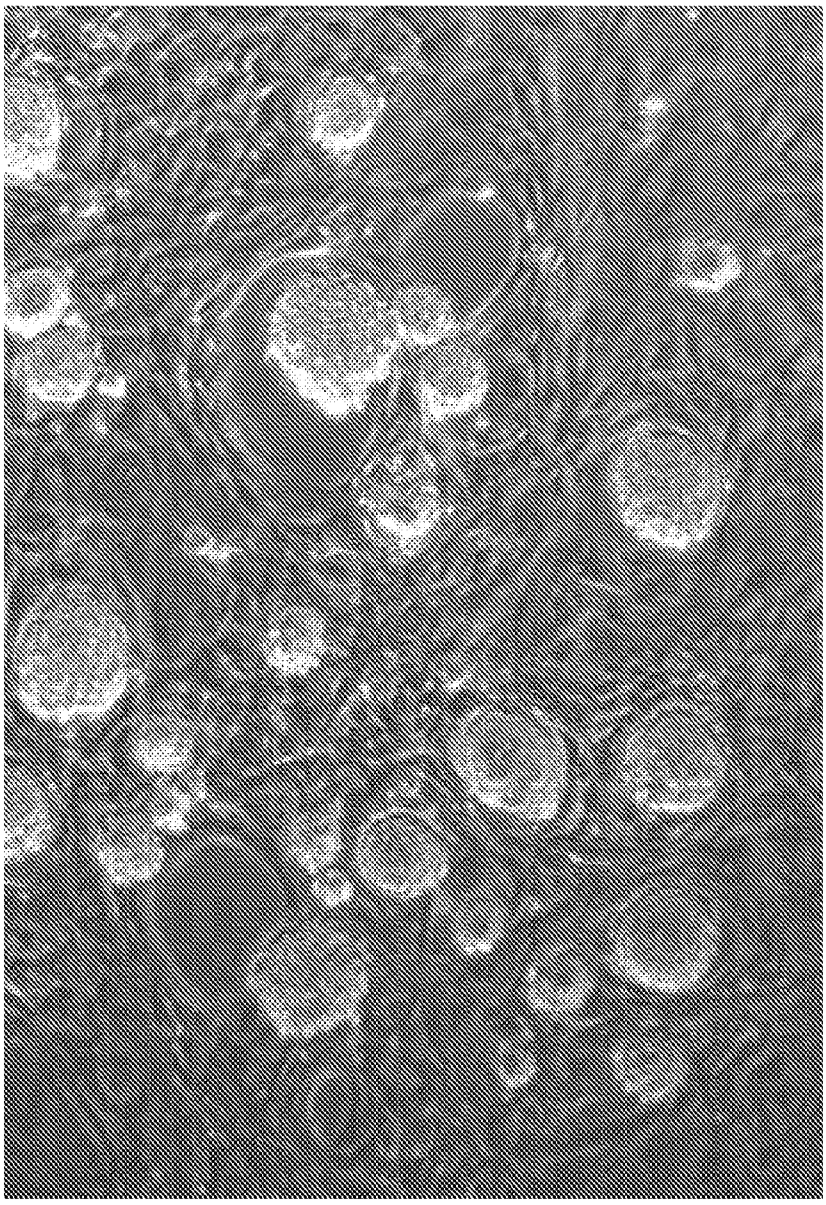


FIG. 1

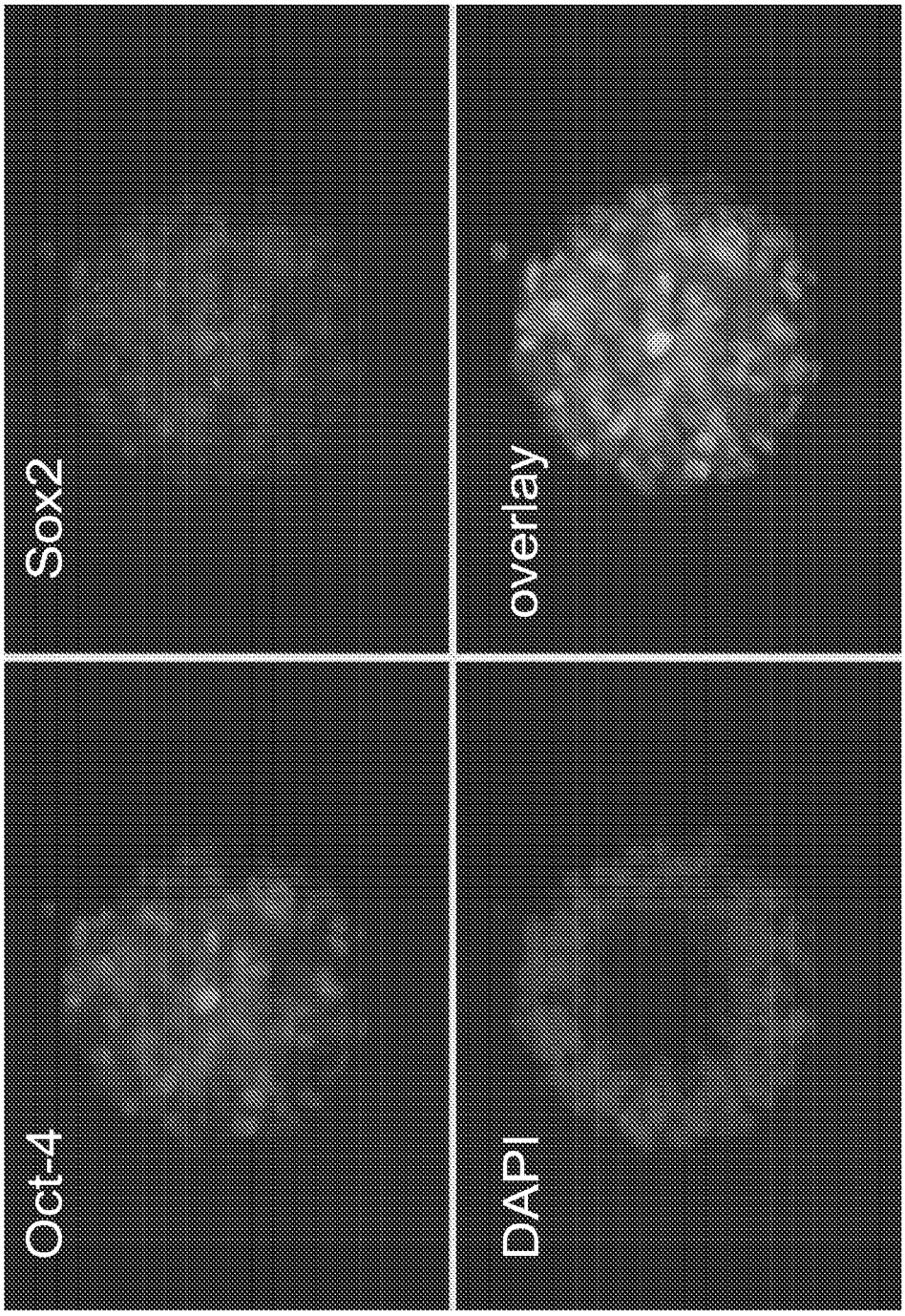


FIG. 2B FIG. 2D

FIG. 2A FIG. 2C

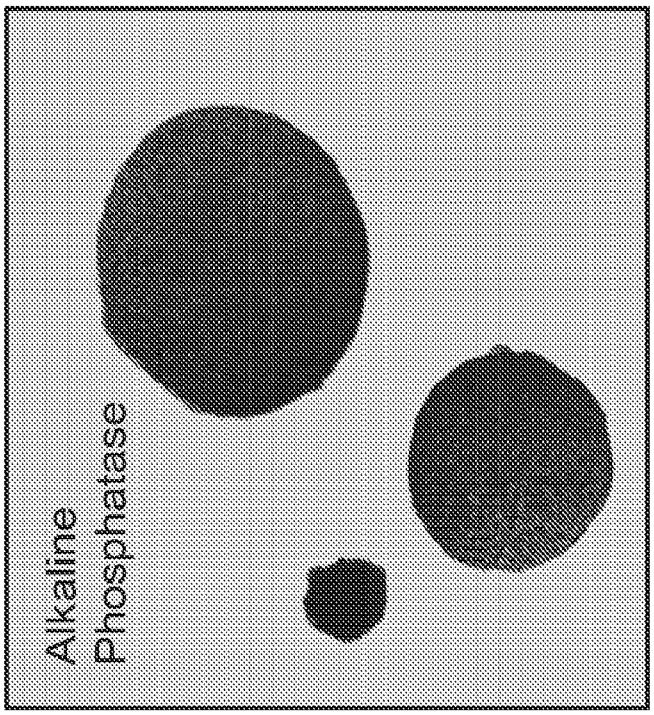


FIG. 3

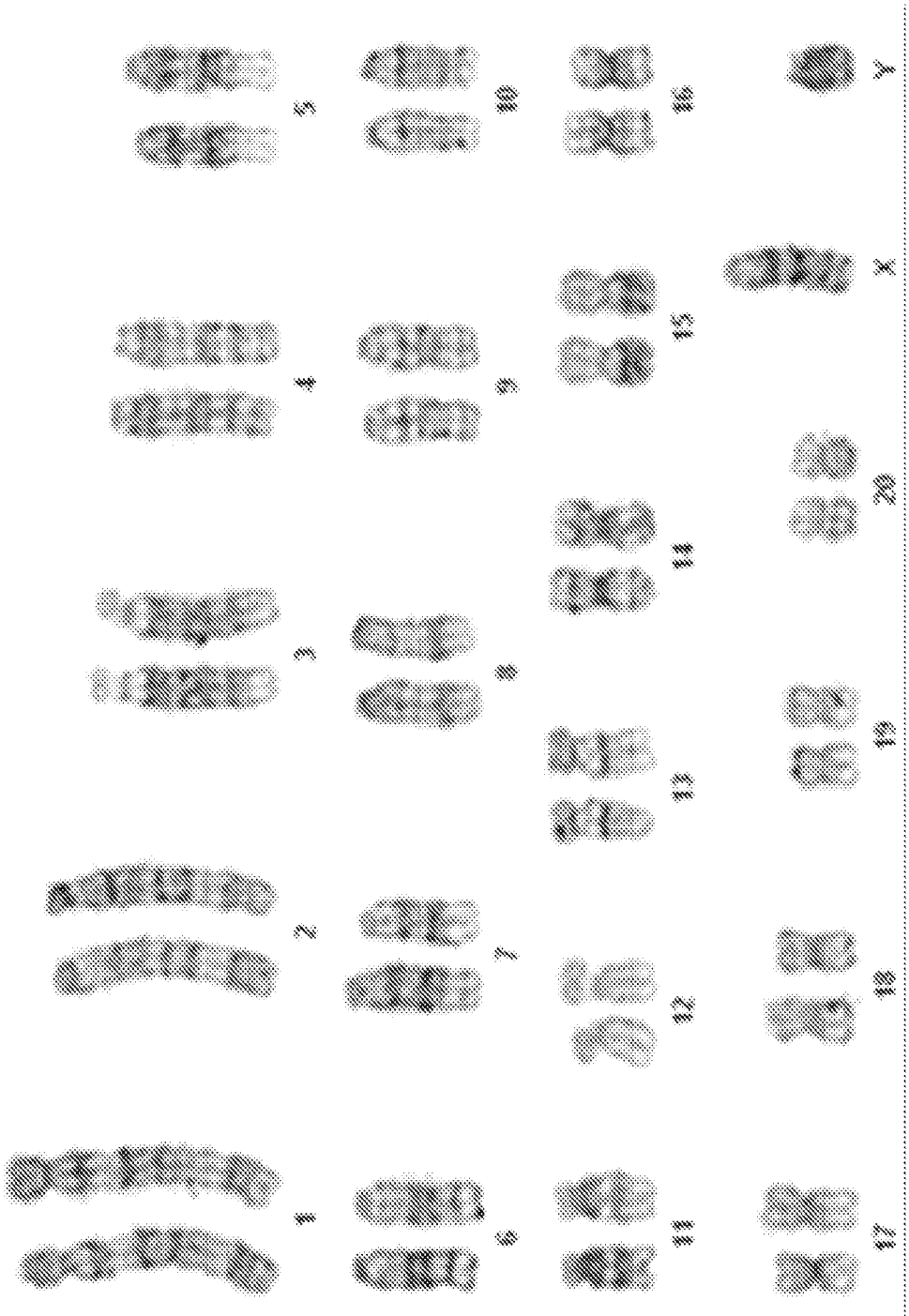


FIG. 4



Cell Results: 42, XY

FIG. 5A

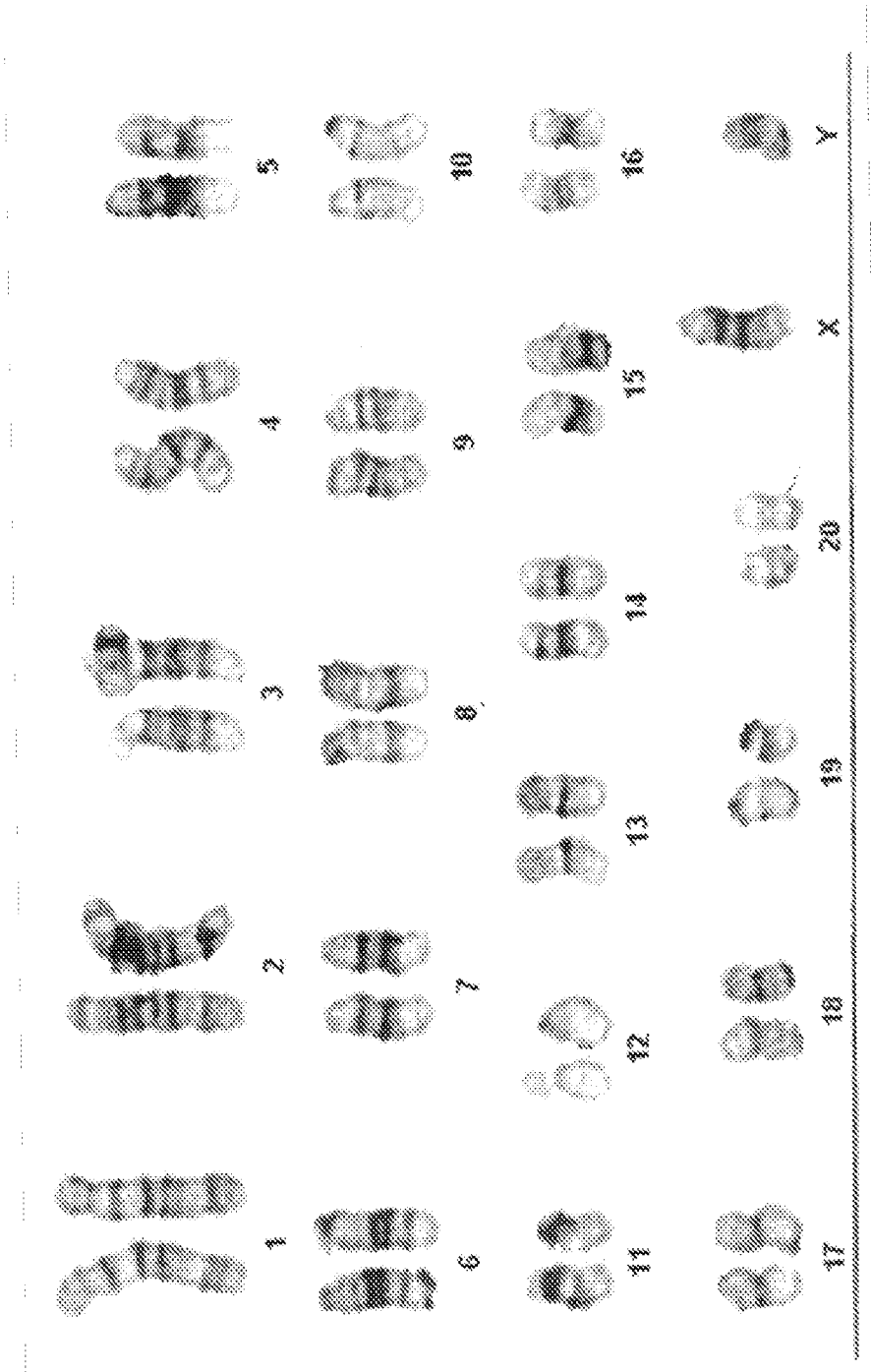


FIG. 5B



Cell Results: 42, XY

FIG. 6A

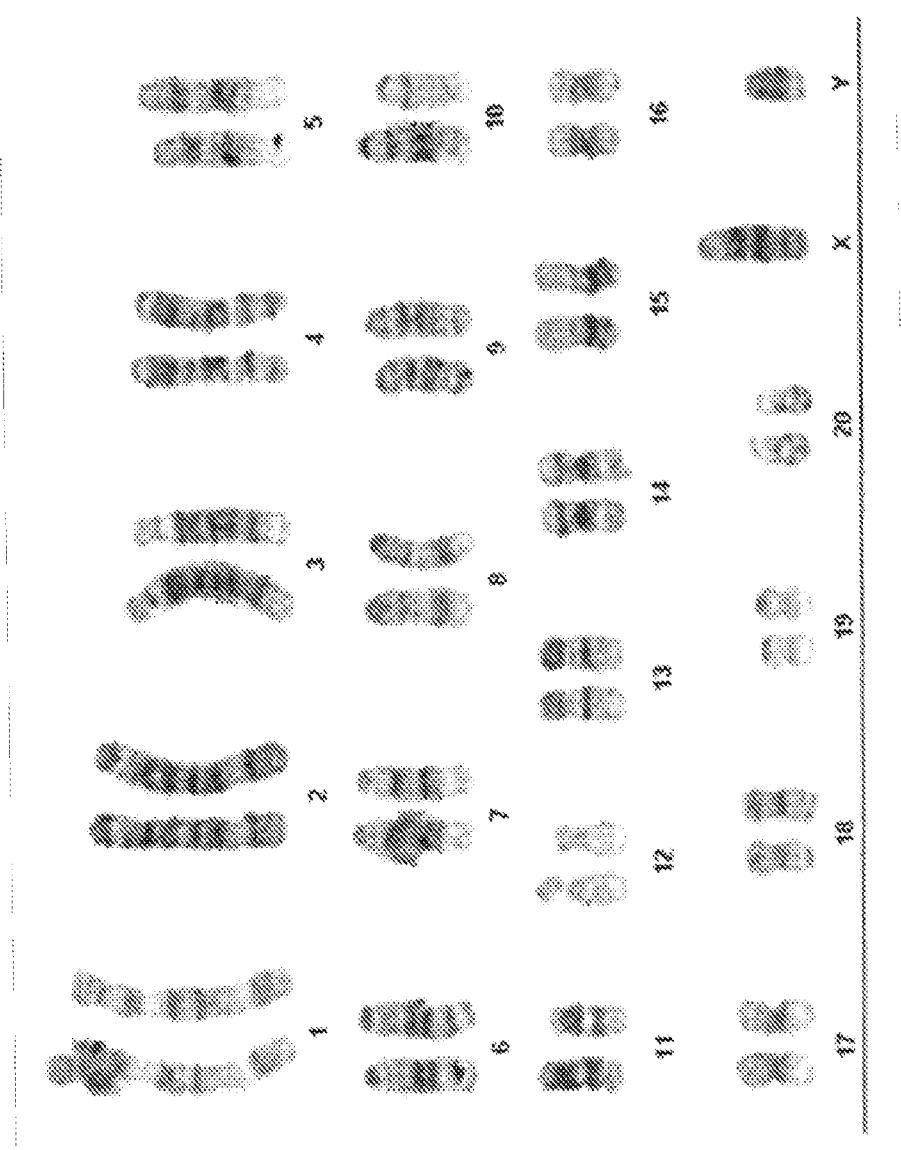


FIG. 6B



FIG. 7A



FIG. 7B

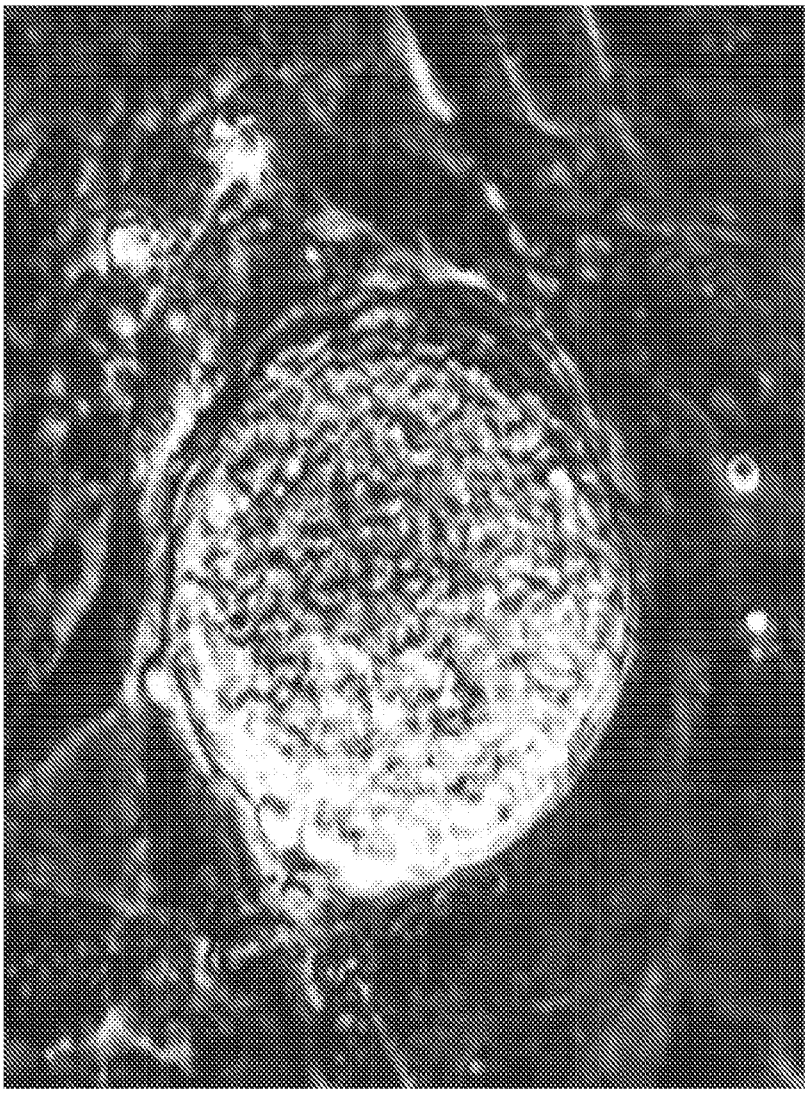


FIG. 8

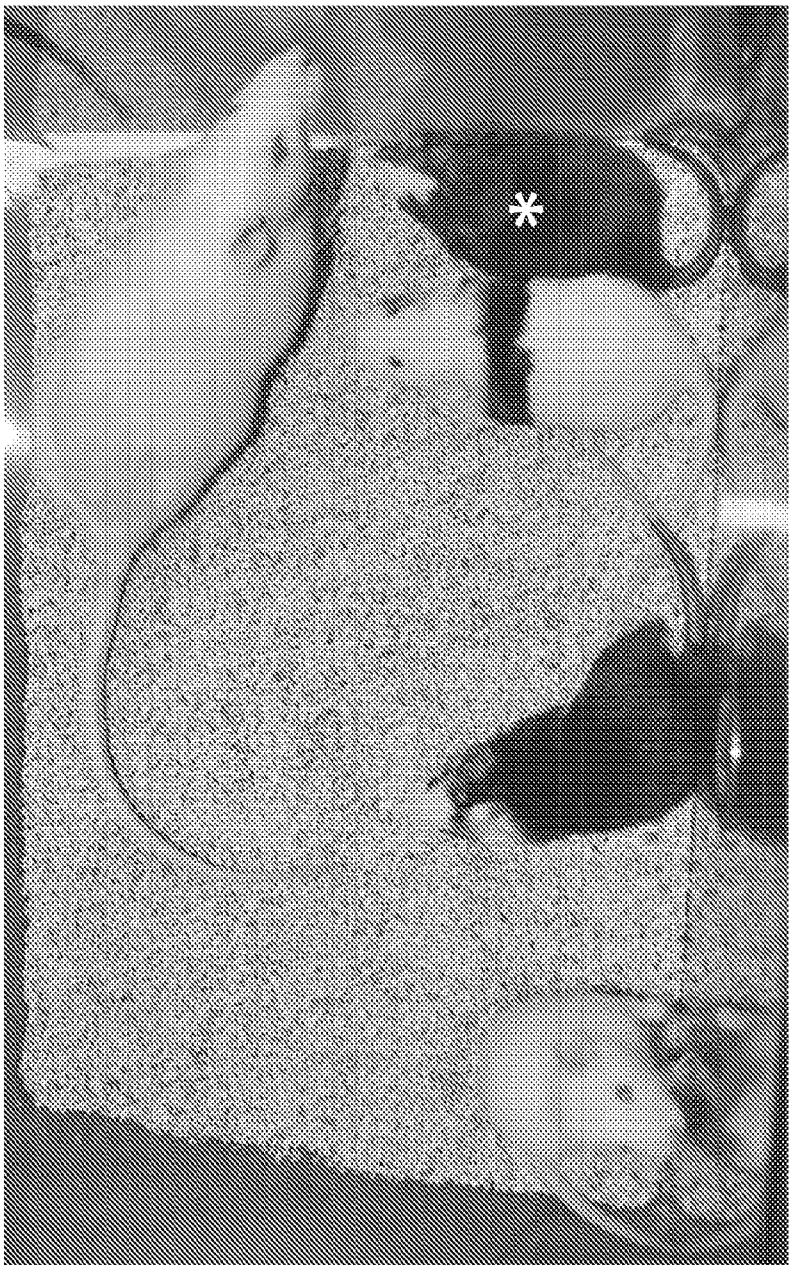


FIG. 9

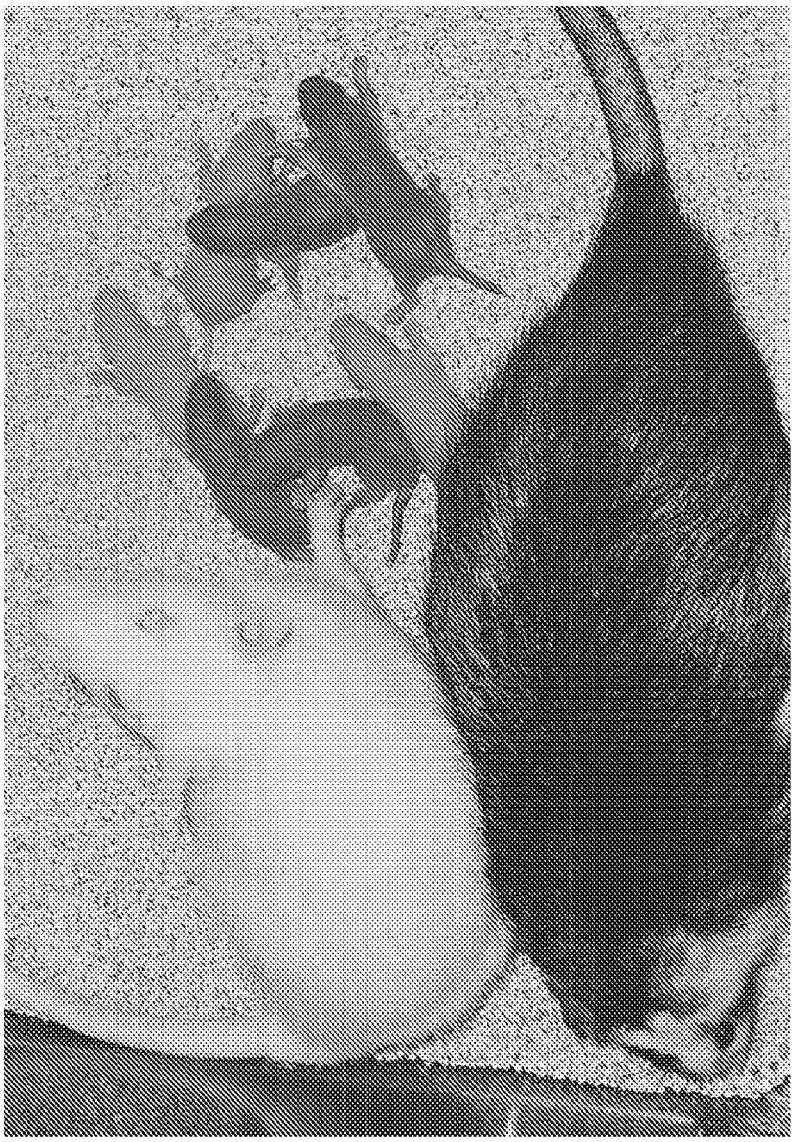


FIG. 10

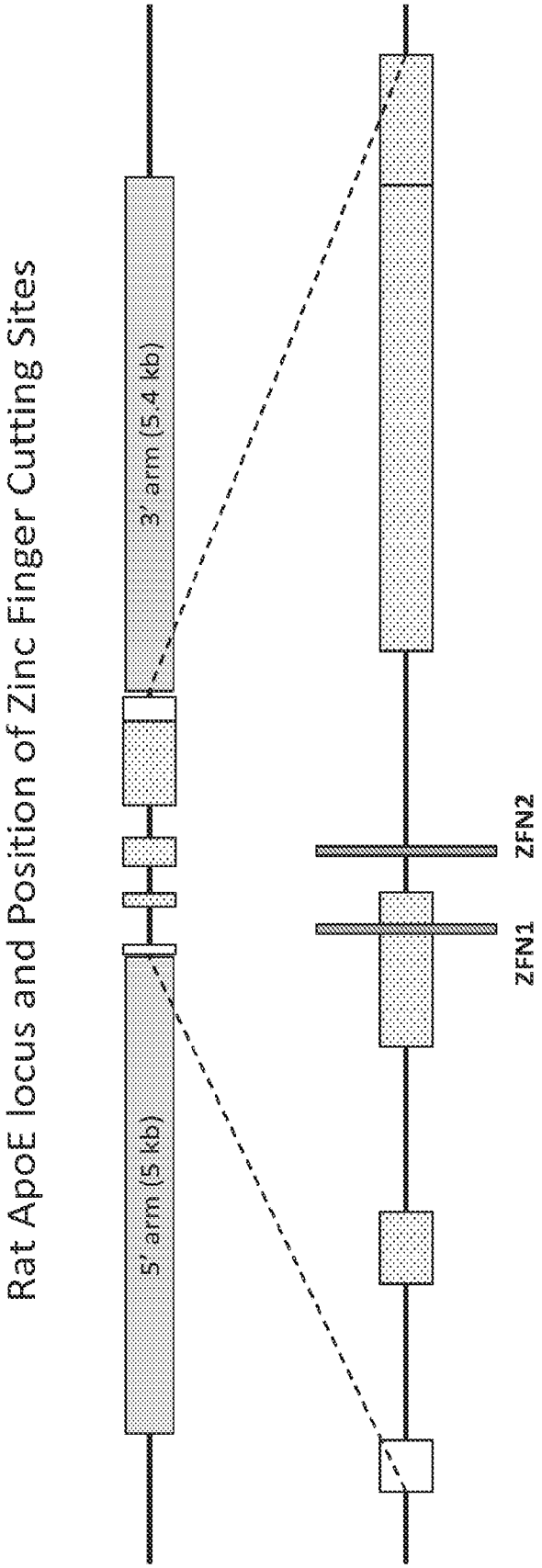
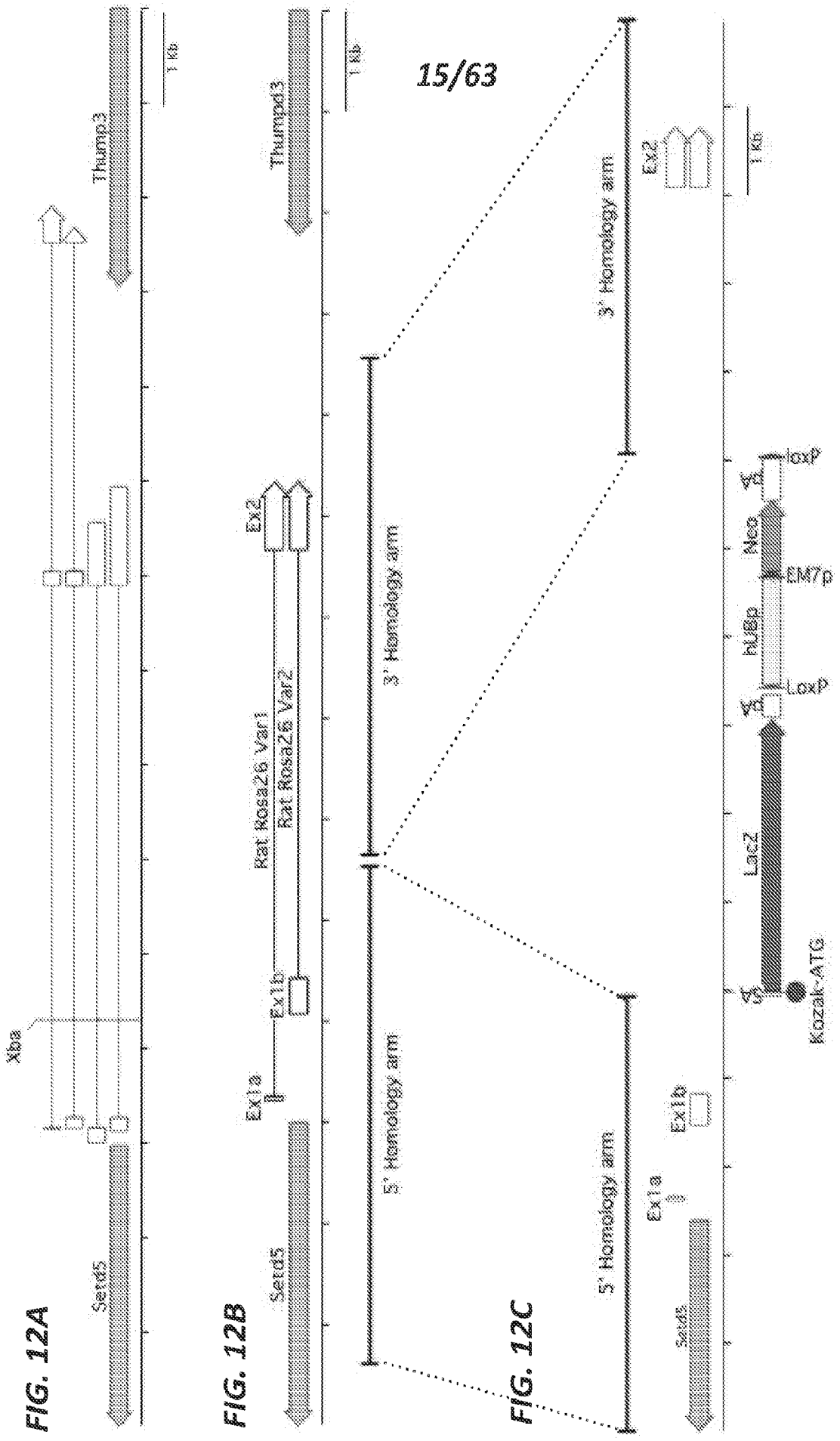

Rat ApoE locus and Position of Zinc Finger Cutting Sites
3' arm (5.4 kb)
5' arm (5 kb)
ZFN2
ZFN1

FIG. 11


15/63
FIG. 12A
Xba
Thump3
Sard5
1 Kb
FIG. 12B
Ex1a
Ex1b
Ex2
Rat Rosa26 Var1
Rat Rosa26 Var2
5' Homology arm
3' Homology arm
FIG. 12C
LoxP
Neo
EM7p
hUBp
LacZ
Kozak-ATG

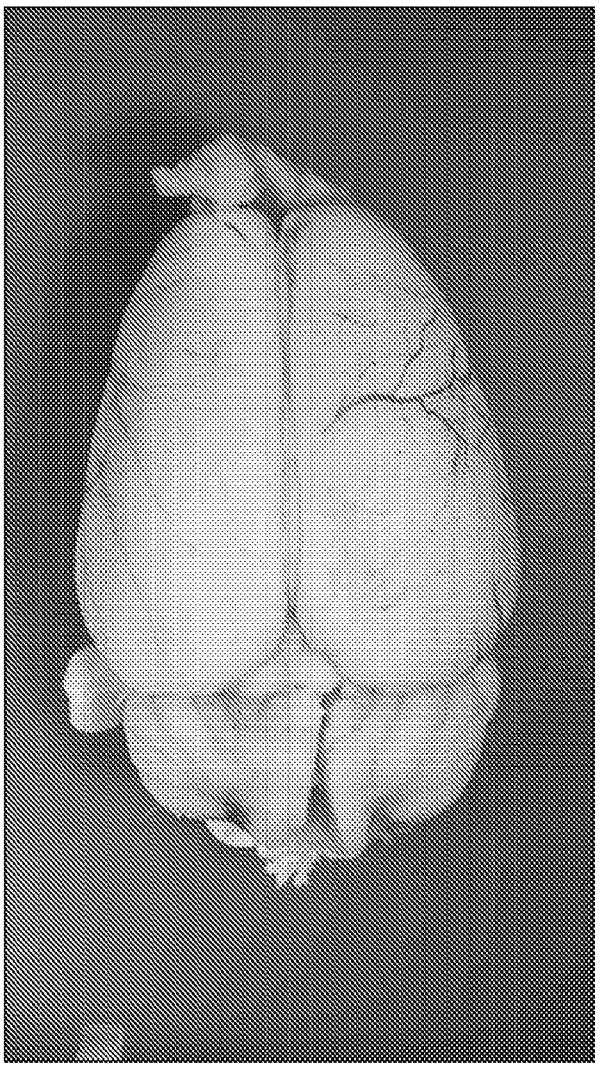


FIG. 13A

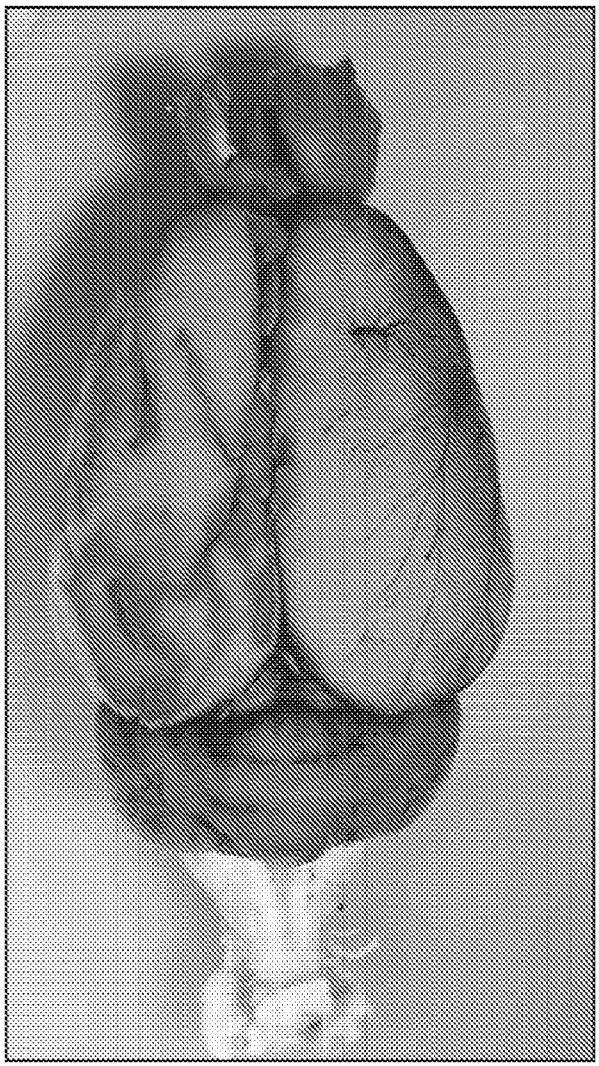


FIG. 13B

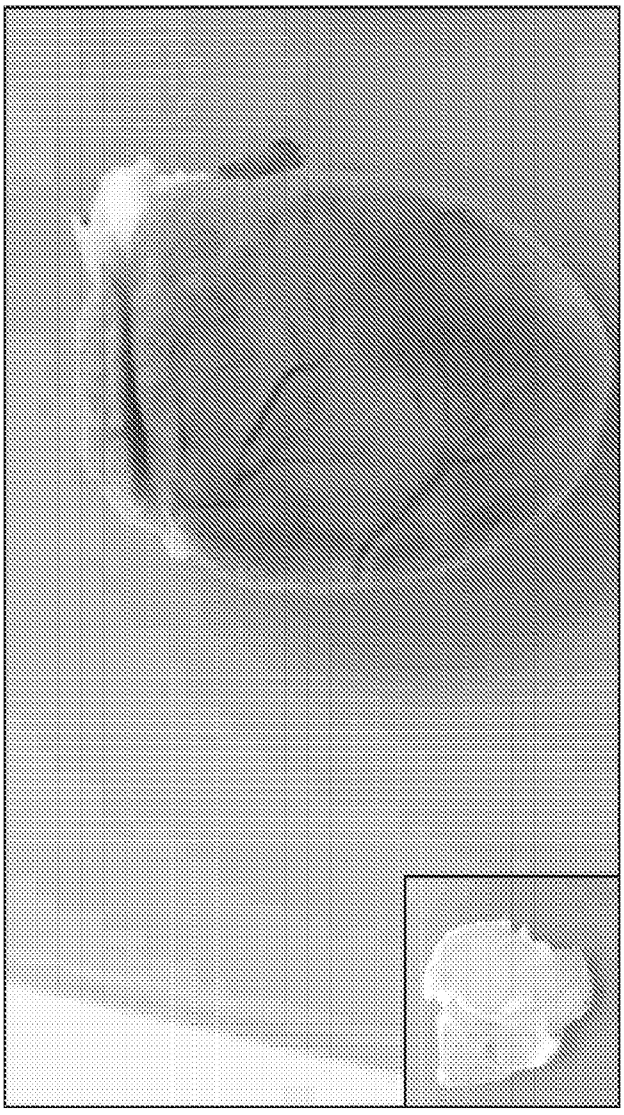


FIG. 13C

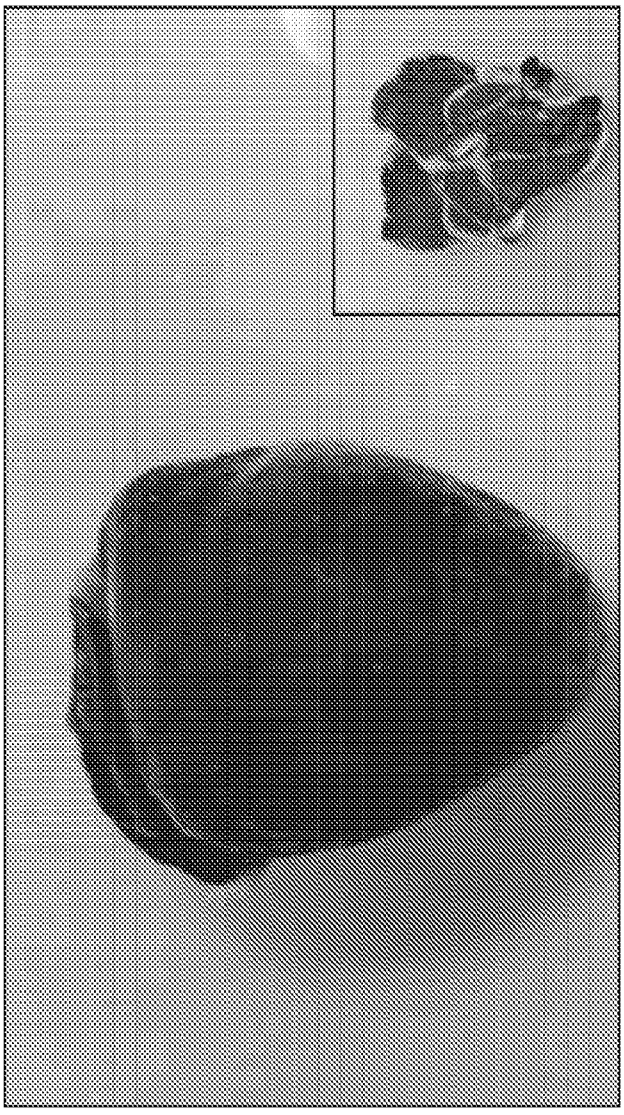


FIG. 13D



FIG. 13E

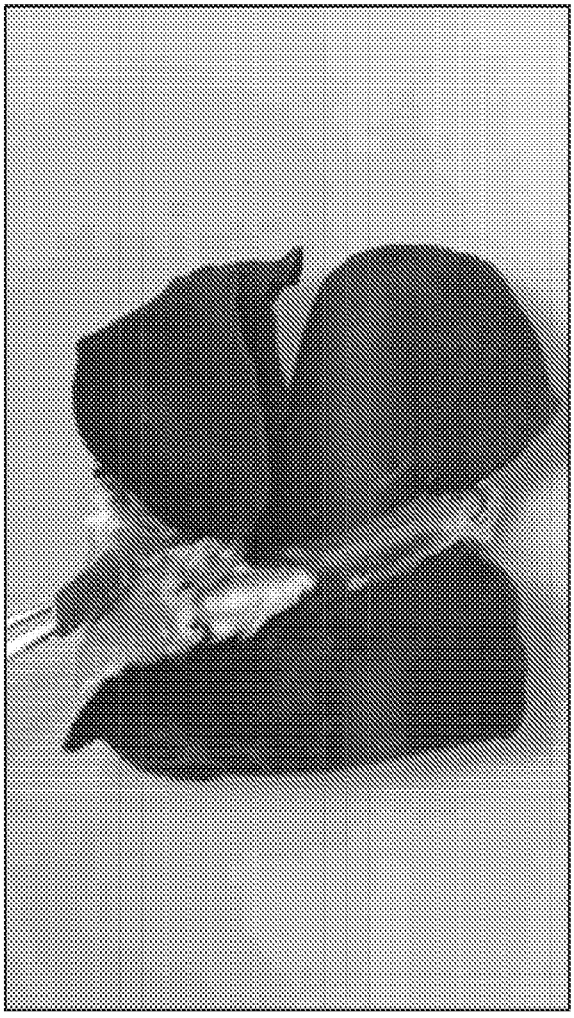


FIG. 13F

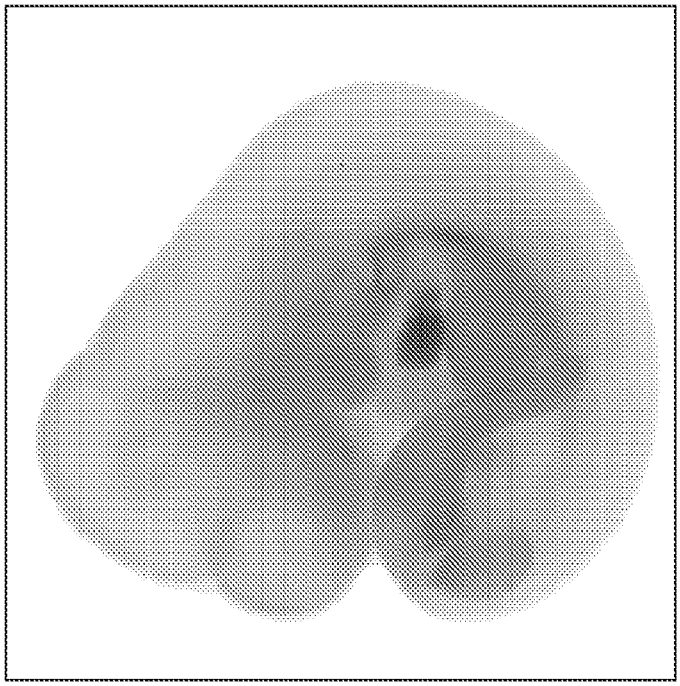


FIG. 13H

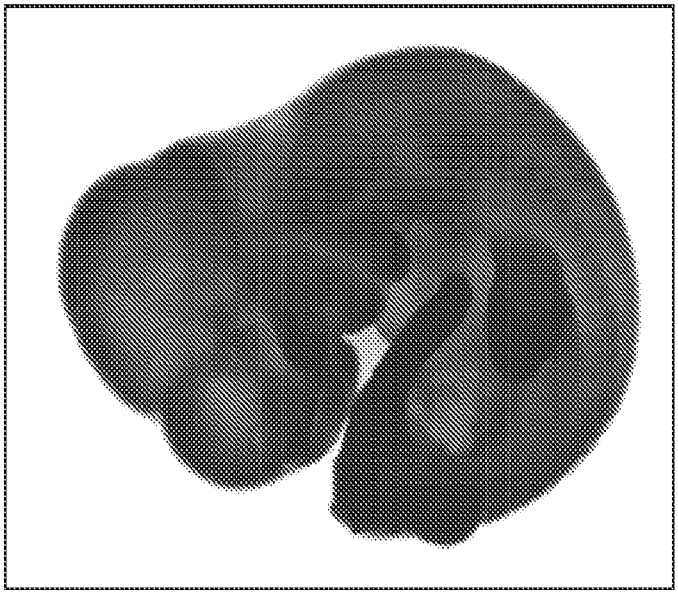


FIG. 13G

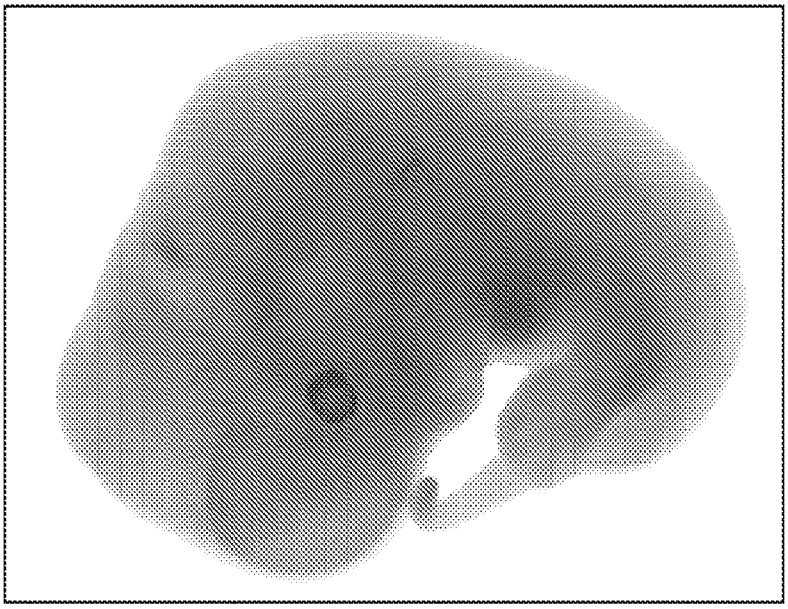


FIG. 13J

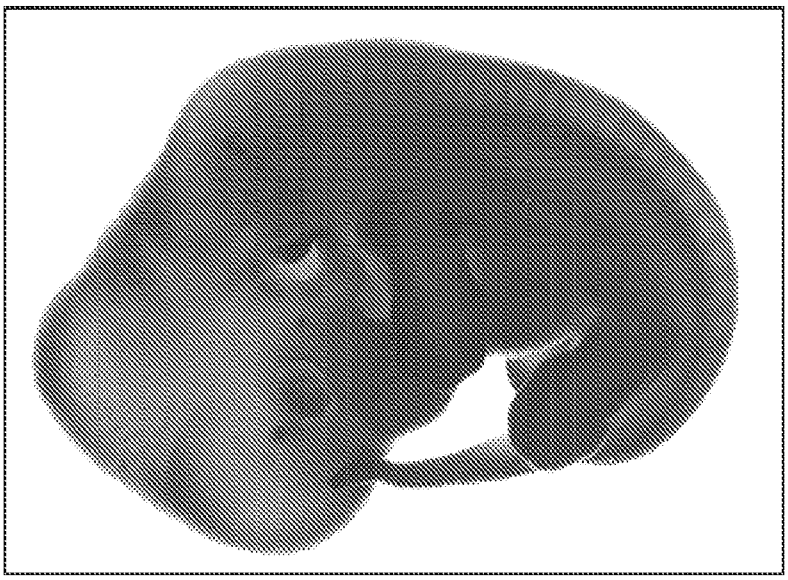


FIG. 13I

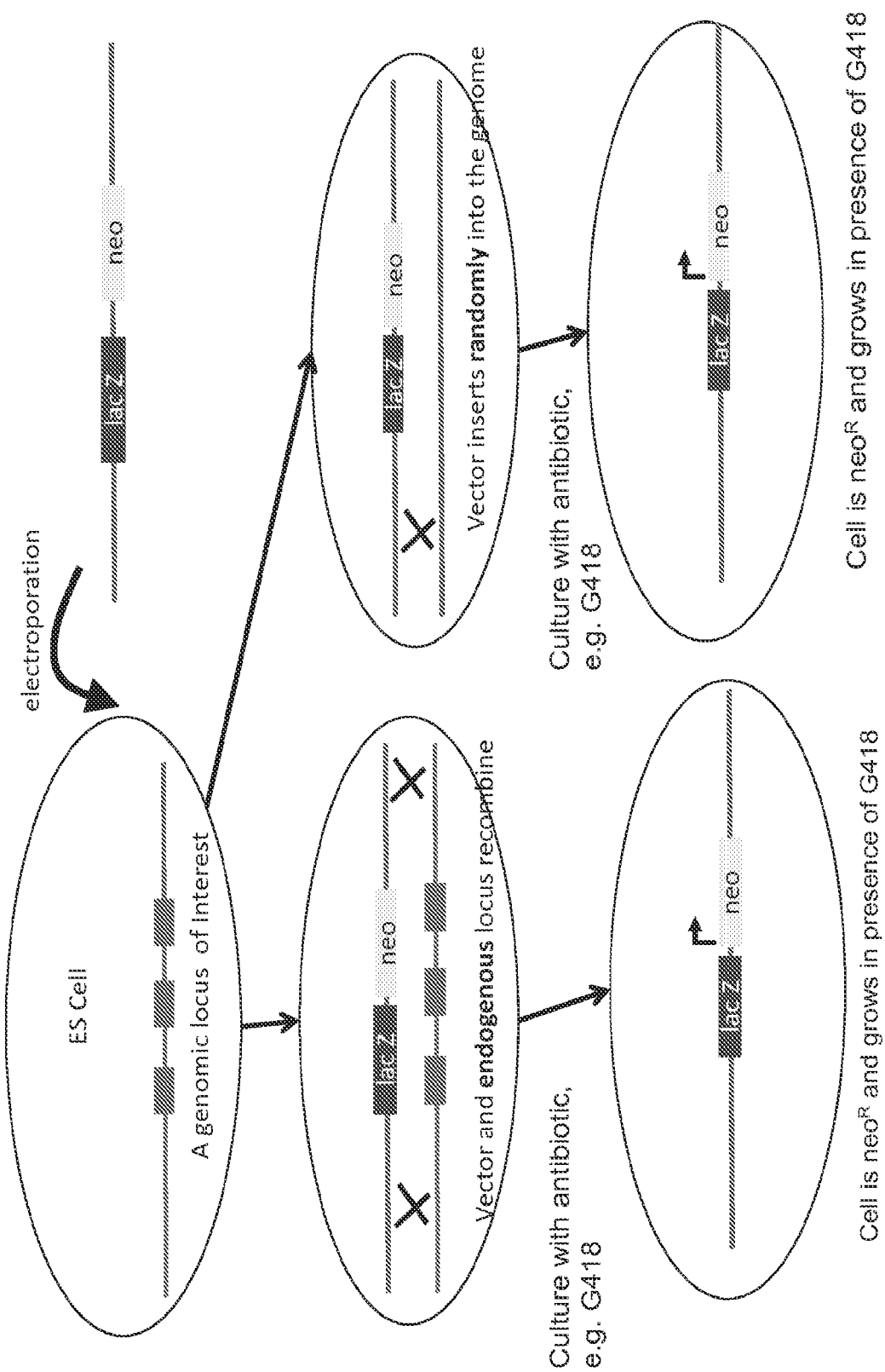

ES Cell
A genomic locus of Interest
electroporation
lacZ
neo
Vector and endogenous locus recombine
Vector inserts randomly into the genome
Culture with antibiotic, e.g. G418
Culture with antibiotic, e.g. G418
Cell is neoR and grows in presence of G418 typically 1-20% (at best) of insertions
Cell is neoR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

FIG. 14

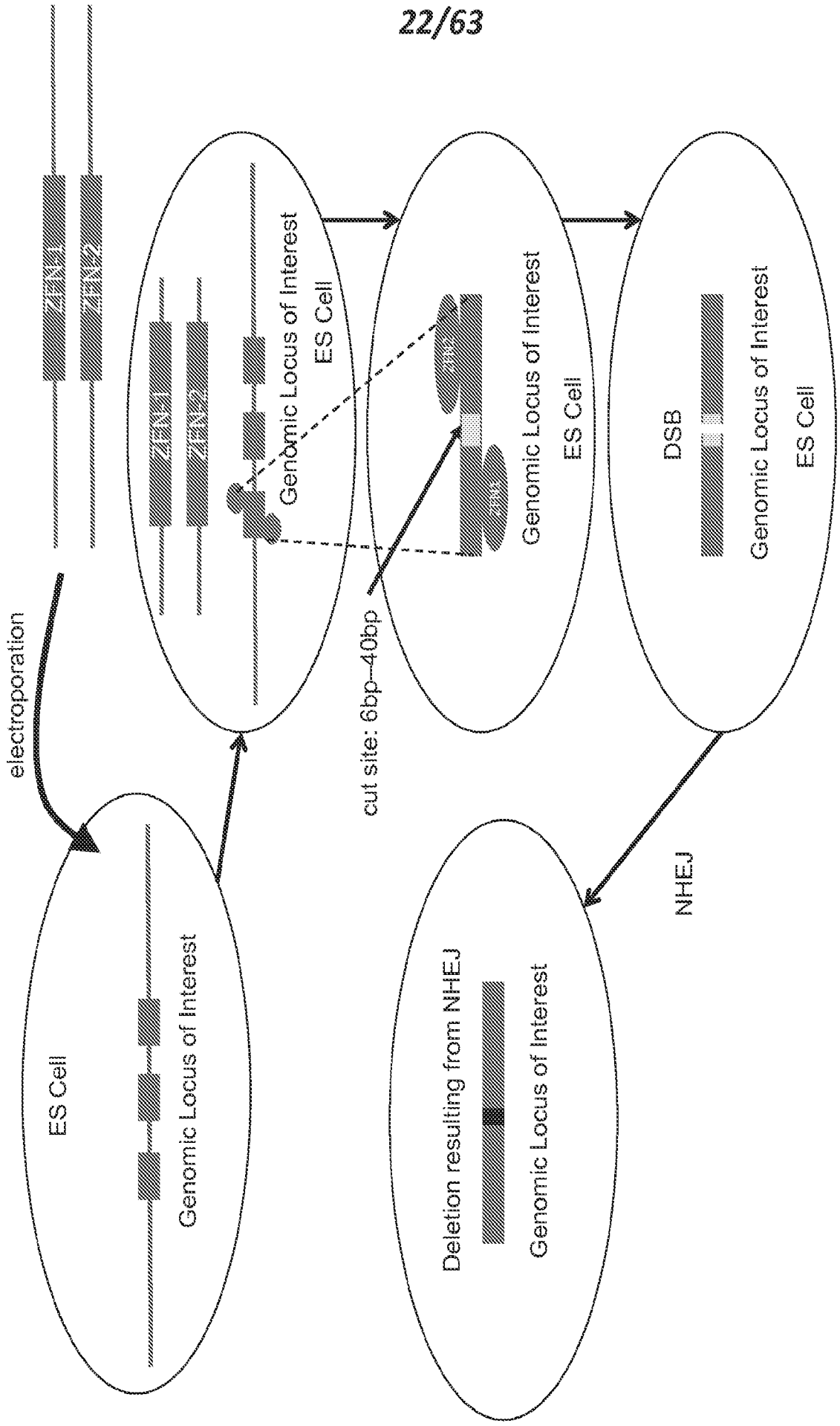

ZFN1
ZFN2
Genomic Locus of Interest
ES Cell
electroporation
cut site: 6bp–40bp
DSB
NHEJ
Deletion resulting from NHEJ

FIG. 15

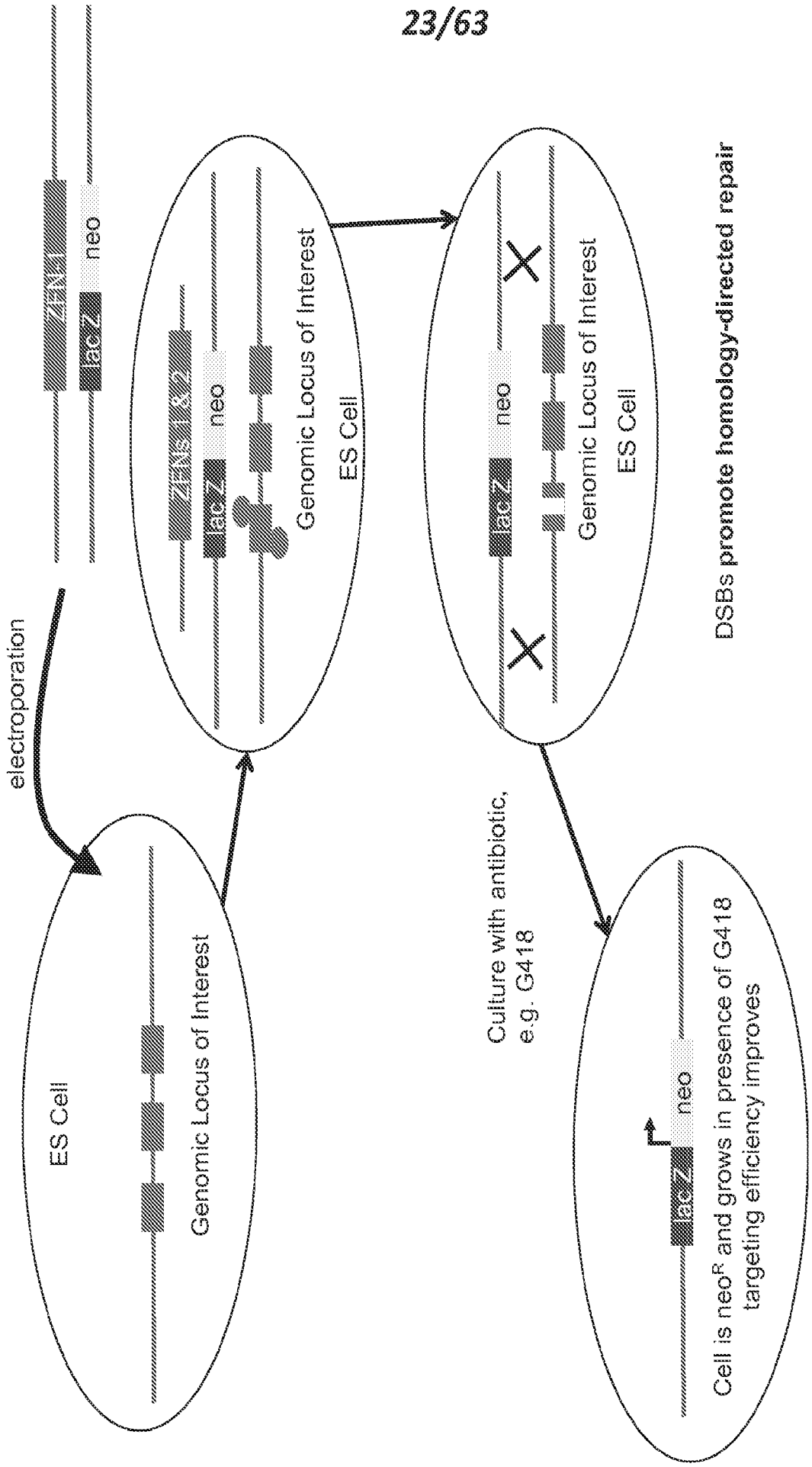

ZFN1
lacZ
neo
electroporation
ES Cell
Genomic Locus of Interest
ZFN1&2
lacZ
neo
ES Cell
Genomic Locus of Interest
DSBs promote homology-directed repair
lacZ
neo
ES Cell
Genomic Locus of Interest
Culture with antibiotic, e.g. G418
lacZ
neo
Cell is neo^R and grows in presence of G418
targeting efficiency improves

FIG. 16



FIG. 17

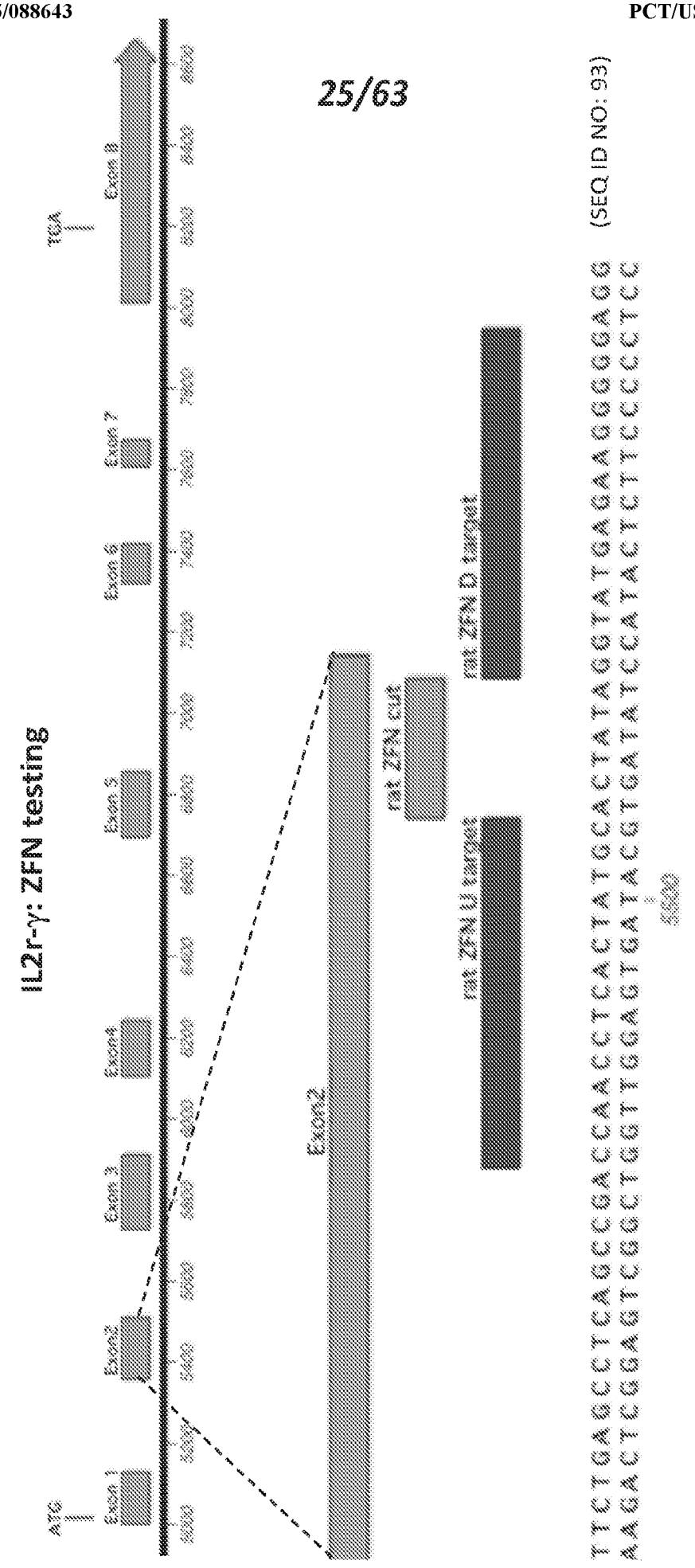
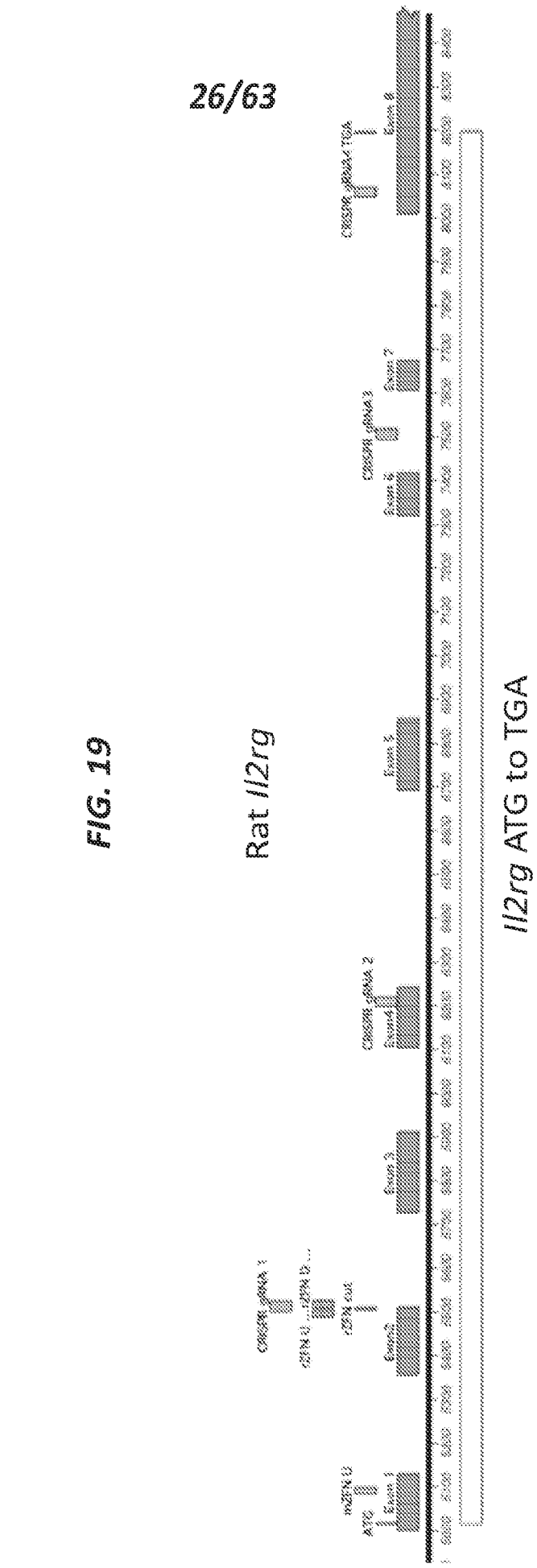

IL2r-γ: ZFN testing
ATG
TGA
Exon 1
Exon2
Exon 3
Exon4
Exon 5
Exon 6
Exon 7
Exon 8
Exon2
rat ZFN cut
rat ZFN D target
rat ZFN U target
5000
6000
6200
6400
6600
6800
7000
7200
7400
7600
7800
8000
8200
8400
8600
8800
TTCTGAGGCCTCAGCCCGACCAACCCTCACTATGCACTATAGGTATGAGAAGGGGGAGGG (SEQ ID NO: 93)
AAGACTCCGGAGTCGGCTTGGTTGGAGTGATACGTGATATCCATACTCTTCCCCCCTCC

FIG. 18

FIG. 19



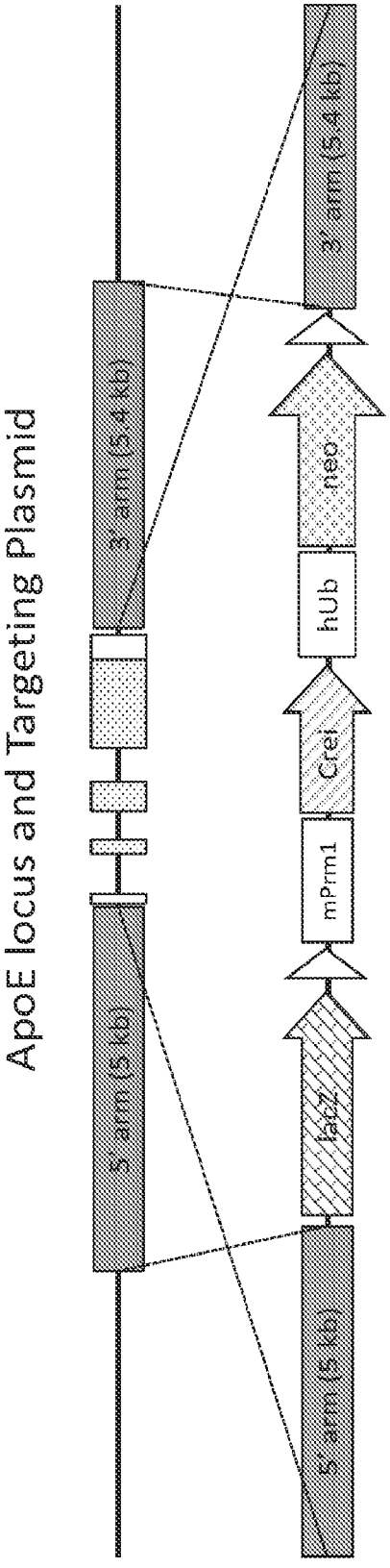

ApoE locus and Targeting Plasmid
5' arm (5 kb)
lacZ
mPrm1
Cre
hUb
neo
3' arm (5.4 kb)
5' arm (5 kb)
3' arm (5.4 kb)

FIG. 20

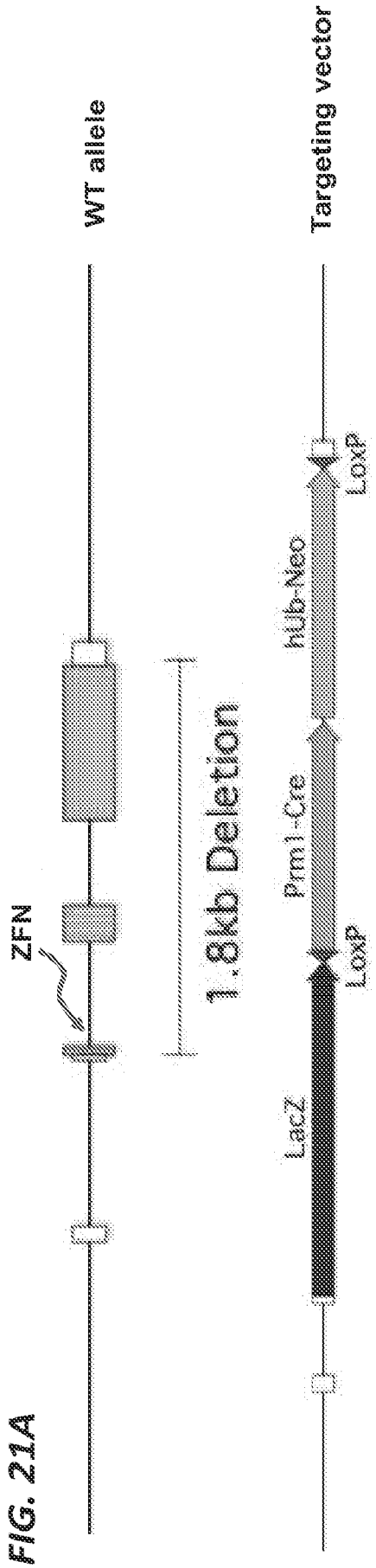


FIG. 21A

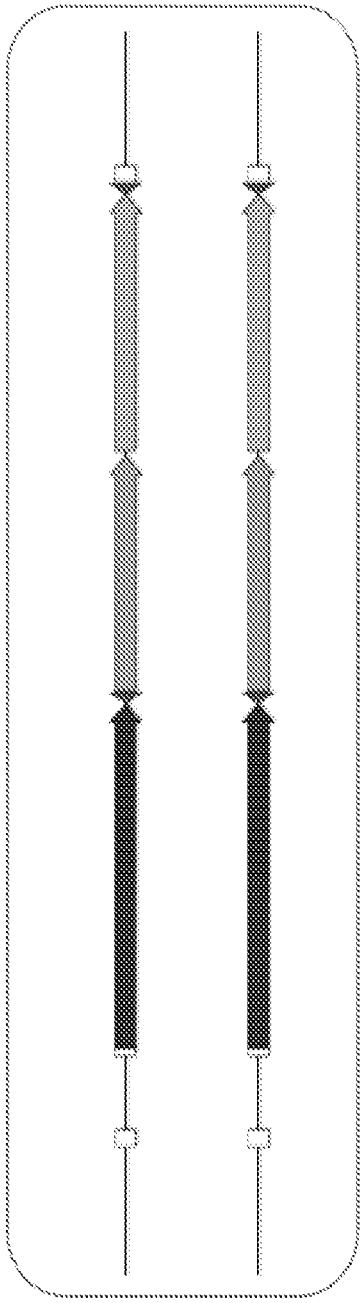


FIG. 21B

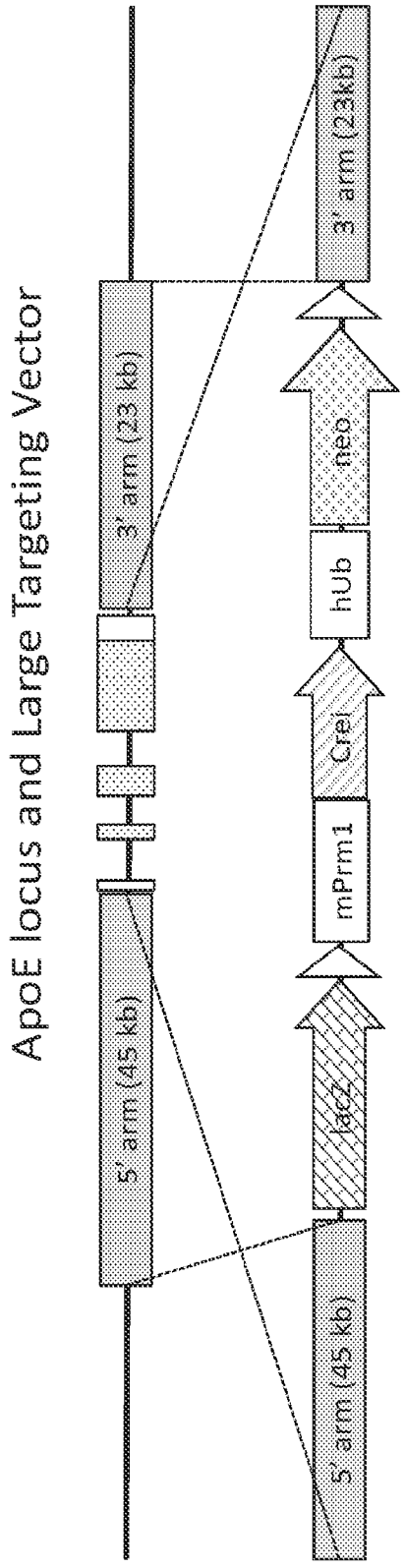

ApoE locus and Large Targeting Vector
5' arm (45 kb)
3' arm (23 kb)
lacZ
mPrm1
Cre
hUb
neo
3' arm (23kb)
5' arm (45 kb)

FIG. 22

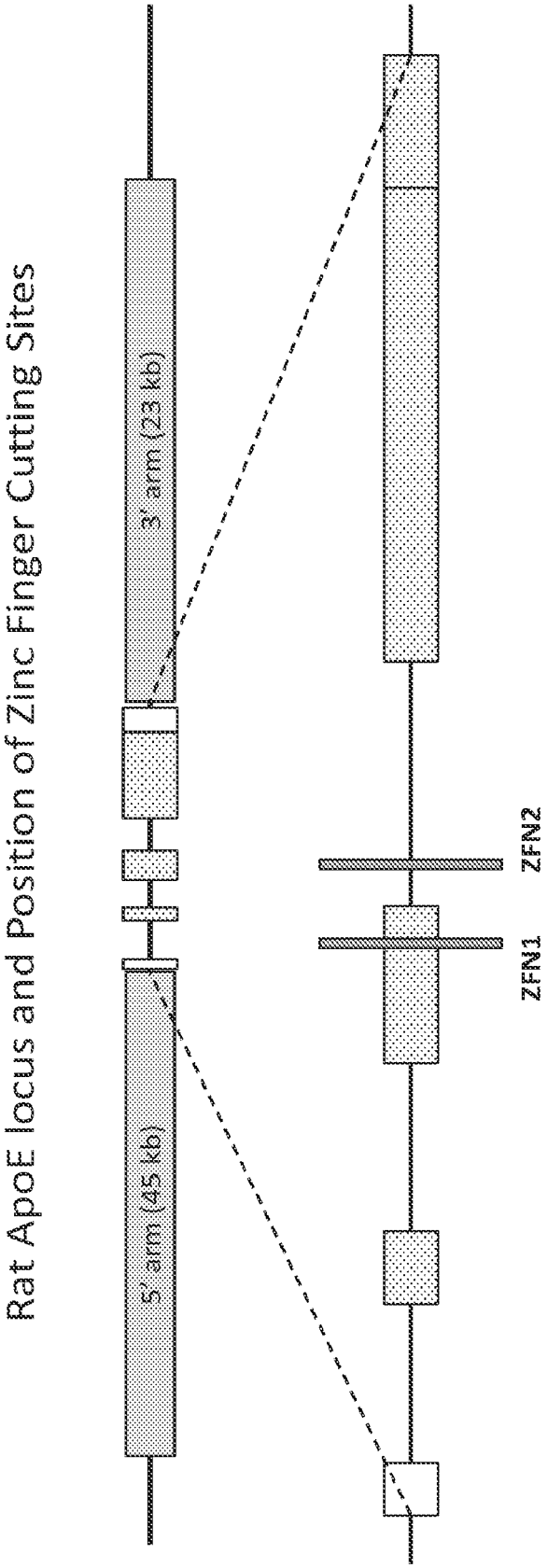

Rat ApoE locus and Position of Zinc Finger Cutting Sites
5' arm (45 kb)
3' arm (23 kb)
ZFN1
ZFN2

FIG. 23

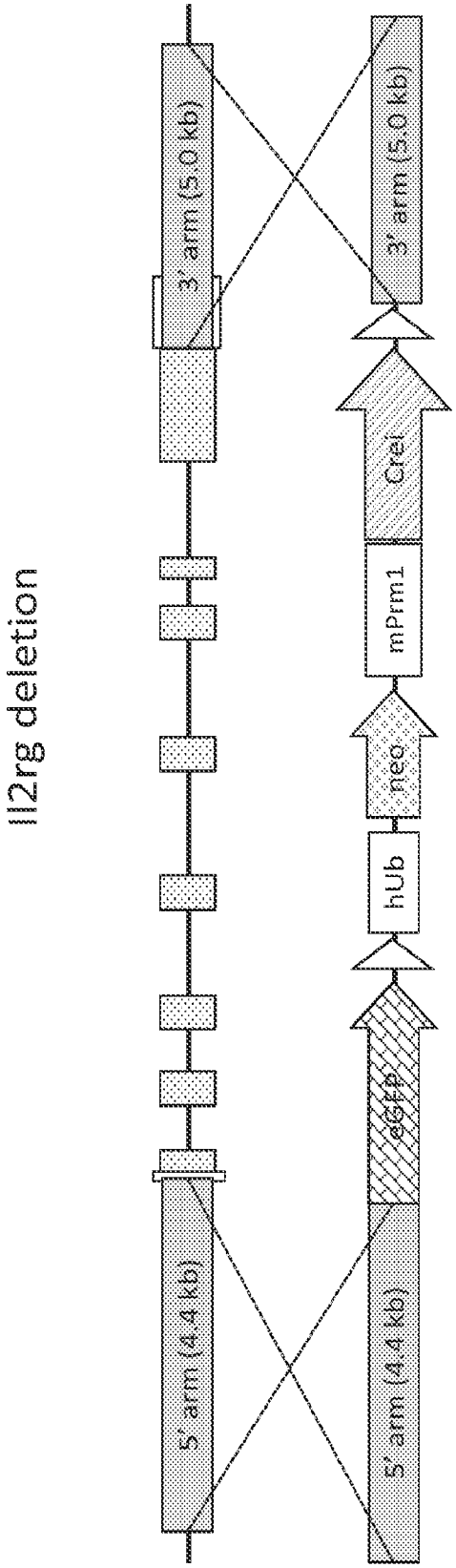

Il2rg deletion
5' arm (4.4 kb)
3' arm (5.0 kb)
eGFP
hUb
neo
mPrm1
Cre
5' arm (4.4 kb)
3' arm (5.0 kb)

FIG. 24

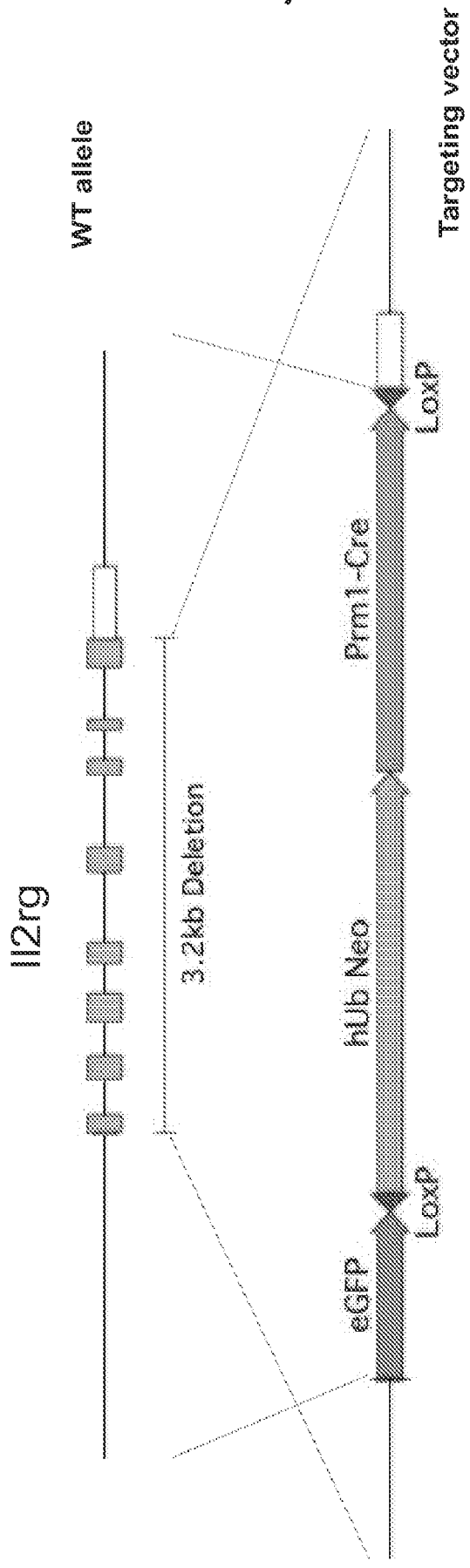

WT allele
Targeting vector
Il2rg
3.2kb Deletion
Prm1-Cre
hUb Neo
eGFP
LoxP
LoxP

FIG. 25

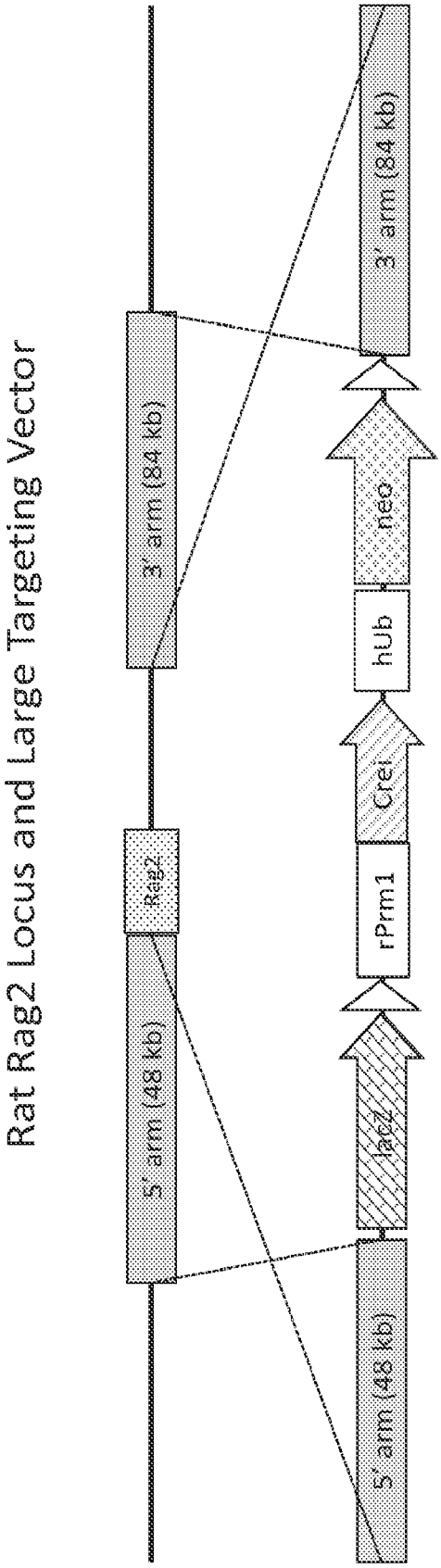

Rat Rag2 Locus and Large Targeting Vector
3' arm (84 kb)
Rag2
5' arm (48 kb)
3' arm (84 kb)
neo
hUb
Cre1
rPrm1
lacZ
5' arm (48 kb)

FIG. 26

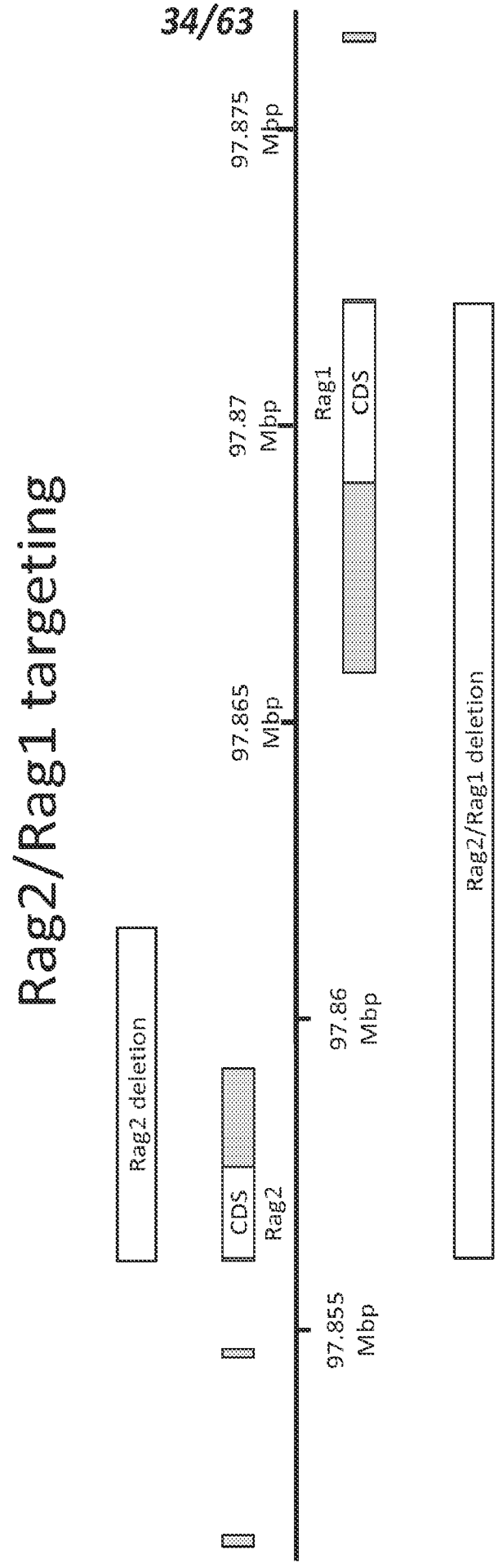

Rag2/Rag1 targeting
Rag2 deletion
CDS
Rag2
Rag1
CDS
Rag2/Rag1 deletion
97.855 Mbp
97.86 Mbp
97.865 Mbp
97.87 Mbp
97.875 Mbp

FIG. 27

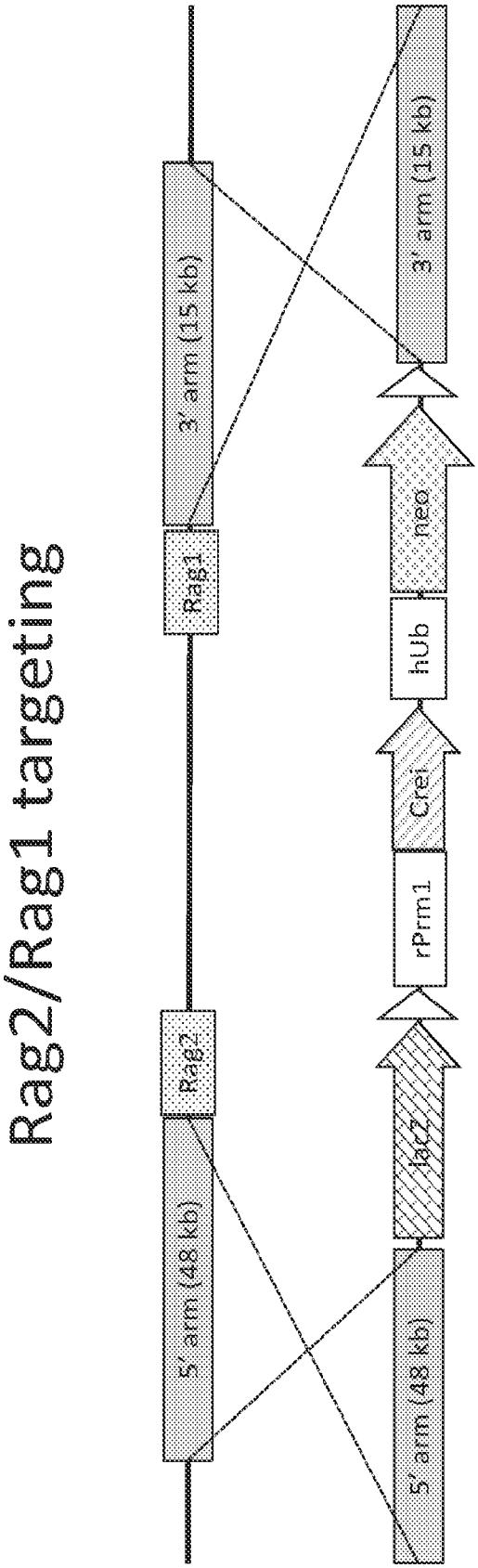
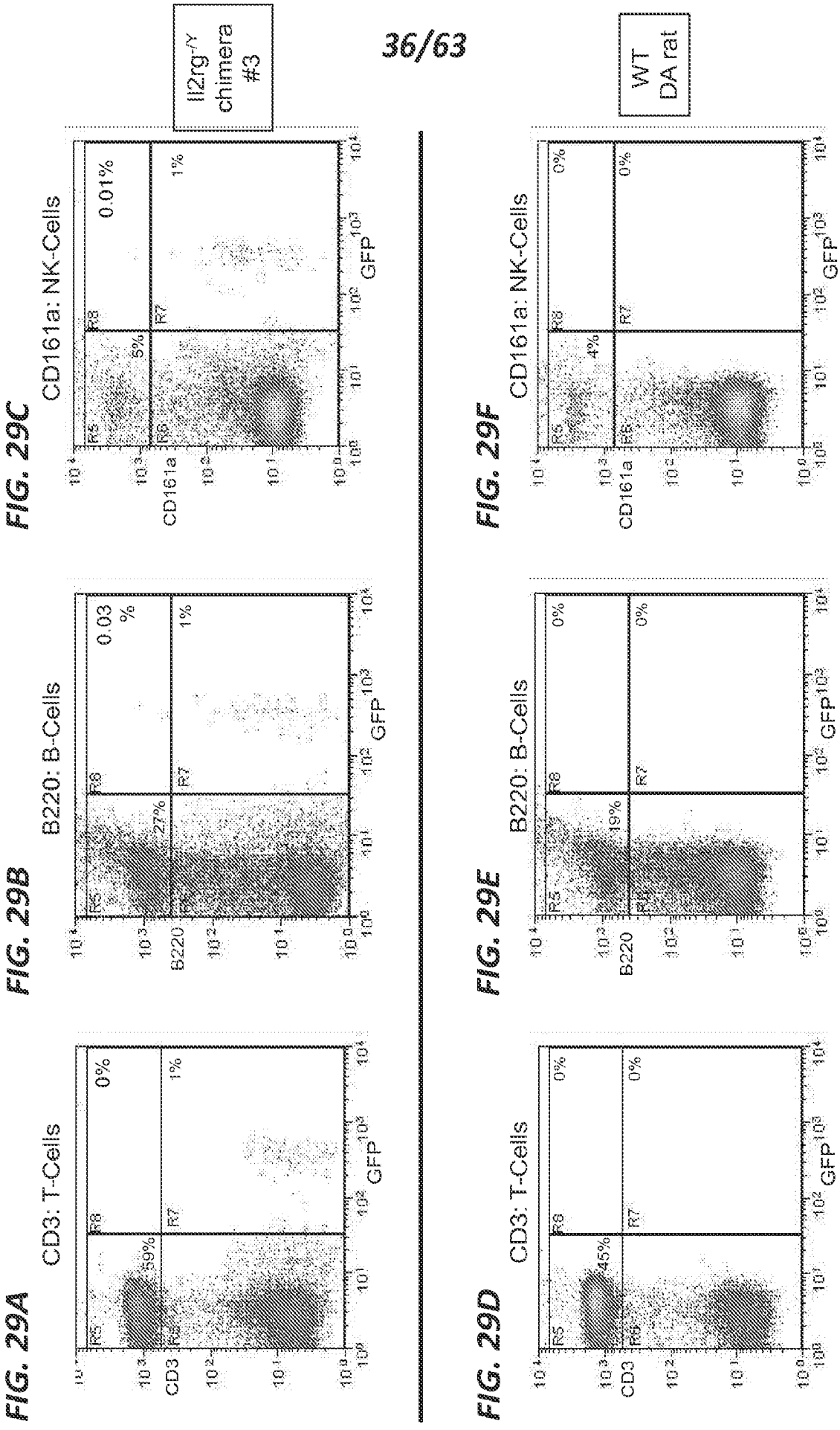

Rag2/Rag1 targeting
5' arm (48 kb)
Rag2
3' arm (15 kb)
Rag1
5' arm (48 kb)
iaC2
rPrm1
Crei
hUb
neo
3' arm (15 kb)

FIG. 28


FIG. 29A
CD3: T-Cells
CD3
GFP
R5
R6
R8
R7
59%
0%
1%
FIG. 29B
B220: B-Cells
B220
GFP
R5
R6
R8
R7
27%
0.03 %
1%
FIG. 29C
CD161a: NK-Cells
CD161a
GFP
R5
R6
R8
R7
5%
0.01%
1%
Il2rg-/Y chimera #3
FIG. 29D
CD3: T-Cells
CD3
GFP
R5
R6
R8
R7
45%
0%
0%
FIG. 29E
B220: B-Cells
B220
GFP
R5
R6
R8
R7
19%
0%
0%
FIG. 29F
CD161a: NK-Cells
CD161a
GFP
R5
R6
R8
R7
4%
0%
0%
WT DA rat

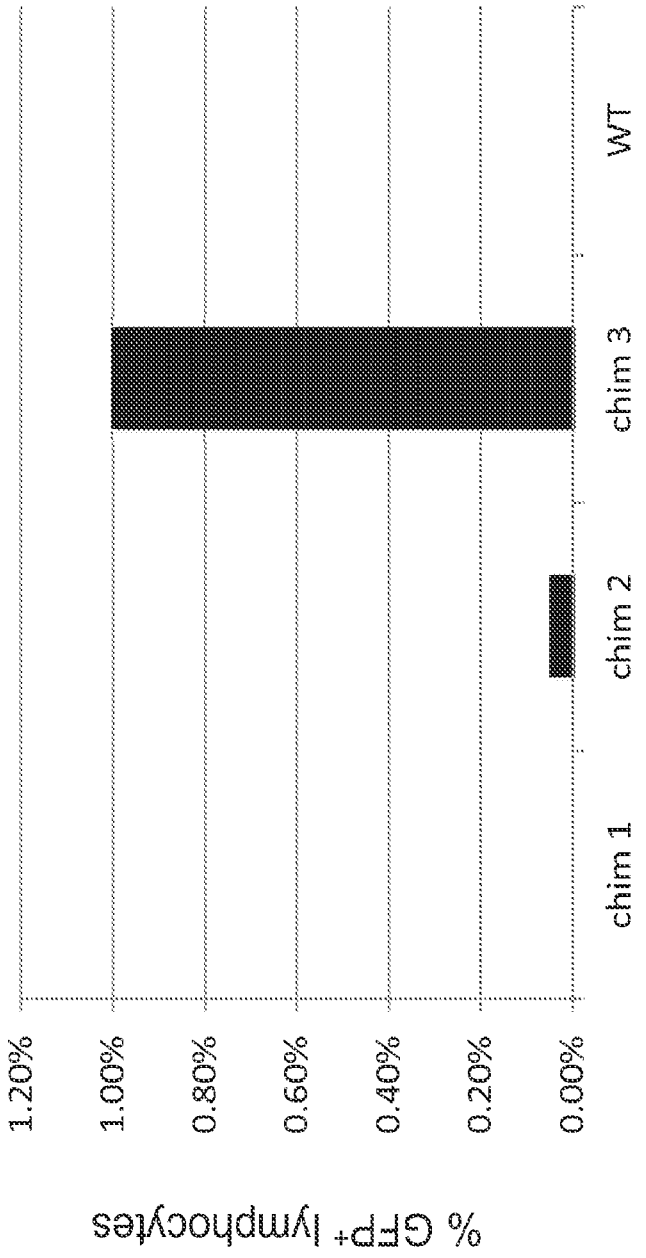

% GFP+ lymphocytes
1.20%
1.00%
0.80%
0.60%
0.40%
0.20%
0.00%
chim 1
chim 2
chim 3
WT

FIG. 30

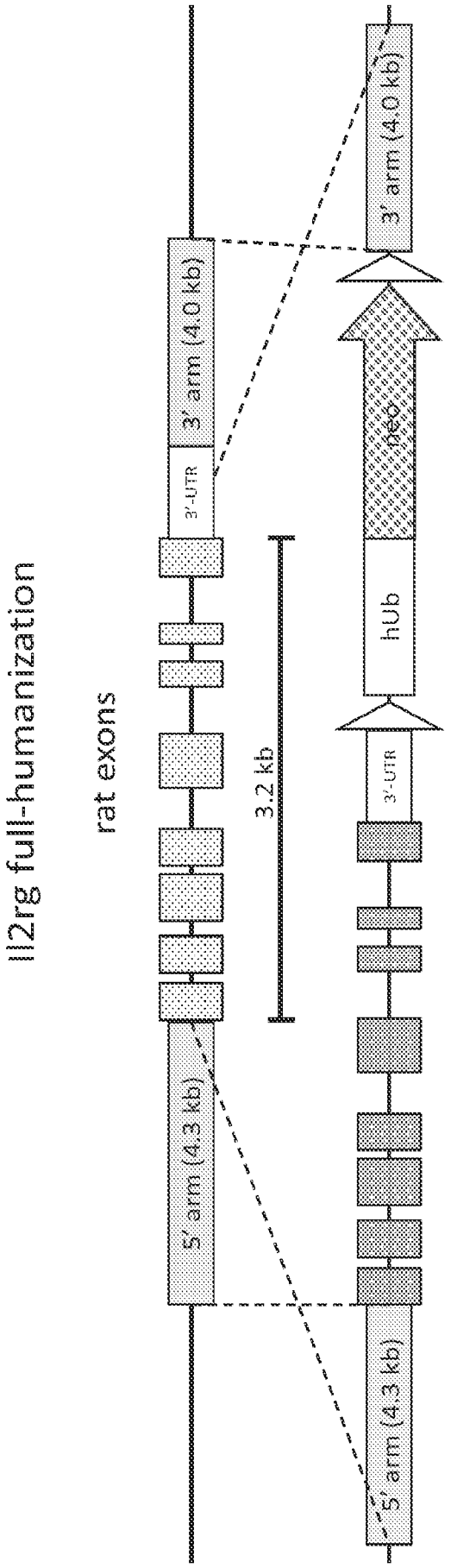

Il2rg full-humanization
rat exons
5' arm (4.3 kb)
3' arm (4.0 kb)
3'-UTR
3.2 kb
hUb
neo
3'-UTR
5' arm (4.3 kb)
3' arm (4.0 kb)
human exons

FIG. 31

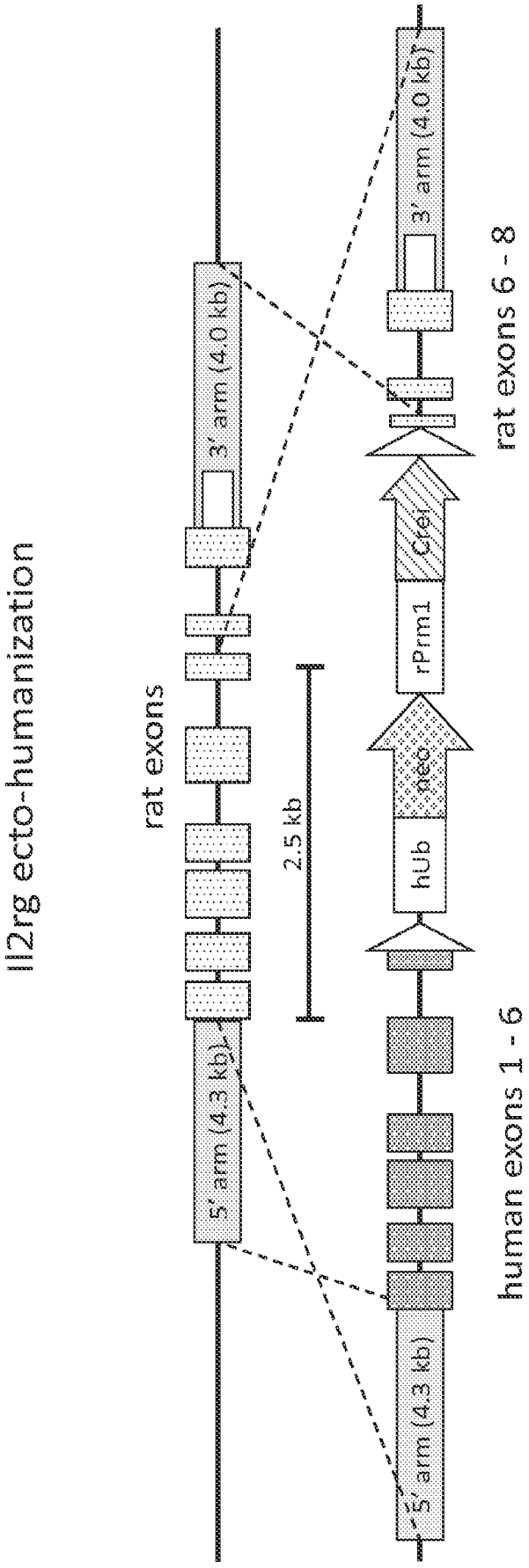

Il2rg ecto-humanization
rat exons
5' arm (4.3 kb)
2.5 kb
3' arm (4.0 kb)
human exons 1 - 6
hUb
neo
rPrm1
Cre
rat exons 6 - 8
3' arm (4.0 kb)
5' arm (4.3 kb)

FIG. 32

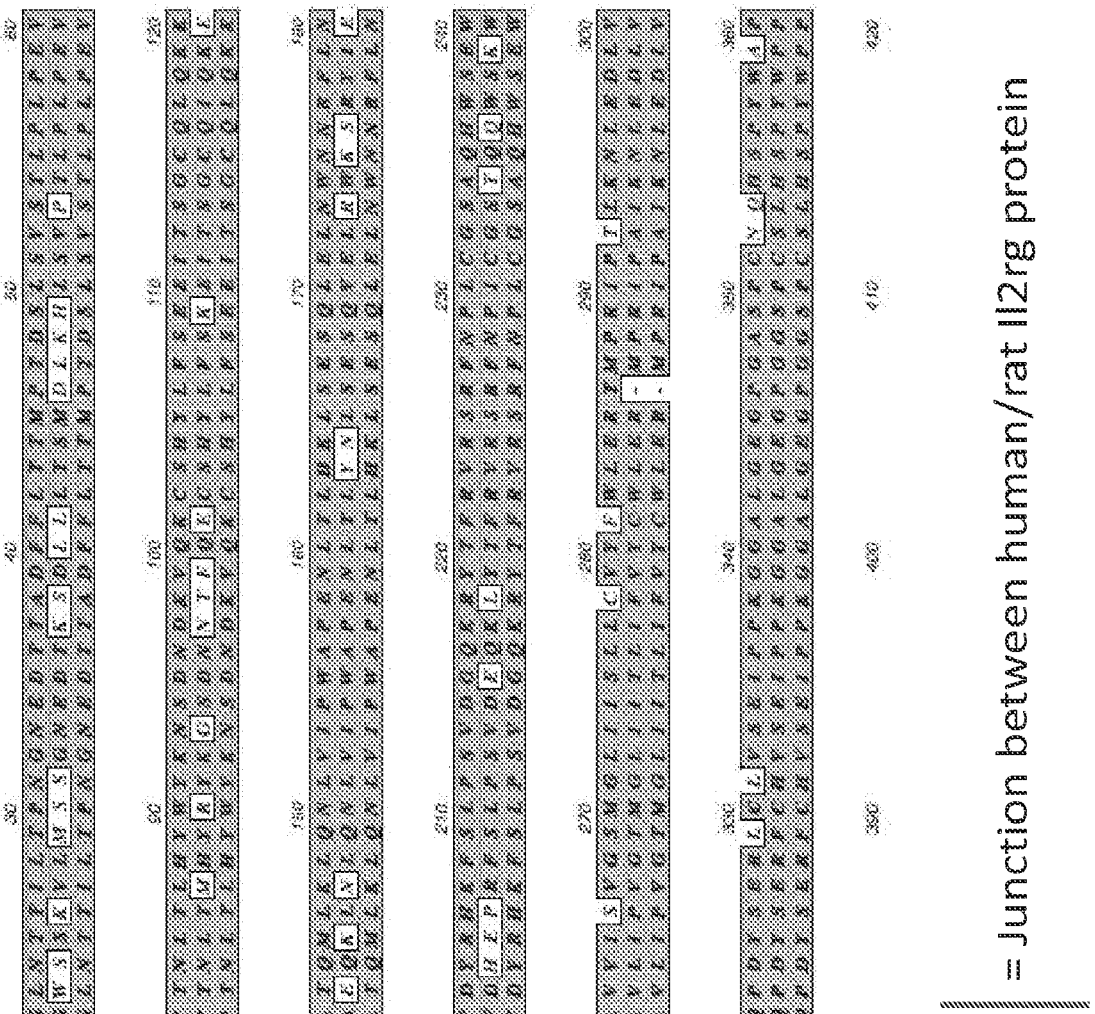
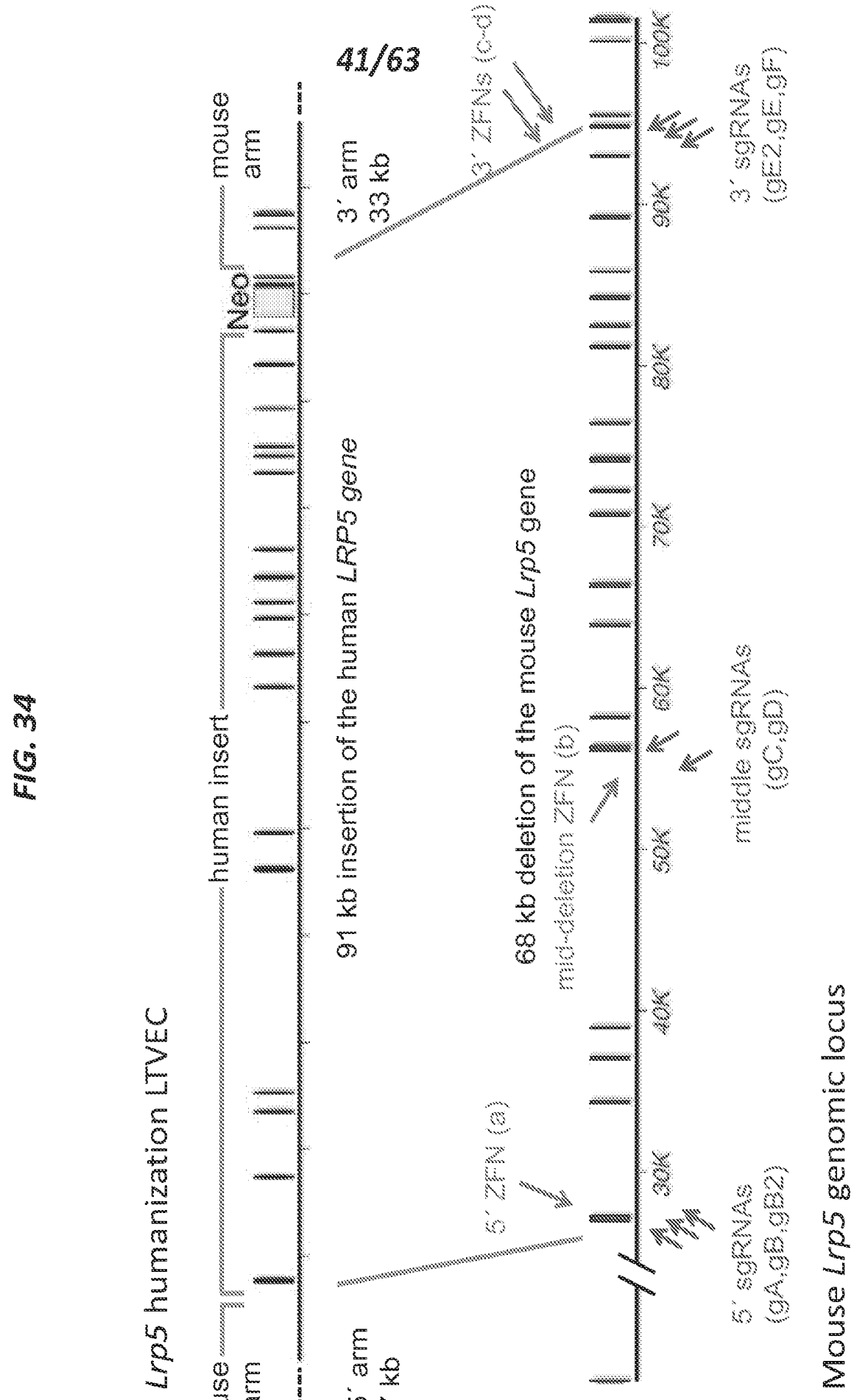

Hsa_Il2rg_NP_000197.1
Rno_Il2rg_NP_543165.1
Rsgn_Il2rg_human
= Junction between human/rat Il2rg protein
(SEQ ID NO: 20)
(SEQ ID NO: 21)
(SEQ ID NO: 22)

FIG. 33

FIG. 34


Lrp5 humanization LTVEC
mouse arm
human insert
mouse arm
Neo
5´ arm 7 kb
91 kb insertion of the human LRP5 gene
3´ arm 33 kb
3´ ZFNs (c-d)
68 kb deletion of the mouse Lrp5 gene
mid-deletion ZFN (b)
5´ ZFN (a)
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

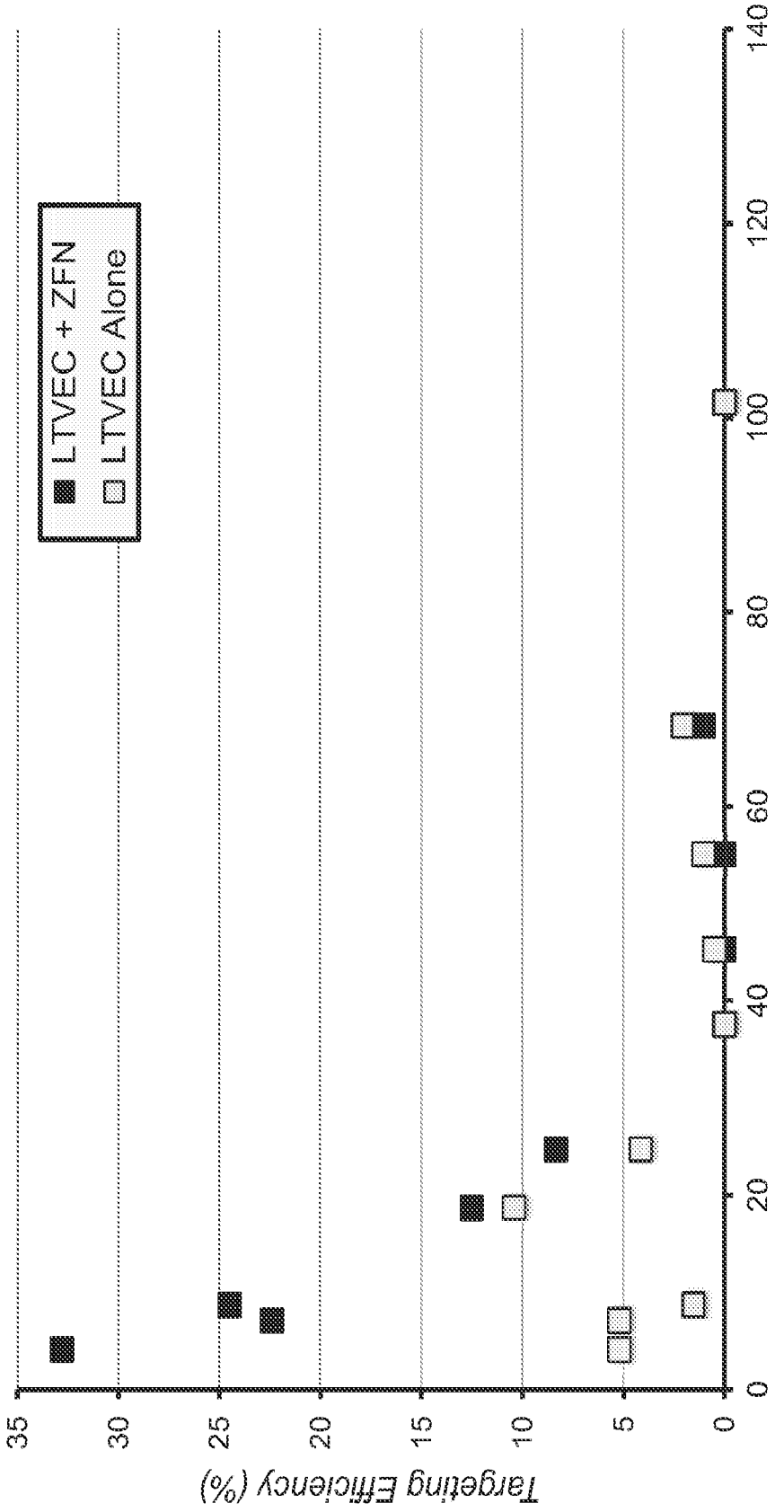

LTVEC + ZFN
LTVEC Alone
Targeting Efficiency (%)
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
140
Mouse Gene Deletion Size (kb)

FIG. 35A

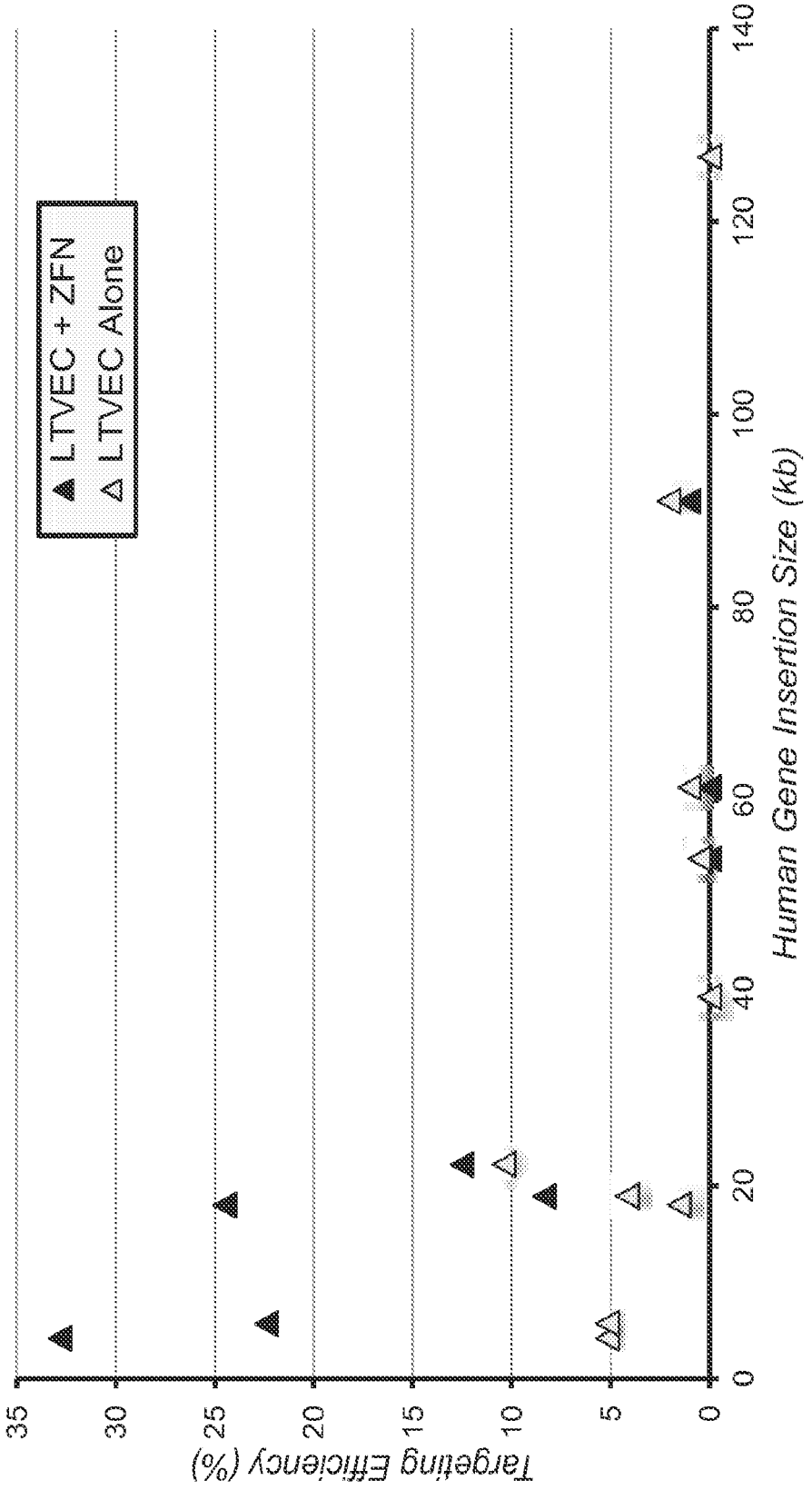

▲ LTVEC + ZFN
△ LTVEC Alone
Targeting Efficiency (%)
0
5
10
15
20
25
30
35
0
20
40
60
80
100
120
140
Human Gene Insertion Size (kb)

FIG. 35B

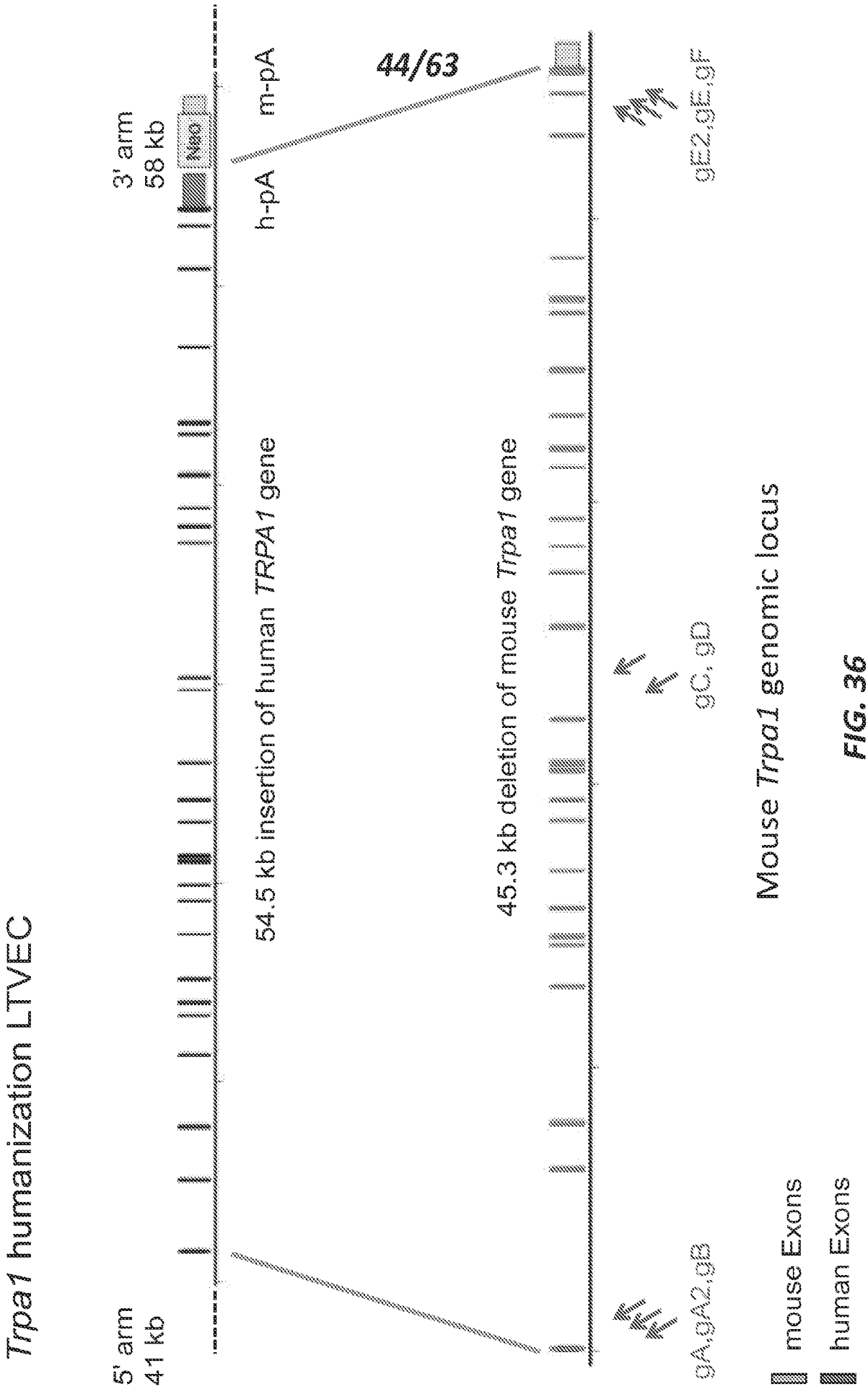

Trpa1 humanization LTVEC
5' arm
41 kb
3' arm
58 kb
Neo
h-pA
m-pA
44/63
54.5 kb insertion of human TRPA1 gene
45.3 kb deletion of mouse Trpa1 gene
gA,gA2,gB
gC, gD
gE2,gE,gF
Mouse Trpa1 genomic locus
mouse Exons
human Exons

FIG. 36

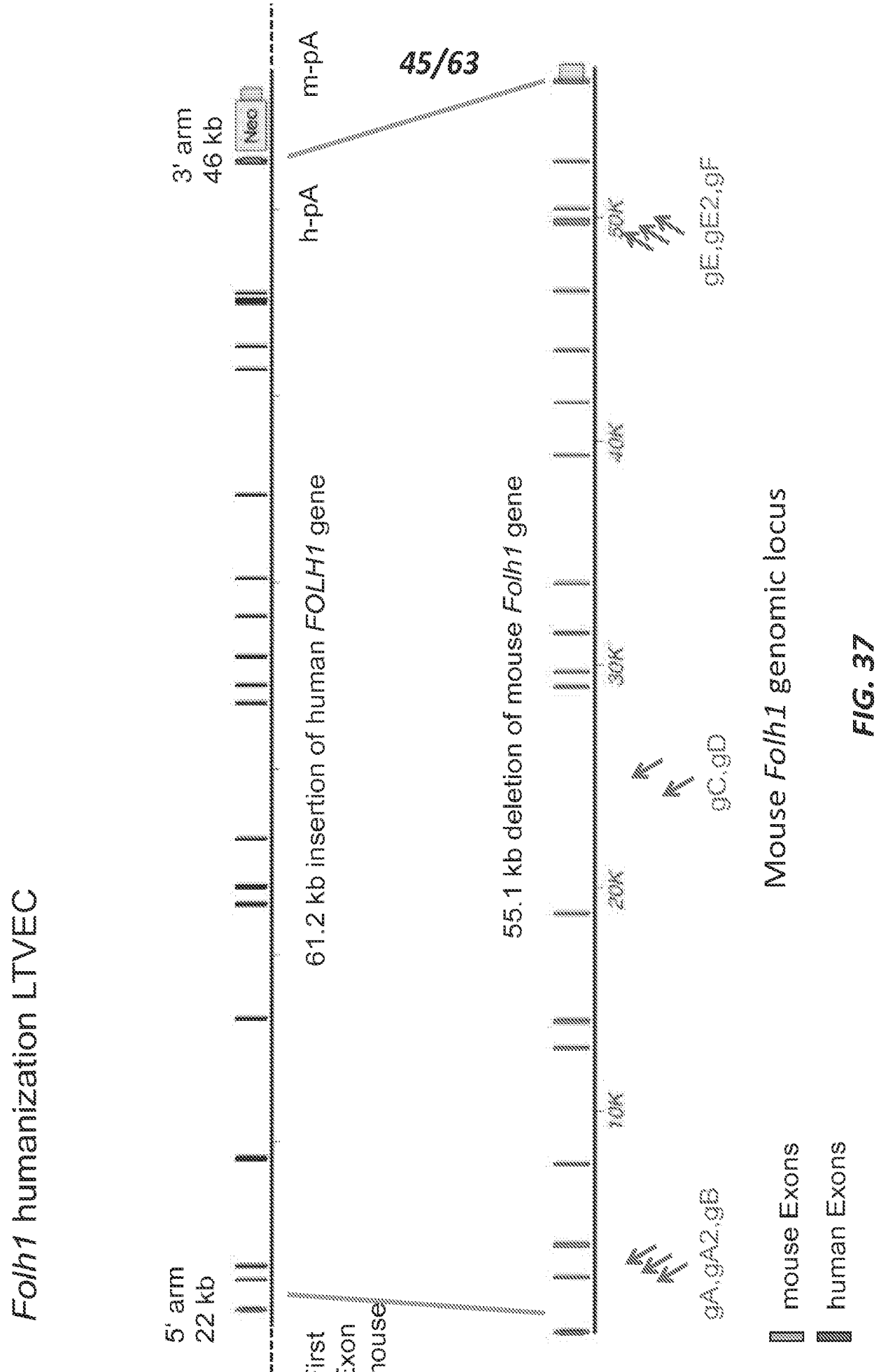

Folh1 humanization LTVEC
5' arm
22 kb
First
Exon
mouse
61.2 kb insertion of human FOLH1 gene
h-pA
3' arm
46 kb
Neo
m-pA
45/63
55.1 kb deletion of mouse Folh1 gene
10K
20K
30K
40K
50K
gA,gA2,gB
gC,gD
gE,gE2,gF
Mouse Folh1 genomic locus
mouse Exons
human Exons

FIG. 37

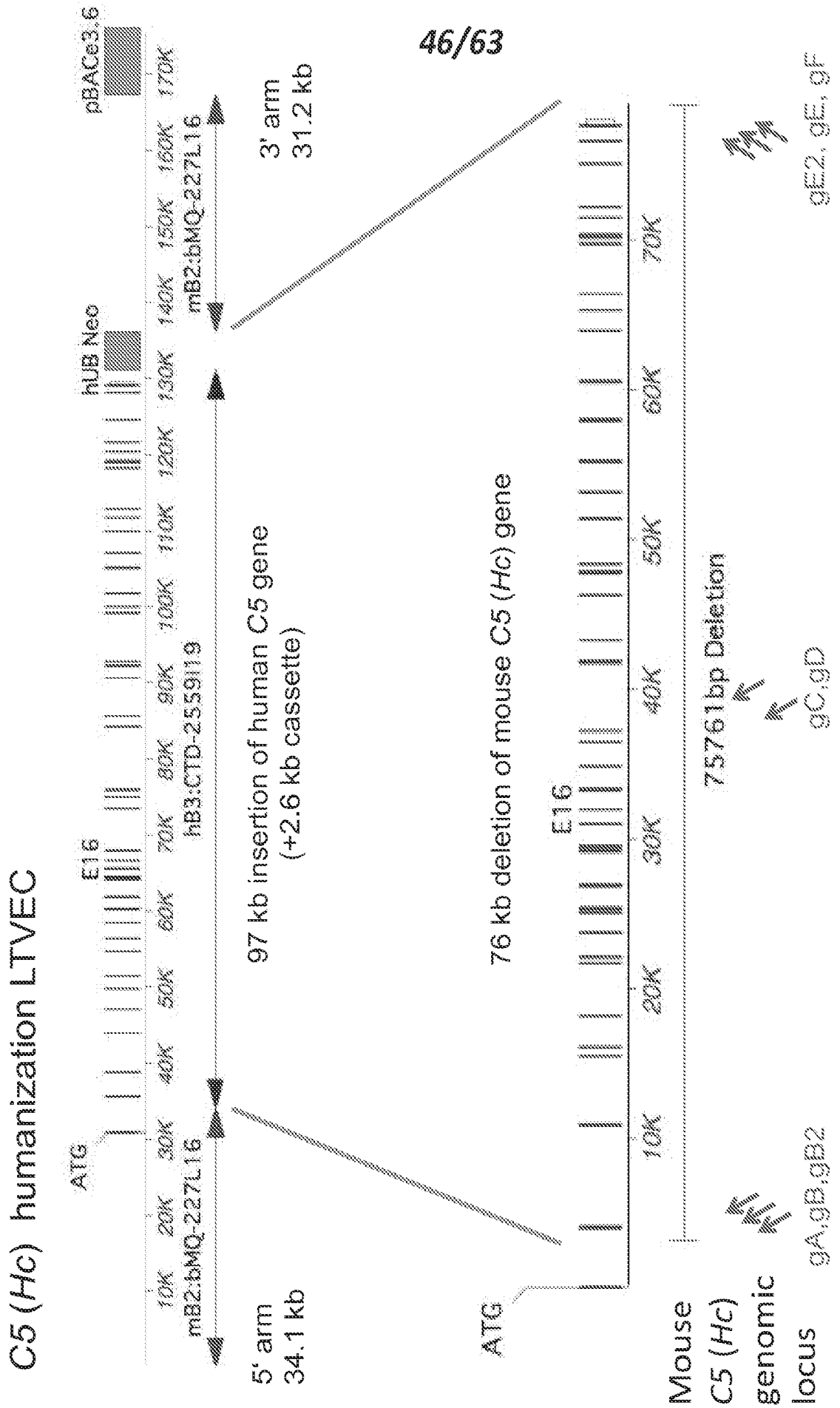

C5 (Hc) humanization LTVEC
pBACe3.6
hUB Neo
E16
ATG
10K 20K 30K 40K 50K 60K 70K 80K 90K 100K 110K 120K 130K 140K 150K 160K 170K
mB2:bMQ-227L16
hB3:CTD-2559I19
mB2:bMQ-227L16
3' arm
31.2 kb
97 kb insertion of human C5 gene
(+2.6 kb cassette)
5' arm
34.1 kb
76 kb deletion of mouse C5 (Hc) gene
E16
ATG
10K 20K 30K 40K 50K 60K 70K
757761bp Deletion
Mouse
C5 (Hc)
genomic
locus
gA,gB,gB2
gC,gD
gE2, gE, gF

FIG. 38

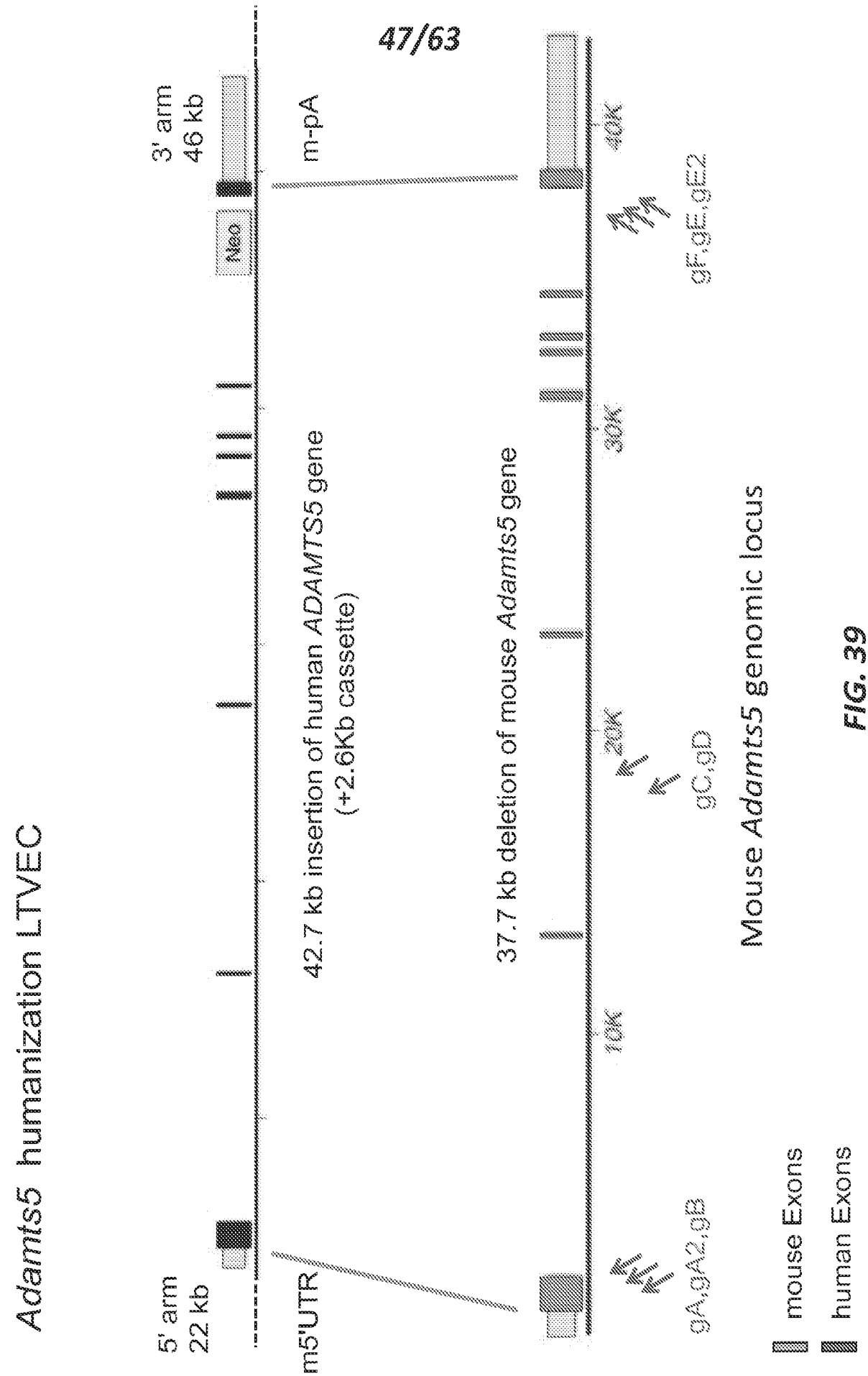

Adamts5 humanization LTVEC
5' arm
22 kb
m5'UTR
42.7 kb insertion of human ADAMTS5 gene
(+2.6Kb cassette)
37.7 kb deletion of mouse Adamts5 gene
3' arm
46 kb
Neo
m-pA
10K
20K
30K
40K
gA,gA2,gB
gC,gD
gF,gE,gE2
Mouse Adamts5 genomic locus
mouse Exons
human Exons

FIG. 39

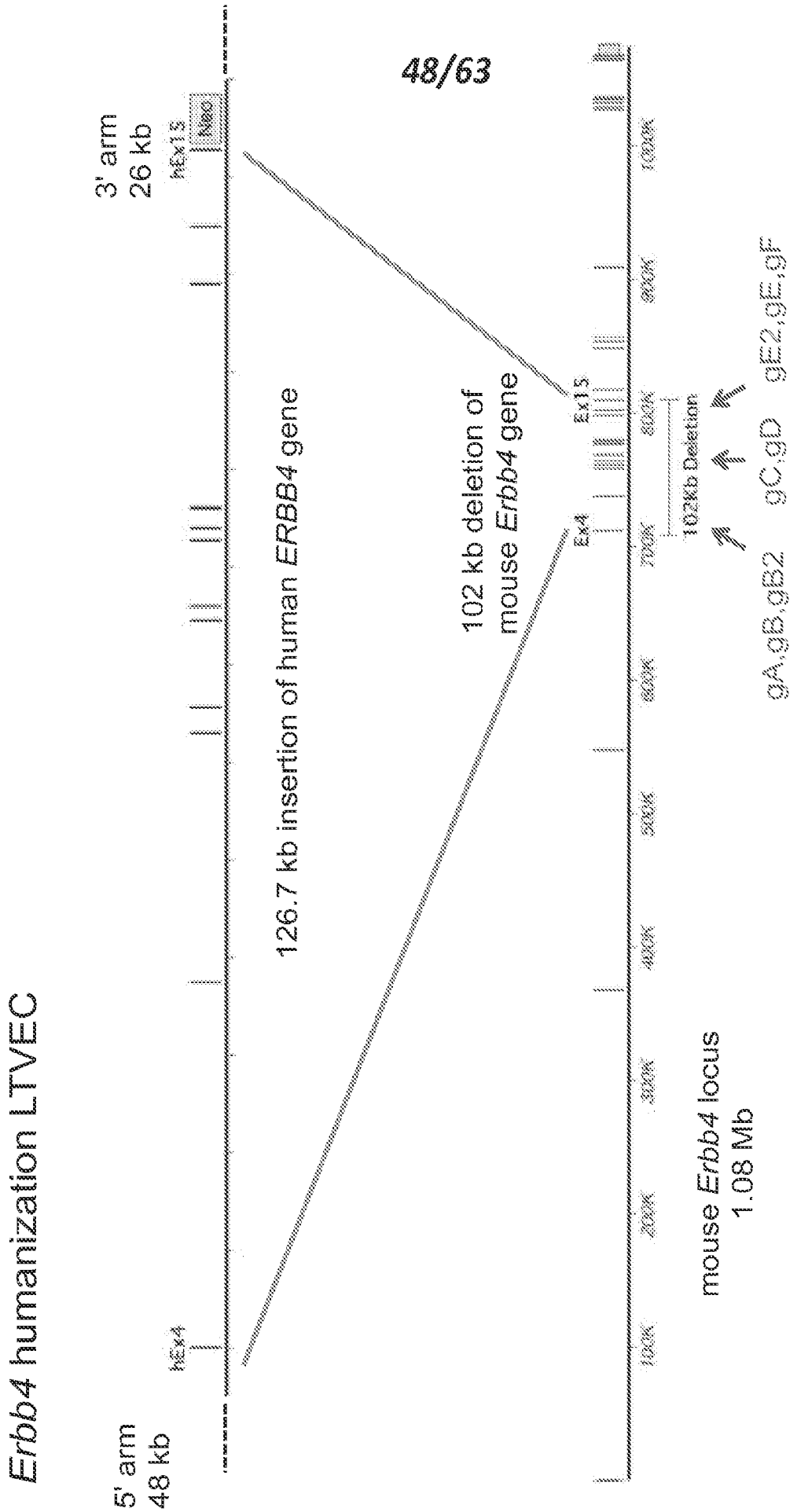

Erbb4 humanization LTVEC
5' arm
48 kb
hEx4
3' arm
26 kb
hEx15
Neo
126.7 kb insertion of human ERBB4 gene
102 kb deletion of
mouse Erbb4 gene
Ex4
Ex15
102Kb Deletion
gA,gB,gB2
gC,gD
gE2,gE,gF
mouse Erbb4 locus
1.08 Mb
Mouse Erbb4 genomic locus

FIG. 40

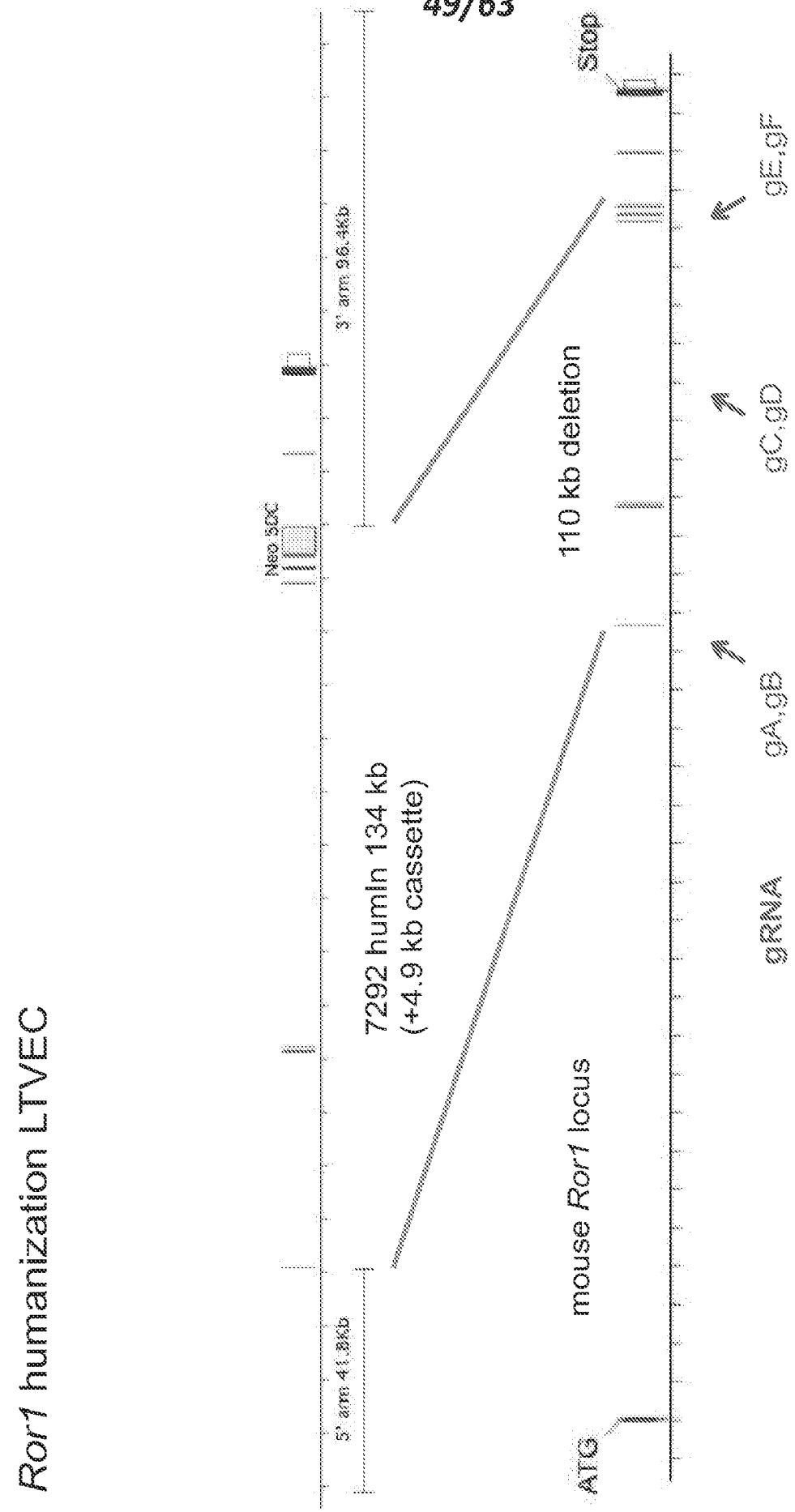

Ror1 humanization LTVEC
5' arm 41.8kb
Neo SDC
3' arm 96.4kb
7292 humIn 134 kb
(+4.9 kb cassette)
mouse Ror1 locus
110 kb deletion
ATG
Stop
gRNA
gA,gB
gC,gD
gE,gF

FIG. 41

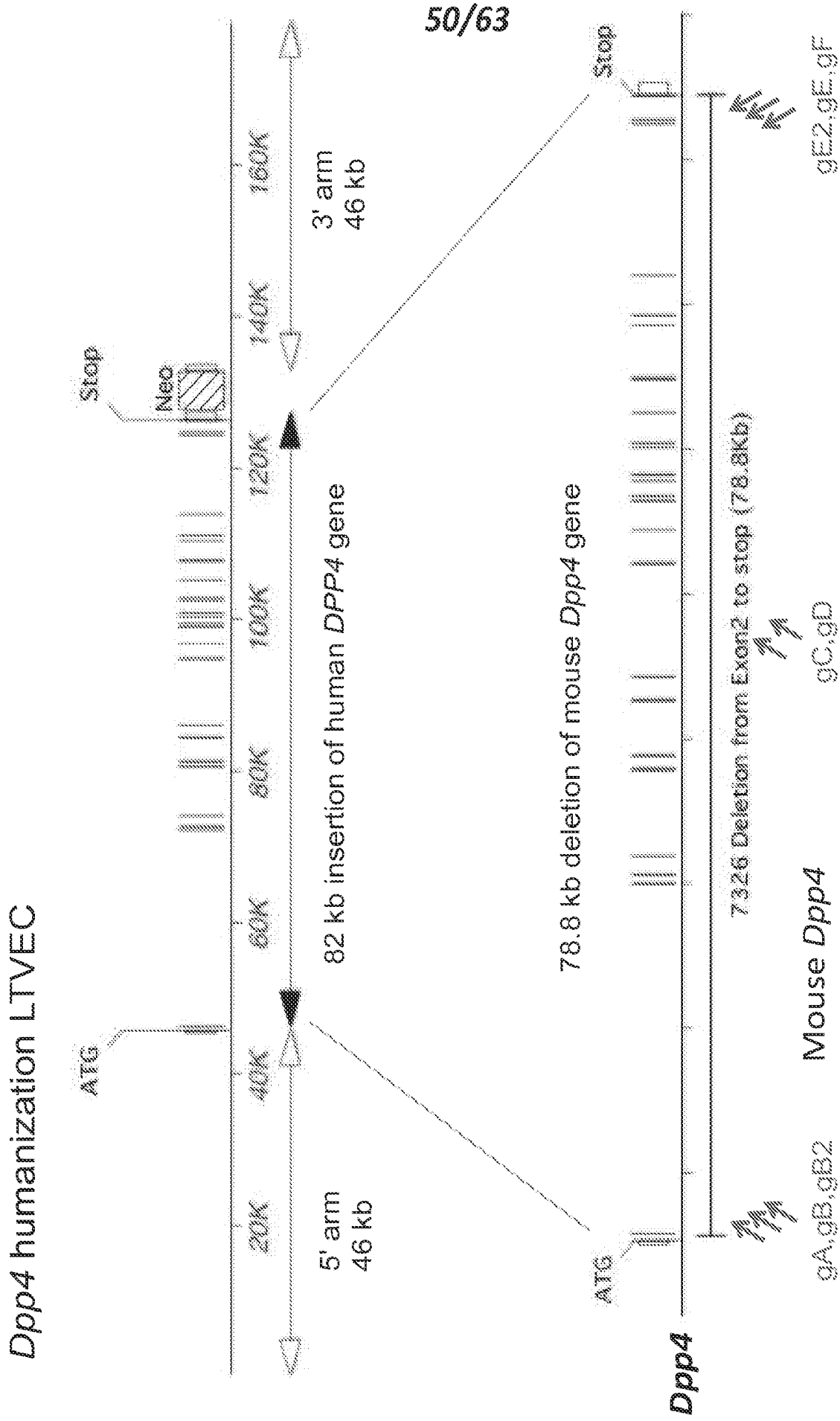

Dpp4 humanization LTVEC
ATG
Stop
Neo
20K
40K
60K
80K
100K
120K
140K
160K
5' arm
46 kb
3' arm
46 kb
82 kb insertion of human DPP4 gene
78.8 kb deletion of mouse Dpp4 gene
7326 Deletion from Exon2 to stop (78.8Kb)
gA,gB,gB2
gC,gD
gE2,gE,gF
Mouse Dpp4
genomic locus
ATG
Stop
Dpp4

FIG. 42

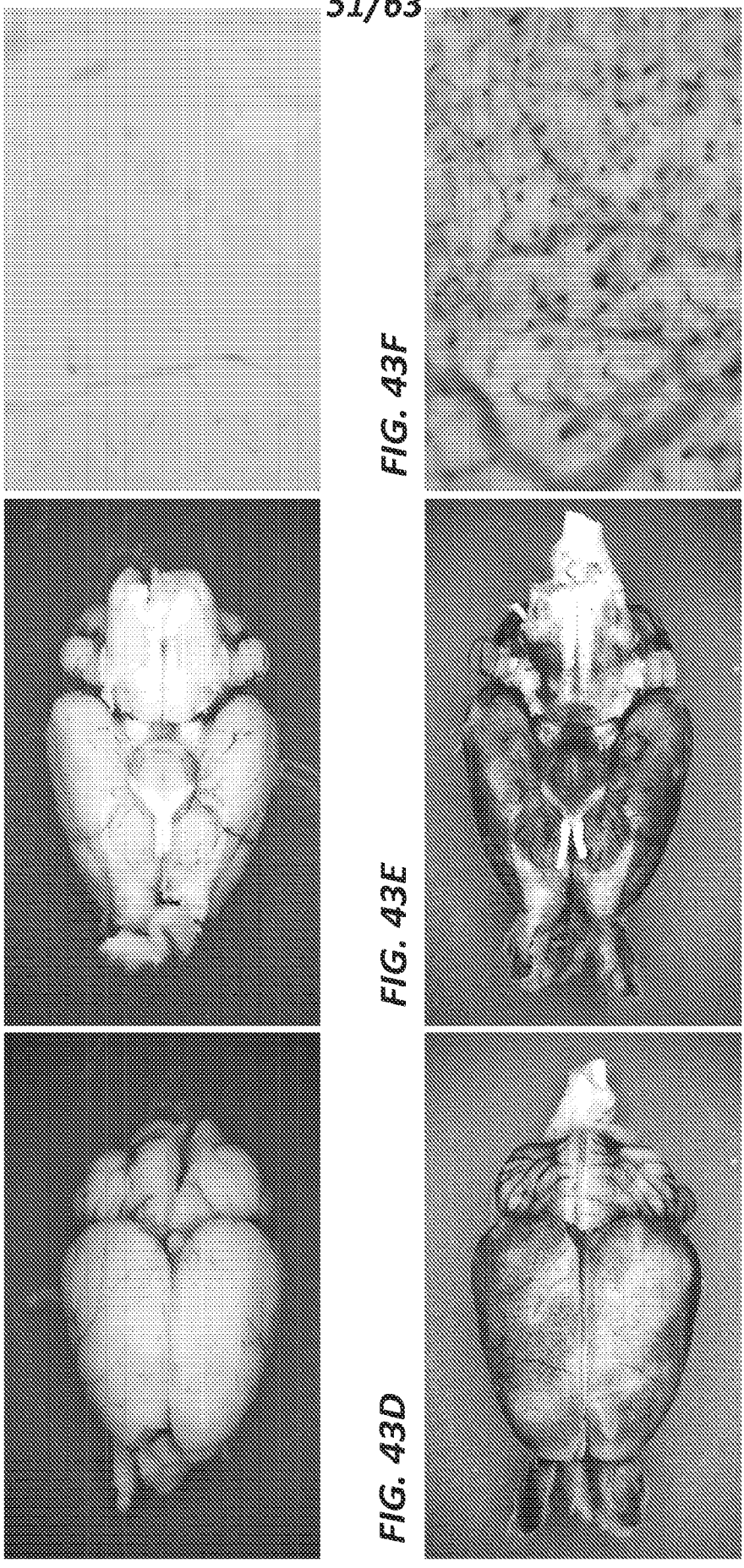
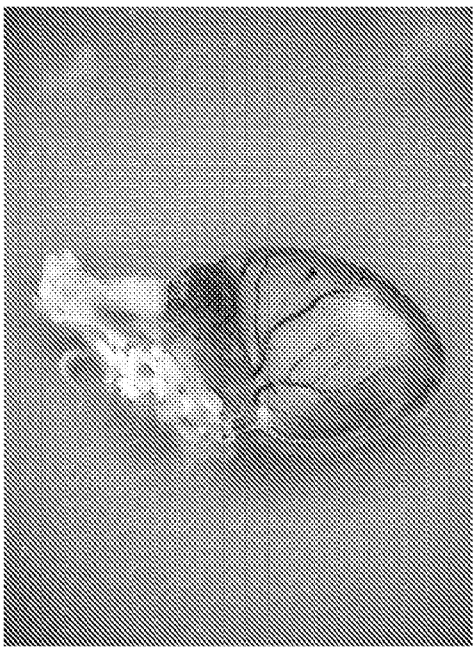

FIG. 43A
FIG. 43B
FIG. 43C
FIG. 43D
FIG. 43E
FIG. 43F
Wild type
ApoE+/-

FIG. 44A

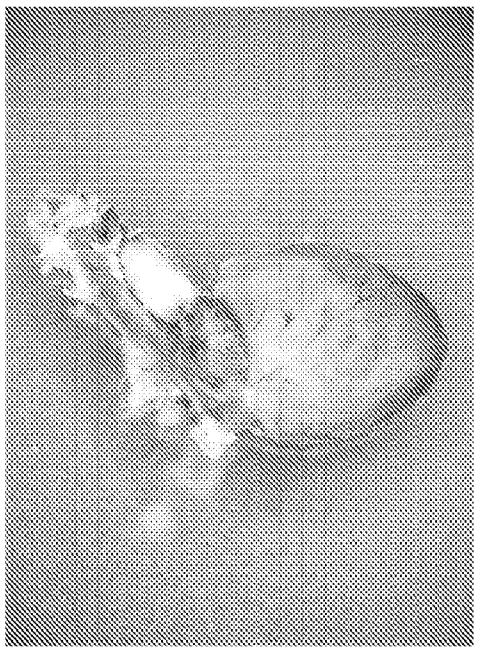


Wild type

FIG. 44B



FIG. 44C

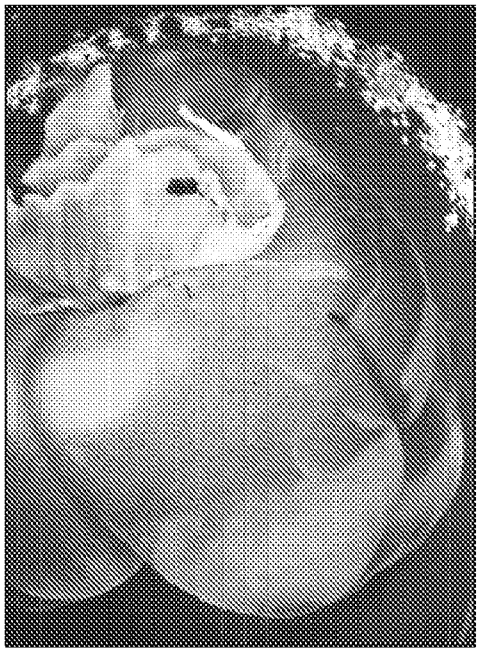


ApoE$^{+/-}$

FIG. 44D



FIG. 45A



Wild type

FIG. 45B

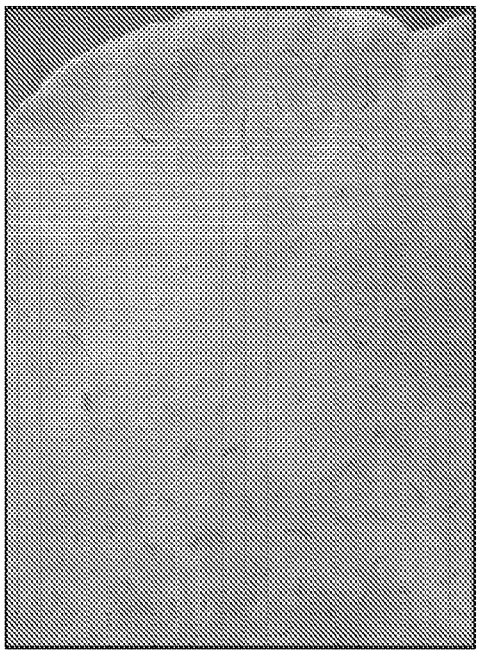
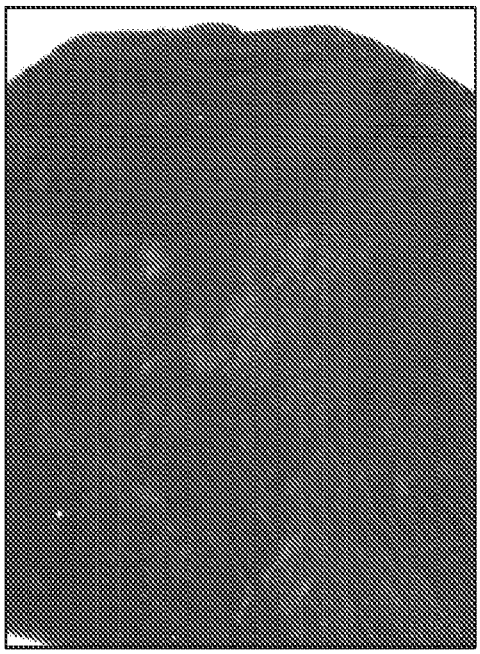


FIG. 45C



ApoE$^{+/-}$

FIG. 45D



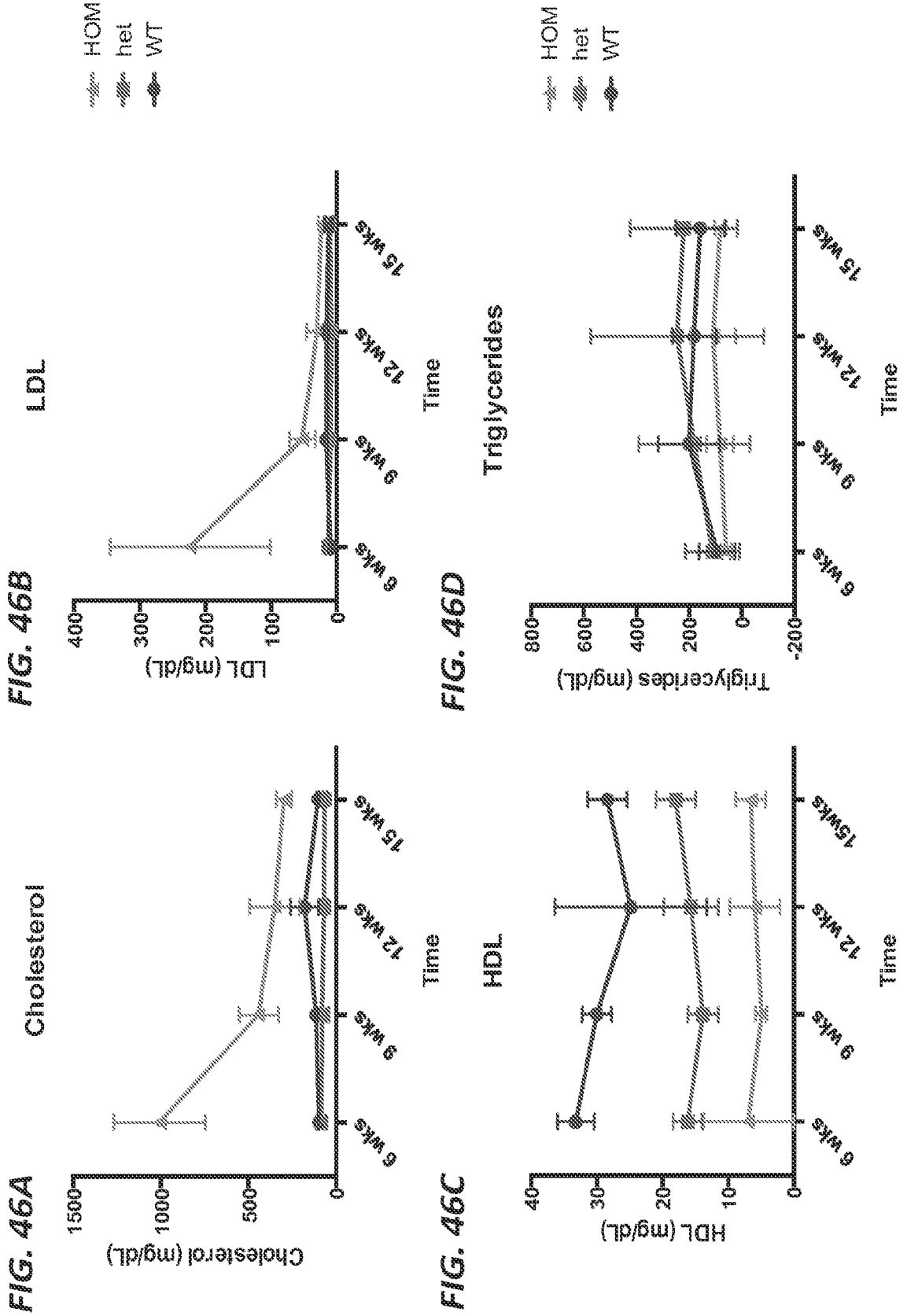

FIG. 46A
Cholesterol
Cholesterol (mg/dL)
1500
1000
500
0
6 wks
9 wks
12 wks
15 wks
Time
FIG. 46B
LDL
LDL (mg/dL)
400
300
200
100
0
HOM
het
WT
FIG. 46C
HDL
HDL (mg/dL)
40
30
20
10
0
FIG. 46D
Triglycerides
Triglycerides (mg/dL)
800
600
400
200
0
-200

FIG. 47

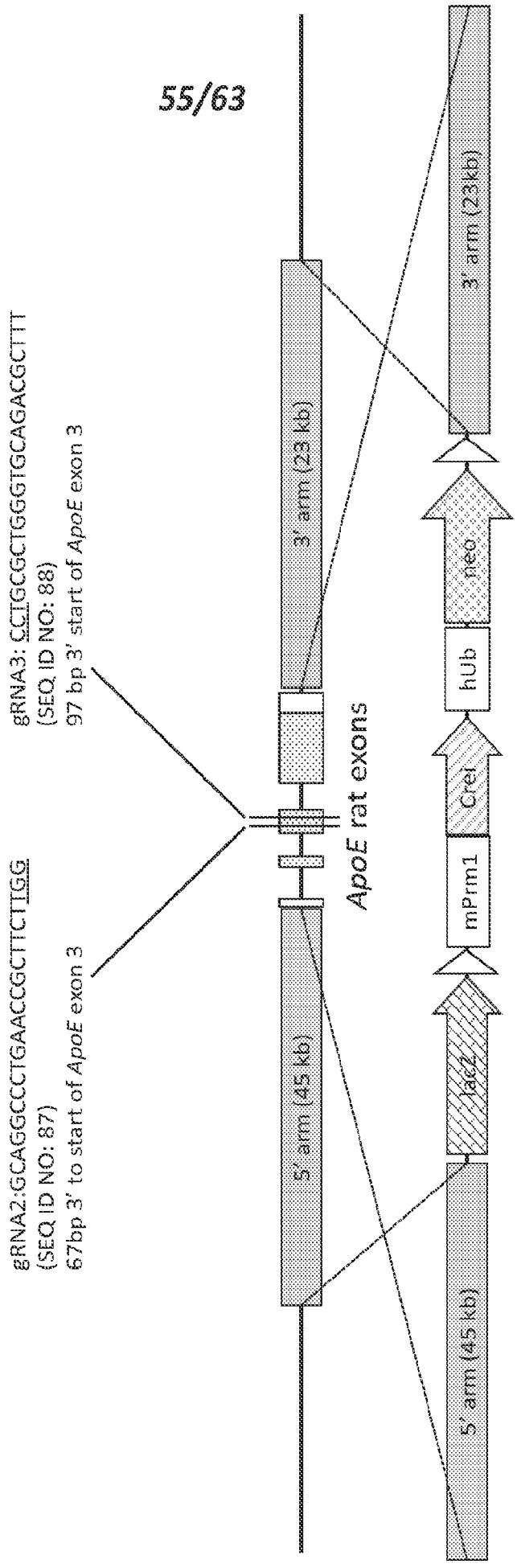

gRNA2:GCAGGCCCTGAACCGCTTCTTGG
(SEQ ID NO: 87)
67bp 3' to start of ApoE exon 3
gRNA3: CCTGCGCTGGGTGCAGACGCTTT
(SEQ ID NO: 88)
97 bp 3' start of ApoE exon 3
5' arm (45 kb)
3' arm (23 kb)
ApoE rat exons
lacZ
mPrm1
Crei
hUb
neo
3' arm (23kb)
5' arm (45 kb)

FIG. 48

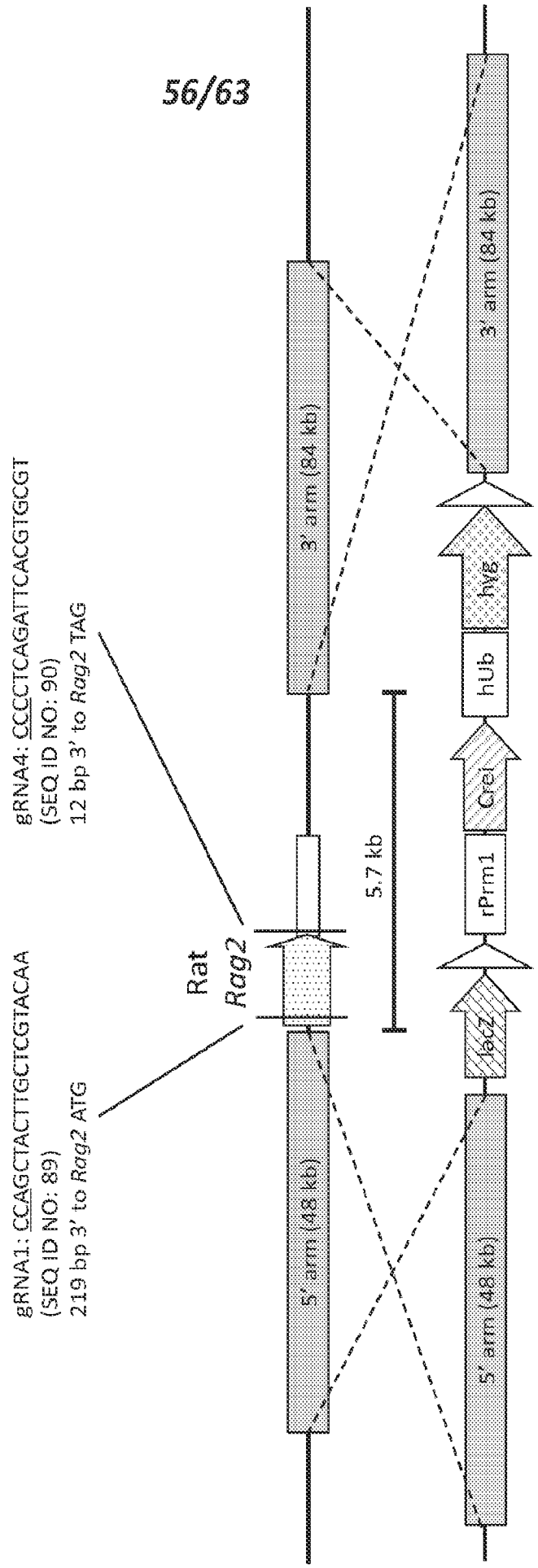
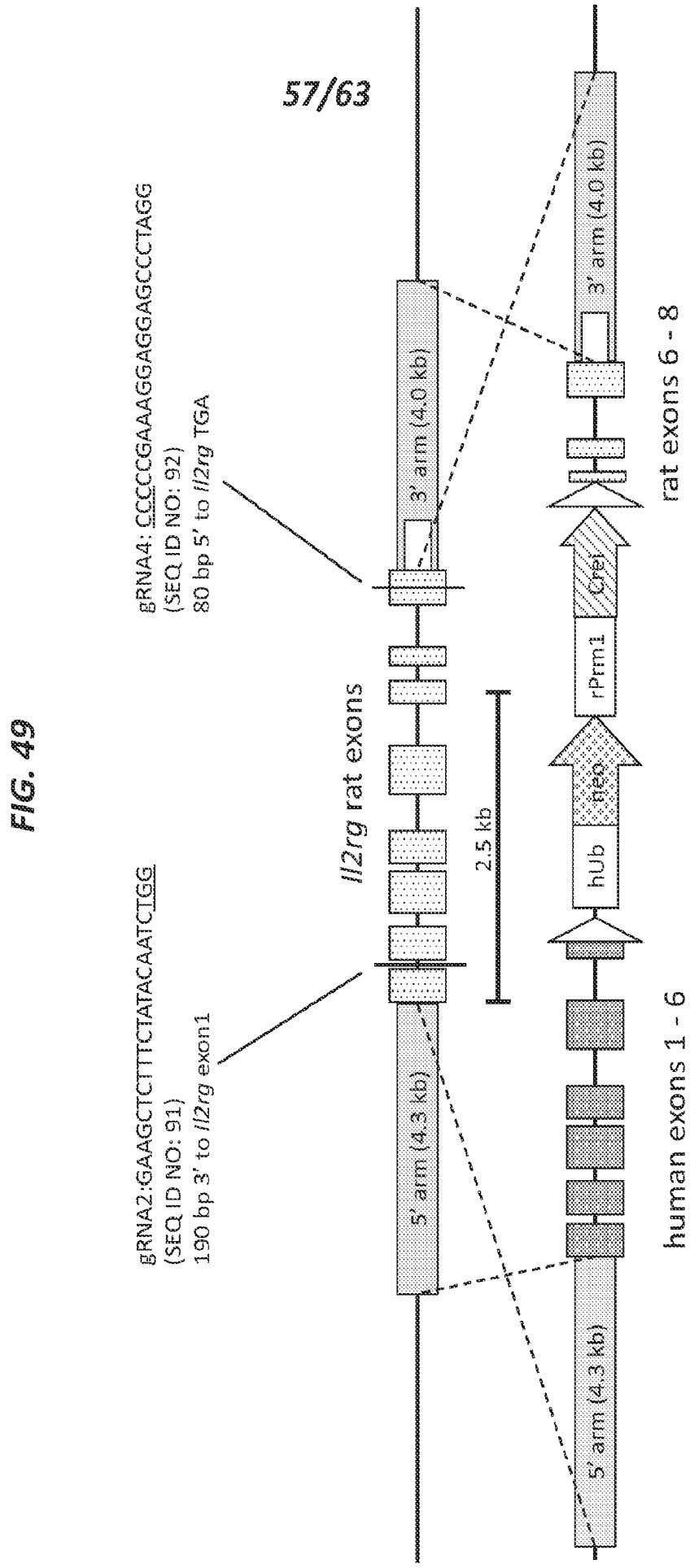

gRNA1: CCAGCTACTTGCTCGTACAA
(SEQ ID NO: 89)
219 bp 3' to Rag2 ATG
gRNA4: CCCCTCAGATTCACGTGCGT
(SEQ ID NO: 90)
12 bp 3' to Rag2 TAG
Rat
Rag2
5' arm (48 kb)
3' arm (84 kb)
5.7 kb
lacZ
rPrm1
Cre
hUb
hyg
5' arm (48 kb)
3' arm (84 kb)

FIG. 49


gRNA4: CCCCCGAAAGGAGGAGCCCTAGG
(SEQ ID NO: 92)
80 bp 5' to Il2rg TGA
gRNA2:GAAGCTCTTTCTATACAATCTGG
(SEQ ID NO: 91)
190 bp 3' to Il2rg exon1
Il2rg rat exons
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

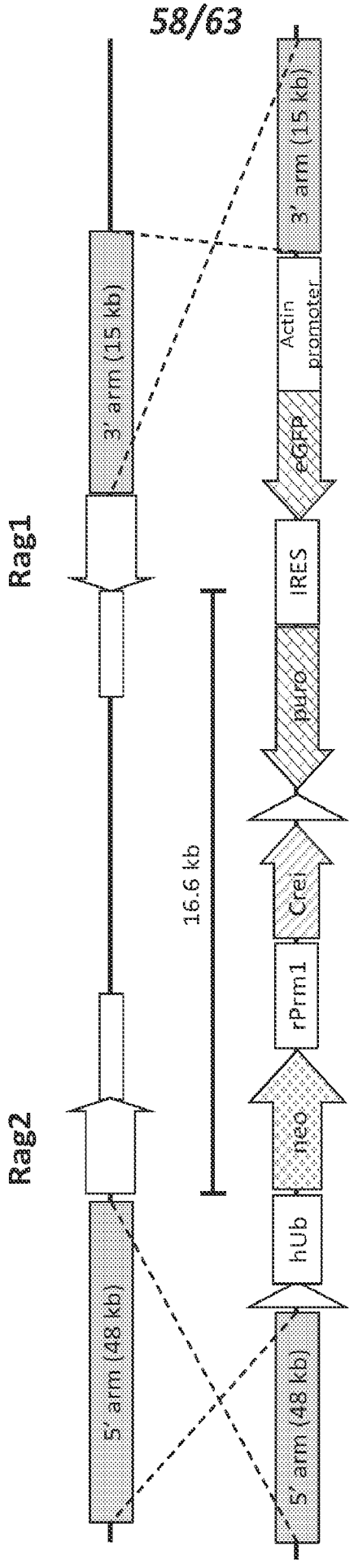

Rag1
Rag2
3' arm (15 kb)
5' arm (48 kb)
16.6 kb
hUb
neo
rPrm1
Cre1
IRES
puro
Actin promoter
eGFP
3' arm (15 kb)
5' arm (48 kb)

FIG. 50

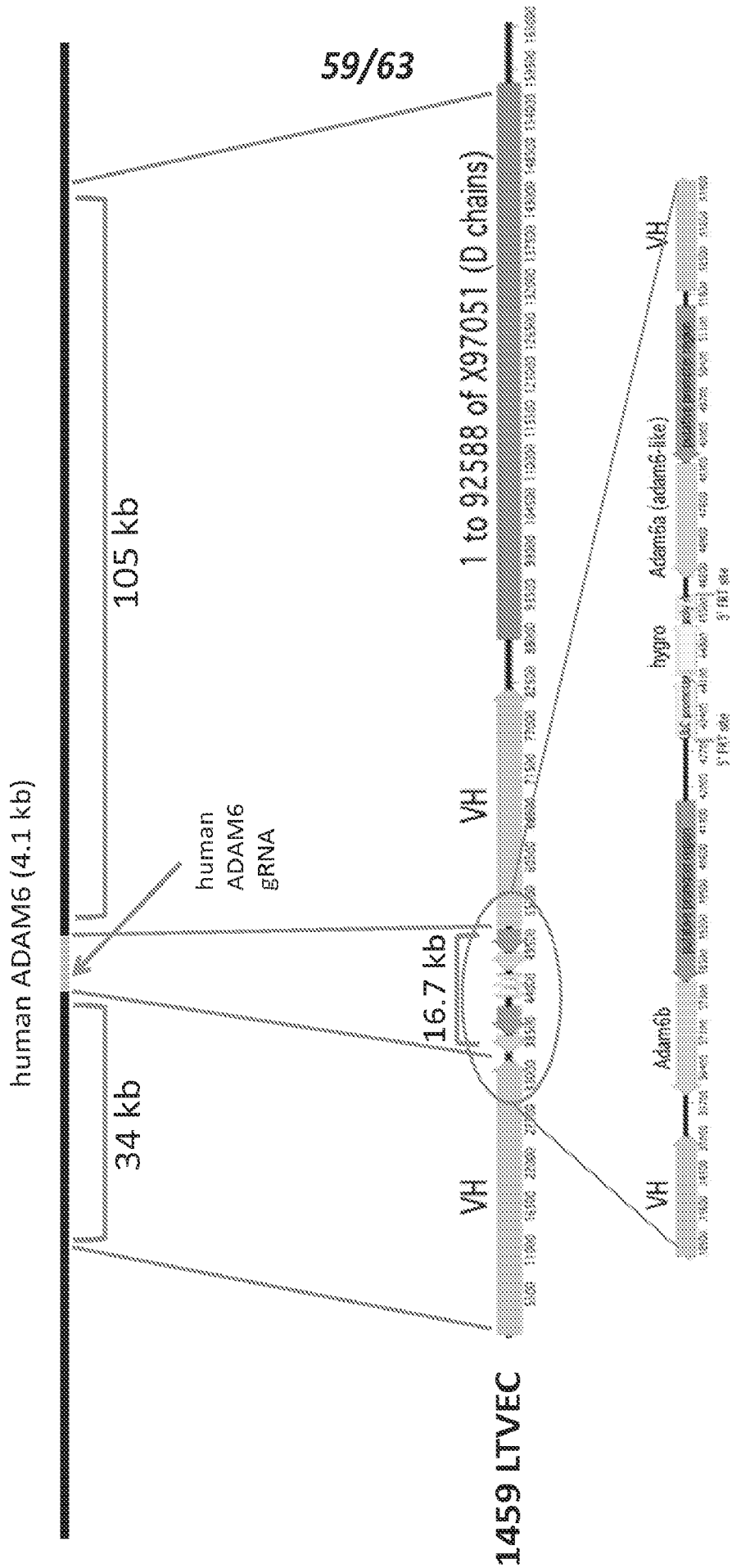

human ADAM6 (4.1 kb)
105 kb
34 kb
human ADAM6 gRNA
16.7 kb
VH
VH
1 to 92588 of X97051 (D chains)
1459 LTVEC
VH
Adam6b
hygro
Adam6a (adam6-like)
VH

FIG. 51

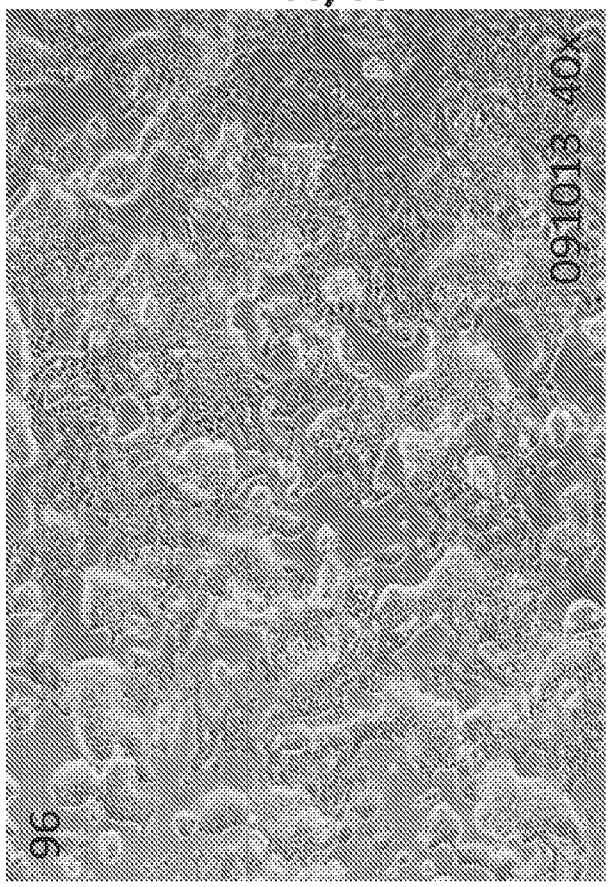


FIG. 52B

12 days in 2i

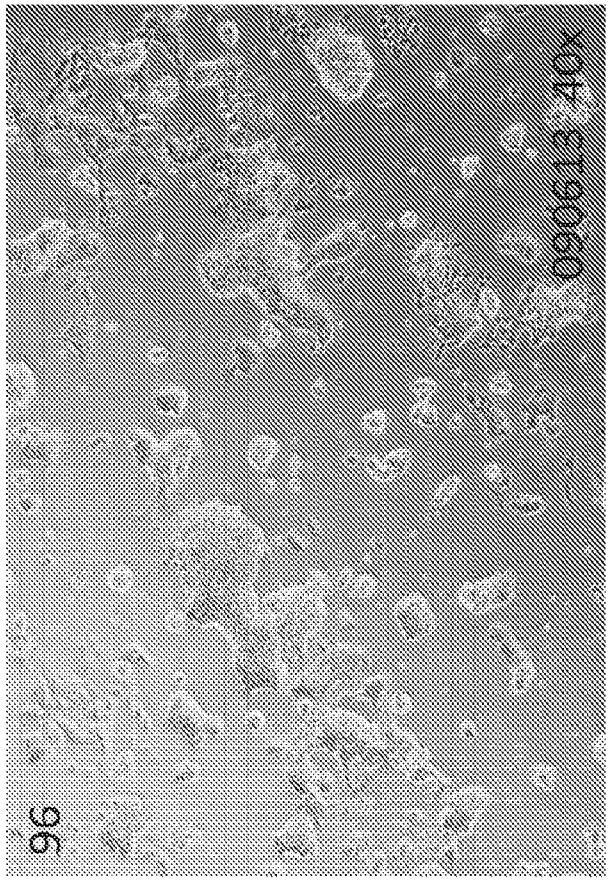


FIG. 52A

8 days in 2i

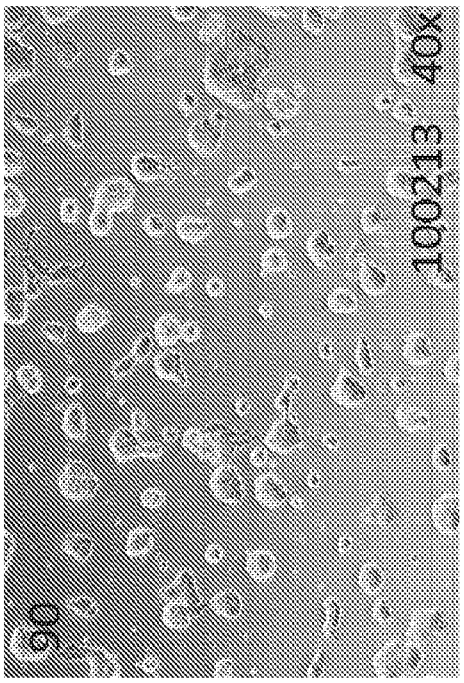


FIG. 53A

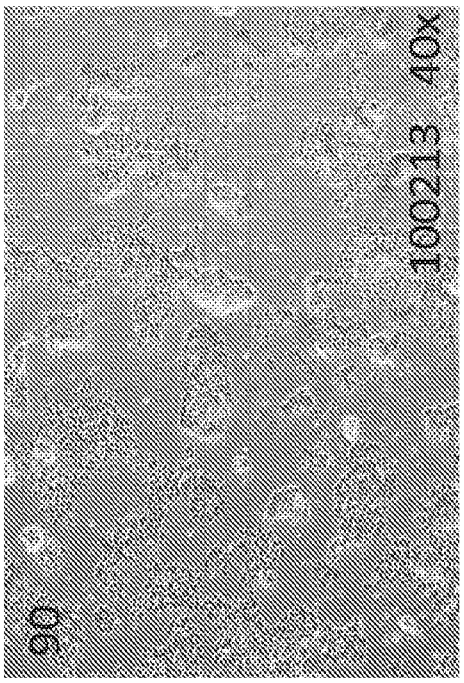


FIG. 53B

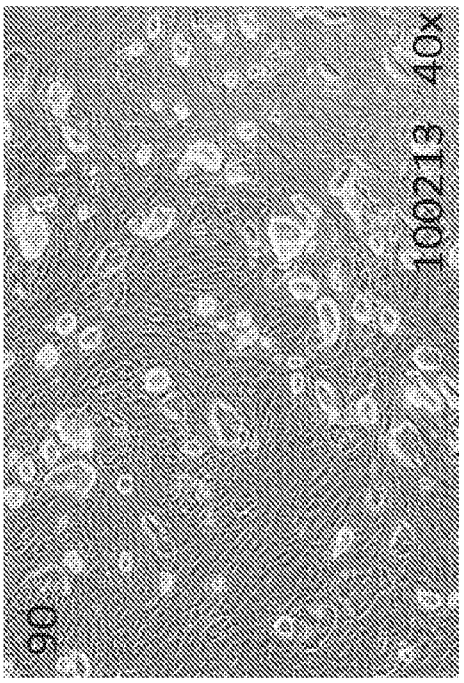


FIG. 53C

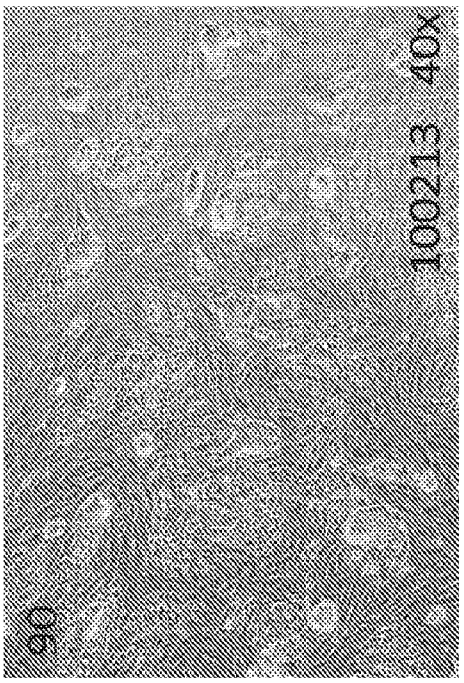


FIG. 53D

mTeSR-hLIF Matrigel hVG2i

mTeSR-hLIF NuFF feeder hVG2i

FIG. 54C

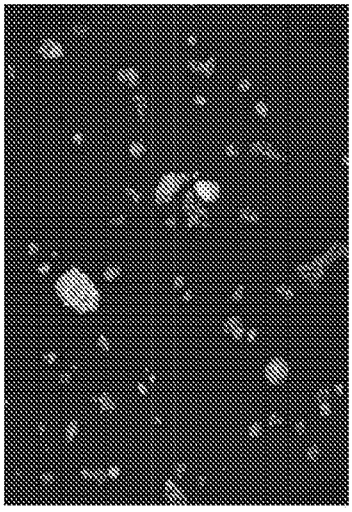


FIG. 54B

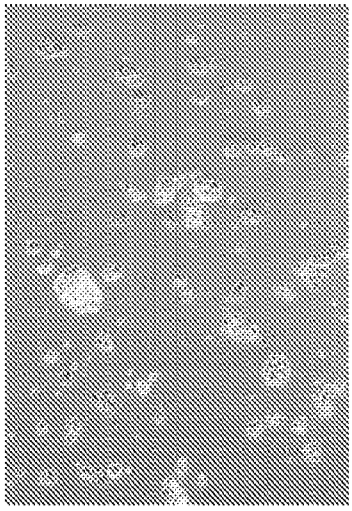
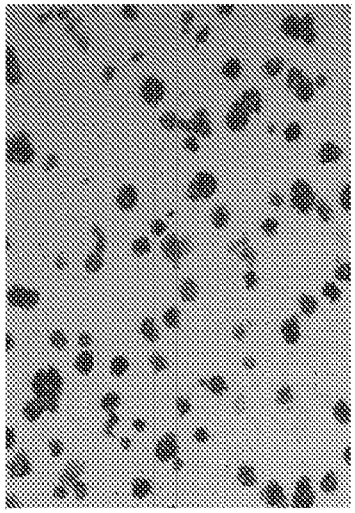
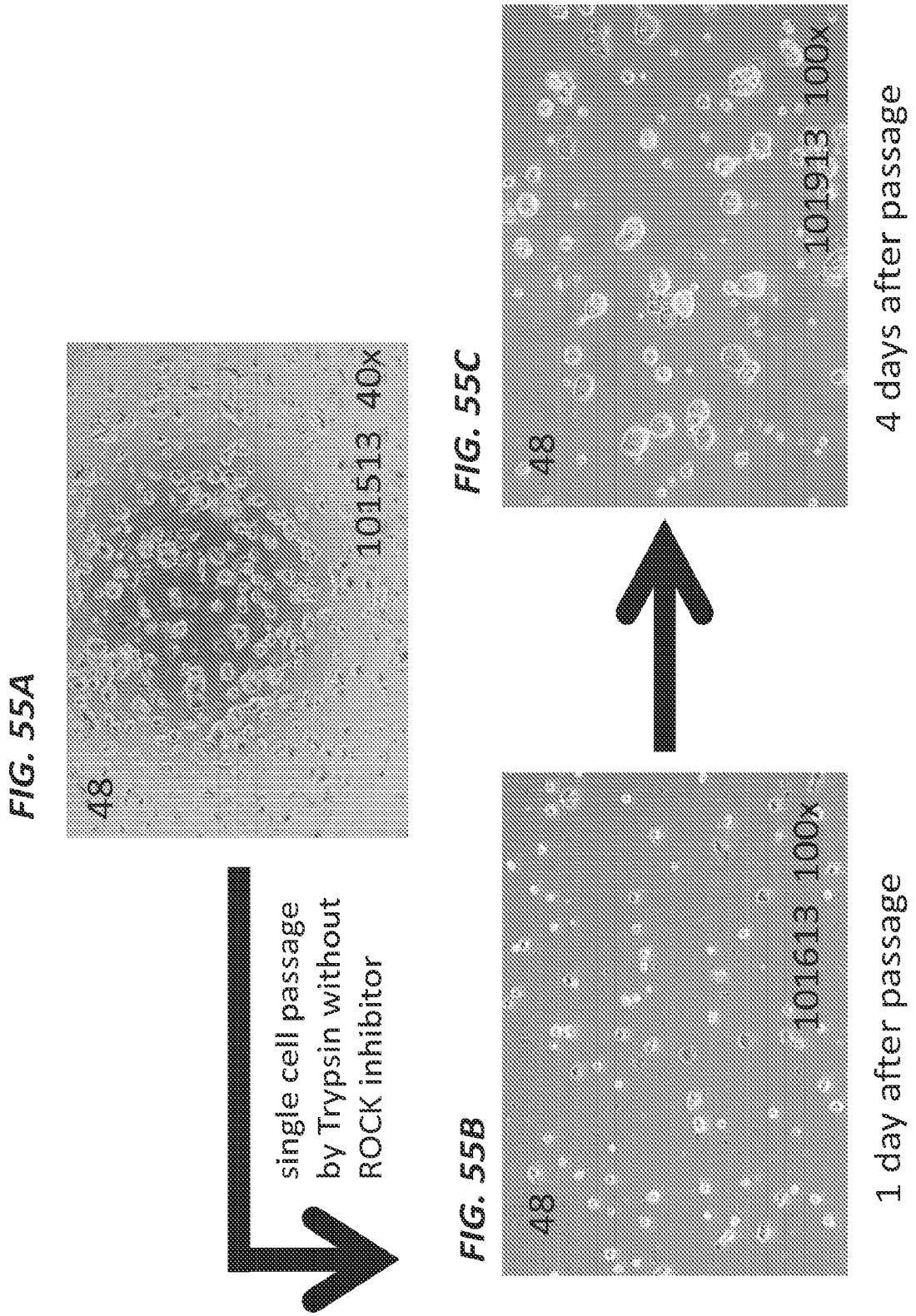


FIG. 54A




FIG. 55A
48
101513 40x
single cell passage
by Trypsin without
ROCK inhibitor
FIG. 55B
48
101613 100x
1 day after passage
FIG. 55C
48
101913 100x
4 days after passage

SEQUENCE LISTING

<110> Frendewey, David
Auerbach, Wojtek
Lai, Ka-Man Venus
Mujica, Alexander O.
Lee, Jeffrey D.
Droguett, Gustavo
Trzaska, Sean
Hunt, Charleen
Gagliardi, Anthony
Kuno, Junko
Valenzuela, David M.
Yancopoulos, George D.

<120> Methods and Compositions for the
Targeted Modification of a Genome

<130> 057766/453460

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<151> 2013-12-11

<150> US 62/017,416
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guide RNA (gRNA)

<220>
<221> misc_feature
<222> 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,
19, 20, 21
<223> n = A,T,C or G

<400> 1
gnnnnnnnnn nnnnnnnnnn ngg 23

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<211> 80
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<220>

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<400> 2
guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc cguuaucaac uugaaaaagu 60
ggcaccgagu cggugcuuuu 80

<210> 3
<211> 42
<212> RNA
<213> Artificial Sequence

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<400> 3
guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc cg 42

<210> 4
<211> 30
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<213> Artificial Sequence

<220>
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<400> 4
guuuuagagc uagaaauagc aaguuaaaau 30

<210> 5
<211> 33
<212> RNA
<213> Artificial Sequence

<220>
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<400> 5
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<210> 6
<211> 26
<212> RNA
<213> Artificial Sequence

<220>
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<400> 6
gaguccgagc agaagaagaa guuuua 26

<210> 7
<211> 12
<212> RNA
<213> Artificial Sequence

<220>
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<400> 7
aaggcuaguc cg 12

<210> 8
<211> 50
<212> RNA
<213> Artificial Sequence

<220>
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<400> 8
aaggcuaguc cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 50

<210> 9
<211> 203
<212> PRT
<213> Mus Musculus

<400> 9
Met Lys Val Leu Ala Ala Gly Ile Val Pro Leu Leu Leu Leu Val Leu
 1   5   10   15
His Trp Lys His Gly Ala Gly Ser Pro Leu Pro Ile Thr Pro Val Asn
            20   25   30
Ala Thr Cys Ala Ile Arg His Pro Cys His Gly Asn Leu Met Asn Gln
        35   40   45
Ile Lys Asn Gln Leu Ala Gln Leu Asn Gly Ser Ala Asn Ala Leu Phe
    50   55   60
Ile Ser Tyr Tyr Thr Ala Gln Gly Glu Pro Phe Pro Asn Asn Val Glu
65   70   75   80
Lys Leu Cys Ala Pro Asn Met Thr Asp Phe Pro Ser Phe His Gly Asn
                85   90   95
Gly Thr Glu Lys Thr Lys Leu Val Glu Leu Tyr Arg Met Val Ala Tyr
            100   105   110
Leu Ser Ala Ser Leu Thr Asn Ile Thr Arg Asp Gln Lys Val Leu Asn
        115   120   125
Pro Thr Ala Val Ser Leu Gln Val Lys Leu Asn Ala Thr Ile Asp Val
    130   135   140
Met Arg Gly Leu Leu Ser Asn Val Leu Cys Arg Leu Cys Asn Lys Tyr
145   150   155   160
Arg Val Gly His Val Asp Val Pro Pro Val Pro Asp His Ser Asp Lys
                165   170   175
Glu Ala Phe Gln Arg Lys Lys Leu Gly Cys Gln Leu Leu Gly Thr Tyr
            180   185   190
Lys Gln Val Ile Ser Val Val Val Gln Ala Phe
        195   200

<210> 10
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> ZFN1a binding site

<400> 10
caggccctga accgc 15

<210> 11
<211> 6
<212> DNA
<213> Artificial Sequence

<220>
<223> ZFN1 cutting site

<400> 11
ttctgg 6

<210> 12
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> ZFN1b binding site

<400> 12
gattacctgc gctggg 16

<210> 13
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
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<400> 13
ttcaccctcc gcacc 15

<210> 14
<211> 7
<212> DNA
<213> Artificial Sequence

<220>
<223> ZFN2 cutting site

<400> 14
tgctgag 7

<210> 15
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> ZF21b binding site

<400> 15
tatccagatc caggggtt 18

<210> 16
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> conserved domain of a family of homing nucleases

<400> 16
Leu Ala Gly Leu Ile Asp Ala Asp Gly
1 5

<210> 17
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> ZFN1a binding site

<400> 17
caggccctga accgc 15

<210> 18
<211> 6
<212> DNA
<213> Artificial Sequence

<220>
<223> ZFN1 cutting site

<400> 18
ttctgg 6

<210> 19
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> ZFN1b binding site

<400> 19
gattacctgc gctggg 16

<210> 20
<211> 369
<212> PRT
<213> Homo sapiens

<400> 20

Met	Leu	Lys	Pro	Ser	Leu	Pro	Phe	Thr	Ser	Leu	Leu	Phe	Leu	Gln	Leu
1				5				10					15		
Pro	Leu	Leu	Gly	Val	Gly	Leu	Asn	Thr	Thr	Ile	Leu	Thr	Pro	Asn	Gly
			20					25					30		
Asn	Glu	Asp	Thr	Thr	Ala	Asp	Phe	Phe	Leu	Thr	Thr	Met	Pro	Thr	Asp
		35					40					45			
Ser	Leu	Ser	Val	Ser	Thr	Leu	Pro	Leu	Pro	Glu	Val	Gln	Cys	Phe	Val
	50					55					60				
Phe	Asn	Val	Glu	Tyr	Met	Asn	Cys	Thr	Trp	Asn	Ser	Ser	Ser	Glu	Pro
65					70					75					80
Gln	Pro	Thr	Asn	Leu	Thr	Leu	His	Tyr	Trp	Tyr	Lys	Asn	Ser	Asp	Asn
			85					90					95		
Asp	Lys	Val	Gln	Lys	Cys	Ser	His	Tyr	Leu	Phe	Ser	Glu	Glu	Ile	Thr
		100					105					110			
Ser	Gly	Cys	Gln	Leu	Gln	Lys	Lys	Glu	Ile	His	Leu	Tyr	Gln	Thr	Phe
	115					120					125				
Val	Val	Gln	Leu	Gln	Asp	Pro	Arg	Glu	Pro	Arg	Arg	Gln	Ala	Thr	Gln
130					135					140					
Met	Leu	Lys	Leu	Gln	Asn	Leu	Val	Ile	Pro	Trp	Ala	Pro	Glu	Asn	Leu
145				150					155						160
Thr	Leu	His	Lys	Leu	Ser	Glu	Ser	Gln	Leu	Glu	Leu	Asn	Trp	Asn	Asn
			165					170					175		
Arg	Phe	Leu	Asn	His	Cys	Leu	Glu	His	Leu	Val	Gln	Tyr	Arg	Thr	Asp
		180					185					190			
Trp	Asp	His	Ser	Trp	Thr	Glu	Gln	Ser	Val	Asp	Tyr	Arg	His	Lys	Phe
	195					200					205				
Ser	Leu	Pro	Ser	Val	Asp	Gly	Gln	Lys	Arg	Tyr	Thr	Phe	Arg	Val	Arg
210					215					220					
Ser	Arg	Phe	Asn	Pro	Leu	Cys	Gly	Ser	Ala	Gln	His	Trp	Ser	Glu	Trp
225				230					235						240
Ser	His	Pro	Ile	His	Trp	Gly	Ser	Asn	Thr	Ser	Lys	Glu	Asn	Pro	Phe
			245					250					255		
Leu	Phe	Ala	Leu	Glu	Ala	Val	Val	Ile	Ser	Val	Gly	Ser	Met	Gly	Leu
		260					265					270			
Ile	Ile	Ser	Leu	Leu	Cys	Val	Tyr	Phe	Trp	Leu	Glu	Arg	Thr	Met	Pro
	275					280					285				
Arg	Ile	Pro	Thr	Leu	Lys	Asn	Leu	Glu	Asp	Leu	Val	Thr	Glu	Tyr	His
290					295					300					
Gly	Asn	Phe	Ser	Ala	Trp	Ser	Gly	Val	Ser	Lys	Gly	Leu	Ala	Glu	Ser
305				310					315						320
Leu	Gln	Pro	Asp	Tyr	Ser	Glu	Arg	Leu	Cys	Leu	Val	Ser	Glu	Ile	Pro
			325					330					335		
Pro	Lys	Gly	Gly	Ala	Leu	Gly	Glu	Gly	Pro	Gly	Ala	Ser	Pro	Cys	Asn
		340					345					350			
Gln	His	Ser	Pro	Tyr	Trp	Ala	Pro	Pro	Cys	Tyr	Thr	Leu	Lys	Pro	Glu
	355					360					365				
Thr															

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<211> 368
<212> PRT
<213> Rattus norvegicus

<400> 21
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Leu Leu Leu Arg Val Gly Trp Ser Ser Lys Val Leu Met Ser Ser Gly
            20   25   30
Asn Glu Asp Thr Lys Ser Asp Leu Leu Leu Thr Ser Met Asp Leu Lys
        35   40   45
His Leu Ser Val Pro Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val
    50   55   60
Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro
65   70   75   80
Gln Pro Thr Asn Leu Thr Met His Tyr Arg Tyr Lys Gly Ser Asp Asn
                85   90   95
Asn Thr Phe Gln Glu Cys Ser His Tyr Leu Phe Ser Lys Glu Ile Thr
            100   105   110
Ser Gly Cys Gln Ile Gln Lys Glu Asp Ile Gln Leu Tyr Gln Thr Phe
        115   120   125
Val Val Gln Leu Gln Asp Pro Gln Lys Pro Gln Arg Arg Ala Glu Gln
    130   135   140
Lys Leu Asn Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu
145   150   155   160
Thr Leu Tyr Asn Leu Ser Glu Ser Gln Val Glu Leu Arg Trp Lys Ser
                165   170   175
Arg Tyr Ile Glu Arg Cys Leu Gln Tyr Leu Val Gln Tyr Arg Ser Asn
            180   185   190
Arg Asp Arg Ser Trp Thr Glu Gln Ile Val Asp His Glu Pro Arg Phe
        195   200   205
Ser Leu Pro Ser Val Asp Glu Gln Lys Leu Tyr Thr Phe Arg Val Arg
    210   215   220
Ser Arg Phe Asn Pro Ile Cys Gly Ser Thr Gln Gln Trp Ser Lys Trp
225   230   235   240
Ser Gln Pro Ile His Trp Gly Ser His Thr Ala Glu Glu Asn Pro Ser
                245   250   255
Leu Phe Ala Leu Glu Ala Val Leu Ile Pro Val Gly Thr Met Gly Leu
            260   265   270
Ile Ile Thr Leu Ile Phe Val Tyr Cys Trp Leu Glu Arg Met Pro Arg
        275   280   285
Ile Pro Ala Ile Lys Asn Leu Glu Asp Leu Val Thr Glu Tyr His Gly
    290   295   300
Asn Phe Ser Ala Trp Ser Gly Val Ser Lys Gly Leu Thr Glu Ser Leu
305   310   315   320
Gln Pro Asp Tyr Ser Glu Arg Phe Cys His Val Ser Glu Ile Pro Pro
                325   330   335
Lys Gly Gly Ala Leu Gly Glu Gly Pro Gly Gly Ser Pro Cys Ser Leu
            340   345   350
His Ser Pro Tyr Trp Pro Pro Pro Cys Tyr Ser Leu Lys Pro Glu Ala
        355   360   365

<210> 22
<211> 368
<212> PRT
<213> Artificial Sequence

<220>
<223> chimeric IL-2 receptor gamma comprising the rat
      IL-2 receptor gamma protein having the ecto domain
      of IL-2 gamma receptor from human

<400> 22
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Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly
            20                  25                  30
Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp
        35                  40                  45
Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val
    50                  55                  60
Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro
65                  70                  75                  80
Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn
                85                  90                  95
Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr
            100                 105                 110
Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe
        115                 120                 125
Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln
    130                 135                 140
Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu
145                 150                 155                 160
Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn
                165                 170                 175
Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp
            180                 185                 190
Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe
        195                 200                 205
Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg
    210                 215                 220
Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp
225                 230                 235                 240
Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Pro Phe
                245                 250                 255
Leu Phe Ala Leu Glu Ala Val Leu Ile Pro Val Gly Thr Met Gly Leu
            260                 265                 270
Ile Ile Thr Leu Ile Phe Val Tyr Cys Trp Leu Glu Arg Met Pro Arg
        275                 280                 285
Ile Pro Ala Ile Lys Asn Leu Glu Asp Leu Val Thr Glu Tyr His Gly
    290                 295                 300
Asn Phe Ser Ala Trp Ser Gly Val Ser Lys Gly Leu Thr Glu Ser Leu
305                 310                 315                 320
Gln Pro Asp Tyr Ser Glu Arg Phe Cys His Val Ser Glu Ile Pro Pro
                325                 330                 335
Lys Gly Gly Ala Leu Gly Glu Gly Pro Gly Gly Ser Pro Cys Ser Leu
            340                 345                 350
His Ser Pro Tyr Trp Pro Pro Pro Cys Tyr Ser Leu Lys Pro Glu Ala
        355                 360                 365
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<210> 23
<211> 23
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<213> Artificial Sequence

<220>
<223> a genomic target sequence that is linked to a guide RNA (gRNA)

<220>
<221> misc_feature
<222> (2)...(21)
<223> n= A, T, C or G

<220>
<221> misc_feature
<222> (0)...(0)
<223> n can be from 1-20 nucleotides

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<210> 24
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<220>
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<400> 25
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<210> 26
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