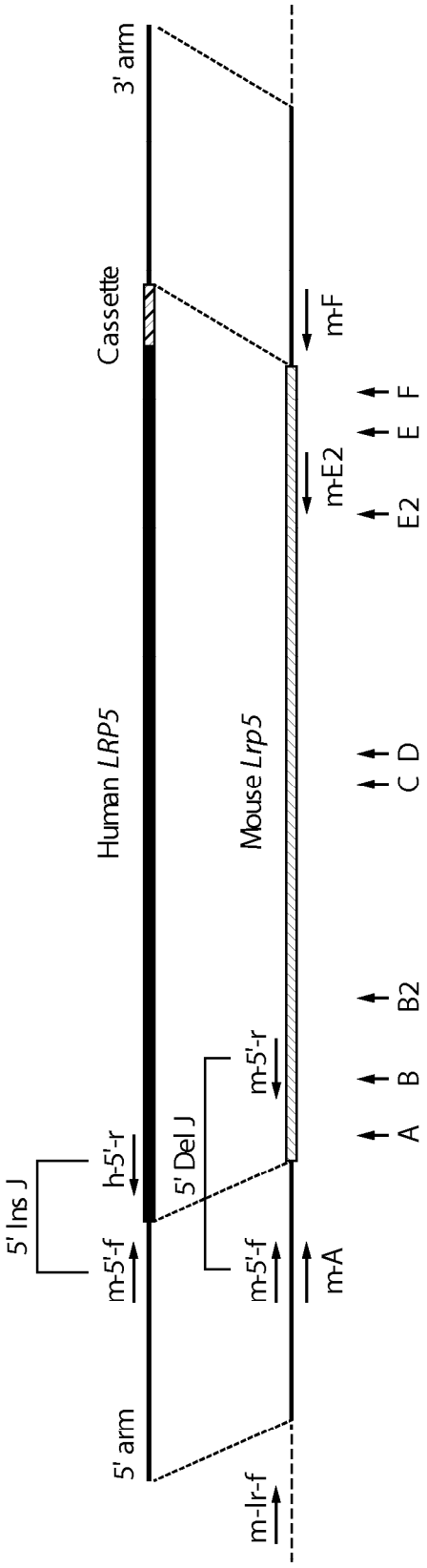

3' arm
Cassette
m-F
m-E2
E2
E F
Human LRP5
Mouse Lrp5
C D
A B B2
m-5'-r
5' Del J
h-5'-r
5' Ins J
m-5'-f
m-5'-f
m-A
5' arm
m-lr-f

FIG. 1



FIG. 2A

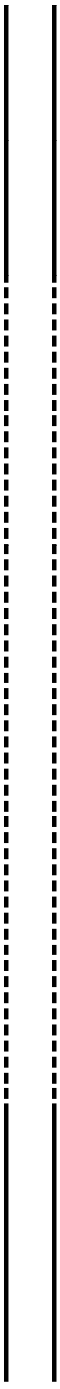


FIG. 2B

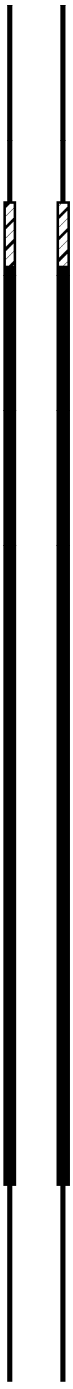


FIG. 2C



FIG. 2D

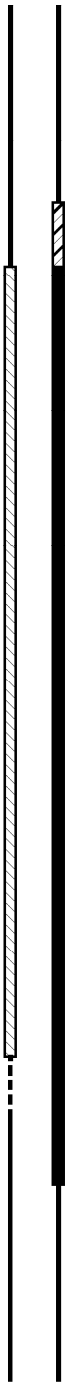


FIG. 2E

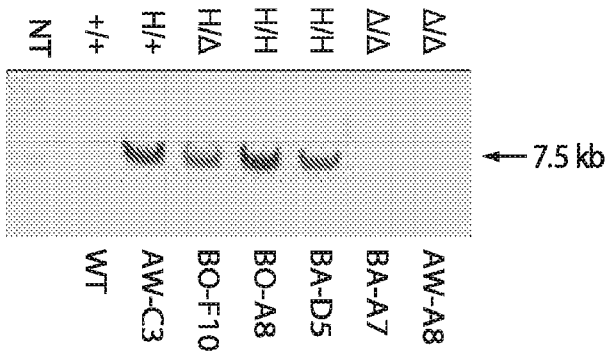

NT
+/+
H/+
H/Δ
H/H
H/H
Δ/Δ
Δ/Δ
7.5 kb
WT
AW-C3
BO-F10
BO-A8
BA-D5
BA-A7
AW-A8

FIG. 3A

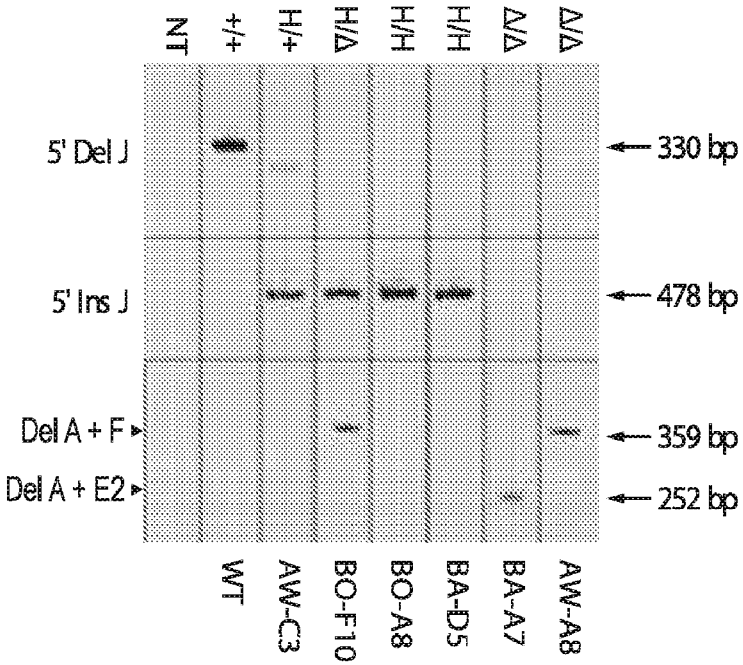

NT
+/+
H/+
H/Δ
H/H
H/H
Δ/Δ
Δ/Δ
5' Del J
5' Ins J
Del A + F
Del A + E2
330 bp
478 bp
359 bp
252 bp
WT
AW-C3
BO-F10
BO-A8
BA-D5
BA-A7
AW-A8

FIG. 3B

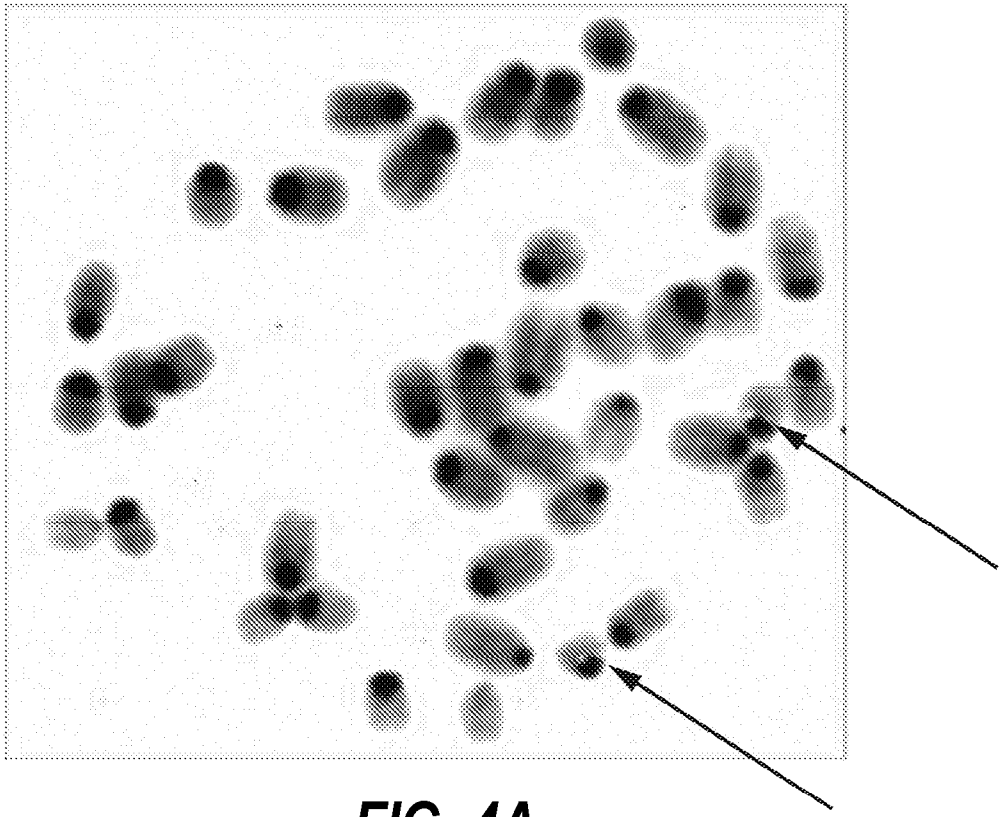


FIG. 4A

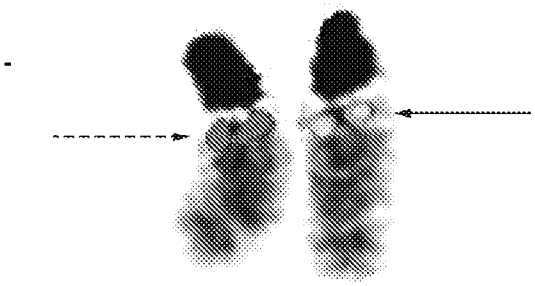


FIG. 4B



FIG. 4C

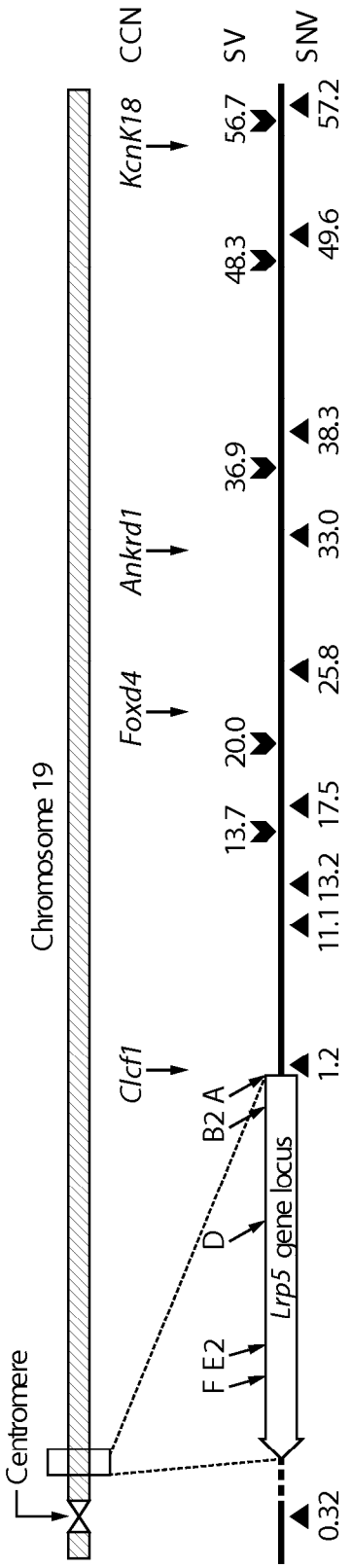

CCN
SV
SNV
KcnK18
Ankrd1
Foxd4
Clcf1
Chromosome 19
Centromere
F E2
D
B2 A
Lrp5 gene locus
0.32
1.2
11.1
13.2
13.7
17.5
20.0
25.8
33.0
36.9
38.3
48.3
49.6
56.7
57.2

FIG. 5

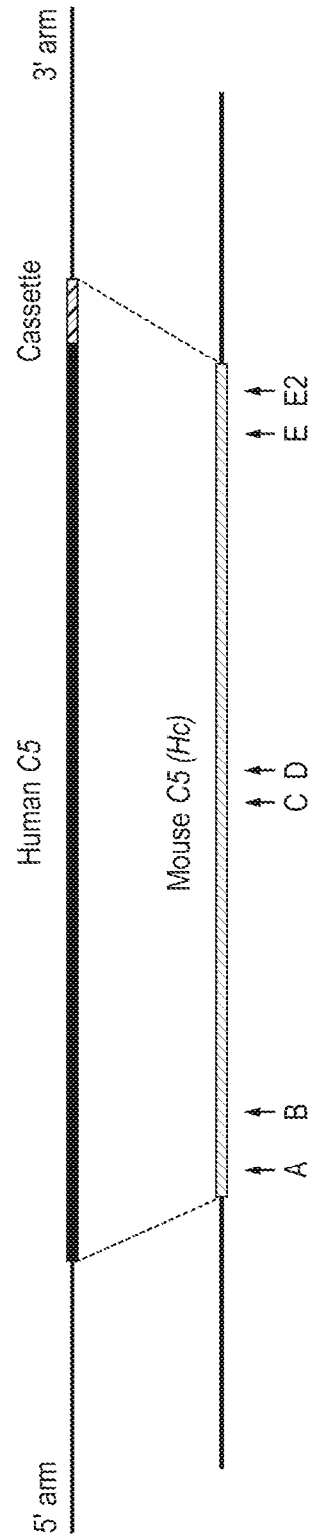


FIG. 6

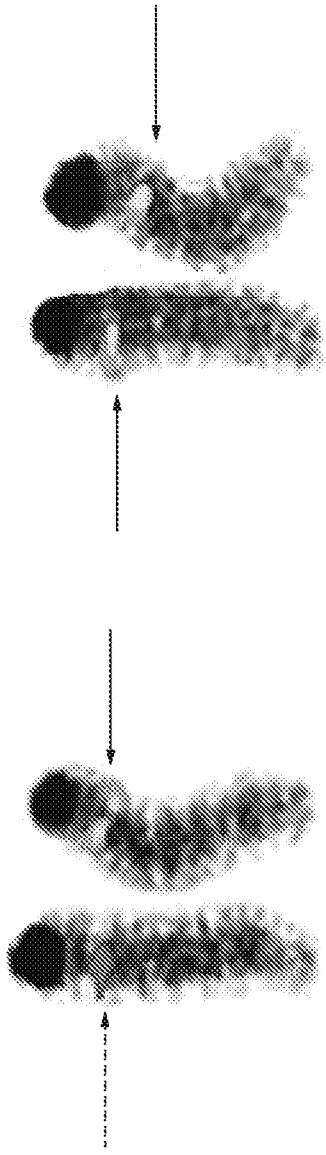


FIG. 7B

FIG. 7A

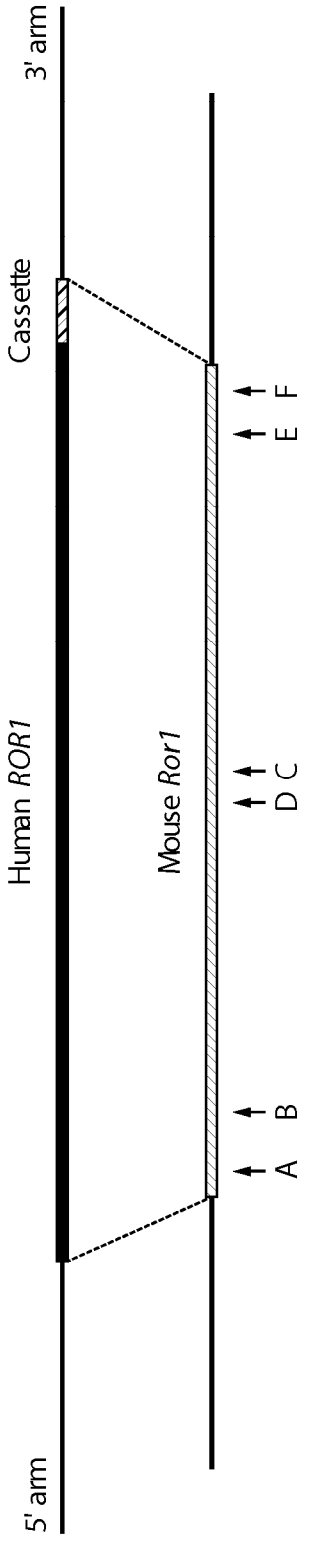

5' arm
Human ROR1
Cassette
3' arm
Mouse Ror1
A B
D C
E F

FIG. 8

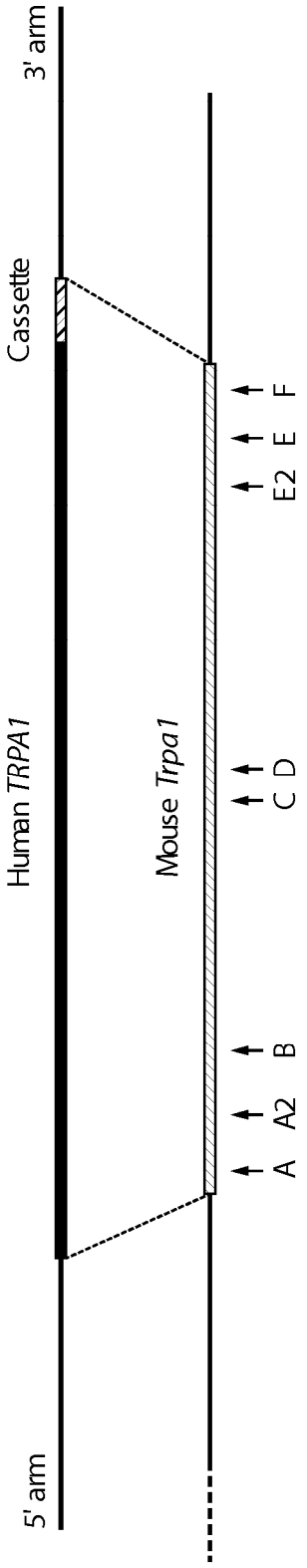

5' arm
Human TRPA1
Cassette
3' arm
Mouse Trpa1
A A2 B
C D
E2 E F

FIG. 9

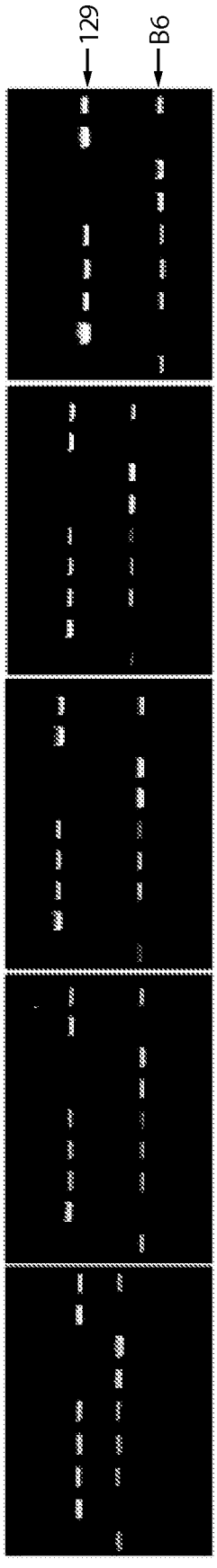


FIG. 10E

FIG. 10D

FIG. 10C

FIG. 10B

FIG. 10A

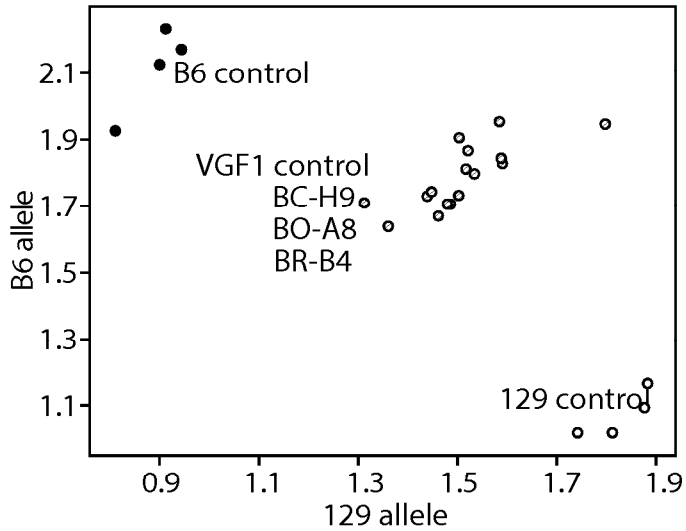


FIG. 11A

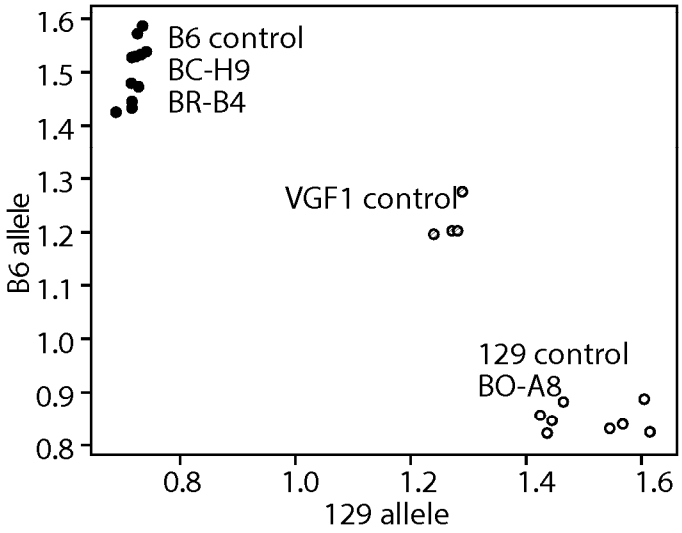


FIG. 11B

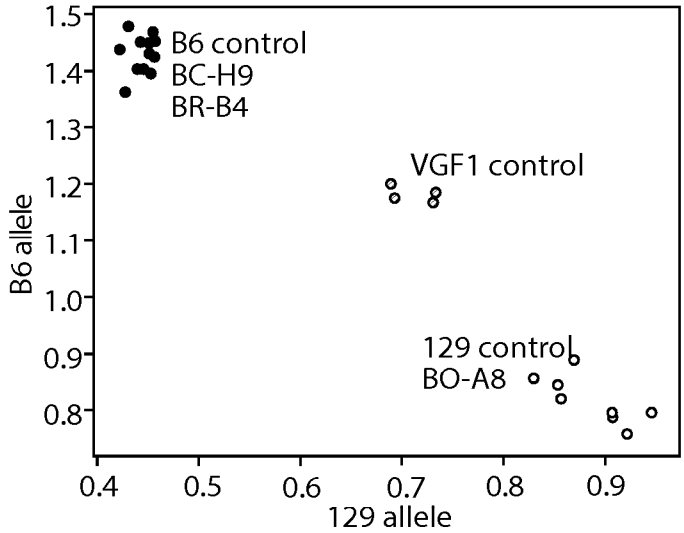


FIG. 11C

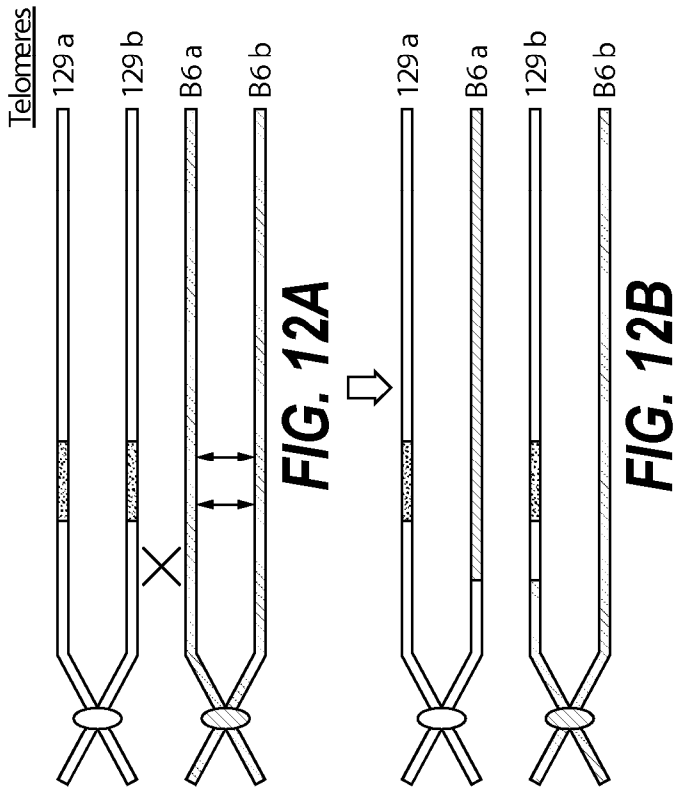


FIG. 12A

FIG. 12B

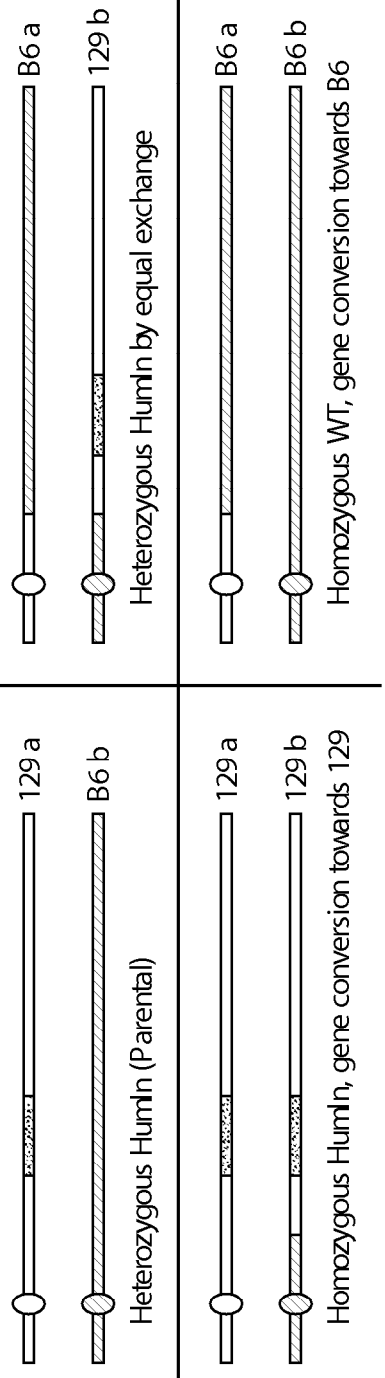


FIG. 12C

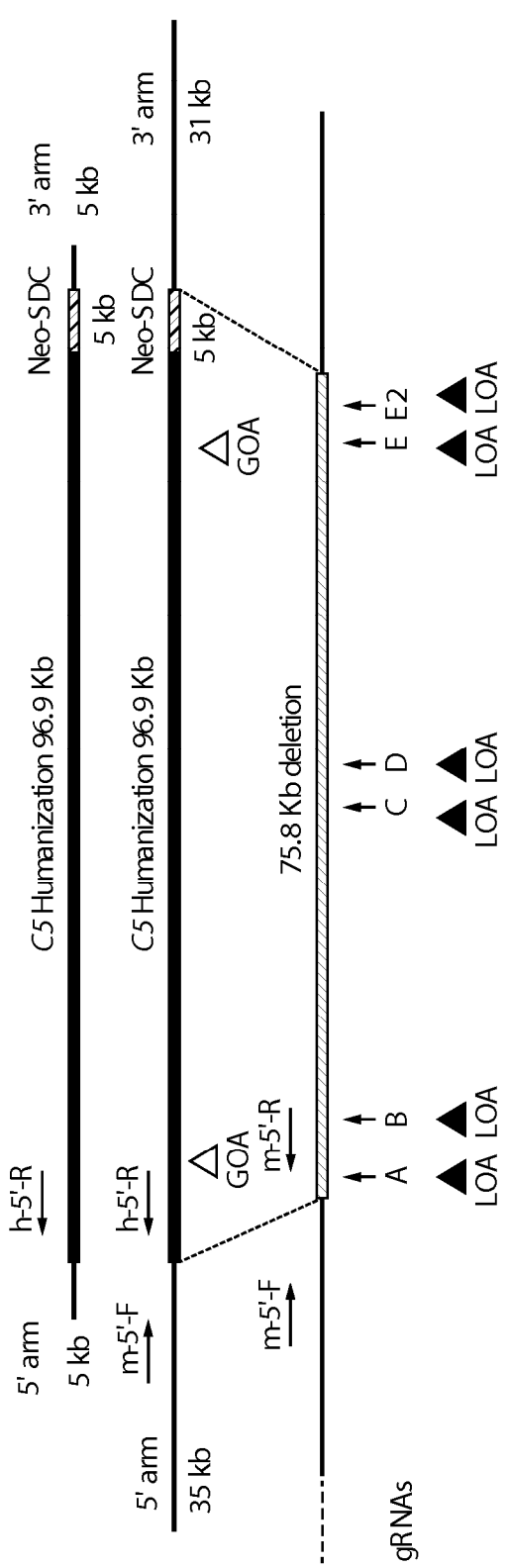

sTVEC
5' arm
5 kb
h-5'-R
m-5'-F
Neo-SDC
3' arm
5 kb
LTVEC
5' arm
35 kb
h-5'-R
C5 Humanization 96.9 Kb
Neo-SDC
5 kb
3' arm
31 kb
GOA
m-5'-R
m-5'-F
75.8 Kb deletion
gRNAs
A B
C D
E E2
LOA LOA
LOA LOA
LOA LOA

FIG. 13

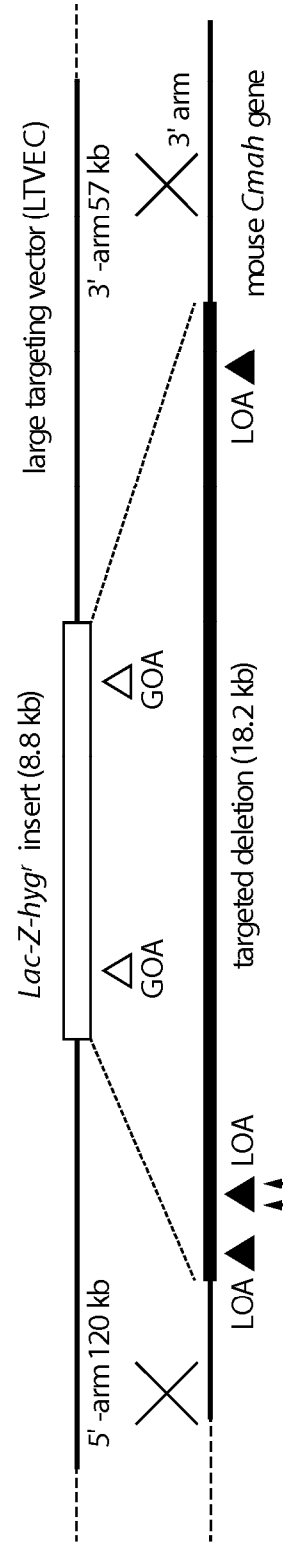

large targeting vector (LTVEC)
5' -arm 120 kb
Lac-Z-hygr insert (8.8 kb)
GOA
GOA
3' -arm 57 kb
3' arm
LOA
LOA
targeted deletion (18.2 kb)
mouse Cmah gene
gRNA sites
A B

FIG. 14

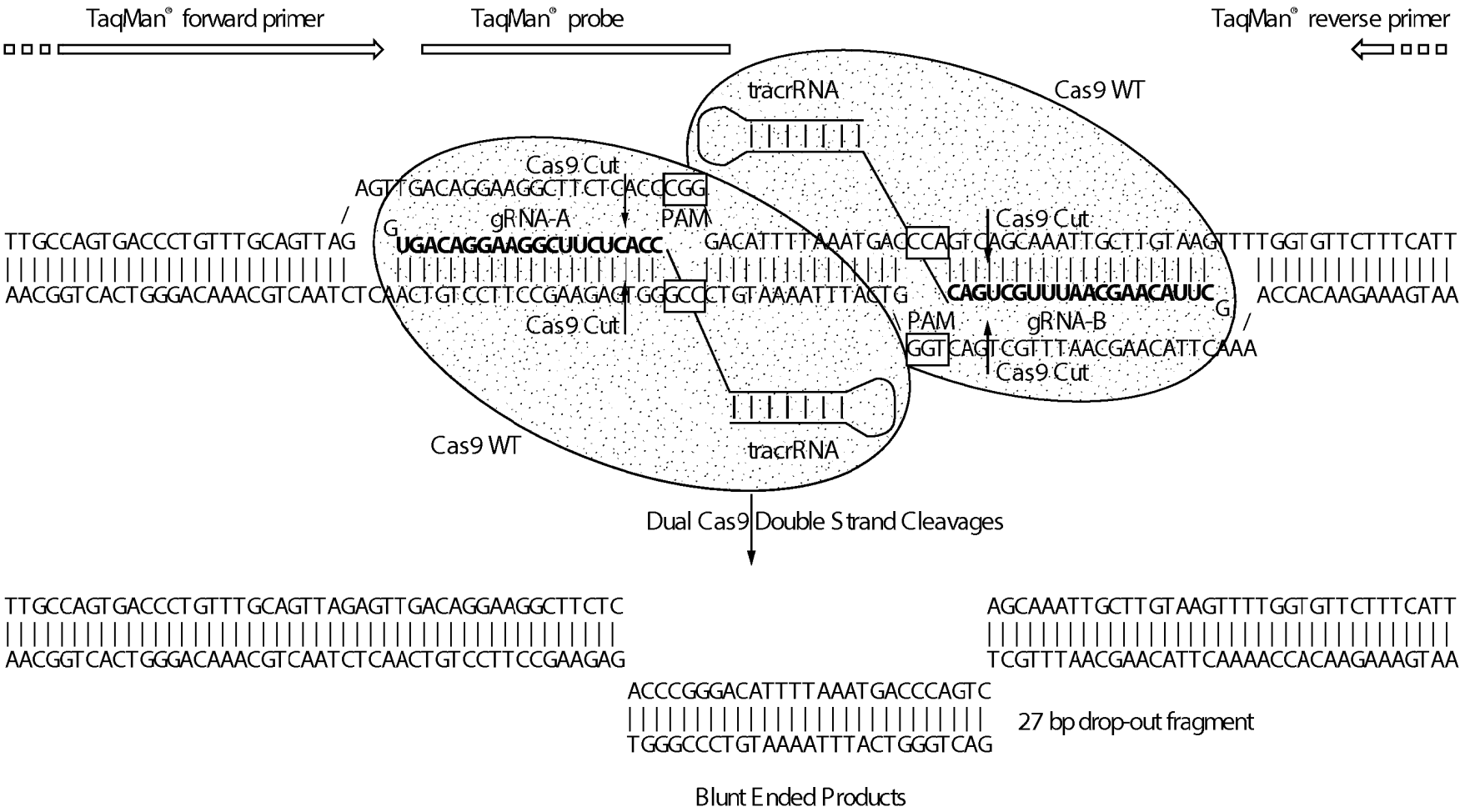

TaqMan® forward primer
TaqMan® probe
TaqMan® reverse primer
tracrRNA
Cas9 WT
Cas9 Cut
AGTTGACAGGAAGGCTTCTCACCCGG
gRNA-A
PAM
TTGCCAGTGACCCTGTTTGCAGTTAG
UGACAGGAAGGCUUCUCACC
GACATTTTAAATGACCCAGTCAGCAAATTGCTTGTAAGTTTTGGTGTTCTTTCATT
AACGGTCACTGGGACAAACGTCAATCTCAACTGTCCTTCCGAAGAGTGGGCCCTGTAAAATTTACTG
CAGUCGUUUAACGAACAUUC
ACCACAAGAAAGTAA
Cas9 Cut
PAM
gRNA-B
GGTCAGTCGTTTAACGAACATTCAAA
Cas9 Cut
Cas9 WT
tracrRNA
Dual Cas9 Double Strand Cleavages
TTGCCAGTGACCCTGTTTGCAGTTAGAGTTGACAGGAAGGCTTCTC
AACGGTCACTGGGACAAACGTCAATCTCAACTGTCCTTCCGAAGAG
AGCAAATTGCTTGTAAGTTTTGGTGTTCTTTCATT
TCGTTTAACGAACATTCAAAACCACAAGAAAGTAA
ACCCGGGACATTTTAAATGACCCAGTC
TGGGCCCTGTAAAATTTACTGGGTCAG
27 bp drop-out fragment
Blunt Ended Products

FIG. 15

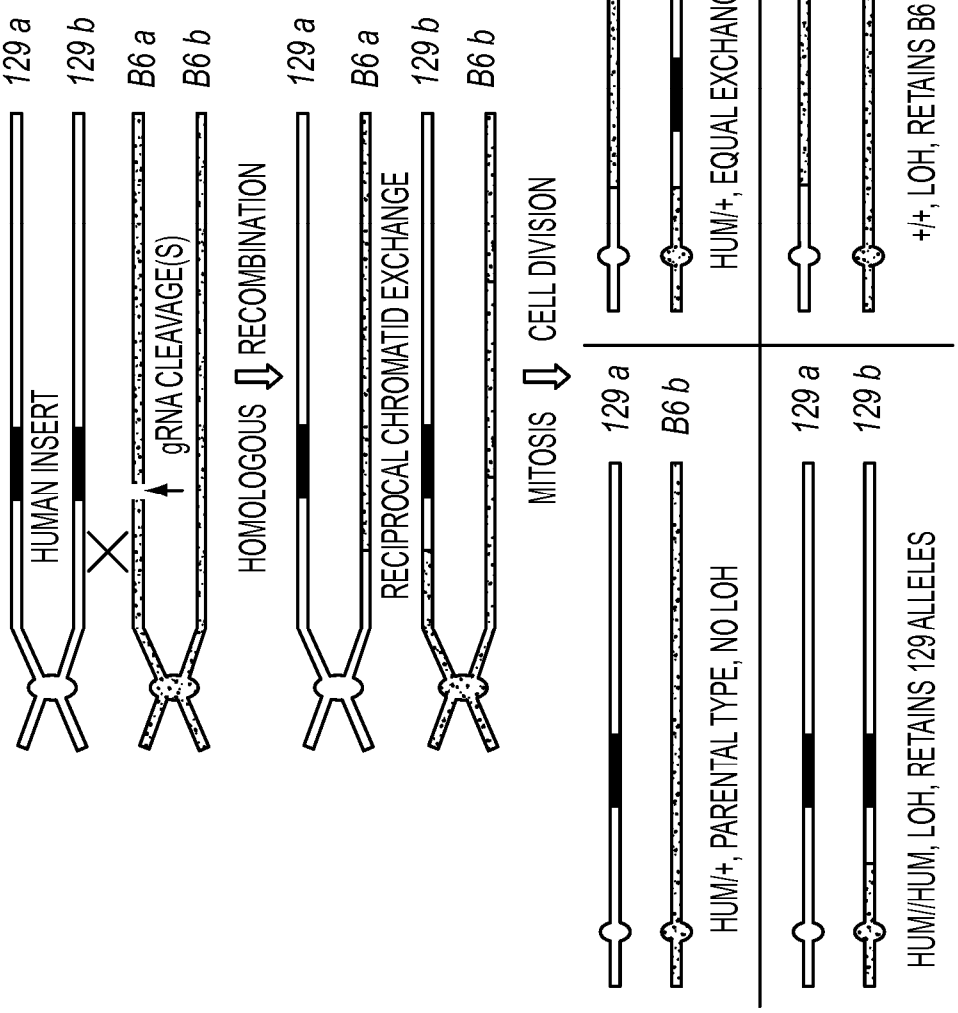

129 a
129 b
B6 a
B6 b
HUMAN INSERT
gRNA CLEAVAGE(S)
HOMOLOGOUS ⇩ RECOMBINATION
129 a
B6 a
129 b
B6 b
RECIPROCAL CHROMATID EXCHANGE
MITOSIS ⇩ CELL DIVISION
129 a
B6 b
HUM/+, PARENTAL TYPE, NO LOH
B6 a
129 b
HUM/+, EQUAL EXCHANGE, NO LOH
129 a
129 b
HUM//HUM, LOH, RETAINS 129 ALLELES
B6 a
B6 b
+/+, LOH, RETAINS B6 ALLELES

FIG. 16A

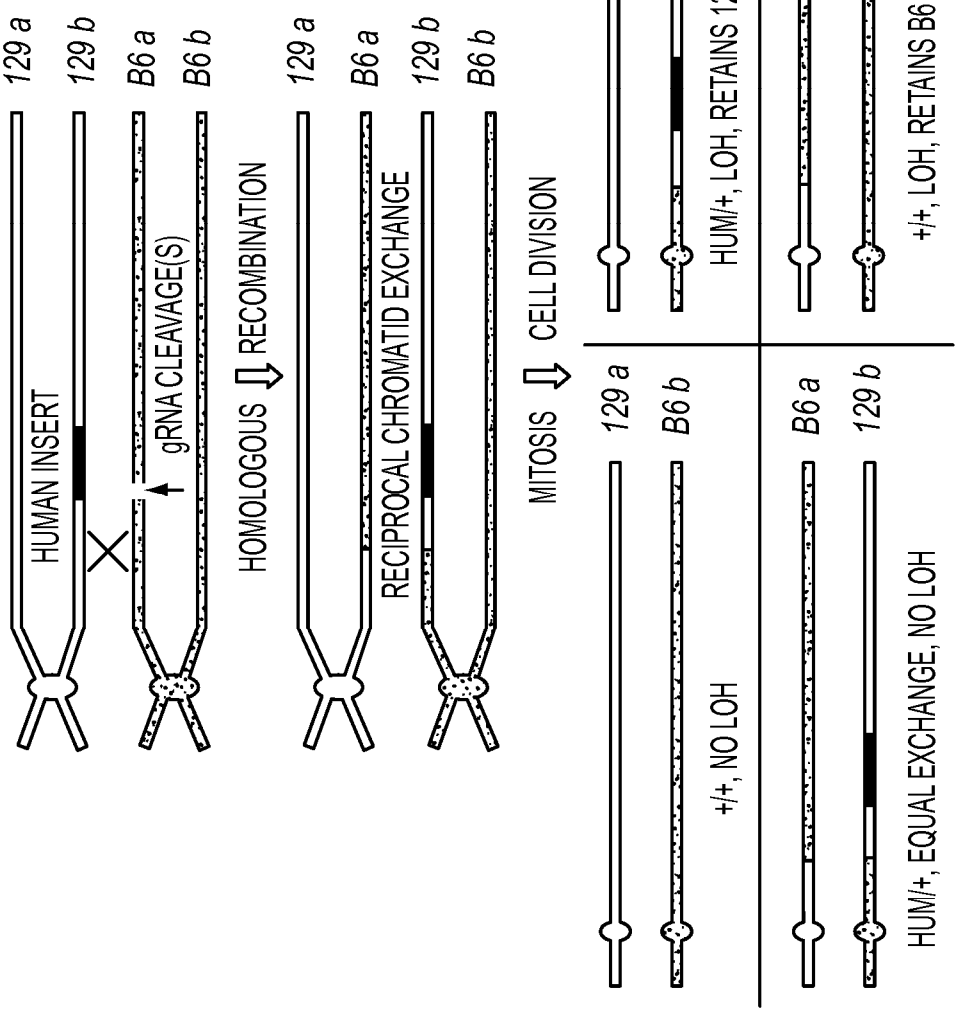

129 a
129 b
B6 a
B6 b
HUMAN INSERT
gRNA CLEAVAGE(S)
HOMOLOGOUS ⇩ RECOMBINATION
RECIPROCAL CHROMATID EXCHANGE
MITOSIS ⇩ CELL DIVISION
+/+, NO LOH
HUM/+, LOH, RETAINS 129 ALLELES
HUM/+, EQUAL EXCHANGE, NO LOH
+/+, LOH, RETAINS B6 ALLELES

FIG. 16B

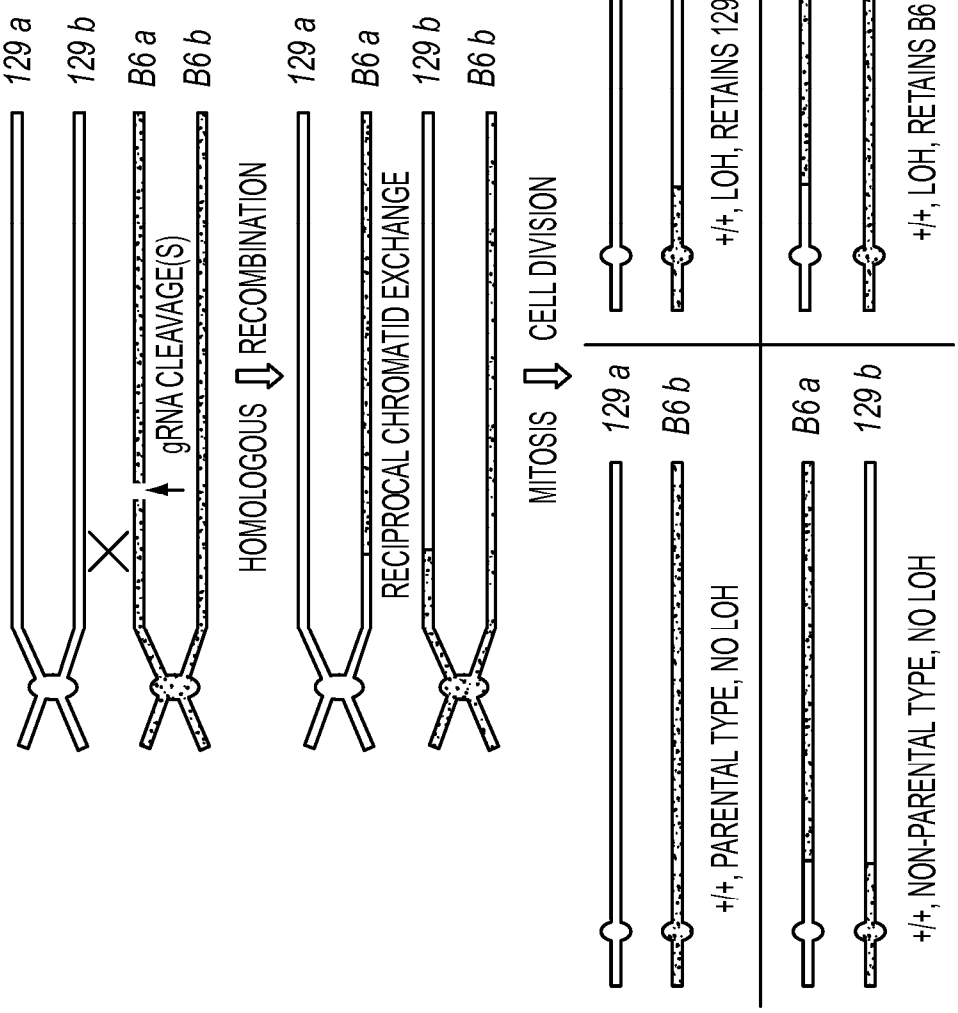

129 a
129 b
B6 a
B6 b
gRNA CLEAVAGE(S)
HOMOLOGOUS ⇩ RECOMBINATION
129 a
B6 a
RECIPROCAL CHROMATID EXCHANGE
129 b
B6 b
MITOSIS ⇩ CELL DIVISION
129 a
B6 b
+/+, PARENTAL TYPE, NO LOH
B6 a
129 b
+/+, NON-PARENTAL TYPE, NO LOH
129 a
129 b
+/+, LOH, RETAINS 129 ALLELES
B6 a
B6 b
+/+, LOH, RETAINS B6 ALLELES

FIG. 16C

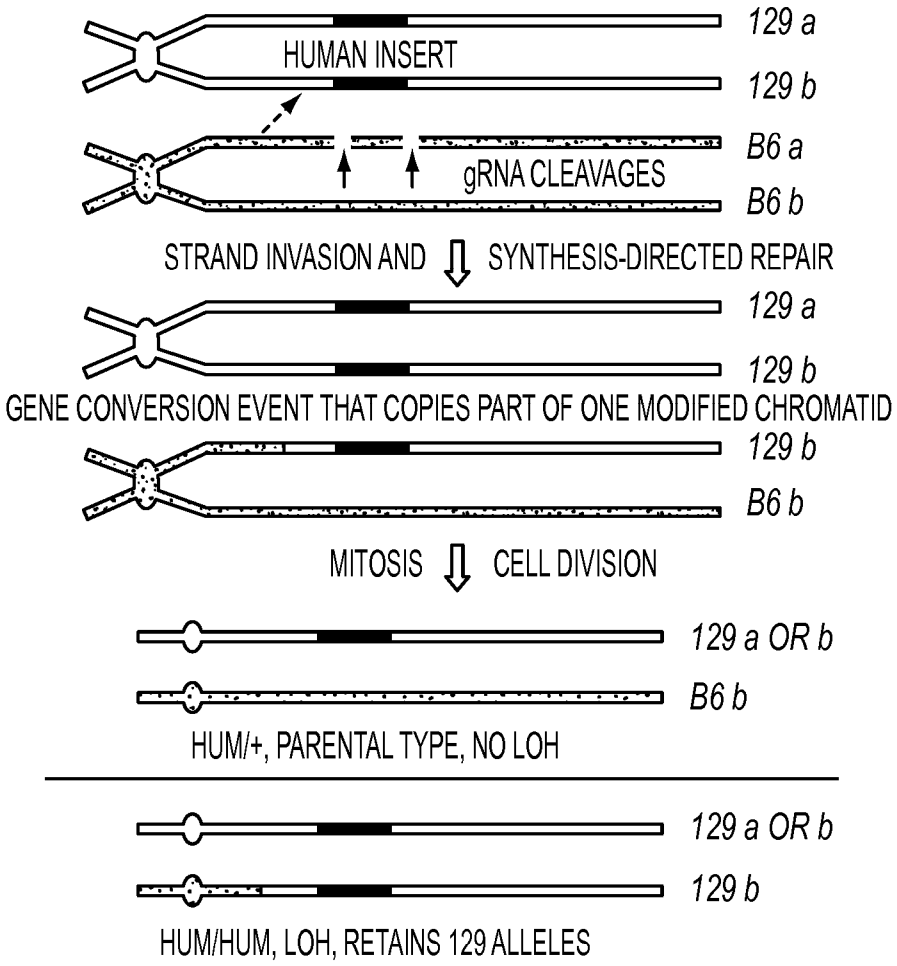

129 a
HUMAN INSERT
129 b
B6 a
gRNA CLEAVAGES
B6 b
STRAND INVASION AND SYNTHESIS-DIRECTED REPAIR
129 a
129 b
GENE CONVERSION EVENT THAT COPIES PART OF ONE MODIFIED CHROMATID
129 b
B6 b
MITOSIS CELL DIVISION
129 a OR b
B6 b
HUM/+, PARENTAL TYPE, NO LOH
129 a OR b
129 b
HUM/HUM, LOH, RETAINS 129 ALLELES

FIG. 16D

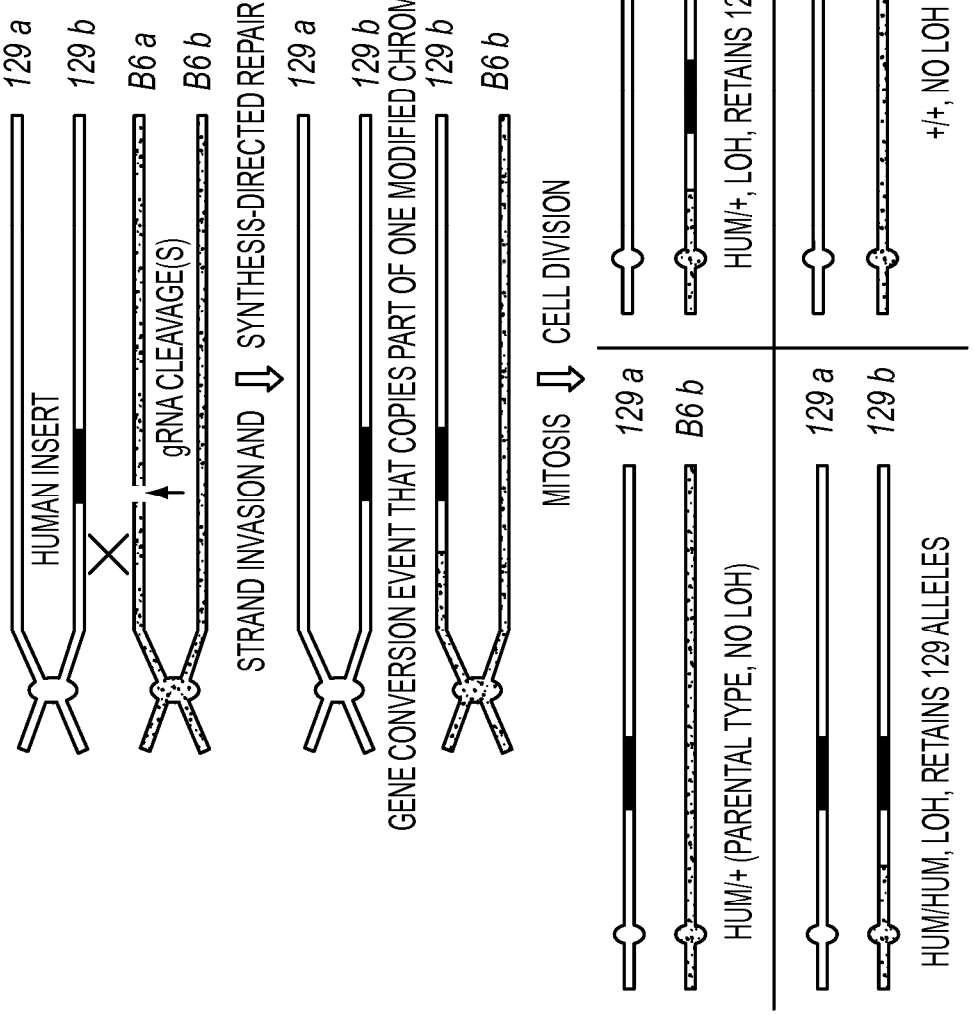

129 a
129 b
HUMAN INSERT
B6 a
B6 b
gRNA CLEAVAGE(S)
STRAND INVASION AND ⇩ SYNTHESIS-DIRECTED REPAIR
129 a
129 b
GENE CONVERSION EVENT THAT COPIES PART OF ONE MODIFIED CHROMATID
129 b
B6 b
MITOSIS ⇩ CELL DIVISION
129 a
B6 b
HUM/+ (PARENTAL TYPE, NO LOH)
129 a
129 b
HUM/HUM, LOH, RETAINS 129 ALLELES
129 a
129 b
HUM/+, LOH, RETAINS 129 ALLELES
129 a
B6 b
+/+, NO LOH

FIG. 16E

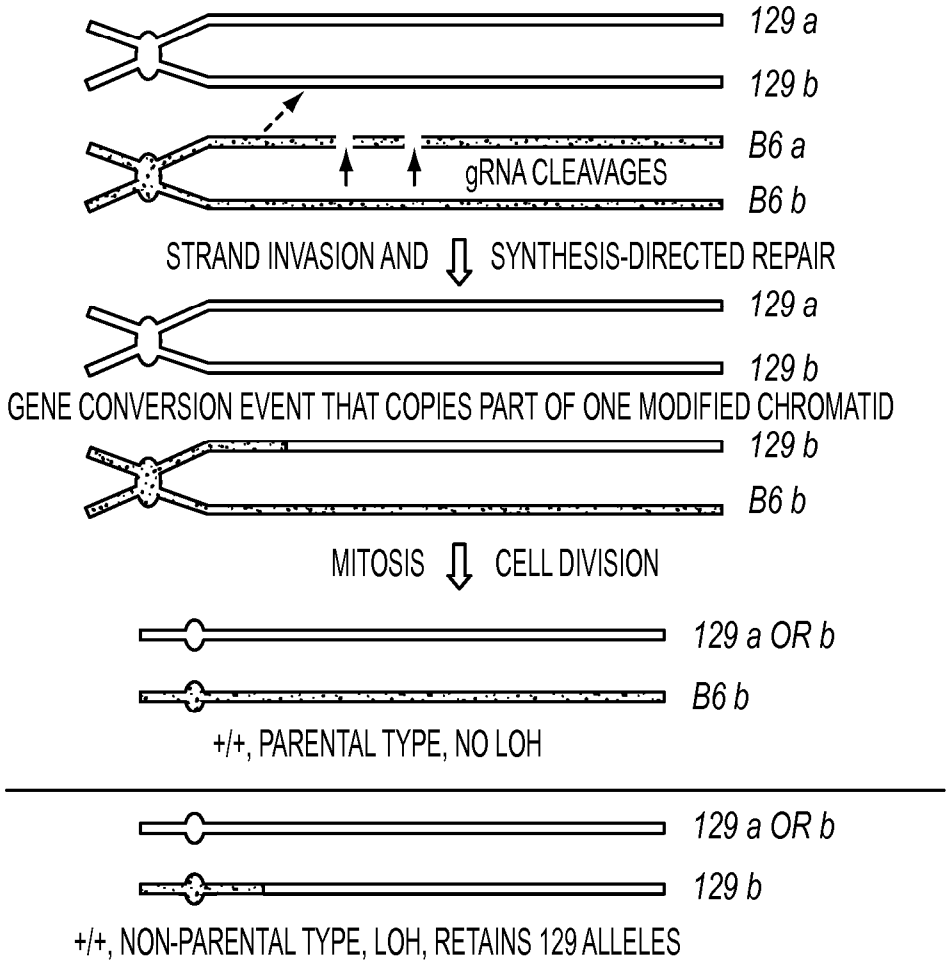

129 a
129 b
B6 a
gRNA CLEAVAGES
B6 b
STRAND INVASION AND SYNTHESIS-DIRECTED REPAIR
129 a
129 b
GENE CONVERSION EVENT THAT COPIES PART OF ONE MODIFIED CHROMATID
129 b
B6 b
MITOSIS CELL DIVISION
129 a OR b
B6 b
+/+, PARENTAL TYPE, NO LOH
129 a OR b
129 b
+/+, NON-PARENTAL TYPE, LOH, RETAINS 129 ALLELES

FIG. 16F

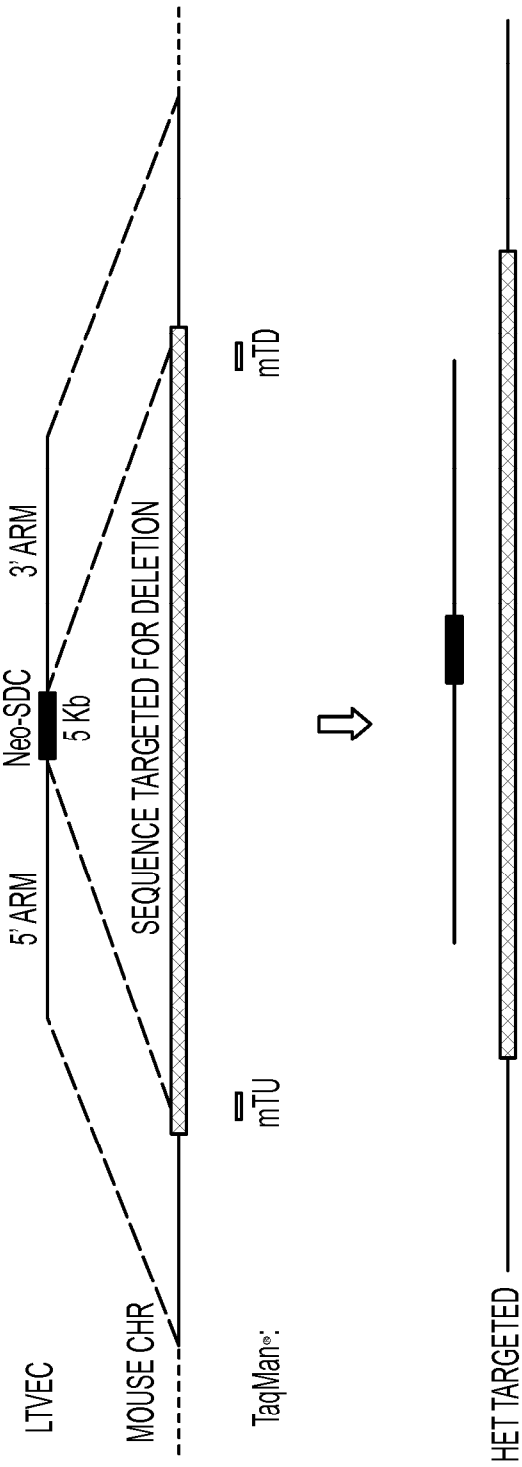

LTVEC
MOUSE CHR
5' ARM
Neo-SDC
5 Kb
3' ARM
SEQUENCE TARGETED FOR DELETION
TaqMan®:
mTU
mTD
HET TARGETED

FIG. 17A

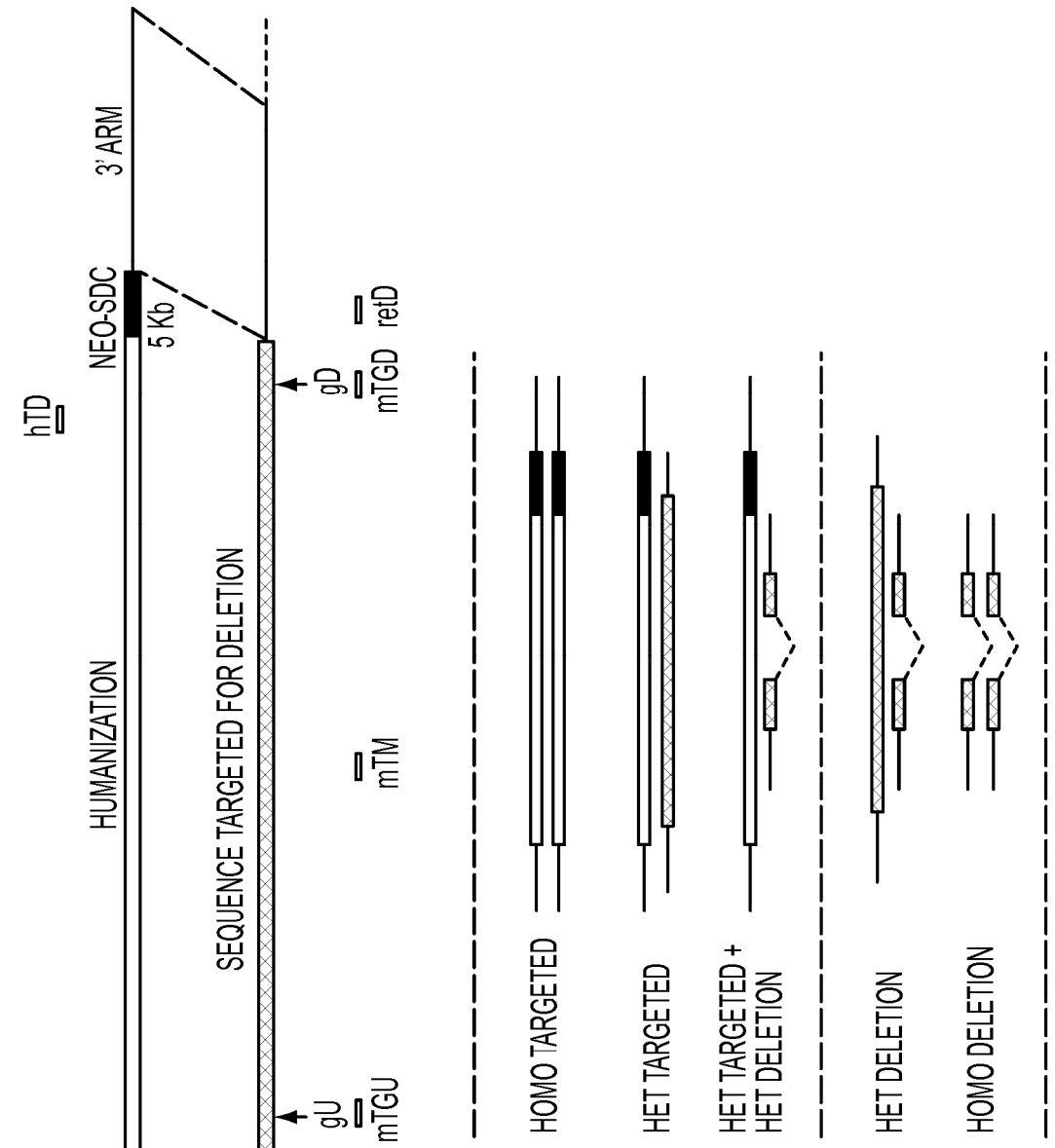

TaqMan®:
hTU
hTD
LTVEC
5' ARM
HUMANIZATION
NEO-SDC
3' ARM
5 Kb
MOUSE CHR
SEQUENCE TARGETED FOR DELETION
gRNA:
gU
gD
TaqMan®:
retU
mTGU
mTM
mTGD
retD
HOMO TARGETED
HET TARGETED
HET TARGETED + HET DELETION
HET DELETION
HOMO DELETION

FIG. 17B

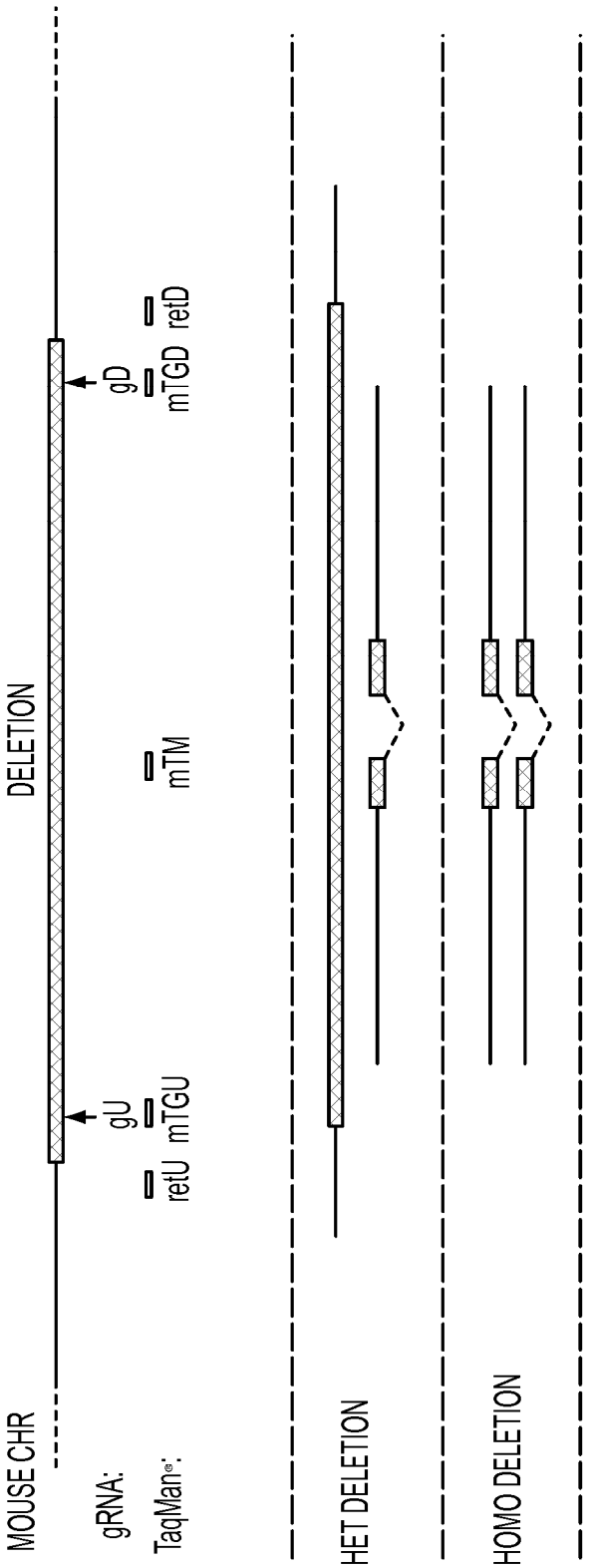

MOUSE CHR
DELETION
gRNA:
gU
gD
TaqMan®:
retU
mTGU
mTM
mTGD
retD
HET DELETION
HOMO DELETION

FIG. 17C

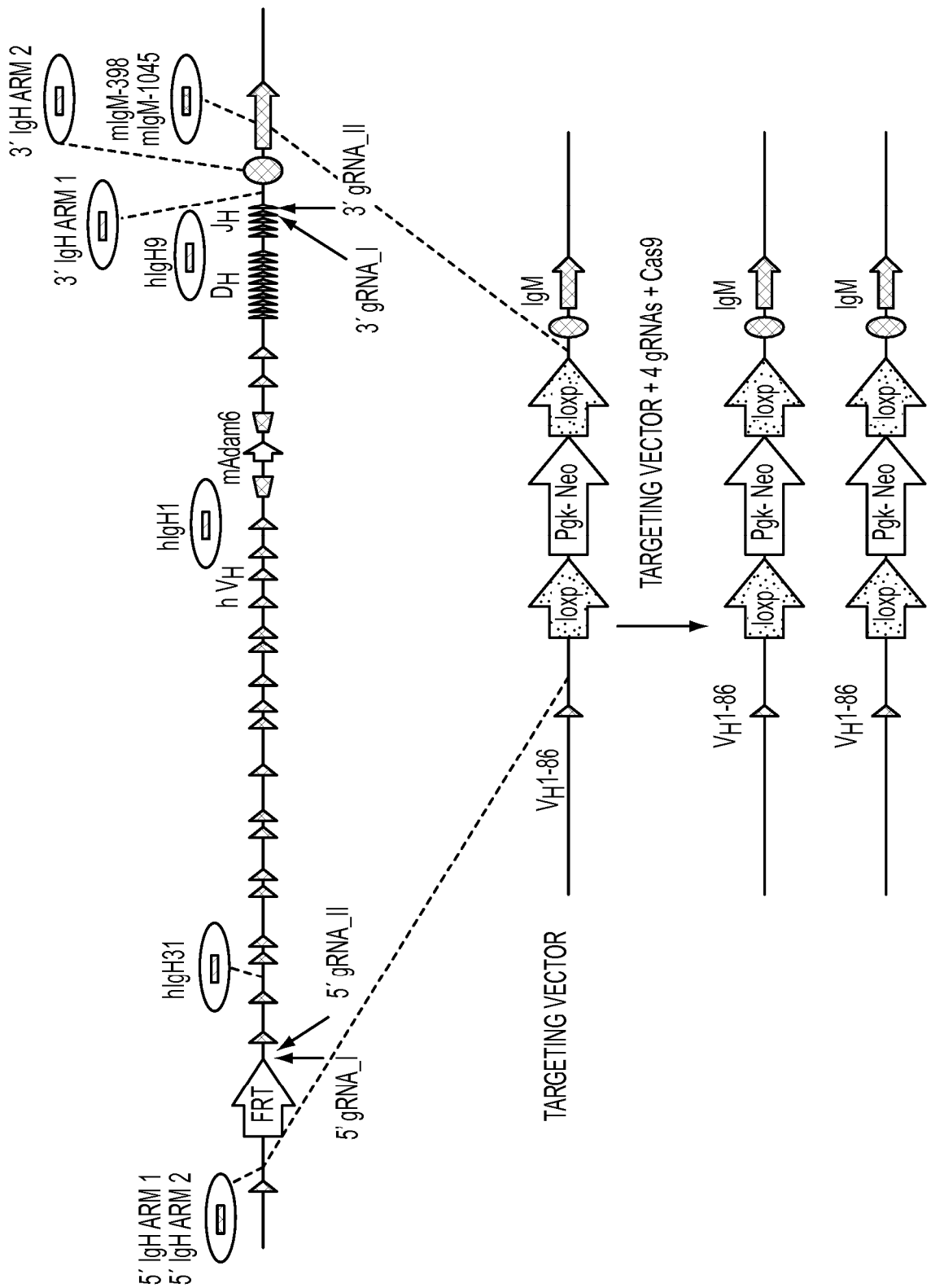

3´ IgH ARM 2
3´ IgH ARM 1
mIgM-398
mIgM-1045
hIgH9
DH
JH
3´ gRNA_I
3´ gRNA_II
hIgH1
h VH
mAdam6
hIgH31
5´ gRNA_II
5´ gRNA_I
FRT
5´ IgH ARM 1
5´ IgH ARM 2
TARGETING VECTOR
VH1-86
IgM
loxp
Pgk- Neo
loxp
TARGETING VECTOR + 4 gRNAs + Cas9
IgM
loxp
Pgk- Neo
loxp
VH1-86
IgM
loxp
Pgk- Neo
loxp
VH1-86

FIG. 18