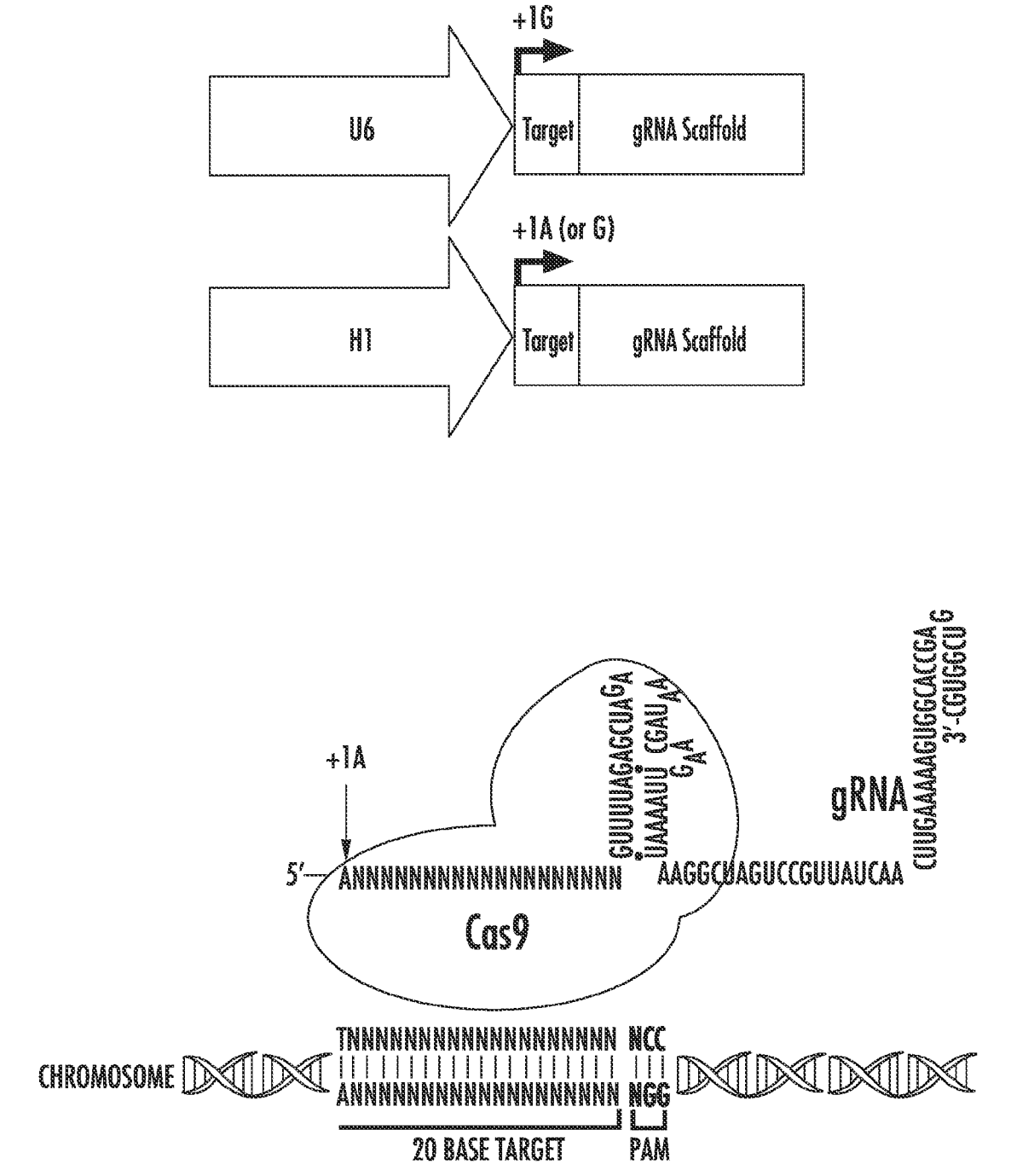

+1G
U6
Target
gRNA Scaffold
+1A (or G)
H1
Target
gRNA Scaffold
+1A
5'
ANNNNNNNNNNNNNNNNNNN
Cas9
gRNA
AAGGCUAGUCCGUUAUCAA
CHROMOSOME
TNNNNNNNNNNNNNNNNNNN
ANNNNNNNNNNNNNNNNNNN
NCC
NGG
20 BASE TARGET
PAM

FIG. 1A

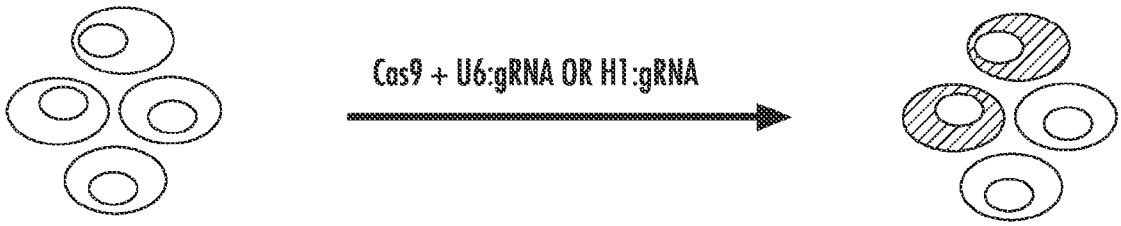

Cas9 + U6:gRNA OR H1:gRNA

FIG. 1B

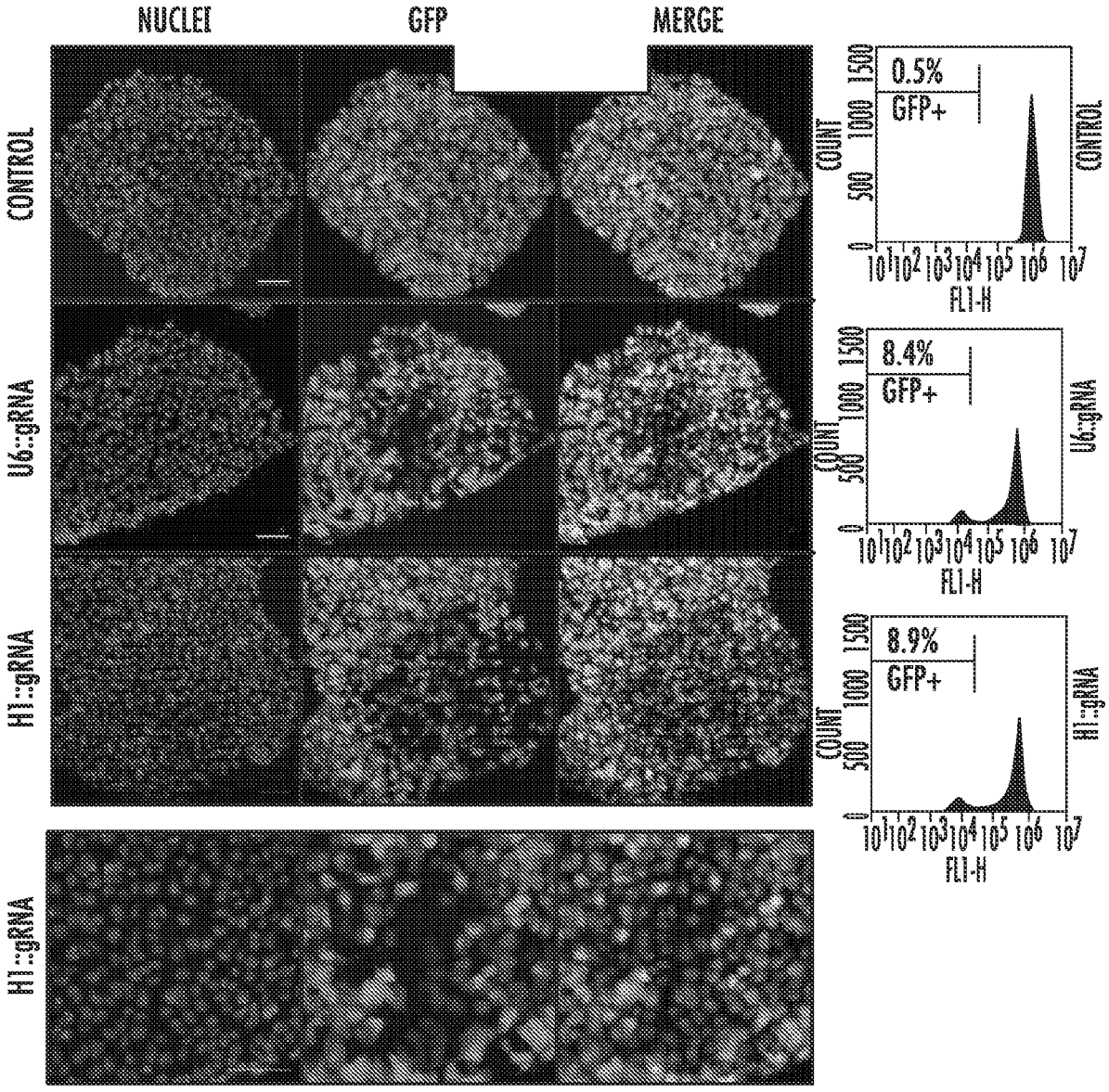

NUCLEI
GFP
MERGE
CONTROL
U6::gRNA
H1::gRNA
H1::gRNA
0.5%
GFP+
8.4%
GFP+
8.9%
GFP+
COUNT
FL1-H
CONTROL
U6::gRNA
H1::gRNA

FIG. 1C

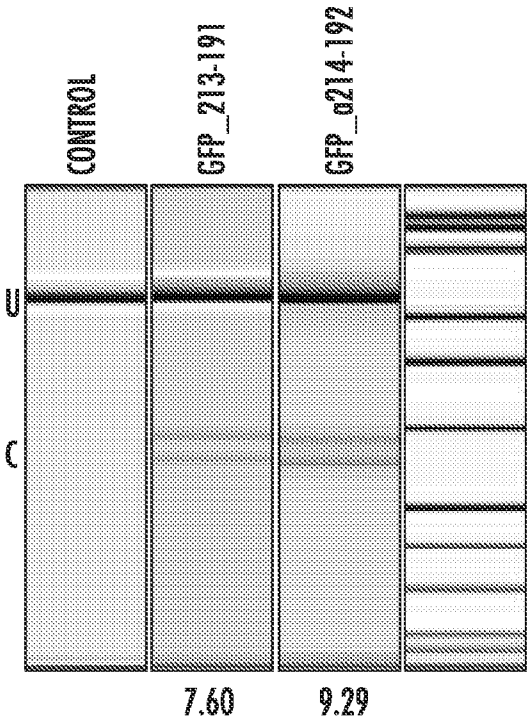


FIG. 1D

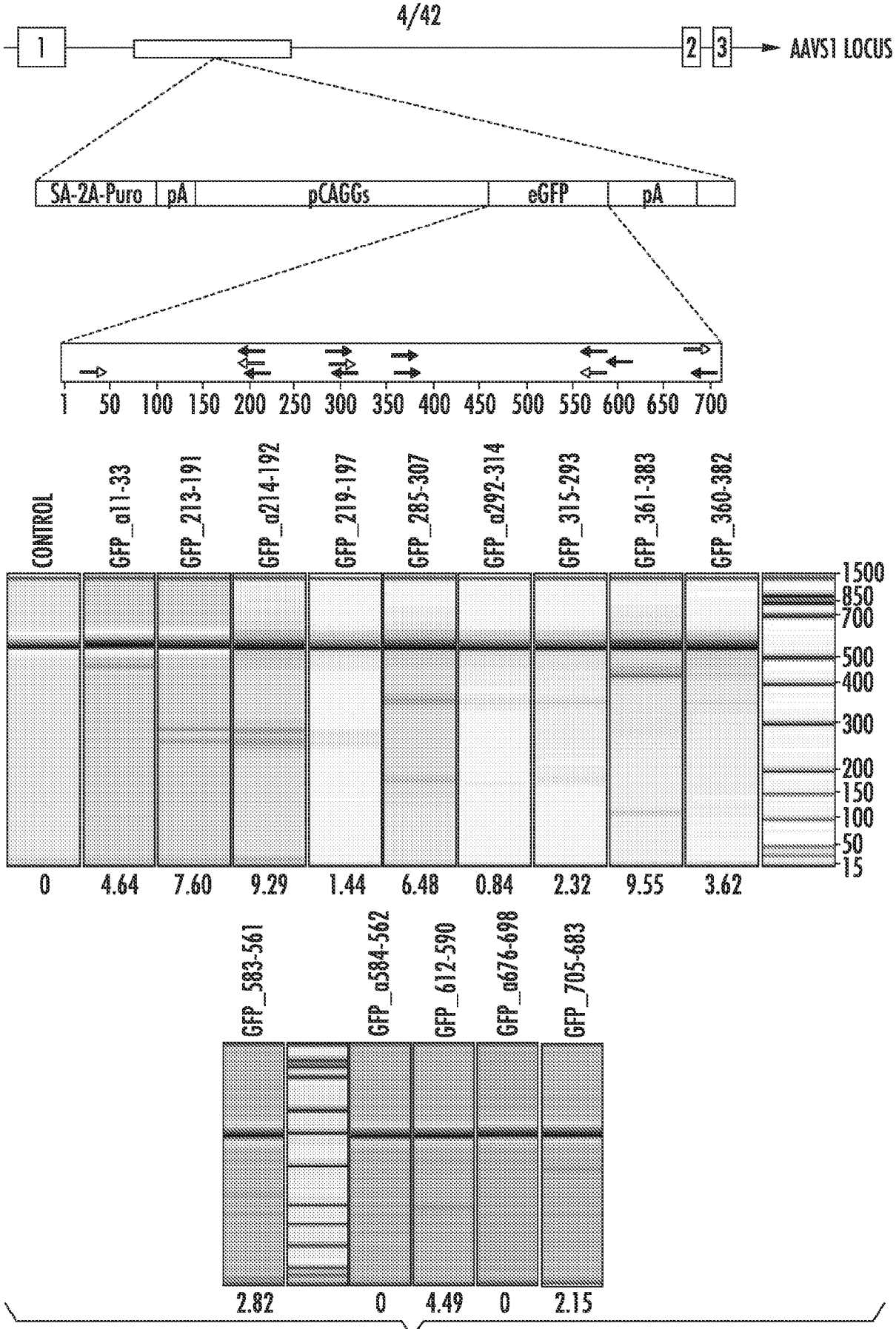

1
2
3
AAVS1 LOCUS
SA-2A-Puro
pA
pCAGGs
eGFP
pA
1 50 100 150 200 250 300 350 400 450 500 550 600 650 700
CONTROL
GFP_a11-33
GFP_213-191
GFP_a214-192
GFP_219-197
GFP_285-307
GFP_a292-314
GFP_315-293
GFP_361-383
GFP_360-382
1500
850
700
500
400
300
200
150
100
50
15
0
4.64
7.60
9.29
1.44
6.48
0.84
2.32
9.55
3.62
GFP_583-561
GFP_a584-562
GFP_612-590
GFP_a676-698
GFP_705-683
2.82
0
4.49
0
2.15

FIG. 2

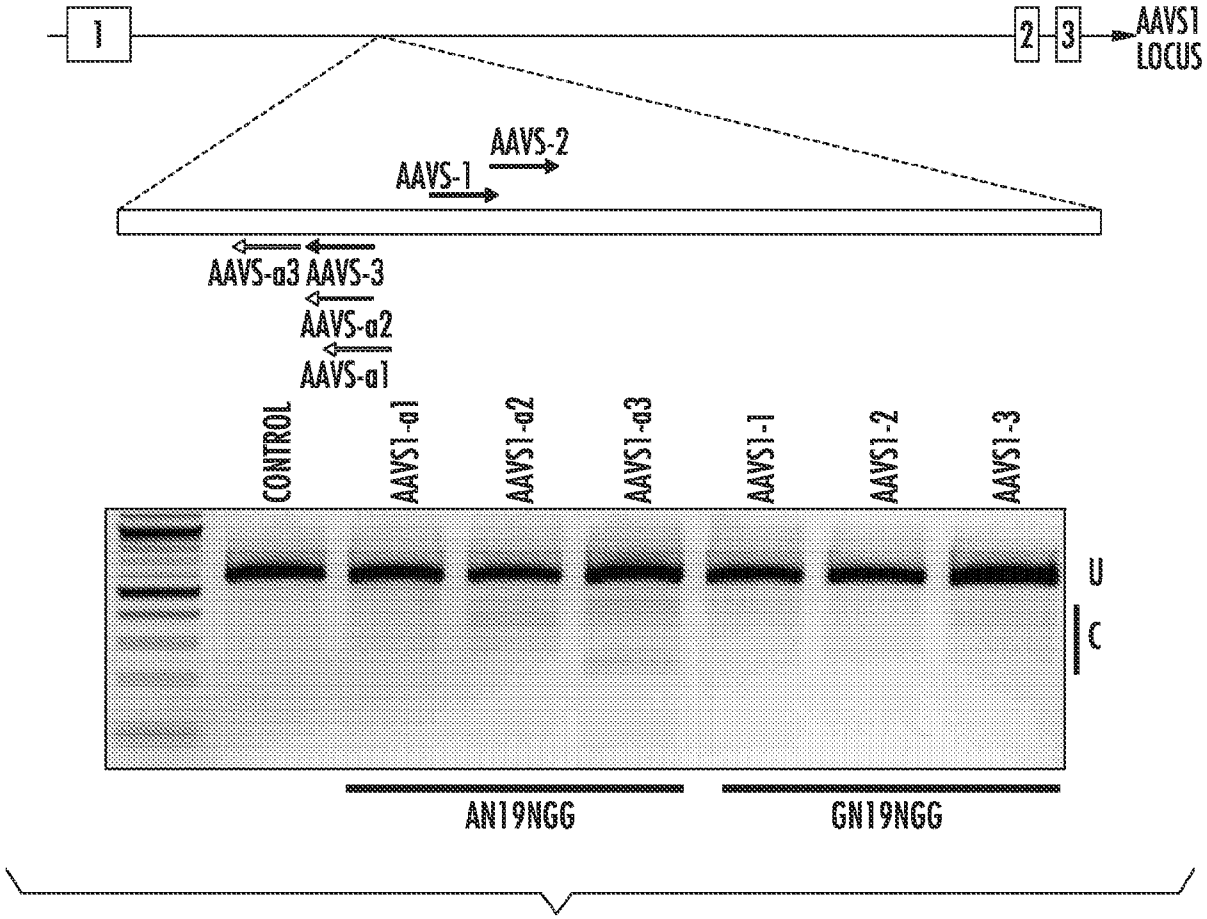

1
2
3
AAVS1
LOCUS
AAVS-2
AAVS-1
AAVS-α3
AAVS-3
AAVS-α2
AAVS-α1
CONTROL
AAVS1-α1
AAVS1-α2
AAVS1-α3
AAVS1-1
AAVS1-2
AAVS1-3
U
C
AN19NGG
GN19NGG

FIG. 3A

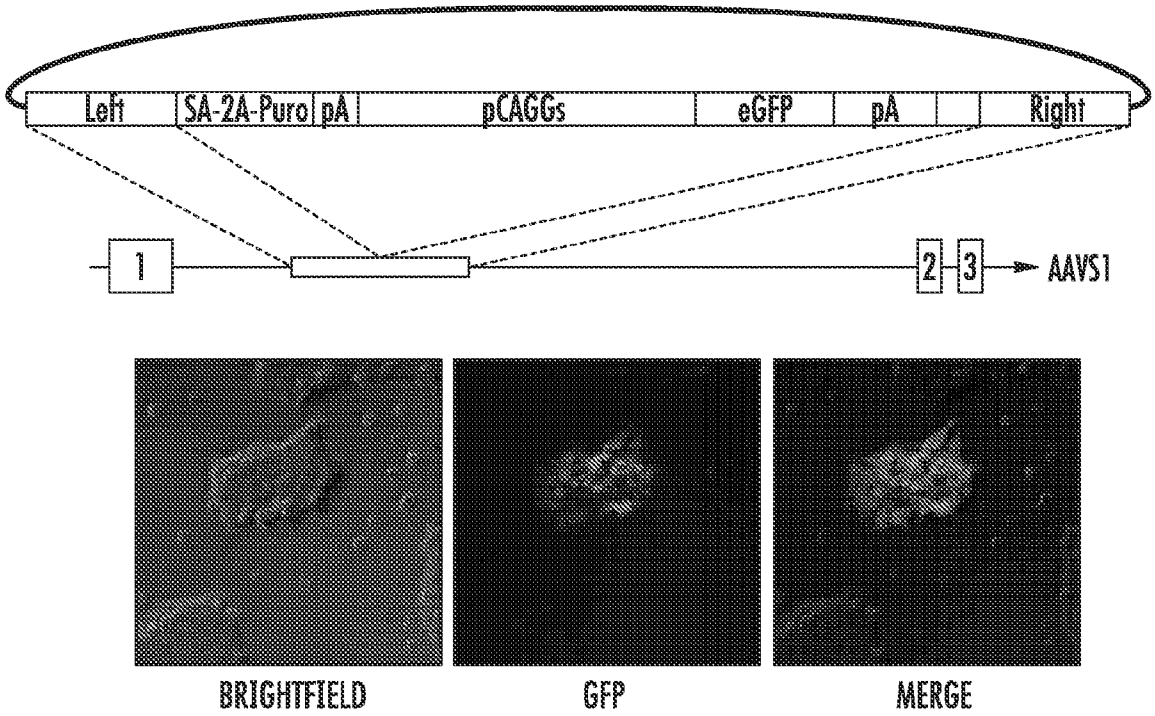

Left
SA-2A-Puro
pA
pCAGGs
eGFP
pA
Right
1
2
3
AAVS1
BRIGHTFIELD
GFP
MERGE

FIG. 3B

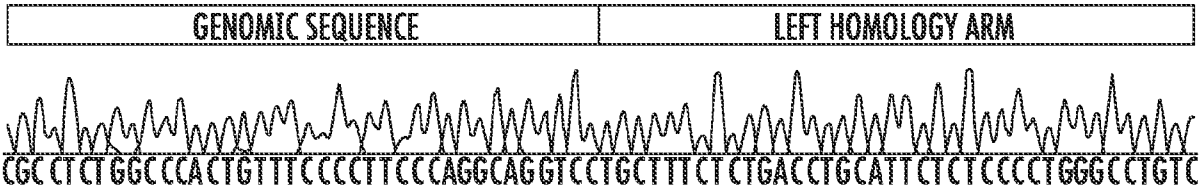

GENOMIC SEQUENCE
LEFT HOMOLOGY ARM
CGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTCTCTCCCCTGGGCCTGTC

FIG. 3C

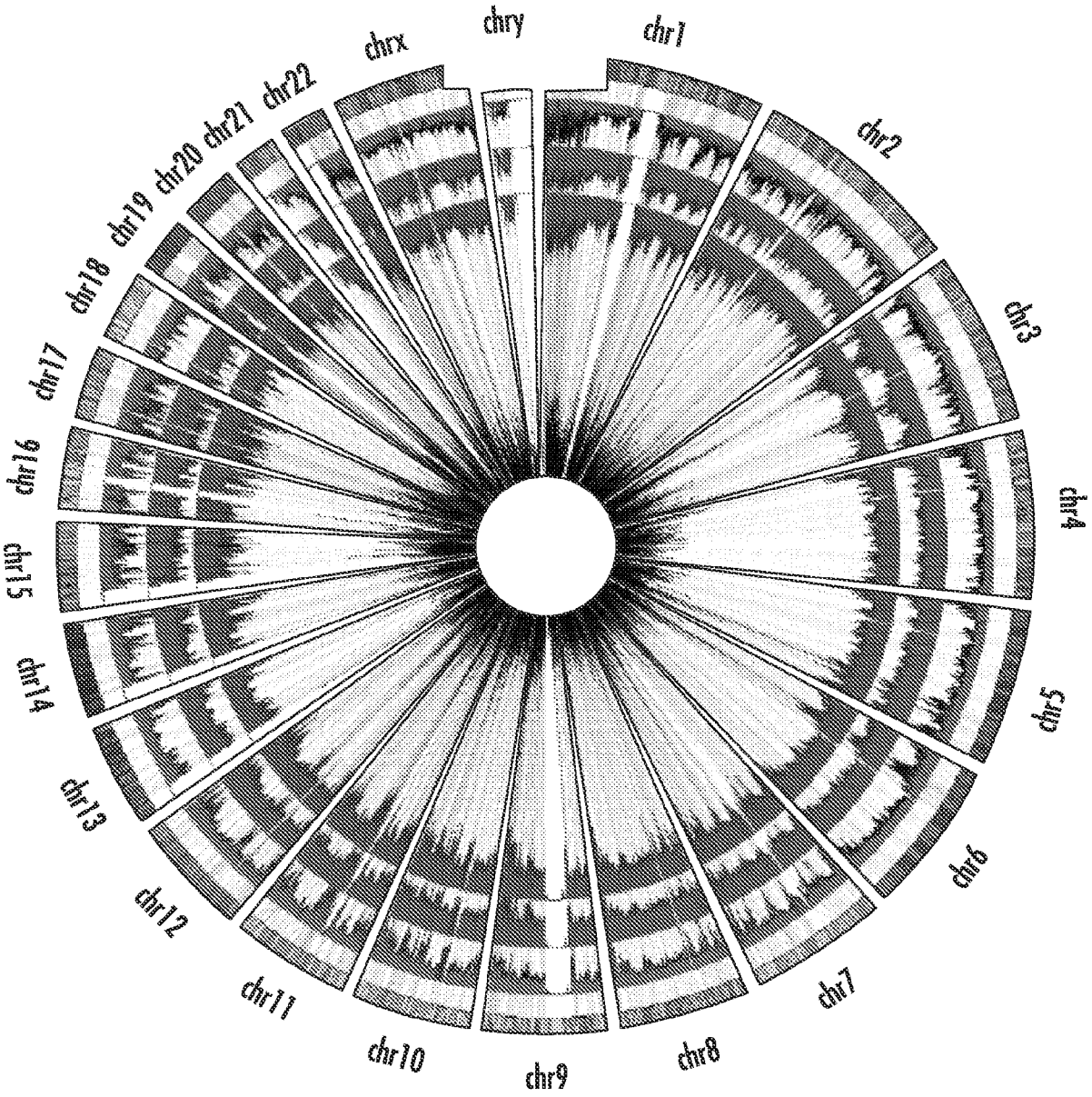

chry
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr20
chr21
chr22
chrx

FIG. 4A

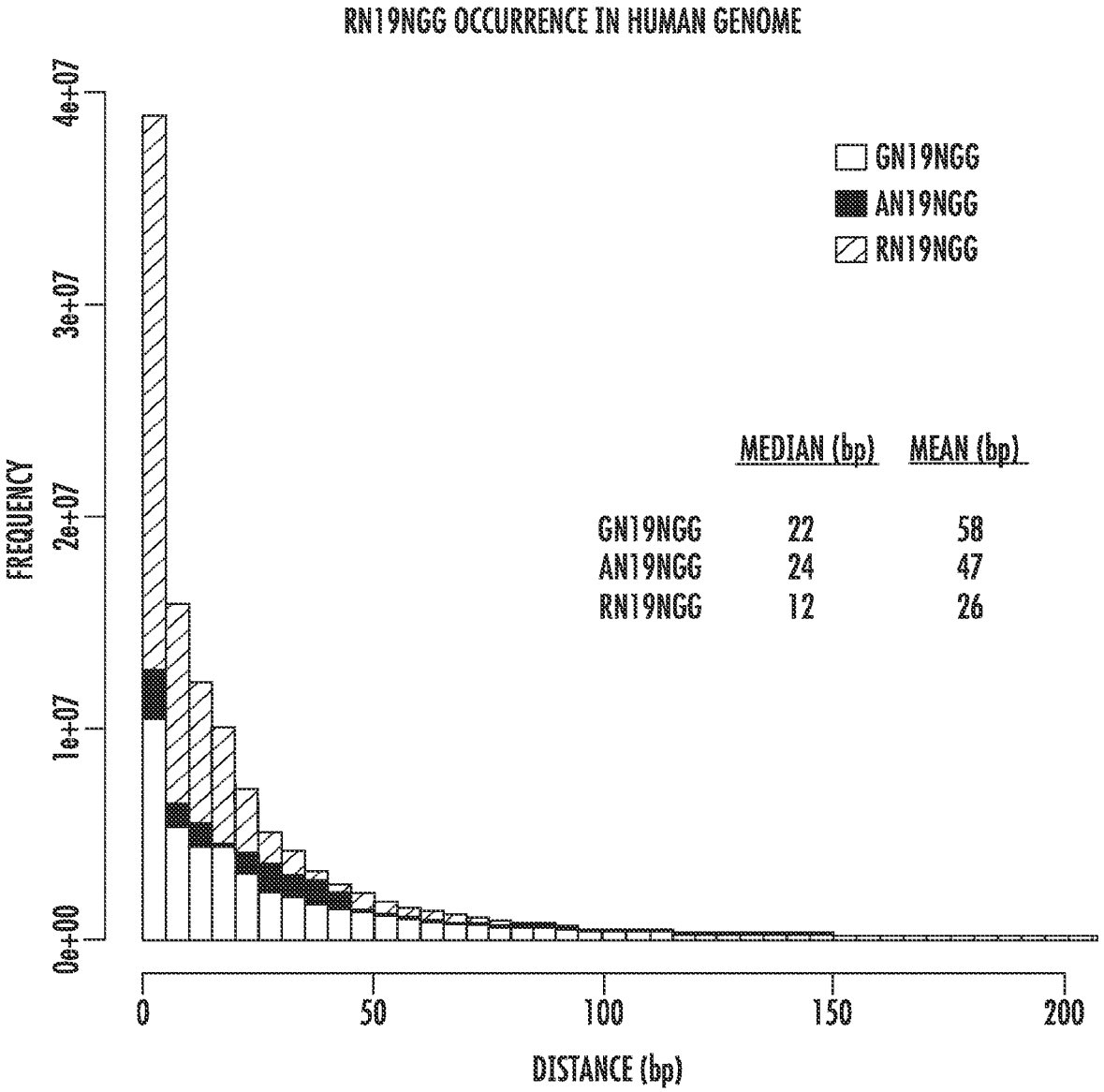

RN19NGG OCCURRENCE IN HUMAN GENOME
GN19NGG
AN19NGG
RN19NGG
MEDIAN (bp)
MEAN (bp)
GN19NGG 22 58
AN19NGG 24 47
RN19NGG 12 26
FREQUENCY
0e+00
1e+07
2e+07
3e+07
4e+07
0
50
100
150
200
DISTANCE (bp)

FIG. 4B

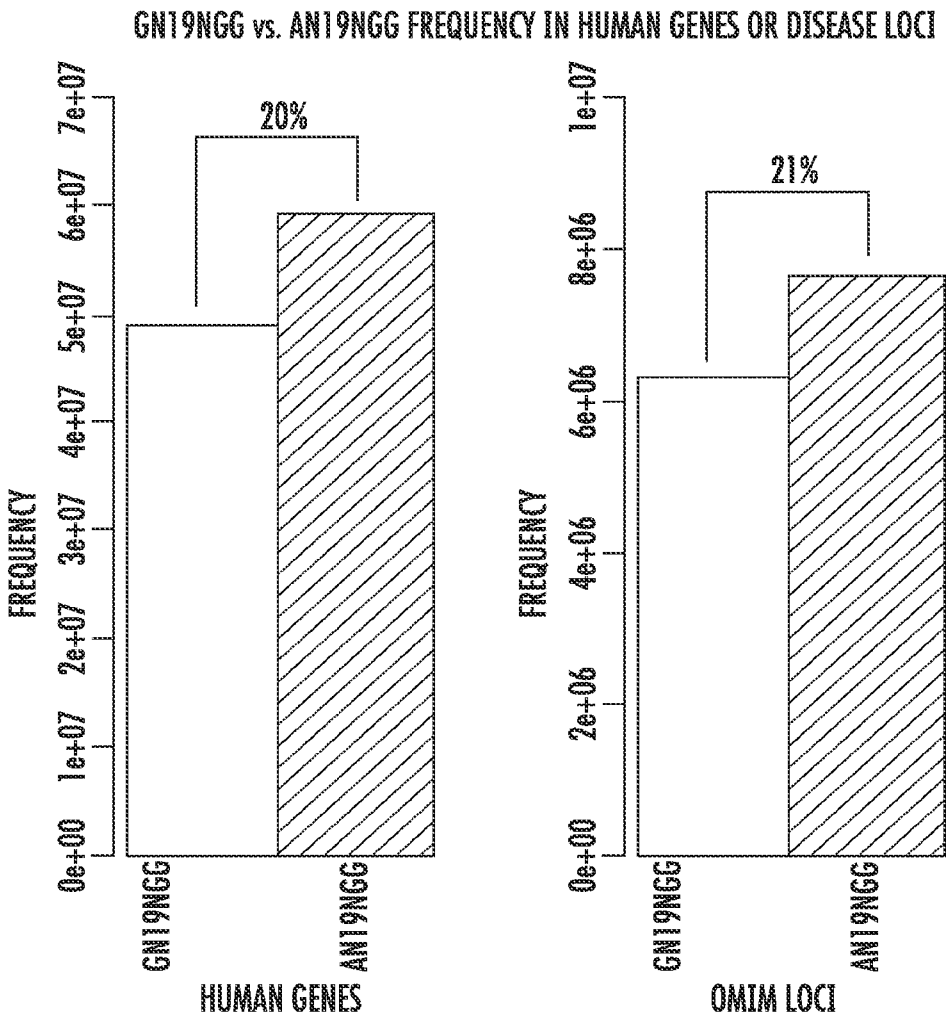

GN19NGG vs. AN19NGG FREQUENCY IN HUMAN GENES OR DISEASE LOCI
FREQUENCY
0e+00
1e+07
2e+07
3e+07
4e+07
5e+07
6e+07
7e+07
20%
GN19NGG
AN19NGG
HUMAN GENES
FREQUENCY
0e+00
2e+06
4e+06
6e+06
8e+06
1e+07
21%
GN19NGG
AN19NGG
OMIM LOCI

FIG. 4C

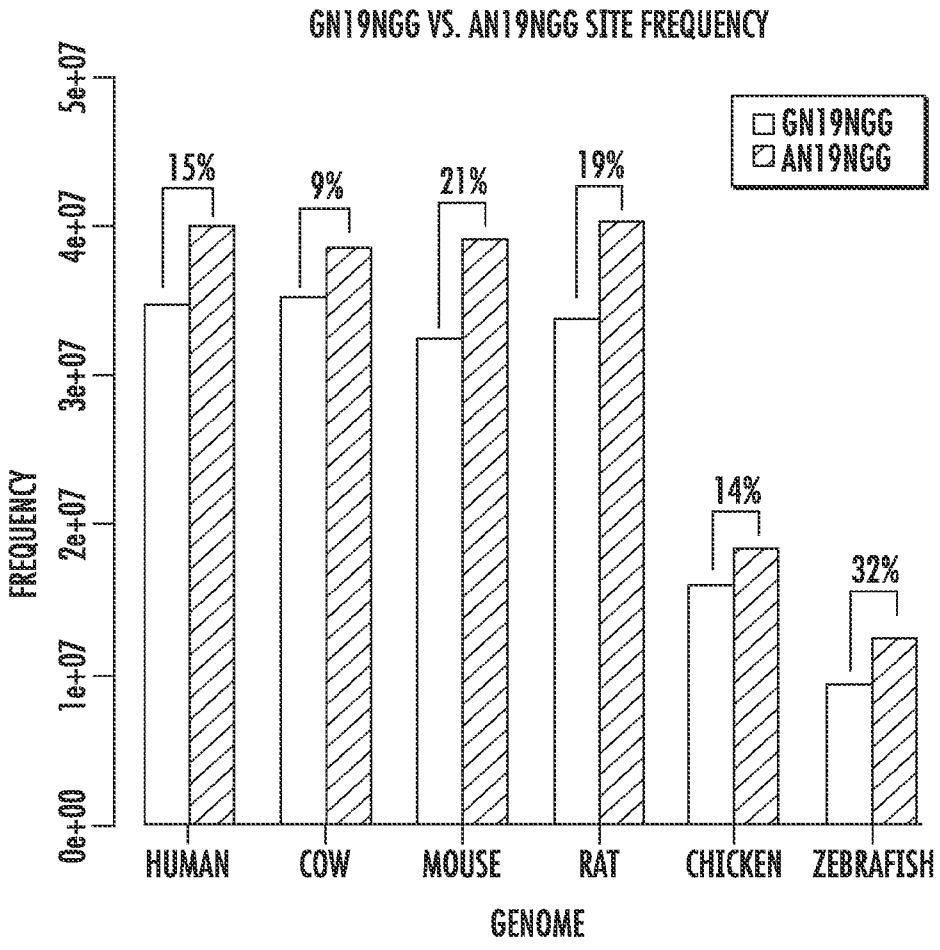

GN19NGG VS. AN19NGG SITE FREQUENCY
GN19NGG
AN19NGG
FREQUENCY
0e+00
1e+07
2e+07
3e+07
4e+07
5e+07
15%
9%
21%
19%
14%
32%
HUMAN
COW
MOUSE
RAT
CHICKEN
ZEBRAFISH
GENOME

FIG. 4D

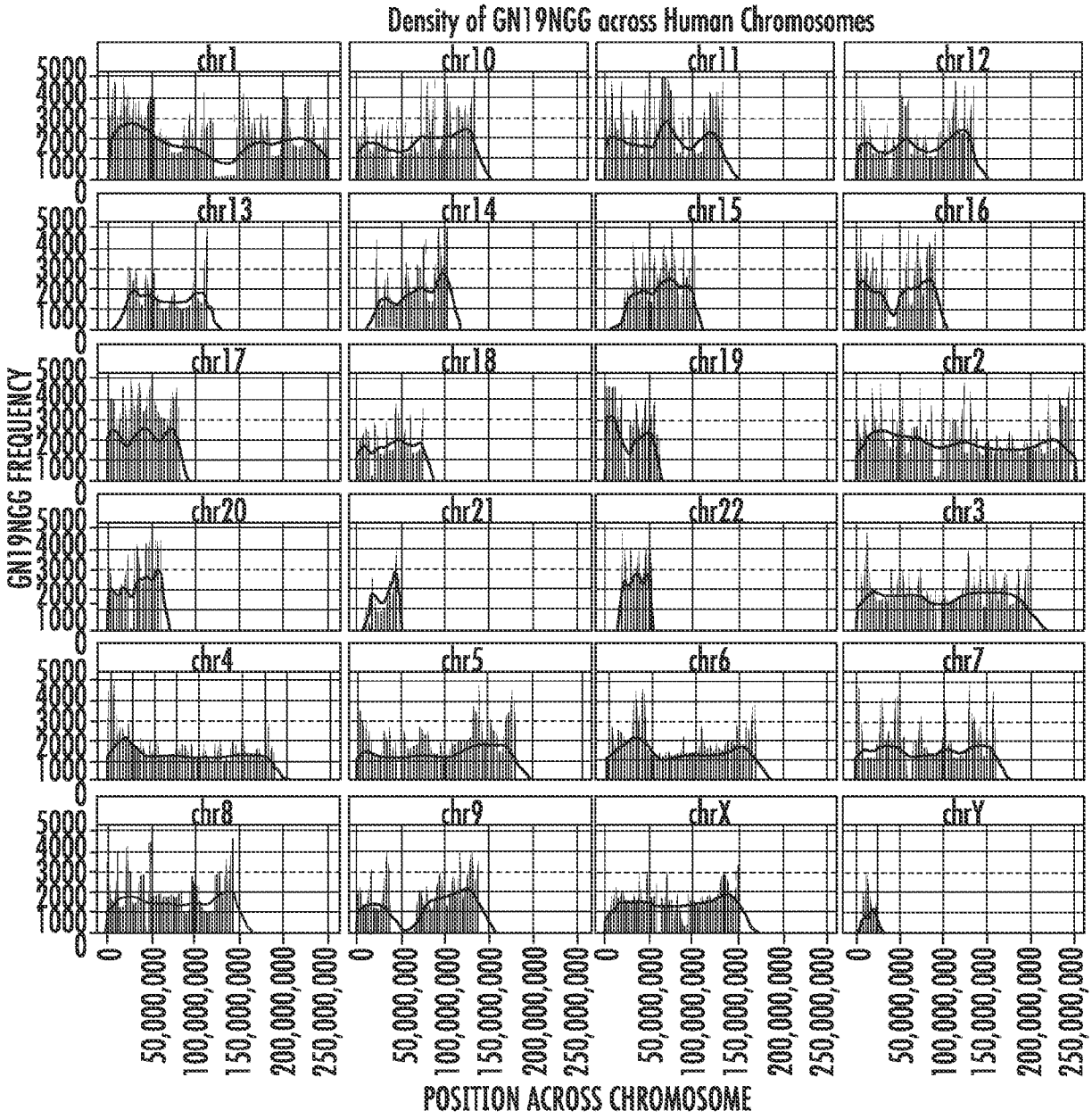

Density of GN19NGG across Human Chromosomes
chr1
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr2
chr20
chr21
chr22
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chrX
chrY
GN19NGG FREQUENCY
0
10000
20000
30000
40000
50000
0
50,000,000
100,000,000
150,000,000
200,000,000
250,000,000
POSITION ACROSS CHROMOSOME

FIG. 5A

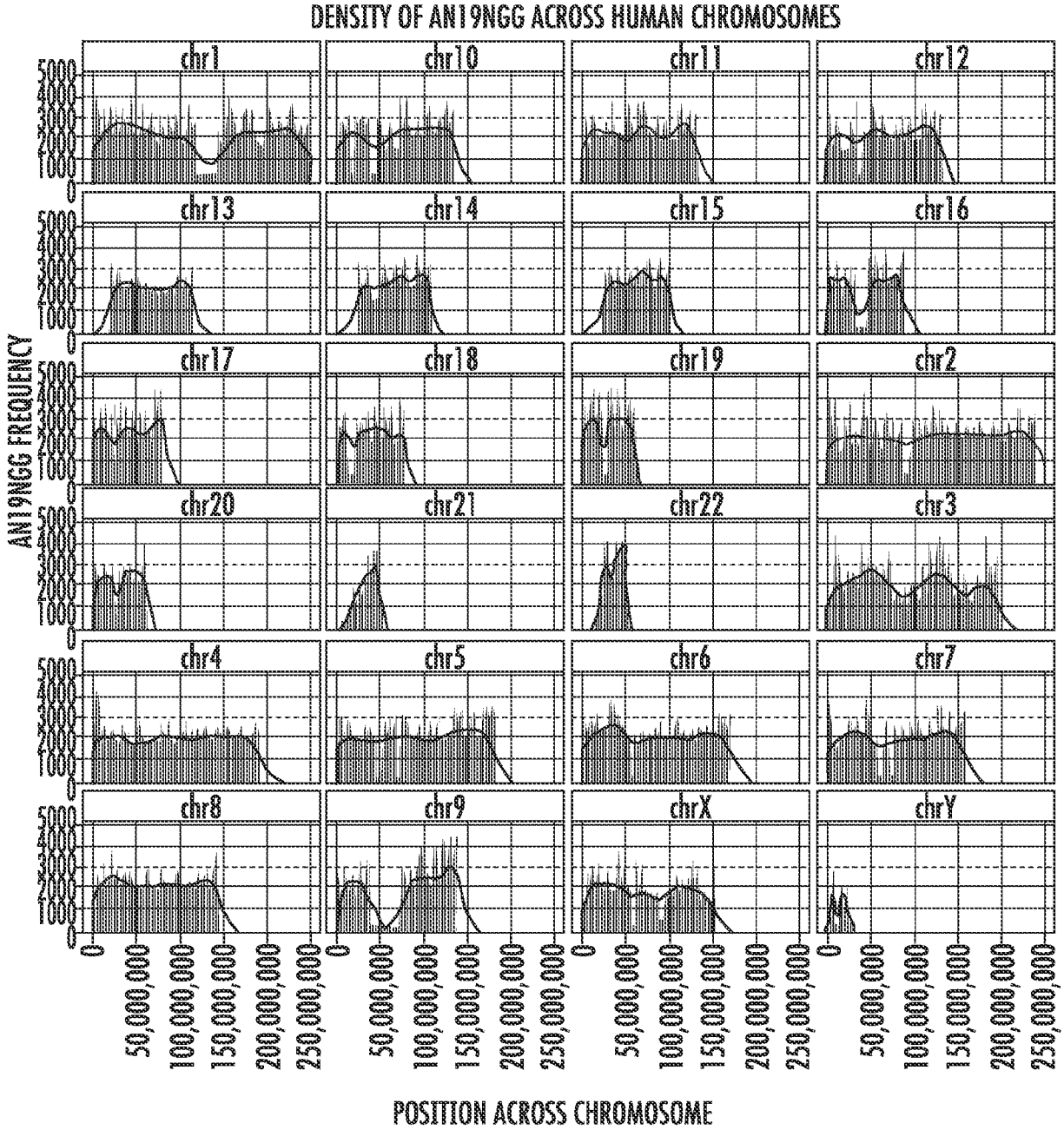

DENSITY OF AN19NGG ACROSS HUMAN CHROMOSOMES
AN19NGG FREQUENCY
chr1
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr2
chr20
chr21
chr22
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chrX
chrY
0
1000
2000
3000
4000
5000
0
50,000,000
100,000,000
150,000,000
200,000,000
250,000,000
POSITION ACROSS CHROMOSOME

FIG. 5B

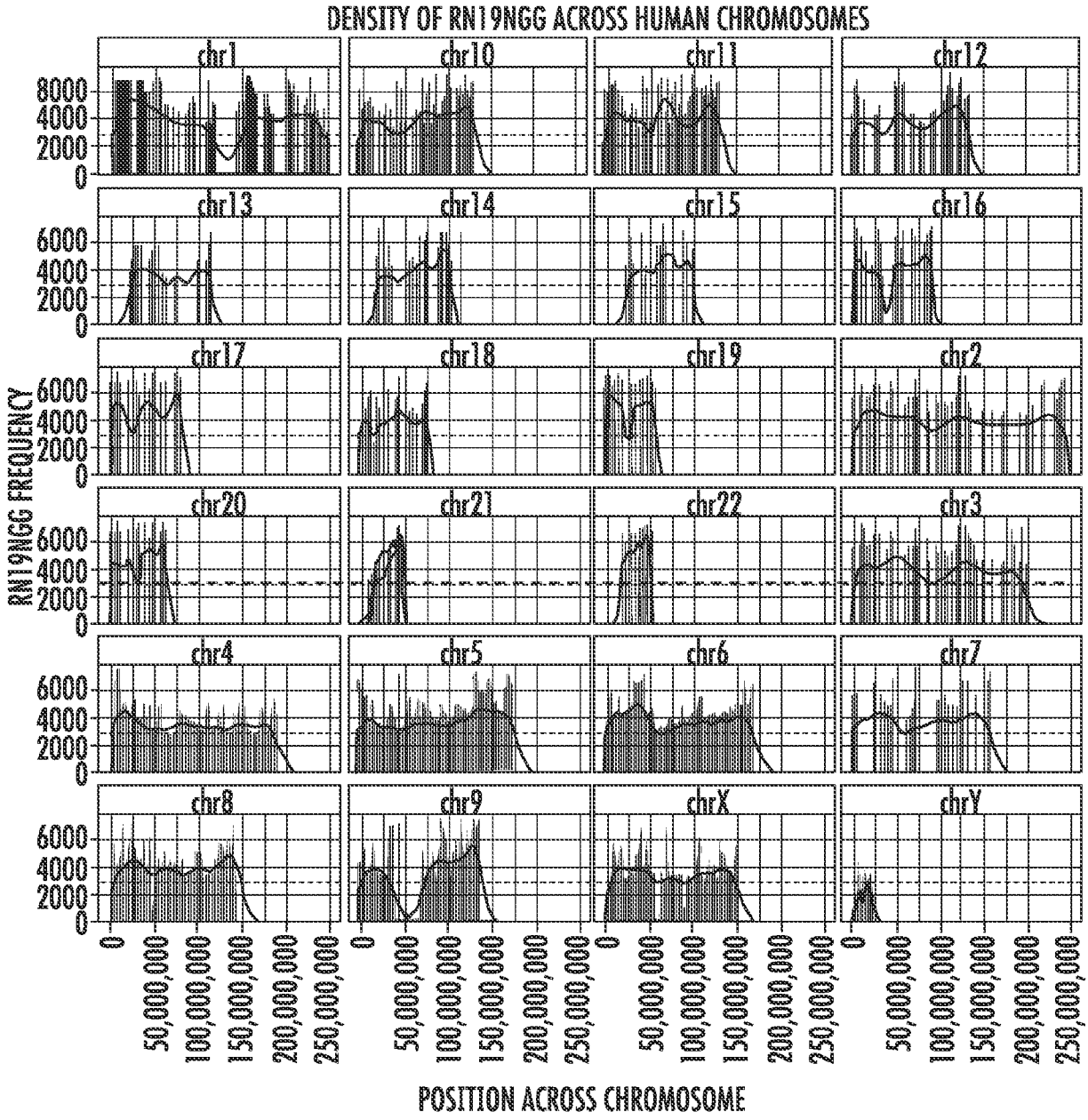

DENSITY OF RN19NGG ACROSS HUMAN CHROMOSOMES
chr1
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr2
chr20
chr21
chr22
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chrX
chrY
RN19NGG FREQUENCY
0
2000
4000
6000
8000
0
50,000,000
100,000,000
150,000,000
200,000,000
250,000,000
POSITION ACROSS CHROMOSOME

FIG. 5C

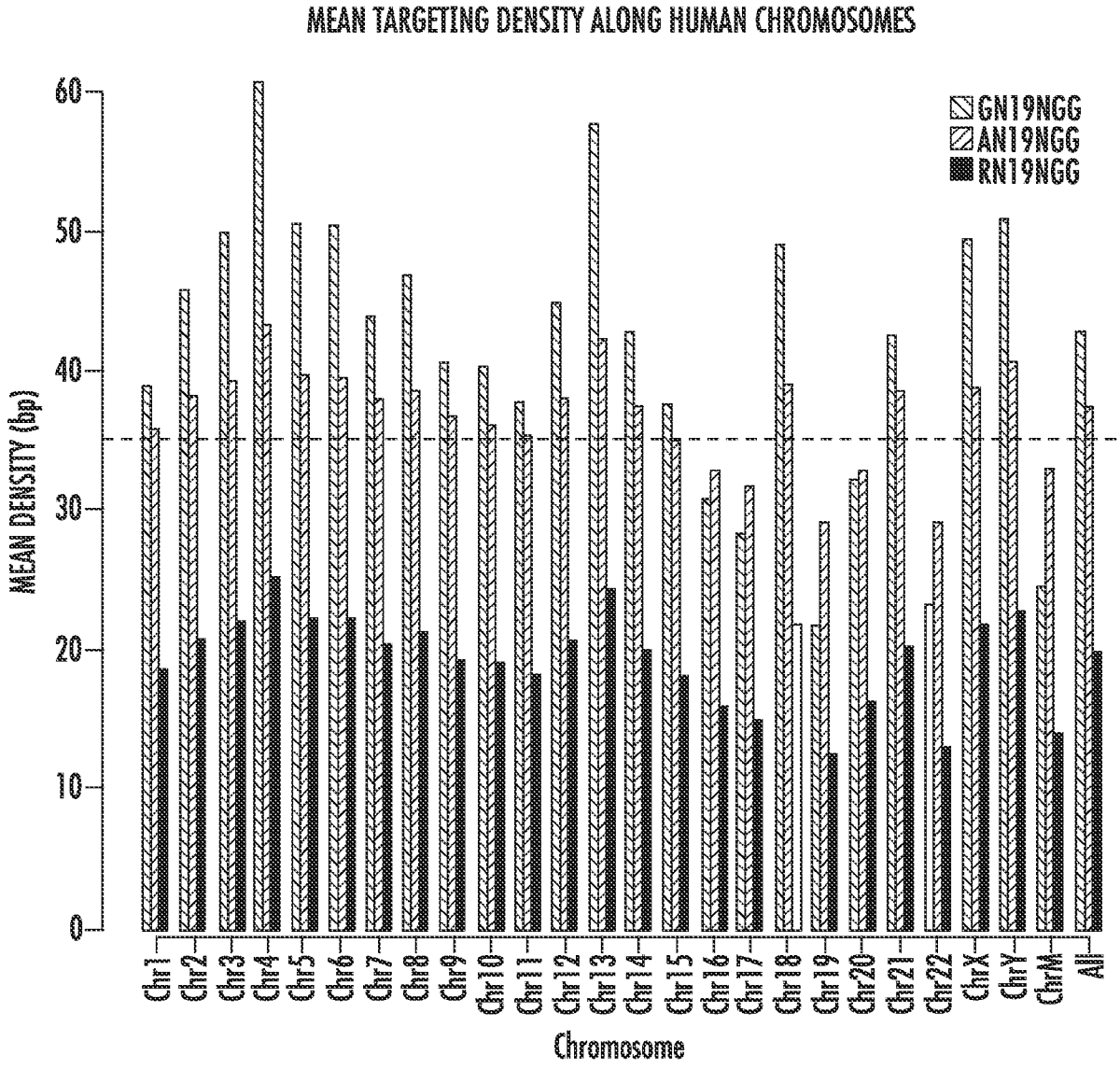

MEAN TARGETING DENSITY ALONG HUMAN CHROMOSOMES
GN19NGG
AN19NGG
RN19NGG
MEAN DENSITY (bp)
0
10
20
30
40
50
60
Chr1
Chr2
Chr3
Chr4
Chr5
Chr6
Chr7
Chr8
Chr9
Chr10
Chr11
Chr12
Chr13
Chr14
Chr15
Chr16
Chr17
Chr18
Chr19
Chr20
Chr21
Chr22
ChrX
ChrY
ChrM
All
Chromosome

FIG. 5D

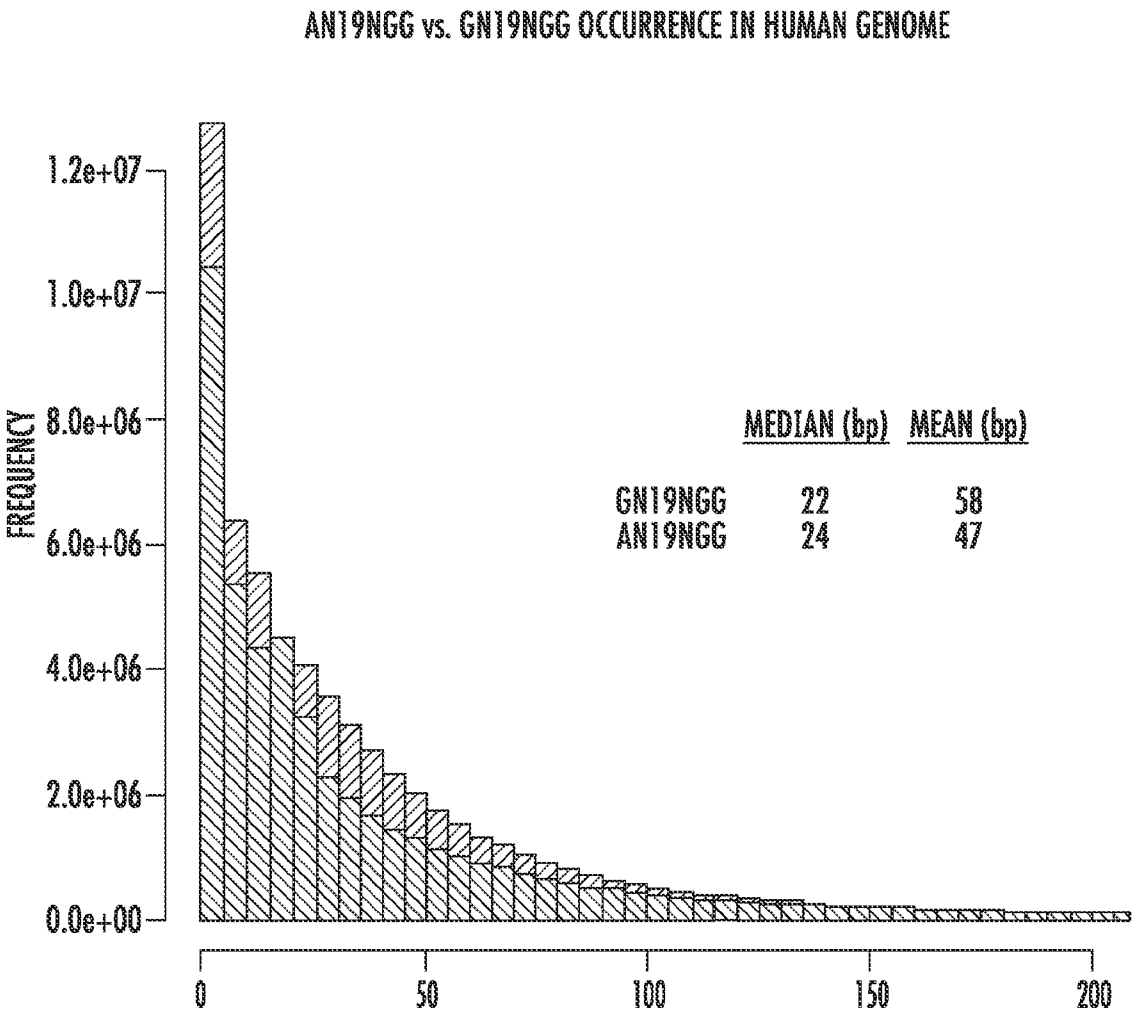

AN19NGG vs. GN19NGG OCCURRENCE IN HUMAN GENOME
FREQUENCY
1.2e+07
1.0e+07
8.0e+06
6.0e+06
4.0e+06
2.0e+06
0.0e+00
0
50
100
150
200
MEDIAN (bp)
MEAN (bp)
GN19NGG 22 58
AN19NGG 24 47

FIG. 5E

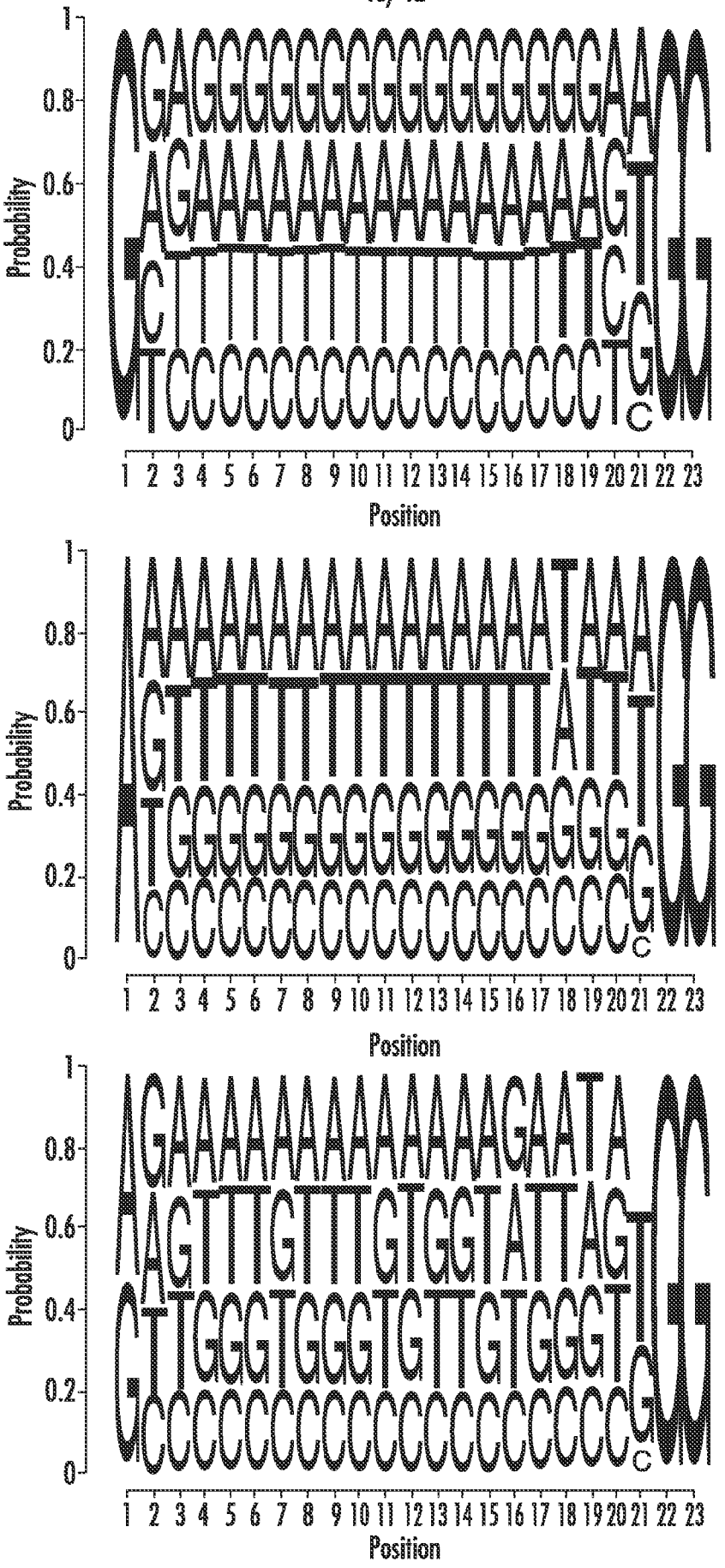

Probability
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Position

FIG. 5F

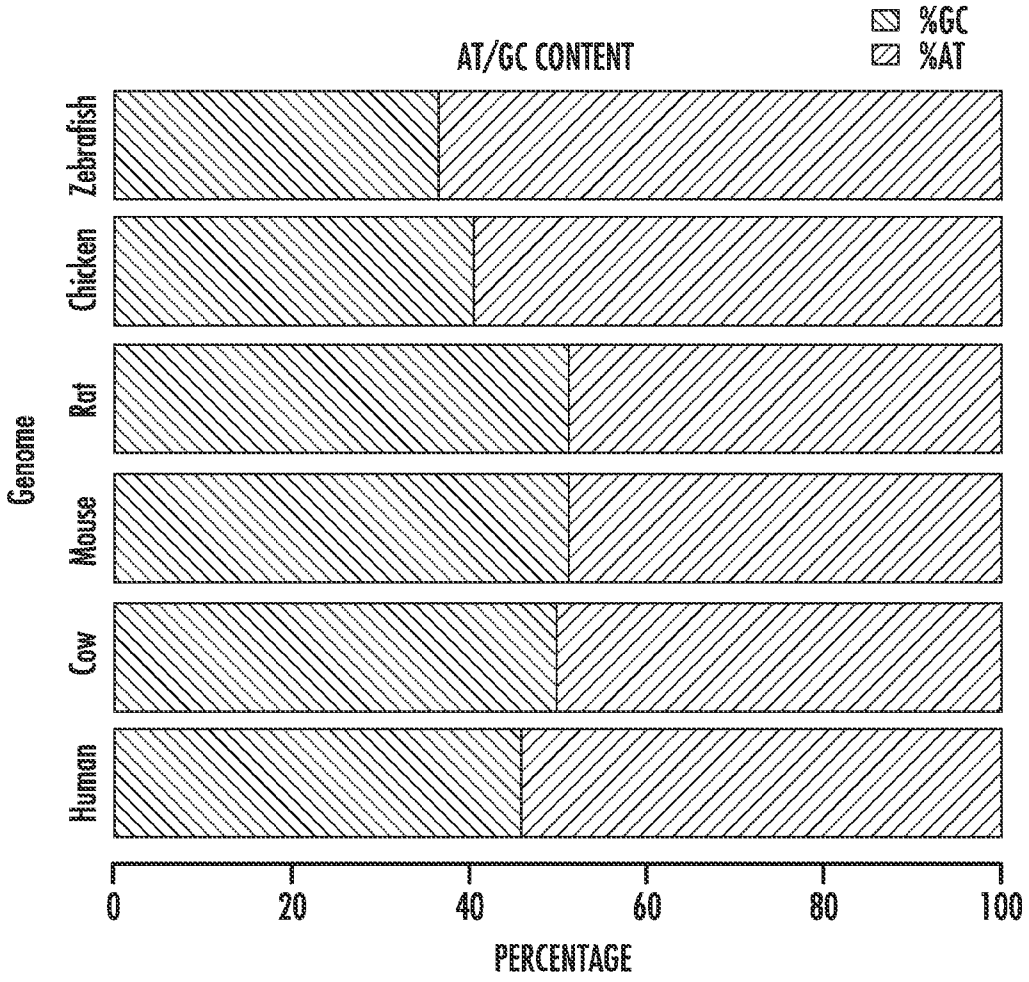

%GC
%AT
AT/GC CONTENT
Zebrafish
Chicken
Rat
Mouse
Cow
Human
Genome
0
20
40
60
80
100
PERCENTAGE

FIG. 6A

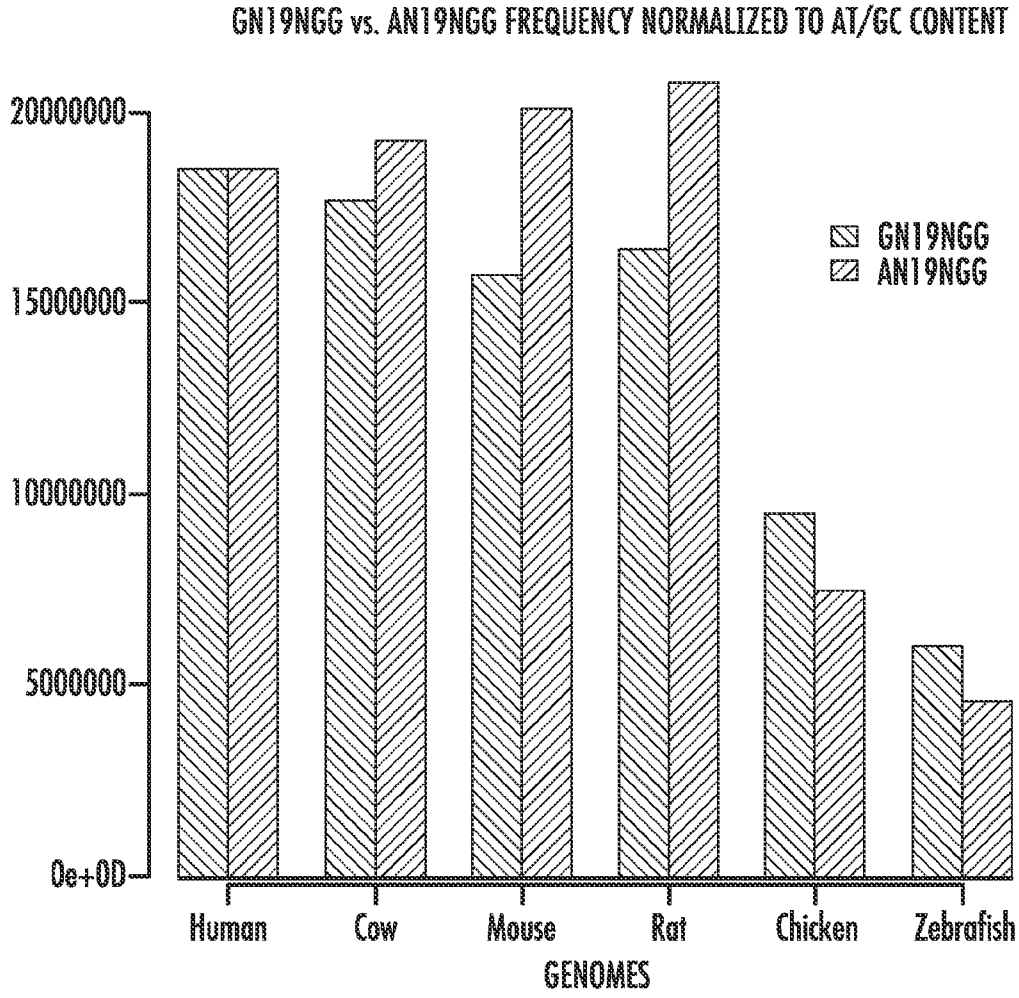

GN19NGG vs. AN19NGG FREQUENCY NORMALIZED TO AT/GC CONTENT
20000000
15000000
10000000
5000000
0e+0D
GN19NGG
AN19NGG
Human
Cow
Mouse
Rat
Chicken
Zebrafish
GENOMES

FIG. 6B

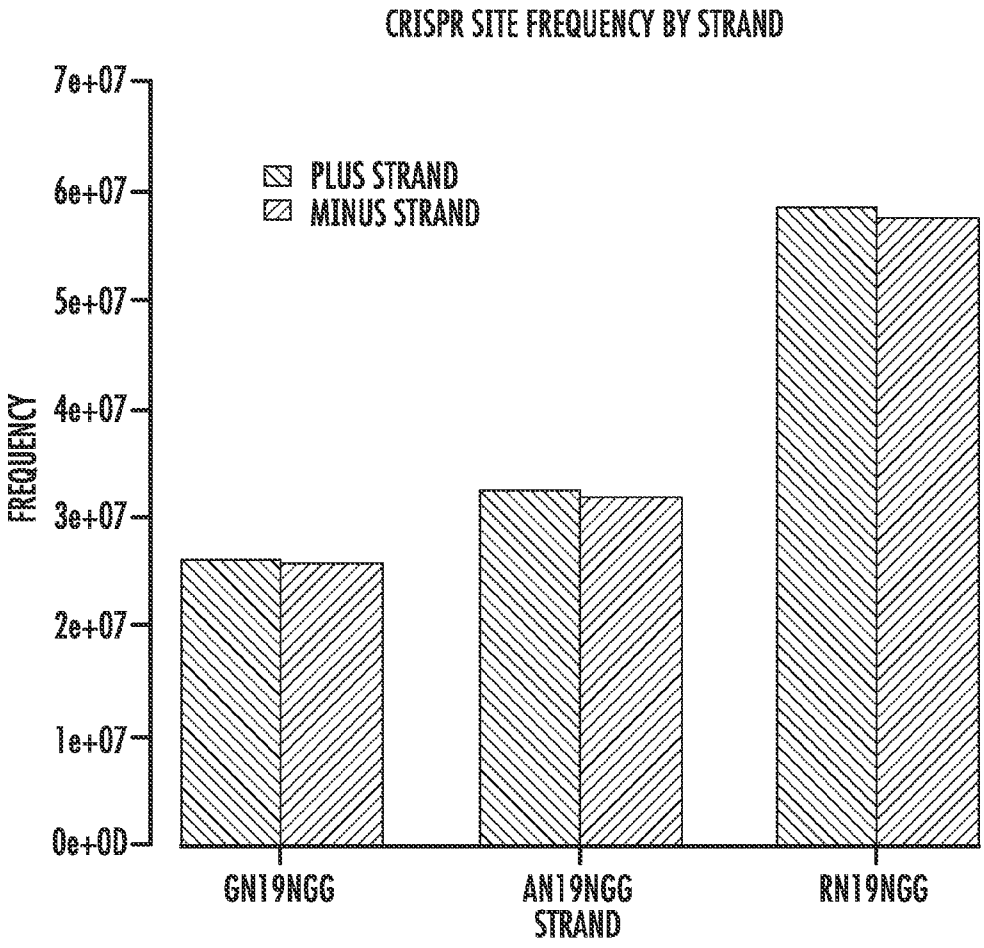

CRISPR SITE FREQUENCY BY STRAND
PLUS STRAND
MINUS STRAND
FREQUENCY
7e+07
6e+07
5e+07
4e+07
3e+07
2e+07
1e+07
0e+00
GN19NGG
AN19NGG
RN19NGG
STRAND

FIG. 6C

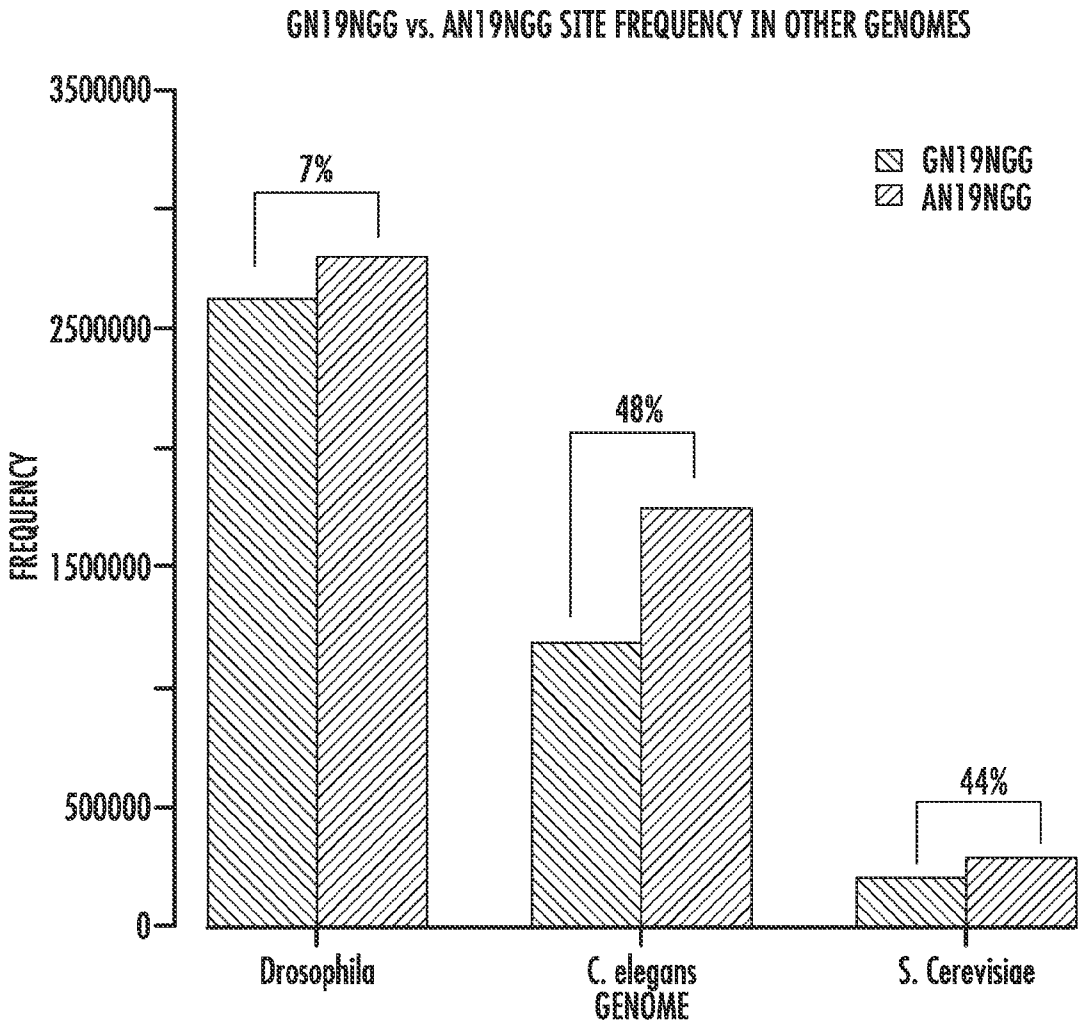

GN19NGG vs. AN19NGG SITE FREQUENCY IN OTHER GENOMES
3500000
2500000
1500000
500000
0
FREQUENCY
7%
48%
44%
GN19NGG
AN19NGG
Drosophila
C. elegans
S. Cerevisiae
GENOME

FIG. 6D

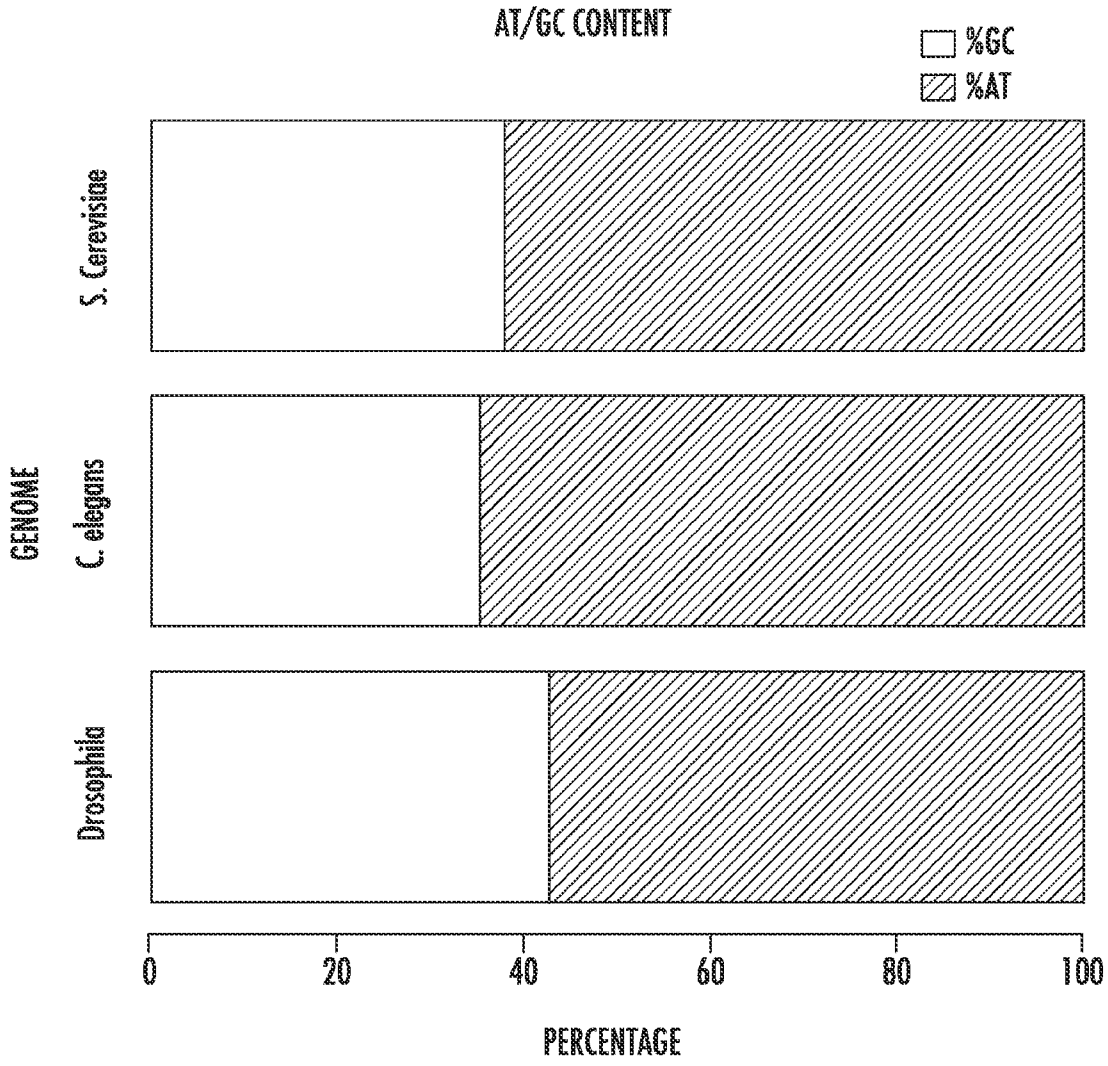

AT/GC CONTENT
%GC
%AT
GENOME
S. Cerevisiae
C. elegans
Drosophila
0
20
40
60
80
100
PERCENTAGE

FIG. 6E

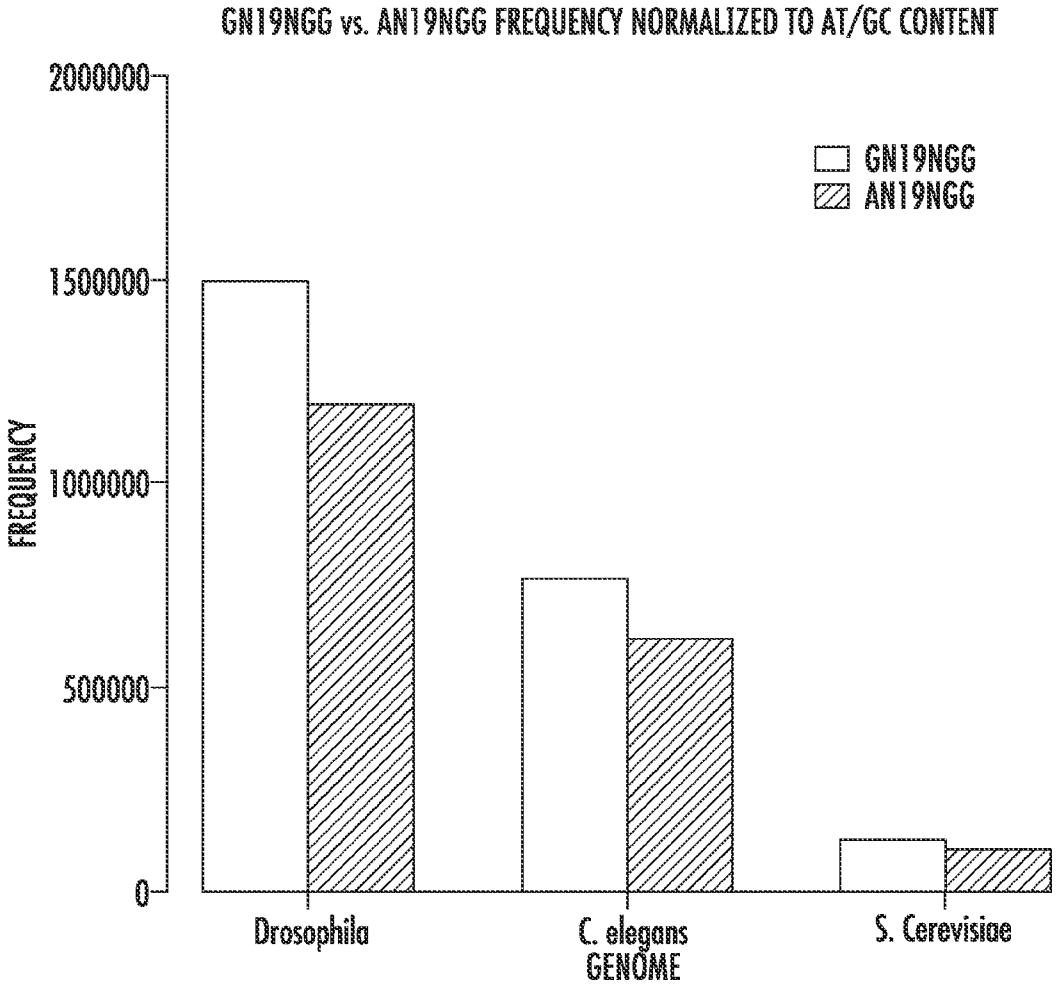

GN19NGG vs. AN19NGG FREQUENCY NORMALIZED TO AT/GC CONTENT
2000000
1500000
1000000
500000
0
FREQUENCY
GN19NGG
AN19NGG
Drosophila
C. elegans
S. Cerevisiae
GENOME

FIG. 6F

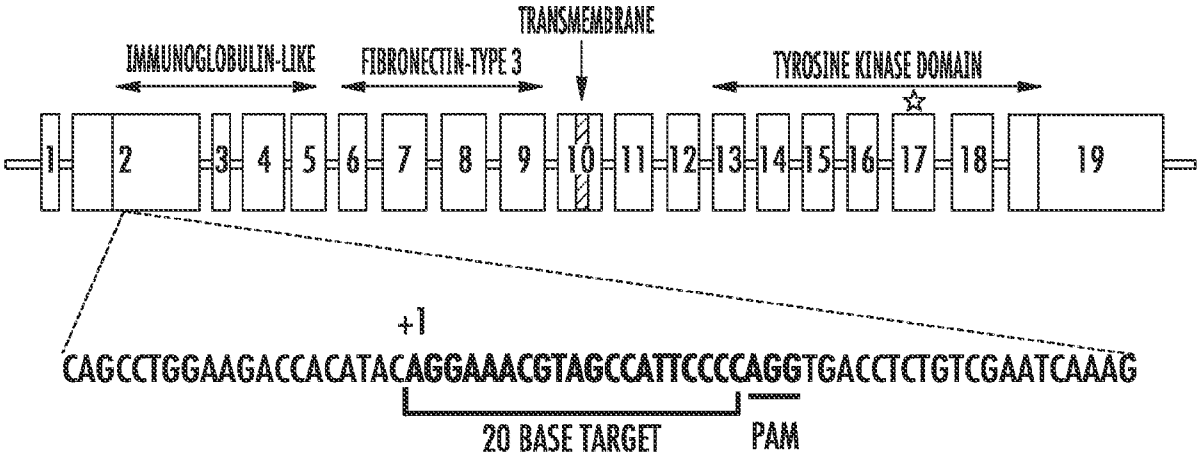

TRANSMEMBRANE
IMMUNOGLOBULIN-LIKE
FIBRONECTIN-TYPE 3
TYROSINE KINASE DOMAIN
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
+1
CAGCCTGGAAGACCACATACAGGAAACGTAGCCATTCCCCAGGTGACCTCTGTCGAATCAAAG
20 BASE TARGET
PAM

FIG. 7A

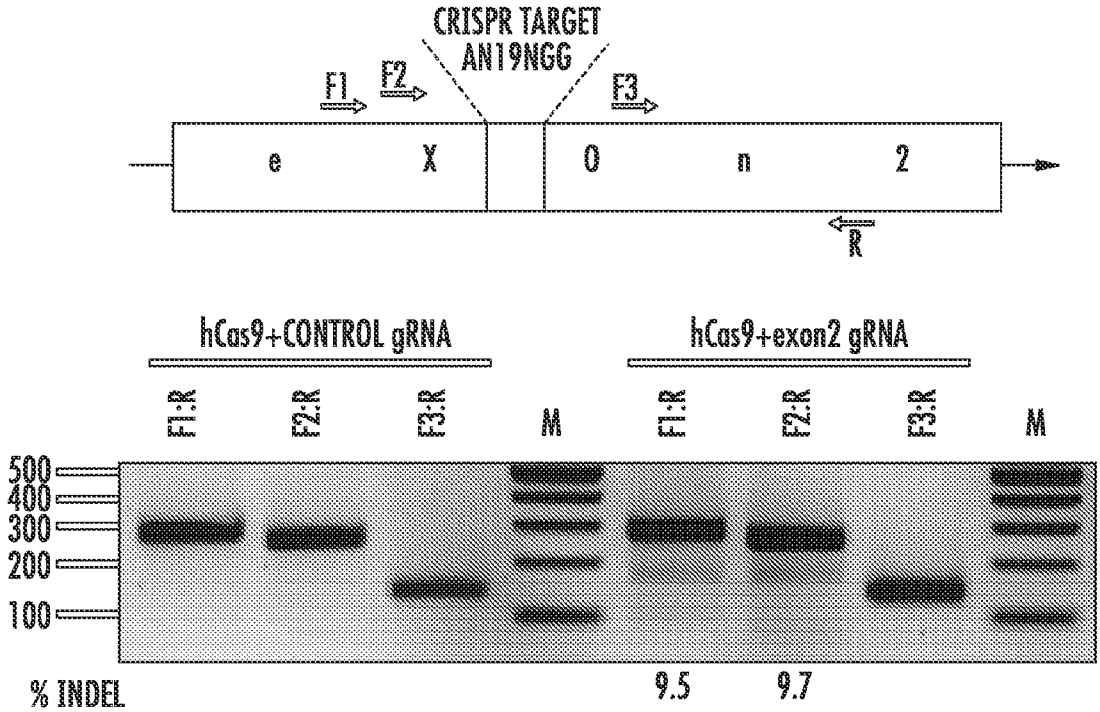

CRISPR TARGET
AN19NGG
F1
F2
F3
e X 0 n 2
R
hCas9+CONTROL gRNA
hCas9+exon2 gRNA
F1:R
F2:R
F3:R
M
F1:R
F2:R
F3:R
M
500
400
300
200
100
% INDEL
9.5
9.7

FIG. 7B

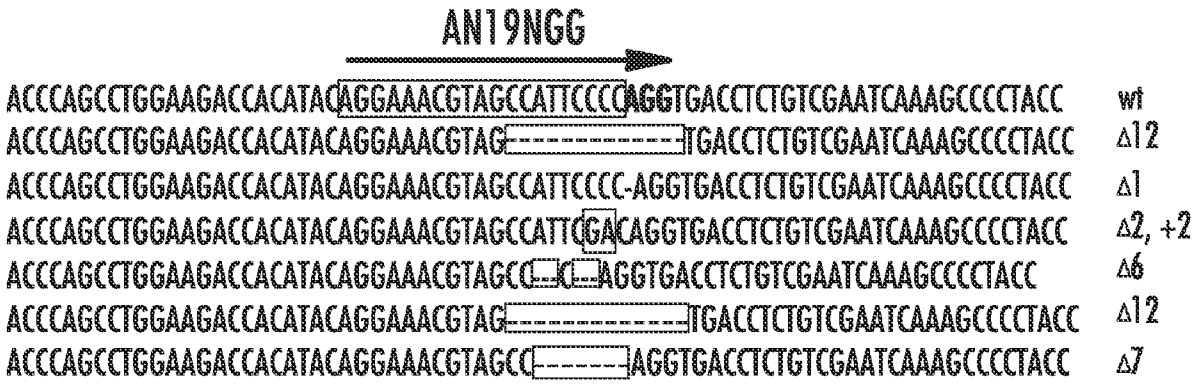

AN19NGG
ACCCAGCCTGGAAGACCACATACAGGAAACGTAGCCATTCCCCAGGTGACCTCTGTCGAATCAAAGCCCCTACC wt
ACCCAGCCTGGAAGACCACATACAGGAAACGTAG----------TGACCTCTGTCGAATCAAAGCCCCTACC Δ12
ACCCAGCCTGGAAGACCACATACAGGAAACGTAGCCATTCCCC-AGGTGACCTCTGTCGAATCAAAGCCCCTACC Δ1
ACCCAGCCTGGAAGACCACATACAGGAAACGTAGCCATTCGACAGGTGACCTCTGTCGAATCAAAGCCCCTACC Δ2, +2
ACCCAGCCTGGAAGACCACATACAGGAAACGTAGCC--C--AGGTGACCTCTGTCGAATCAAAGCCCCTACC Δ6
ACCCAGCCTGGAAGACCACATACAGGAAACGTAG----------TGACCTCTGTCGAATCAAAGCCCCTACC Δ12
ACCCAGCCTGGAAGACCACATACAGGAAACGTAGCC------AGGTGACCTCTGTCGAATCAAAGCCCCTACC Δ7

FIG. 7C

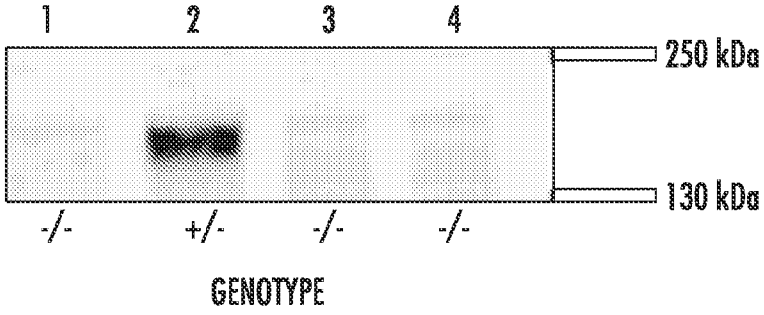

1
2
3
4
250 kDa
130 kDa
-/-
+/-
-/-
-/-
GENOTYPE

FIG. 7D

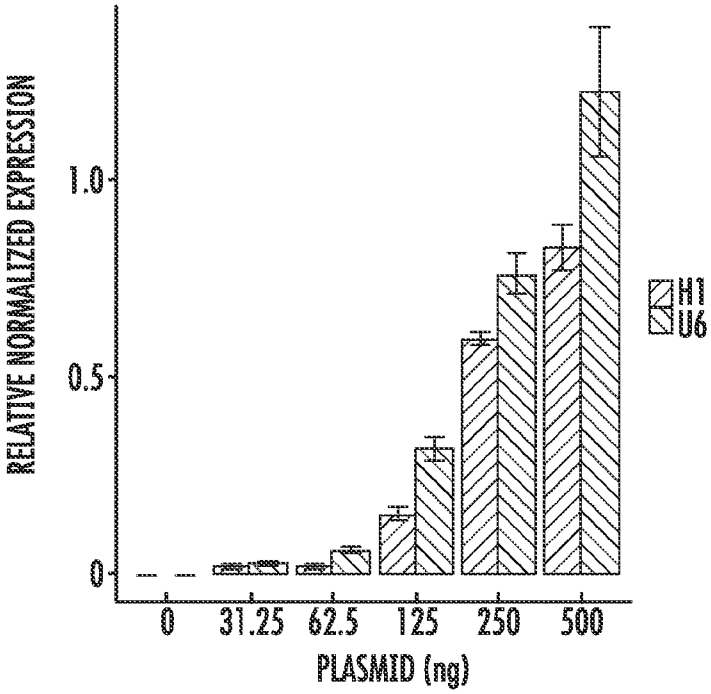

RELATIVE NORMALIZED EXPRESSION
1.0
0.5
0
0
31.25
62.5
125
250
500
PLASMID (ng)
H1
U6

FIG. 8A

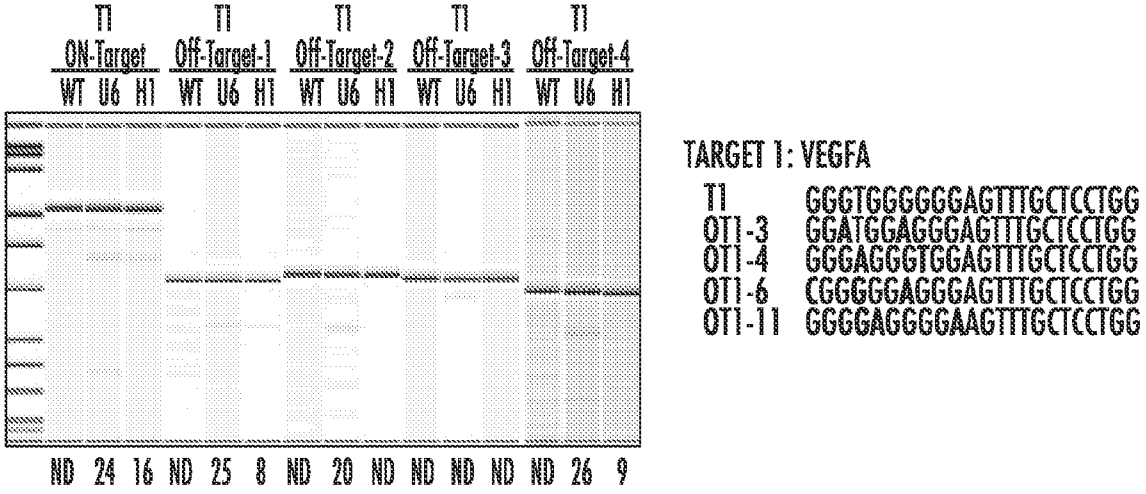
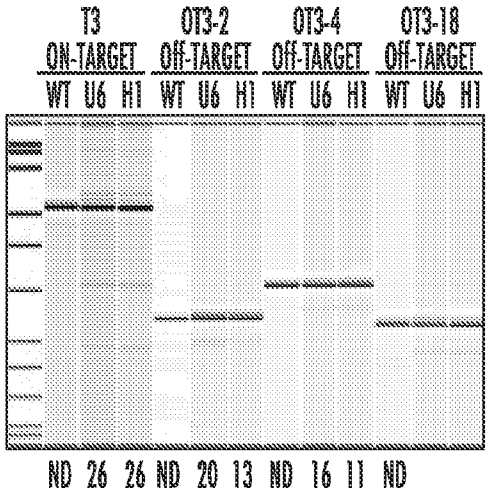

T1
ON-Target
Off-Target-1
Off-Target-2
Off-Target-3
Off-Target-4
WT U6 H1
ND 24 16 ND 25 8 ND 20 ND ND ND ND ND 26 9
TARGET 1: VEGFA
T1 GGGTGGGGGGAGTTTGCTCCTGG
OT1-3 GGATGGAGGGAGTTTGCTCCTGG
OT1-4 GGGAGGGTGGAGTTTGCTCCTGG
OT1-6 CGGGGGAGGGAGTTTGCTCCTGG
OT1-11 GGGGAGGGGAAGTTTGCTCCTGG

FIG. 8B



TARGET 3: VEGFA

T3	GGTGAGTGAGTGTGTGCGTGTGG
OT3 1	CCTCACTCACTCTCTCTCTCACC
OT3-2	AGTGAGTGAGTGTGTGTGTGGGG
OT3-4	GCTGAGTGAGTGTATGCGTGTGG
OT3-18	TGTGGGTGAGTGTGTGCGTGAGG

FIG. 8C

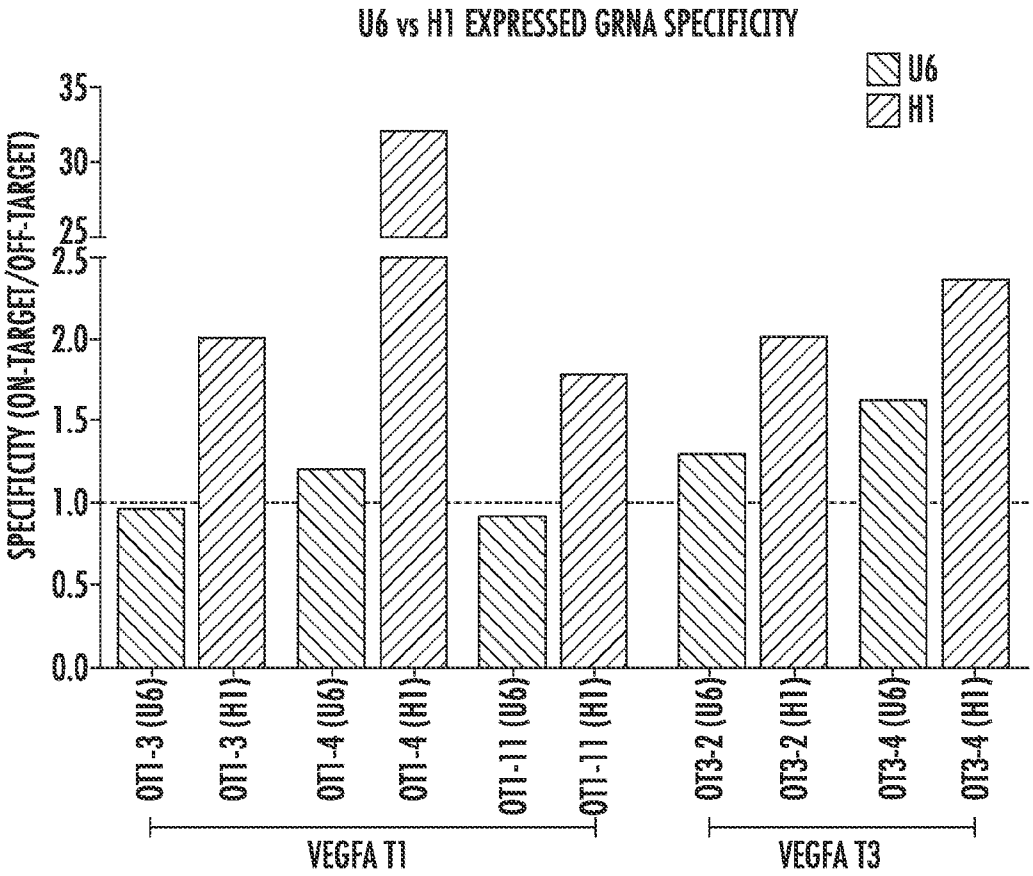


FIG. 8D

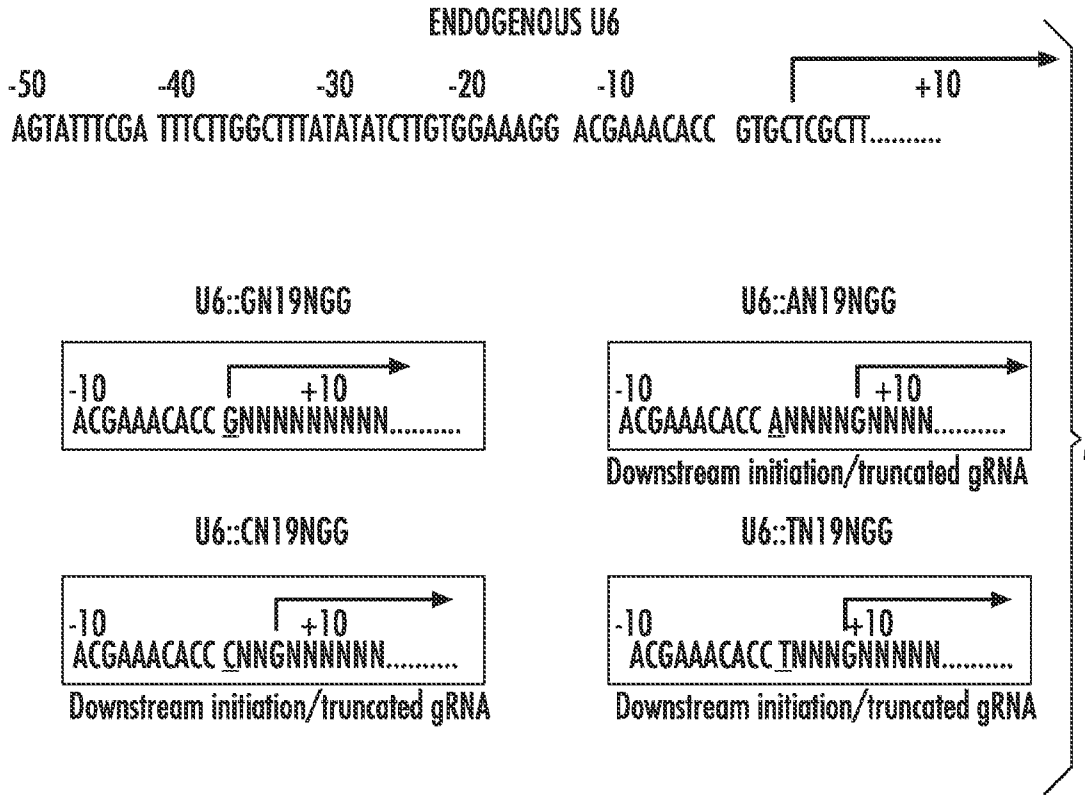

ENDOGENOUS U6
-50 -40 -30 -20 -10 +10
AGTATTTCGA TTTCTTGGCTTTATATATCTTGTGGAAAGG ACGAAACACC GTGCTCGCTT.....
U6::GN19NGG
-10 +10
ACGAAACACC GNNNNNNNNN.....
U6::AN19NGG
-10 +10
ACGAAACACC ANNNNGNNNN.....
Downstream initiation/truncated gRNA
U6::CN19NGG
-10 +10
ACGAAACACC CNNGNNNNNN.....
Downstream initiation/truncated gRNA
U6::TN19NGG
-10 +10
ACGAAACACC TNNNGNNNNN.....
Downstream initiation/truncated gRNA

FIG. 9A

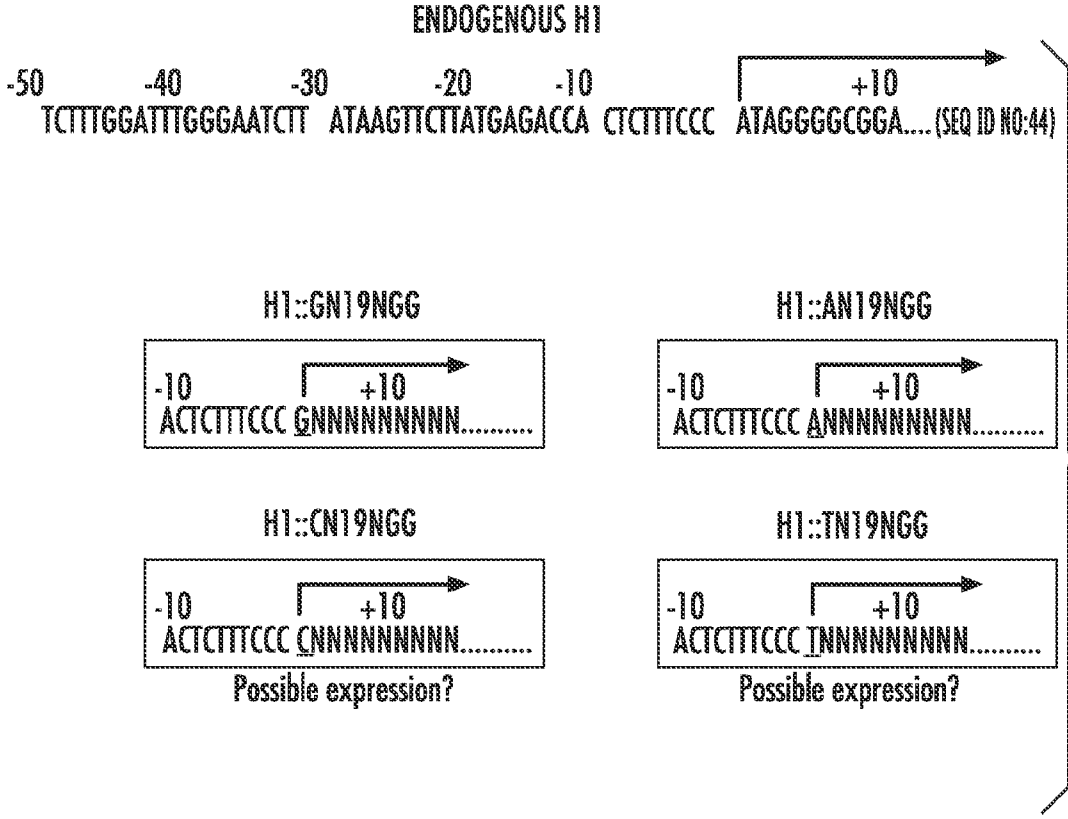

ENDOGENOUS H1
-50 -40 -30 -20 -10 +10
TCTTTGGATTTGGGAATCTT ATAAGTTCTTATGAGACCA CTCTTTCCC ATAGGGGCGGA.... (SEQ ID NO:44)
H1::GN19NGG
-10 +10
ACTCTTTCCC GNNNNNNNNN.....
H1::AN19NGG
-10 +10
ACTCTTTCCC ANNNNNNNNN.....
H1::CN19NGG
-10 +10
ACTCTTTCCC CNNNNNNNNN.....
Possible expression?
H1::TN19NGG
-10 +10
ACTCTTTCCC TNNNNNNNNN.....
Possible expression?

FIG. 9B

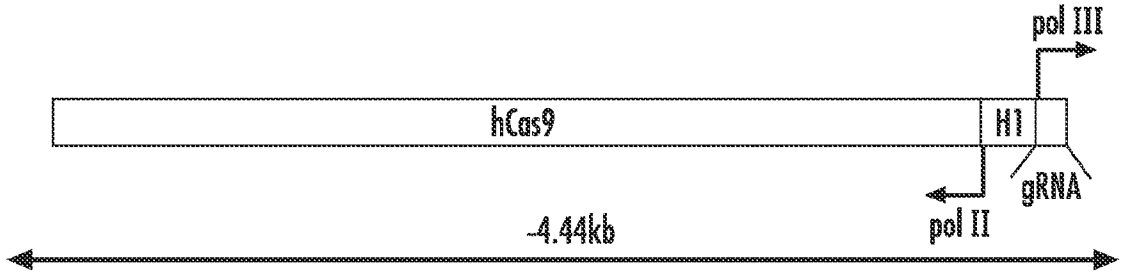


FIG. 10A

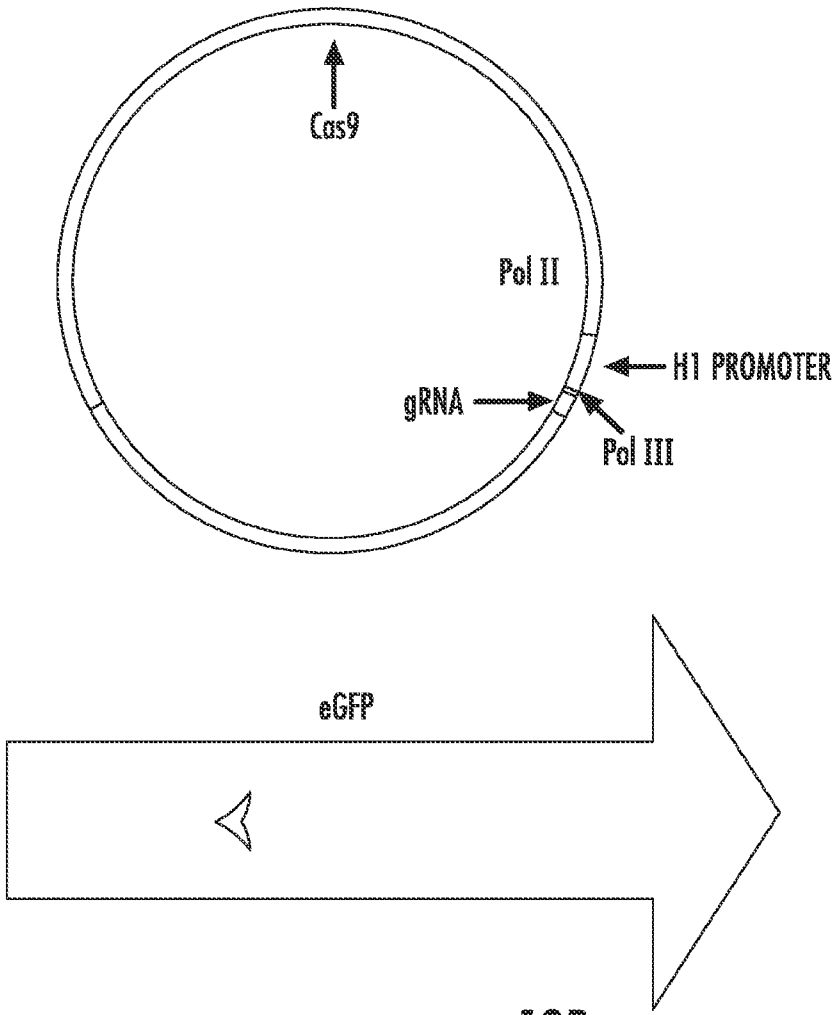


FIG. 10B

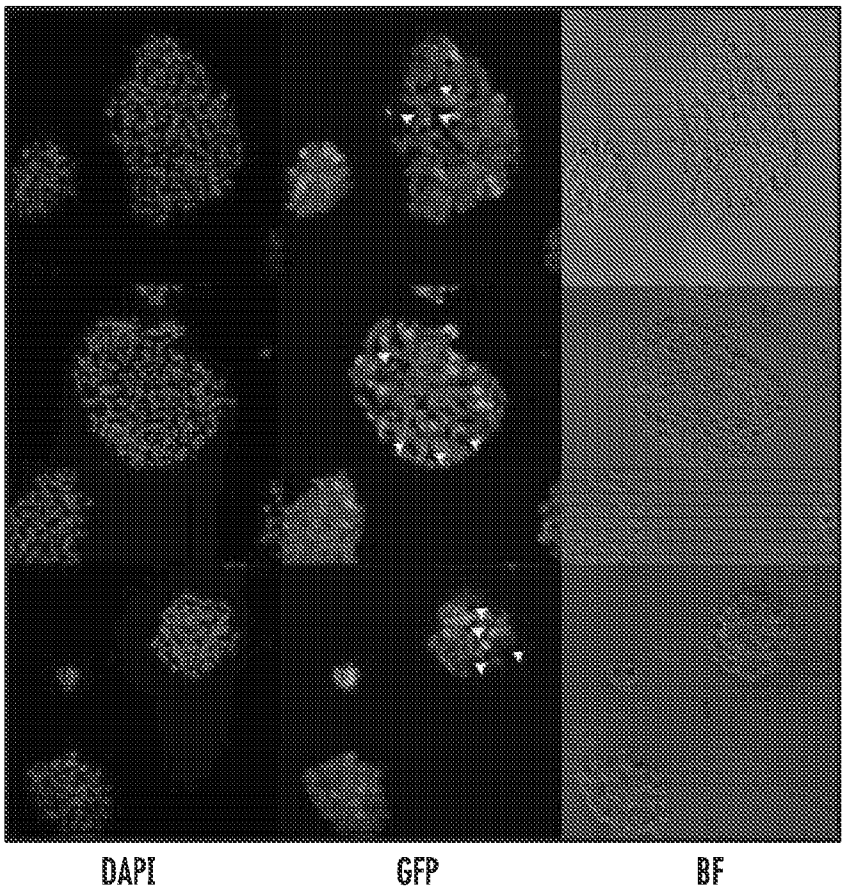


FIG. 10C

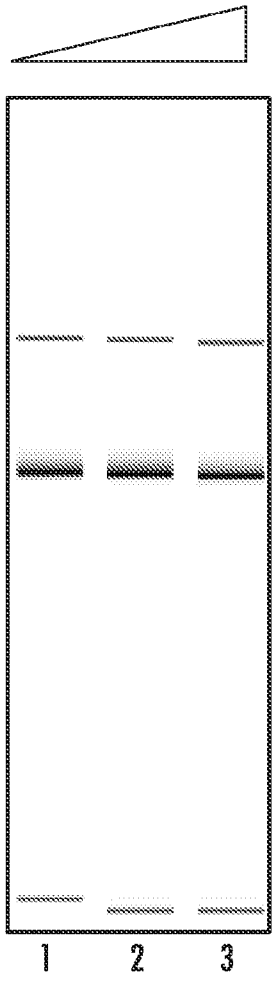


FIG. 10D

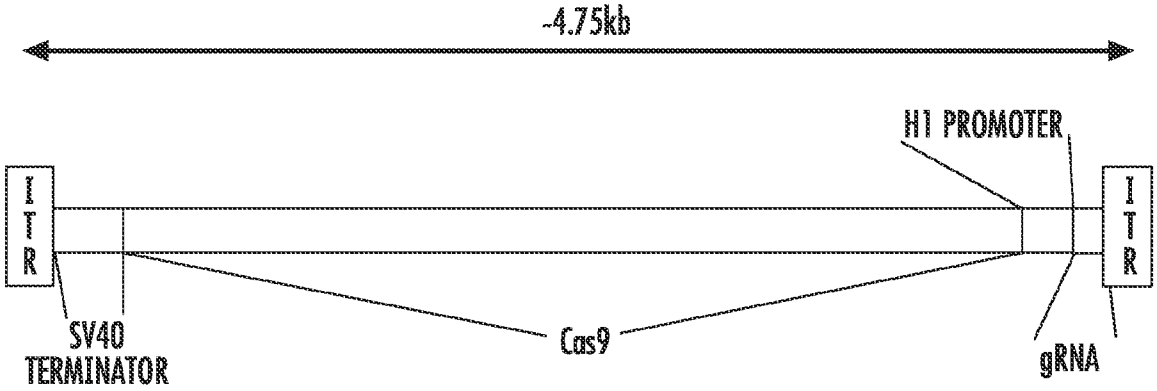


FIG. 10E

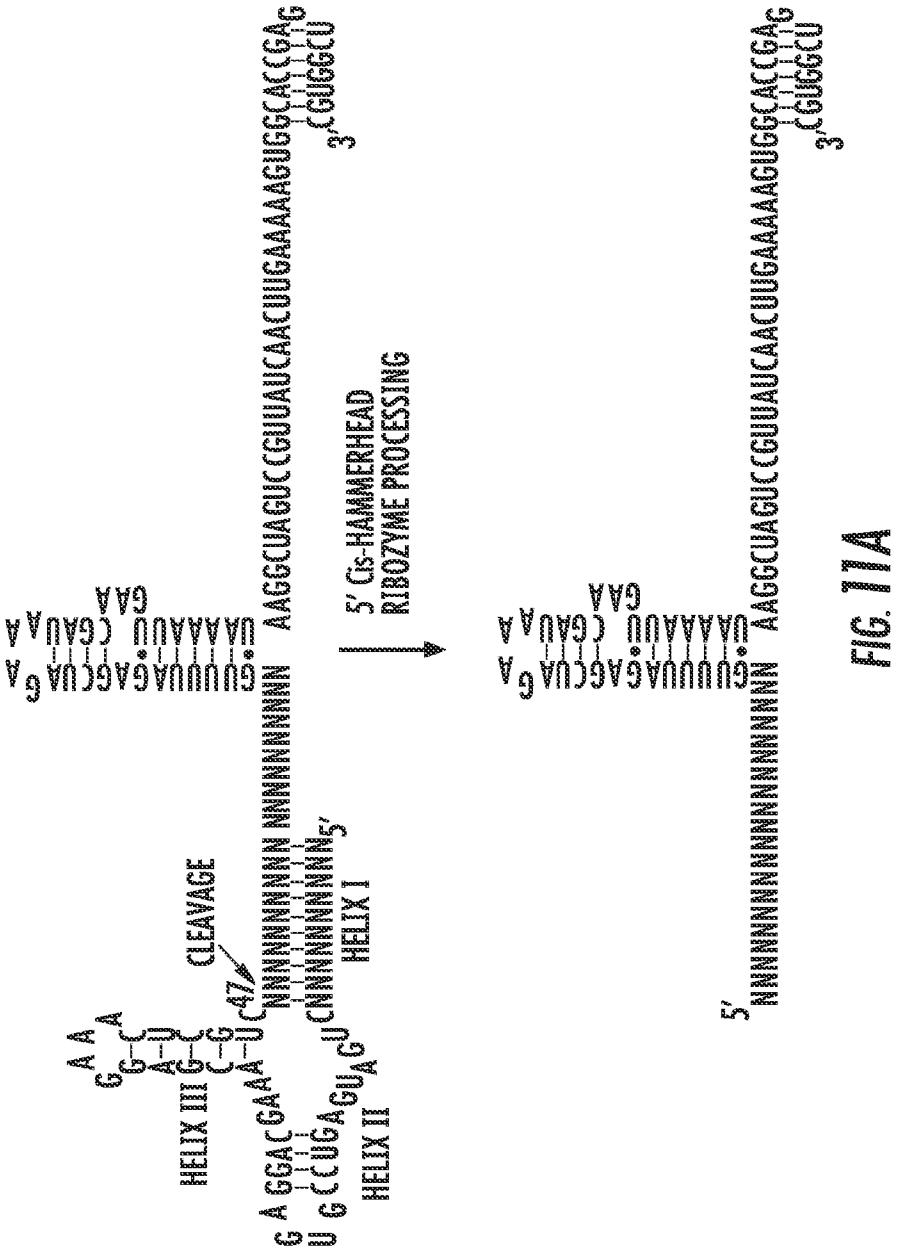

5' Cis-HAMMERHEAD
RIBOZYME PROCESSING
HELIX III
CLEAVAGE
HELIX II
HELIX I
5'
3'

FIG. 17A

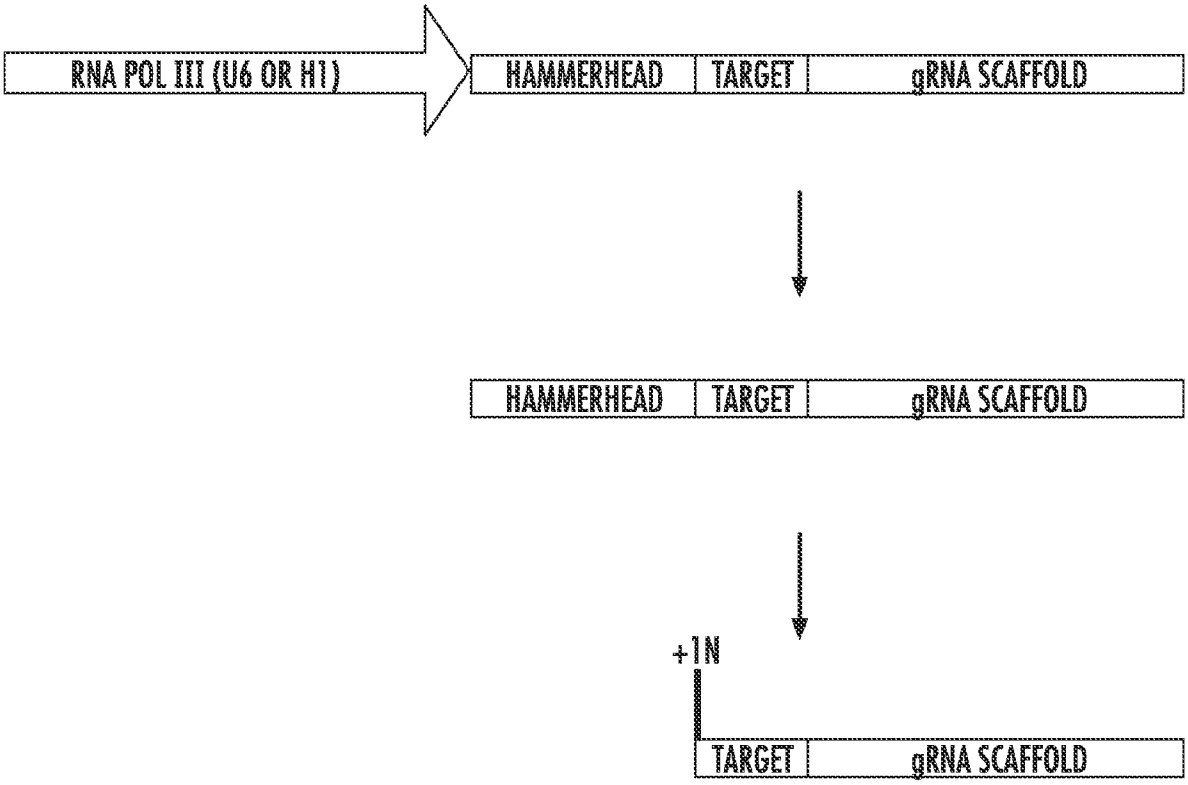

RNA POL III (U6 OR H1)
HAMMERHEAD
TARGET
gRNA SCAFFOLD
HAMMERHEAD
TARGET
gRNA SCAFFOLD
+1N
TARGET
gRNA SCAFFOLD

FIG. 11B

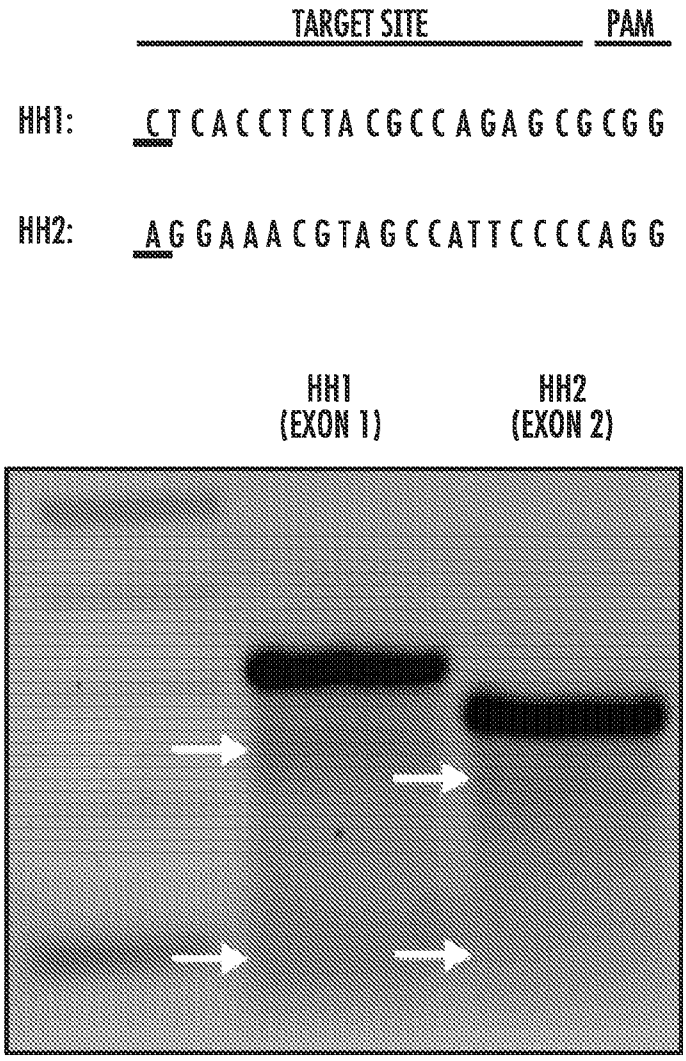

TARGET SITE
PAM
HH1:
CTCACCTCTACGCCAGAGCGCGG
HH2:
AGGAAACGTAGCCATTCCCCAGG
HH1
(EXON 1)
HH2
(EXON 2)

FIG. 11C

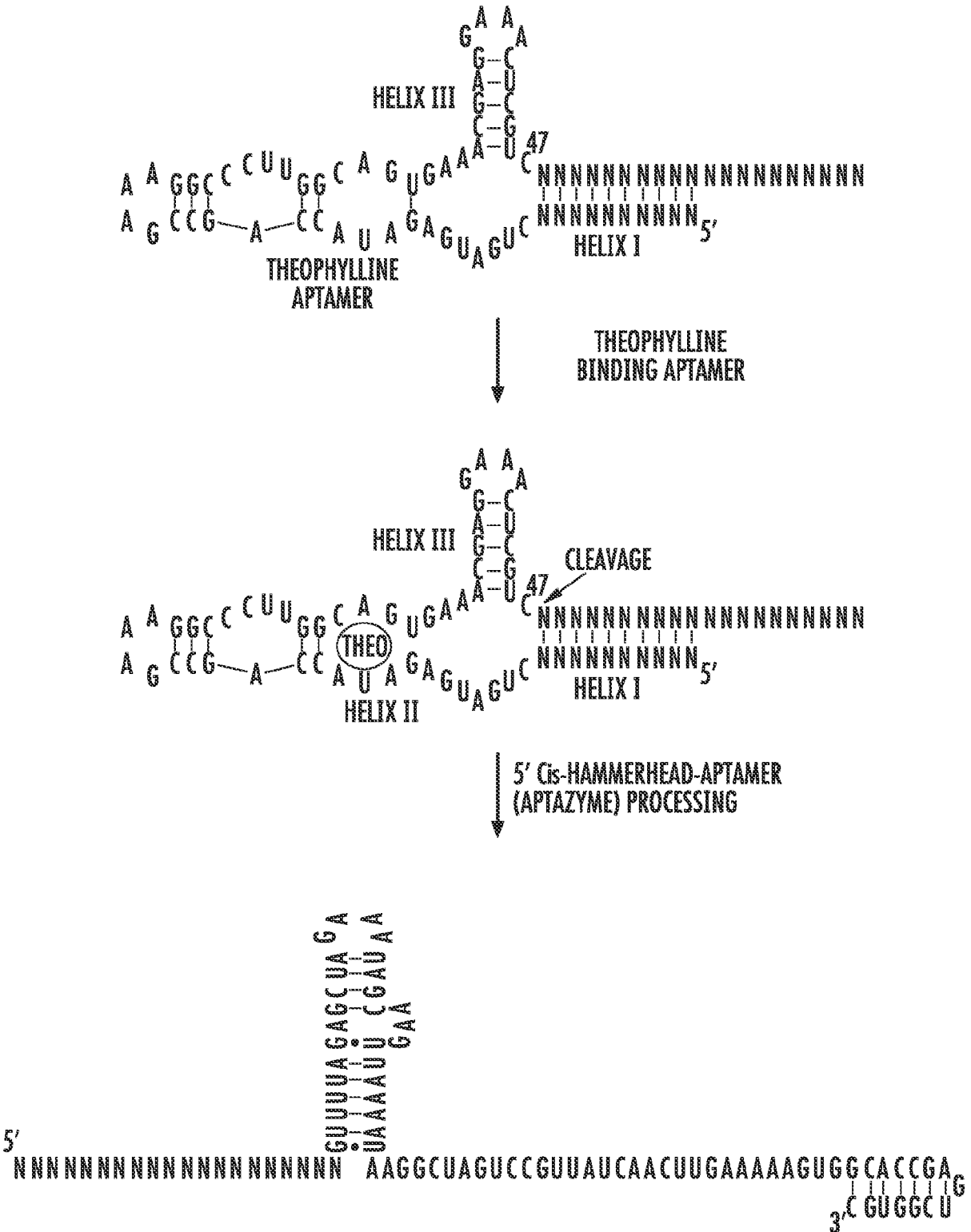

HELIX III
47
HELIX I
5′
THEOPHYLLINE
APTAMER
THEOPHYLLINE
BINDING APTAMER
HELIX III
CLEAVAGE
47
THEO
HELIX II
HELIX I
5′
5′ Cis-HAMMERHEAD-APTAMER
(APTAZYME) PROCESSING
5′
3′

FIG. 12

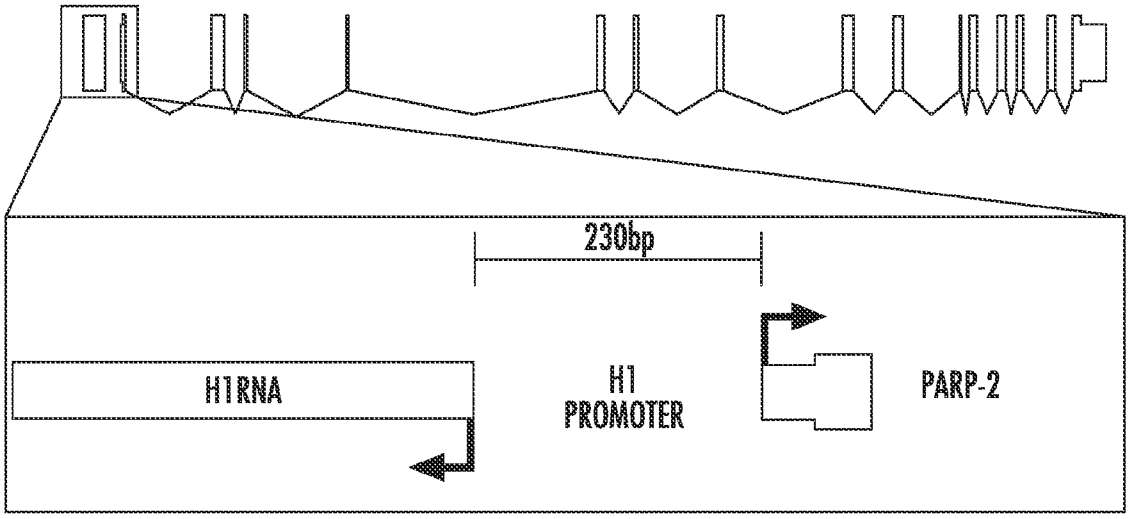


FIG. 13

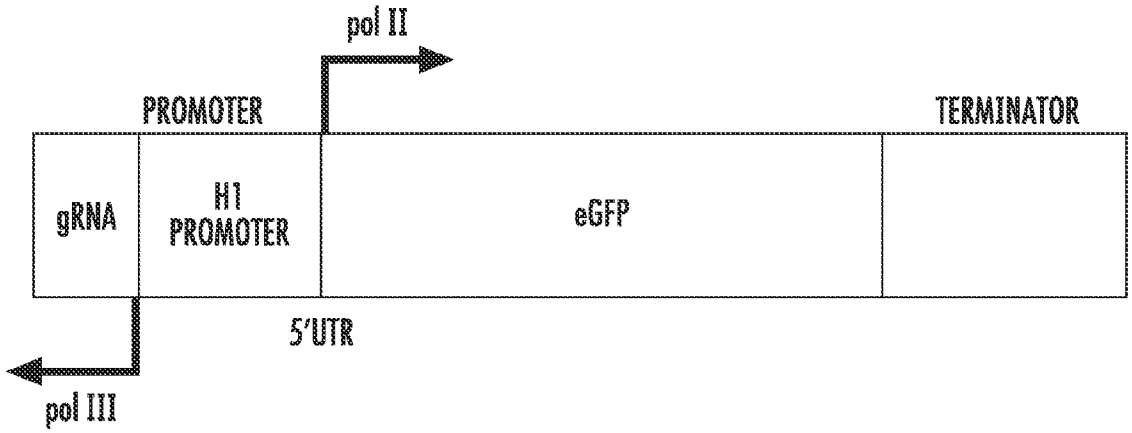

pol II
PROMOTER
TERMINATOR
gRNA
H1
PROMOTER
eGFP
5'UTR
pol III

FIG. 14

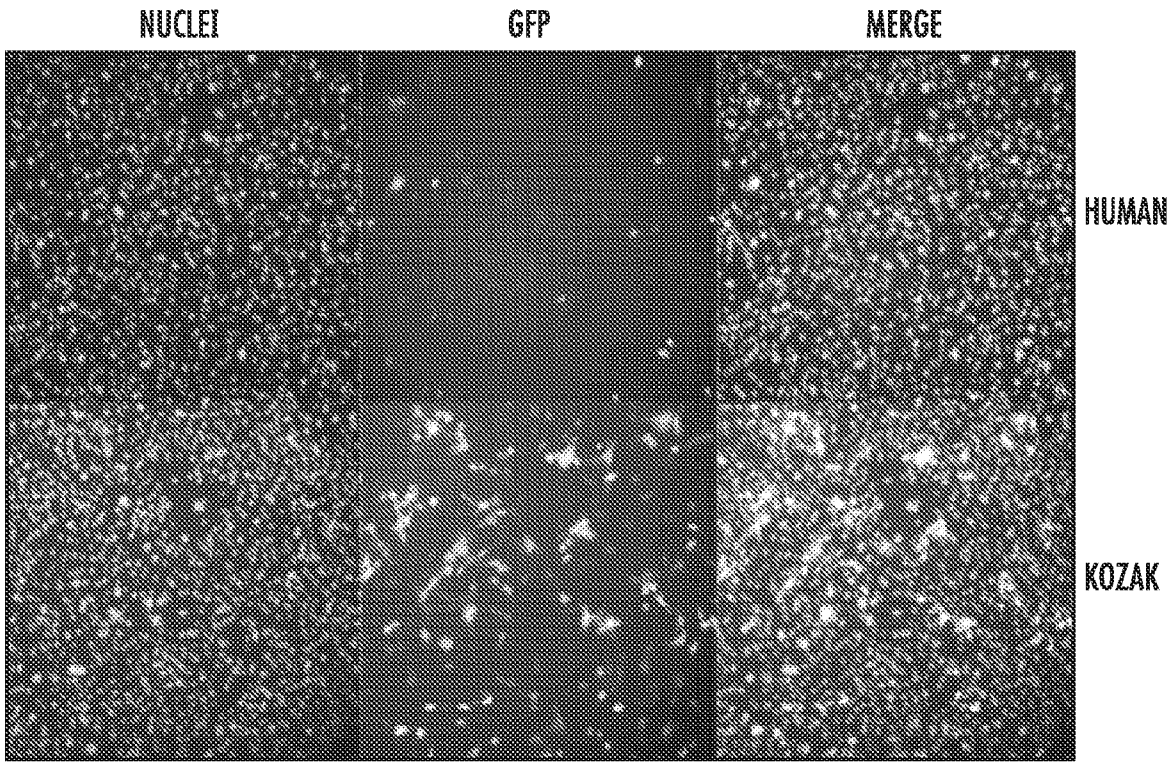


FIG. 15

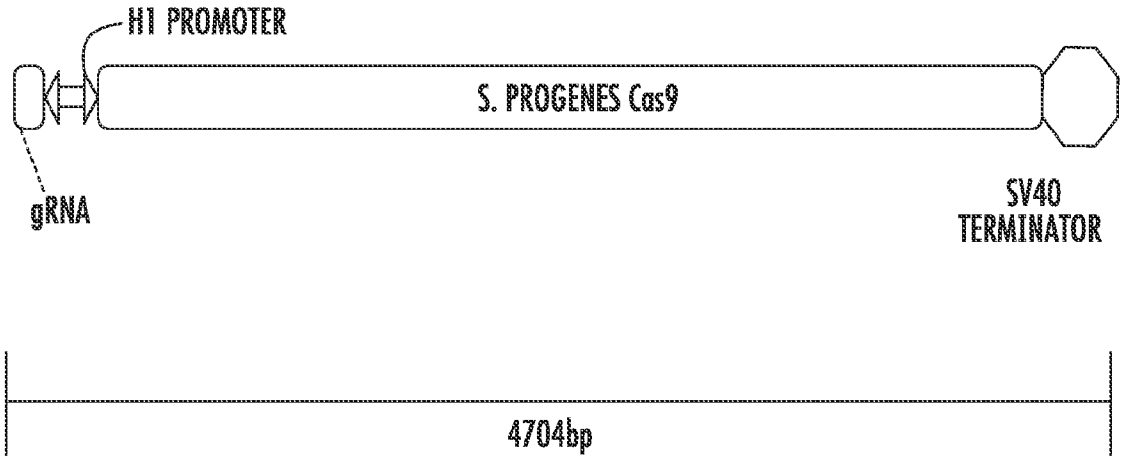


FIG. 16A

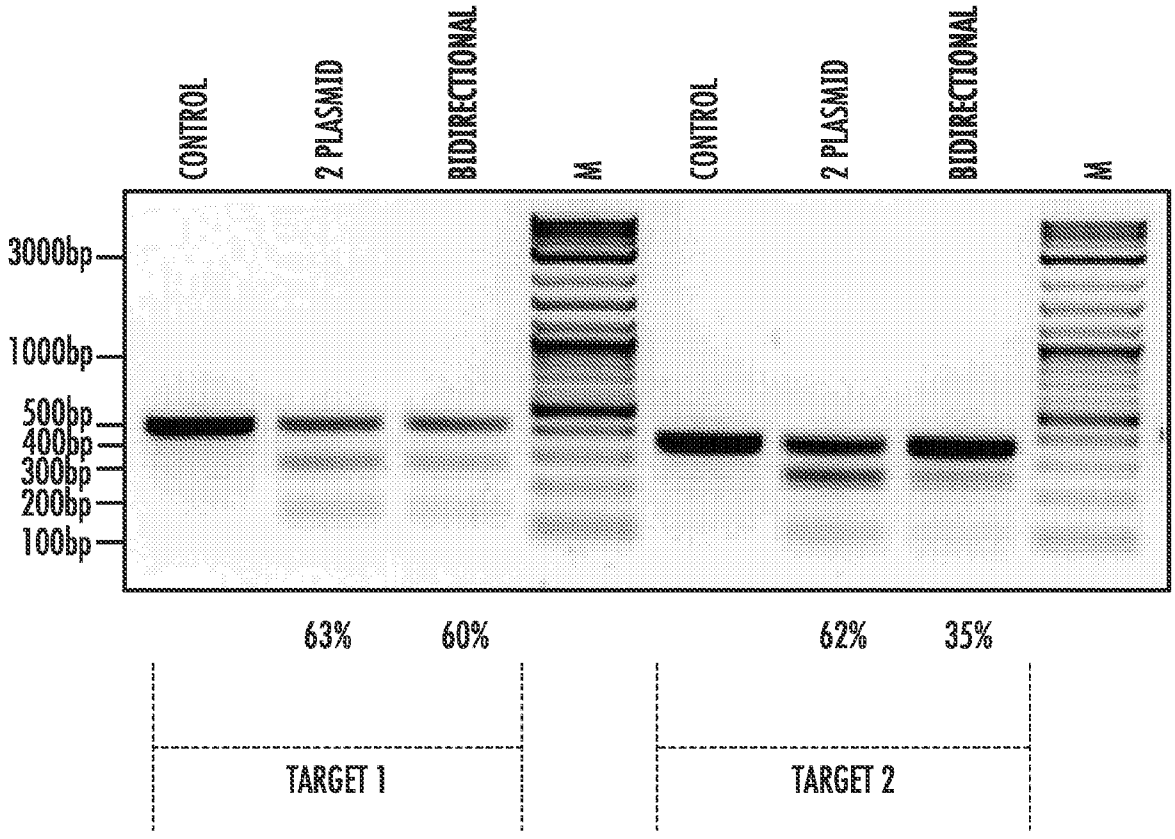


FIG. 16B

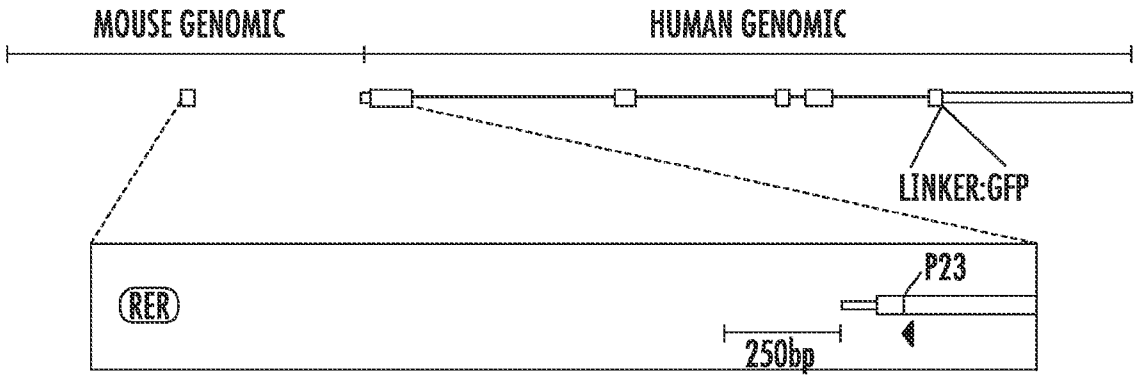


FIG. 17

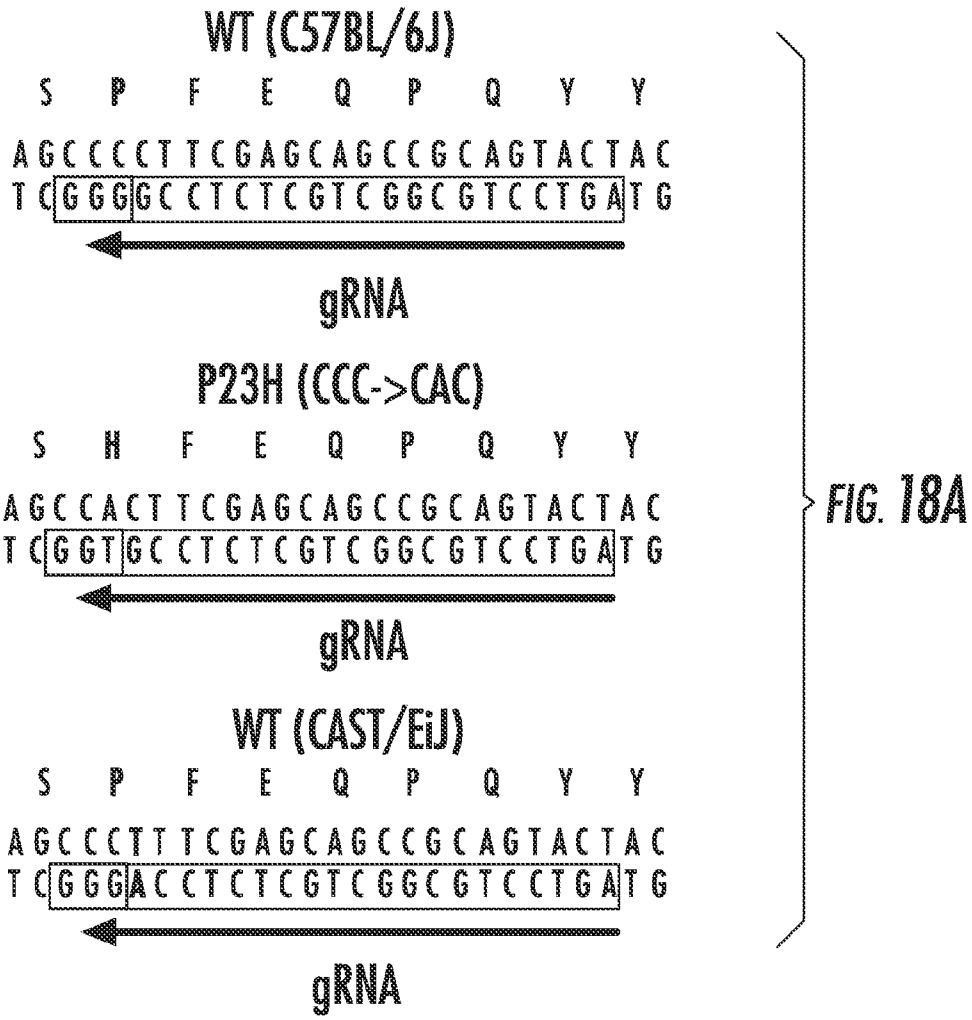


FIG. 18A

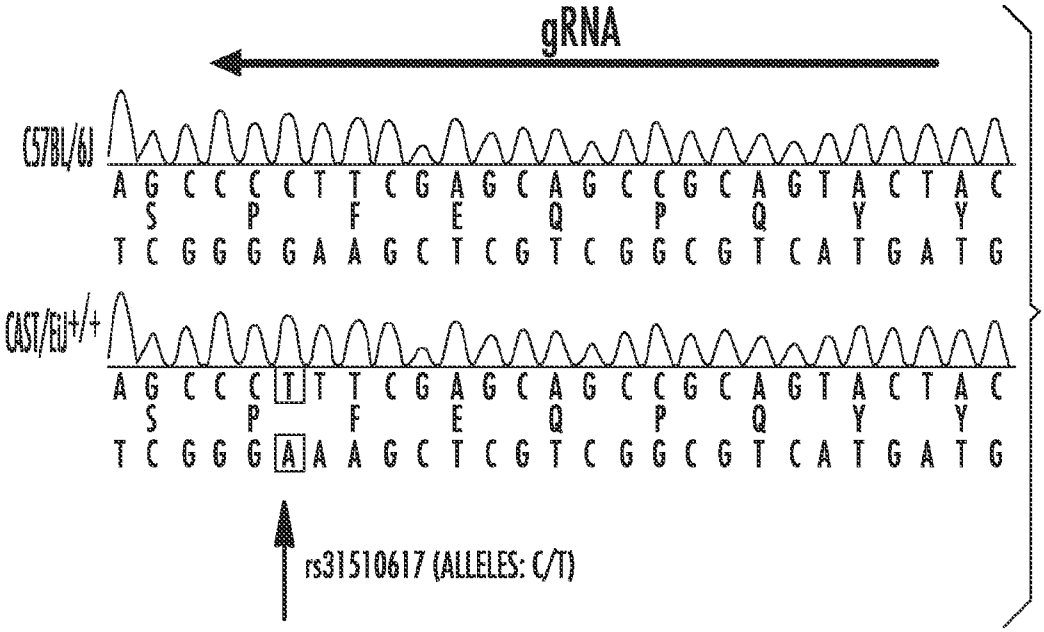


FIG. 18B

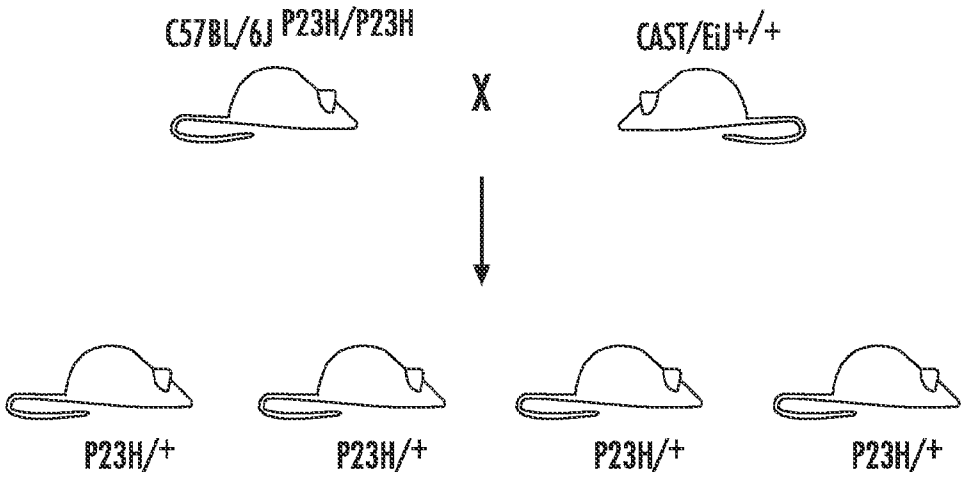


FIG. 18C

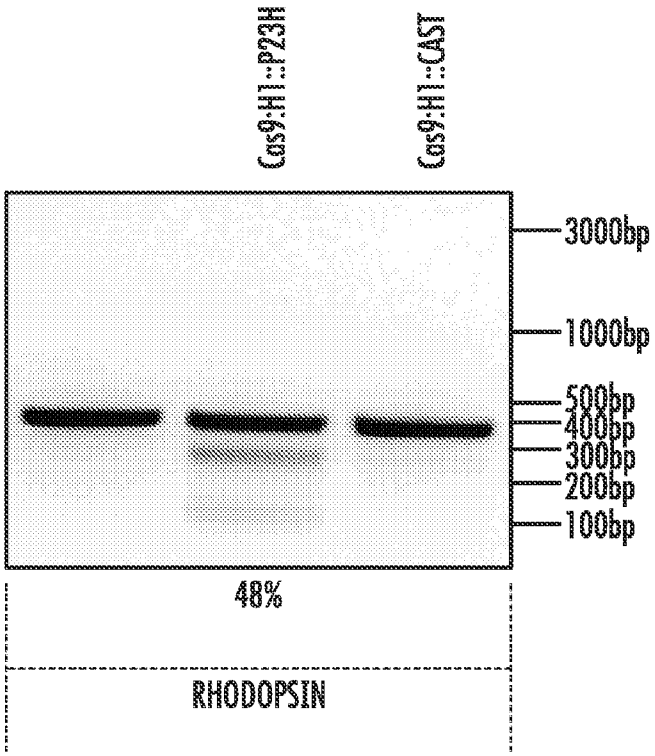

Cas9:H1::P23H
Cas9:H1::CAST
3000bp
1000bp
500bp
400bp
300bp
200bp
100bp
48%
RHODOPSIN

FIG. 19