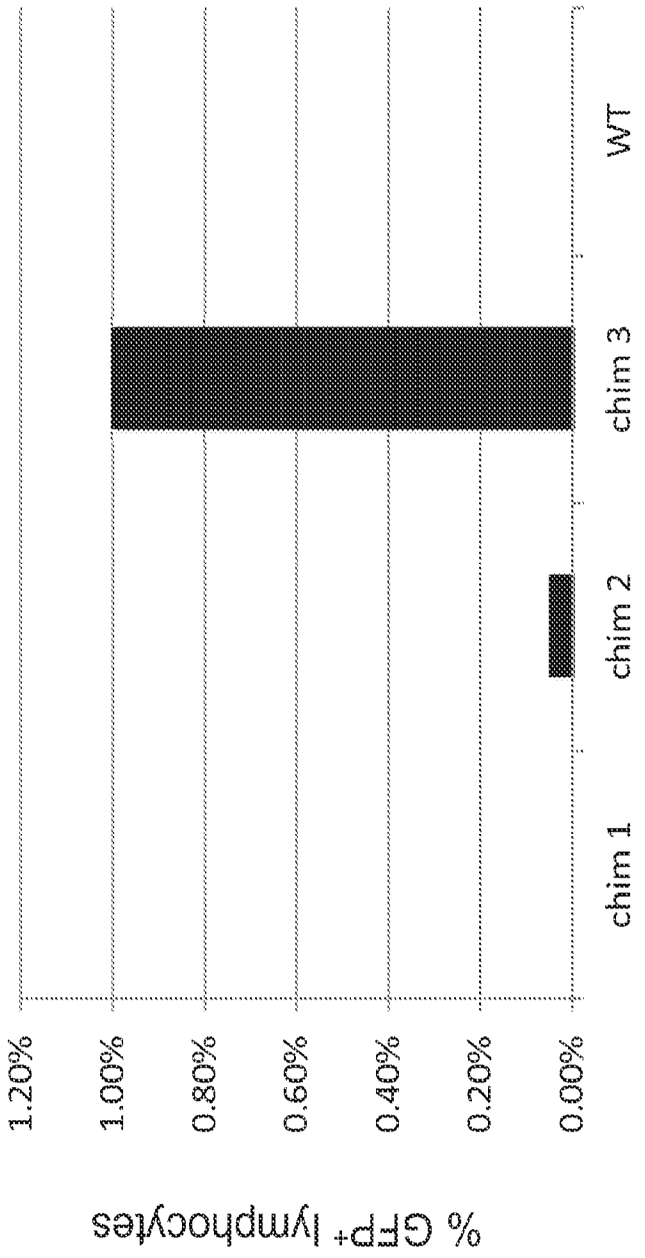

% GFP+ lymphocytes
1.20%
1.00%
0.80%
0.60%
0.40%
0.20%
0.00%
chim 1
chim 2
chim 3
WT

FIG. 30

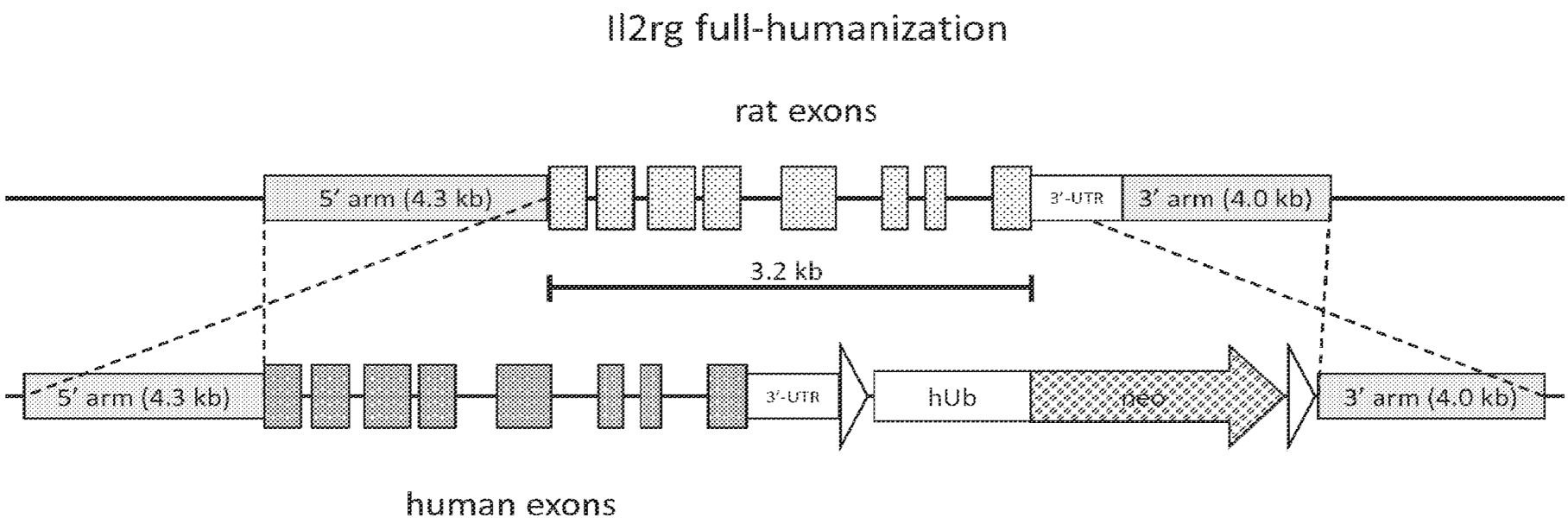

Il2rg full-humanization
rat exons
5′ arm (4.3 kb)
3′-UTR
3′ arm (4.0 kb)
3.2 kb
5′ arm (4.3 kb)
3′-UTR
hUb
neo
3′ arm (4.0 kb)
human exons

FIG. 31

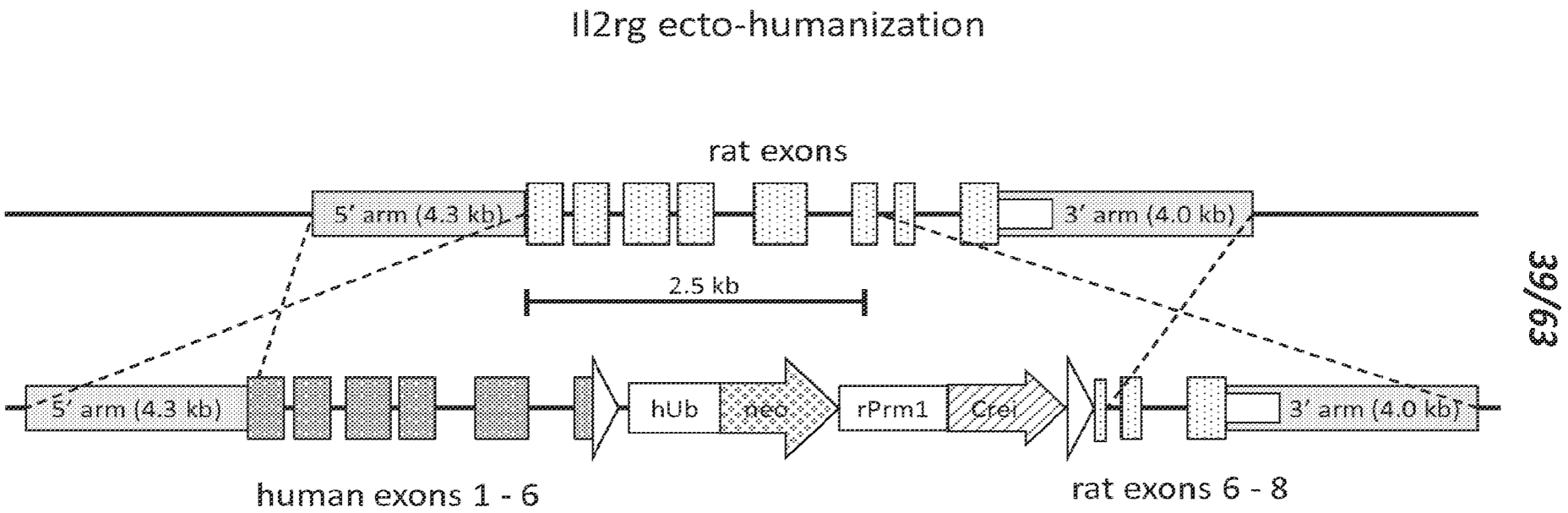

Il2rg ecto-humanization
rat exons
5' arm (4.3 kb)
3' arm (4.0 kb)
2.5 kb
5' arm (4.3 kb)
hUb
neo
rPrm1
Crei
3' arm (4.0 kb)
human exons 1 - 6
rat exons 6 - 8

FIG. 32

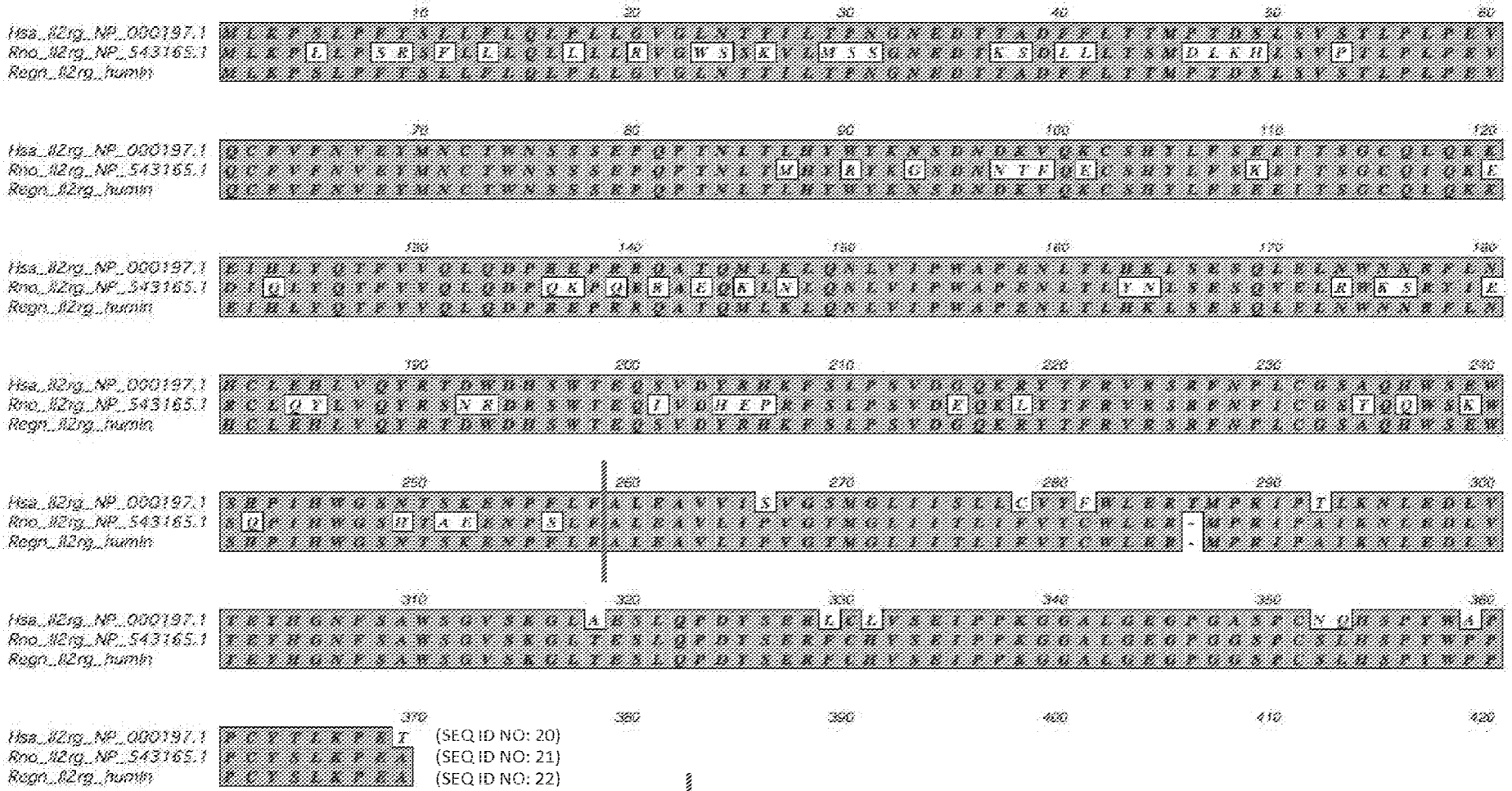
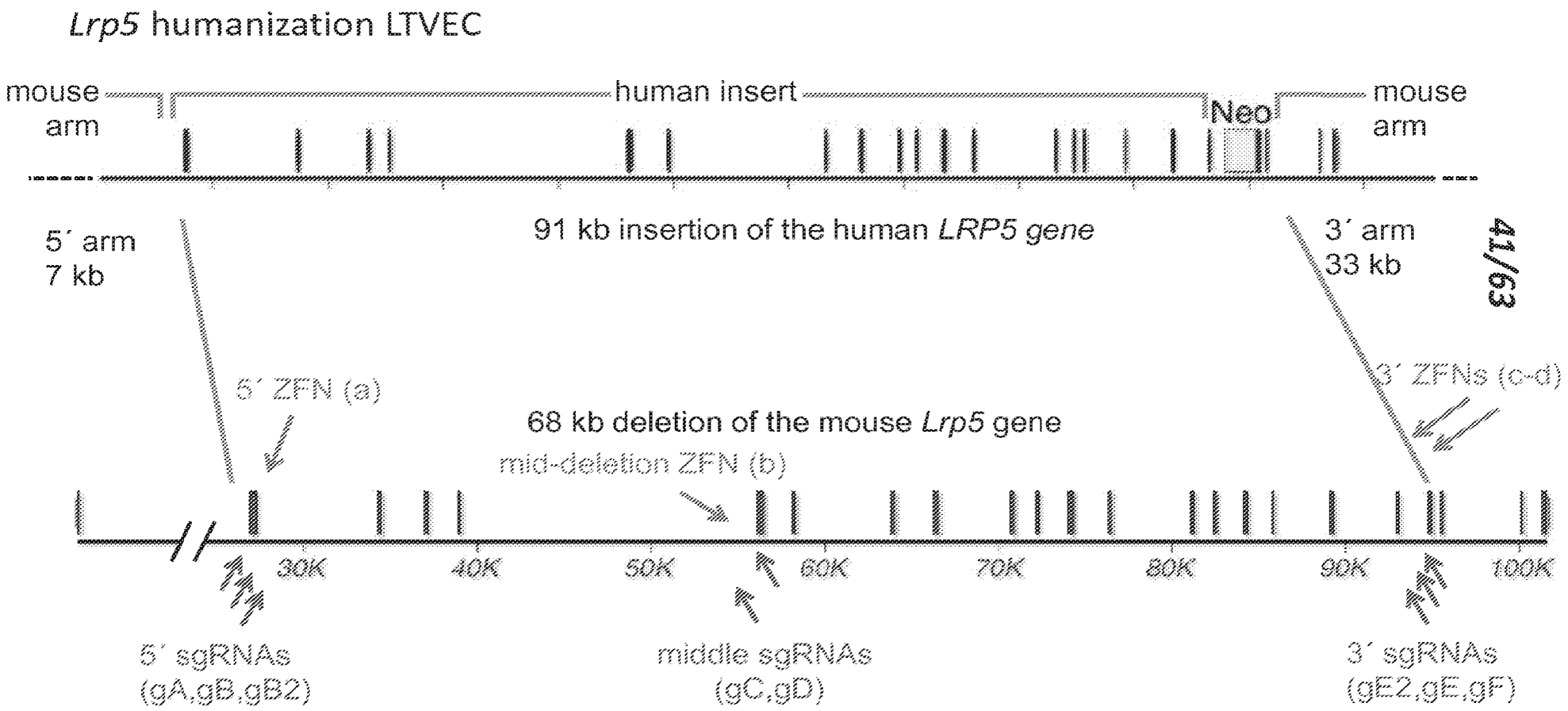

Hsa_Il2rg_NP_000197.1
Rno_Il2rg_NP_543165.1
Regn_Il2rg_humin
(SEQ ID NO: 20)
(SEQ ID NO: 21)
(SEQ ID NO: 22)
= Junction between human/rat Il2rg protein

FIG. 33

FIG. 34


Lrp5 humanization LTVEC
mouse arm
human insert
Neo
mouse arm
5′ arm 7 kb
91 kb insertion of the human *LRP5 gene*
3′ arm 33 kb
41/63
5′ ZFN (a)
68 kb deletion of the mouse *Lrp5* gene
mid-deletion ZFN (b)
3′ ZFNs (c-d)
30K
40K
50K
60K
70K
80K
90K
100K
5′ sgRNAs (gA,gB,gB2)
middle sgRNAs (gC,gD)
3′ sgRNAs (gE2,gE,gF)
Mouse *Lrp5* genomic locus

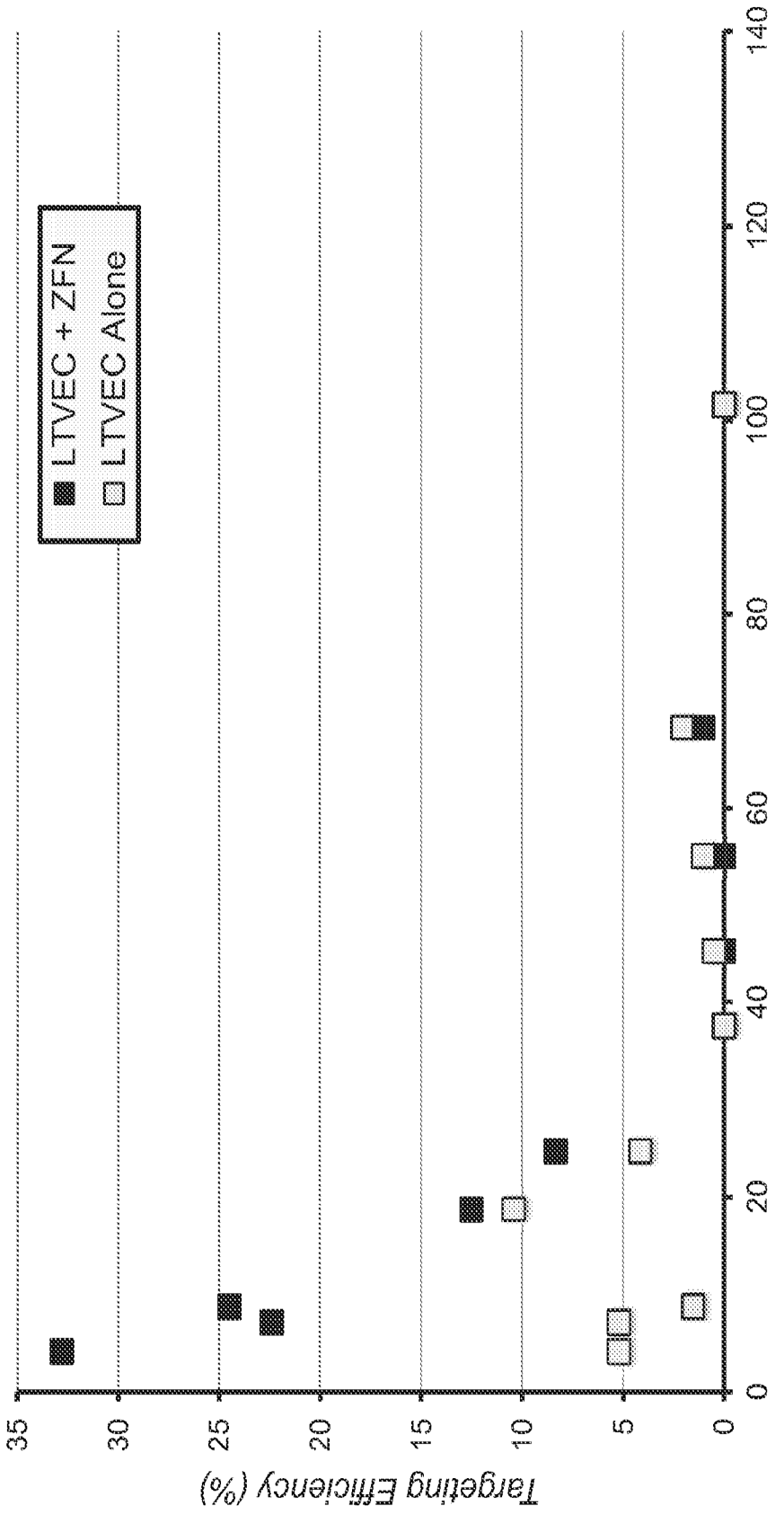

LTVEC + ZFN
LTVEC Alone
Targeting Efficiency (%)
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
140
Mouse Gene Deletion Size (kb)

FIG. 35A

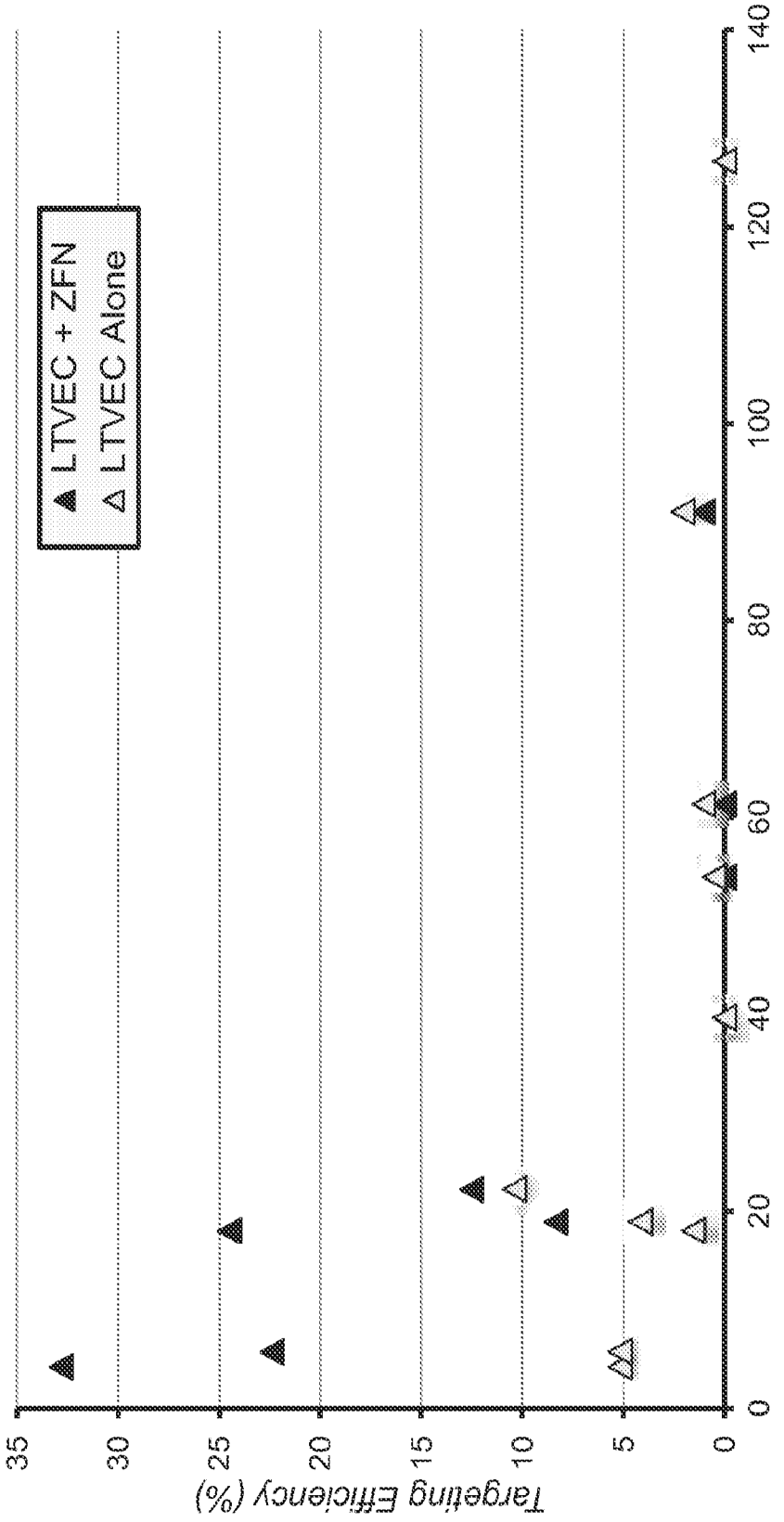

LTVEC + ZFN
LTVEC Alone
Targeting Efficiency (%)
Human Gene Insertion Size (kb)
0
5
10
15
20
25
30
35
0
20
40
60
80
100
120
140

FIG. 35B

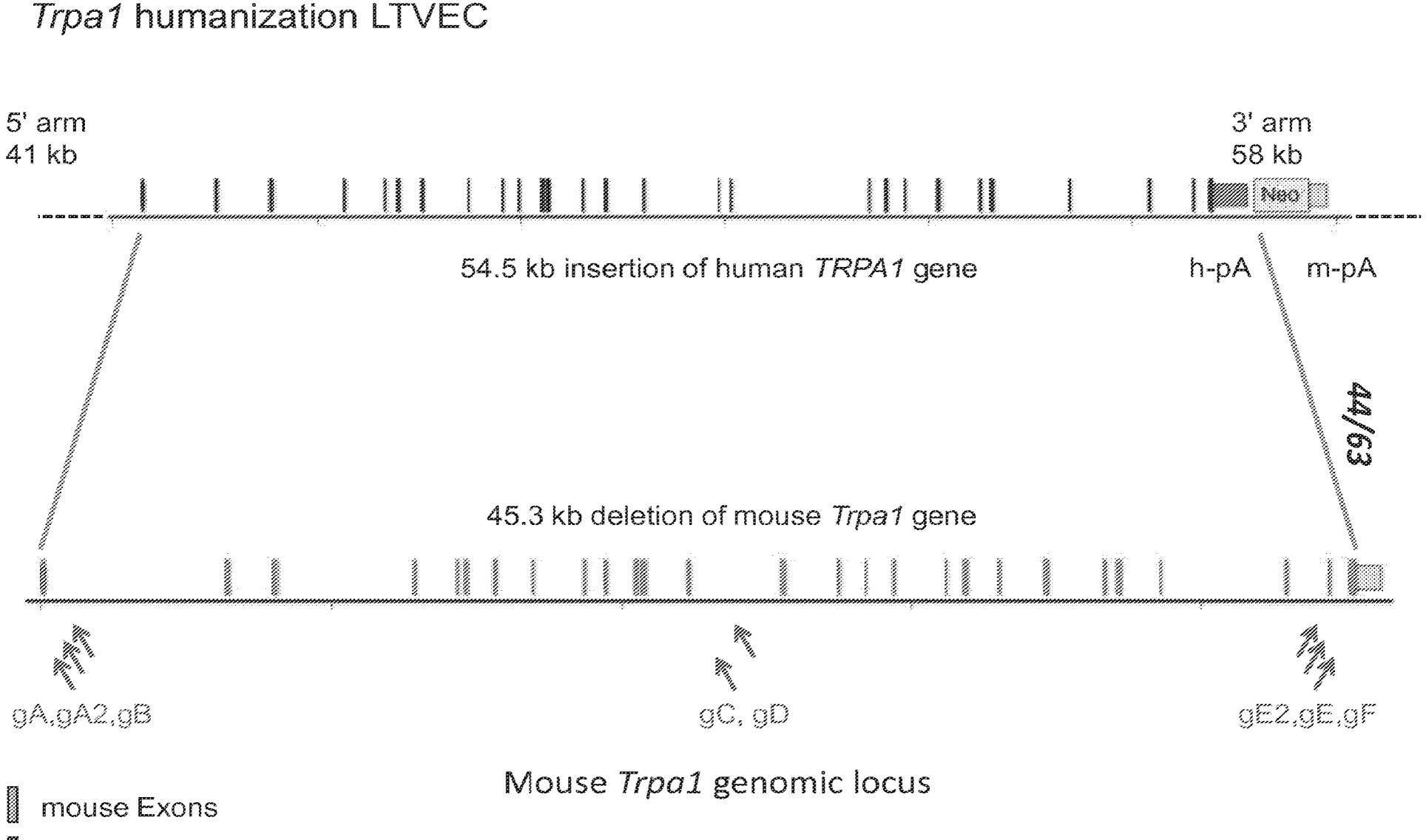

Trpa1 humanization LTVEC
5' arm
41 kb
3' arm
58 kb
Neo
54.5 kb insertion of human *TRPA1* gene
h-pA
m-pA
45.3 kb deletion of mouse *Trpa1* gene
gA,gA2,gB
gC, gD
gE2,gE,gF
Mouse *Trpa1* genomic locus
mouse Exons
human Exons

FIG. 36

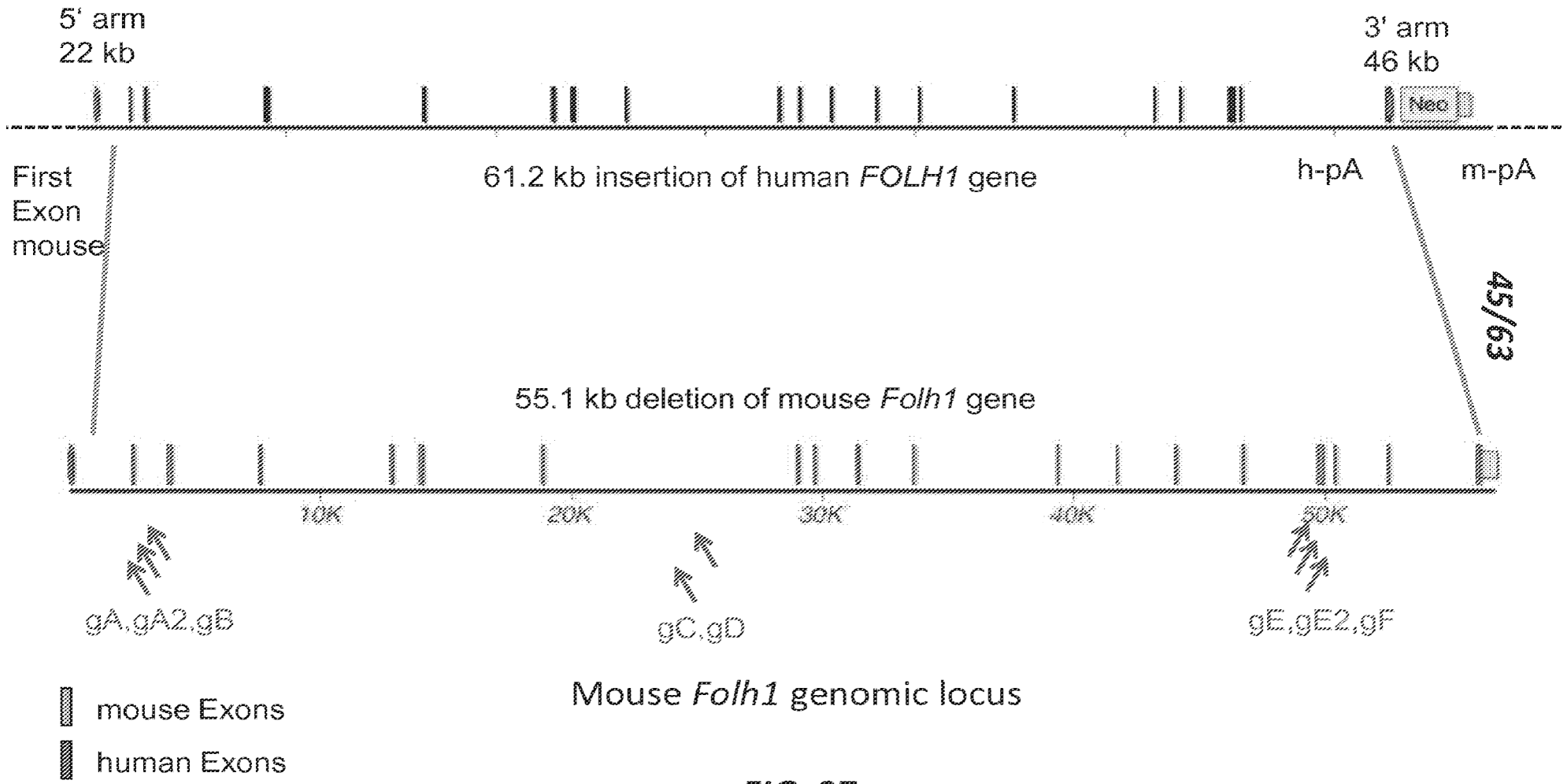
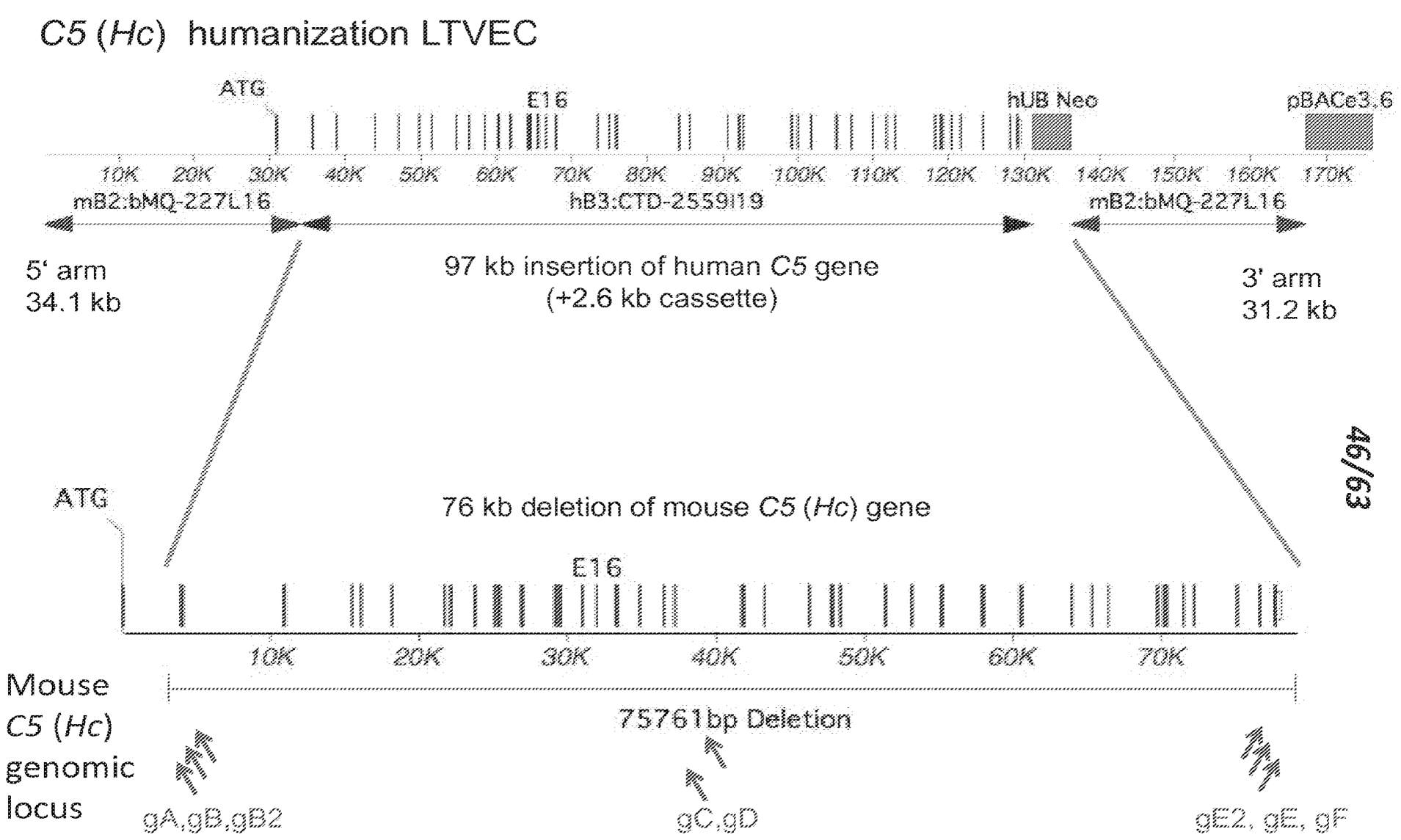

Folh1 humanization LTVEC
5' arm
22 kb
3' arm
46 kb
Neo
First
Exon
mouse
61.2 kb insertion of human *FOLH1* gene
h-pA
m-pA
55.1 kb deletion of mouse *Folh1* gene
10K
20K
30K
40K
50K
gA,gA2,gB
gC,gD
gE,gE2,gF
mouse Exons
human Exons
Mouse *Folh1* genomic locus

FIG. 37


C5 (Hc) humanization LTVEC
ATG
E16
hUB Neo
pBACe3.6
10K 20K 30K 40K 50K 60K 70K 80K 90K 100K 110K 120K 130K 140K 150K 160K 170K
mB2:bMQ-227L16
hB3:CTD-2559I19
mB2:bMQ-227L16
5' arm
34.1 kb
97 kb insertion of human C5 gene
(+2.6 kb cassette)
3' arm
31.2 kb
ATG
76 kb deletion of mouse C5 (Hc) gene
E16
10K 20K 30K 40K 50K 60K 70K
Mouse
C5 (Hc)
genomic
locus
75761bp Deletion
gA,gB,gB2
gC,gD
gE2, gE, gF

46/63

FIG. 38

Adamts5 humanization LTVEC

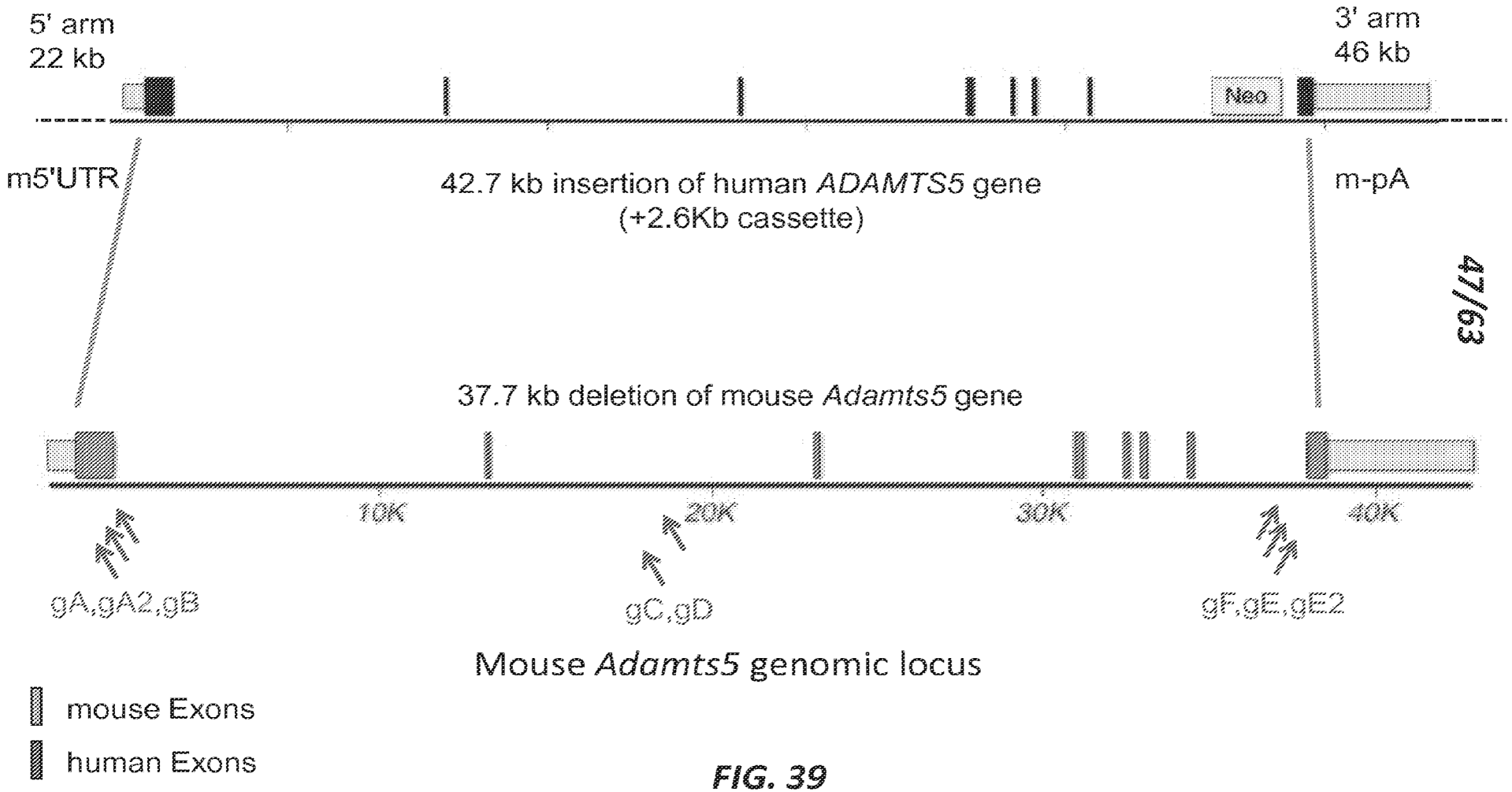
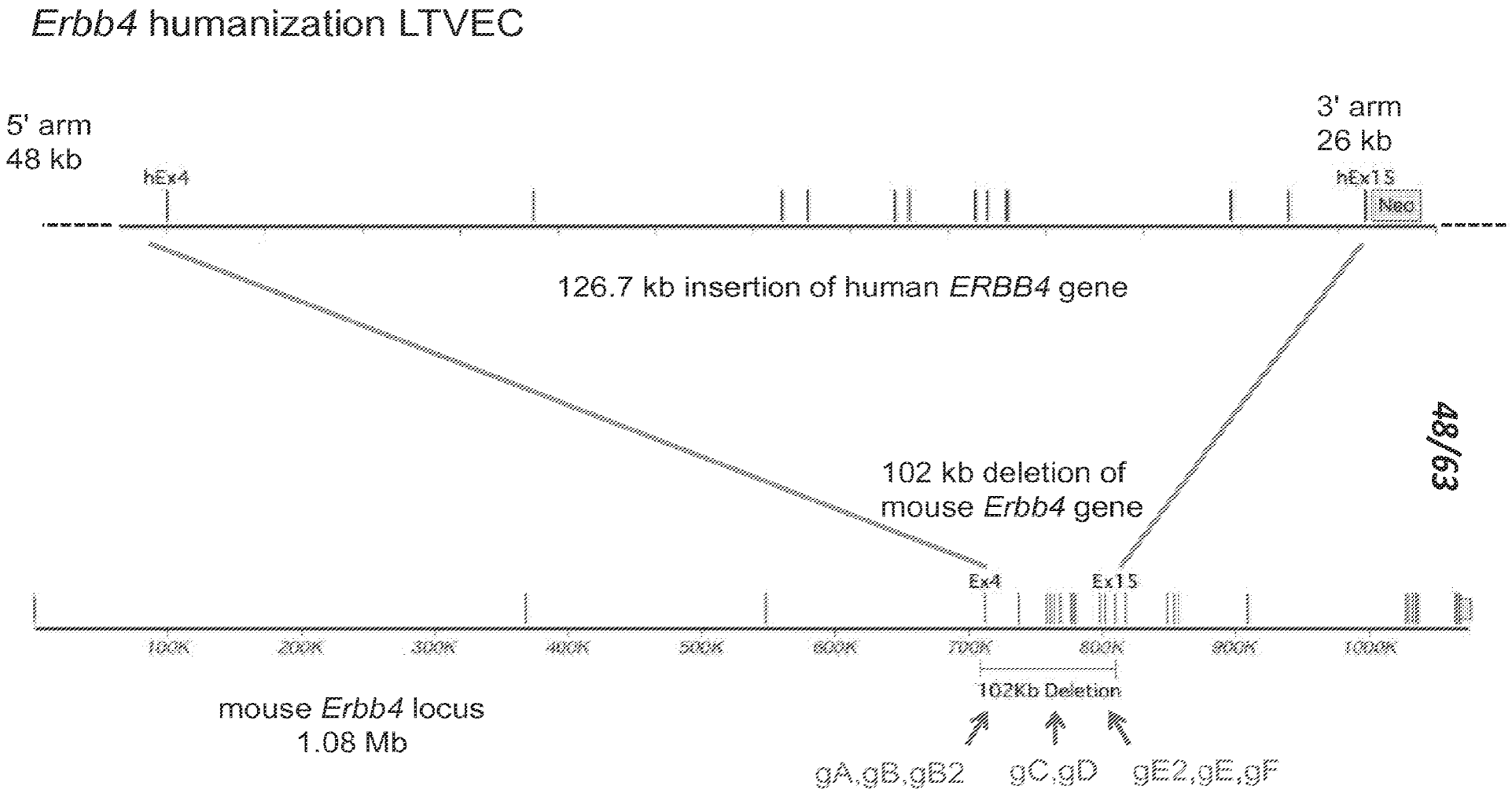


FIG. 39


Erbb4 humanization LTVEC
5' arm
48 kb
3' arm
26 kb
hEx4
hEx15
Neo
126.7 kb insertion of human *ERBB4* gene
102 kb deletion of
mouse *Erbb4* gene
Ex4
Ex15
100K
200K
300K
400K
500K
600K
700K
800K
900K
1000K
102Kb Deletion
mouse *Erbb4* locus
1.08 Mb
gA,gB,gB2
gC,gD
gE2,gE,gF
Mouse *Erbb4* genomic locus

48/63

FIG. 40

Ror1 humanization LTVEC

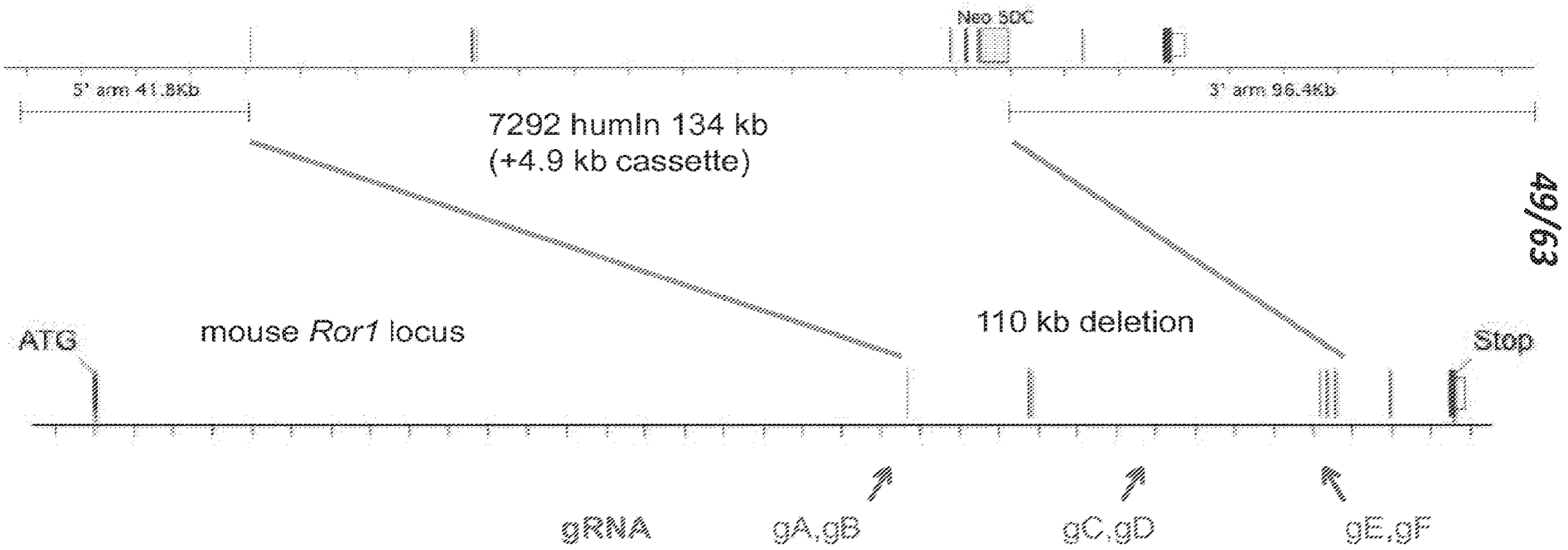
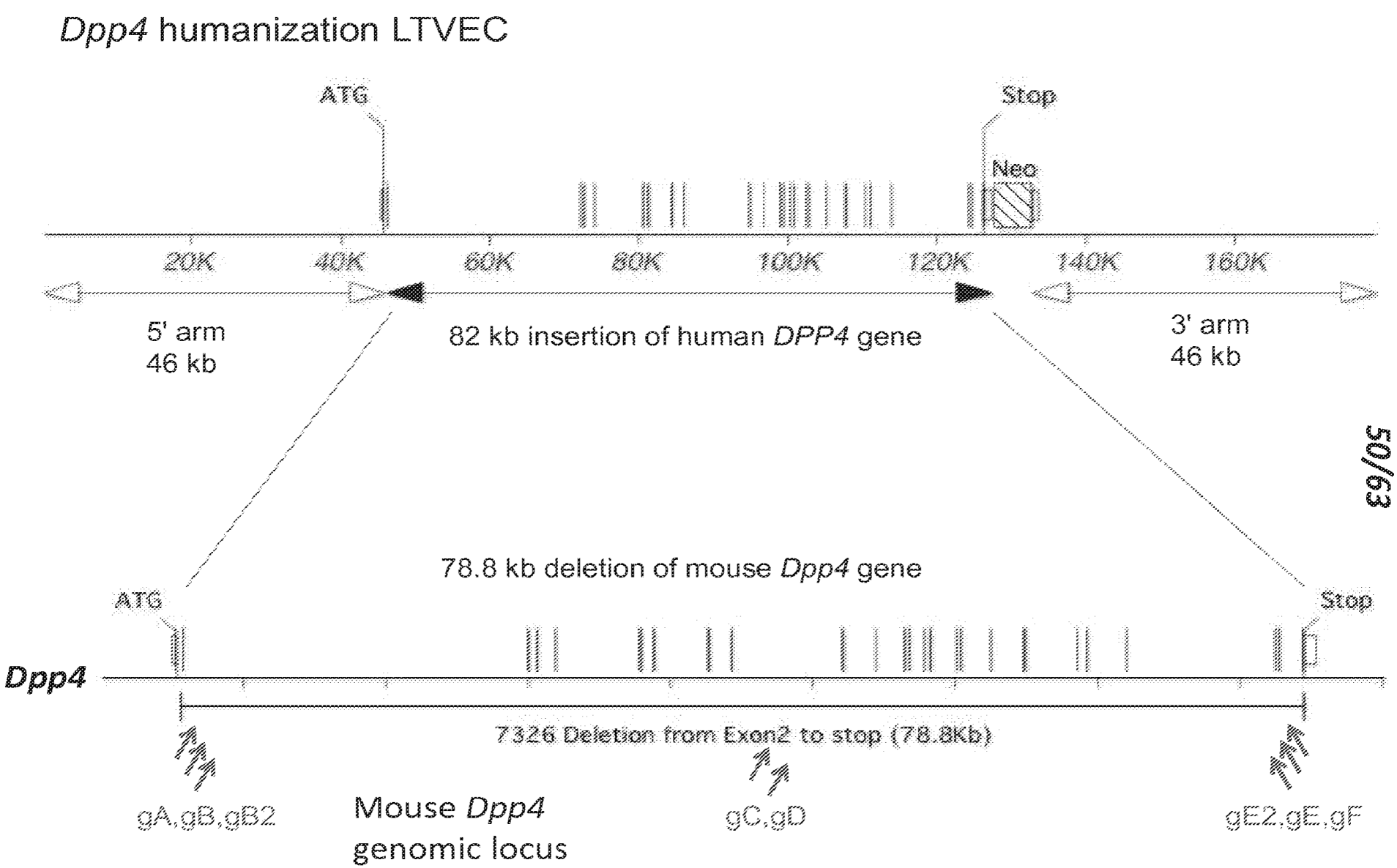


FIG. 41


Dpp4 humanization LTVEC
ATG
Stop
Neo
20K
40K
60K
80K
100K
120K
140K
160K
5' arm
46 kb
82 kb insertion of human *DPP4* gene
3' arm
46 kb
78.8 kb deletion of mouse *Dpp4* gene
ATG
Stop
Dpp4
7326 Deletion from Exon2 to stop (78.8Kb)
gA,gB,gB2
Mouse *Dpp4*
genomic locus
gC,gD
gE2,gE,gF

50/63

FIG. 42

FIG. 43A

FIG. 43B

FIG. 43C

Wild type

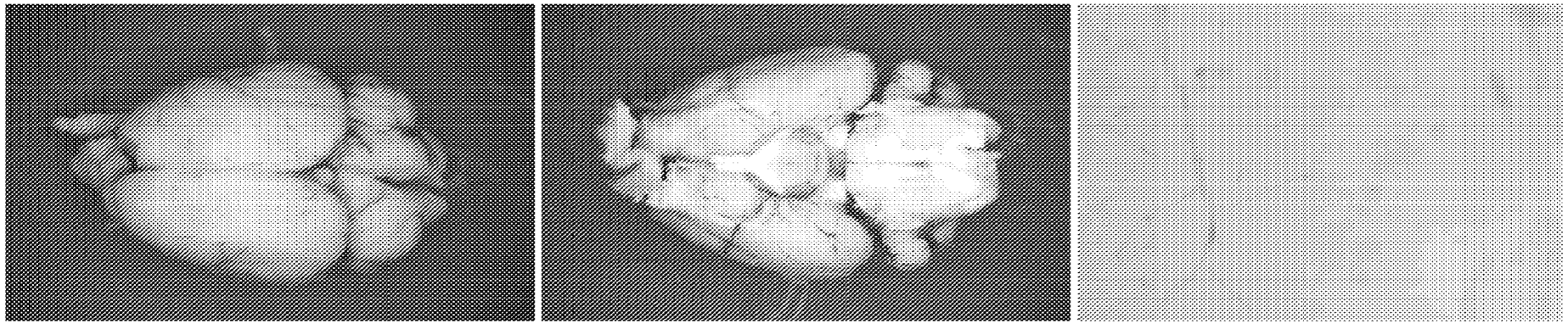


FIG. 43D

FIG. 43E

FIG. 43F

ApoE$^{+/-}$

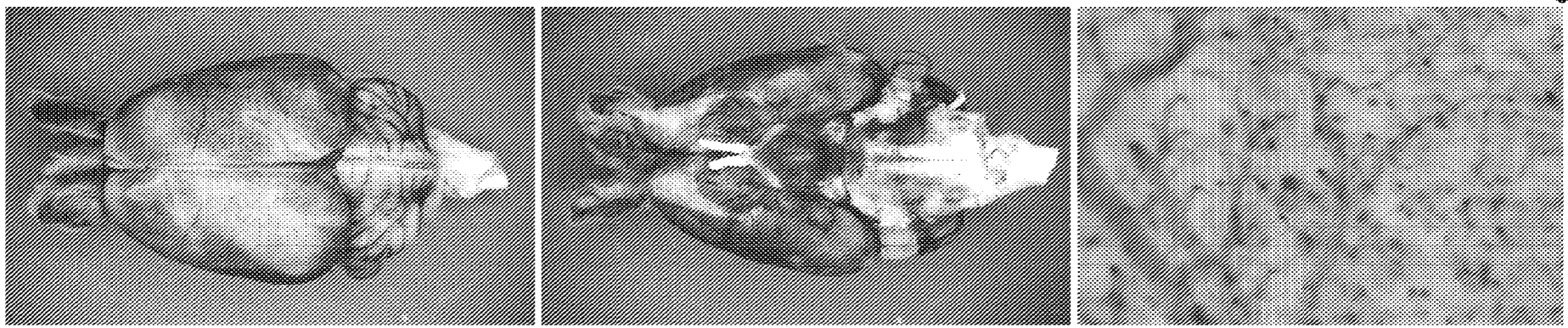


FIG. 44A

FIG. 44B

Wild type

FIG. 44C

FIG. 44D

ApoE$^{+/-}$

FIG. 45A

FIG. 45B

Wild type

FIG. 45C

FIG. 45D

ApoE$^{+/-}$

FIG. 46A

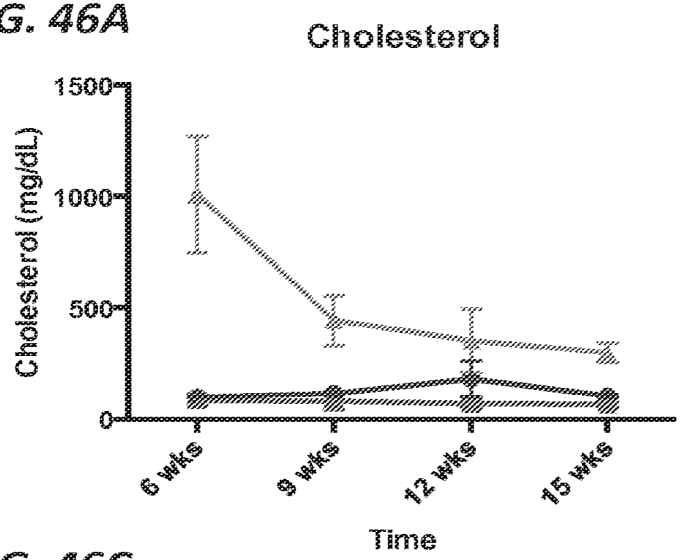


FIG. 46B

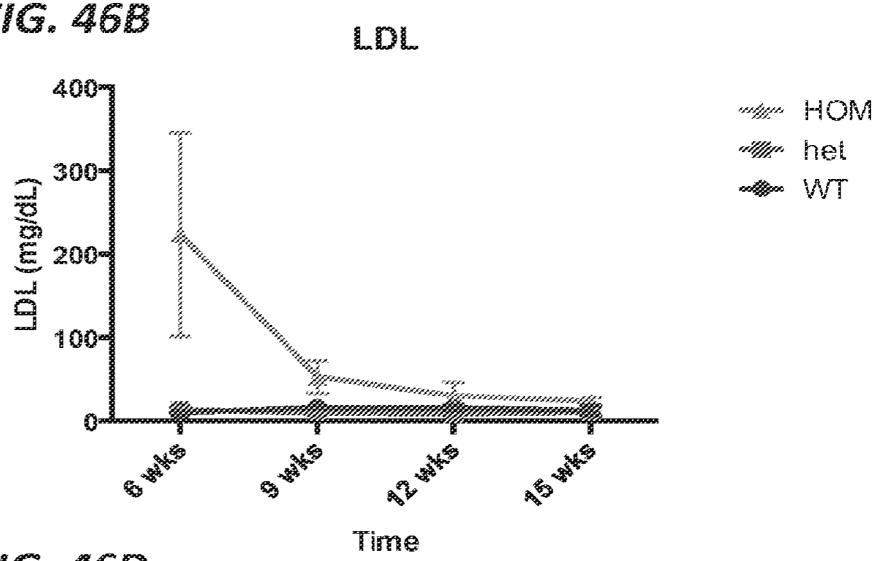


FIG. 46C

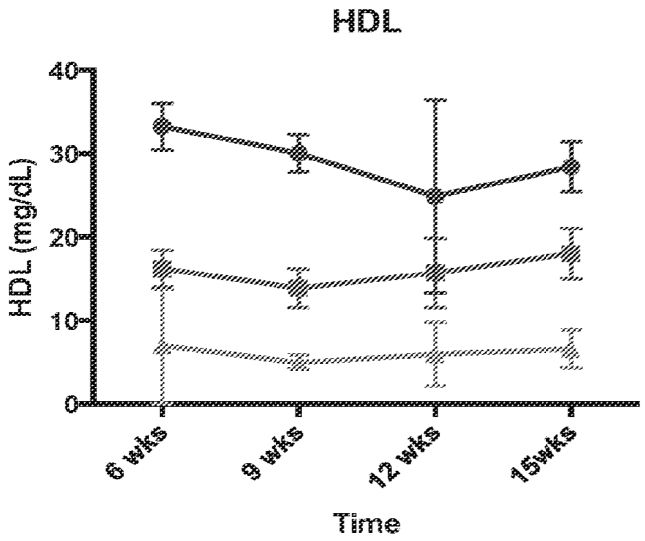


FIG. 46D

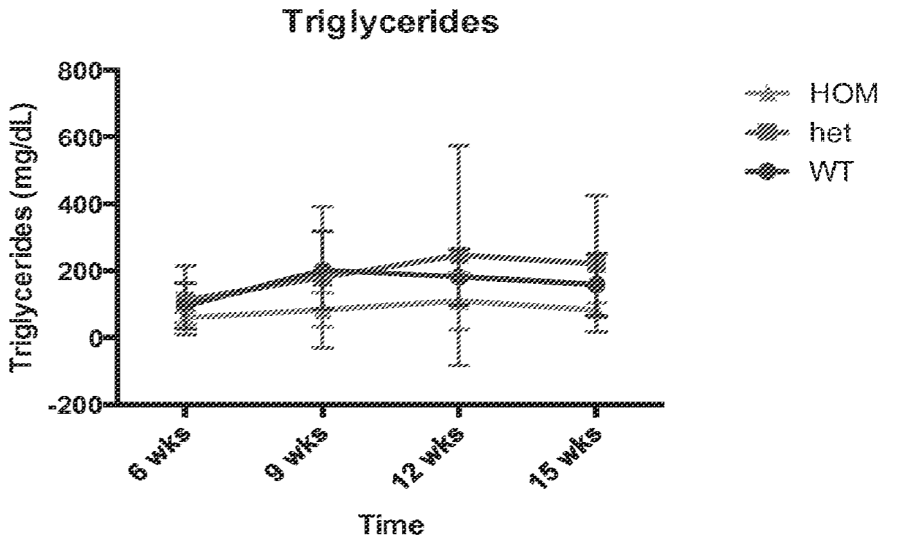


FIG. 47

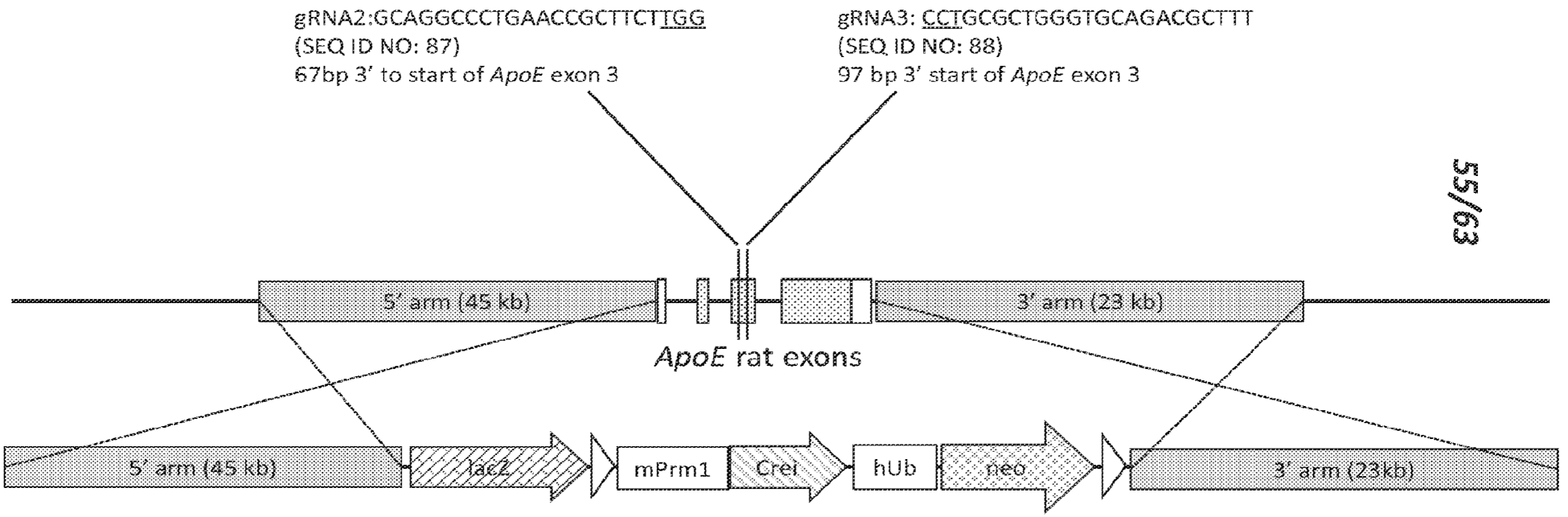

gRNA2:GCAGGCCCTGAACCGCTTCTTGG
(SEQ ID NO: 87)
67bp 3' to start of ApoE exon 3
gRNA3: CCTGCGCTGGGTGCAGACGCTTT
(SEQ ID NO: 88)
97 bp 3' start of ApoE exon 3
5' arm (45 kb)
3' arm (23 kb)
ApoE rat exons
5' arm (45 kb)
lacZ
mPrm1
Crei
hUb
neo
3' arm (23kb)

FIG. 48

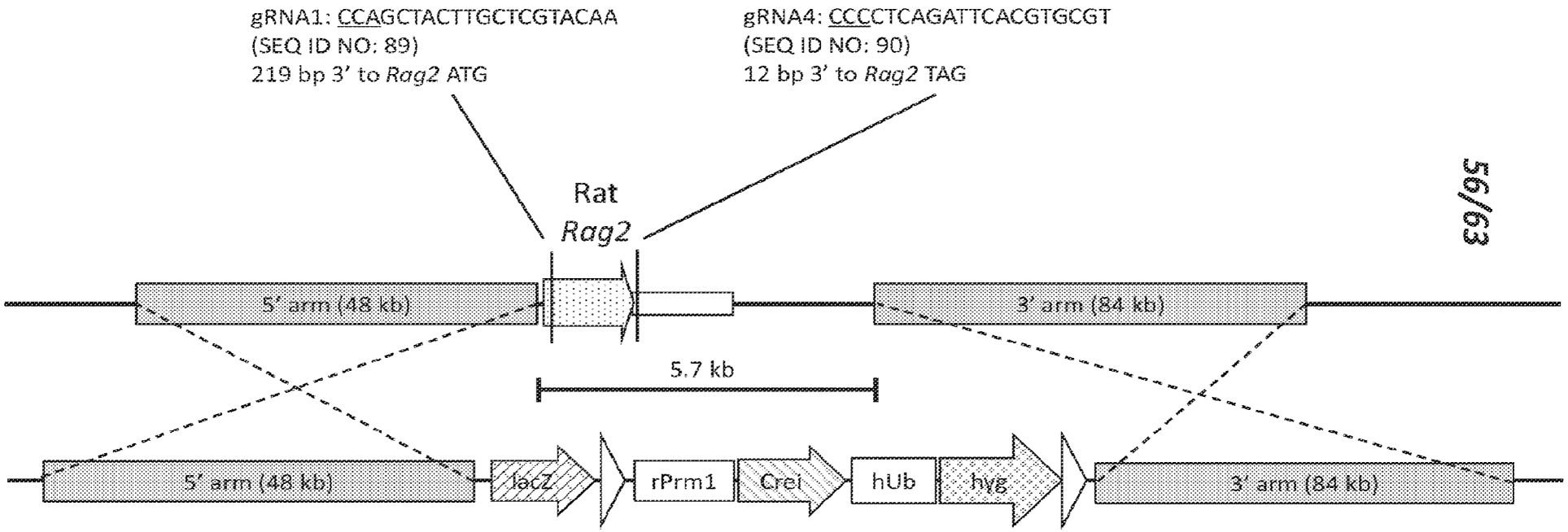
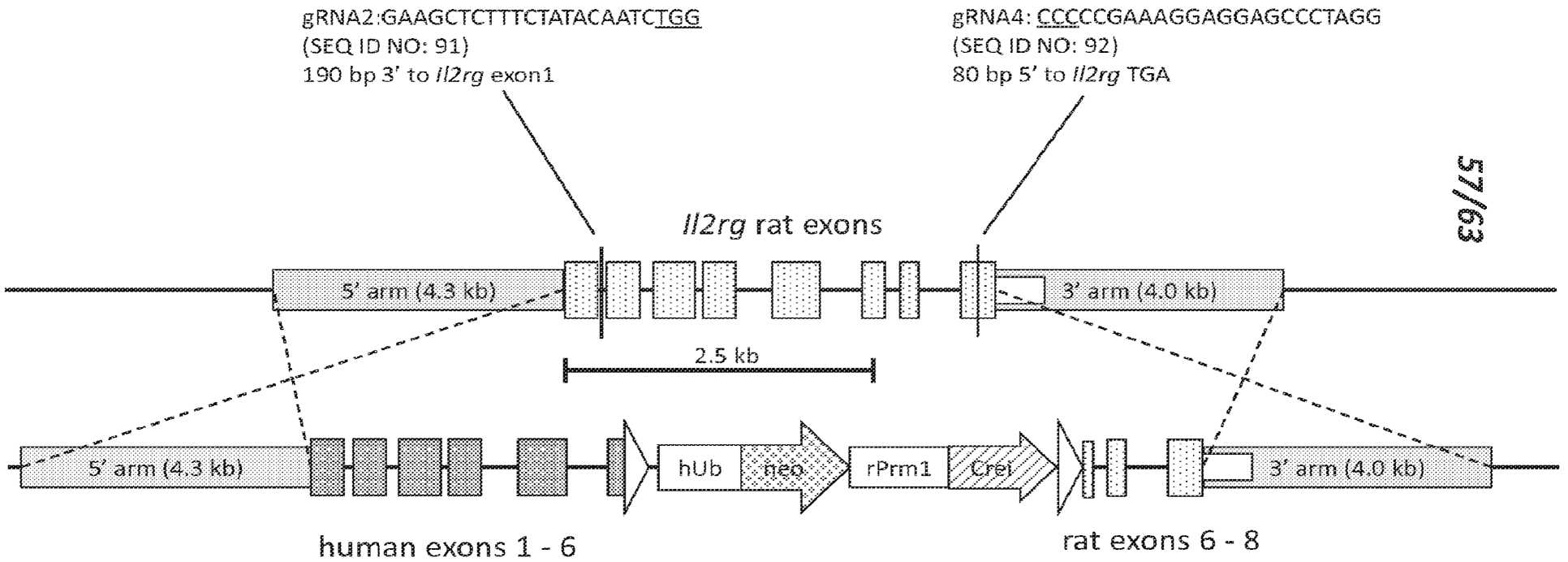

gRNA1: CCAGCTACTTGCTCGTACAA
(SEQ ID NO: 89)
219 bp 3' to *Rag2* ATG
gRNA4: CCCCTCAGATTCACGTGCGT
(SEQ ID NO: 90)
12 bp 3' to *Rag2* TAG
Rat
Rag2
5' arm (48 kb)
3' arm (84 kb)
5.7 kb
5' arm (48 kb)
lacZ
rPrm1
Crei
hUb
hyg
3' arm (84 kb)

FIG. 49


gRNA2:GAAGCTCTTTCTATACAATCTGG
(SEQ ID NO: 91)
190 bp 3' to *Il2rg* exon1
gRNA4: CCCCCGAAAGGAGGAGCCCTAGG
(SEQ ID NO: 92)
80 bp 5' to *Il2rg* TGA
Il2rg rat exons
5' arm (4.3 kb)
3' arm (4.0 kb)
2.5 kb
5' arm (4.3 kb)
hUb
neo
rPrm1
Cre
3' arm (4.0 kb)
human exons 1 - 6
rat exons 6 - 8

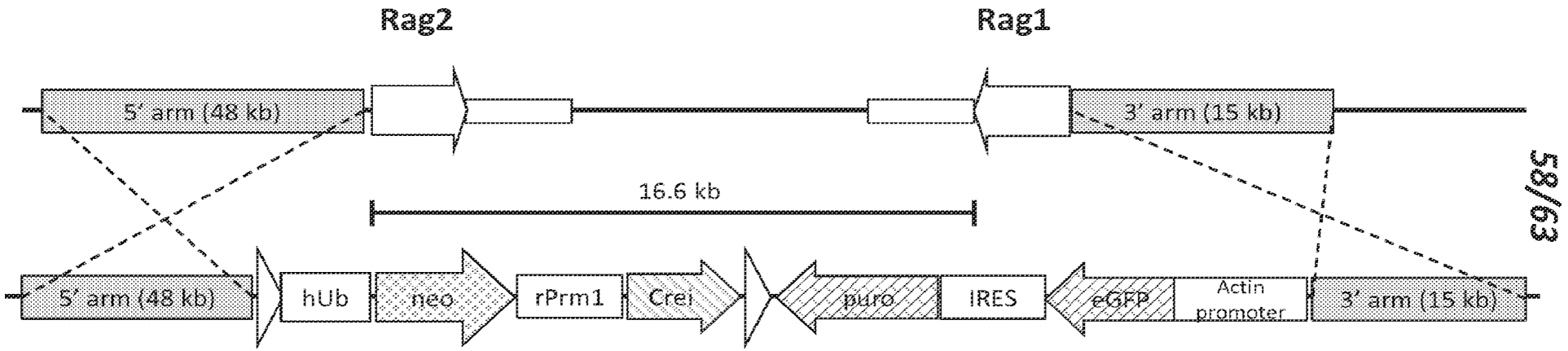

Rag2
Rag1
5' arm (48 kb)
3' arm (15 kb)
16.6 kb
5' arm (48 kb)
hUb
neo
rPrm1
Crei
puro
IRES
eGFP
Actin promoter
3' arm (15 kb)

FIG. 50

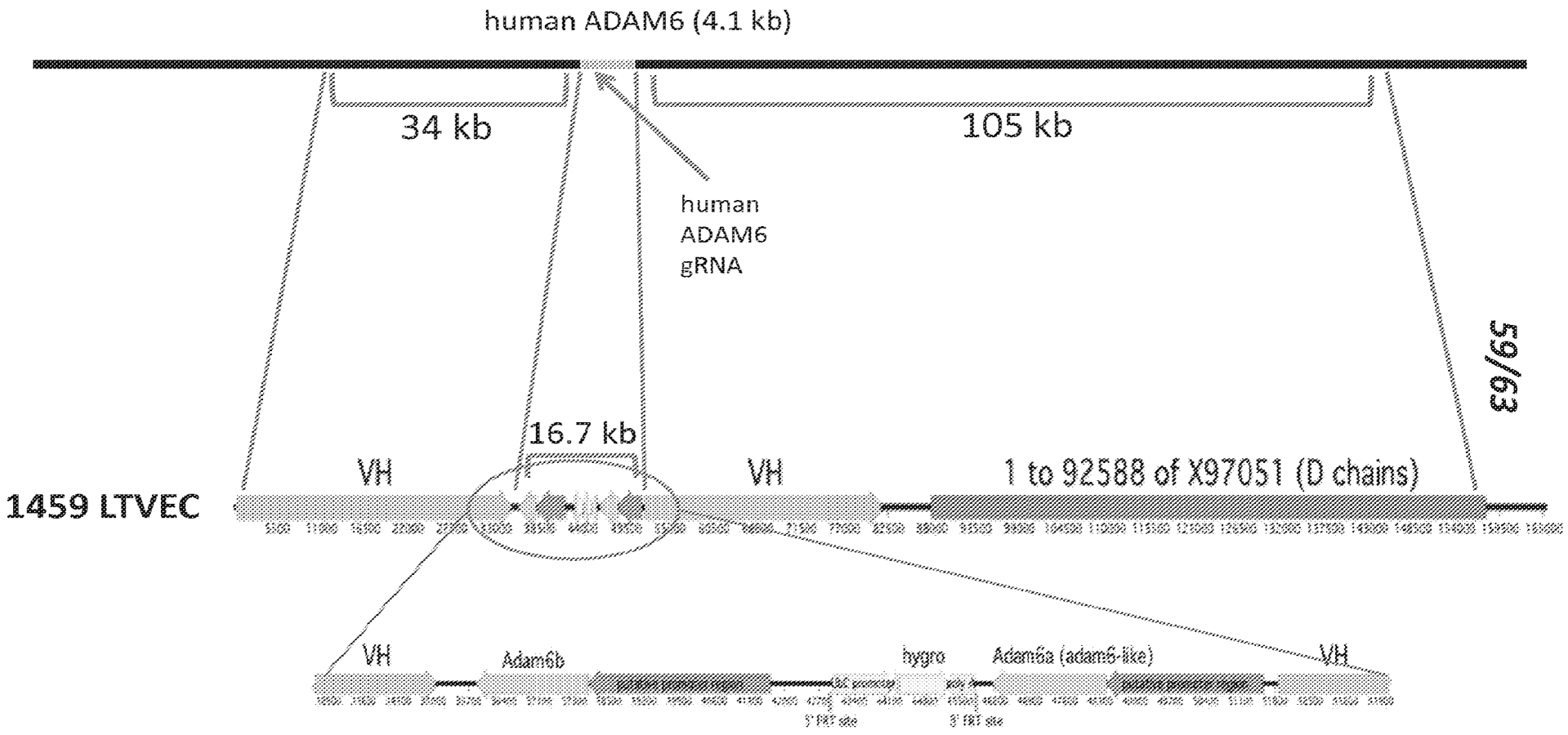
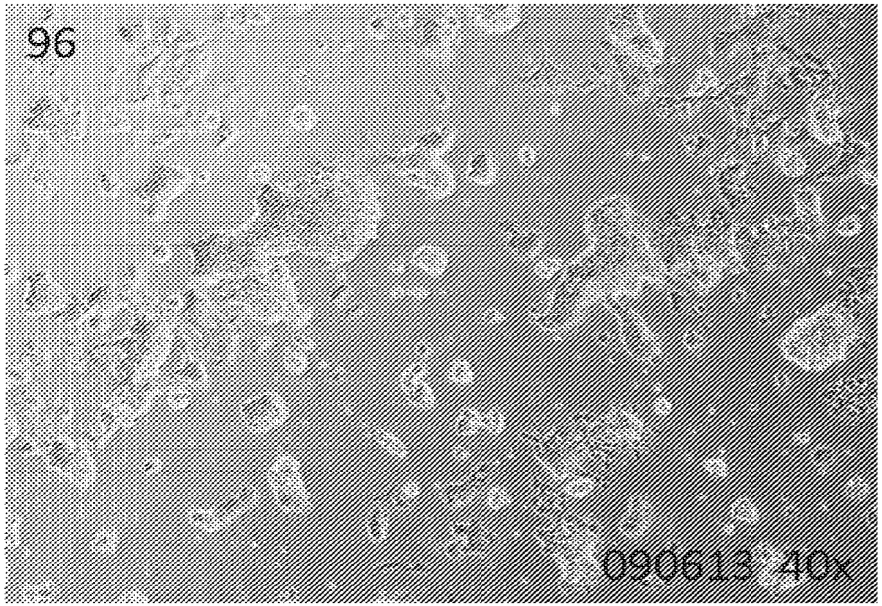

human ADAM6 (4.1 kb)
34 kb
105 kb
human
ADAM6
gRNA
16.7 kb
VH
VH
1 to 92588 of X97051 (D chains)
1459 LTVEC
VH
Adam6b
hygro
Adam6a (adam6-like)
VH
5' FRT site
3' FRT site

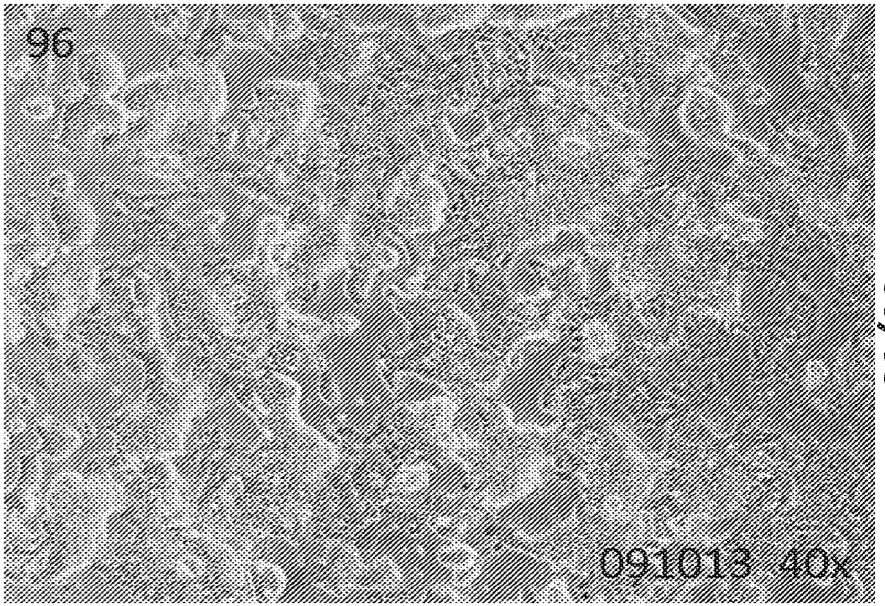
FIG. 51

FIG. 52A



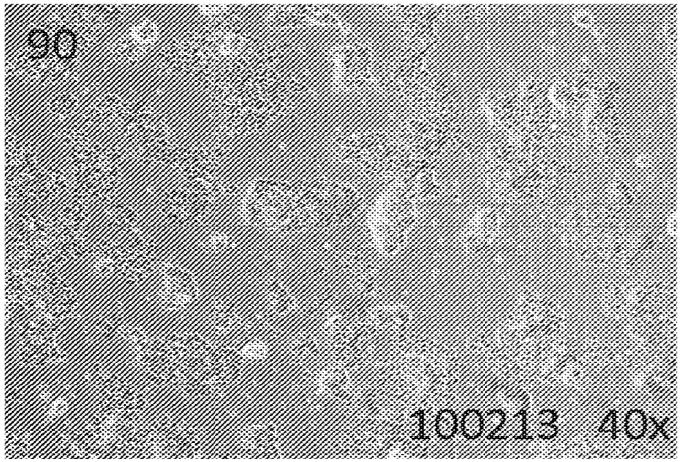
8 days in 2i

FIG. 52B



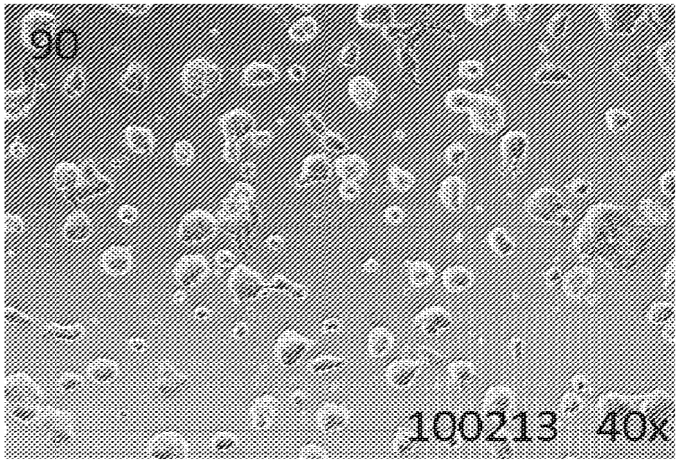
12 days in 2i

FIG. 53A



mTeSR-hLIF

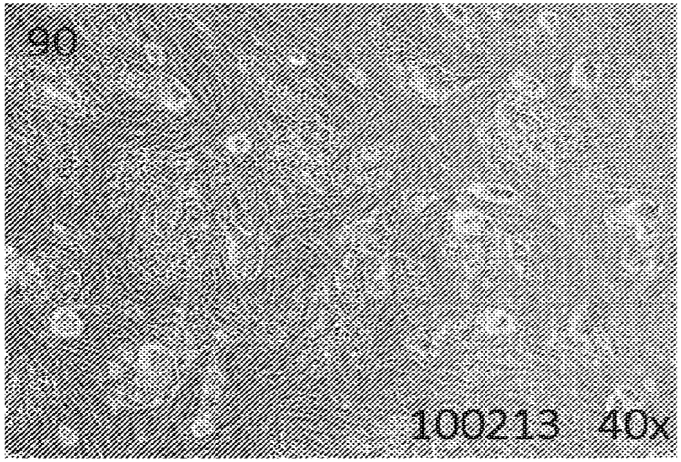
FIG. 53B



hVG2i

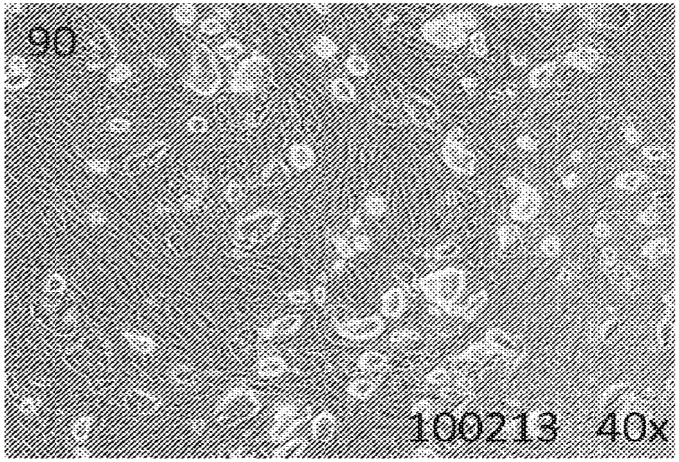
Matrigel

FIG. 53C



mTeSR-hLIF

FIG. 53D



hVG2i

NuFF feeder

FIG. 54C

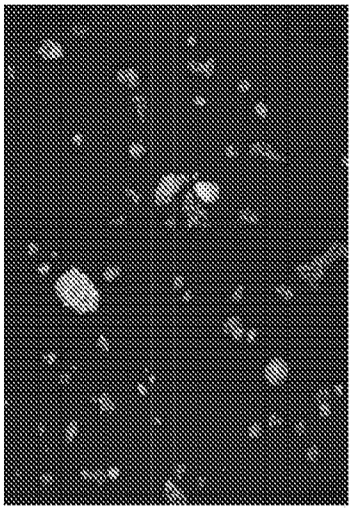


FIG. 54B

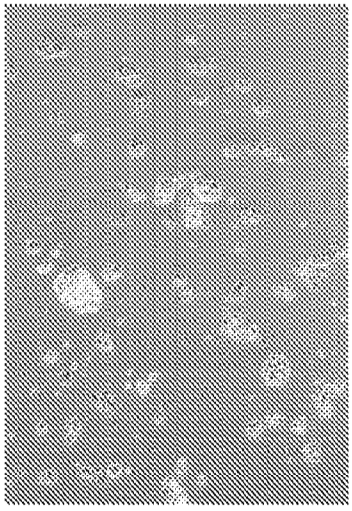


FIG. 54A

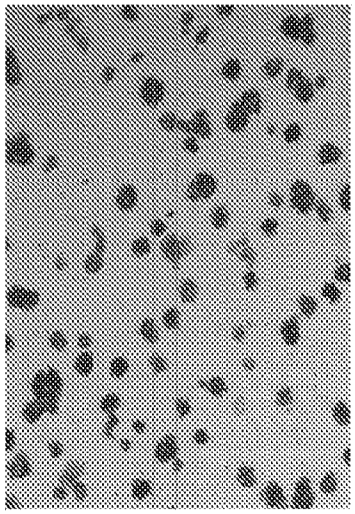
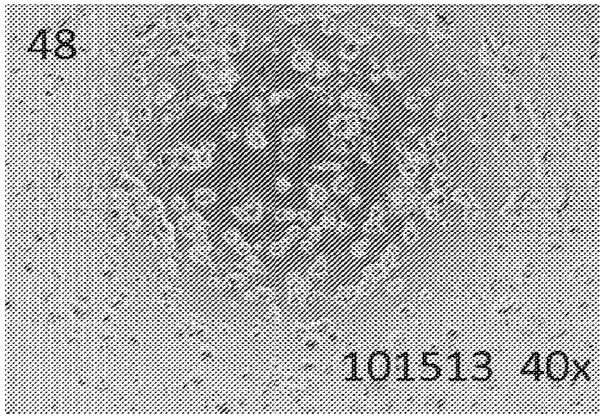
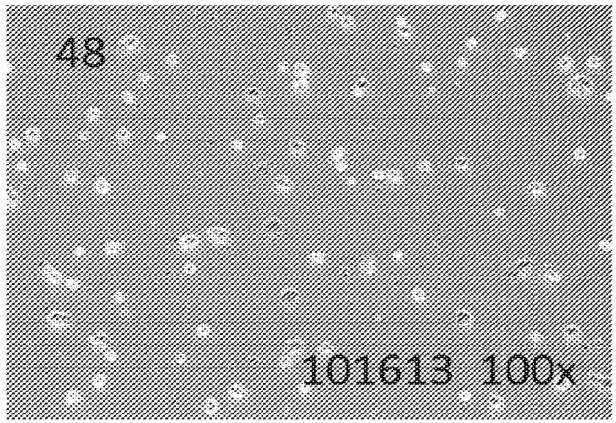


FIG. 55A



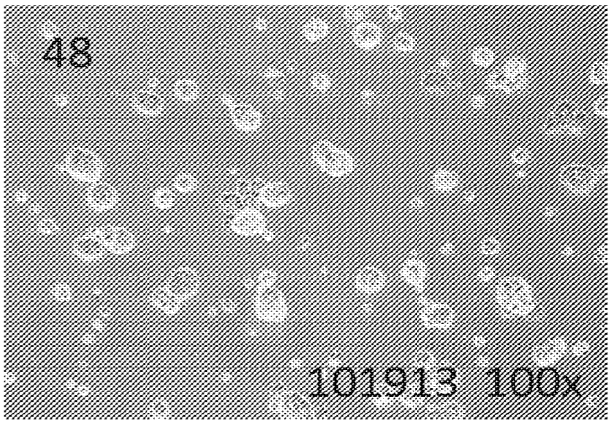
single cell passage
by Trypsin without
ROCK inhibitor

FIG. 55B



1 day after passage

FIG. 55C



4 days after passage