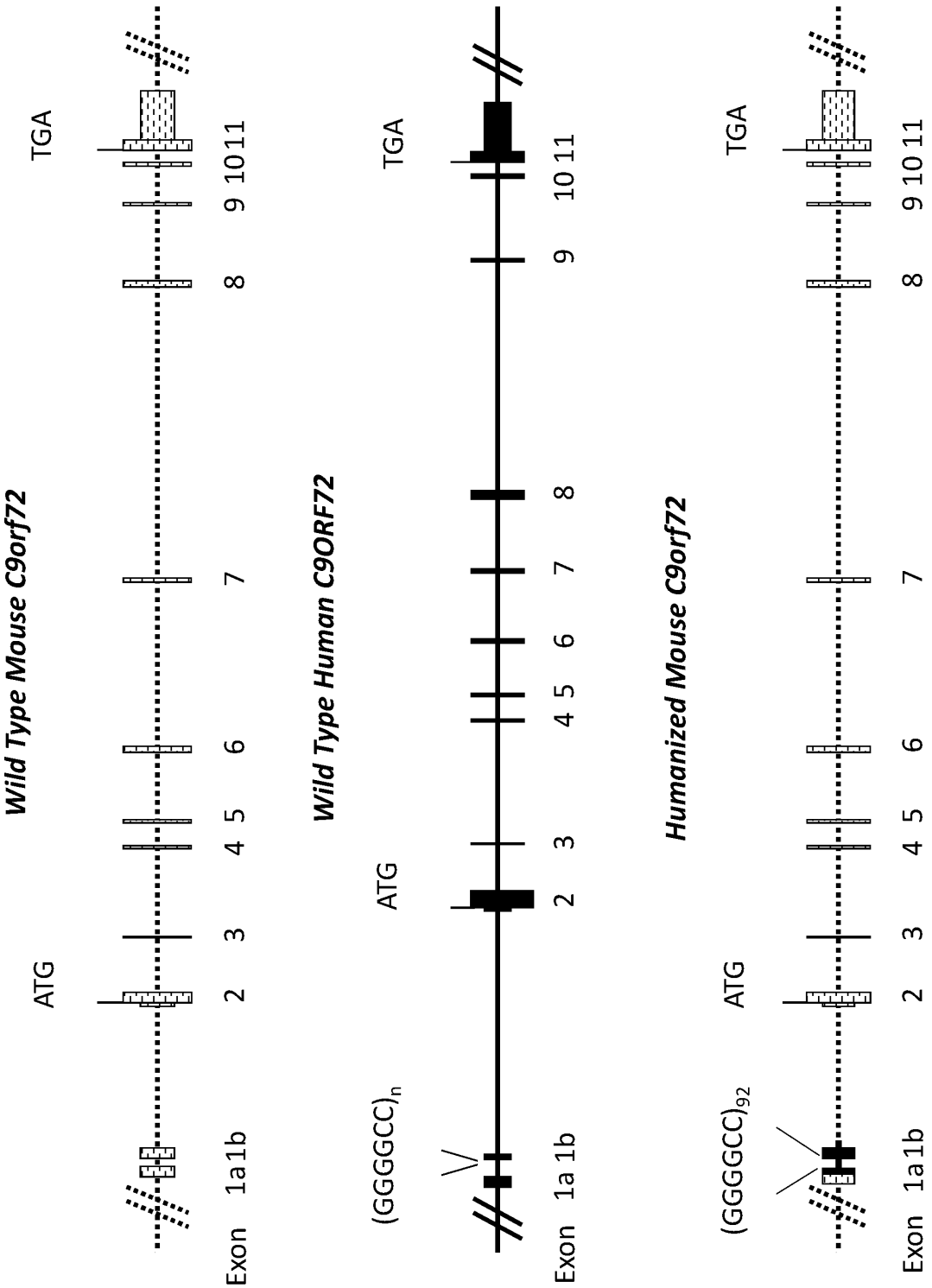

Wild Type Mouse C9orf72
ATG
TGA
Exon 1a 1b 2 3 4 5 6 7 8 9 10 11
Wild Type Human C9ORF72
(GGGGCC)n
ATG
TGA
Exon 1a 1b 2 3 4 5 6 7 8 9 10 11
Humanized Mouse C9orf72
(GGGGCC)92
ATG
TGA
Exon 1a 1b 2 3 4 5 6 7 8 9 10 11

FIG. 1

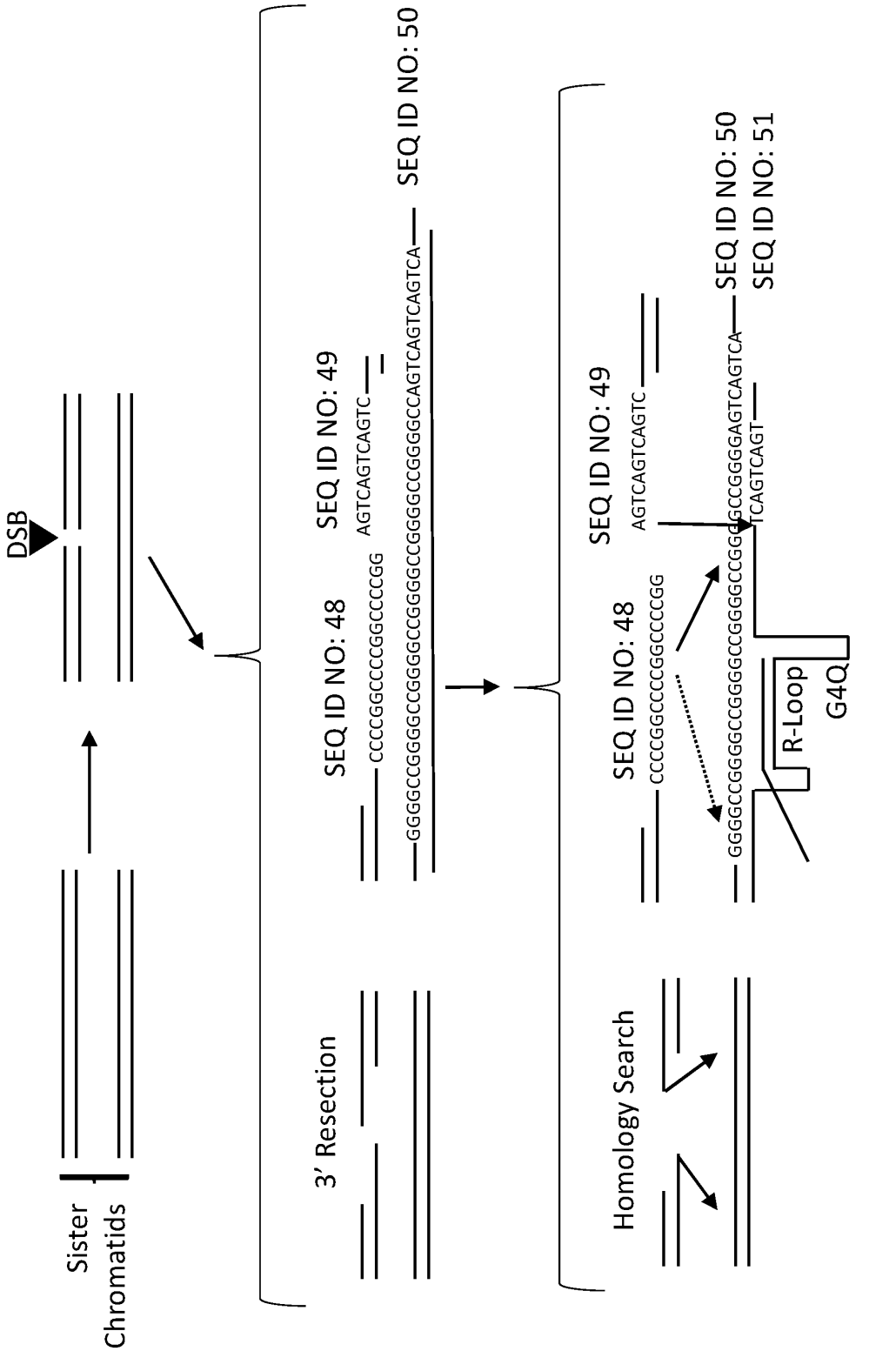

Sister Chromatids
DSB
3' Resection
SEQ ID NO: 48
CCCCGGCCCCGGCCCCGG
SEQ ID NO: 49
AGTCAGTCAGTC
SEQ ID NO: 50
GGGGCCGGGGCCGGGGCCGGGGCCGGGGCCAGTCAGTCAGTCA
Homology Search
SEQ ID NO: 48
CCCCGGCCCCGGCCCCGG
SEQ ID NO: 49
AGTCAGTCAGTC
SEQ ID NO: 50
GGGGCCGGGGCCGGGGCCGGGGCCGGGGCCGGGGAGTCAGTCA
SEQ ID NO: 51
TCAGTCAGT
R-Loop
G4Q

FIG. 2

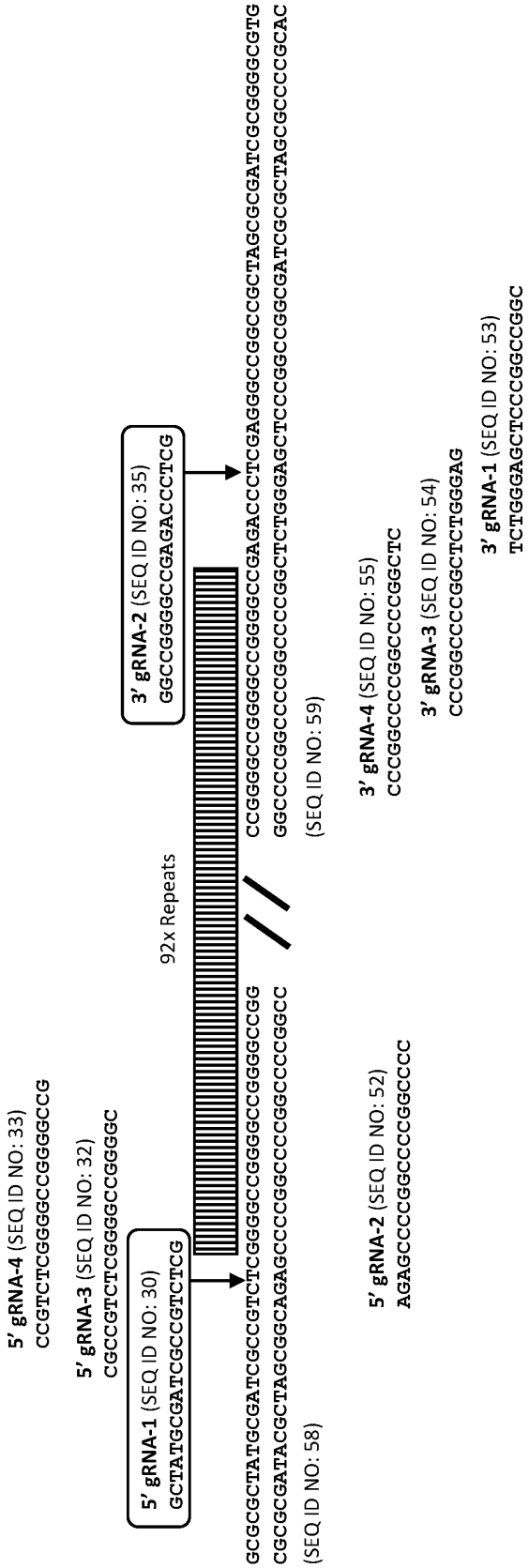

5' gRNA-4 (SEQ ID NO: 33)
CCGTCTCGGGGCCGGGGCCG
5' gRNA-3 (SEQ ID NO: 32)
CGCCGTCTCGGGGCCGGGGC
5' gRNA-1 (SEQ ID NO: 30)
GCTATGCGATCGCCGTCTCG
3' gRNA-2 (SEQ ID NO: 35)
GGCCGGGGCCGAGACCCTCG
92x Repeats
GCGCGCTATGCGATCGCCGTCTCGGGGCCGGGGCCGGG
CGCGCGATACGCTAGCGGCAGAGCCCCGGCCCCGGCCCCGGCC
(SEQ ID NO: 58)
CCGGGGCCGGGGCCGGGGCCGAGACCCTCGAGGGCCGGCCGCTAGCGCGATCGCGGGGCGTG
GGCCCCGGCCCCGGCCCCGGCTCTGGGAGCTCCCGGCCGGCGATCGCGCTAGCGCCCCGCAC
(SEQ ID NO: 59)
5' gRNA-2 (SEQ ID NO: 52)
AGAGCCCCGGCCCCGGCCCC
3' gRNA-4 (SEQ ID NO: 55)
CCCGGCCCCGGCCCCGGCTC
3' gRNA-3 (SEQ ID NO: 54)
CCCGGCCCCGGCTCTGGGAG
3' gRNA-1 (SEQ ID NO: 53)
TCTGGGAGCTCCCCGGCCGGC

FIG. 3

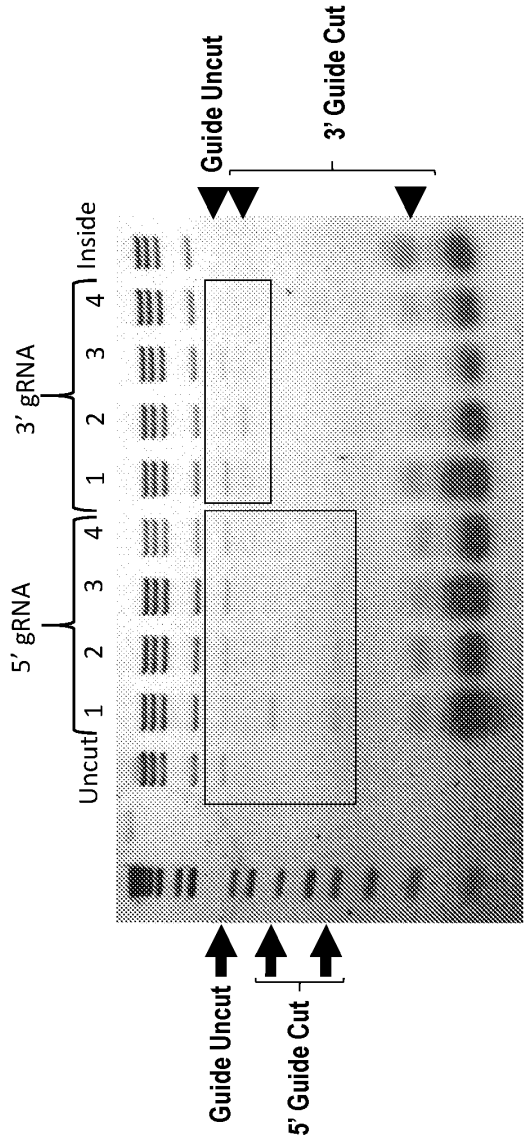

Guide Uncut
3' Guide Cut
5' gRNA
3' gRNA
Uncut
Inside
1
2
3
4
Guide Uncut
5' Guide Cut

FIG. 4

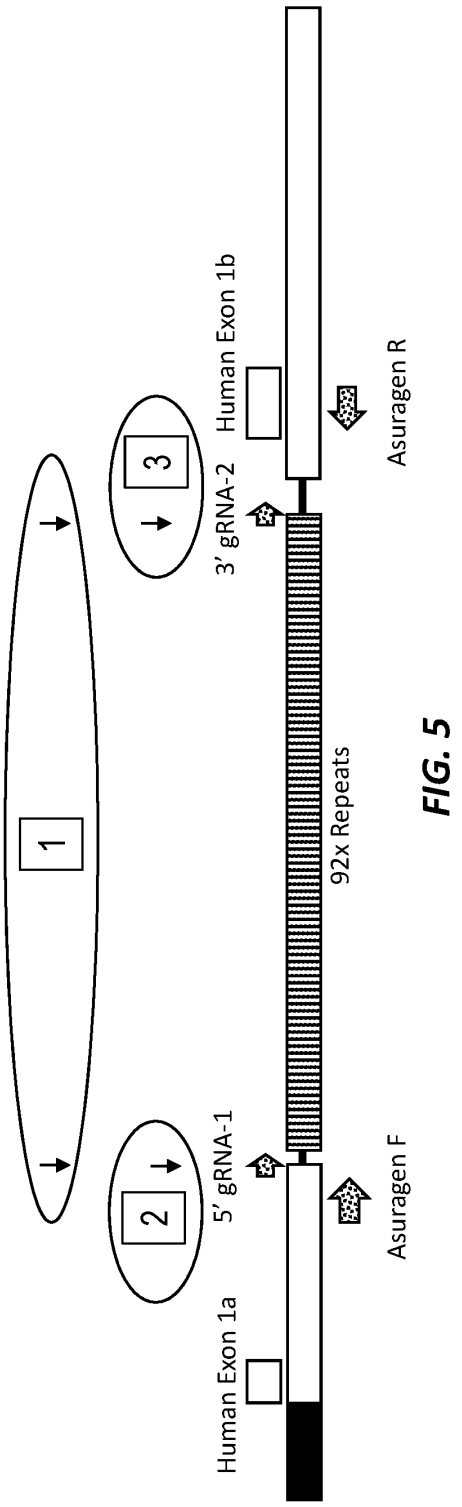

1
2
3
Human Exon 1a
5' gRNA-1
3' gRNA-2
Human Exon 1b
92x Repeats
Asuragen F
Asuragen R

FIG. 5

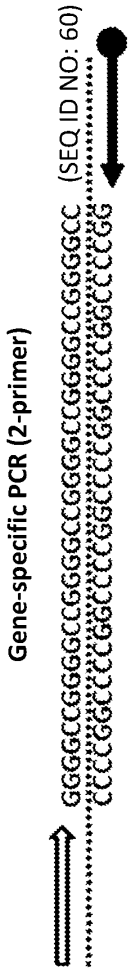

Gene-specific PCR (2-primer)
GGGGCCGGGGCCGGGGCCGGGGCCGGGGCCGGGGCC (SEQ ID NO: 60)
CCCCGGCCCCGGCCCCGGCCCCGGCCCCGGCCCCGGG

FIG. 6A

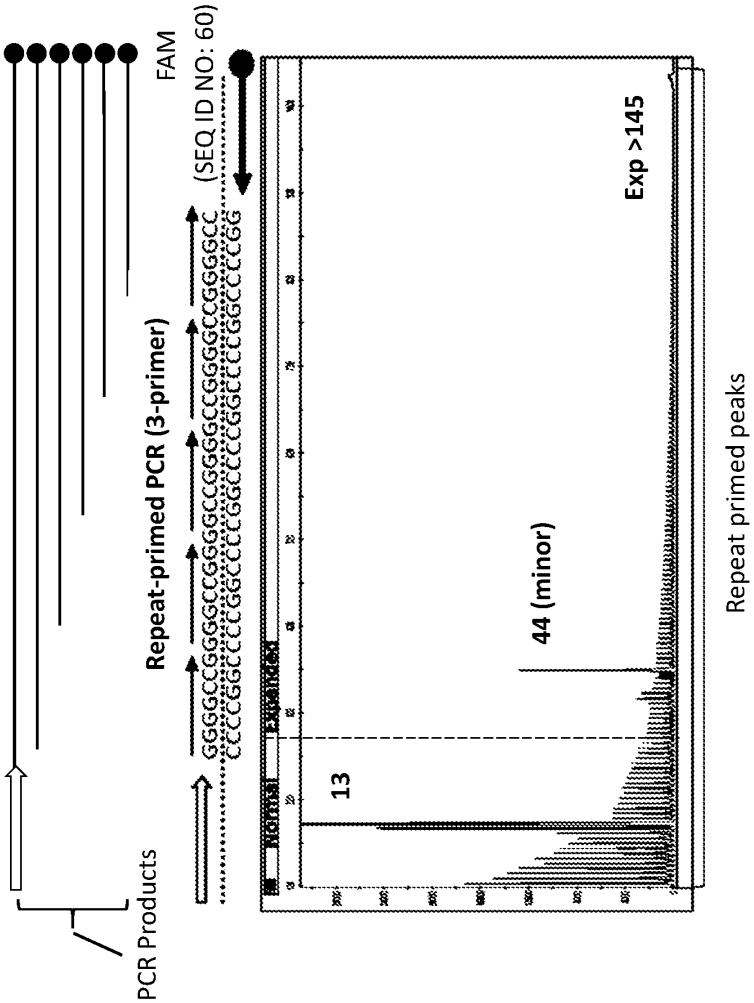

Repeat-primed PCR (3-primer)
FAM
(SEQ ID NO: 60)
PCR Products
Normal
Expanded
13
44 (minor)
Exp >145
Repeat primed peaks

FIG. 6B

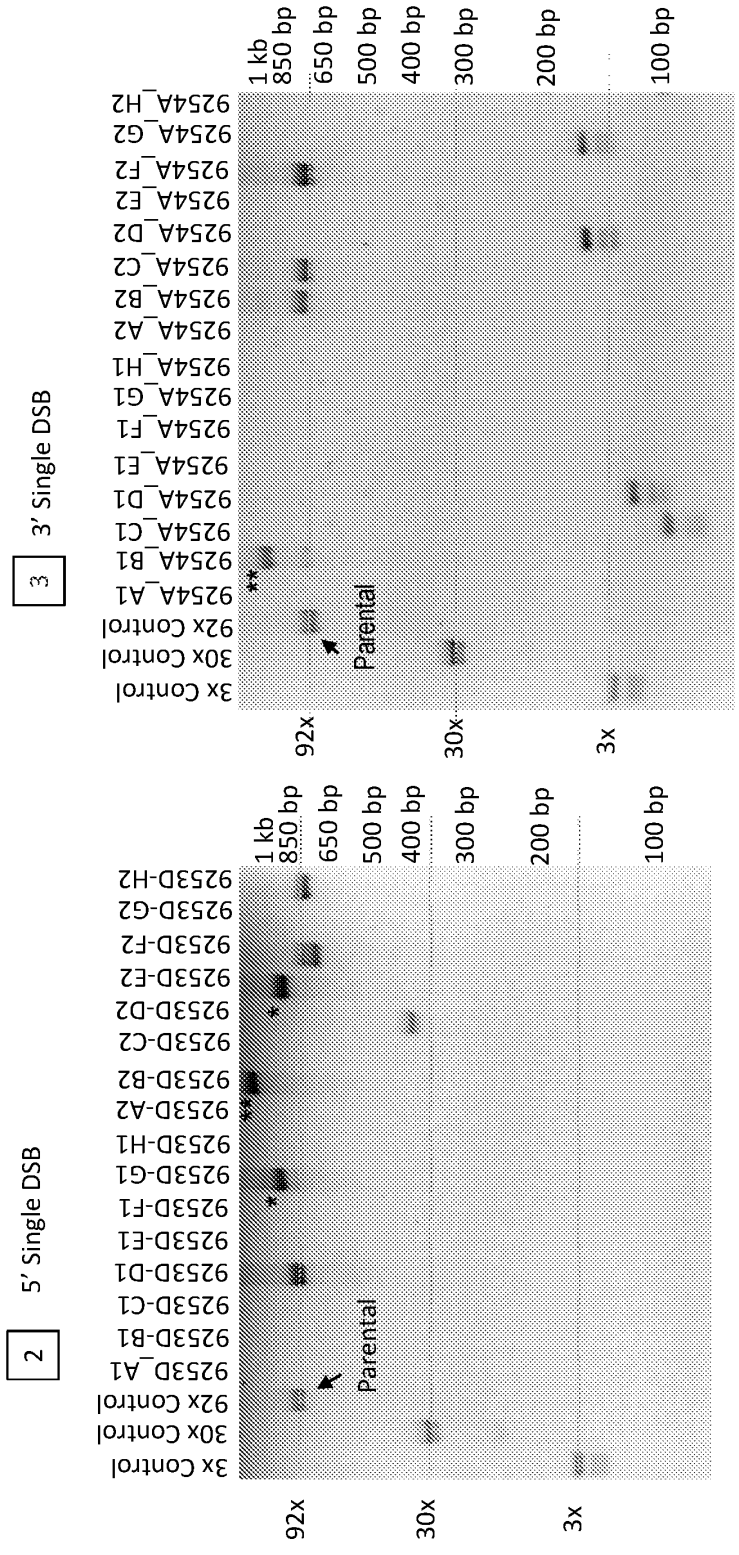

2
5' Single DSB
3x Control
30x Control
92x Control
9253D_A1
9253D-B1
9253D-C1
9253D-D1
9253D-E1
9253D-F1
9253D-G1
9253D-H1
9253D-A2
9253D-B2
9253D-C2
9253D-D2
9253D-E2
9253D-F2
9253D-G2
9253D-H2
Parental
92x
30x
3x
1 kb
850 bp
650 bp
500 bp
400 bp
300 bp
200 bp
100 bp

FIG. 7A

3
3' Single DSB
3x Control
30x Control
92x Control
9254A_A1
9254A_B1
9254A_C1
9254A_D1
9254A_E1
9254A_F1
9254A_G1
9254A_H1
9254A_A2
9254A_B2
9254A_C2
9254A_D2
9254A_E2
9254A_F2
9254A_G2
9254A_H2
Parental
92x
30x
3x
1 kb
850 bp
650 bp
500 bp
400 bp
300 bp
200 bp
100 bp

FIG. 7B

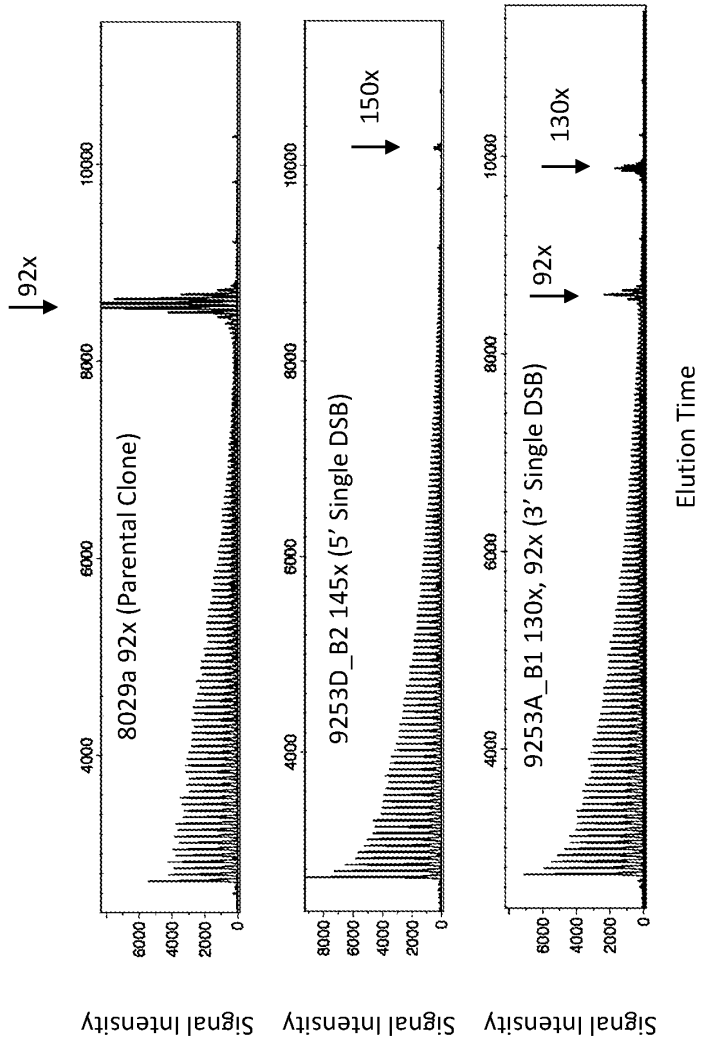

92x
8029a 92x (Parental Clone)
9253D_B2 145x (5' Single DSB)
150x
9253A_B1 130x, 92x (3' Single DSB)
92x
130x
Signal Intensity
Signal Intensity
Signal Intensity
Elution Time

FIG. 8

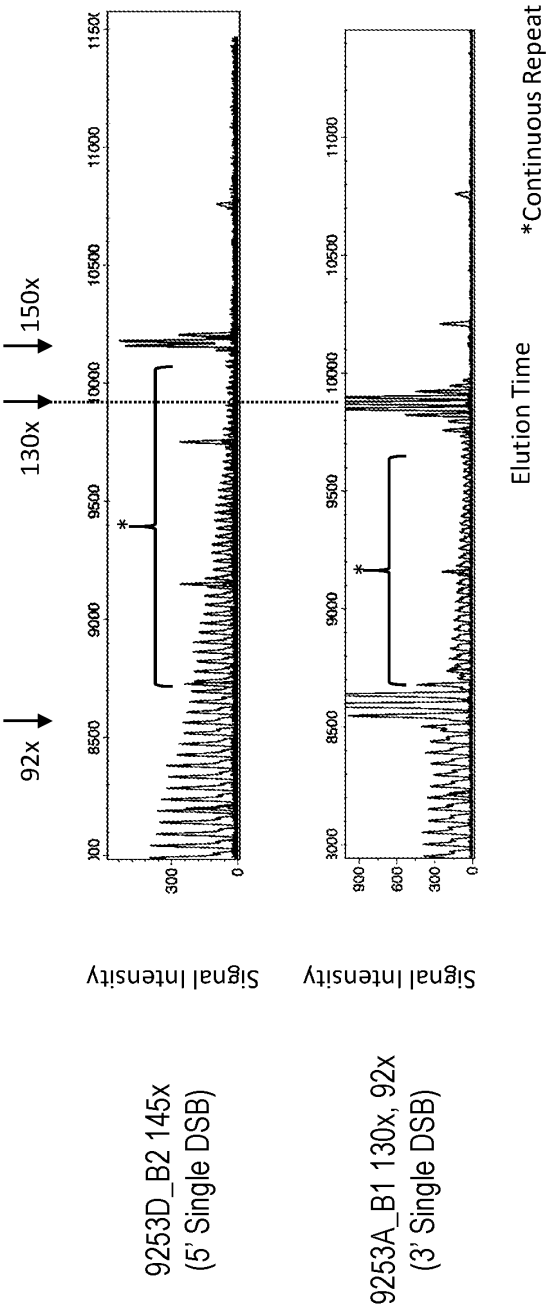

92x
130x
150x
9253D_B2 145x
(5' Single DSB)
9253A_B1 130x, 92x
(3' Single DSB)
Signal Intensity
Signal Intensity
Elution Time
*Continuous Repeat

FIG. 9

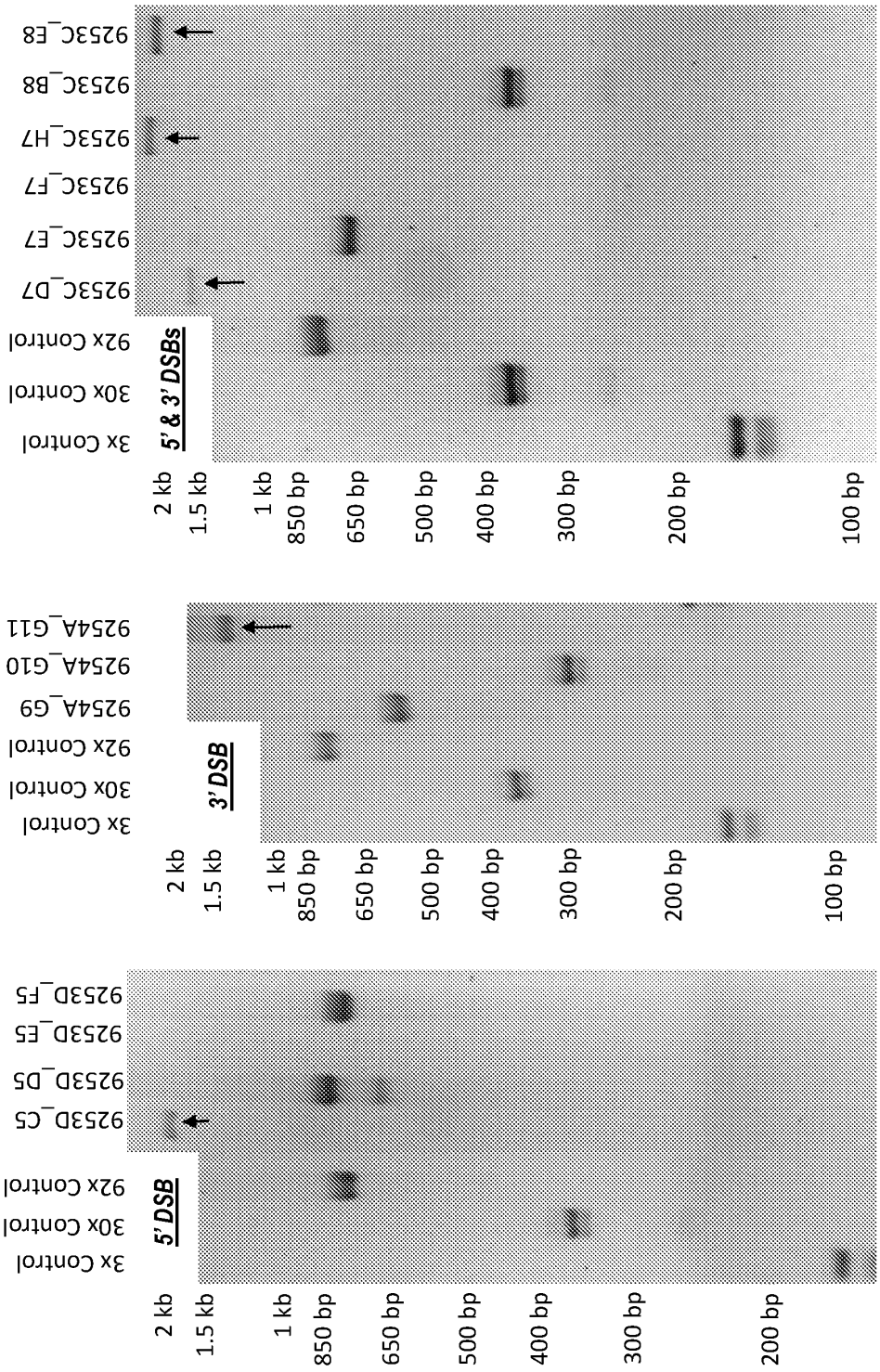

5' DSB
3x Control
30x Control
92x Control
9253D_C5
9253D_D5
9253D_E5
9253D_F5
3' DSB
3x Control
30x Control
92x Control
9254A_G9
9254A_G10
9254A_G11
5' & 3' DSBs
3x Control
30x Control
92x Control
9253C_D7
9253C_E7
9253C_F7
9253C_H7
9253C_B8
9253C_E8
2 kb
1.5 kb
1 kb
850 bp
650 bp
500 bp
400 bp
300 bp
200 bp
100 bp

FIG. 10

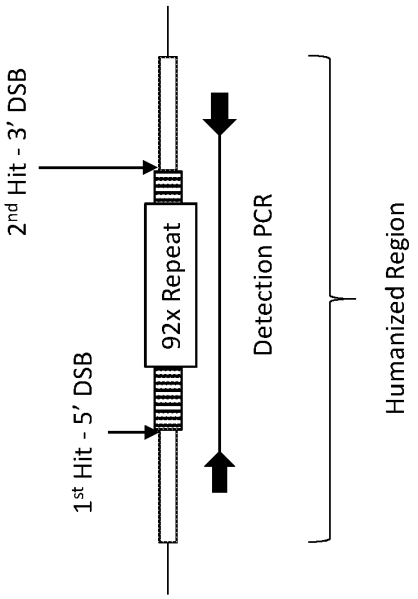

2nd Hit - 3' DSB
1st Hit - 5' DSB
92x Repeat
Detection PCR
Humanized Region

FIG. 11A

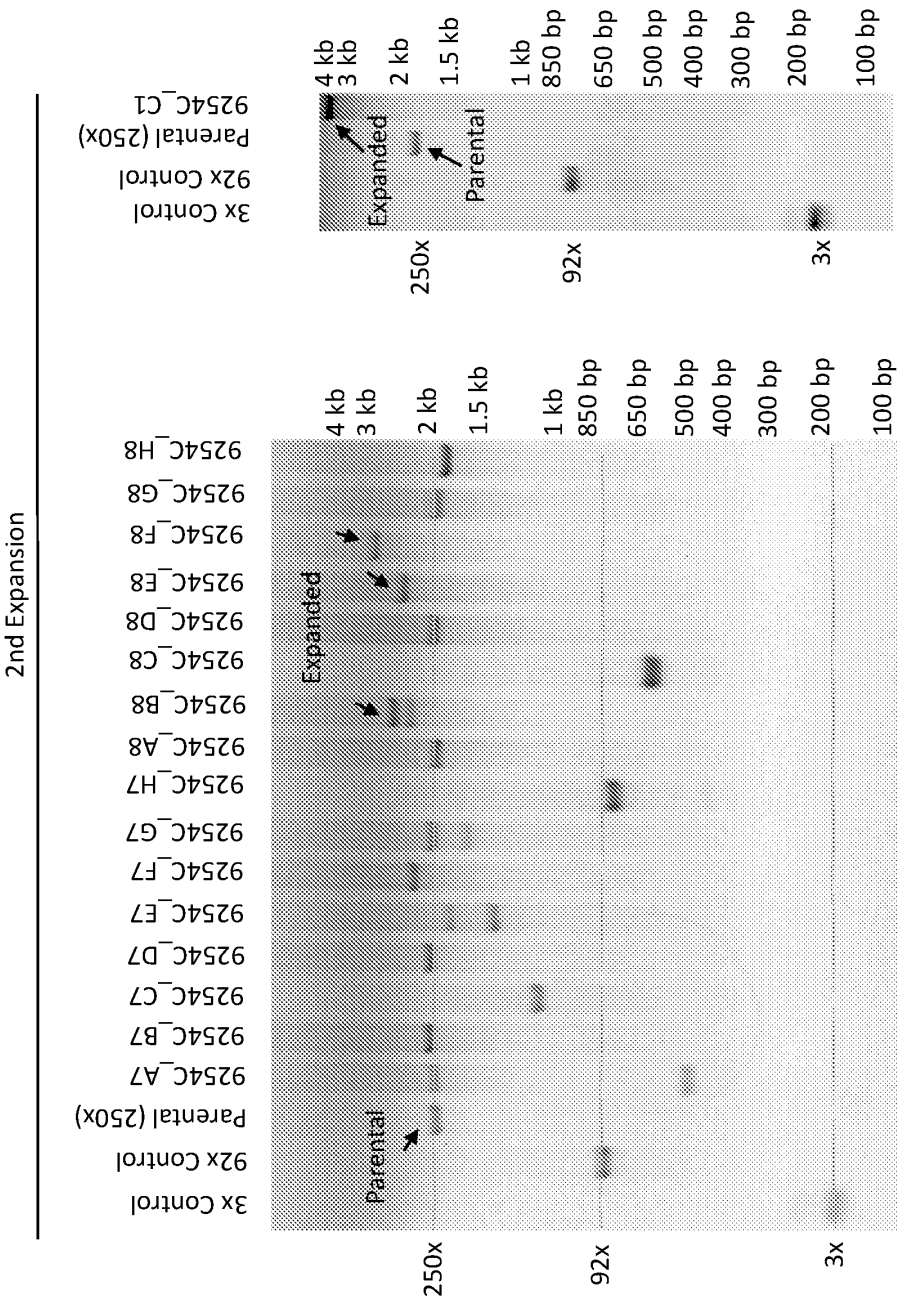

2nd Expansion
3x Control
92x Control
Parental (250x)
9254C_A7
9254C_B7
9254C_C7
9254C_D7
9254C_E7
9254C_F7
9254C_G7
9254C_H7
9254C_A8
9254C_B8
9254C_C8
9254C_D8
9254C_E8
9254C_F8
9254C_G8
9254C_H8
Parental
Expanded
250x
92x
3x
4 kb
3 kb
2 kb
1.5 kb
1 kb
850 bp
650 bp
500 bp
400 bp
300 bp
200 bp
100 bp
3x Control
92x Control
Parental (250x)
9254C_C1
Expanded
Parental

FIG. 11B

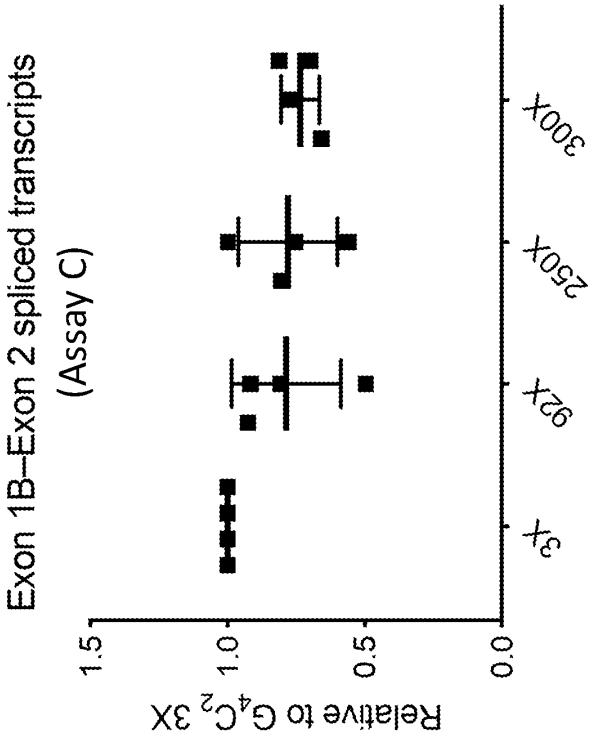


FIG. 12B

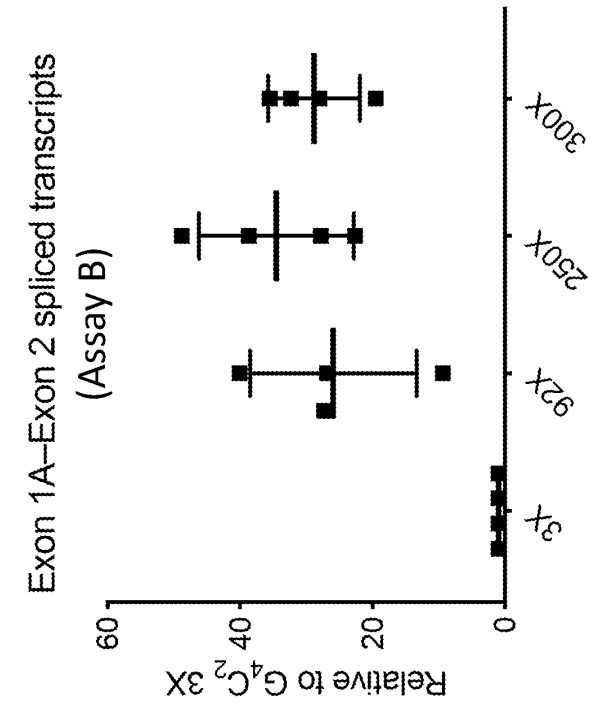


FIG. 12A

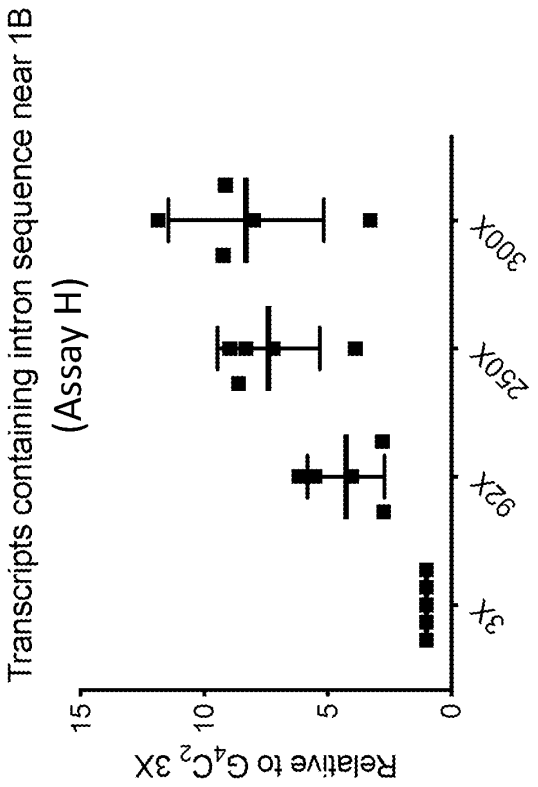


FIG. 12D

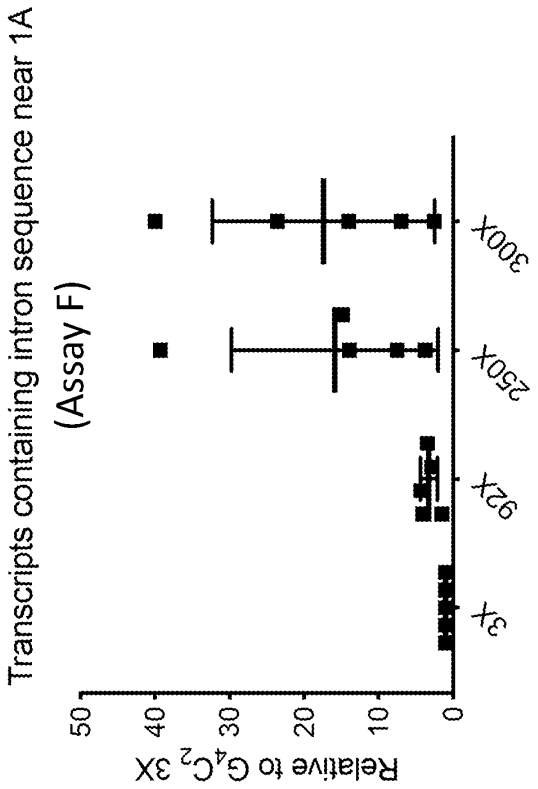


FIG. 12C

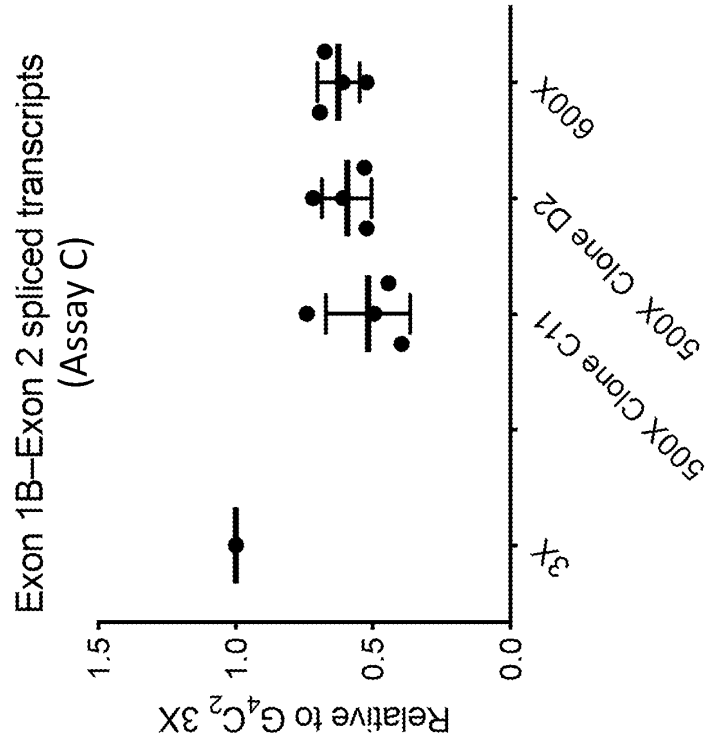


FIG. 12F

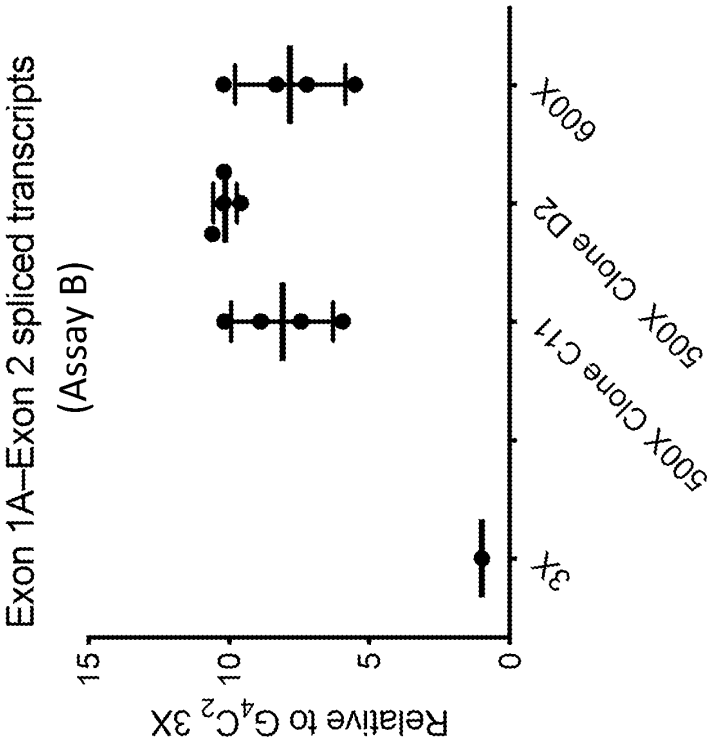


FIG. 12E

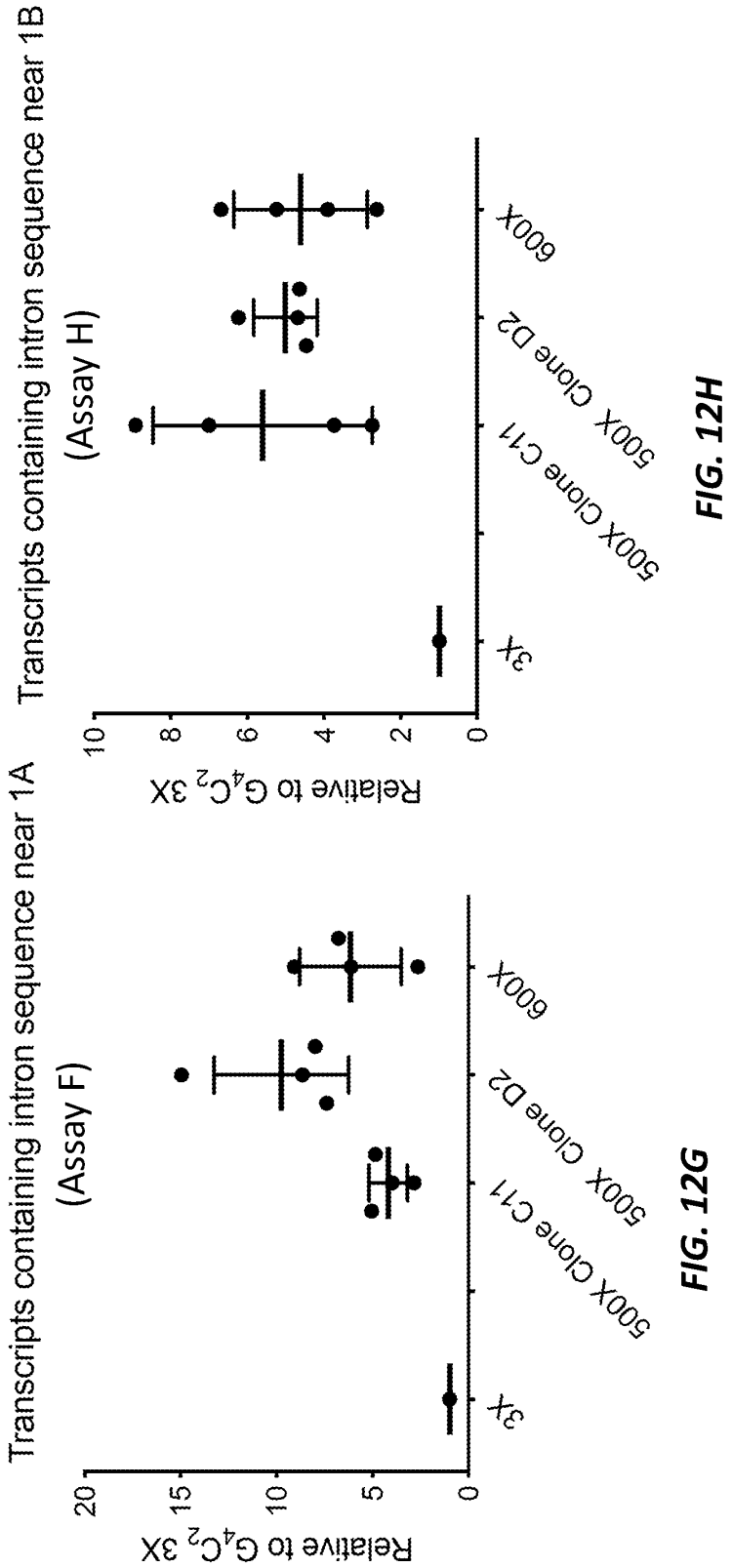


FIG. 12G

FIG. 12H

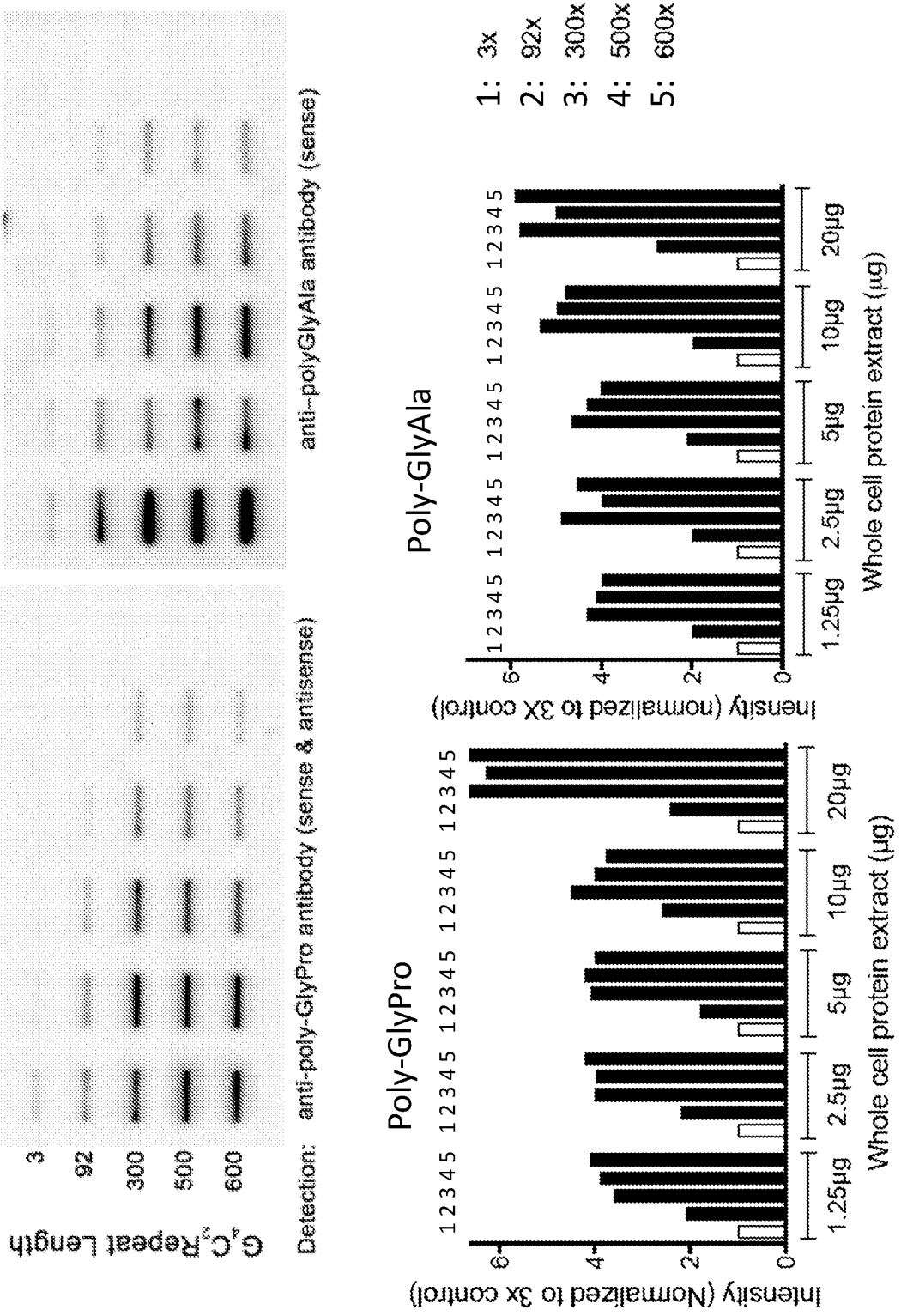

Whole Cell Extract Protein (µg)
20 10 5 2.5 1.25 0 20 10 5 2.5 1.25 0
3
92
300
500
600
G4C2 Repeat Length
Detection: anti-poly-GlyPro antibody (sense & antisense)
anti-polyGlyAla antibody (sense)
Poly-GlyPro
Poly-GlyAla
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
1.25µg 2.5µg 5µg 10µg 20µg
Whole cell protein extract (µg)
Intensity (Normalized to 3x control)
Intensity (normalized to 3X control)
0 2 4 6
1: 3x
2: 92x
3: 300x
4: 500x
5: 600x

FIG. 13

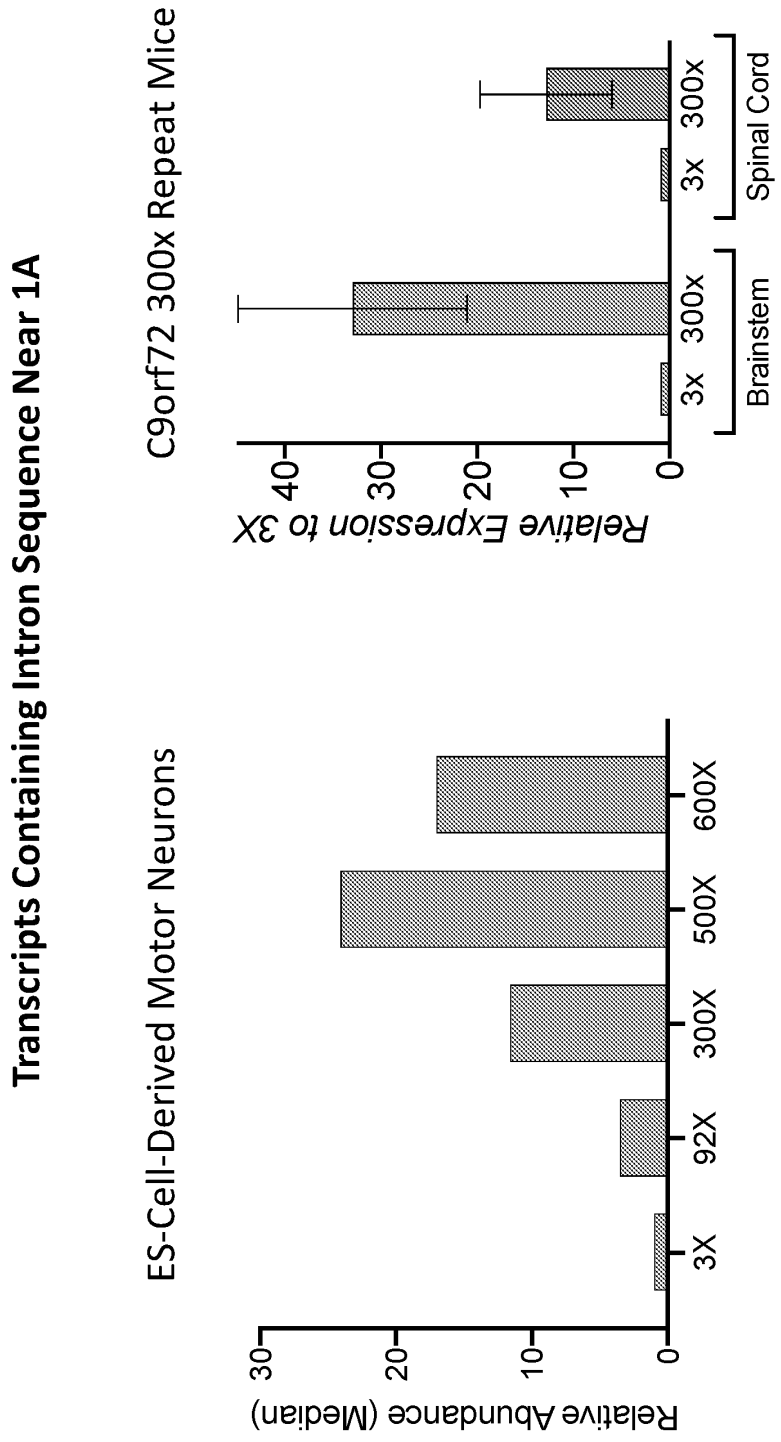


FIG. 14A

FIG. 14B

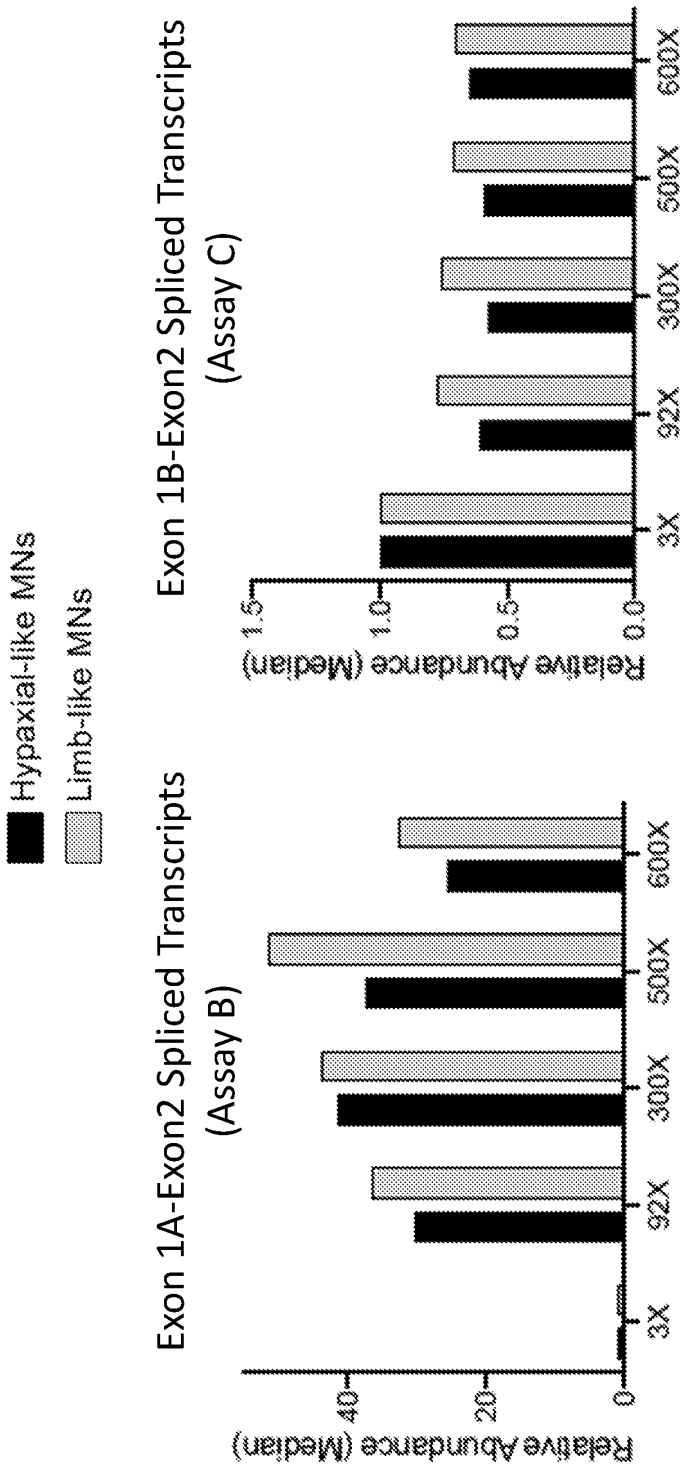


FIG. 15A

FIG. 15B

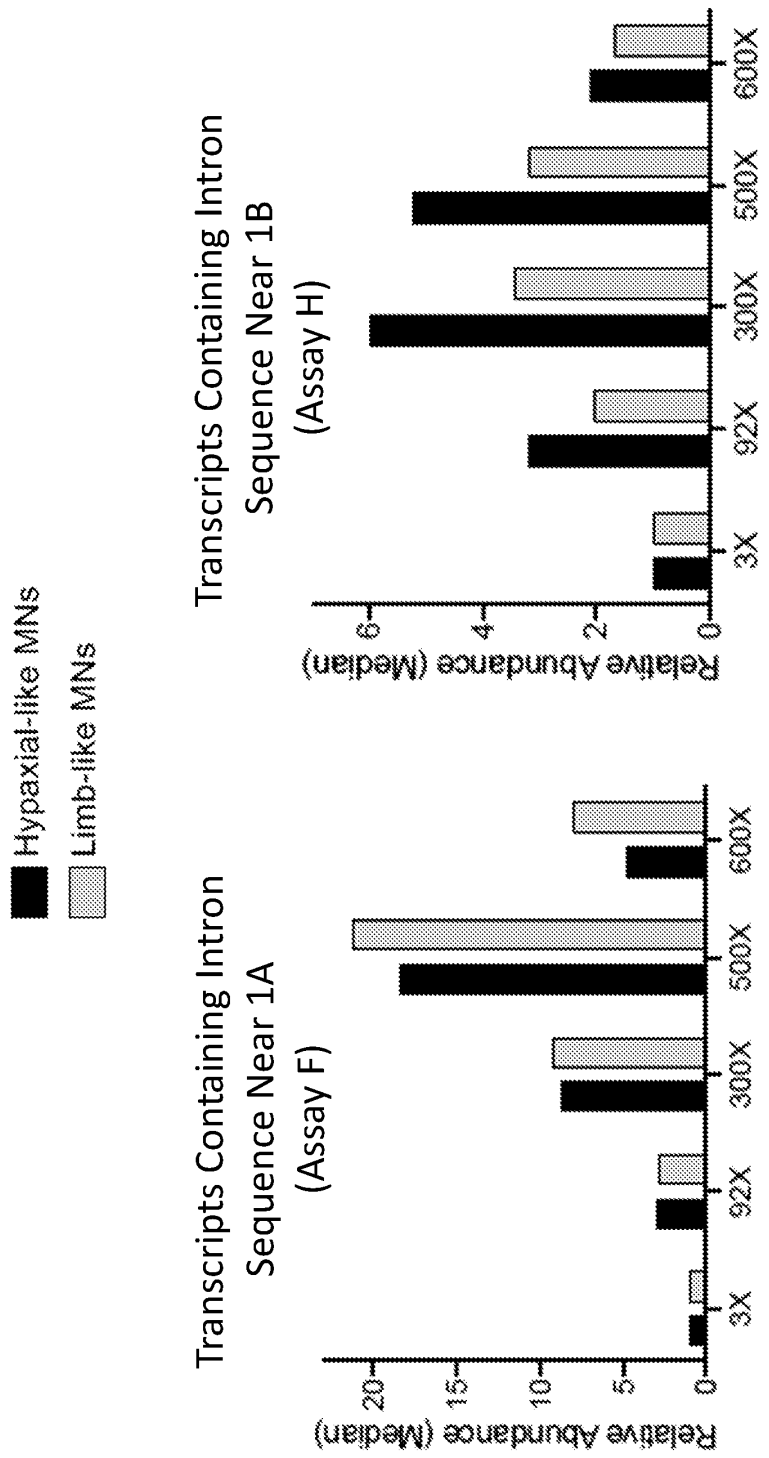


FIG. 15C

FIG. 15D

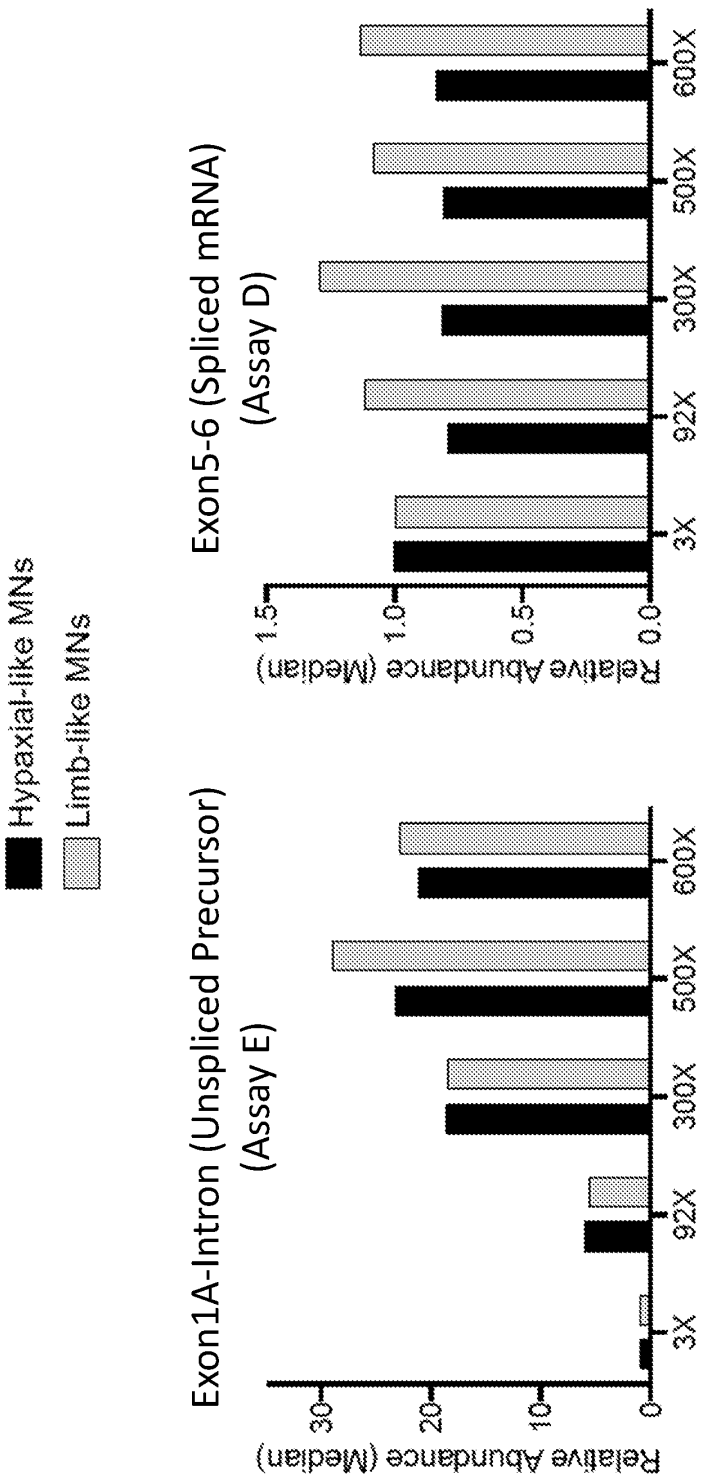


FIG. 15E

FIG. 15F

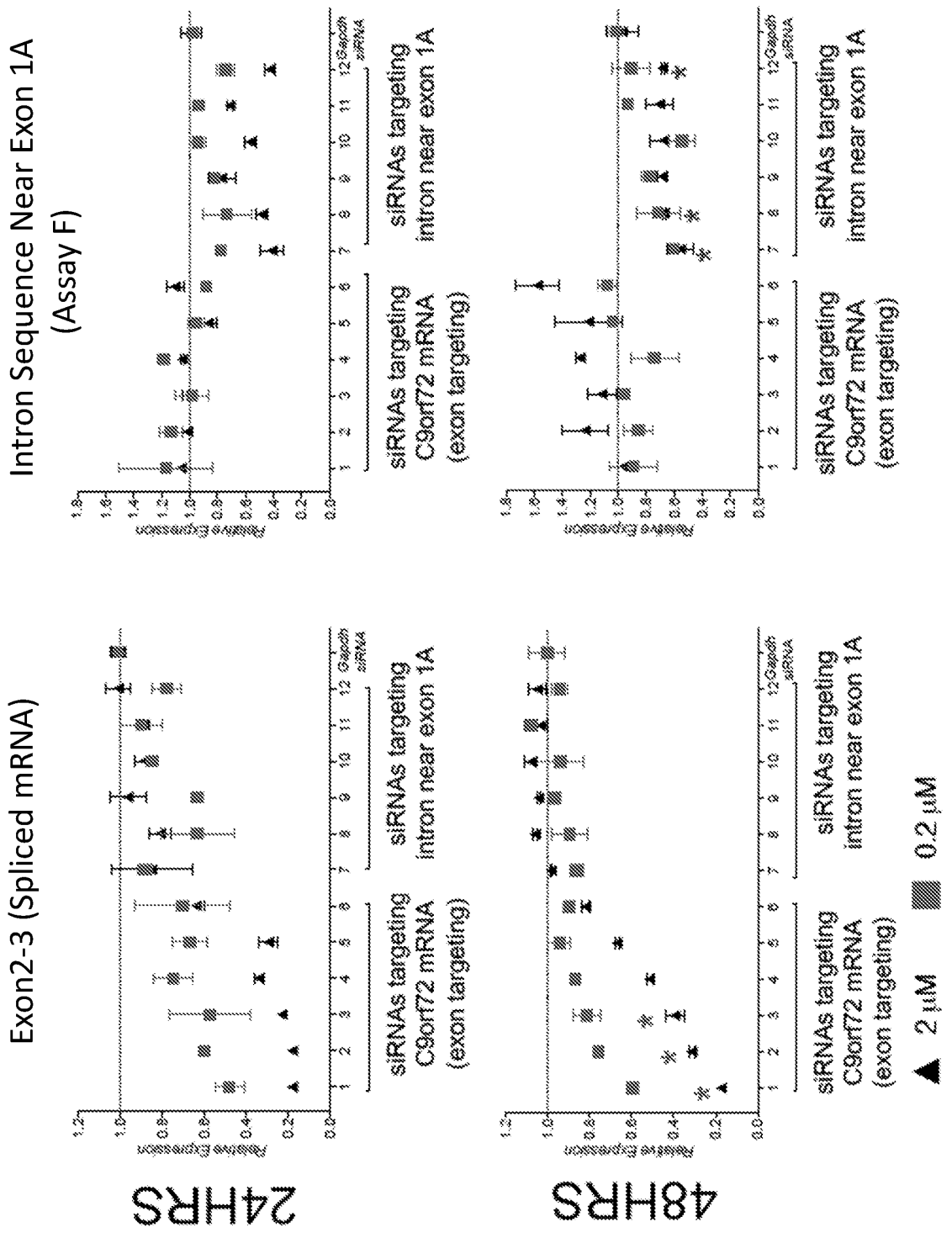


FIG. 16A

FIG. 16B

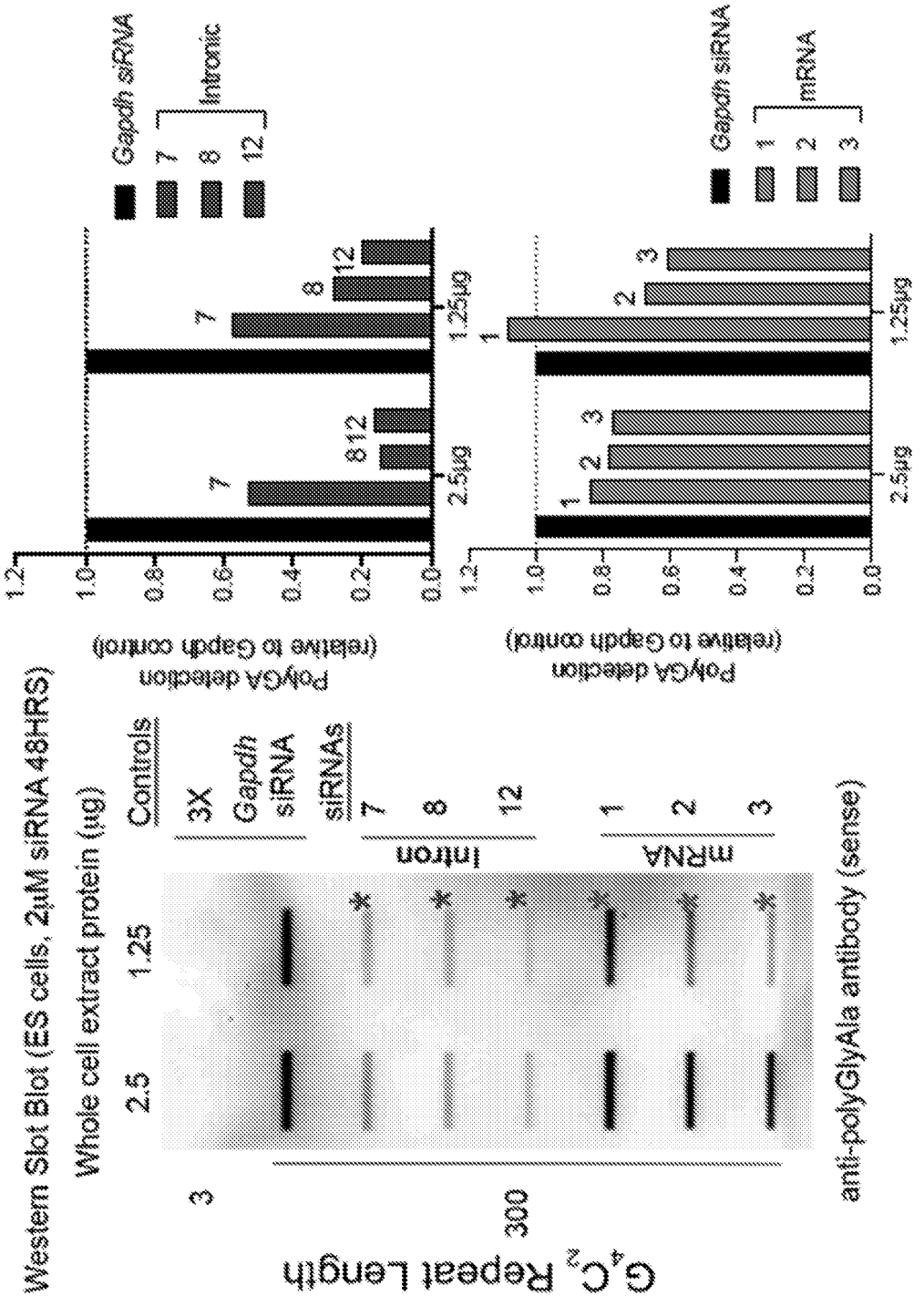


FIG. 17A

FIG. 17B

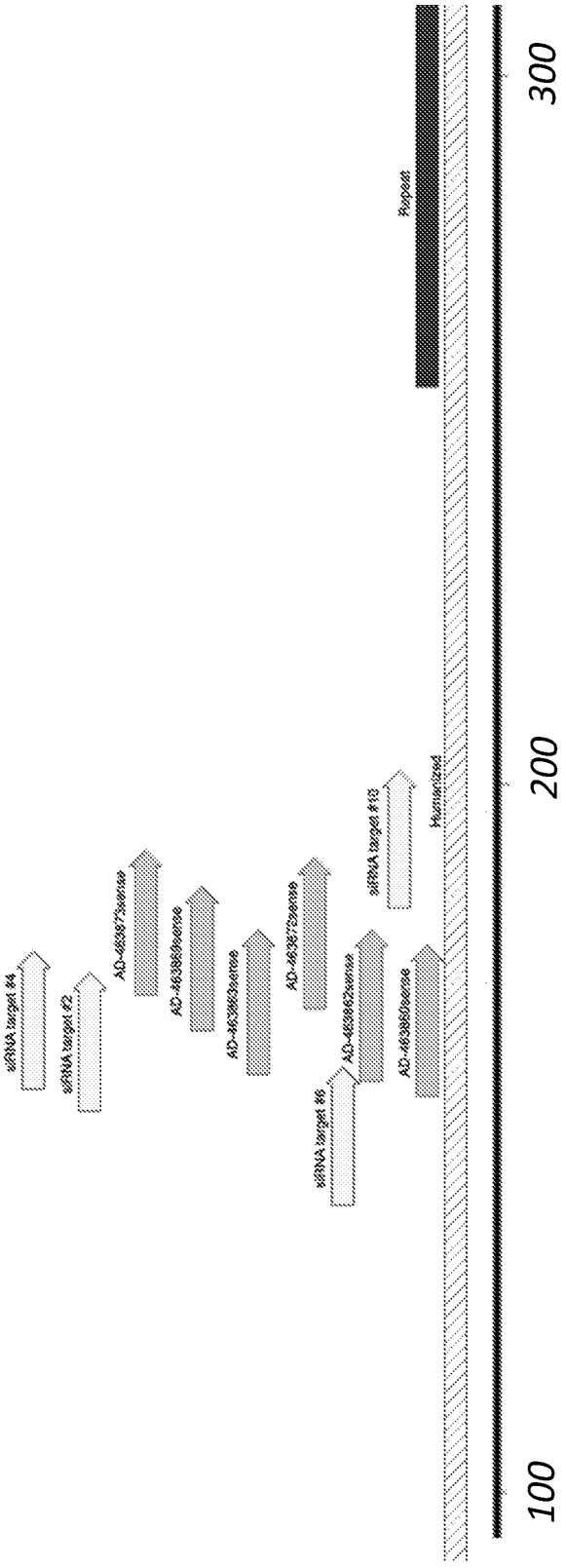

siRNA target #4
siRNA target #2
AD-463873sense
AD-463869sense
AD-463863sense
AD-463872sense
siRNA target #6
AD-463862sense
AD-463860sense
siRNA target #13
Intergenic
Exon1
100
200
300

FIG. 18A

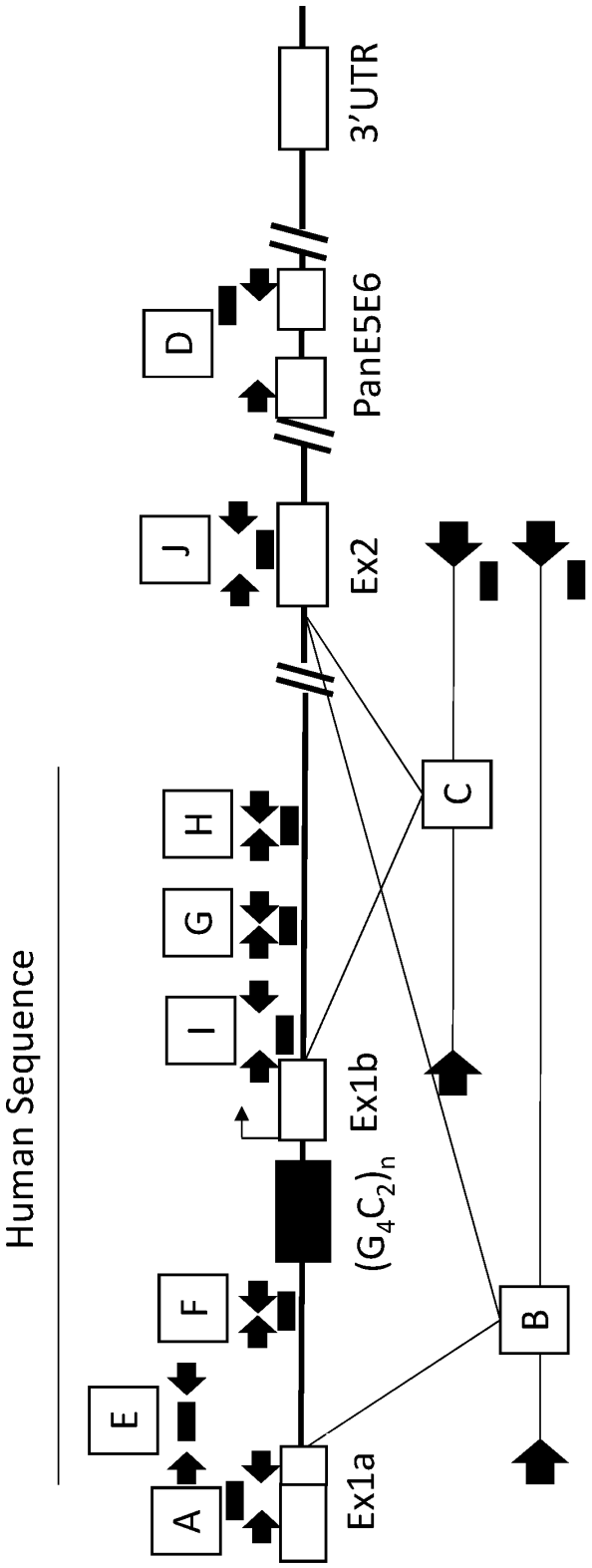

Human Sequence
A
E
F
Ex1a
$(G_4C_2)_n$
Ex1b
I
G
H
C
B
J
Ex2
D
PanE5E6
3'UTR

FIG. 18B

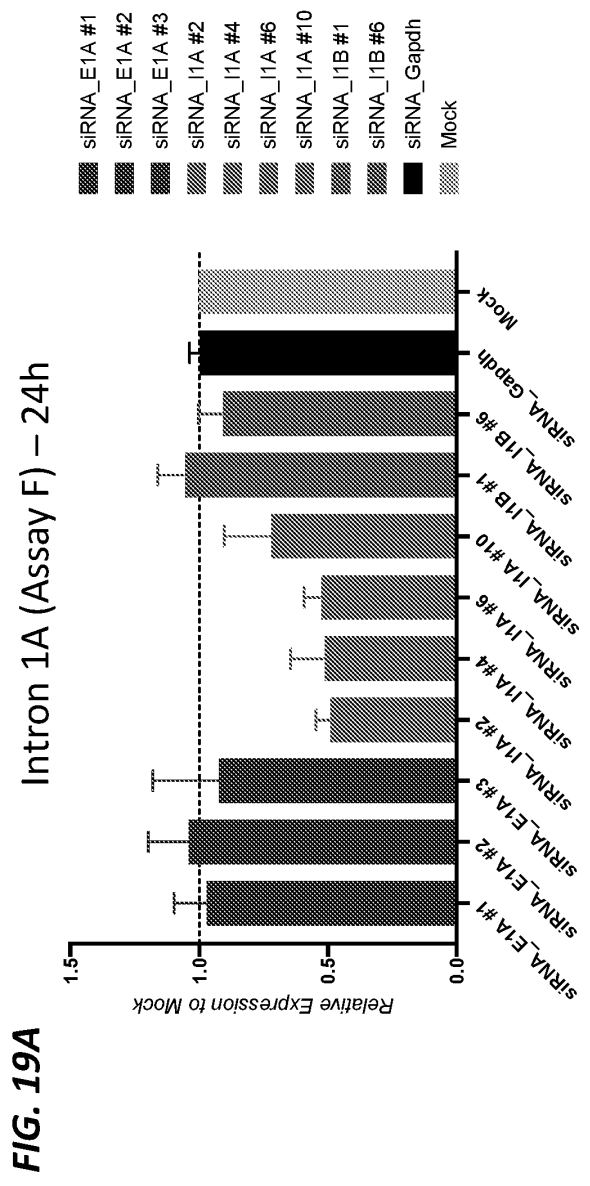


FIG. 19A

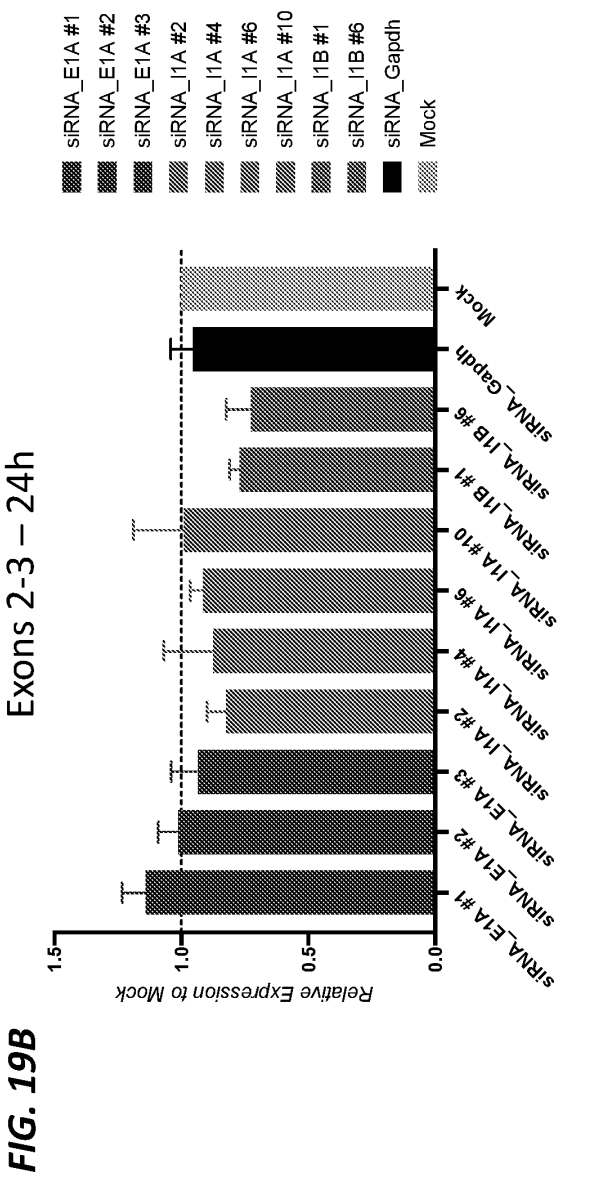


FIG. 19B

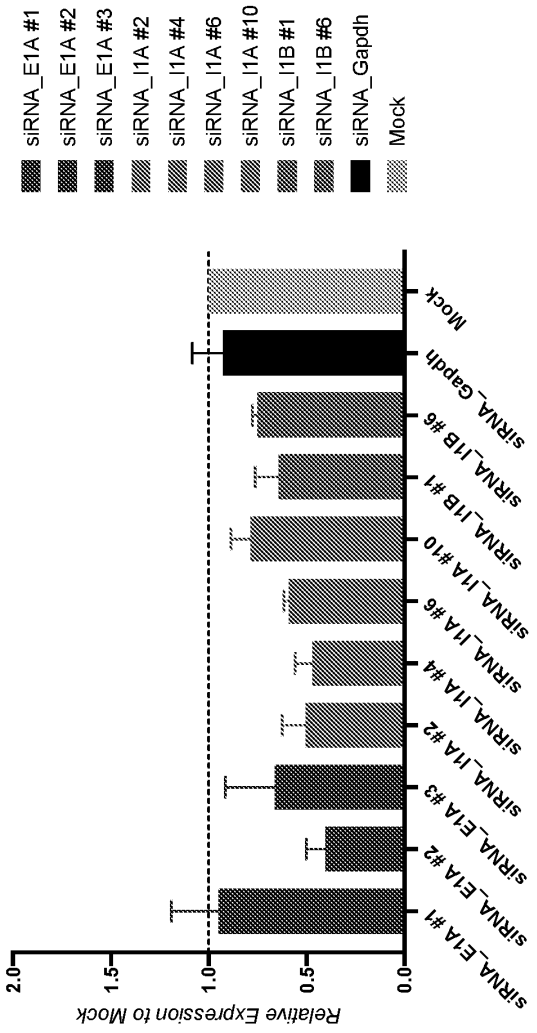

Exons 1a-2 (Assay B) – 24h
Relative Expression to Mock
2.0
1.5
1.0
0.5
0.0
siRNA_E1A #1
siRNA_E1A #2
siRNA_E1A #3
siRNA_I1A #2
siRNA_I1A #4
siRNA_I1A #6
siRNA_I1A #10
siRNA_I1B #1
siRNA_I1B #6
siRNA_Gapdh
Mock

FIG. 19C

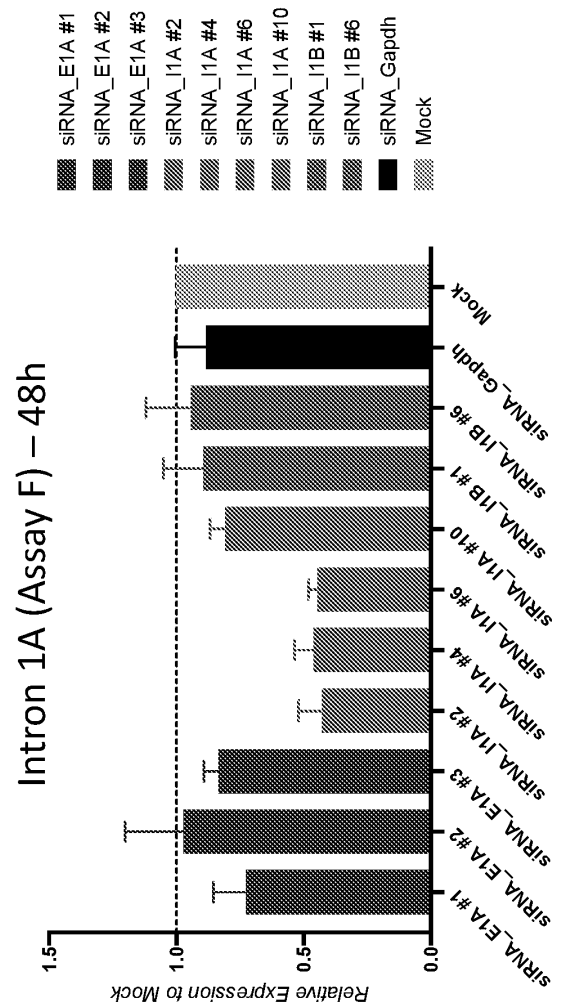


FIG. 20A

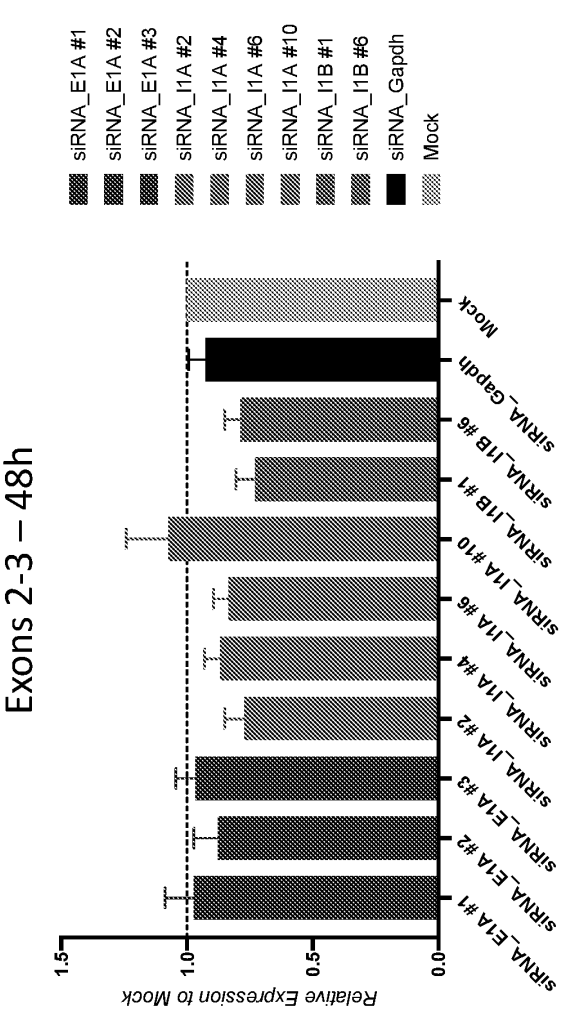


FIG. 20B

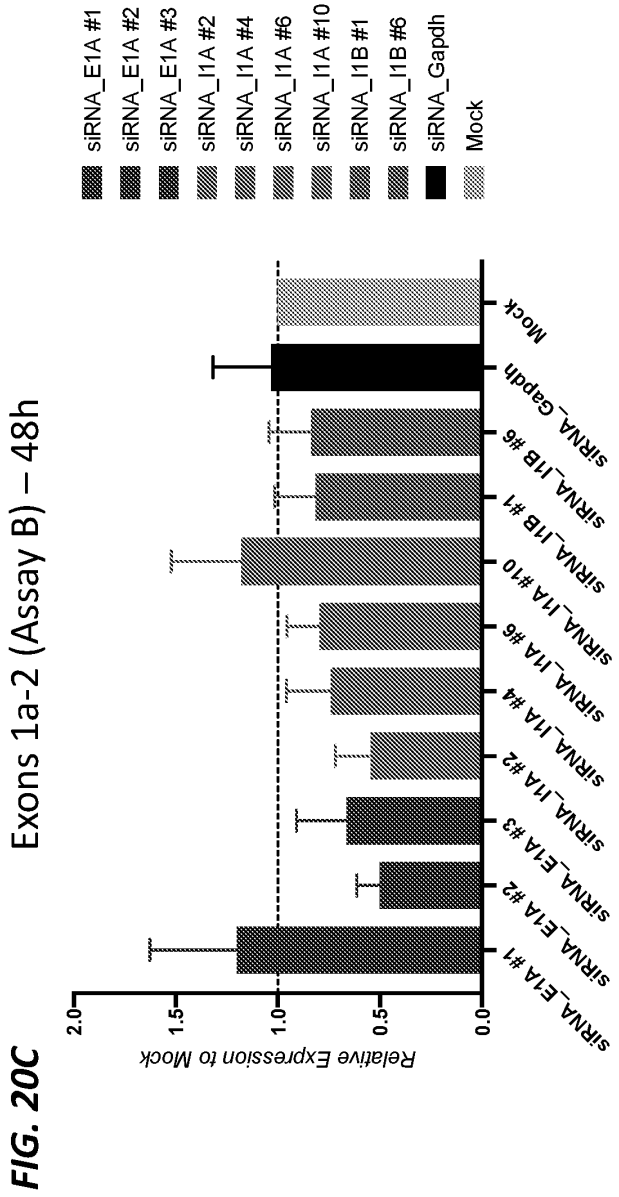

siRNA_E1A #1
siRNA_E1A #2
siRNA_E1A #3
siRNA_I1A #2
siRNA_I1A #4
siRNA_I1A #6
siRNA_I1A #10
siRNA_I1B #1
siRNA_I1B #6
siRNA_Gapdh
Mock
Exons 1a-2 (Assay B) – 48h
Relative Expression to Mock
2.0
1.5
1.0
0.5
0.0
siRNA_E1A #1
siRNA_E1A #2
siRNA_E1A #3
siRNA_I1A #2
siRNA_I1A #4
siRNA_I1A #6
siRNA_I1A #10
siRNA_I1B #1
siRNA_I1B #6
siRNA_Gapdh
Mock

FIG. 20C

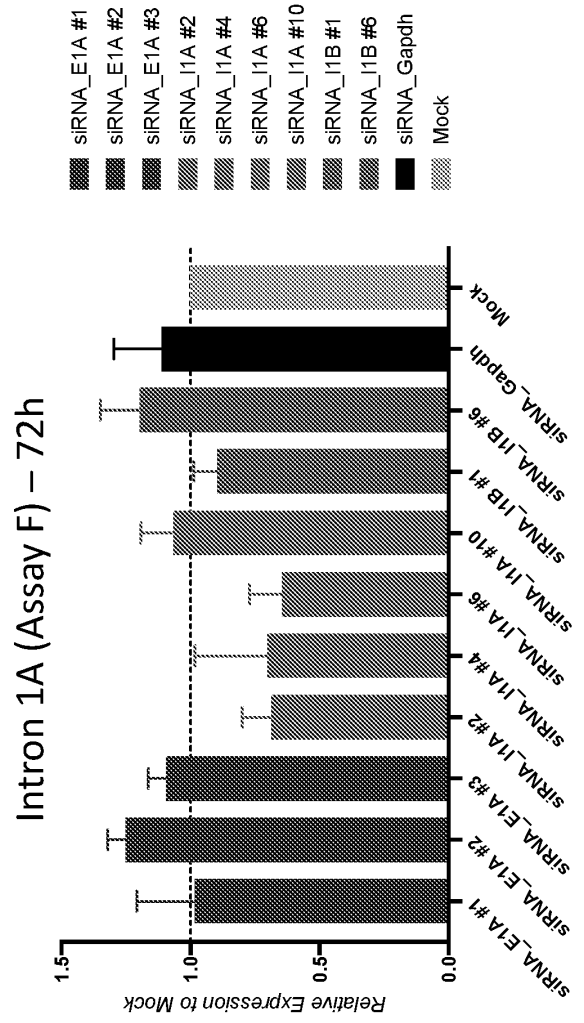


FIG. 21A

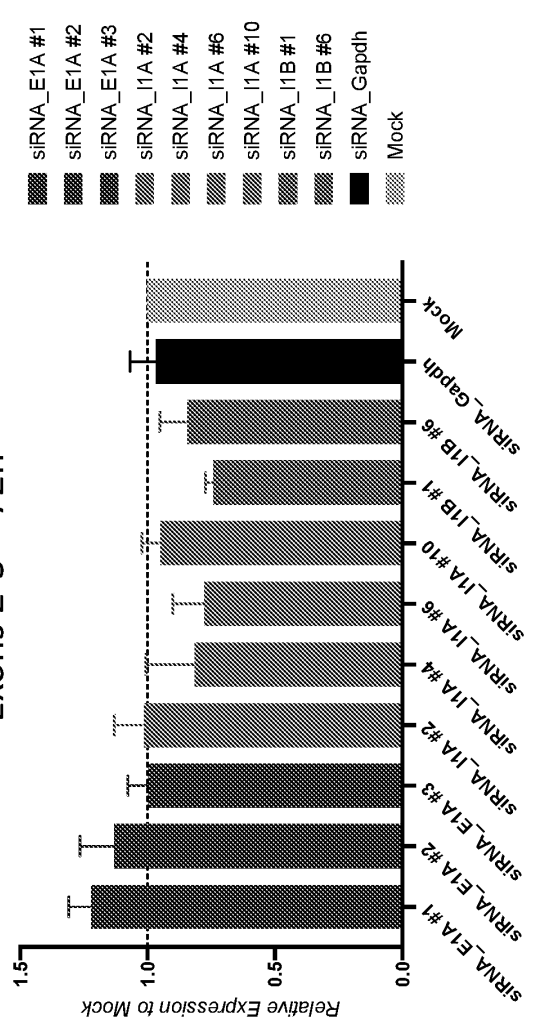


FIG. 21B

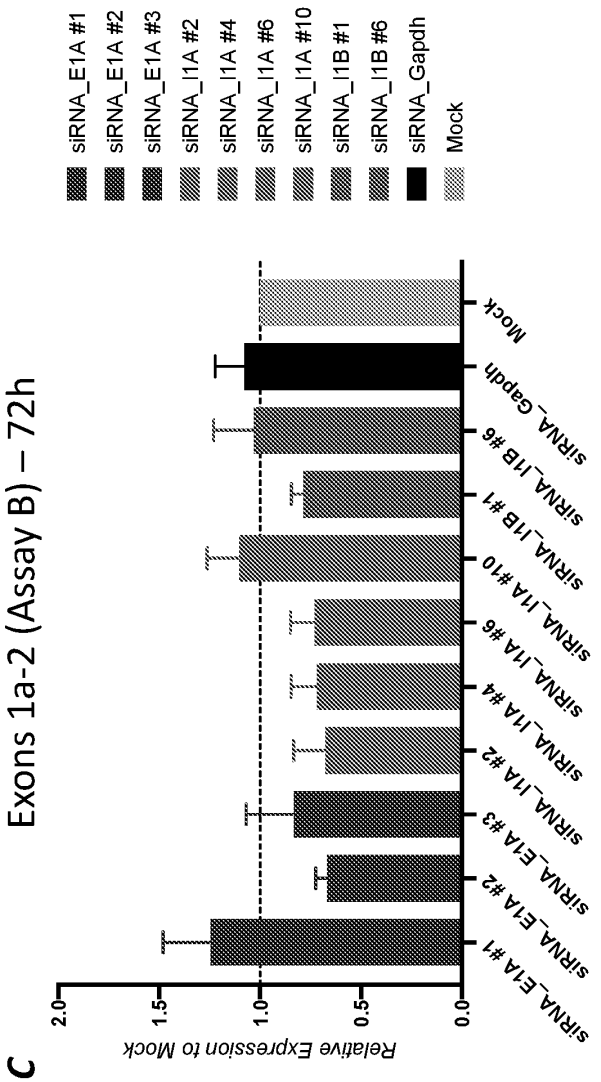

Exons 1a-2 (Assay B) – 72h
Relative Expression to Mock
2.0
1.5
1.0
0.5
0.0
siRNA_E1A #1
siRNA_E1A #2
siRNA_E1A #3
siRNA_I1A #2
siRNA_I1A #4
siRNA_I1A #6
siRNA_I1A #10
siRNA_I1B #1
siRNA_I1B #6
siRNA_Gapdh
Mock

FIG. 21C

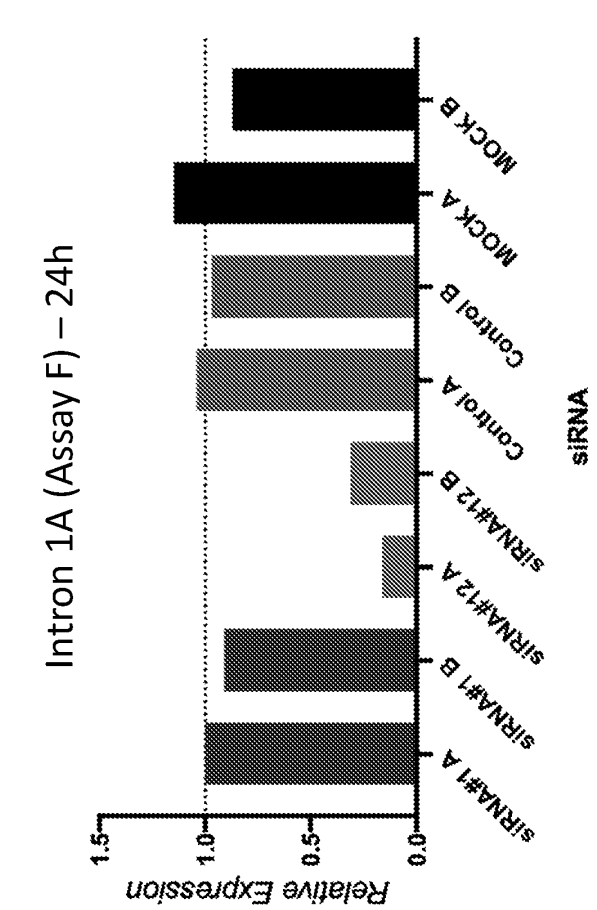


FIG. 22A

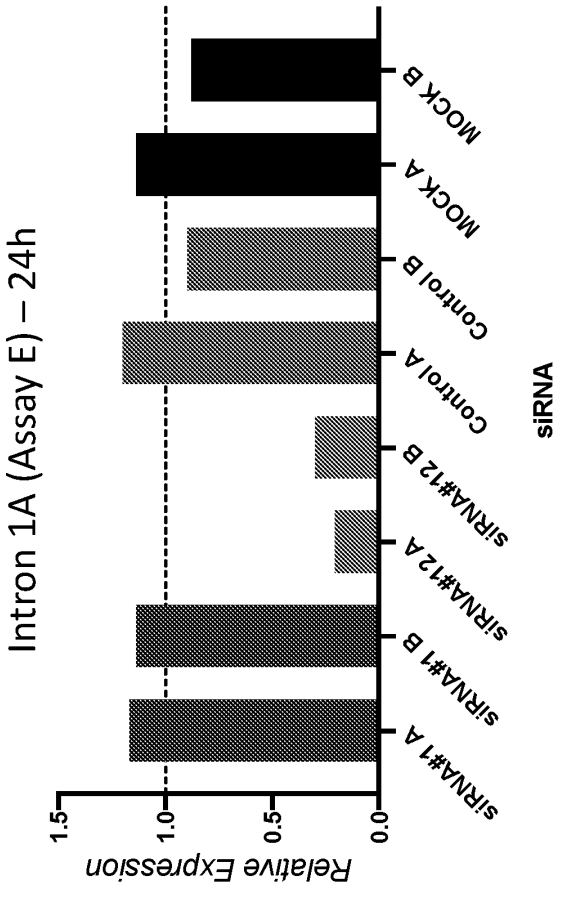


FIG. 22B

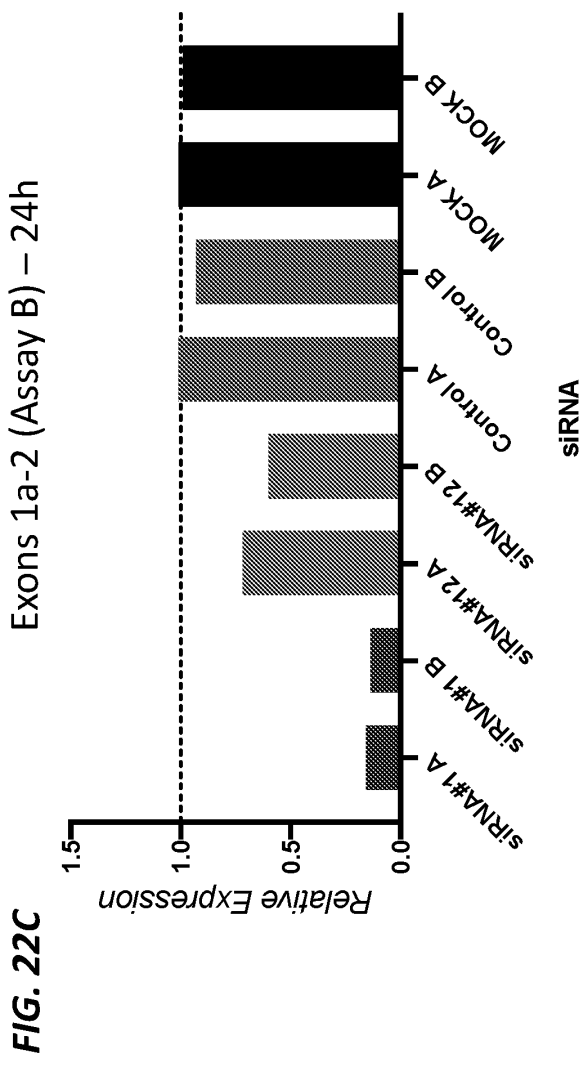


FIG. 22C

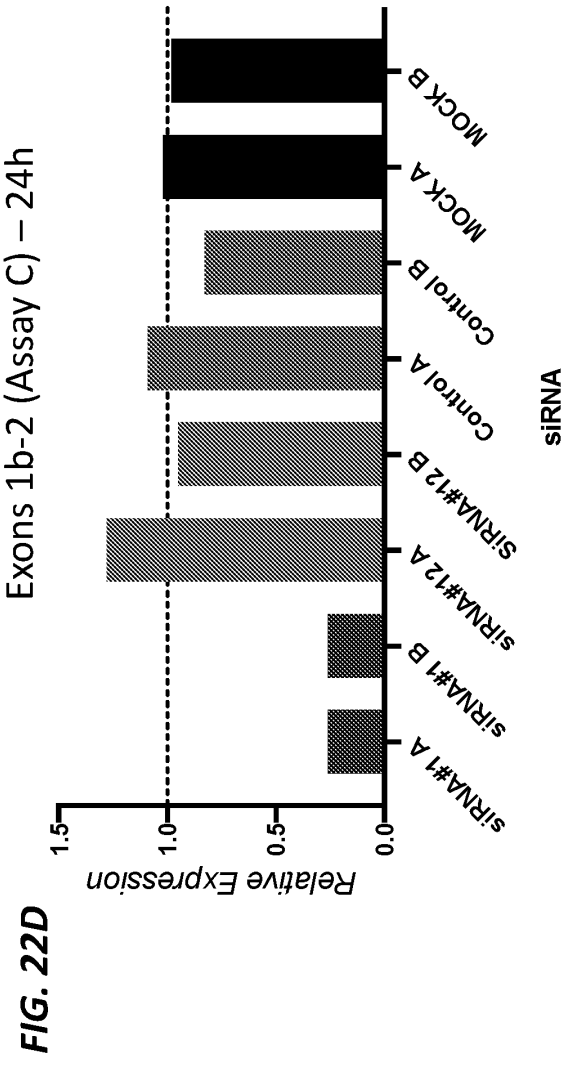


FIG. 22D

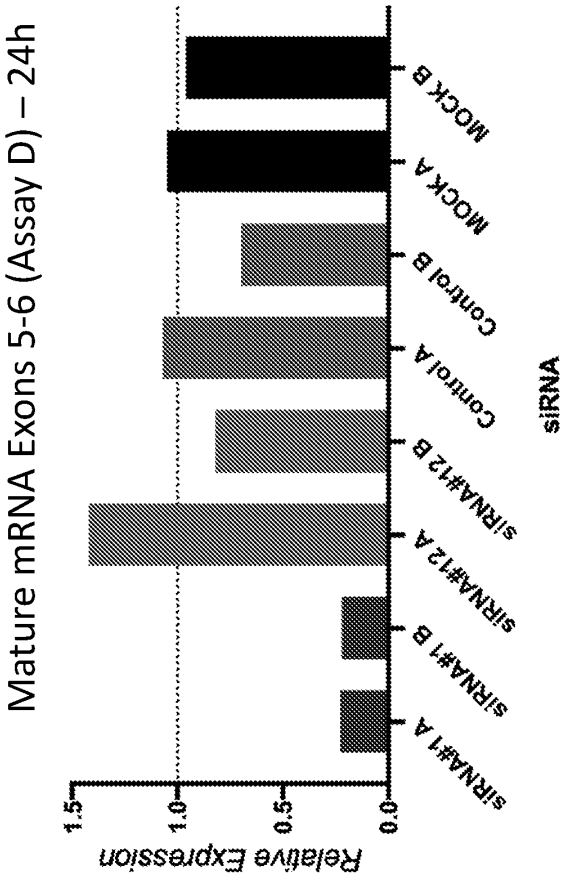

Mature mRNA Exons 5-6 (Assay D) – 24h
Relative Expression
1.5
1.0
0.5
0.0
siRNA#1 A
siRNA#1 B
siRNA#12 A
siRNA#12 B
Control A
Control B
MOCK A
MOCK B

FIG. 22E

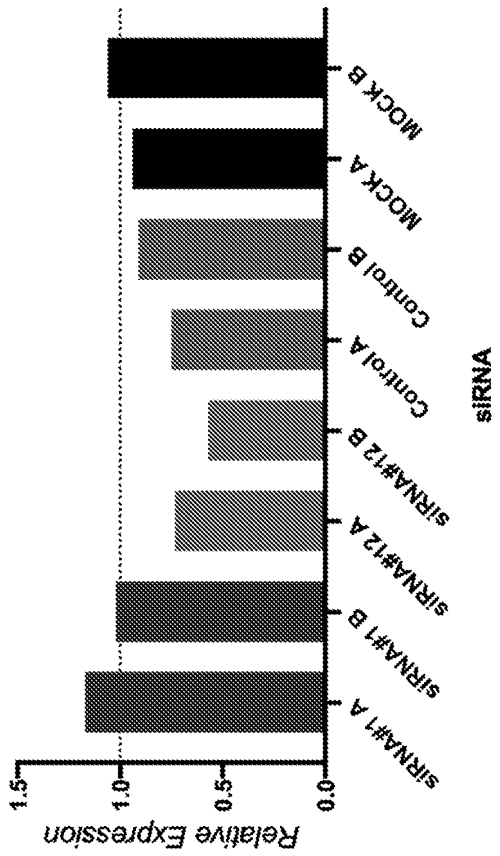


FIG. 23A

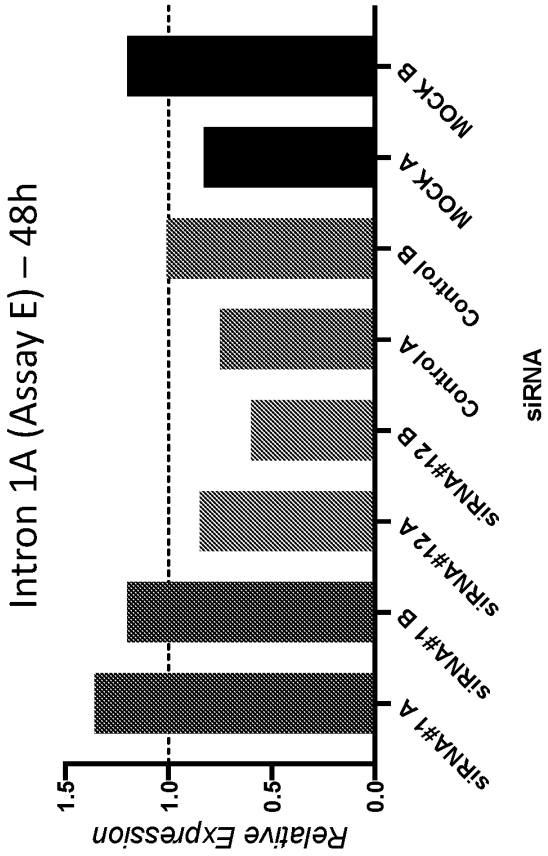


FIG. 23B

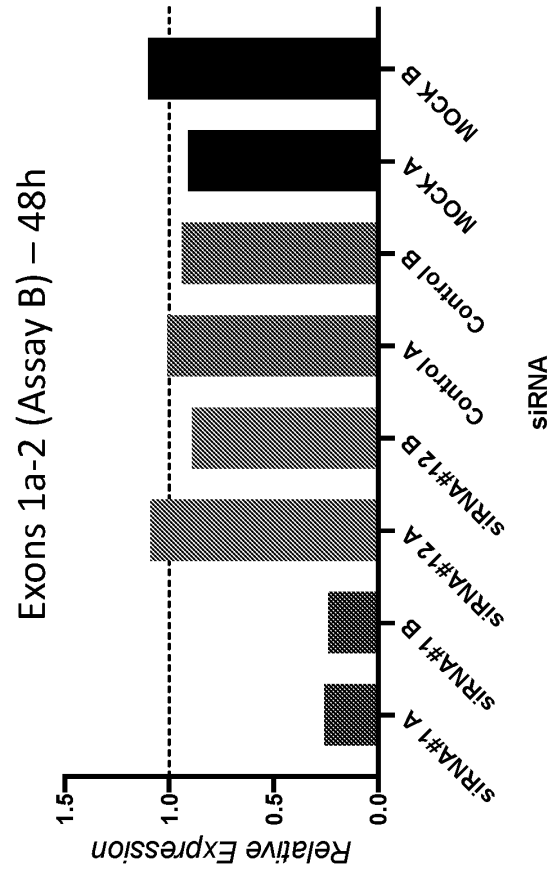


FIG. 23C

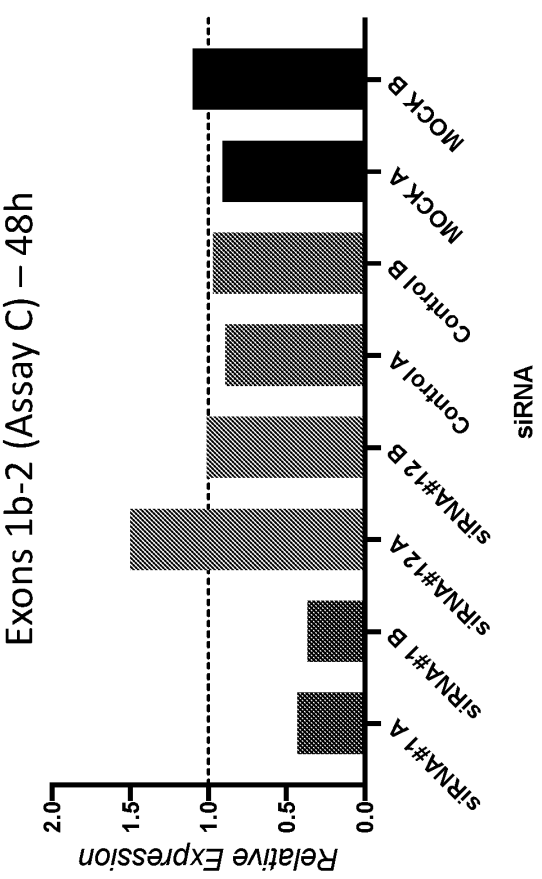


FIG. 23D

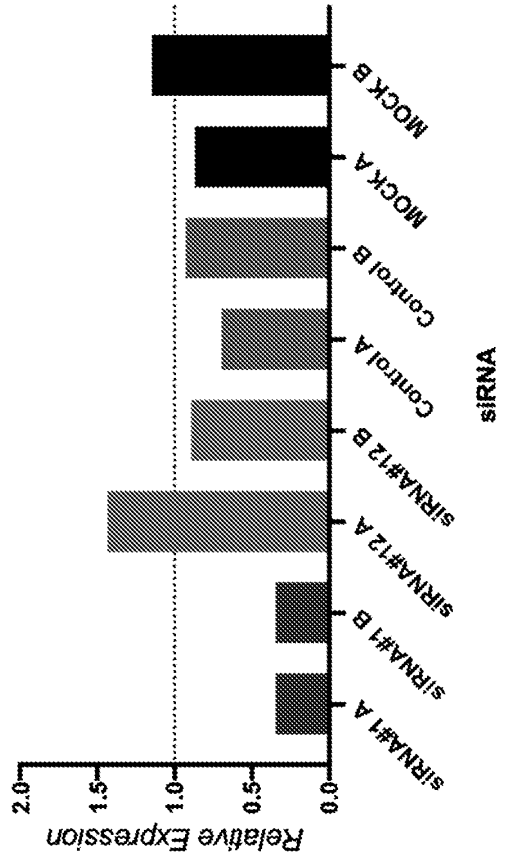

Mature mRNA Exons 5-6 (Assay D) – 48h
Relative Expression
2.0
1.5
1.0
0.5
0.0
siRNA
siRNA#1 A
siRNA#1 B
siRNA#12 A
siRNA#12 B
Control A
Control B
MOCK A
MOCK B

FIG. 23E