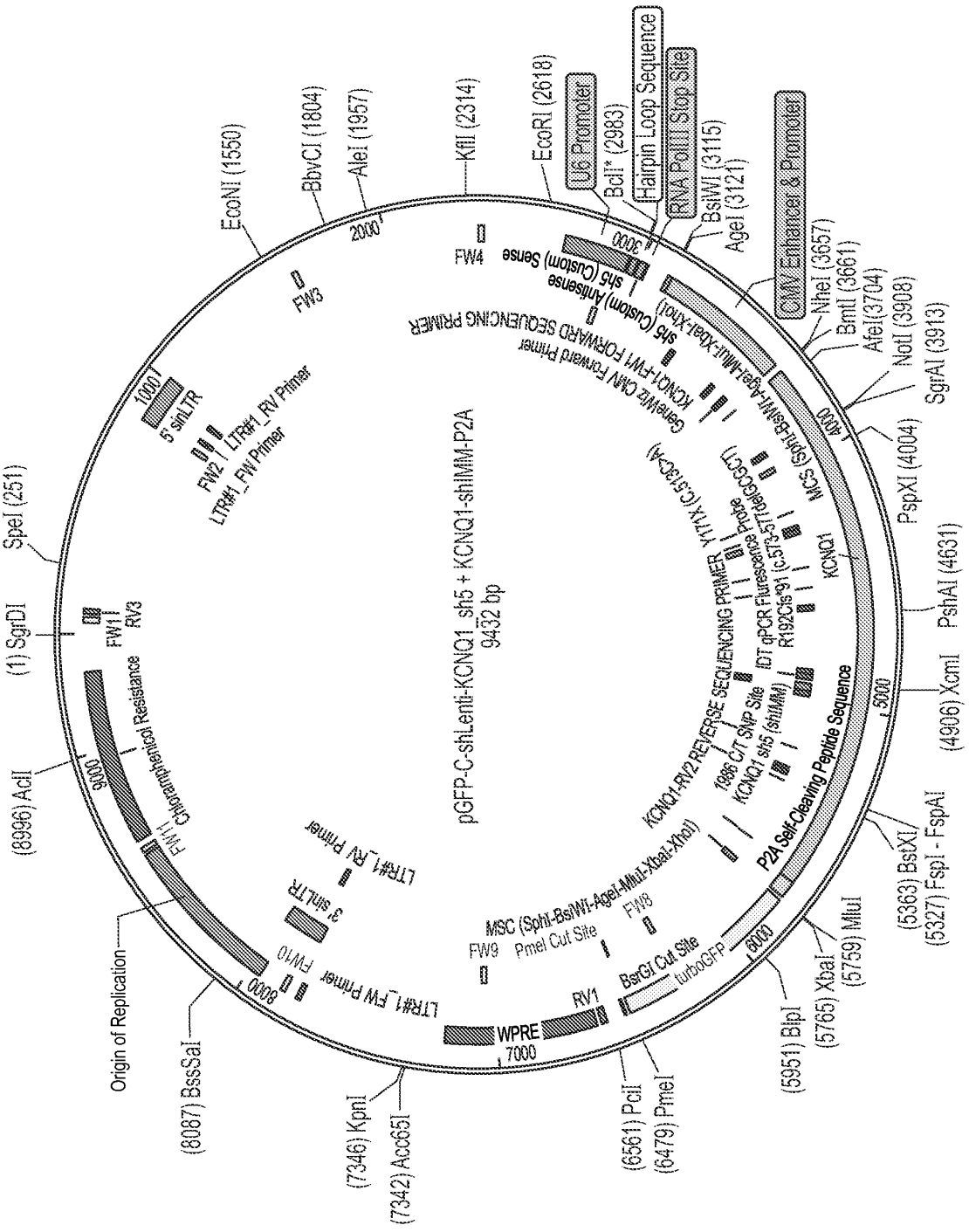

pGFP-C-shLenti-KCNQ1_sh5 + KCNQ1-shIMM-P2A
9432 bp
CMV Enhancer & Promoter
U6 Promoter
Hairpin Loop Sequence
RVA PolIII Stop Site
sh5 (Custom) Sense
sh5 (Custom) Antisense
KCNQ1-FW1 FORWARD SEQUENCING PRIMER
GeneWiz CMV Forward Primer
MCS (SphI-SbfI-MluI-AgeI-NheI-BmtI-XbaI-XhoI)
KCNQ1
IDT qPCR Fluorescence Probe (c.573-577delCCGCT)
R192Cfs*91
KCNQ1 sh5 (shIMM)
1886 C/T SNP Site
KCNQ1-RV2 REVERSE SEQUENCING PRIMER
P2A Self-Cleaving Peptide Sequence
turboGFP
BsrGI Cut Site
MSC (SphI-BsiWI-AgeI-MluI-XbaI-XhoI)
PmeI Cut Site
WPRE
3' shLTR
LTR#1_RV Primer
LTR#1_FW Primer
Origin of Replication
Chloramphenicol Resistance
5' shLTR
LTR#2 LTR#1_RV Primer
LTR#1_FW Primer
FW1
FW2
FW3
FW4
FW8
FW9
FW10
FW11
RV1
RV3
1000
2000
3000
4000
5000
6000
7000
8000
9000
(1) SgrDI
SpeI (251)
(8996) AclI
(8087) BssSaI
(7346) KpnI
(7342) Acc65I
(6561) PciI
(6479) PmeI
(5951) BlpI
(5765) XbaI
(5759) MluI
(5363) BstXI
(5327) FspI - FspAI
(4906) XcmI
PshAI (4631)
PspXI (4004)
SgrAI (3913)
NotI (3908)
AfeI(3704)
BmtI (3661)
NheI (3657)
AgeI (3121)
BsiWI (3115)
BclI* (2983)
EcoRI (2618)
KflI (2314)
AleI (1957)
BbvCI (1804)
EcoNI (1550)

FIG. 1A

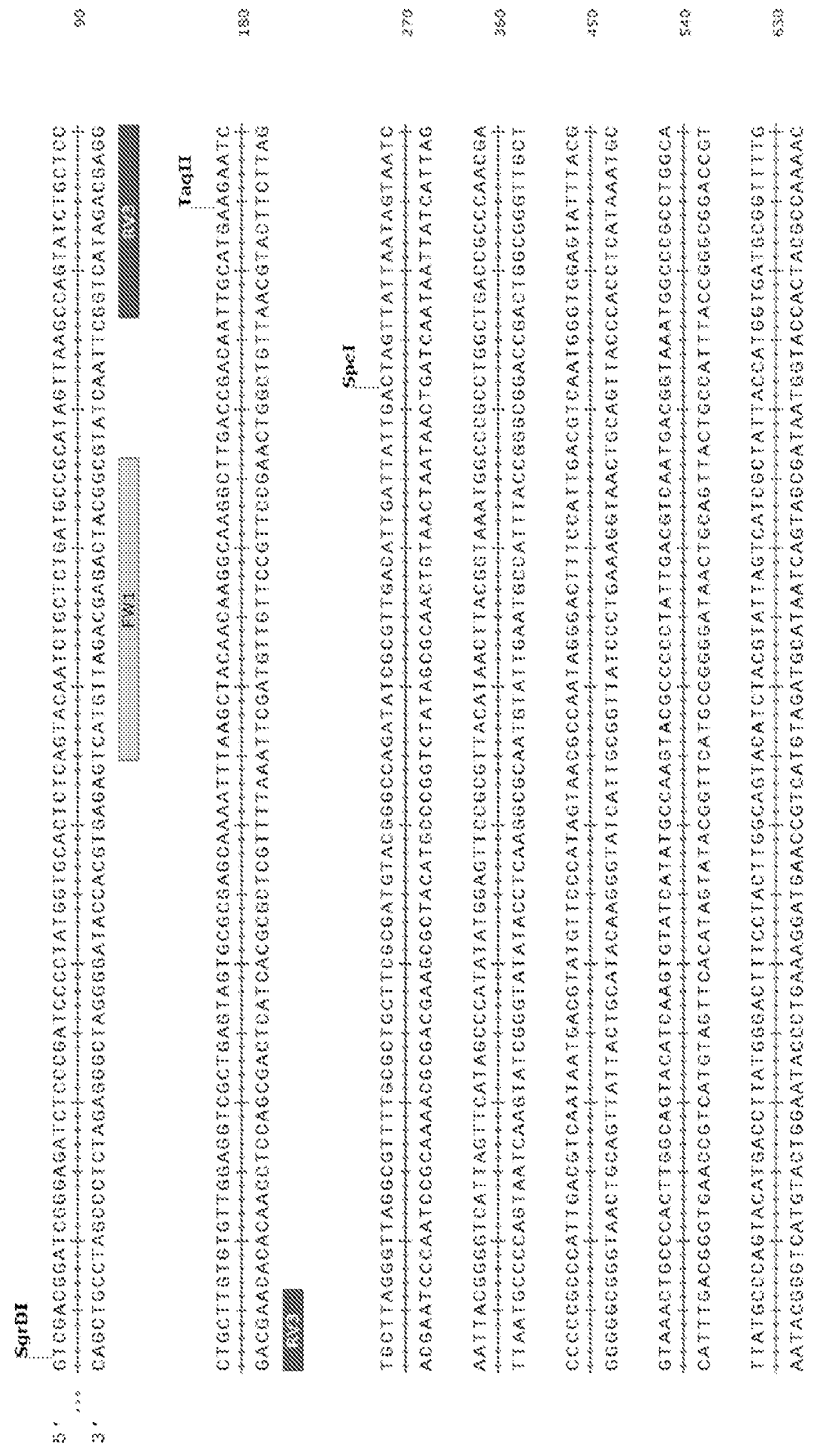


FIG. 1B

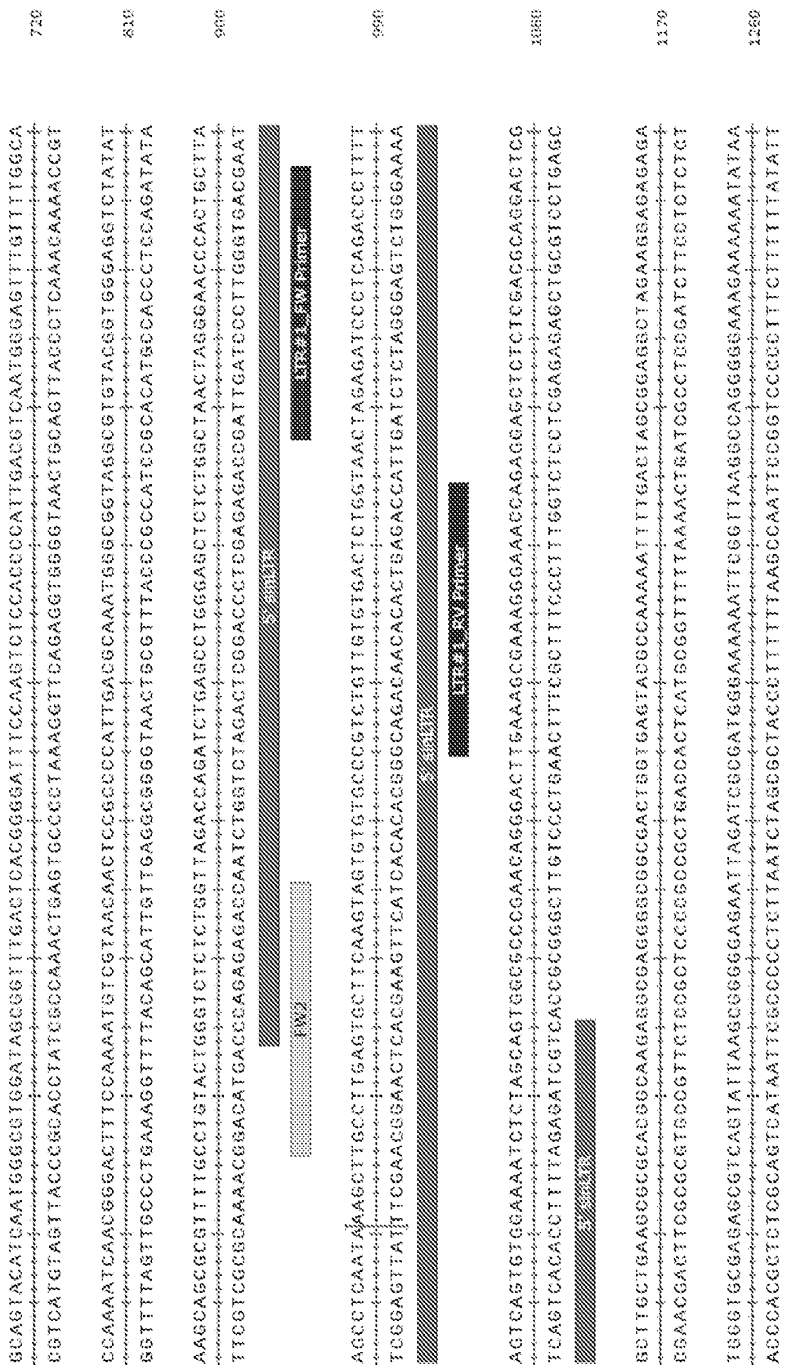


FIG. 1B – cont.

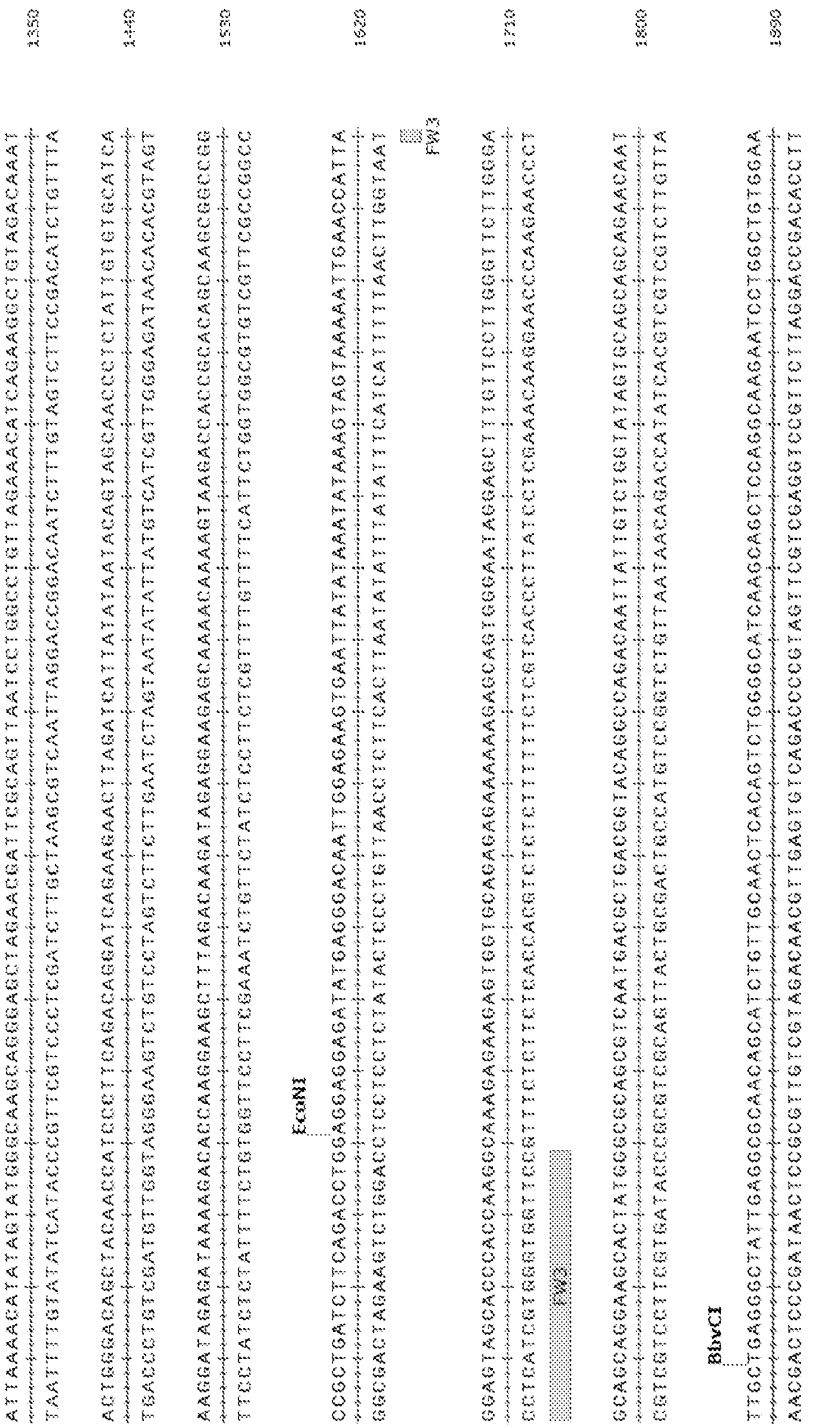


FIG. 1B – cont.

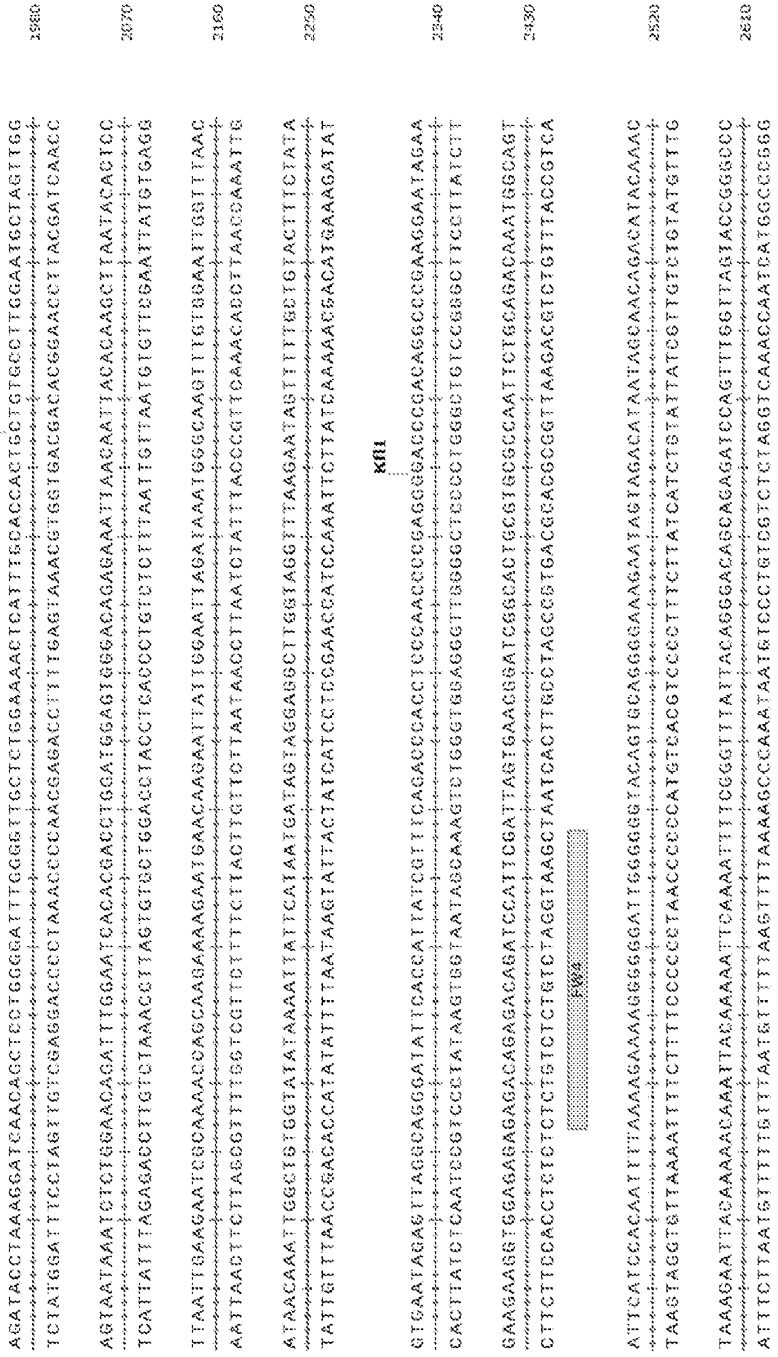


FIG. 1B – cont.


EcoRI
U6 Promoter
GeneWiz Universal Primer U6-FW
BclI*
RNA PolIII Stop Site

FIG. 1B – cont.

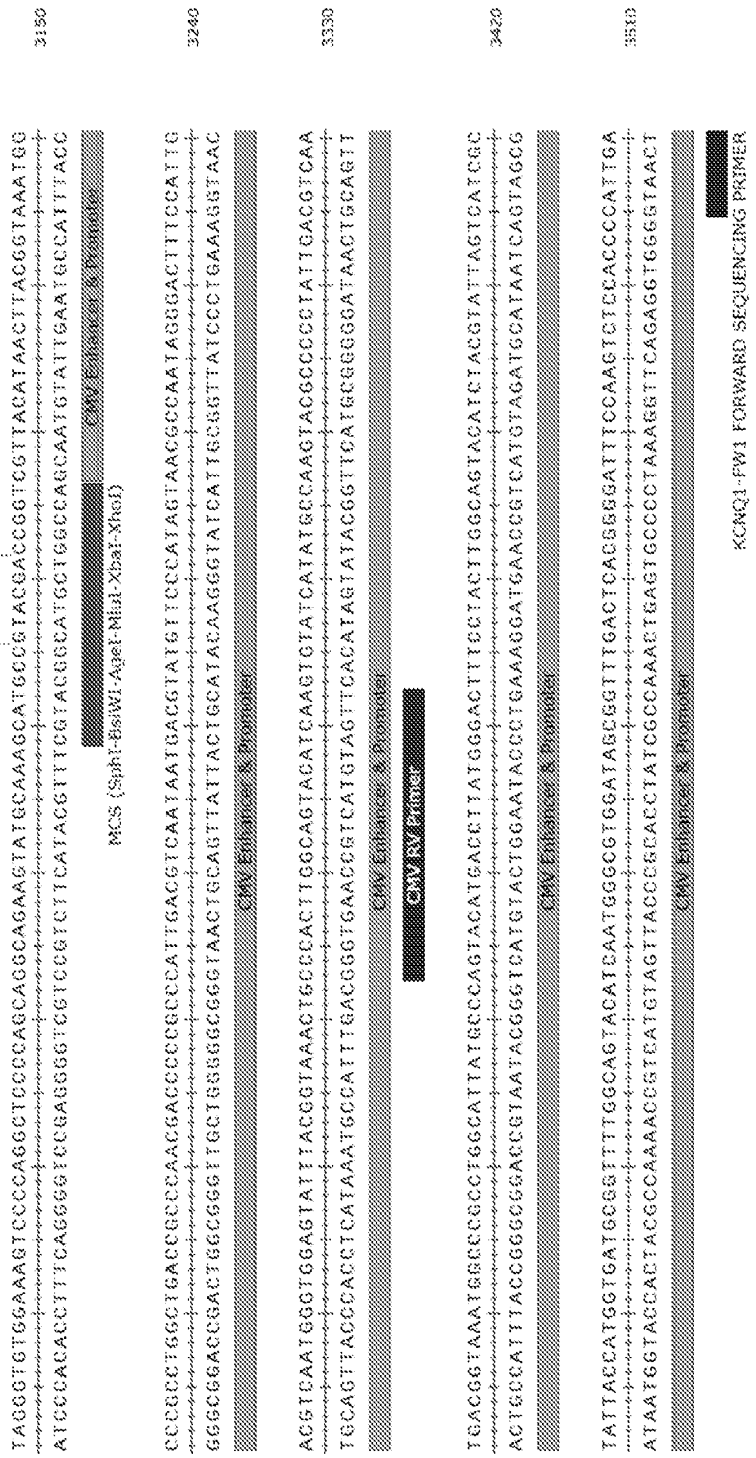

BsiWI
AgeI
3150
3240
3330
3420
3510
MCS (SphI-BsiWI-AgeI-MluI-XbaI-XhoI)
CMV Enhancer & Promoter
CMV Enhancer & Promoter
CMV 5'UTR Intron
CMV Enhancer & Promoter
CMV Enhancer & Promoter
KCMQ1-FW1 FORWARD SEQUENCING PRIMER

FIG. 1B – cont.

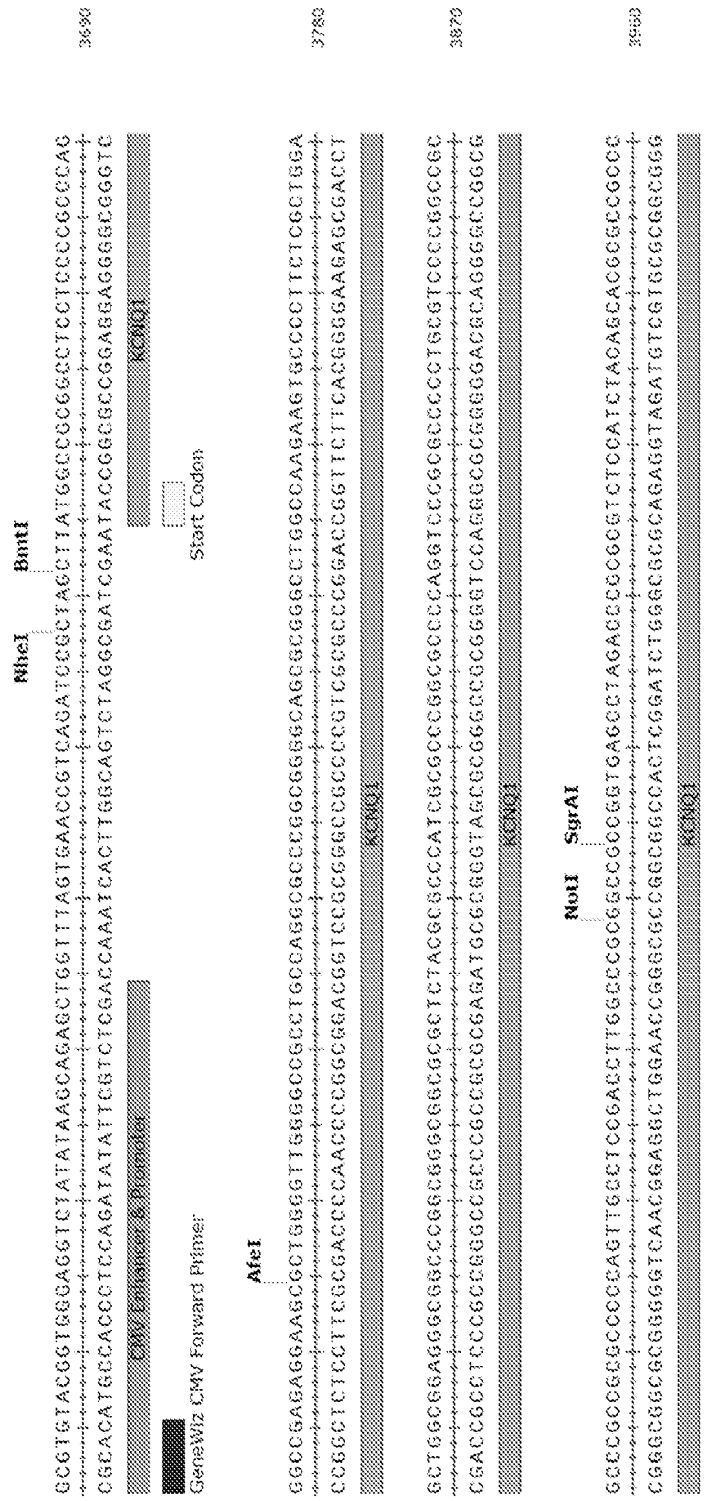

NheI
BmtI
3690
CMV Enhancer & Promoter
KCNQ1
Start Codon
GeneWiz CMV Forward Primer
AfeI
3780
KCNQ1
3870
KCNQ1
NotI
SgrAI
3960
KCNQ1
KCNQ1-FW2 FORWARD SEQUENCING PRIMER

FIG. 1B – cont.


PspXI
4858
4148
4238
4328
KCNQ1
KCNQ1-RV1 REVERSE SEQUENCING PRIMER
Codon 171
Y171X (c.513C>A)
R19...
Codon 192
KCNQ1-FW3 FORWARD SEQUENCING PRIMER
KCNQ1-RV2 REVERSE SEQUENCING PRIMER

FIG. 1B – cont.

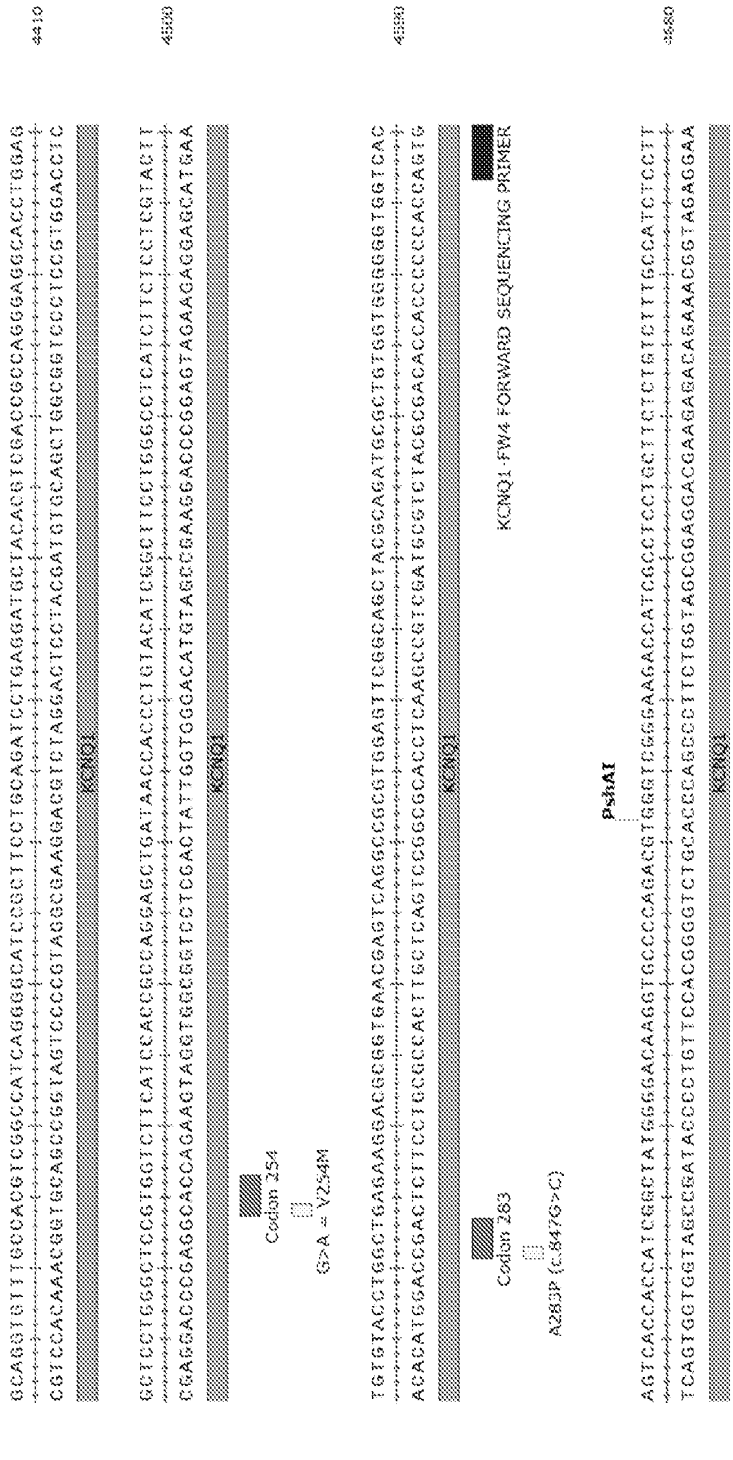

4410
4530
4590
4650
KCNQ1
Codon 254
G>A = V254M
Codon 283
A283P (c.847G>C)
PshAI
KCNQ1-FW4 FORWARD SEQUENCING PRIMER
KCNQ1-FW4 FORWARD SEQUENCING PRIMER

FIG. 1B – cont.

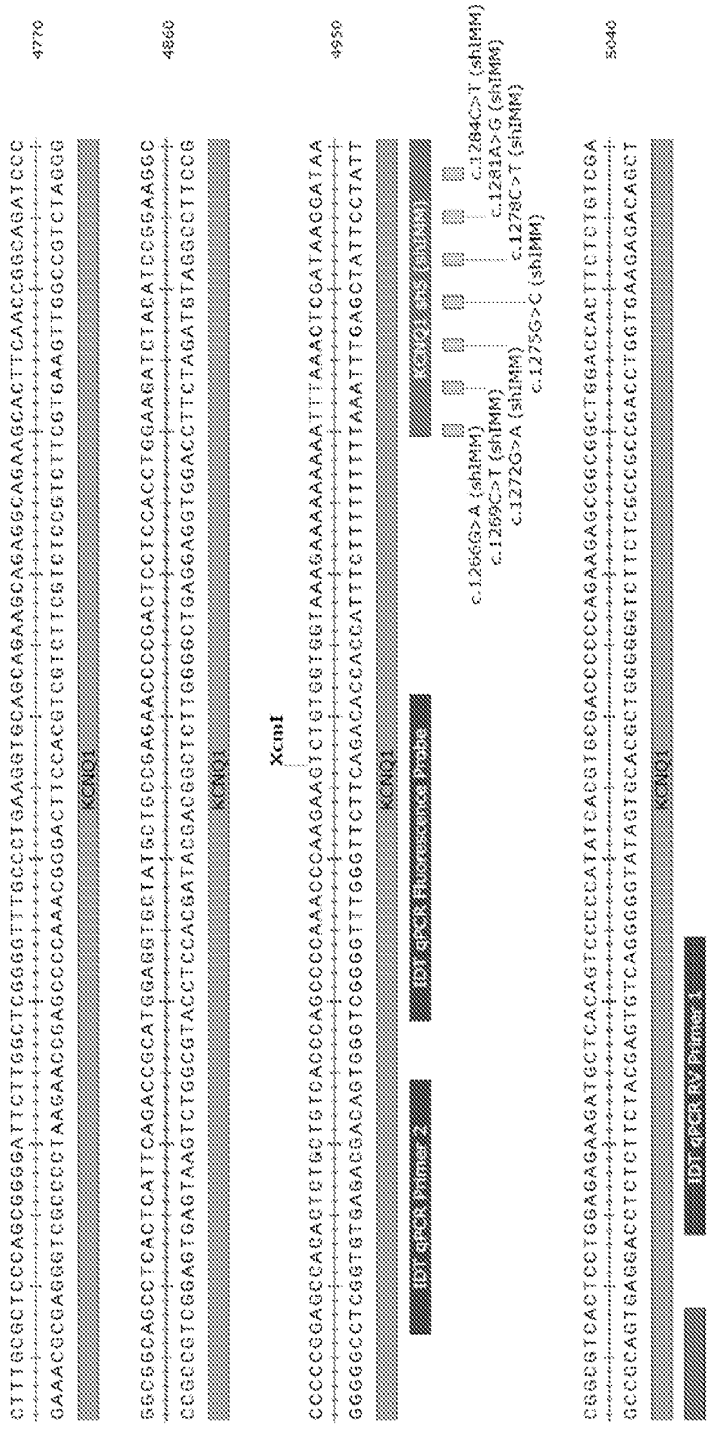

4770
4860
XcmI
4950
5040
KCNQ1
c.1266G>A (shIMM)
c.1269C>T (shIMM)
c.1272G>A (shIMM)
c.1275G>C (shIMM)
c.1278C>T (shIMM)
c.1281A>G (shIMM)
c.1284C>T (shIMM)
KCNQ1 sh3 (shIMM)
c.1293G>C (shIMM)
c.1296G>C (shIMM)
c.1287T>C (shIMM)

FIG. 1B – cont.


5130
5220
5310
5400
KCNQ1
Codon 518
R518X (c.1552C>T)
c.1638 G/A SNP Site
FspI
FspAI
BstXI
Codon 567
T>G - 15675
KCNQ1-FW6 FORWARD SEQUENCING PRIMER

FIG. 1B – cont.

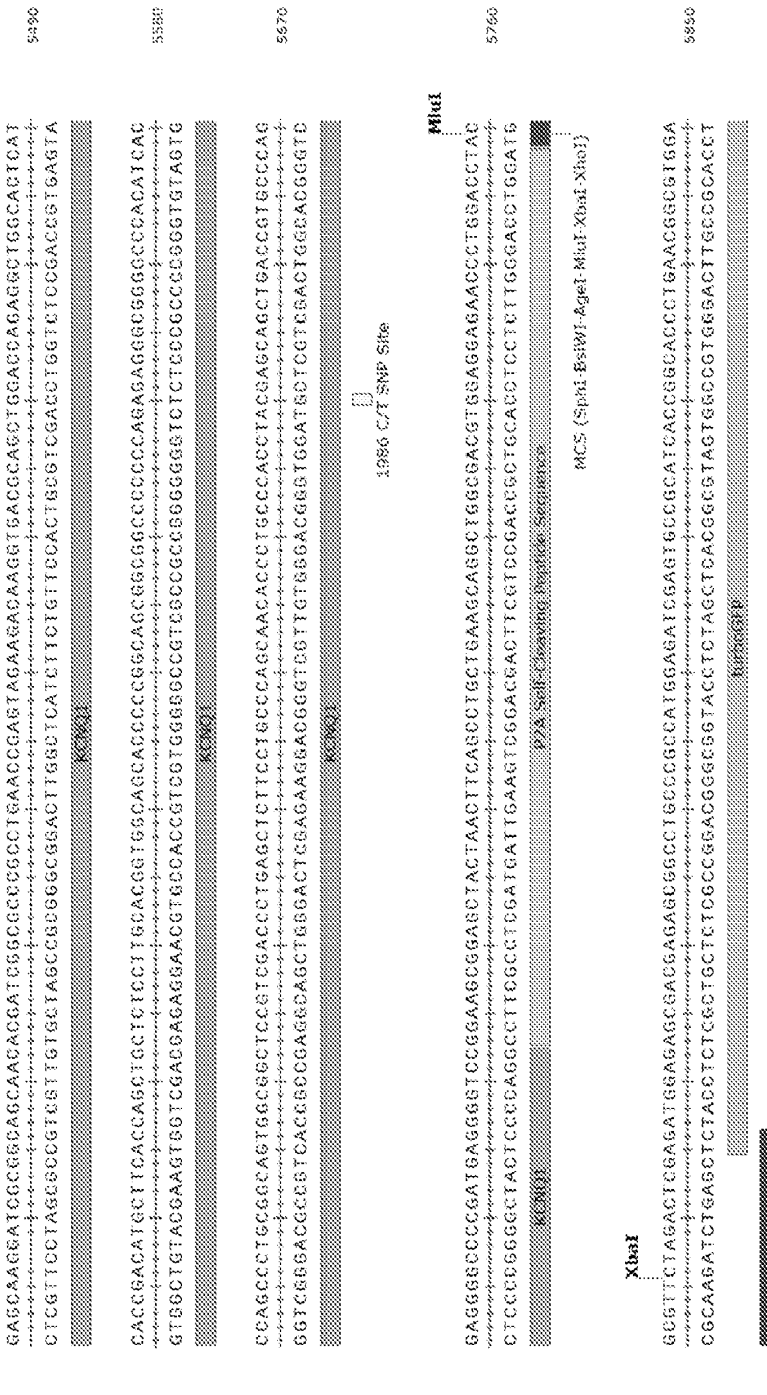

5490
5580
5670
1986 C/T SNP Site
MluI
5760
PYA Self-Cleaving Peptide Sequence
MCS (SphI-BsiWI-AgeI-MluI-XbaI-XhoI)
5850
XbaI
MCS (SphI-BsiWI-AgeI-MluI-XbaI-XhoI)

FIG. 1B – cont.

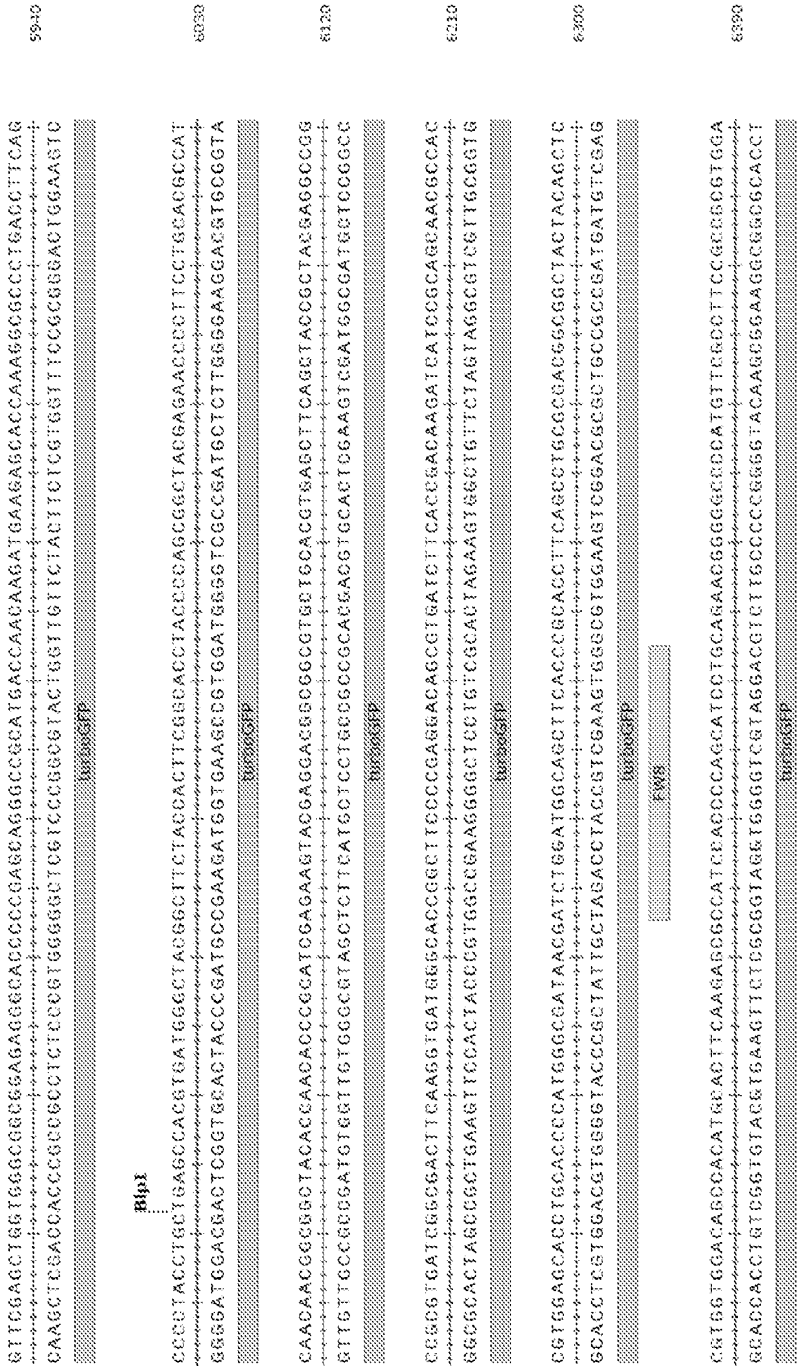


FIG. 1B – cont.

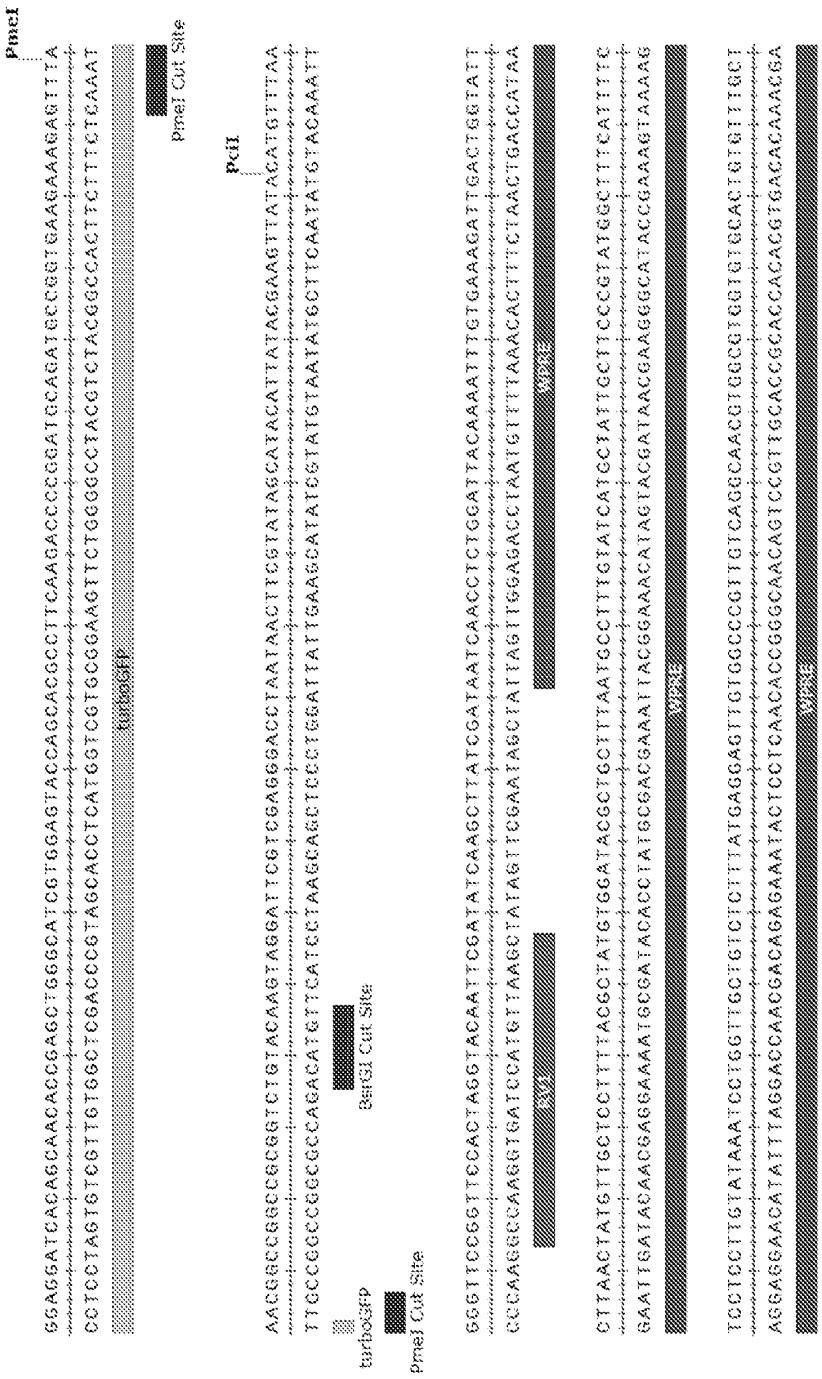

PmeI
6480
PmeI Cut Site
turboGFP
PciI
6570
6660
6750
6840
turboGFP
PmeI Cut Site
BerGI Cut Site
WPRE
WPRE
WPRE

FIG. 1B – cont.

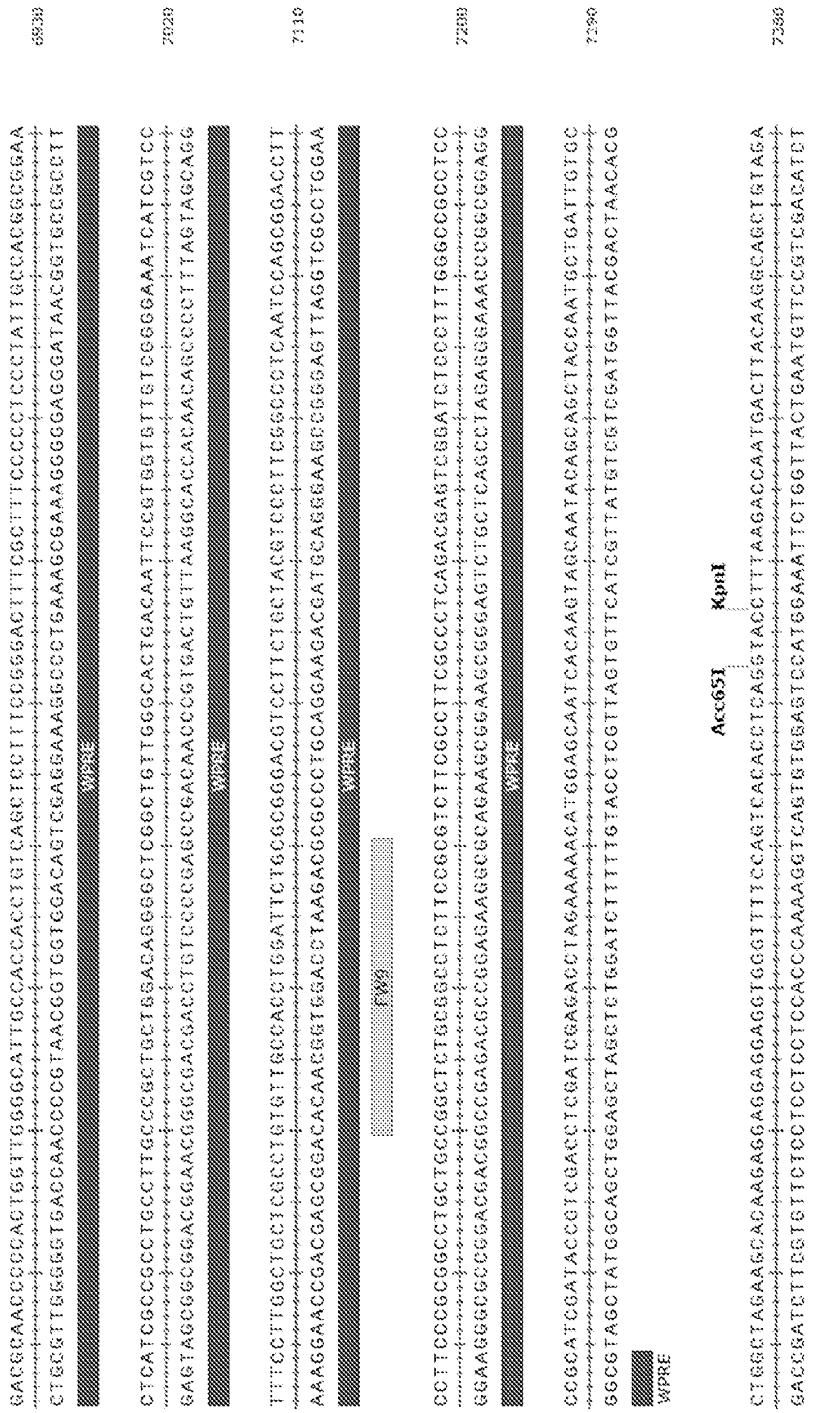


FIG. 1B – cont.

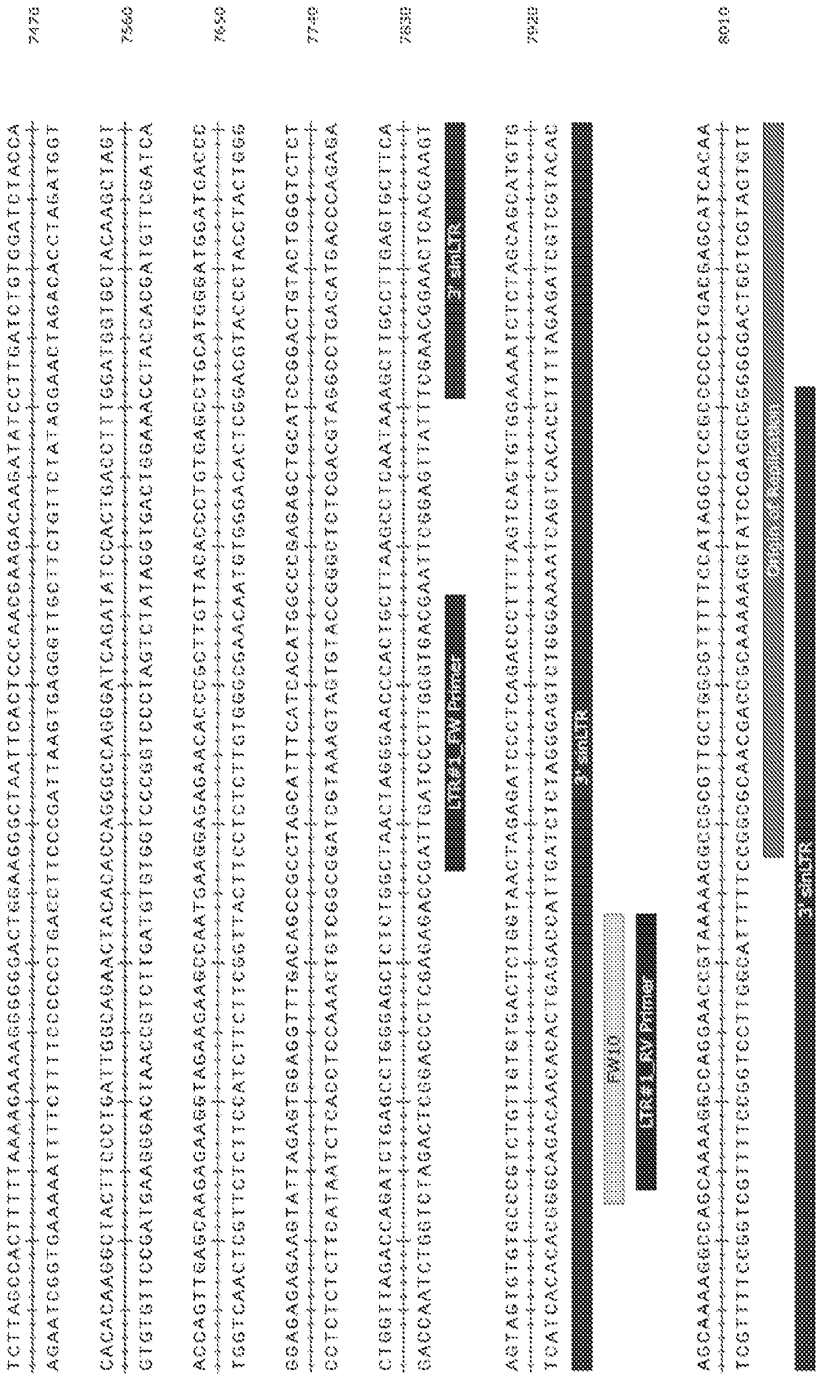

7470
7560
7650
7740
7830
7920
8010
3' sinLTR
3' sinLTR
FWD
GTR5.1 RV Primer
3' sinLTR
3' sinLTR

FIG. 1B – cont.

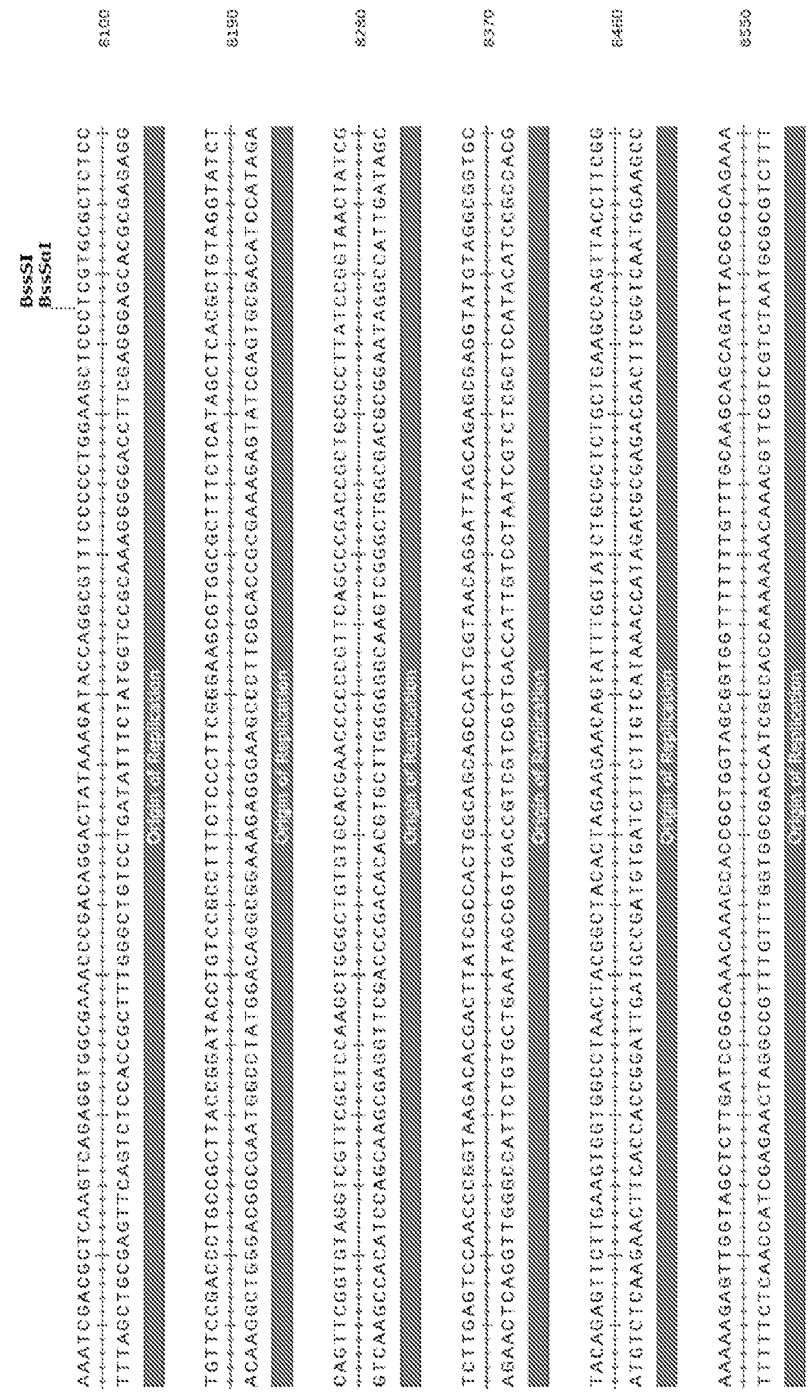


FIG. 1B – cont.

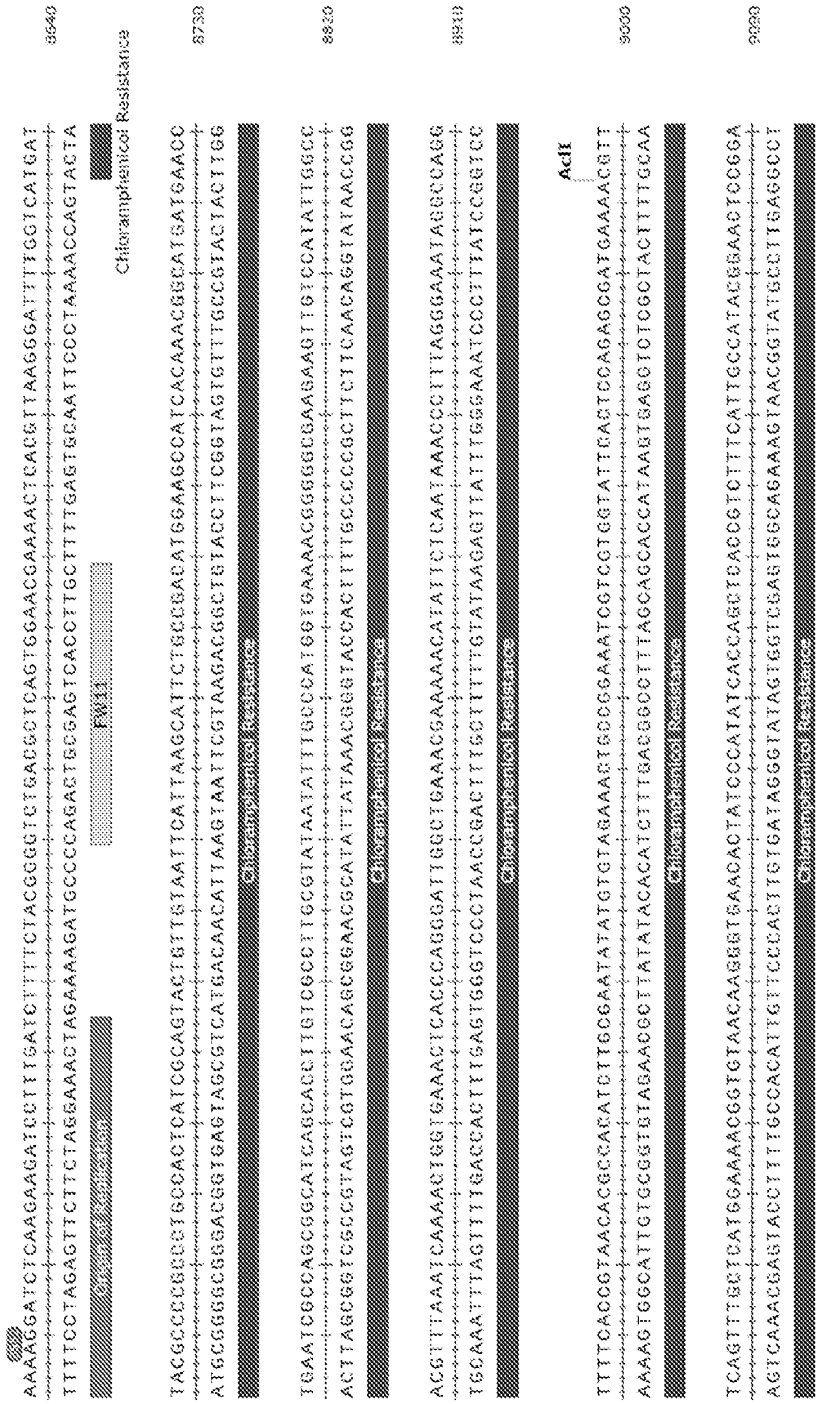

8640
FW11
Chloramphenicol Resistance
8730
Chloramphenicol Resistance
8820
Chloramphenicol Resistance
8910
Chloramphenicol Resistance
AclI
9000
Chloramphenicol Resistance
9090
Chloramphenicol Resistance

FIG. 1B – cont.

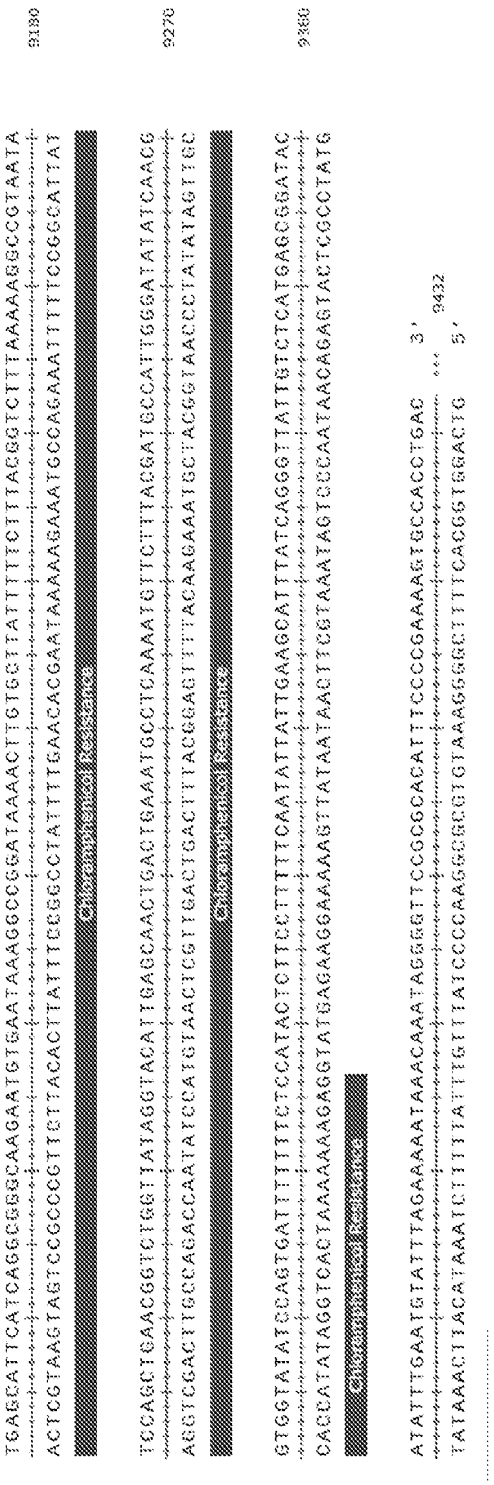


FIG. 1B – cont.

KCNQ1 (NM_000218.3)
sh#5: GTTCAAGCTGGACAAAGACAATGGGGTGA (SEQ ID NO:102)
shIMM: ATTTAAACTCGATAAGGATAACGGCGTCA (SEQ ID NO:103)

Nucleic Acid (WT):

ATGGCCGCGGCCTCCTCCCGCCCAGGGCCGAGAGGAAGCGCTGGGGTTGGGGCCGCCTGCCAGGCGCCCGGCGGGGCAGCGCGGGCCTGGCCAAG
AAGTGCCCCTTCTCGCTGGAGCTGGCGGAGGGCGGCCCGGCGGGCGGCGCGCTCTACGCGCCCATCGCGCCCGGCGCCCCAGGTCCCGCGCCCCCT
GCGTCCCCGGCCGCGCCCGCCGCGCCCCCAGTTGCCTCCGACCTTGGCCCGCGGCCGCGGTGAGCCTAGACCCGCGCGTCTCCATCTACAGCACG
CGCCGCCCGGTGTTGGCGCGCACCCACGTCCAGGGCCGCGTCTACAACTTCCTCGAGCGTCCCCACCGGCTGGAAATGCTTCGTTTACCACTTCGCC
GTCTTCCTCATCGTCCTGGTCTGCCTCATCTTCAGCGTGCTGTCCACCATCGAGCAGTATGCCGCCCTGGCCACGGGGACTCTCTTCTGGATGGAG
ATCGTGCTGGTGGTGTTCTTCGGGACGGAGTACGTGGTCCGCCTCTGGTCCGGCTGCCGCAGCAAGTACGTGGGGCCTCTGGGGGCGGCTGCGC
TTTGCCCCGGAAGCCCATTTCCATCATCGACCTCATCGTGGTCGTGGCCTCCTCTGCGTGGGCTCCAAGGGCGCAGGTGTTTGCCACG
TCGGCCATCAGGGGCATCCGCTTCCTGCAGATCCTGAGGATGCTACACGTCGACCGCCAGGGAGGCACCTGGAGGCTCCTGGGCTCCGTGGTCTTC
ATCCACCGCCAGGAGCTGATAACCACCCTGTACATCGGCTTCCTGGGCCTCATCTTCTCCTCGTACTTTGTGTACCTGGCTGAGAAGGACGCGGTG
AACGAGTCAGGCCGCGTGGAGTTCGGCAGCTACGCAGATGCGCTGTGGTGGGGGGTGGTCACAGTCACCACCATCGGCTATGGGGACAAGGTGCCC
CAGACGTGGGTCGGGAAGACCATCGCCTCCTTCTCTGTCTTTGCCATCTCCTTCTTTGCGCTCCCAGCGGGGATTCTTGGCTCGGGGTTTGCC
CTGAAGGTGCAGCAGAAGCAGAAGCACTTCAACCGGCAGATCCCGGCGGCAGCCTCACTCATTCAGACCGCATGGAGGTGCTATGCTGCC
GAGAACCCCGACTCCTCCACCTGGAAGATCTACATCCGGAAGGCCCCCCGGAGCCACACTCTGCTGTCACCCAGCCCCCAAACCCAAGAAGTCTGTG
GTGGTAAAGAAAAAAAAGTTCAAGCTGGACAAAGACAATGGGGTGACTCCTGGAGAGAAGATGCTCACAGTCCCCCATATCACGTGCGACCCCCCA
GAAGAGCGGCGGCTGGACCACTTCTCTGTCGACGGCTATAAGGAAGAGGCCCAACACTGCTGGAAGTGAGCATGCCCCCATTTCATG
AGAACCAACAGCTTCGCCGAGGACCTGGACCTGGAAGGGGAGACTCTGCTGACACCCATCACCCACATCTCACAGCTGCGGGAACACCATCGGGCC
ACCATTAAGGTCATTCGACGCATGCAGTACTTTGTGGCCAAGAAGAAATTCCAGCAAGCGCGGAAGCCTTACGATGTGCGGGACGTCATTGAGCAG
TACTCGCAGGGCCACCTCAACCTCATGGTGCGCATCAAGGAGCTGCAGAGGAGGCTGGACCAGTCCATTGGGAAGCCCTCACTGTTCATCTCCGTC
TCAGAAAAGAGCAAGGATCGCGGCAACAACGATCGGCGCCCGCCTGAACCGAGTAGAAGACAAGGTGACGCAGCTGGACCAGAGGCTGGCACTC
ATCACCGACATGCTTCACCAGCTGCTCTCCTTGCACGGTGGCAGCACCCCCGGCAGCGGCGCCCCCCAGAGAGGGCGGGGCCCACATCACCCAG
CCCTGCGGCAGTGGCGGCTCCGTCGACCCTGAGCTCTTCCTGCCCAGCAACACCCTGCCCACCTACGAGCAGCTGACCGTGCCCAGGAGGGGCCCC
GATGAGGGGTCCTGA (SEQ ID NO:1030)

FIG. 1C

Nucleic Acid (shIMM):

ATGGCCGCGGCCTCCTCCCCGCCCAGGGCCGAGAGGAAGCGCTGGGTTGGGGCCGCCTGCCAGGCGCCCGGCGGGCAGCGCGGCCTGGCCAAGAAGTGC
CCCTTCTCGCTGGAGCTGGCGGAGGGCGGCCCGGCGGGCGGCGCGCTCTACGCGCCCATCGCGCCCGGCGCCCCCAGGTCCCGCGCCCCCTGCGTCCCCGGCC
GCGCCCGCCGCGCCCCCCAGTTGCCTCCGACCTTGGCCCGCGGCCGGCCGGTGAGCCTAGACCCGCGCGTCTCCATCTACAGCACGCGCCGCCCGGTGTTGGCG
CGCACCCACGTCCAGGGCCGCGTCTACAACTTCCTCGAGCGTCCCCACCGGCTGGAAATGCTTCGTTTTACCACTTCGCCGTCTTCCTCATCGTCCTGGTCTGC
CTCATCTTCAGCGTGCTGTCCACCATCGAGCAGTATGCCGCCCTGGCCACGGGGACTCTCTTCTTGGATGGAGATCGTGCTGGTGTTCTTCGGGACGGAG
TACGTGGTCCGCCTCTGGTCCGCCGGCTGCCGCAGCAAGTACGTGGGCCTCTGGGGGCGGCTGCGCTTTGCCCGGAAGCCCATTTCCATCATCGACCTCATC
GTGGTCGTGGCCTCCATGGTGGTCCTCTGCGTGGGCTCCAAGGGGCAGGTGTTTGCCACGTCGGCCATCAGGGCATCCGCTTCCTGCAGATCCTGAGGATG
CTACACGTCGACCGCCAGGGAGGCACCTGGAGGCTCCGTGGTCTTCATCCACCGCCAGGAGCTGATAACCACCCTGTACATCGGCTTCCTGGGC
CTCATCTTCTCCTCGTACTTTGTGTACCTGGCTGAGAAGGACGCGGTGAACGAGTCAGGCCGCGTGGAGTTCGGCAGCTACGCAGATGCGCTGTGGTGGGGG
GTGGTCACAGTCACCACCATCGGCTATGGGGACAAGGTGCCCCAGACGTGGGTCGGGAAGACCATCGCCTCCTGCTTCTCTGTCTTTGCCATCTCCTTCTTT
GCGCTCCCCAGCGGGGATTCTTGGCTCGGGGTTTGCCCTGAAGGTGCAGCAGAAGGCAGAGGCAGAAGCACTTCAACCGGCAGATCCCGGCGGCAGCCTCACTC
ATTCAGACCGCATGGAGGTGCTATGCTGCCGAGAACCCCGACTCCTCCACCTGGAAGATCTACATCCGGAAGGCCCCCCGGAGCCACACTCTGCTGTCACCC
AGCCCCAAACCCAAGAAGTCTGTGGTGGTAAAGAAAAAAAAATTTAAACTCGATAAGGATAACGGCGTCACTCCTGGAGAGAAGATGCTCACAGTCCCCCAT
ATCACGTGCGACCCCCCCAGAAGAGCGGCGGCTGGACCACTTCTCTGTCGACGGCTATGACAGTTCTGTAAGGAAGAGCCCAACACTGCTGGAAGTGAGCATG
CCCCATTTCATGAGAACCAACAGCTTCGCCGAGGACCTGGACCTGGAAGGGGAGACTCTGCTGACACCCATCACCCACATCTCACAGCTGCGGGAACACCAT
CGGGCCACCATTAAGGTCATTCGACGCATGCAGTACTTTGTGGCCAAGAAGAAATTCCAGCAAGCGCGGAAGCCTTACGATGTGCGGGACGTCATTGAGCAG
TACTCGCAGGGCCACCTCAACCTCATGGTGCGCATCAAGGAGGAGGCTGCAGAGGAGGCTGGACCAGTCCATTGGGAAGCCCTCACTGTTCATCTCCGTCTCAGAA
AAGAGCAAGGATCGCGGCAGCAACACGATCGGCGCCCGCCTGAACCGAGTAGAAGACAAGGTGACGCAGCTGGACCAGAGGCTGGCACTCATCACCGACATG
CTTCACCAGCTGCTCTCCTTGCACGGTGGCAGCACCCCCGGCAGCGGCGCCCCCCAGAGAGGGCGGGGCCCACATCACCCAGCCCTGCGGCAGTGGCGGC
TCCGTCGACCCTGAGCTCTTCCTGCCCAGCAACACCCTGCCCACCTACGAGCAGCTGACCGTGCCCAGGAGGGGCCCCGATGAGGGGTCCTGA (SEQ ID
NO:1031)

Amino Acid

MAAASSPPRAERKRWGWGRLPGARRGSAGLAKKCPFSLELAEGGPAGGALYAPIAPGAPGPAPPASPAAPAAPPVASDLGPRPPVSLDPRVSIYSTRRPVLA
RTHVQGRVYNFLERPTGWKCFVYHFAVFLIVLVCLIFSVLSTIEQYAALATGTLFWMEIVLVVFFGTEYVVRLWSAGCRSKYVGLWGRLRFARKPISIIDLI
VVVASMVVLCVGSKGQVFATSAIRGIRFLQILRMLHVDRQGGTWRLLGSVVFIHRQELITTLYIGFLGLIFSSYFVYLAEKDAVNESGRVEFGSYADALWWG
VVTVTTIGYGDKVPQTWVGKTIASCFSVFAISFFALPAGILGSGFALKVQQKQRQKHFNRQIPAAASLIQTAWRCYAAENPDSSTWKIYIRKAPRSHTLLSP
SPKPKKSVVVKKKKFKLDKDNGVTPGEKMLTVPHITCDPPEERRLDHFSVDGYDSSVRKSPTLLEVSMPHFMRTNSFAEDLDLEGETLLTPITHISQLREHH
RATIKVIRRMQYFVAKKKFQQARKPYDVRDVIEQYSQGHLNLMVRIKELQRRLDQSIGKPSLFISVSEKSKDRGSNTIGARLNRVEDKVTQLDQRLALITDM
LHQLLSLHGGSTPGSGGPPREGGAHITQPCGSGGSVDPELFLPSNTLPTYEQLTVPRRGPDEGS (SEQ ID NO:1032)

FIG. 1C – cont.

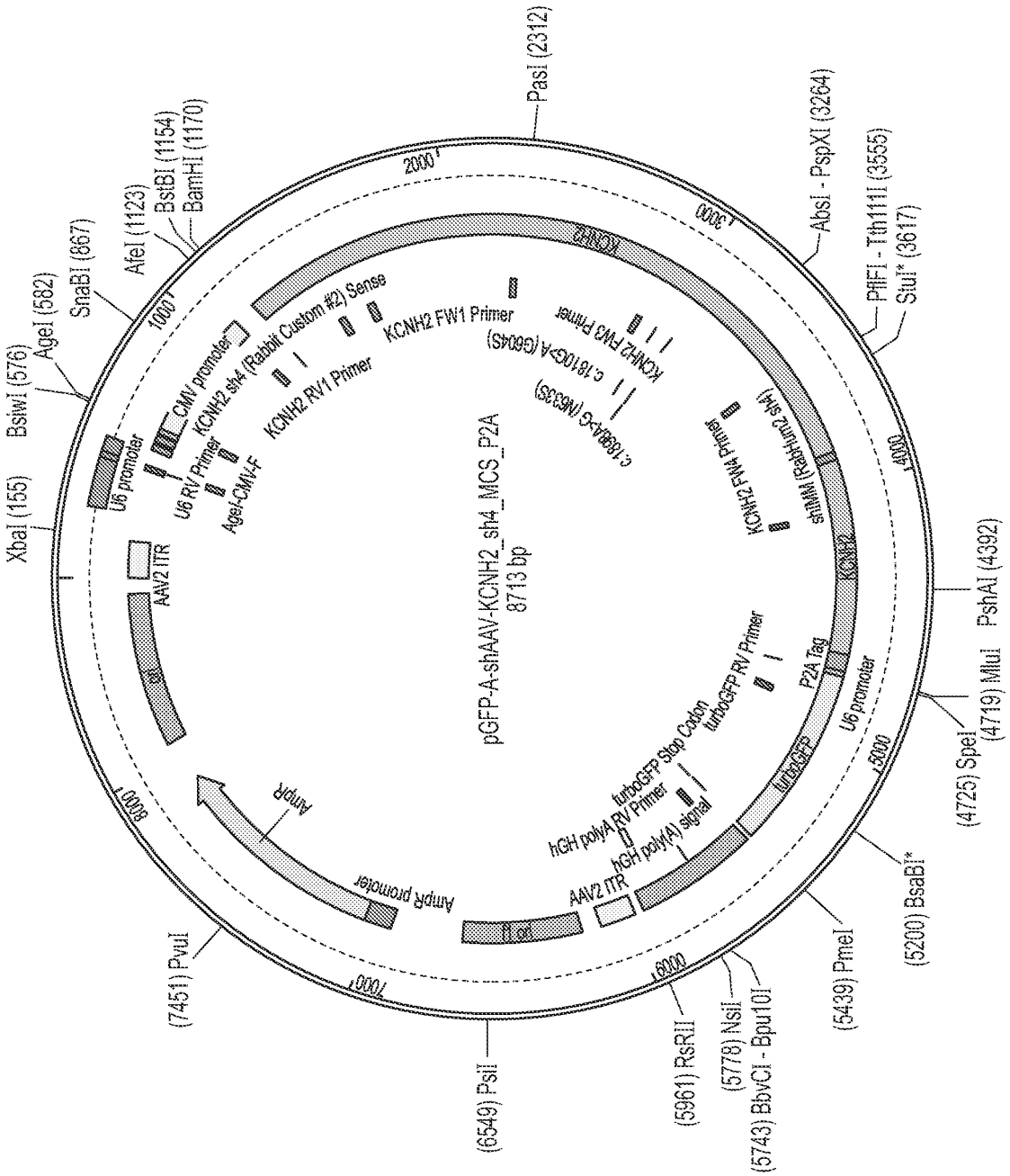

pGFP-A-shAAV-KCNH2_sh4_MCS_P2A
8713 bp
1000
2000
3000
4000
5000
6000
7000
8000
PasI (2312)
AbsI - PspXI (3264)
PflFI - Tth111I (3555)
StuI* (3617)
PshAI (4392)
MluI (4719)
SpeI (4725)
BsaBI* (5200)
PmeI (5439)
NsiI (5778)
BbvCI - Bpu10I (5743)
RsrII (5961)
PsiI (6549)
PvuI (7451)
XbaI (155)
BsiwI (576)
AgeI (582)
SnaBI (867)
AfeI (1123)
BstBI (1154)
BamHI (1170)
KCNH2
U6 promoter
CMV promoter
KCNH2 sh4 (Rabbit Custom #2) Sense
KCNH2 FW1 Primer
KCNH2 RV1 Primer
U6 RV Primer
AgeI-CMV-F
c.1809A>G (N603S)
c.1810C>A (G604S)
KCNH2 FW3 Primer
KCNH2 FW4 Primer
shMM (Rabbit Custom #2) sh4
AAV2 ITR
P2A Tag
turboGFP
turboGFP RV Primer
turboGFP Stop Codon
hGH poly(A) signal
hGH polyA RV Primer
ori
AmpR
AmpR promoter

FIG. 2A

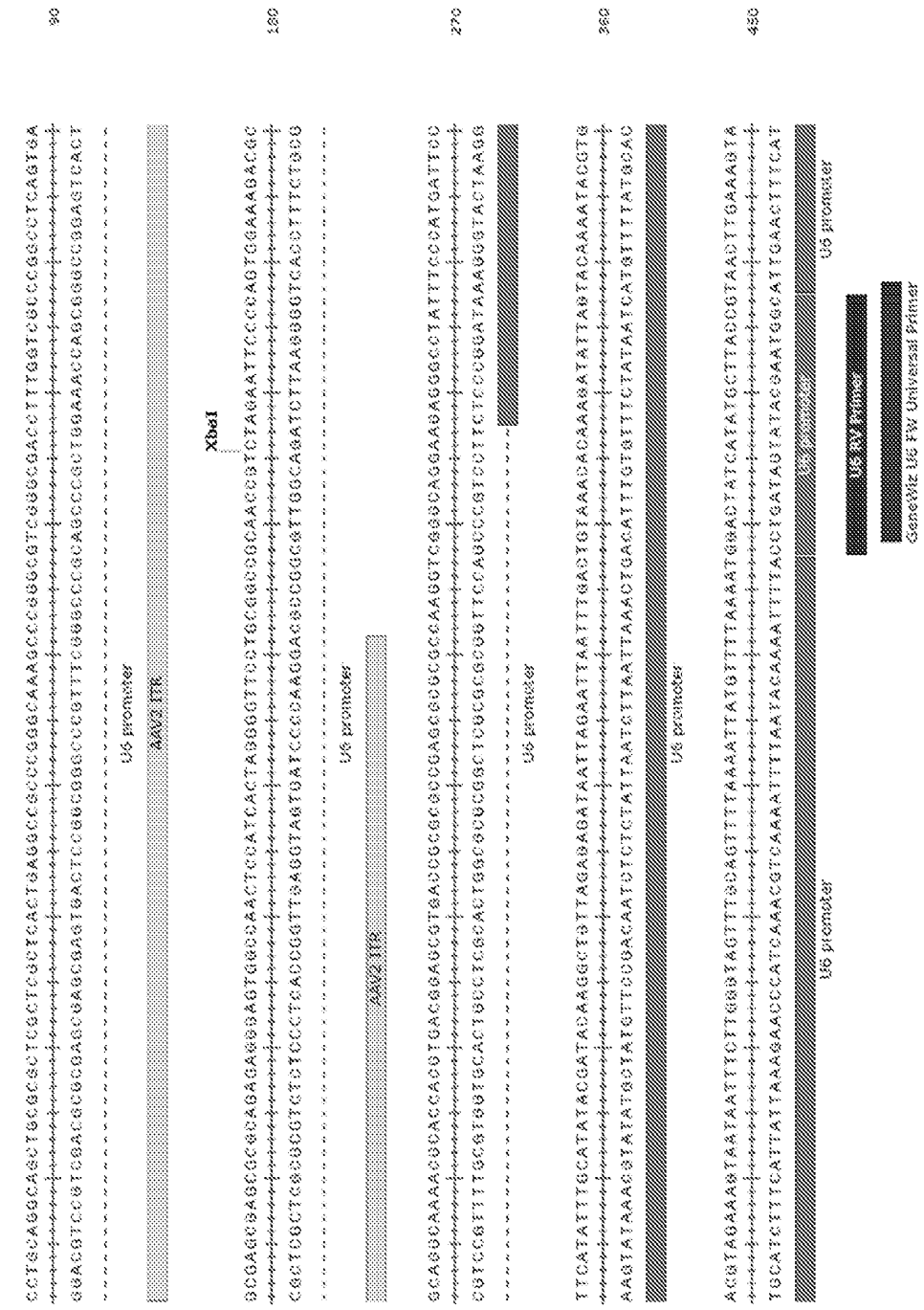

5'
3'
90
180
270
360
450
U6 promoter
AAV2 ITR
XbaI
U6 promoter
AAV2 ITR
U6 promoter
U6 promoter
U6 promoter
U6 promoter
U6 FW Primer
GeneWiz U6 FW Universal Primer

FIG. 2B


540
630
720
810
U6 promoter
KCNH2 sh4 (Rabbit Custom #2) Antisense
CMV promoter
BsiWI
AgeI

FIG. 2B – cont.

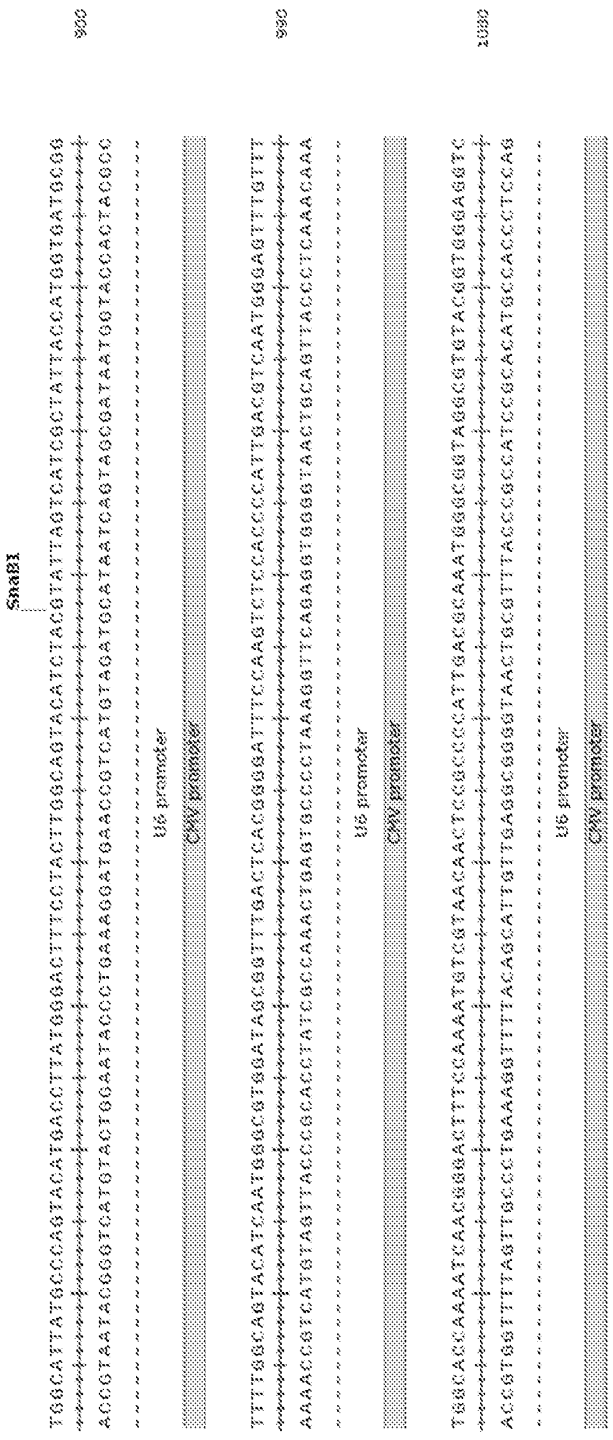

SnaBI
AflI
BstBI
CMV FW Primer (GeneWiz Universal Primer)
U6 promoter
CMV promoter

FIG. 2B – cont.

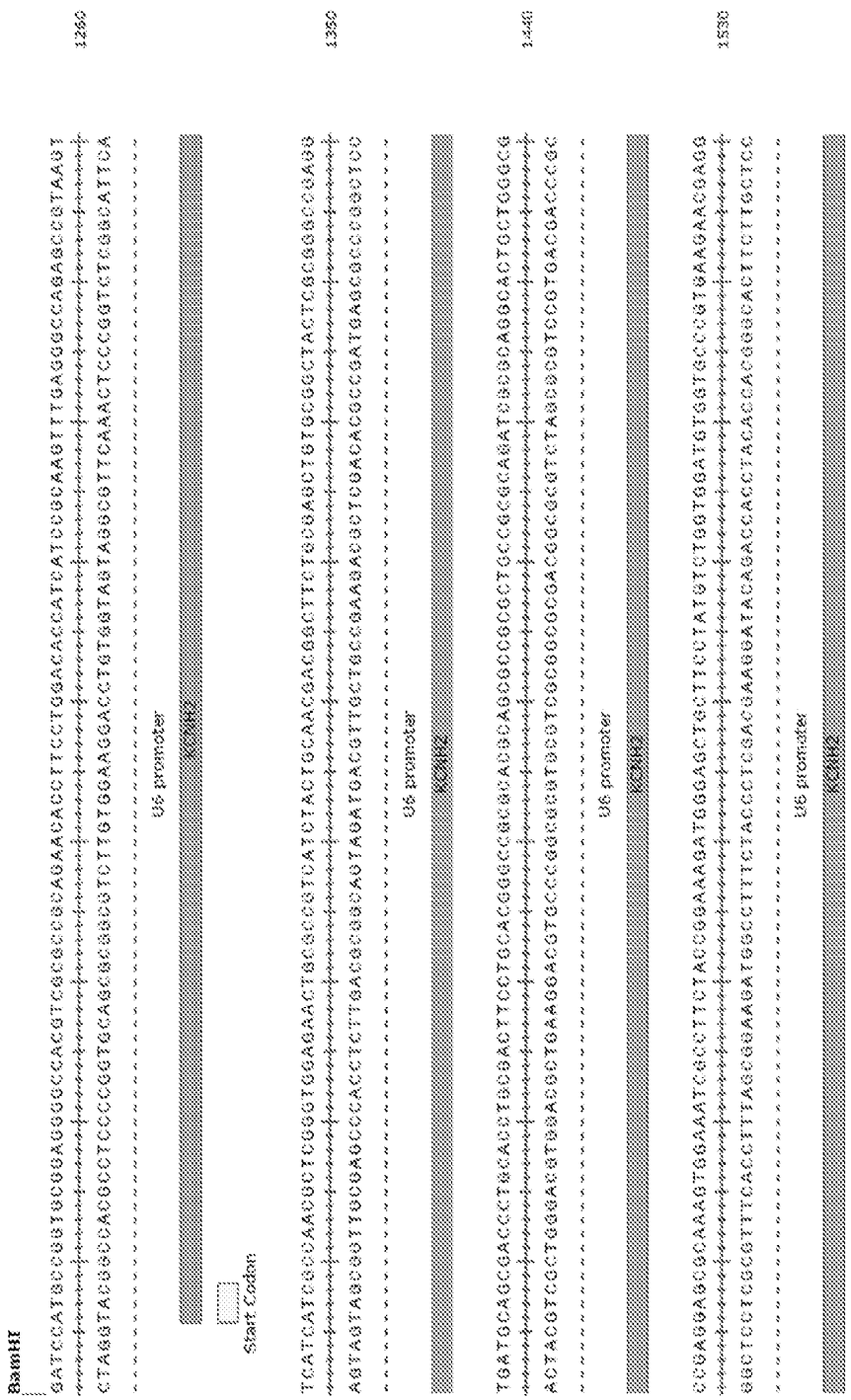

BamHI
1260
1350
1440
1530
U6 promoter
KCNH2
Start Codon
KCNH2 Exon 2

FIG. 2B – cont.


1620
1710
1800
1890
1980
US promoter
US promoter
US promoter
US promoter
US promoter

FIG. 2B – cont.

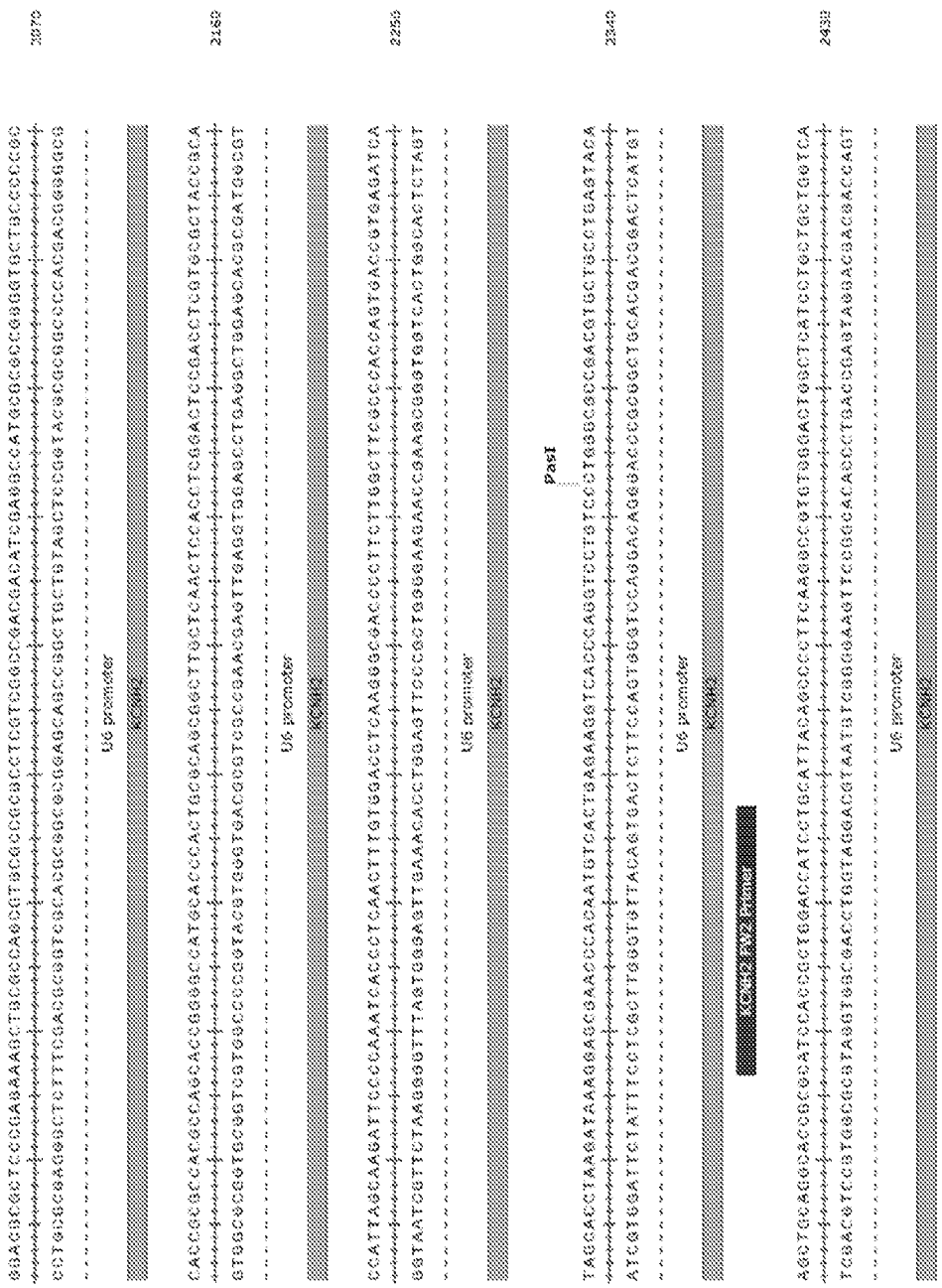

U6 promoter
KCNK6
PasI
U6 promoter
KCNK6
U6 promoter
KCNK6
U6 promoter
KCNK6
U6 promoter
KCNK6

FIG. 2B – cont.

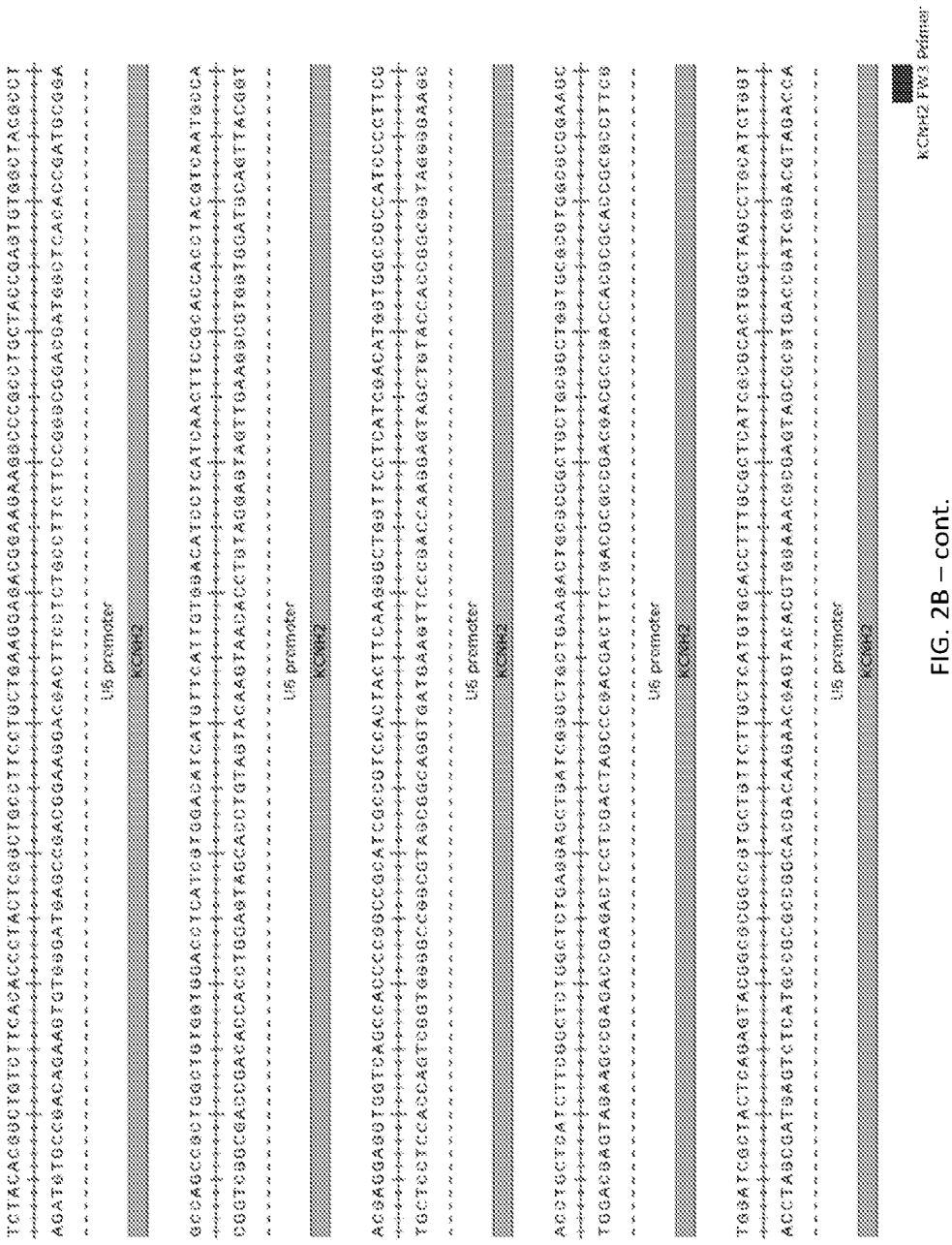


FIG. 2B – cont.

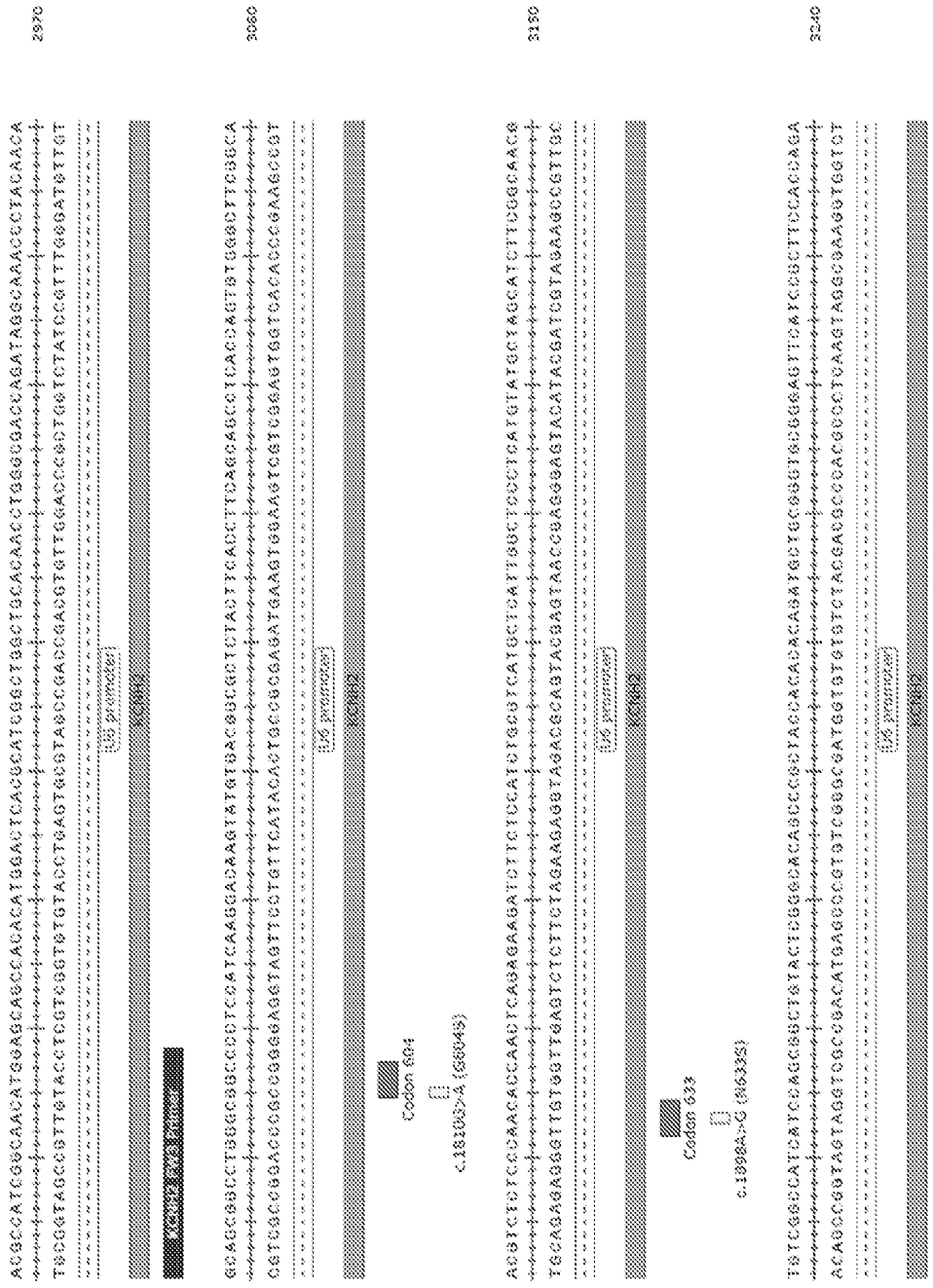

2970
3060
3150
3240
U6 promoter
KCNH2
Codon 604
c.1810G>A (G604S)
Codon 633
c.1898A>G (M633S)

FIG. 2B – cont.

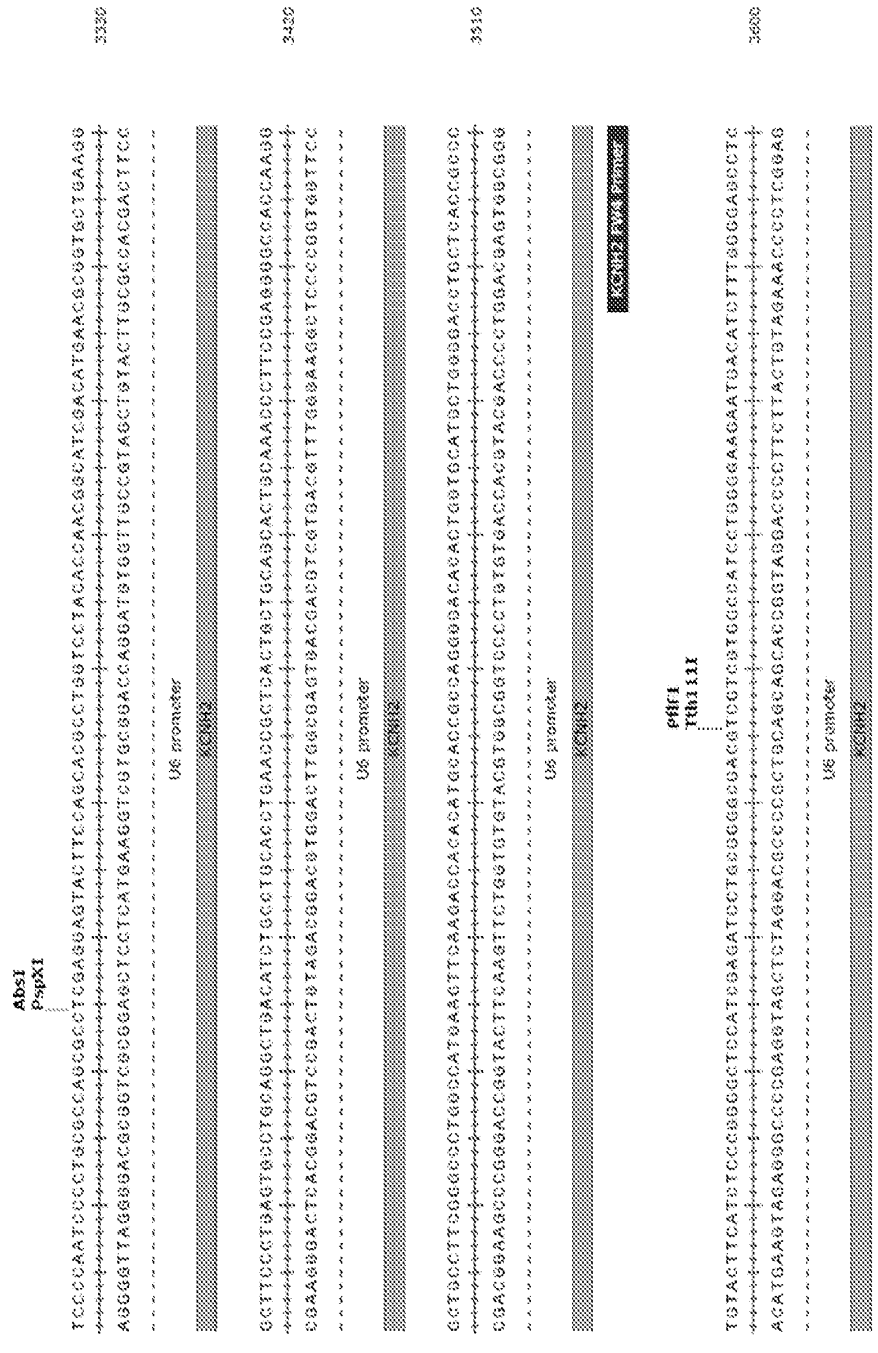

AbsI
PspXI
3330
3420
3510
3600
U6 promoter
KCNH2
KCNH2 FW4 Primer

FIG. 2B – cont.


3680
3780
3870
3960
4050
U6 promoter
U6 promoter
U6 promoter
U6 promoter
U6 promoter

FIG. 2B – cont.



FIG. 2B – cont.


4590
4680
4770
4860
4950
U6 promoter
U6 promoter
U6 promoter
U6 promoter
U6 promoter
MluI
SpeI
turboGFP Start Codon

FIG. 2B – cont.



FIG. 2B – cont.

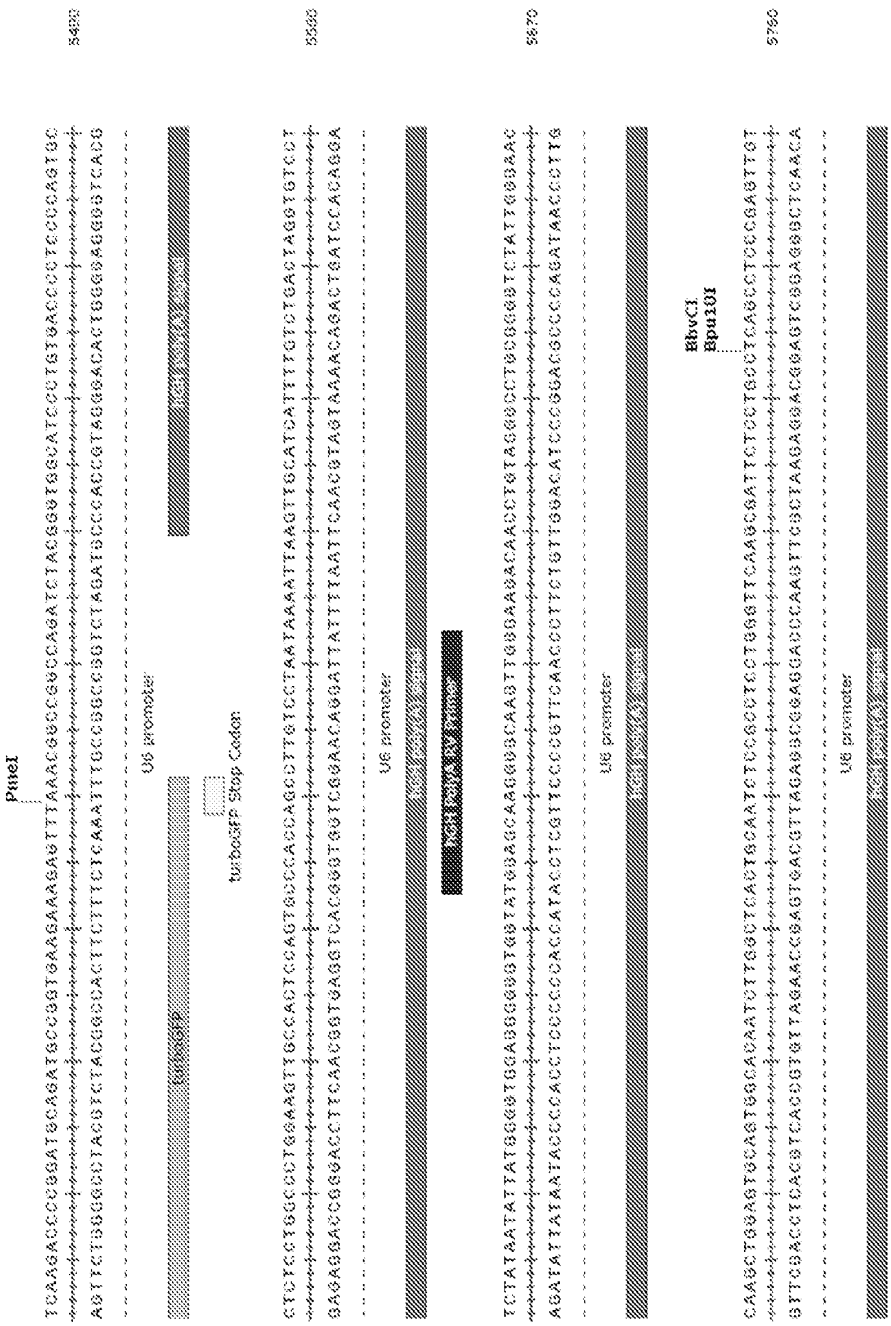

PineI
U6 promoter
turboGFP Stop Codon
U6 promoter
U6 promoter
BbvCI
Bpu10I
U6 promoter

FIG. 2B – cont.



FIG. 2B – cont.



FIG. 2B – cont.

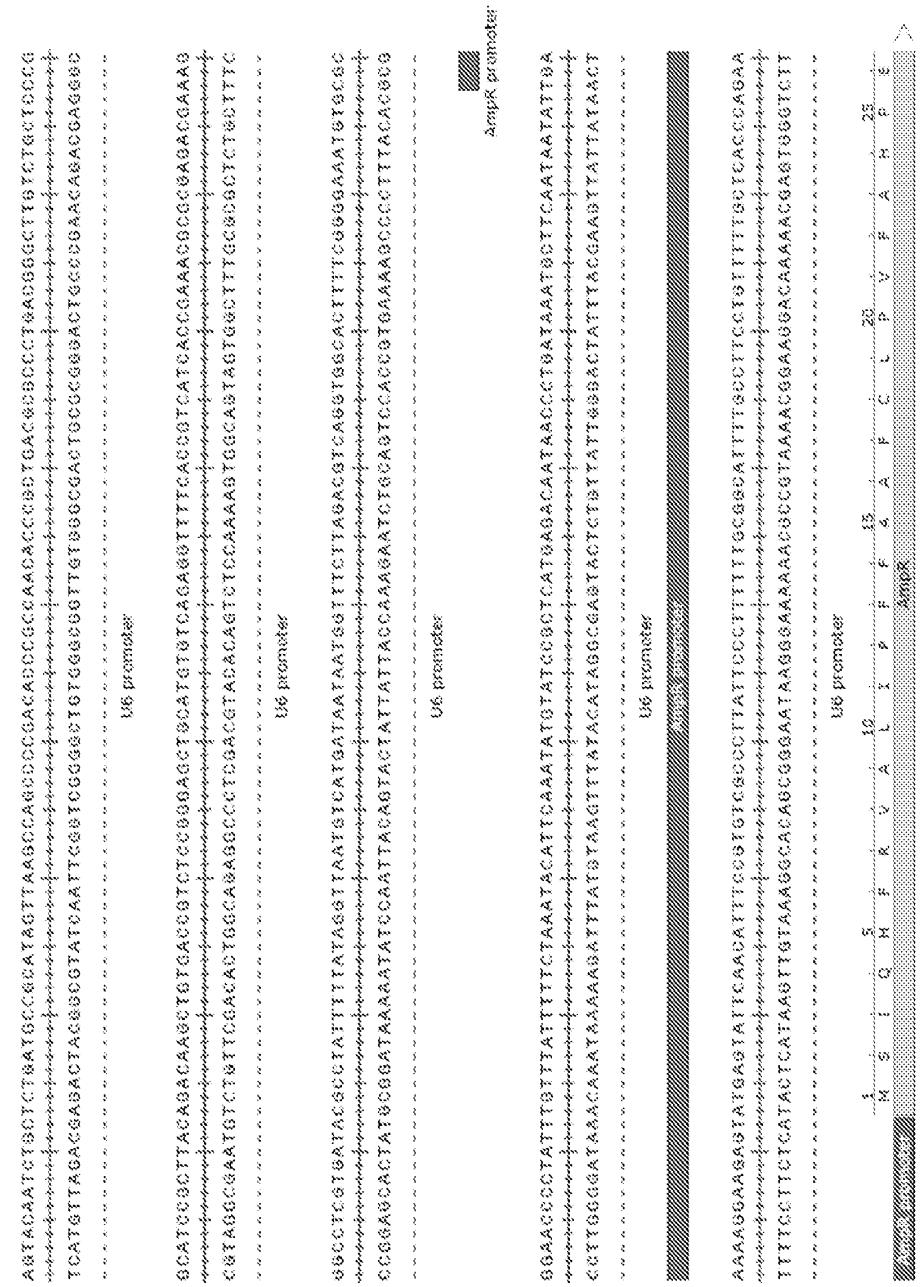

6750
6840
6930
7020
7110
U6 promoter
U6 promoter
U6 promoter
AmpR promoter
U6 promoter
AmpR
M S I Q H F R V A L I P F F A F C L P V F A H P E

FIG. 2B – cont.

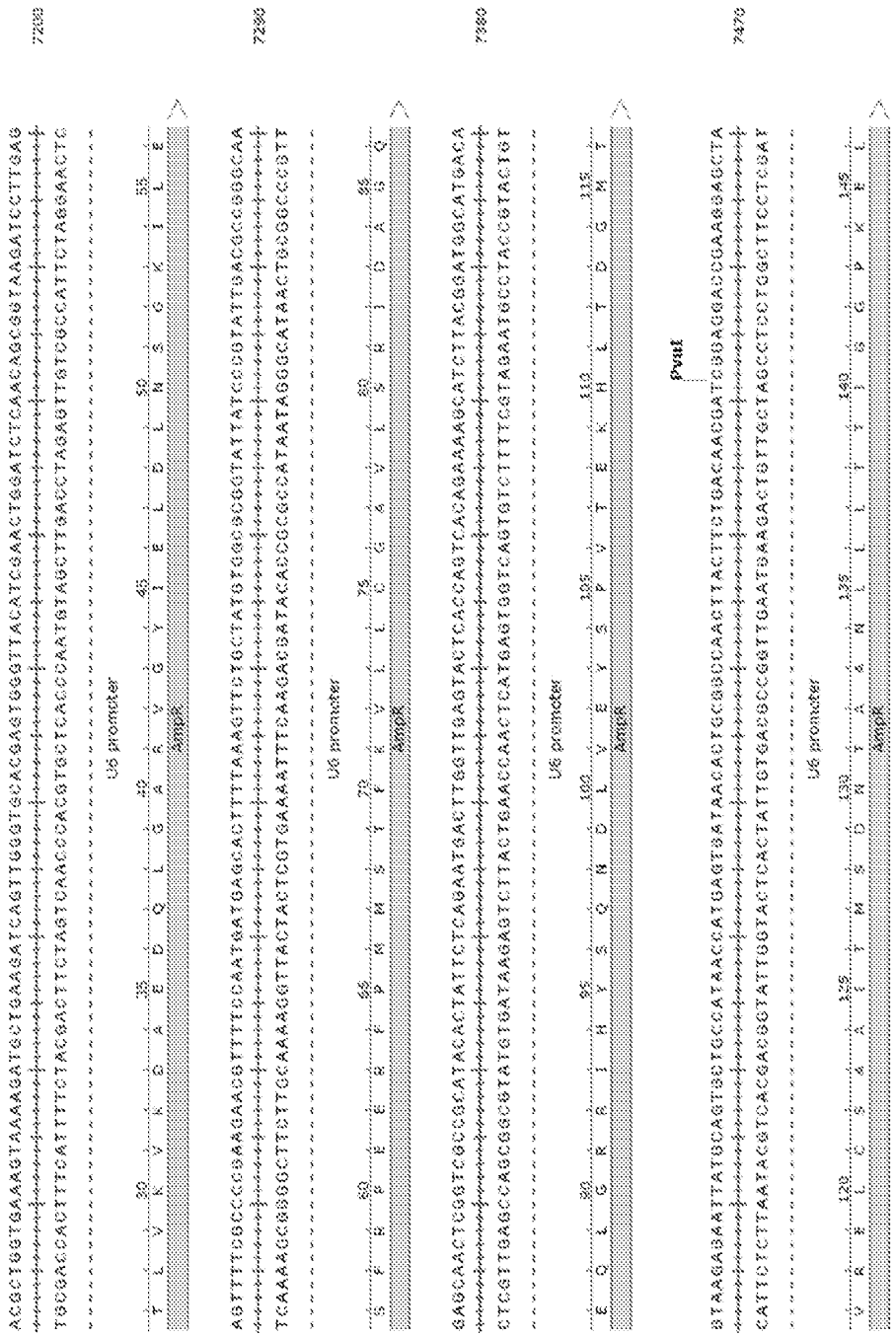


FIG. 2B – cont.

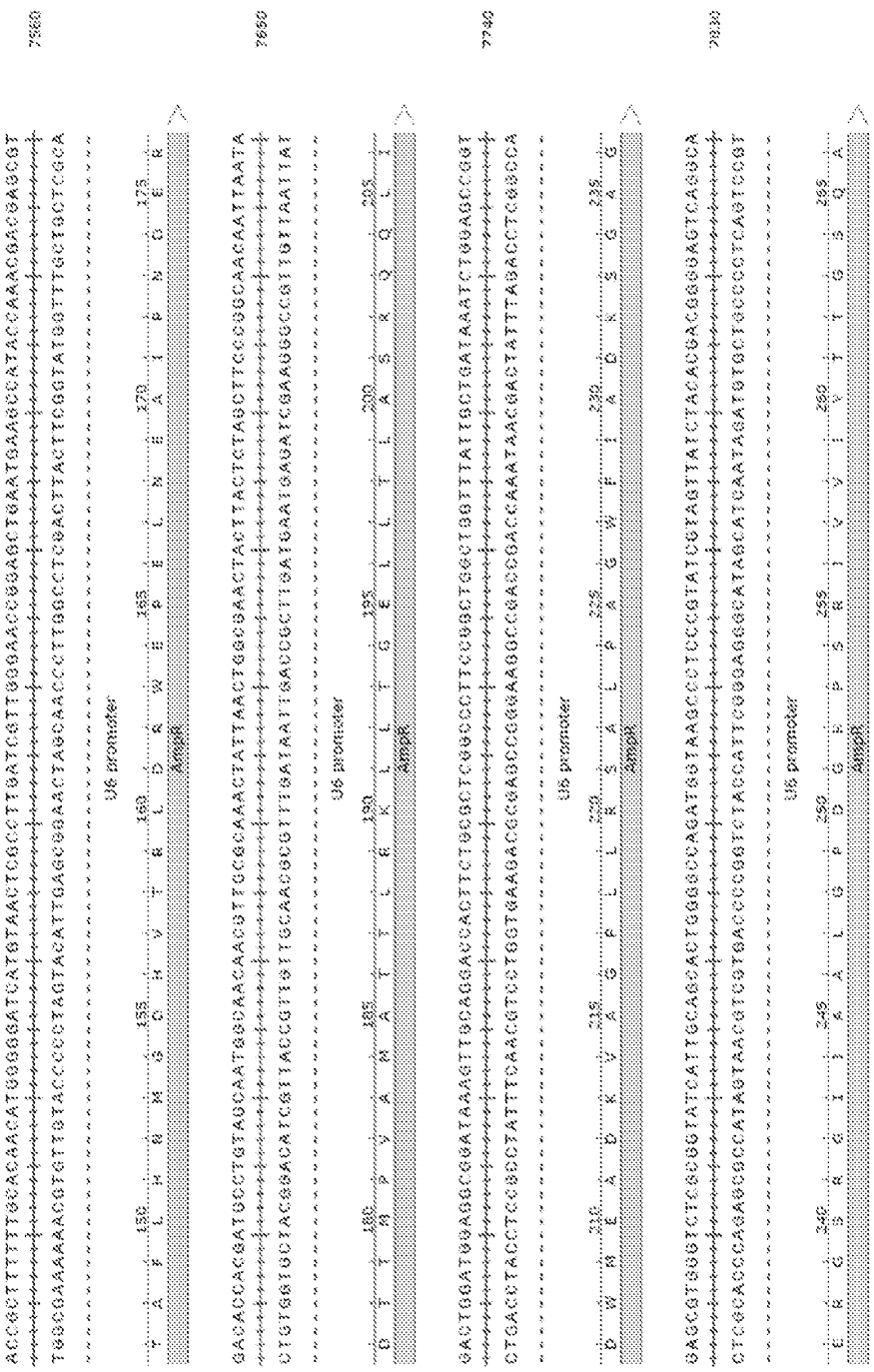


FIG. 2B – cont.

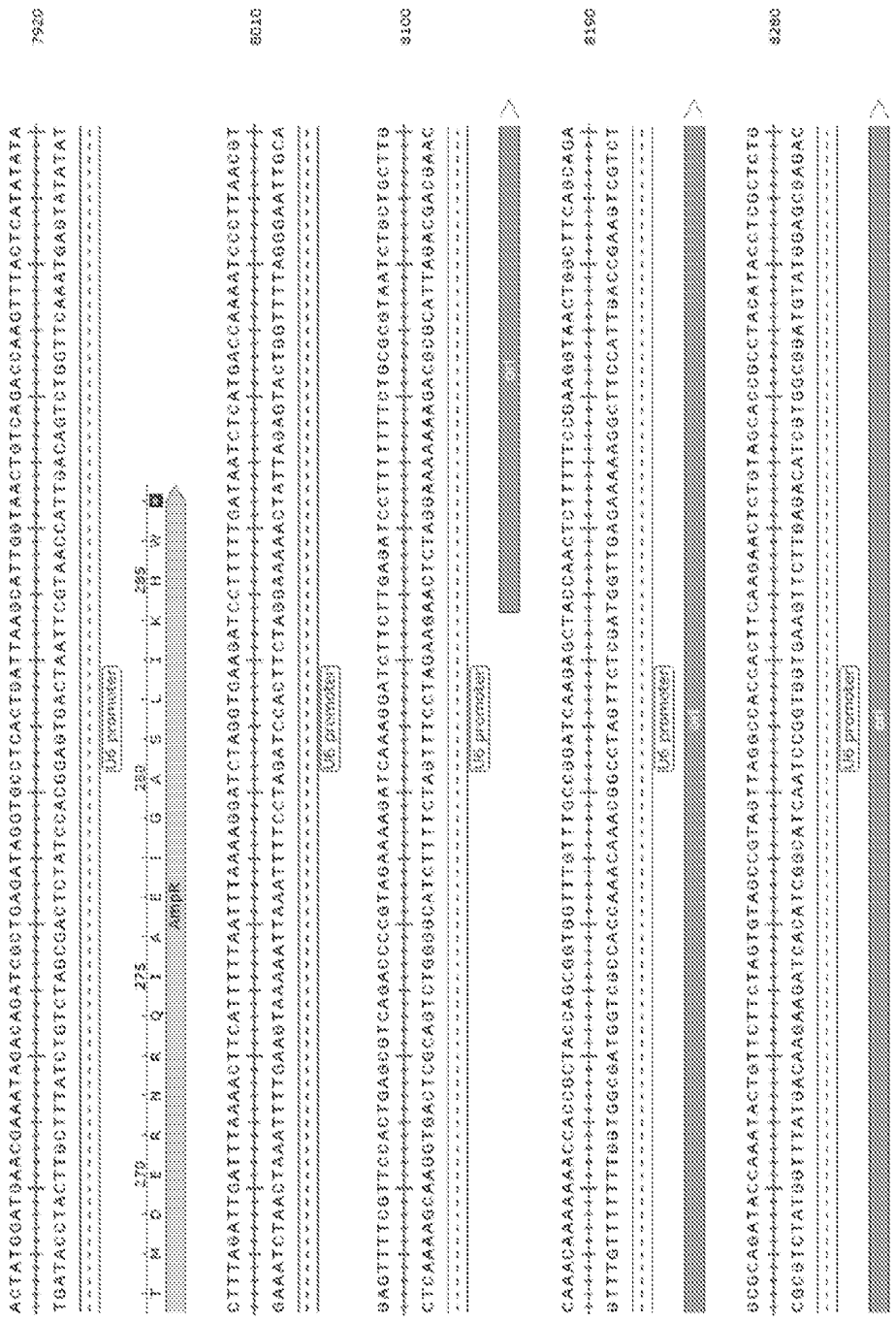


FIG. 2B – cont.


U6 promoter
U6 promoter
U6 promoter
U6 promoter
U6 promoter
5'
3'
5'

FIG. 2B – cont.

KCNH2 (NM_000238.4)
RAB_sh#4: CACGGAGCAGCCAGGGGAGGTGTCGGCCT (SEQ ID NO:27)
shIMM : TACCGAACAACCTGGCGAAGTCTCCGCGT (SEQ ID NO:29)

Nucleic Acid (WT)

ATGCCGGTGCGGAGGGCCACGTCGCGCCGCAGAACACCTTCCTGGACACCATCATCCGCAAGTTTGAGGGCCAGAGCCGTAAGTTCATCATCGCCAACGCTCGGGTGGAGAACTGCGC
CGTCATCTACTGCAAACGACGGCTTCTGCGAGCTGTGCGGCTACTCGCGGGGCCGAGGTGATGCAGCGACCTGCACCTGCGACTTCCTGCACGGGCCGCGCACGCCAGCGCCGCTGCCG
CGCAGATCGCGCAGGCACTGCTGGGCGCCGAGGAGCGCAAAGTGGAAATCGCCTTCTACCGGAAAGATGGGAGCTGCTTCCTATGTCTGGTGGATGTGGTGCCCGTGAAGAACGAGGAT
GGGGCTGTCATCATGTTCATCCTCAATTTCGAGGTGGTGATGGAGAAGGACATGGTGGGGTCCCCGGCTCATGACACCAACCGGGGCGCCCCGGGGCCGTGGCTGGCCCCAGGCCG
CGCCAAGACCTTCCGCCTGAAGCTGCCCGCGCTGCGGTCGGTGCGGCGCGGCGCCCCGGGGGCCGTGGTGGTGACGTGG
ACCTGACGCCCGCGCACCCAGCGAGTCGCTGGCCCTGGACGAAGTGACAGCCATGGACAACCACGTGGCAGGGCTCGGCGCCGGAGAGGCGGCGTGCGCTGGTGGGTCCCGGC
TCTCCGCCCCCGCAGCGCGCCCGGCCAGCTCCCATCGCCCCGGGCGCACACGCCTCAACCCCGACGCCTCGGCTCCAGCTGCAGCCTGGCCCGGACGCCTCCCGAGAAAGCTGCGCCAG
CGTGCGCCGCGCCTCGTCGGCCAGACATCGAGGCCATGCGCGCCGCGCCACGCCAGCACCGGGGCCATGCACCCACTGCGCAGCGGCTTGCTCAACT
CCACCTCGGACTCCGACCTCGTGCGCTACCGCACCATTAGCAAGATTCCCCAAATCACCCTCAACTTTGTGGACCTCAAGGGCGACCCCTTCTTGGCTTCGCCCACCAGTGACCGTGAG
ATCATAGCACCTAAGATAAAGGAGCGAACCCACAATGTCACTGAGAAGGTCACCCAGGTCCTGTCCCTGGGCGCCGACTGCTCGTCATTTCGACCTGATCCAGCGCGCCGCTACCGCA
CTGGACCATCCTGCATTACAGCCCCTTCAAGGCCGTGTGGGACTGGCTCATCCTGCTGCTGGTCATCTACACGGCTGTCTTCACACCCTACTCGGCTGCCTTCCTGCTGAAGGAGACGG
AAGAAGGCCCCGCCTGCTACCGAGTGTGGCTACGCCTGCCAGCCGCTGGTGTGGTGGACCTCATCGTGGACATCATGTTCATTGTGGACATCCTCATCAACTTCCGCACCACCTACGTC
AATGCCAACGAGGAGGTGGTCAGCCACCCCGGCCGCATCGCCGTCCACTACTTCAAGGGCTGGTTCCTCATCGACATTGGTGGCCGCCATCCCCTTCGACCTGCTCATCTTCGGCTCTGG
CTCTGAGGAGCTGATCGGGCTGCTGAAGACTGCGCGGCTGGTGCGCGTGGCGCGGAAGCTGGATCGCTACTCAGAGTACGGCGCGGCCGTGCTGTTCTTGCTCATGTGCA
CCTTTGCGCTCATCGCGCACTGGCTAGCCTGCATCTGGTACGCCATCGGCAACATGGAGCAGCCACACATGGACTCACGCATCGGCTGGCTGCACAACCTGGGCGACCAGATAGGCAAA
CCCTACAACAGCAGCAGCGCCTGGGCGGCCCTCCATCAAGGACAAGTATGTGACGGCGCTCTACTTCACCTTCAGCAGCCTCACCAGTGTGGCTTCGGCAACGTCTCTCCCAACACCAA
CTCAGAGAAGATCTTCTCCATCTGCGTCATGCTCATTGGCTCCCTCATGTATGCTAGCATCTTCGGCAACGTGTCGGCCATCATCCAGCGGCTGTACTCGGGCACAGCCCGCTACCACA
CACAGATGCTGCGGGTGCGGGAGTTCATCCGCTTCCACCAGATCCCCAATCCCCTGCGCCAGCGCCTCGAGGAGTACTTCCAGCACGCCTGGTCCTACACCAACGGCATCGACATGAAC
GCGGTGCTGAAGGGCTTCCCTGAGTGCCTGCAGGCTGACATCTGCCTGCACCTGAACCGCTCACTGCTGCAGCACTGCTACTTCATCTCCGGGGCCTGCTTCGGGCCCT
GGCCATGAAGTTCAAGACCACACACATGCACCGCCAGGGGACACACTGGTGCATGCTGGGGACTGCTCACCGCCCTGTACTTCATCTCCGGGGCCCTCCATCGAGATCCTGCGGGGCGACG
TCGTCGTGGCCATCCTGGGGAAGAATGACATCTTTGGGGAGCCTCTGAACCTGTATGCAAGGCTGGCAAGTCGAACGGGGATGTGCGGGCCCTCACCTACTGTGACCTACACACAAGATC
CATCGGGACGACCTGCTGGAGGTGCTGGACATGTACCTGAGTTCTCCGACCACTTCTGGTCCAGCCTGGAGATCACCTTCAACCTGCGAGATACCAACATGATCCCGGGCTCCCCCGG
CAGTACGGAGTTAGAGGGTGGCTTCAGTCGGCAACGCAAGCGCAAGTTGTCCTTCCGCAGGCGCACGGACAAGGACAGGGAGGTGTCGGCCTTGGGGCCGGGCCGGG
CGGGGGCAGGGCCGAGTAGCCGGGGCCGGCCGGGGGGGAGAGCCCGTCCAGTGGCCCCTCCAGCCCTGAGAGCATGAGGATGAGGGCCCAGGCCGCAGTCCCAGCCCC
CTCCGCCTGGTGCCCTTCTCCAGCCCCAGGCCCCCCGGGTGGGGAGCCCCTGATGGAGGACTGCGAGAAGAGCAGCGACACTTGCAACCCCCTGTCAGGCGCCTTCTC
AGGAGTGTCCAACATTTTCAGCTTCTGGGGGGACAGTCGGGGCCGCCAGTACCAGGAGCTCCCTCGATGCCCCGCCCCCACACCCCAGCCTCCTCAACATCCCCCTCCTCCAGCCCGGGTC
GGCGGCCCCGGGGCGAGTGGAGAGGCAGGCTGGATGCCCTCCAGCGCCAGCTCAACAGGCTGGAGACCCGGCTGAGTGCAGACATGGCCACTGTCCTGCAGCTACAGAGGCAGATG
ACGCTGGTCCCGCCCCGCCTACAGTGCTGTGACCACCCCGGGGCCTGGCCCCACTTCCACATCCCCGCTGTTGCCCGTCAGCCCCCTCCCCACACCTCACCTTGGACTCGCTTTCTCAGGT
TTCCCAGTTCATGGCGTGTGAGGAGCTGCCCCCGGGGGCCCCAGAGCTTCCCCAAGAAGGCCCCACACGACGCCTCTCCCTACGGGCCAGCTGGGGGCCCTCACCTCCCAGCCCCTGC
ACAGACACGGCTCGGACCCGGGCAGTTAG (SEQ ID NO:1034)

FIG. 2C

Nucleic Acid (shIMM)

ATGCCGGTGCGGAGGGGCCACGTCGCGCCGCAGAACACCTTCCTGGACACCATCATCCGCAAGTTTGAGGGCCAGAGCCGTAAGTTCATCATCGCCAACGCTC
GGGTGGAGAACTGCCGCCGTCATCTACTGCAACGACGGCTTCTGCGAGCTGTGCGGCTACTCGCGGGCCGAGGTGATGCAGCGACCCTGCACCTGCGACTTCCT
GCACGGGCCGCGCACGCAGCGCCGCTGCCGCGCAGATCGCGCAGGCACTGCTGGGCGCCGAGGAGCGCAAAGTGGAAATCGCCTTCTACCGGAAAGATGGG
AGCTGCTTCCTATGTCTGGTGGATGTGGTCGCCGTGAAGAACGAGGATGGGGCTGTCATCATGTTCATCCTCAATTTCGAGGTGGTGATGGAGAAGGACATGG
TGGGGTCCCCGGCTCATGACACCAACCACCGGGGCCCCCCCACCAGCTGGCTGGCCCCAGGCCGCGCCAAGACCTTCCGCCTGAAGCTGCCCGCGCTGCTGGC
GCTGACGGCCCGGGAGTCGTCGGTGCGGTCGGGCGCGGCGCCCCGGGGGCCGTGGTGGTGGACGTGGACCTGACGCCCGCGGCACCCAGC
AGCGAGTCGCTGGCCCTGGACCCATGGACAACCACACGTGGCAGGGCTCGGGCCCGCGGAGGAGCGGCGTGCGCTGGTGGGTCCCGGCTCTCCGC
CCCGCAGCGCGCCCGGCCAGCTCCCATCGCCCCGGGCGCACAGCCTCAACCCCGACGCCTCGGGCTCCAGCTGCAGCCTGGCCCGGACGCGCTCCCGAGAAAG
CTGCGCCAGCGTGCCGCCGGCCGACGCATCGAGGCCATGCGCGCCGGGGTGCTGCCCCCGCCACCGCCAGCACCGGGGCCATGCACC
CCACTGCGCAGCGGCTTGCTCAACTCCACCTCGGACTCCGACCTCGTGCGCTACCGCACCATTAGCAAGATTCCCCAAATCACCCTCAACTTTGTGGACCTCA
AGGGCGACCCCCTTCTTGGCTTCGCCCACCAGTGACCGTGAGATCATAGCACCTAAGATAAAGGAGCGAACCCACAATGTCACTGAGAAGGTCACCCAGGTCCT
GTCCCTGGGCGCCGACGTGCTGCCTGAGTACAAGCTGCAGGCACCGCGCATCCACCGCTGGACCATCCTGCATTACAGCCCCTTCAAGGCCGTGTGGACTGG
CTCATCCTGCTGCTGGTCATCTACACGGCTGTCTTCACACCCTACTCGGCTGCCTTCCTGCTGAAGGAGACGGAAGAAGGCCCGCCTGCTACCGAGTGTGGCT
ACGCCTGCCAGCCGCTGGCTGTGGTGGACCTCATCGTGGACATCATGTTCATTGTGGACATCCTCATCAACTTCCGCACCACCTACGTCAATGCCAACGAGGA
GGTGGTCAGCCACCCCGGCCGCATCGCCGTCCACTACTTCAAGGGCTGGTTCCTCATCGACATGGTGGCCGCCATCCCCTTCGACCTGCTCATCTTCGGCTCT
GGCTCTGAGGAGCTGATCGGGCTGCTGAAGACTGCGCGGCTGCTGGCGCGTGGCGCGGAAGCTGGATCGCTACTCAGAGTACGGCGCGGCCGTGC
TGTTCTTGCTCATGTGCACCTTTGCGCTCATCGCCACTGGCTAGCCTGCATCTGGTACGCCATCGACAACATGGAGCAGCCACACACATGGACTCACGCATCGG
CTGGCTGCACAACCTGGGCGACCAGATAGGCAAACCCTACACAACAGCGGCCTGGGCGGCCCCTCCATCAAGGACAAGATATGTGACGGCGCTCTACTTCACC
TTCAGCAGCCTCACCAGTGTGGGCTTCGGCAACGTCTCTCCCAACACCAACTCAGAGAAGATCTTCTCCATCTGCGTCATGCTCATTGGCTCCCTCATGTATG
CTAGCATCTTCGGCAACGTGTCGGCCATCATCCAGCGGCTGTACTCGGGCACAGCCCGCTACCACACACAGATGCTGCGGGTGCGGAGTTCATCCGCTTCCA
CCAGATCCCCAATCCCCTGCGCCAGCGCCTCGAGGAGTACTTCCAGCACGCCTGGTCCTACACCAACGGCATCGACATGAACGCGGTGTCGTTCCCT
GAGTGCCTGCAGGCTGACATCTGCCTGCACCTGAACCGCTCACTGCTGCAGCACTGCAAACCCTTCCGAGGGGCCACCAAGGGCTGCCTTCGGGCCCTGGCCA
TGAAGTTCAAGACCACACATGCACCGCCAGGGGACACACTGGTGCATGCTGGGGACCTGCTCACCGCCCTGTACTTCATCTCCCGGGCTCCATCGAGATCCT
GCGGGGCGACGTCGTCGTGGCCATCCTGGGGAAGAATGACATCTTTGGGGAGCCTCTGAACCTGTATGCAAGGCCTGGCAAGTCGAACGGGATGTGCGGGCC
CTCACCTACTGTGACCTACACAAGATCCATCGGGACGACCTGCTGGAGGTGCTTCAGTCGCAAGCGCAAGTTGTCCTTCCGCAG
CCTTCAACCTGCGAGATACCAACATGATCCCGGGCTCCCCCGGCAGTACGGAGTTAGAGGTGGCTTCAGTCGGCAAGCGCAAGTTGTCCTTCCGCAG
GCGCACGGACAAGATACCGAACAACCTGGCGAAGTCTCCGCGTTGGGGCCGGGCCGGGGGCAGGGCCGAGTAGCCGGGGCCGGGGGGCCGTGG
GGGGAGAGCCCGTCCAGTGGCCCCTCCAGCCCTGAGAGCAGTGAGGATGAGGGCCCAGCTCCAGCCCCCTCCGCCTGGTGCCCTTCTCCAGCCCCA
GGCCCCCCGGAGAGCCCGCCGGGTGGGGAGCCCCCTGATGGAGGACTGCGAGAAGAGCAGCGACACTTGCAACCCCCTGTCAGGCGCCTTCTCAGGAGTGTCCAA
CATTTTCAGCTTCTGGGGGGACAGTCGGGGCCGCCAGTACCAGGAGCTCCCTCGATGCCCCGCCCCCCAGCCTCCTCAACATCCCCCTCTCCAGCCCG
GGTCGGCGGCCCCGGGGCGACGTGGAGAGCAGGCTGGATGCCCTCCAGCGCCAGCTCAACAGGCTGGAGACCCGGCTGAGTGCAGACATGGCCACTGTCCTGC
AGCTGCTACAGAGGCAGATGACGCTGGTCCCGCCCGCCTACAGTGCTGTGACCACCCCGGGGCCTGGCCCCACTTCCACATCCCCGCTGTTGCCCGTCAGCCC
CCTCCCCACCCTCACCTTGGACTCGCTTTCTCAGGTTTCCCAGTTCATGGCGTGTGAGGAGCTGCCCCCGGGGGCCCCAGAGCTTCCCCCAAGAAGGCCCCACA
CGACGCCTCTCCCCTACCGGGCCAGCTGGGGGCCCTCACCTCCCAGCCCCTGCACACAGACACGGCTCGGACCCGGGCAGTTAG (SEQ ID NO:1035)

FIG. 2C – cont.

Amino Acid

MPVRRGHVAPQNTFLDTIIRKFEGQSRKFIIANARVENCAVIYCNDGFCELCGYSRAEVMQRPCTCDFLHGPRTQRRAAAQIAQALLGAEERKVEIAF
YRKDGSCFLCLVDVVPVKNEDGAVIMFILNFEVVMEKDMVGSPAHDTNHRGPPTSWLAPGRAKTFRLKLPALLALTARESSVRSGGAGAGAPGAVVV
DVDLTPAAPSSESLALDEVTAMDNHVAGLGPAEERRALVGPGSPPRSAPGQLPSPRAHSLNPDASGSSCSLARTRSRESCASVRRASSADDIEAMRAG
VLPPPPRHASTGAMHPLRSGLLNSTSDSDLVRYRTISKIPQITLNFVDLKGDPFLASPTSDREIIAPKIKERTHNVTEKVTQVLSLGADVLPEYKLQA
PRIHRWTILHYSPFKAVWDWLILLLVIYTAVFTPYSAAFLLKETEEGPPATECGYACQPLAVVDLIVDIMFIVDILINFRTTYVNANEEVVSHPGRIA
VHYFKGWFLIDMVAAIPFDLLIFGSGSEELIGLLKTARLLRLVRVARKLDRYSEYGAAVLFLLMCTFALIAHWLACIWYAIGNMEQPHMDSRIGWLHN
LGDQIGKPYNSSGLGGPSIKDKYVTALYFTFSSLTSVGFGNVSPNTNSEKIFSICVMLIGSLMYASIFGNVSAIIQRLYSGTARYHTQMLRVREFIRF
HQIPNPLRQRLEEYFQHAWSYTNGIDMNAVLKGFPECLQADICLHLNRSLLQHCKPFRGATKGCLRALAMKFKTTHAPPGDTLVHAGDLLTALYFISR
GSIEILRGDVVVAILGKNDIFGEPLNLYARPGKSNGDVRALTYCDLHKIHRDDLLEVLDMYPEFSDHFWSSLEITFNLRDTNMIPGSPGSTELEGGFS
RQRKRKLSFRRRTDKDTEQPGEVSALGPGRAGAGPSSRGRPGGPWGESPSSGPSSPESSEDEGPGRSSSPLRLVPFSSPRPPGEPPGGEPLMEDCEKS
SDTCNPLSGAFSGVSNIFSFWGDSRGRQYQELPRCPAPTPSLLNIPLSSPGRRPRGDVESRLDALQRQLNRLETRLSADMATVLQLLQRQMTLVPPAY
SAVTTPGPGPTSTSPLLPVSPLPTLTLDSLSQVSQFMACEELPPGAPELPQEGPTRRLSLPGQLGALTSQPLHRHGSDPGS (SEQ ID NO:1036)

FIG. 2C – cont.

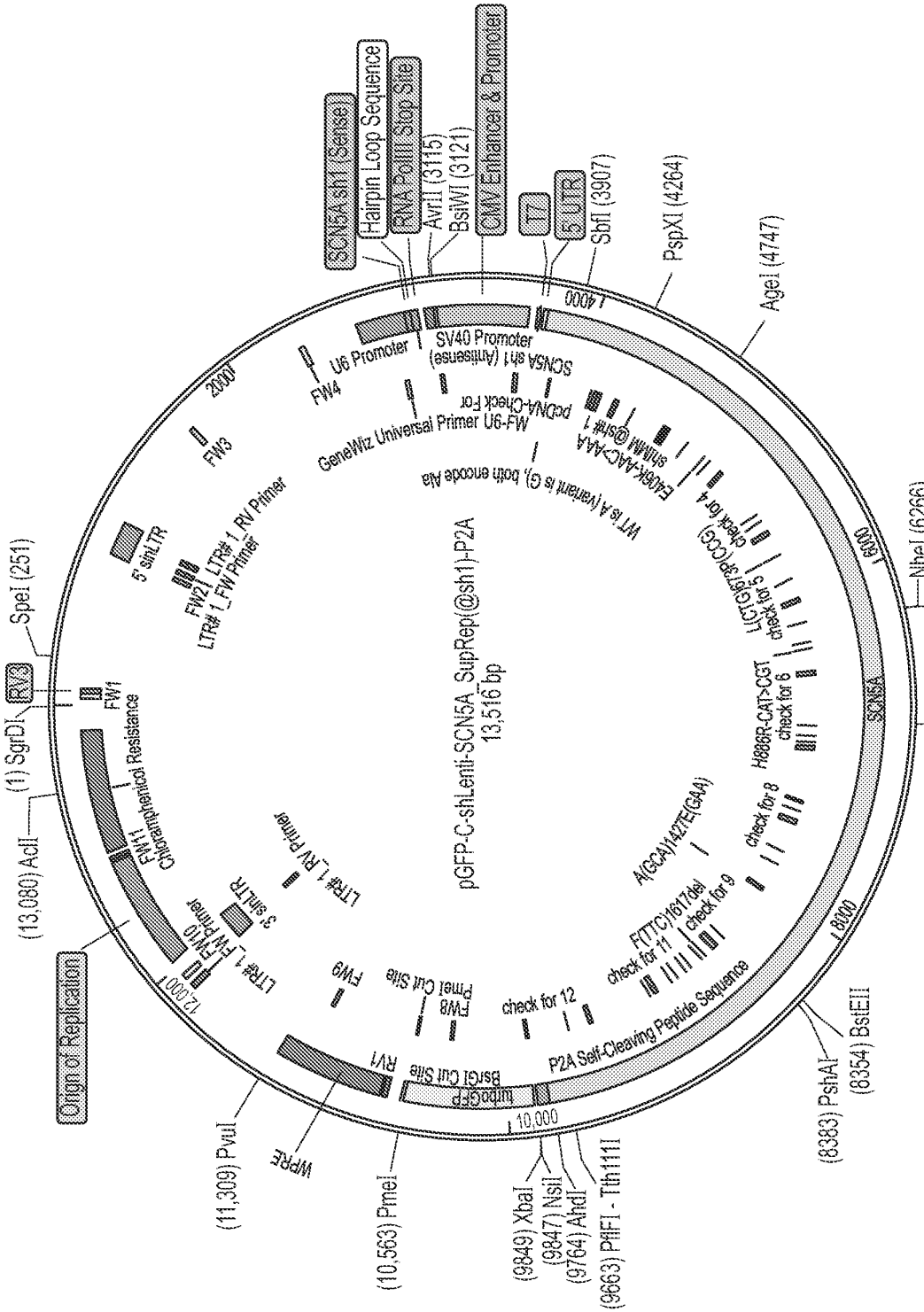

pGFP-C-shLenti-SCN5A_SupRep(@sh1)-P2A
13,516 bp
SCN5A sh1 (Sense)
Hairpin Loop Sequence
RNA PolIII Stop Site
AvrII (3115)
BsiWI (3121)
CMV Enhancer & Promoter
T7
5' UTR
SbfI (3907)
PspXI (4264)
AgeI (4747)
NheI (6266)
BmtI (6270)
SmaI (6848)
TspMI - XmaI (6846)
BstEII (8354)
PshAI (8383)
TTh111I (9663)
PflFI (9764)
NsiI (9847)
XbaI (9849)
PmeI (10,563)
WPRE
PvuI (11,309)
AclI (13,080)
SpeI (251)
SV40 Promoter
U6 Promoter
SCN5A sh1 (Antisense)
pcDNA-Check For
GeneWiz Universal Primer U6-FW
FW4
FW3
FW2
5' shLTR
LTR# 1_RV Primer
LTR# 1_FW Primer
RV3
FW1
FW11
Chloramphenicol Resistance
FW10
3' shLTR
LTR# 1_FW Primer
LTR# 1_RV Primer
FW9
PmeI Cut Site
FW8
RV1
BsrGI Cut Site
hrGFP
P2A Self-Cleaving Peptide Sequence
check for 12
check for 11
check for 9
check for 8
check for 6
check for 5
check for 4
SCN5A
Origin of Replication
2000
4000
6000
8000
10,000
12,000

FIG. 3A



FIG. 3B



FIG. 3B – cont.



FIG. 3B – cont.

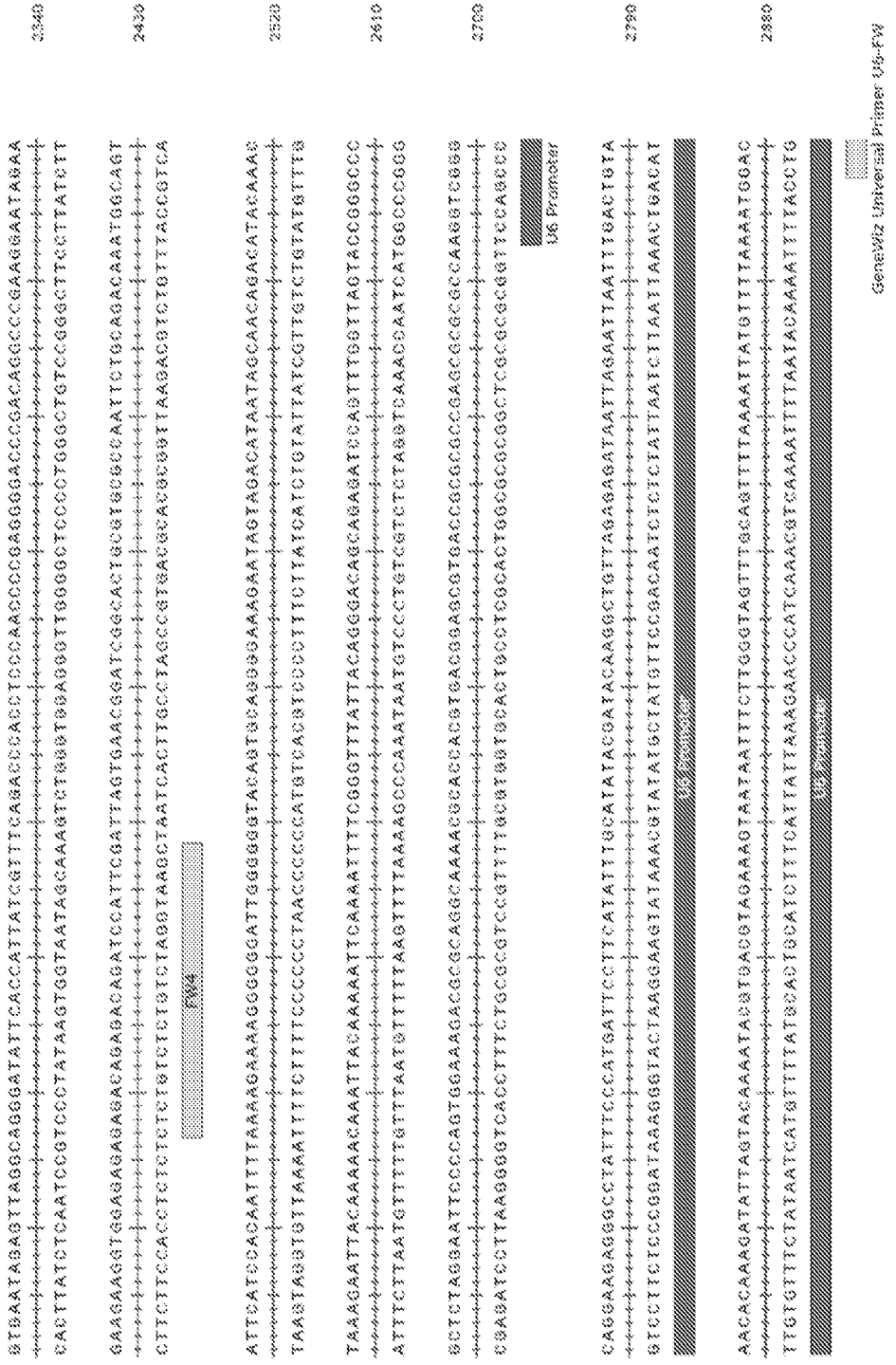

2340
2430
2520
2610
2700
2790
2880
FW4
U6 Promoter
GeneWiz Universal Primer U6-FW

FIG. 3B – cont.

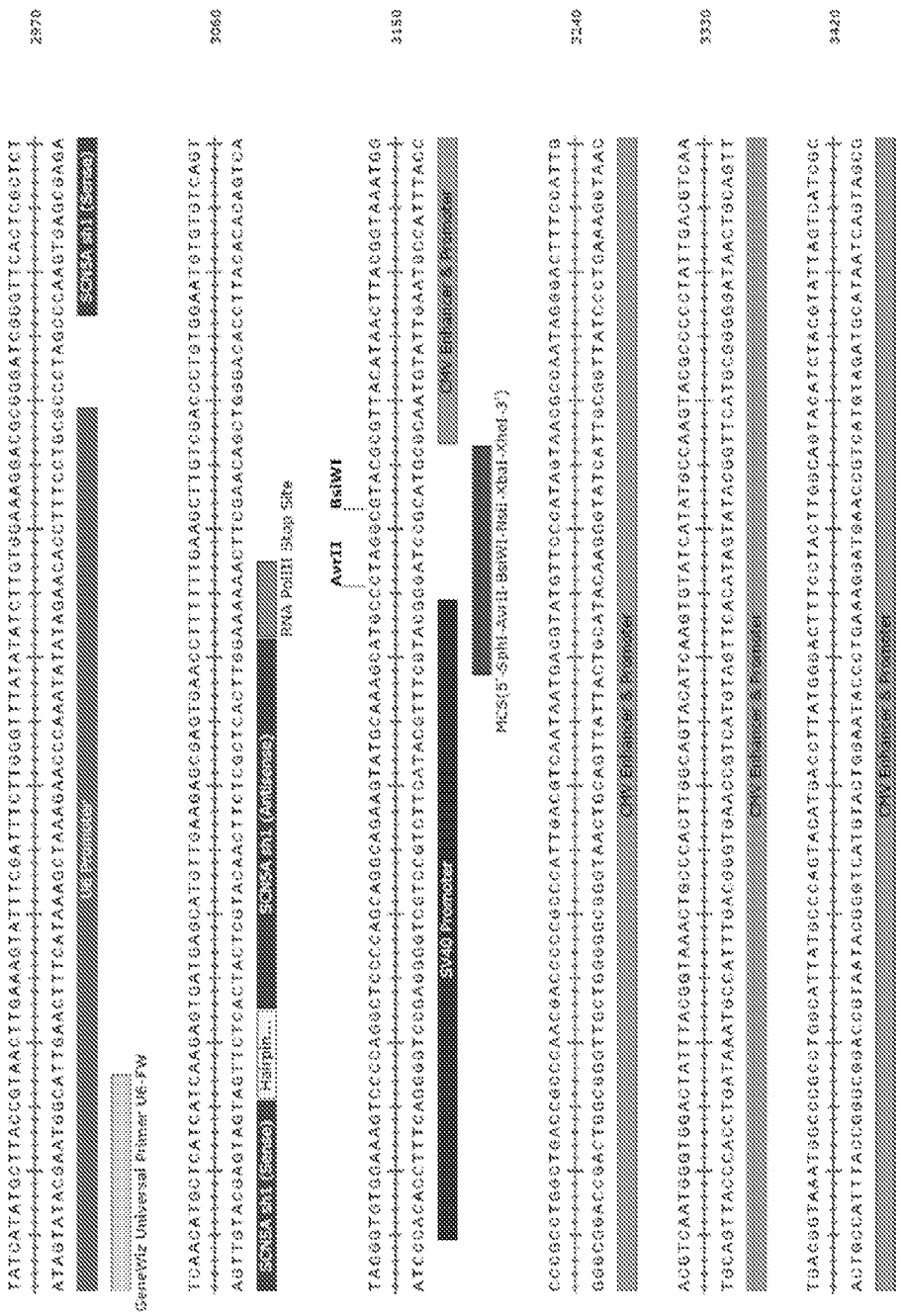

2970
3060
3150
3240
3330
3420
GeneWiz Universal Primer U6-FW
Hairpin...
RNA PolIII Stop Site
AvrII
BsiWI
MCS(5'-SphI-AvrII-BsiWI-NsiI-XbaI-XhoI-3')
CMV Enhancer & Promoter

FIG. 3B – cont.

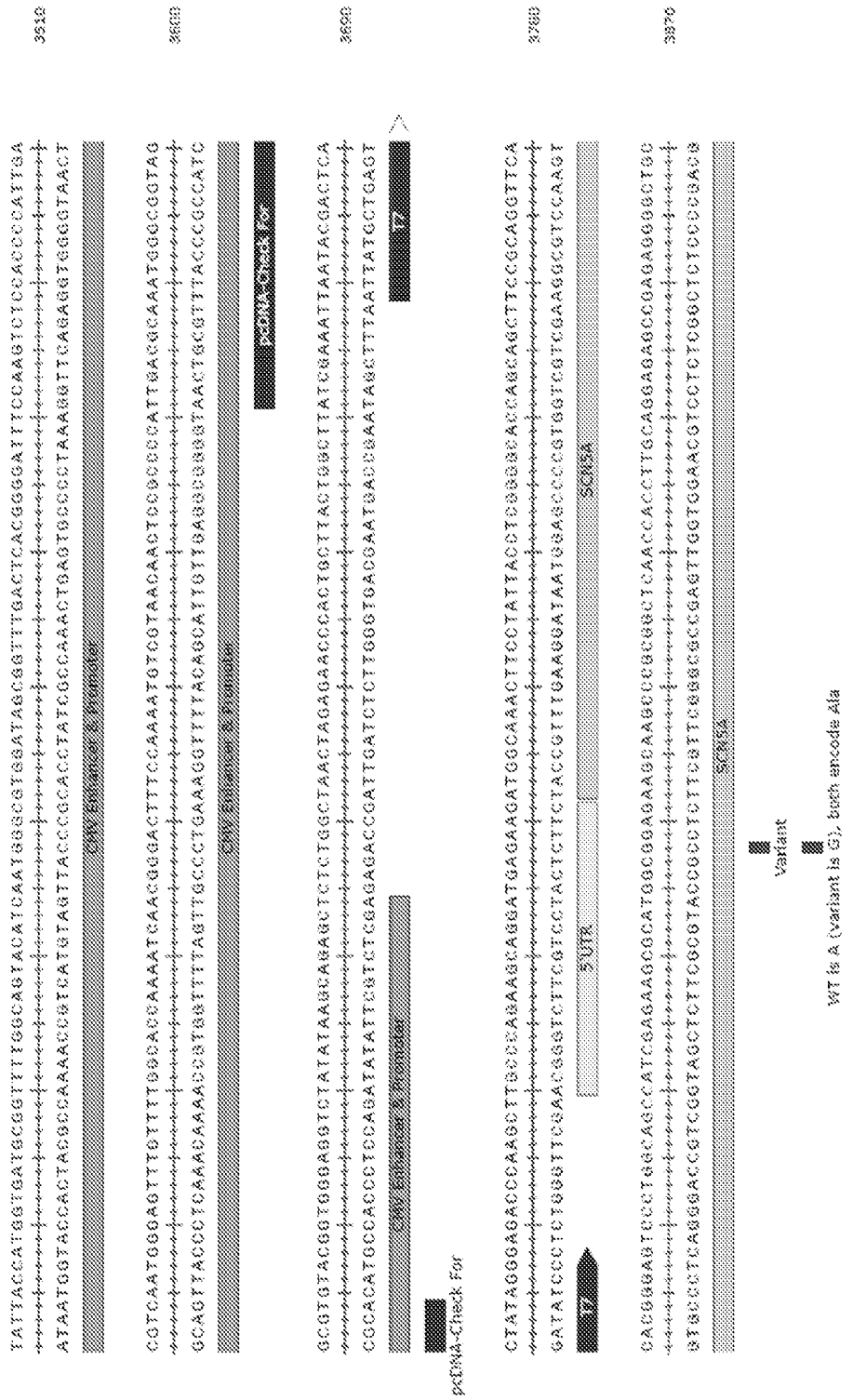

3510
3600
3690
3760
3870
CMV Enhancer & Promoter
pcDNA-Check For
T7
5'UTR
SCNSA
Variant
WT is A (variant is G), both encode Ala

FIG. 3B – cont.

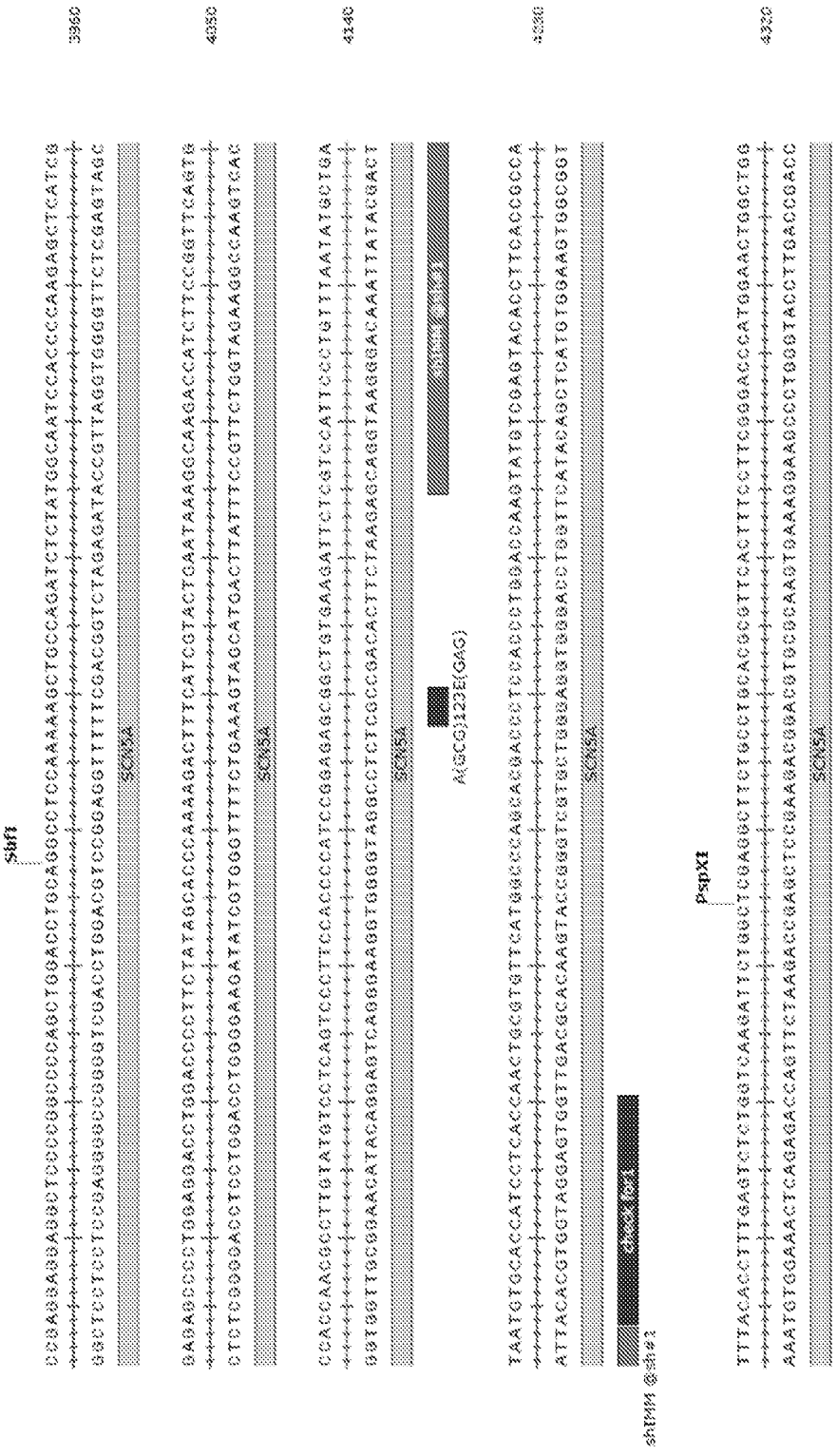

3960
4050
4140
4230
4320
SbfI
PspXI
SCN5A
A(GCG)123E(GAG)
check_for1
rev-1
shIMM @sh#1

FIG. 3B – cont.


4440
4500
4560
4620
4680
4740
4800
SCN5A
R(CGA)222>G(GGA)
AgeI
A359D

FIG. 3B – cont.

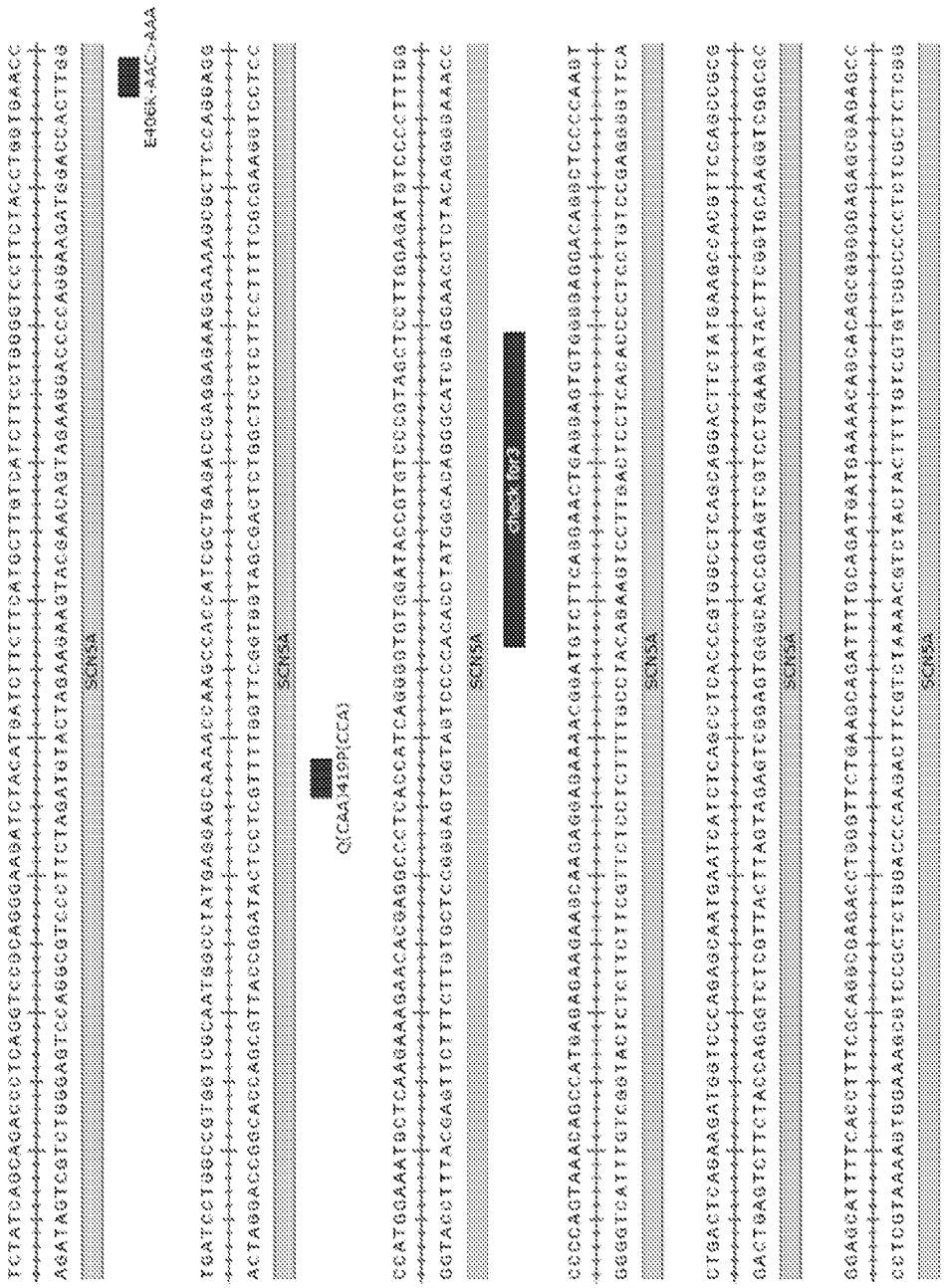

4950
5040
5130
5220
5310
5400
SCN5A
E406K-AAC>AAA
Q(CAA)419P(CCA)

FIG. 3B – cont.

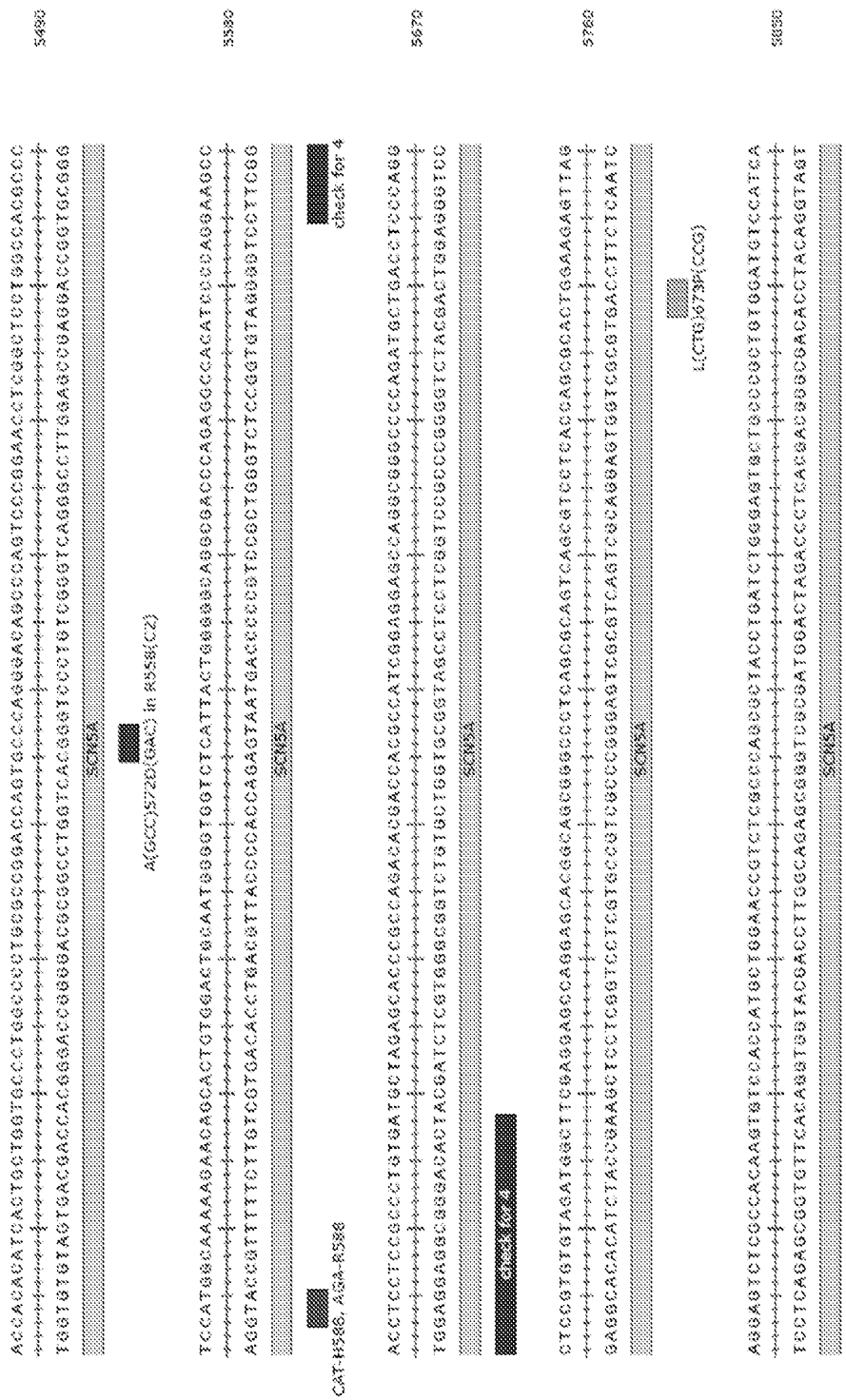
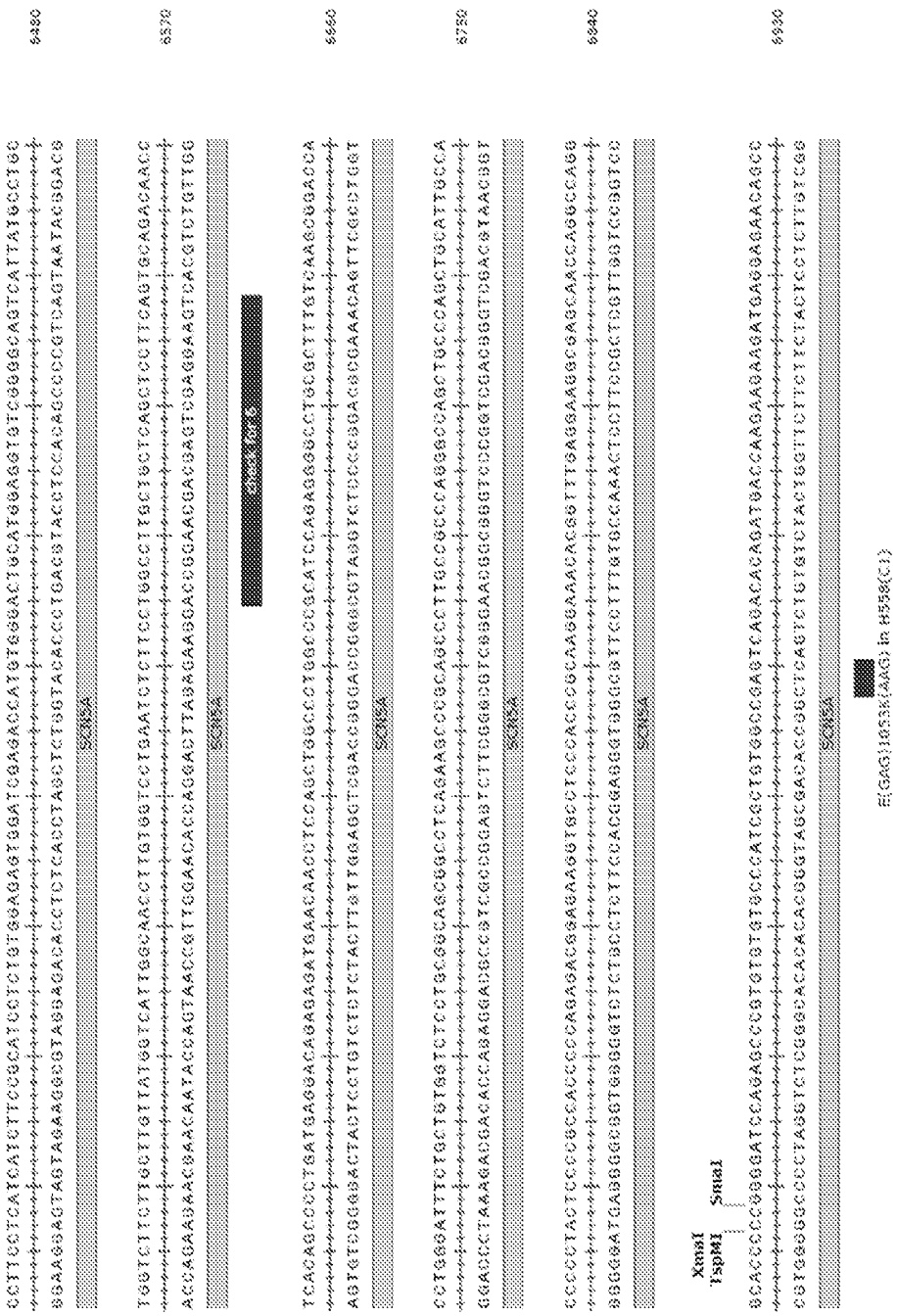

5490
5580
5670
5760
5850
SCN5A
A(GCC)572D(GAC) in R558(C2)
CAT-H586, AGA-R588
check for 4
L(CTG)673P(CCG)

FIG. 3B – cont.


5940
6030
6120
6210
6300
6390
SCN5A
T(ACA)7331(ATA)
check for 5
NheI
BantI
L(CTC)828del
D(GAC)872N(AAC)
H886R-CAT>CGT

FIG. 3B – cont.


6480
6520
6560
6600
6640
6680
6720
6760
6800
6840
6880
6920
6960
7000
XmaI
TspMI
SmaI
SCN5A
SCN5A
SCN5A

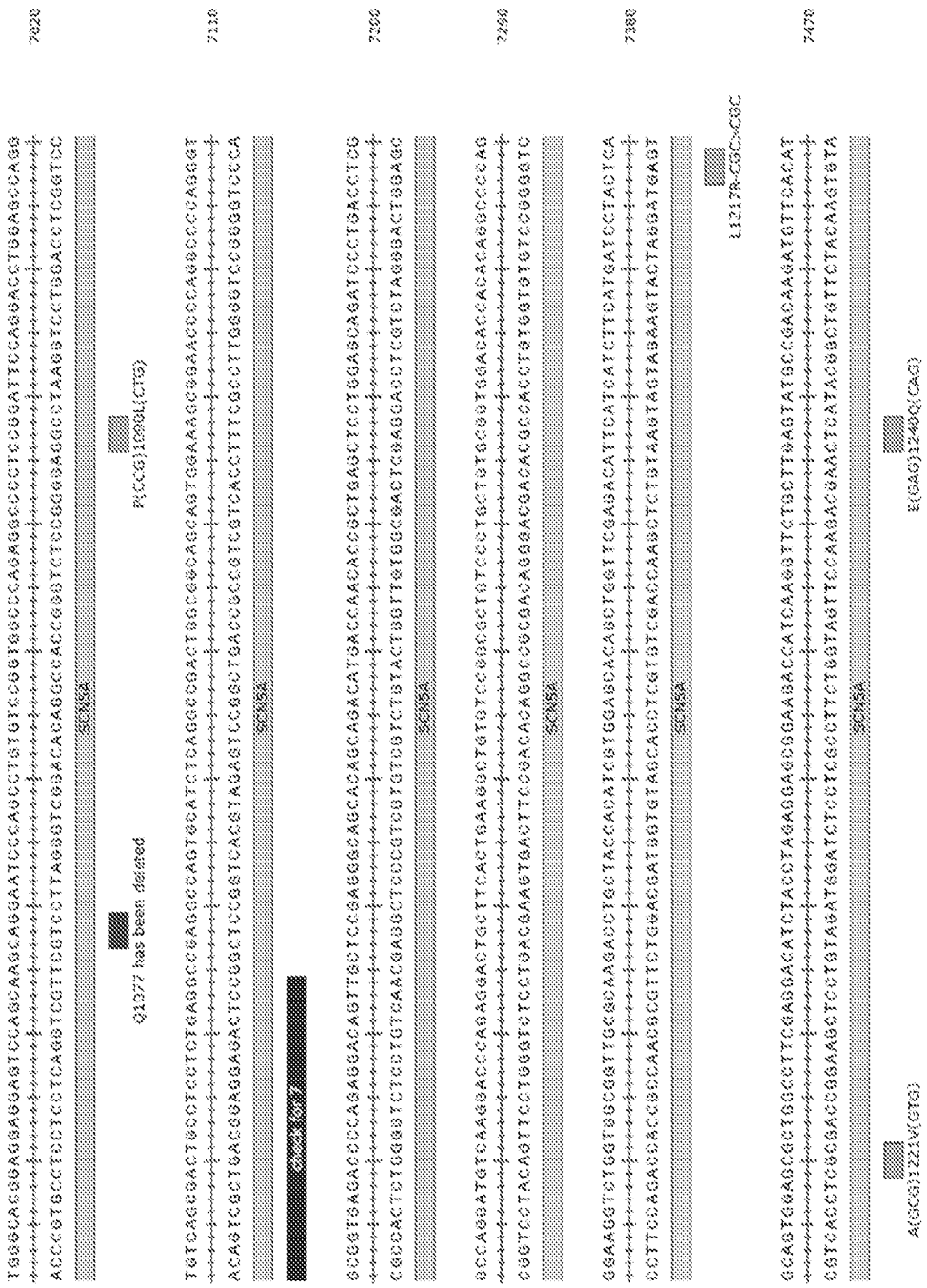

7020
7110
7200
7290
7380
7470
SCN5A
Q1077 has been deleted
P(CCG)1090L(CTG)
check for 7
L1217R-CGC>CGC
A(GCG)1221V(GTG)
E(GAG)1240Q(CAG)

FIG. 3B – cont.

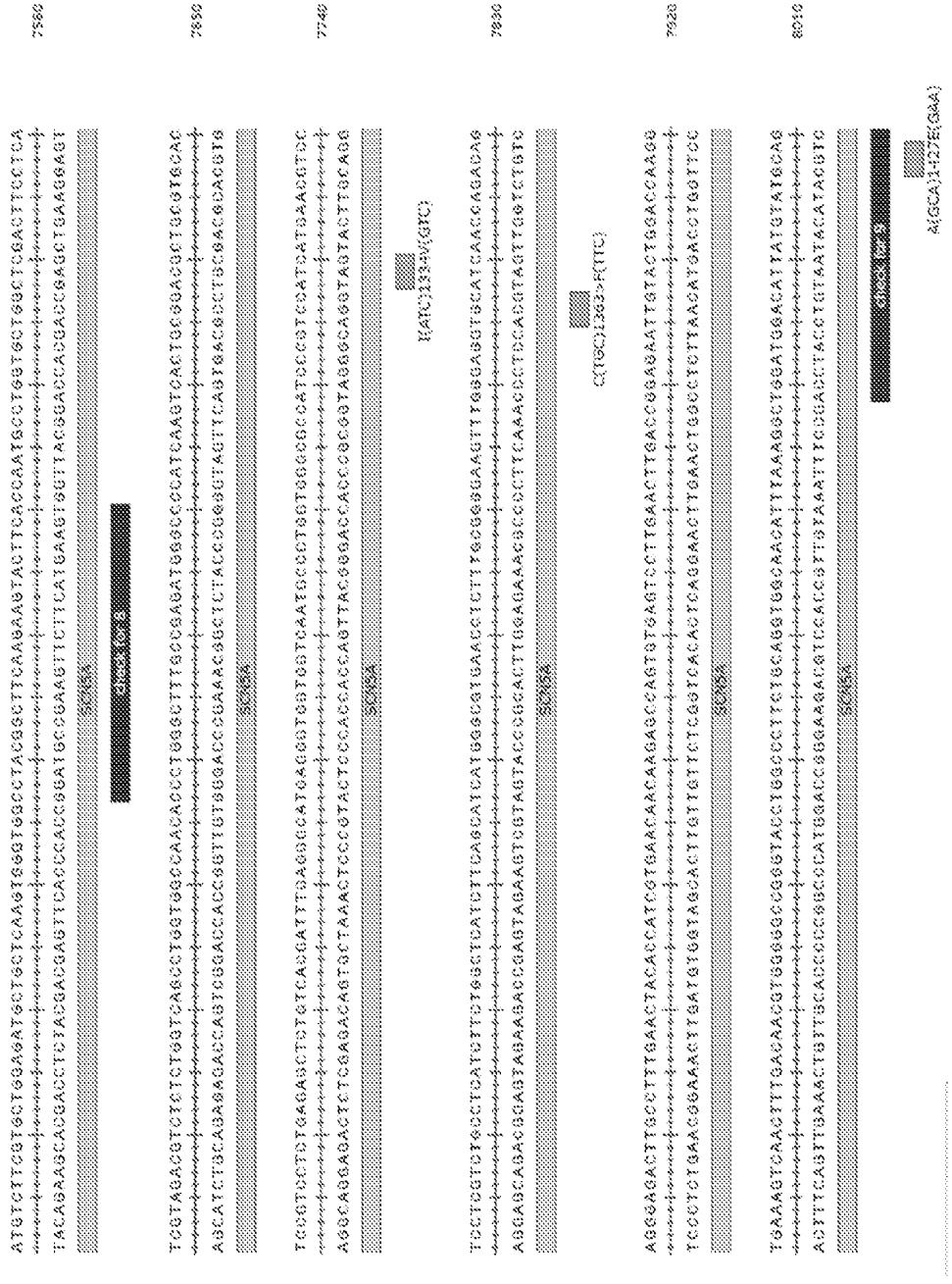

7560
7650
7740
7830
7920
8010
SCN5A
check for B
check for S
A(GCA)1+127E(GAA)

FIG. 3B – cont.


8100
8190
8280
8370
8460
8550
check for 9
SCN5A
BstEII
PshAI
N(AAC)1558S(AGC)
check for 10

FIG. 3B – cont.

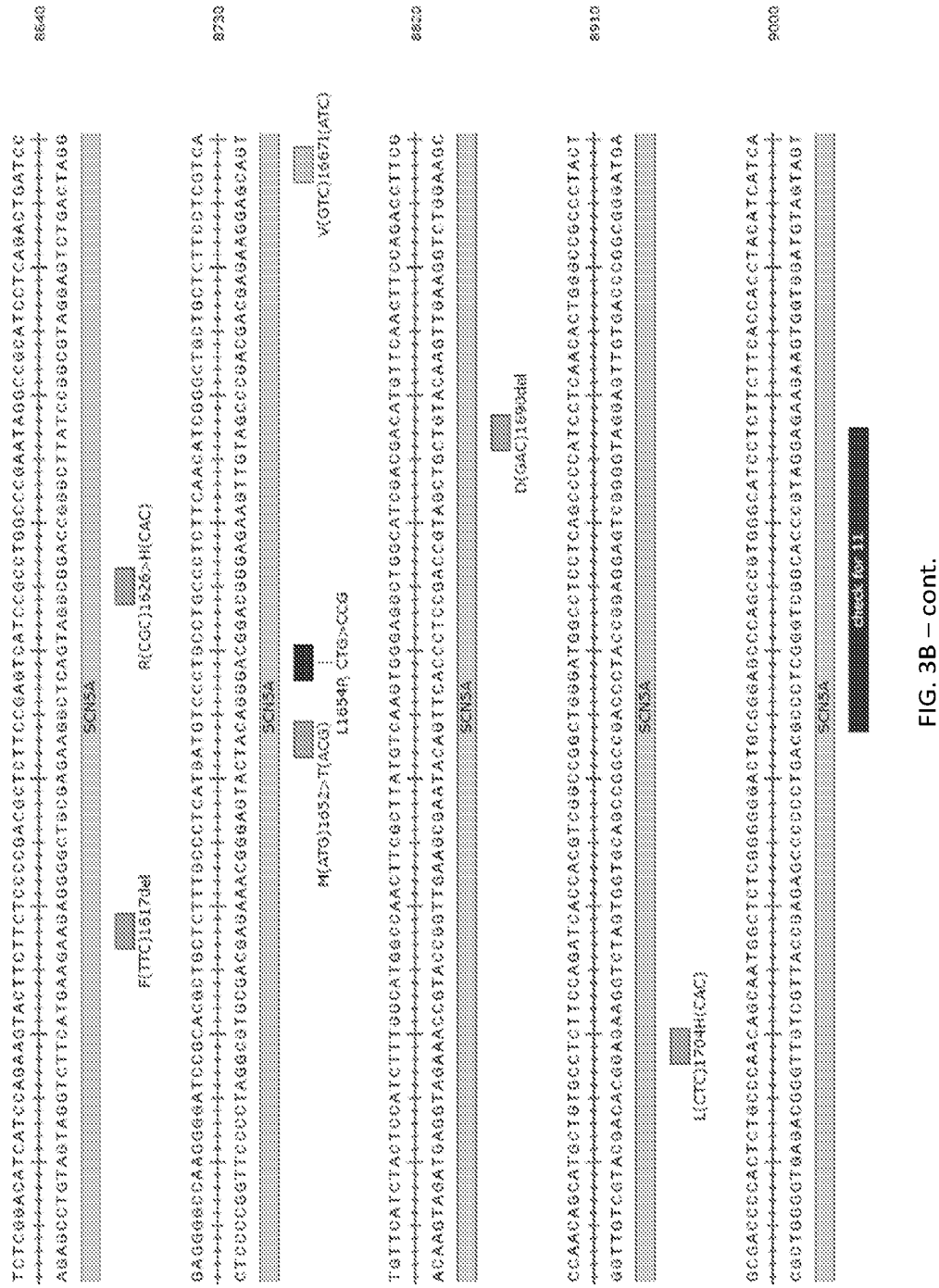

8640
8730
8820
8910
9000
SCN5A
F(TTC)1817del
R(CGC)1826>H(CAC)
M(ATG)1852>T(ACG)
L1834fs CTG>CCG
V(GTC)1867I(ATC)
D(GAC)1893del
L(CTC)1704H(CAC)
Exon 11

FIG. 3B – cont.


9090
9180
9270
9360
9450
9540
SCN5A
FITC(1760)(TGC)
SCN5A
SCN5A
SCN5A
SCN5A
SCN5A

FIG. 3B – cont.

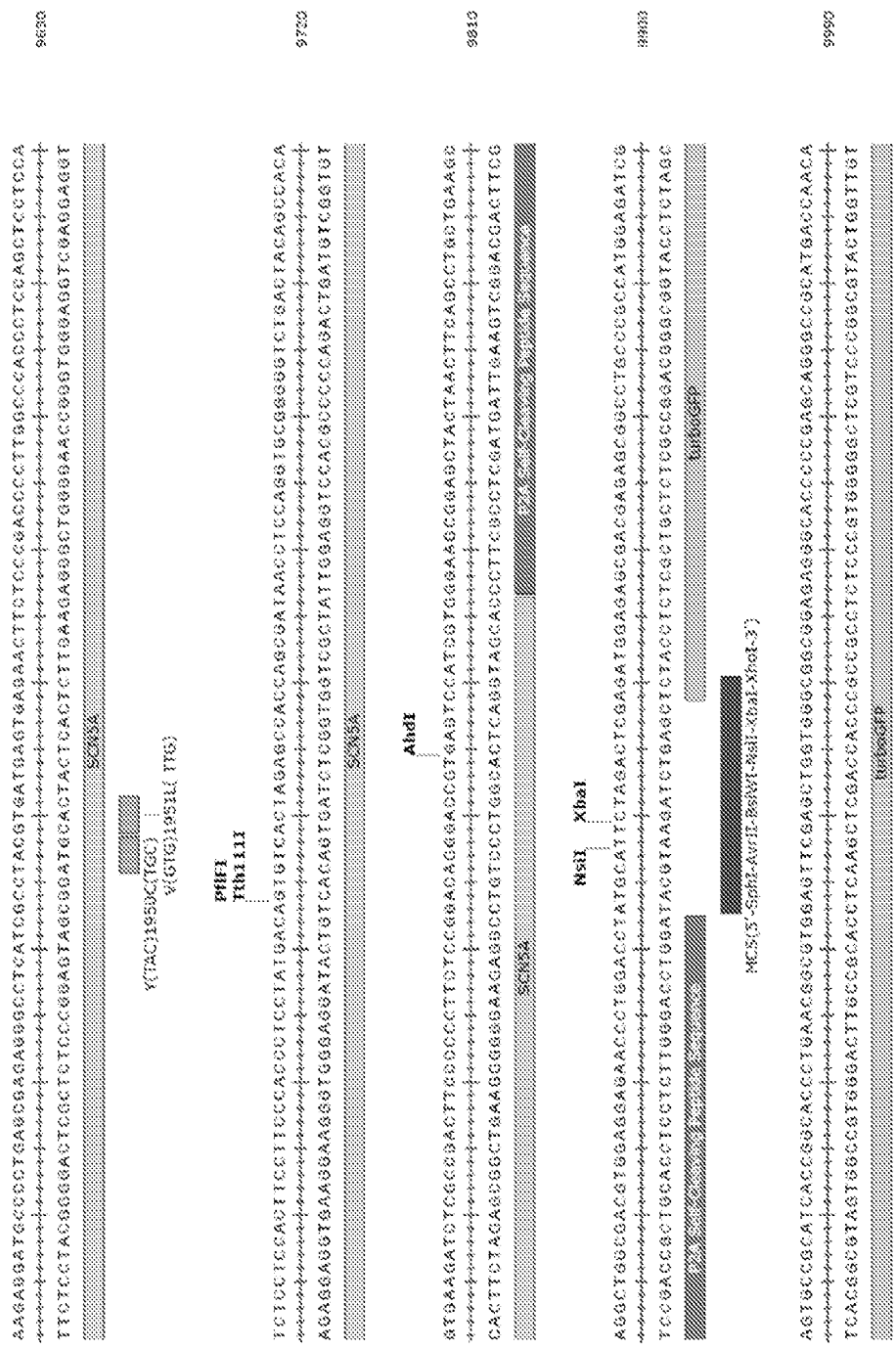

9630
9720
9810
9900
9990
SCN5A
Y(TAC)1950C(TGC)
V(GTG)1951L(TTG)
PflFI
TthI111
AhdI
NsiI
XbaI
MCS(5'-SphI-AvrII-BsiWI-NsiI-XbaI-XhoI-3')
turboGFP

FIG. 3B – cont.


18,080
18,170
18,260
18,350
18,440
18,530
turboGFP
turboGFP
turboGFP
turboGFP
turboGFP
turboGFP
FWB

FIG. 3B – cont.

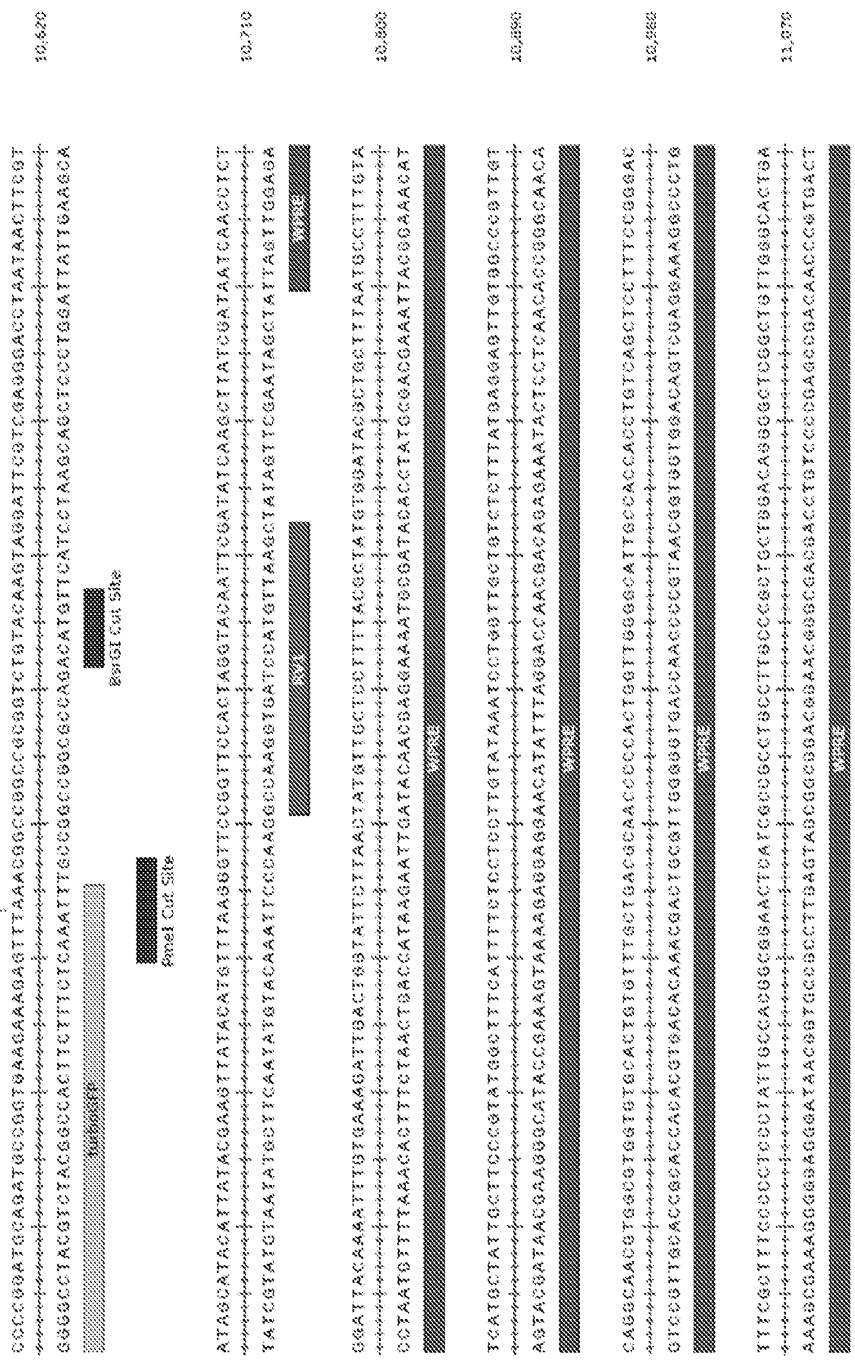


FIG. 3B – cont.



FIG. 3B – cont.



FIG. 3B – cont.

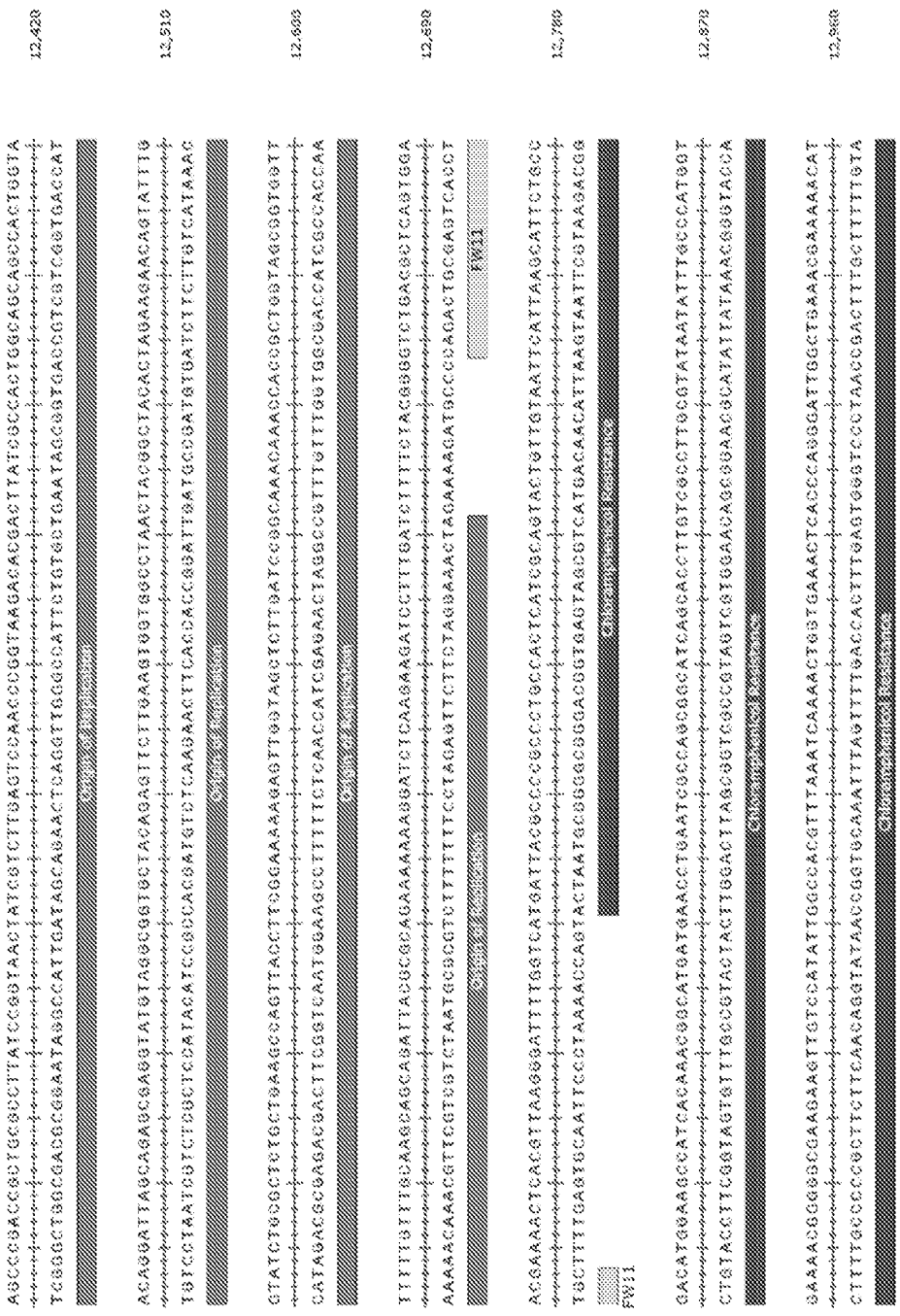


FIG. 3B – cont.



FIG. 3B – cont.

SCN5A (NM_000335.5)
sh#4: GGTTCACTCGCTCTTCAACATGCTCATCA (SEQ ID NO:30)
shIMM: CGTACATTCCCTGTTTAATATGCTGATTA (SEQ ID NO:32)

Nucleic Acid (WT)

ATGGCAAACTTCCTATTACCTCGGGGCACCAGCAGCTTCCGCAGGTTCACACGGGAGTCCCTGGCAGCCATCGAGAAGCGCATGGCAGAGAAGCAAGCCCGCGGCTCAACCACCTTG
CAGGAGAGCCGAGAGGGGCTGCCCGAGGAGGAGGCTCCCCGGCCCCAGCTGGACCTGCAGGCCTCCAAAAAGCTGCCAGATCTCTATGGCAATCCACCCCAAGAGCTCATCGAGAG
CCCCTGGAGGACCTGGACCCCTTCTATAGCACCCAAAGACTTTCATCGTACTGAATAAAGGCAAGACCATCTTCCGGTTCAGTGCCACCAACGCCTTGTATGTCCTCAGTCCCCTTC
CACCCCATCCGGAGAGCGGCTGTGAAGATTCTGGTTCACTCGCTCTTCAACATGCTCATCATGTGCACCATCCTCACCAACTGCGTGTTCATGGCCCAGCACGACCCTCCACCCTGG
ACCAAGTATGTCGAGTACACCTTTACACCTTTGAGTCTCTGGTCAAGATTCTGGCTCGAGGCTTCTGCCTGCACGCGTTCACTTTCCTTCGGGACCCATGGAACTGG
CTGGACTTTAGTGTGATTATCATGGCATACACAACTGAATTTGTGGACCTGGGCAATGTCTCAGCCTTACGCACCTTCCGAGTCCTCCGGGCCCTGAAAACTATATCAGTCATTTCA
GGGCTGAAGACCATCGTGGGGGCCCTGATCCAGTCTGTGATGGTCCTCACAGTCTTCTGCCTCAGCGTCTTTGCCCTCATCGGCCTGCAGCTCTTCATG
GGCAACCTAAGGCACAAGTGCGTGCGCAACTTCACAGCGCTCCGTGGAGGCCGACGGCTTGGTCTGGGAATCCCTGGACCTTTACCTCAGTGATCCAGAA
AATTACCTGCTCAAGAACGGCACCTCTGATGTGTTACTGTGTGGGAACAGCTCTGACGCTGGGACATGTCCGGAGGGCTACCGGTGCCTAAAGGCAGGCGAGAACCCCGACCACGGC
TACACCAGCTTCGATTCCTTTGCCTGGGCCTTTCTTGCACTCTTCCGCCTGATGACGCAGGATCGCCTCTATCAGCAGACCCTCAGGTCCGCAGGGAAGATCTACATG
ATCTTCTTCATGCTTGTCATCTTCCTGGGGTCCTTCTACCTGGTCGTGCTGGCCGTGATCGTCGACAATGGCCTATGAGGAGCAAAACCAAGCCACCATCGCTGAGACCGAGGAG
AAGGAAAAGCGCTTCCAGGAGGCCATGGAAATGCTCAAGAAAGAACACGAGGCCCTCACCATCAGGGGTGTGGATACCGTGTCCCGTAGCTCCTTGGAGATGTCCCCTTTGGCCCCA
GTAAACAGCCATGAGAGAAGAAGCAAGAGGATGTCTTCAGGAACTGAGGAGTGTGGGGAGGACAGGCTCCCCAAGTCTGACTCATCAGAAGATGGTCCCAGAGCAATGAAT
CATCTCAGCCTCACCCGTGGCCTCAGCAGGACTTCTATGAAGCCACGTTCCAGCCGCGGGAGCATTTTCACCTTTCGCAGGCGAGACCTGGGTTCTGAAGCAGATTTTGCAGATGAT
GAAAACAGCACAGCGGGGAGAGCGAGAGCCACCACACATCACTGCTGGTGCCCTGGCCCCTGCGCCGGACCAGTGCCCAGGGACAGCCCAGTCCCGGAACCTCGGCTCCTGGCCAC
GCCCTCCATGGCAAAAAGAACAGCACTGTGGACTGCAATGGGGTGGTCTCATTACTGGGGGCAGGCGACCCACATCCCCAGGAAGCCACCTCCTCCGCCCTGTGATGCTA
GAGCACCCGCCAGACACGACCACGCCATCGGAGGAGCCAGGCGGGCCCCAGATGCTGACCTCCCAGGCTCCGTGTGTAGATGGCTTCGAGGAGCCAGGCACGGCAGCGGGCCCTC
AGCGCAGTCAGCGTCCTCACCAGCGCACTGGAAGAGTTCTCGCCACACAAGTGTCCACCATGCTGGAACCGTCTCGCCCAGCGCTACCTGATCTGGGAGTGCTGCCGCTG
TGGATGTCCATCAAGCAGGGAGTGAAGTTGGTGGTCATGGACCCGTTTACTGACCTCACCATCACTATGTGCATCGTACTCAACACACTCTTCATGGCGCTGGAGCACTACAACATG
ACAAGTGAATTCGAGGAGATGCTGCAGGTCGGAAACCTGGTCTTCACAGGGATTTTCACAGCAGAGATGACCTTCAAGATCATTGCCCTCGACCCCTACTACTCTTCCAACAGGGC
TGGAACATCTTCGACAGCATCATCGTCATCCTTAGCCTCATGGAGCTGGGCCTGTCCCGCATGAGCAACTTGTCGGTGCTCGCTCCTTCCGCCTGCCAGCTGGCC
AAATCATGGCCCACCCTGAACACACTCATCAAGATCATCGGAACCTGACACTGGTGCTAGCCATCATCGTGTTCATCTTTGCTGTGGTGGGCATG
CAGCTCTTTGGCAAGAACTACTCGGAGCTGAGGGACAGCGACTCAGGCCTGCTGCCTCGCTGGCACATGATGGACTTCTTTATGCATTCCTCATCATTCCTCTGTGGA
GAGTGGATCGAGACCATGTGGGACTGCATGGAGGTGTCGGGGCAGTCATTATGCCTGCTTGTTATGGTCATTGGCAACCTGTTGTCCTGAATCTCTTCCTTGGCC
TTGCTGCTCAGCTCCTTCAGTGCAGACAACCTCACAGCCCCTGATGAGGACAGAGAGATGAACAACCTCCAGCTGGCCCTGGCCCGCATCCAGAGGGGCCTGCGCTTTGTCAAGCGG
ACCACCTGGGATTTCTGCTGTGGTCTCCTGCGGCAGCGGCCTCAGAAGCCCGCAGCCCTTGCCGCCCAGGGCCAGCTGCCCAGCTGCATTGCCACCCCCTACTCCCCGCCACCCCCA
GAGACGGAGAAGGTGCCTCCCACCCGCAAGGAAACACGGTTTGAGGAAGGCGAGCAACCAGGCCAGGGCACCCCCGGGGATCCAGAGCCCGTGTGTGTGCCCATCGCTGTGGCCGAG
TCAGACACAGATGACCAAGAAGAAGATGAGGAGAACAGCCTGGGCACGGAGGAGGAGTCCAGCAAGCAGGAATCCCAGCCTGTGTCCGGTGGCCCAGAGGCCCCTCCGGATTCCAGG
ACCTGGAGCCAGGTGTCAGCGACTGCCTCCTCTGAGGCCGAGTGCATCTCAGGCCGACTGGCGGCAGCAGTGGAAAGCGGAACCCCAGGGTGCGGTGAGACCCCA
GAGGACAGTTGCTCCGAGGGCAGCACAGCAGACATGACCAACACCGCTGAGCTCCTGGAGCAGATCCCTGACCTCGGCCAGGATGTCAAGGACCCAGAGGACTGCTTCACTGAAGGC
TGTGTCCGGCGCTGTCCCTGCTGTGCGGTGGACACCACACAGGCCCCAGGGAAGGTCTGGTGGCGGTTGCGCAAGACCTGCTACCACATCGTGGAGCACAGCTGGTTCGAGACATTC
ATCATCTTCATGATCCTACTCAGCAGTGGAGCGCTGGCCTTCGAGGACATCTACCTAGAGGAGCGGAAGACCATCAAGGTTCTGCTTGAGTATGCCGACAAGATGTTCACATATGTC
TTCGTGCTGGAGATGCTGCTCAAGTGGGTGGCCTACGGCTTCAAGAAGTACTTCACCAATGCCTGGTGCTGGCTCGACTTCCTCATCGTAGACGTCTCTCTGGTCAGCCTGGTGGCC

FIG. 3C

AACACCCTGGGCTTTGCCGAGATGGGCCCCATCAAGTCACTGCGGACGCTGCGTGCACTCCGTCCTCTGAGAGCTCTGTCACGATTTGAGGGCATGAGGGTGGTGGTCAATGCCCTGG
TGGCGCCATCCCGTCCATCATGAACGTCCTCCTCGTCTGCCTCATCTTCTGGCTCATCTTCAGCATCATGGCGTGAACCTCTTTGCGGGGAAGTTTGGGAGGTGCATCAACCAGAC
AGAGGGAGACTTGCCTTTGAACTACACCATCGTGAGTCCTTGAACTTGACCGGAGAATTGTACTGGACCAAGGTGAAAGTCAACTTTGACAACGTGGGG
GCCGGGTACCTGGCCCTTCTGCAGGTGGCAACATTATGAAAGGCTGGATGGACATTATGTATGCAGCTGTGGACTCCAGGGGGTATGAAGAGCAGCCTCAGTGGGAATACAACCTCTACA
TGTACATCTATTTTGTCATTTTCATCATCTTTGGTCTTTCTTCACCCTGAACCTCTTTATTGGTGTCATCATTGACAACTTCAACCACAGAAGAAAAAGTTAGGGGGCCAGGACAT
CTTCATGACAGAGGAGCAGAAGAAGTACTACAATGCCCATCCCACGGCCCCTGAACAAGTACCAGGGCTTCATATTCGACATT
GTGACCAAGCAGGCCTTTGACGTCACCATCATGTTTCTTGATCTGCTTGAATATGGTGACCATGAGTCCTGAGAAAATCAACATCTTGGCCAAGATCA
ACCTGCTCTTTGTGGCCATCTTCACAGGCGAGTGTATTGTCAAGCTGGCTGCGCCACTACTTCACCAACAGCTGGAATATCTTCGACTTCGTGTTGTCATCCTCTCCAT
CGTGGGCACTGTGCTCTCGGACATCATCCAGAAGTACTTCTTCTCCCCGACGCTCTTCCGAGTCATCCGGCCTGGCCGAATAGGCCGCATCCTCAGACTGATGCCAAGGGG
ATCCGCACGCTGCTCTTTGCCCTCATGATGTCCCTGCCTCTTCCTCGTCATGTTCATCTACTCCATCTTTGGCATGGCCAACTTCGCTTATG
TCAAGTGGGAGGCTGGCATCGACGACATGTTCAACTTCCAGACCTTCGCCAACAGCATGCTGTGCCTCTTCCAGATCACCACGTCGGCCGGCTGGGATGGCCTCCTCCTCAGCCCCATCCT
CAACACTGGGCCGCCCTACTGCGACCCCACTCTGCCCAACAGCAATGGCTCTCGGGGGGACTGCGGGAGCCCAGCCGTGGGCATCCTCTTCTTCACCACCTACATCATCATCCTCTTC
CTCATCGTGGTCAACATGTACATTGCCATCATCCTGGAGAACTTCAGCGTGGCCACGGAGGAGCACCGAGCCCCTGAGTGAGGACGACTTCGATATGTTCTATGAGATCTGGGAGA
AATTTGACCCAGAGGCCACTCAGTTTATTGAGTATTCGGTCCTGTCTGACTTTGCCGATGCCCTGTCTGAGCCACTCCGTATCGCCAAGCCCAACCAGATAAGCCTCATCAACATGGA
CCTGCCCATGGTGAGTGGGGACCGCATCCATTGCATGGACATTCTCTTTGCCTTCACCAAAAGGGTCCTGGGGGAGATGGACGCCCTGAAGATCCAGATGGAGGAGAAG
TTCATGGCAGCCAACCCATCCAAGATCTCCTACGAGCCCATCACCACCTCCGGCGCAAGCACGAAGAGGTGTCGGCCATGGTTATCCAGAGAGCCTTCCGCAGGCACCTGCTGC
AACGCTCTTTGAAGCATGCCTCCTTCCTCTTCCGTCAGCAGGCGGCCATCTCCGAAGAGGATGCCCCTGAGCGAGAGGGCCTCATCGCCTACGTGATGAGTGAGAACTTCTC
CCGACCCCTTGGCCCACCCTCCAGCTCCTCCATCTCCTTCCCACACCTCCTATGACAGTGTCACTAGAGCCACCAGCGATAACCTCCAGGTGCGGGGGTCTGACTACAGC
CACAGTGAAGATCTCGCCGACTTCCCCCCTTCTCCGGACAGGGACCGTGAGTCCATCGTGTGA (SEQ ID NO:1042)

Nucleic Acid (shIMM)

ATGGCAAACTTCCTATTACCTCGGGGCACCAGCAGTTCCGCAGGTTCACACGGGAGTCCCTGGCAGCCATCGAGAAGCGCATGGCAGAGAAGCAAGCCCGCGGCTCAACCACCTTGC
AGGAGAGCCGAGAGGGGCTGCCCGAGGAGGAGGCTCCCCGGCCCCAGCTGGACCTGCAGGCCTCCAAAAAGCTGCCAGATCTCTATGGCAATCCACCCCAAGAGCTCATCGGAGAGCC
CCTGGAGGACCTGGACCCCTTCTATAGCACCCAAAAGACTTTCATCGTACTGAATAAAGGCAAGACCATCTTCCGGTTCAGTGCCACCAACGCCTTGTATGTCCTCAGTCCCTTCCAC
CCCATCCGGAGAGCGGCTGTGAAGATTCTCGTATTAATATGCTGATTATGTGCACCATCCTCACCAACTGCGTGTTCATGGCCCAGCACGACCCTCCACCCTGGACCA
AGTATGTCGAGTACACCTTCACCGCCATTTACACCTTTGAGTCTCTGGTCAAGATTCTGGCTCGAGGCTTCTGCCTGCACGCGTTCACTTTCCTTCGGGACCCATGGAACTGGCTGGA
CTTTAGTGTGATTATCATGGCATACACAACTGAATTTGTGGACCTGGGCAATGTCTCAGCCTTACGCACCTTCCGAGTCCTCCGGGCCCTGAAAACTATATCAGTCATTTCAGGGCTG
AAGACCATCGTGGGGGCCCTGATCCAGTCTGTGAAGAAGCTGGCTGATGTGATGGTCCTCACAGTCTTCTGCCTCAGCGTCTTTGCCCTCATCGGCCTGCAGCTCTTTCATGGGCAACC
TAAGGCACAAGTGCGTGCGCAACTTCACAGCGCTCAACGGCACCAACGGCTCCGTGGAGGCCGACGGCTTGGTCTGGAATCCCTGGACCTTTACCTCAGTGATCCAGAAAATTACCT
GCTCAAGAACGGCACCTCTGATGTGTTACTGTGTGGGAACAGCTCTGACGCTGGGACATGTCCGGAGGGCTACCGGTGCCTAAAGGCAGGCGAGAACCCCGACCACGGCTACACCAGC
TTCGATTCCTTTGCCTGGGCCTTTCTTGCACTCTTCCGCCTGATGACGCAGGACTGCTGGGAGCGCCTCTATCAGCAGACCTCAGGTCCGCAGGGAAGATCTACATGATCTTCTTCA
TGCTTGTCATCTTCCTGGGGTCCTTCTTACCGGTGGTCGCAATGGCCTATGAGGAGCAAAACCAAGCCACCATCGCTGAGACCGAGGAGAAGGAAAAGCG
CTTCCAGGAGGCCATGGAAATGCTCAAGAAAGAACACGAGGCCCTCACCATCAGGGGTGTGGATACCGTGTCCCGTAGCTCCTTGGAGATGTCCCCTTTGGCCCCAGTAAACAGCCAT
GAGAGAAGAAGCAAGAGGAGAAAACGGATGTCTTCAGGAACTGAGGAGTGTGGGAGGACAGGCTCCCCAAGTCTGACTCAGAAGATGGTCCCAGAGCAATGAATCATCTCAGCCTCA
CCCGTGGCCTCAGCAGGACTTCTATGAAGCCACGTTCCAGCCGCGGGAGCATTTTCACCTTTCGCAGGCGAGACCTGGGTTCTGAAGCAGATTTGCAGATGATGATGAAAACAGCACAGC
GGGGGAGAGCGAGAGCCACCACACATCACTGCTGGTGCCCTGGCCCCTGCGCCGGACCAGTGCCCAGGGACAGCCCAGTCCGGCTCCTGGCCACGCCTCCATGGCAAA
AAGAACAGCACTGTGGACTGCAATGGGGTGGTCTCATTACTGGGGGCAGGCGACCCAGAGGCCACATCCCCAGGAAGCCACCTCCTCCGCCCTGTGATGCTAGAGCACCCGCCAGACA
CGACCACGCCATCGGAGGAGCCAGGCGGGCCCCAGATGCTGACCTCCCAGGCTCCGTGTGTAGATGGCTTCGAGGAGCCAGGAGCGCAGCACGGCAGGGCCCTCAGCGGCAGTCAGCGTCCT
CACCAGCGCACTGGAAGAGTTAGAGGAGTCTCGCCACAAGTGTCCACCATGCTGGAACCGTCTCGCCCAGCGCTACCTGATCTGGGAGTGCTGCCCGCTGTGGATGTCCCATCAAGCAG

FIG. 3C – cont.

GGAGTGAAGTTGGTGGTCATGGACCCGTTTACTGACCTCACCATCACTCATGTGCATCGTACTCAACACACTCTTCATGGCGCTGGAGCACTACAACATGACAAGTGAATTCGAGGAGA
TGCTGCAGGTCGGAAACCTGGTCTTCACAGGAGATTTTCACAGCAGAGATGACCTTCAAGATCATTGCCCTCGACCCCTACTACTTCCAACAGGGCTGGAACATCTTCGACAGCAT
CATCGTCATCCTTAGCCTCATGGAGCTGGGCCTGTCCGCATGAGCAACTTGTCGGTGCTGCGGGTCTTCAAGCTGGCCAAATCATGGCCCACCCTGAAC
ACACTCATCAAGATCATCGGGAACCTGACACTGGTGCTAGCCATCATCGTGTTCATCTTTGCTGTGGTGGGCATGCAGCTCTTTGGCAAGAACTACT
CGGAGCTGAGGGACAGCGACTCAGGCCTGCTGCCTCGCTGCCTTCCTCATCATCTTCCGCATCCTCTGTGGAGATGTGGATCGAGACCATGTGGGA
CTGCATGGAGGTGTCGGGGCAGTCATTATGCCTGCTGTCTTCTTGCTATTGGTCATTGGCAACCTTGTGGTCCTGAATCTCTTCCTGGCCTTGCTCAGCTCCTTCAGTGCA
GACAACCTCACAGCCCCTGATGAGGACAGAGAGATGAACAACCTCCAGCTGGCCCGCATCCAGAGGGGCCTGCGCTTTGTCAAGCGGACCACCTGGGATTTCTGCTGTGGTC
TCCTGCGGCAGCGGCCTCAGAAGCCCGCAGCCTTGCCACCCCCTACTCCCGCCACACCCCAGAGACGGAGAAGGTGCCTCCCACCCG
CAAGGAAACACGGTTTGAGGAAGGCGAGCAACCAGGCCAGGGCACCCCCGGGGATCCAGACCGTGTGTGTGCCCAGTCAGACACAGATGACCAAGAAGAAGAT
GAGGAGAACAGCCTGGGCACGGAGGAGGAGTCCAGCCTGTGTCCGGTGGCCCAGAGGCCCCTCCGGATTCCAGGACCTGGAGCCAGGTGTCAGCGACTGCCT
CCTCTGAGGCCGAGGCCAGTGCATCTCAGGCCGACTGGCGGCAGCAGTGGAAAGCGGAACCCCAGGCCCCAGGGTGCGGTGAGACCCCAGAGGACAGTTGCTCCGAGGGCAGCACAGC
AGACATGACCAACACCGCTGAGCTCCTGGAGCAGATGTCAAGGACCCAGAGGACTGCTTCACTGAAGGCTGTGTCCGGCGCTGTCCCTGCTGTGCGGTG
GACACCACACAGGCCCCAGGGAAGGTCTGGTGGCGGTTGCGCAAGACCTGCTACCACATCGTGGAGCACAGCTGGTTCGAGACATTCATCTTCATGATCCTACTCAGCAGTGGAG
CGCTGGCCTTCGAGGACATCTACCTAGAGGAGCGGAAGACCATCAAGGTTCTGCTTGAGTATGCCGACAAGATGTTCACATATGTCTTCGTGCTCAAGTGGGTGGC
CTACGGCTTCAAGAAGTACTTCACCAATGCCTGGTGCTGGCTCGACTTCCTCATCGTAGACGTCTCTCTGGTCAGCCTGGTGGGCGCCAACACCCTGGGCTTTGCCGAGATGGGCCCCATC
AAGTCACTGCGGACGCTGCGTGCACTCCGTCCTCTGAGAGCTCTGTCACGATTTGAGGGCATGAGGGTGGTGGTCAATGCCCTGGTGGGCGCCATCCCGTCCATCATGAACGTCCTCC
TCGTCTGCCTCATCTTCTGGCTCATCTTCAGCATCATGGGCGTGAACCTCTTTGCGGGGAAGTTTGGGAGGTGCATCAACCAGACACGAGGGAGACTTGCCTTTGAACTACACCATCGT
GAACAACAAGAGCCAGTGTGAGTCCTTGAACTTGACCGGAGAATTGTACTGGACCAAGGTGAAAGTCAACTTTGACAACGTGGGGGCCGGGTACCTGGCCCTTCTGCAGGTGGCAACA
TTTAAAGGCTGGATGGACATTATGTATGCAGCTGTGGACTCCAGGGGGTATGAAGAGCAGCCTCAGTGGGAATACAACCTCTACATGTACATCTATTTTGTCATTTTCATCATCTTTG
GGTCTTTCTTCACCCTGAACCTCTTTATTGGTGTCATCATTGACAACTTCAACCACAGAAGAAAAAGTTAGGGGGCCAGGACATCTTCATGACAGAGGAGCAGAAGGAAGTACTACAA
TGCCATGAAGAAGCTGGGCTCCAAGAAGCCCCAGAAGCCCCTGAACAAGTACCAGGGCTTCATATTCGACATTGTGACCAAGCAGGCCTTTGACGTCACCATCATG
TTTCTGATCTGCTTGAATATGGTGACCATGATGGTGGAGACAGATGACCAAAGTCCTGAGAAAATCAACATCTTGGCCAAGATCTTCTTTGTGGCCATCTTCACAGGCGAGT
GTATTGTCAAGGTGGCTGCCCTGCGCCACTACTTCACCAACAGCTGGAATATCTTCGACTTCGTGGTTGTCATCCTCTCCATCGTGGGCACTGTGCTCTCGGACATCATCCAGAA
GTACTTCTTCTCCCGACGCTCTTCCGAGTCATCCGCCTGGCCCGAATAGGCCGCATCCTCCAGACTGATCCGAGGGGCCAAGGGGATCCGCACGCTGCTCTTTGCCCTCATGATGTCC
CTGCCTGCCCTCTTCAACATCGGGCTGCTGCTCTTCCTCGTCATGTTCATCTACTCCATCTTTGGCATGCAGCTGGCATCGACGACATGTTCA
ACTTCCAGACCTTCGCCAACAGCATGCTGTGCCTCTTCCAGATCACCACGTCGGCCGGCTGGGATGGCCTCCTTCAACACTGGGCCGCCCACTCT
GCCCAACAGCAATGGCTCTCGGGGGGACTGCGGGAGCCCAGCCGTGGGCATCCTCTTCTTCACCACCTACATCATCATCCTTCCTCATCGTGGTCAACATGTACATTGCCATCATC
CTGGAGAACTTCAGCGTGGCCACGGAGGAGAGCACCGAGCCCCTGAGTGAGGACGACTTCGAGATATGTTCTATGAGATCTGGGAGAAATTTGACCCAGAGGCCACTCAGTTTATTGAGT
ATTCGGTCCTGTCTGACTTTGCCGATGCCGTGTCTGAGCCACTCCGTATCGCCAAGCCCAAGCCTCATCAACATGGACCTGCCCATGGTGAGTGGGGACCGCATCCATTG
CATGGACATTTCTCTTTGCCTTCACCAAAAGGGTCCTGGGGGAGATGGACGCCCTGAAGATCCAGATGGAGAGGAGAAGTTCATGGCAGCCAACCCATCCTAC
GAGCCCATCACCACACACTCCGGCGCCAAGCACGAAGAGGTGTCGGCCATGGTTATCCAGAGAGCCTTCCGCAAGCGTCTTTGAAGCATGCCTCCTTCCTCTTCC
GTCAGCAGGCGGGCAGCGGCCTCTCCGAAGAGGATGCCCCTGAGCGAGAGGGCCTCATCGCCTACGTGATGATGAGTGAGAAACTTCTCCCGACCCCTTGGCCCACCCTCCAGCTCCTCCAT
CTCCTCCACTTCCTTCCCACCCTCCTATGACAGTGTCACTAGAGCCACCAGCGATAACCTCCAGGTGCGGGGGTCTGACTACAGCCACAGTGAAGATCTCGCCGACTTCCCCCCCTTCT
CCGGACAGGGACCGTGAGTCCATCGTGTGA (SEQ ID NO:1043)

FIG. 3C – cont.

Amino Acid

MANFLLPRGTSSFRRFTRESLAAIEKRMAEKQARGSTTLQESREGLPEEEAPRPQLDLQASKKLPDLYGNPPQELIGEPLEDLDPFYSTQKTFIVLNKGKTIF
RFSATNALYVLSPFHPIRRAAVKILVHSLFNMLIMCTILTNCVFMAQHDPPPWTKYVEYTFTAIYTFESLVKILARGFCLHAFTFLRDPWNWLDFSVIIMAYT
TEFVDLGNVSALRTFRVLRALKTISVISGLKTIVGALIQSVKKLADVMVLTVFCLSVFALIGLQLFMGNLRHKCVRNFTALNGTNGSVEADGLVWESLDLYLS
DPENYLLKNGTSDVLLCGNSSDAGTCPEGYRCLKAGENPDHGYTSFDSFAWAFLALFRLMTQDCWERLYQQTLRSAGKIYMIFFMLVIFLGSFYLVNLILAVV
AMAYEEQNQATIAETEEKEKRFQEAMEMLKKEHEALTIRGVDTVSRSSLEMSPLAPVNSHERRSKRRKRMSSGTEECGEDRLPKSDSEDGPRAMNHLSLTRGL
SRTSMKPRSSRGSIFTFRRRDLGSEADFADDENSTAGESESHHTSLLVPWPLRRTSAQGQPSPGTSAPGHALHGKKNSTVDCNGVVSLLGAGDPEATSPGSHL
LRPVMLEHPPDTTTPSEEPGGPQMLTSQAPCVDGFEEPGARQRALSAVSVLTSALEELEESRHKCPPCWNRLAQRYLIWECCPLWMSIKQGVKLVVMDPFTDL
TITMCIVLNTLFMALEHYNMTSEFEEMLQVGNLVFTGIFTAEMTFKIIALDPYYYFQQGWNIFDSIIVILSLMELGLSRMSNLSVLRSFRLLRVFKLAKSWPT
LNTLIKIIGNSVGALGNLTLVLAIIVFIFAVVGMQLFGKNYSELRDSDSGLLPRWHMMDFFHAFLIIFRILCGEWIETMWDCMEVSGQSLCLLVFLLVMVIGN
LVVLNLFLALLLSSFSADNLTAPDEDREMNNLQLALARIQRGLRFVKRTTWDFCCGLLRQRPQKPAALAAQGQLPSCIATPYSPPPETEKVPPTRKETRFEE
GEQPGQGTPGDPEPVCVPIAVAESDTDDQEEDEENSLGTEEESSKQESQPVSGGPEAPPDSRTWSQVSATASSEAEASASQADWRQQWKAEPQAPGCGETPED
SCSEGSTADMTNTAELLEQIPDLGQDVKDPEDCFTEGCVRRCPCCAVDTTQAPGKVWWRLRKTCYHIVEHSWFETFIIFMILLSSGALAFEDIYLEERKTIKV
LLEYADKMFTYVFVLEMLLKWVAYGFKKYFTNAWCWLDFLIVDVSLVSLVANTLGFAEMGPIKSLRTLRALRPLRALSRFEGMRVVVNALVGAIPSIMNVLLV
CLIFWLIFSIMGVNLFAGKFGRCINQTEGDLPLNYTIVNNKSQCESLNLTGELYWTKVKVNFDNVGAGYLALLQVATFKGWMDIMYAAVDSRGYEEQPQWEYN
LYMYIYFVIFIIFGSFFTLNLFIGVIIDNFNQQKKKLGGQDIFMTEEQKKYYNAMKKLGSKKPQKPIPRPLNKYQGFIFDIVTKQAFDVTIMFLICLNMVTMM
VETDDQSPEKINILAKINLLFVAIFTGECIVKLAALRHYYFTNSWNIFDFVVVILSIVGTVLSDIIQKYFFSPTLFRVIRLARIGRILRLIRGAKGIRTLLFA
LMMSLPALFNIGLLLFLVMFIYSIFGMANFAYVKWEAGIDDMFNFQTFANSMLCLFQITTSAGWDGLLSPILNTGPPYCDPTLPNSNGSRGDCGSPAVGILFF
TTYIIISFLIVVNMYIAIILENFSVATEESTEPLSEDDFDMFYEIWEKFDPEATQFIEYSVLSDFADALSEPLRIAKPNQISLINMDLPMVSGDRIHCMDILF
AFTKRVLGESGEMDALKIQMEEKFMAANPSKISYEPITTTLRRKHEEVSAMVIQRAFRRHLLQRSLKHASFLFRQQAGSGLSEEDAPERGLIAYVMSENFSR
PLGPPSSSSISSTSFPPSYDSVTRATSDNLQVRGSDYSHSEDLADFPPSPDRDRESIV (SEQ ID NO:1044)

FIG. 3C – cont.

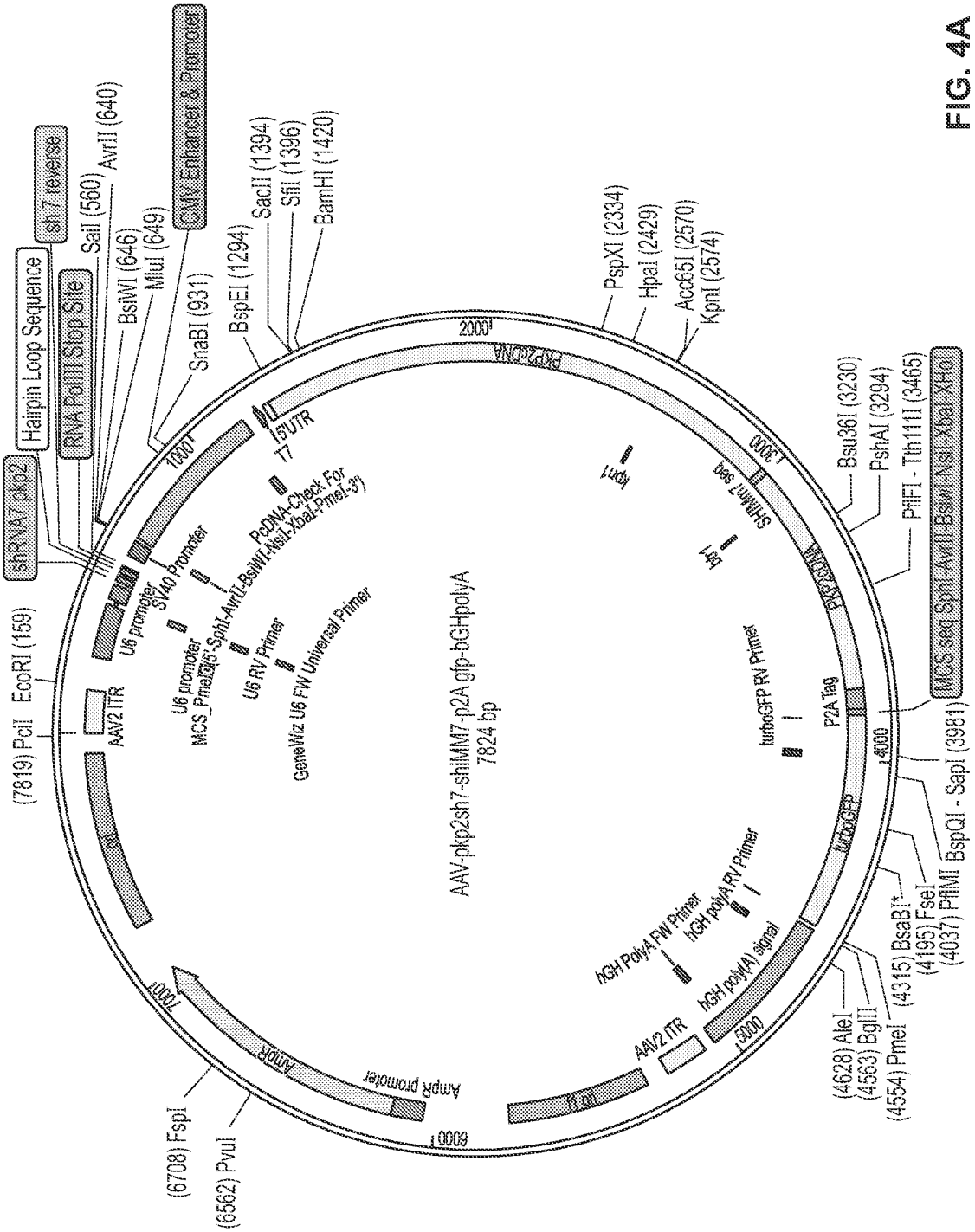

AAV-pkp2sh7-shiMM7-p2A gfp-bGHpolyA
7824 bp
shRNA7 pkp2
Hairpin Loop Sequence
RNA PolIII Stop Site
sh 7 reverse
CMV Enhancer & Promoter
SaiI (560)
AvrII (640)
BsiWI (646)
MluI (649)
SnaBI (931)
BspEI (1294)
SacII (1394)
SfiI (1396)
BamHI (1420)
PspXI (2334)
HpaI (2429)
Acc65I (2570)
KpnI (2574)
Bsu36I (3230)
PshAI (3294)
PflFI - Tth111I (3465)
MCS seq SphI-AvrII-BsiWI-NsiI-XbaI-XHoI
BspQI - SapI (3981)
PflMI
FseI
(4195)
BsaBI*
(4315)
PmeI
(4554)
BglII
(4563)
AleI
(4628)
PvuI
(6562)
FspI
(6708)
(7819) PciI
EcoRI (159)
AAV2 ITR
U6 promoter
SV40 Promoter
U6 promoter
U6 RV Primer
GeneWiz U6 FW Universal Primer
PcDNA-Check For
T7
5'UTR
PKP2cDNA
SHIMM7 seq
turboGFP RV Primer
P2A Tag
turboGFP
hGH PolyA FW Primer
hGH polyA RV Primer
hGH poly(A) signal
AAV2 ITR
AmpR promoter
AmpR
1000
2000
3000
4000
5000
6000
7000

FIG. 4A

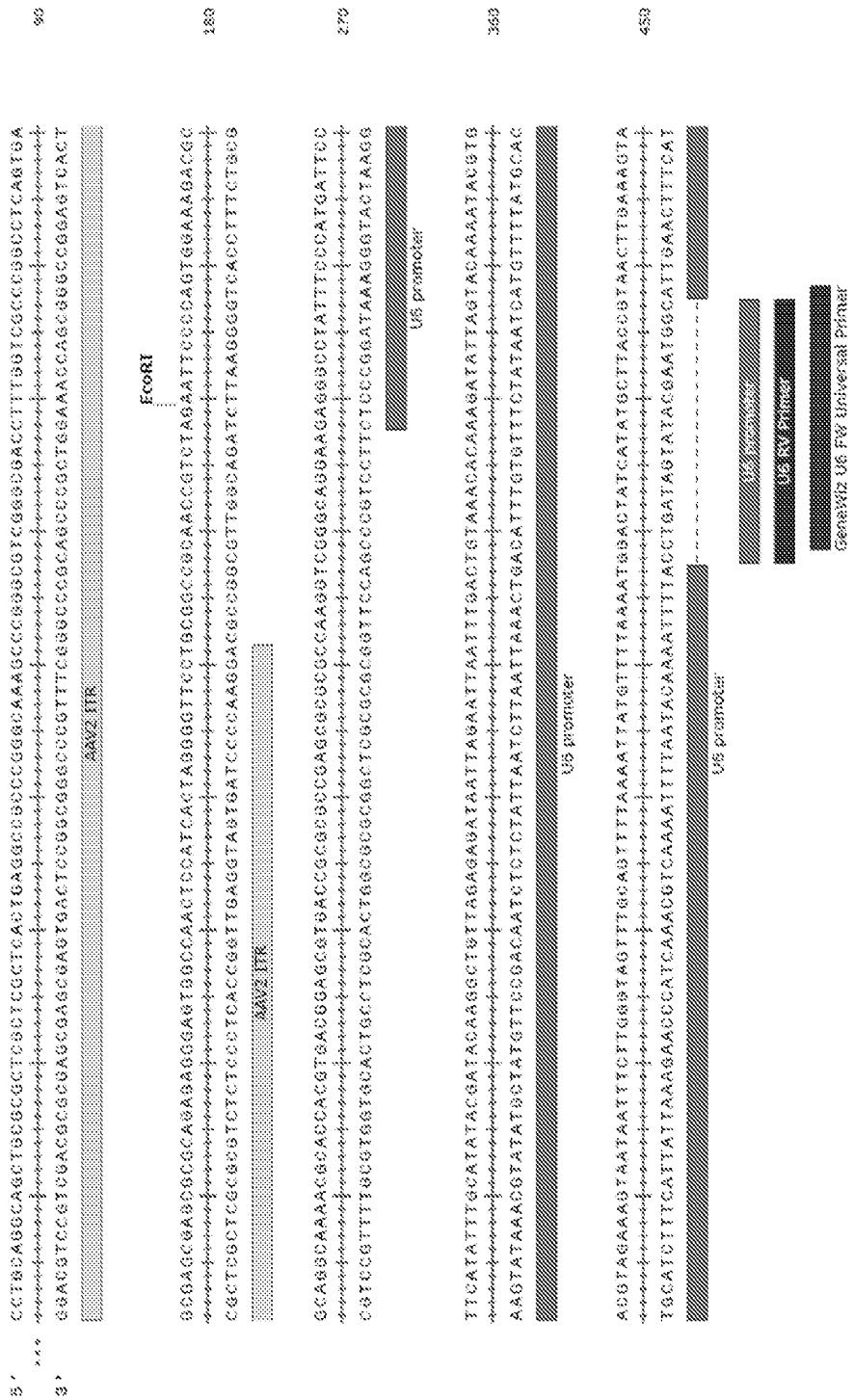

5'
3'
90
180
270
360
450
EcoRI
AAV2 ITR
AAV2 ITR
U6 promoter
U6 promoter
U6 promoter
U6 RV Primer
Genewiz U6 FW Universal Primer

FIG. 4B

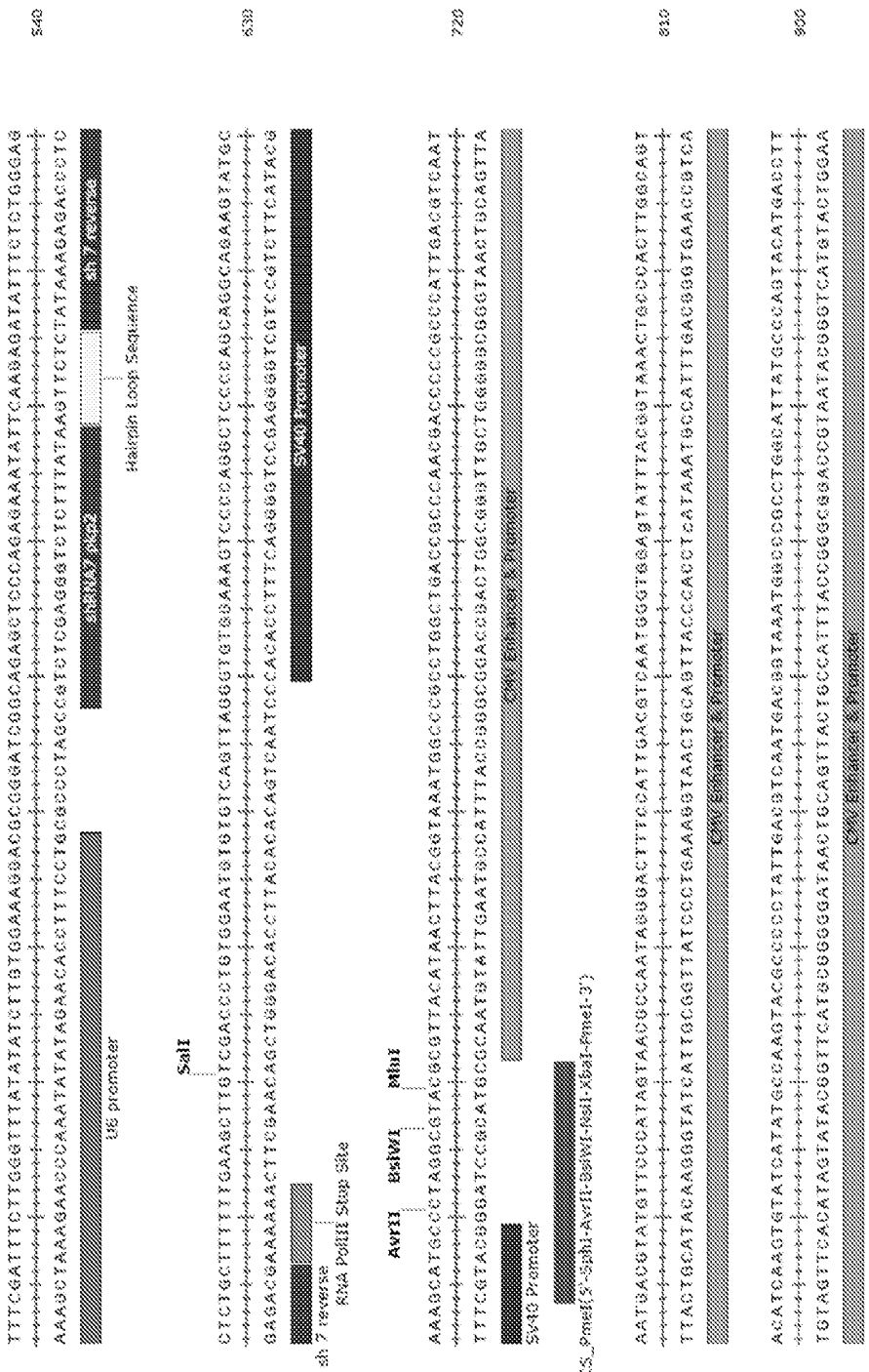

U6 promoter
SalI
Hairpin Loop Sequence
sh 7 reverse
RNA PolIII Stop Site
AvrII
BsiWI
PhuI
SV40 Promoter
MCS_PmeI(5'-SphI-AvrII-BsiWI-HsiII-XbaI-PmeI-3')

FIG. 4B – cont.


SnaBI
CMV Enhancer & Promoter
CMV Enhancer & Promoter
CMV Enhancer & Promoter
gcMAP Check Tag
TF
SUTR
PKPZcDNA
BspEI
SacII
SfiI
BamHI
PKPZcDNA
PKPZcDNA

FIG. 4B – cont.

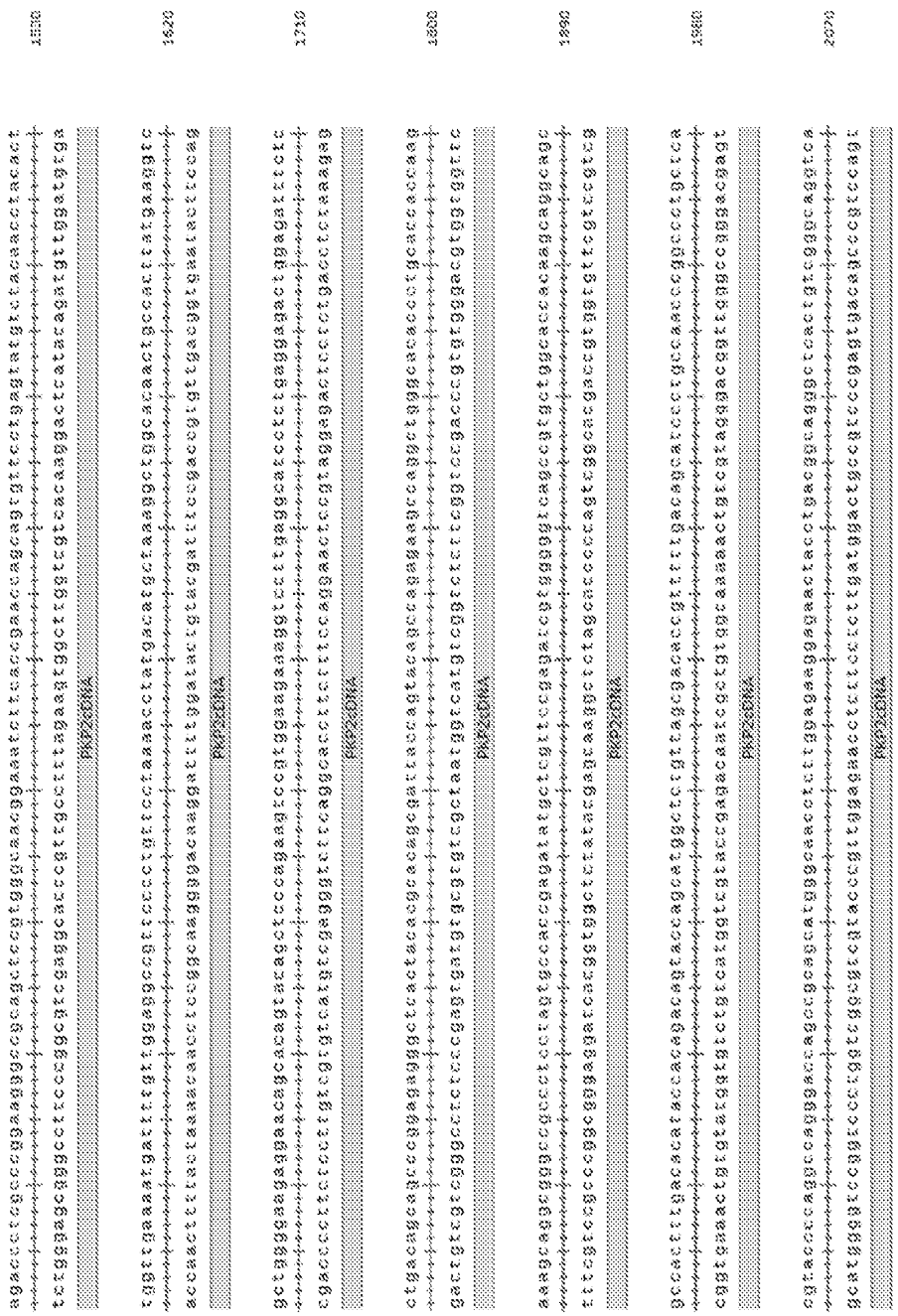


FIG. 4B – cont.

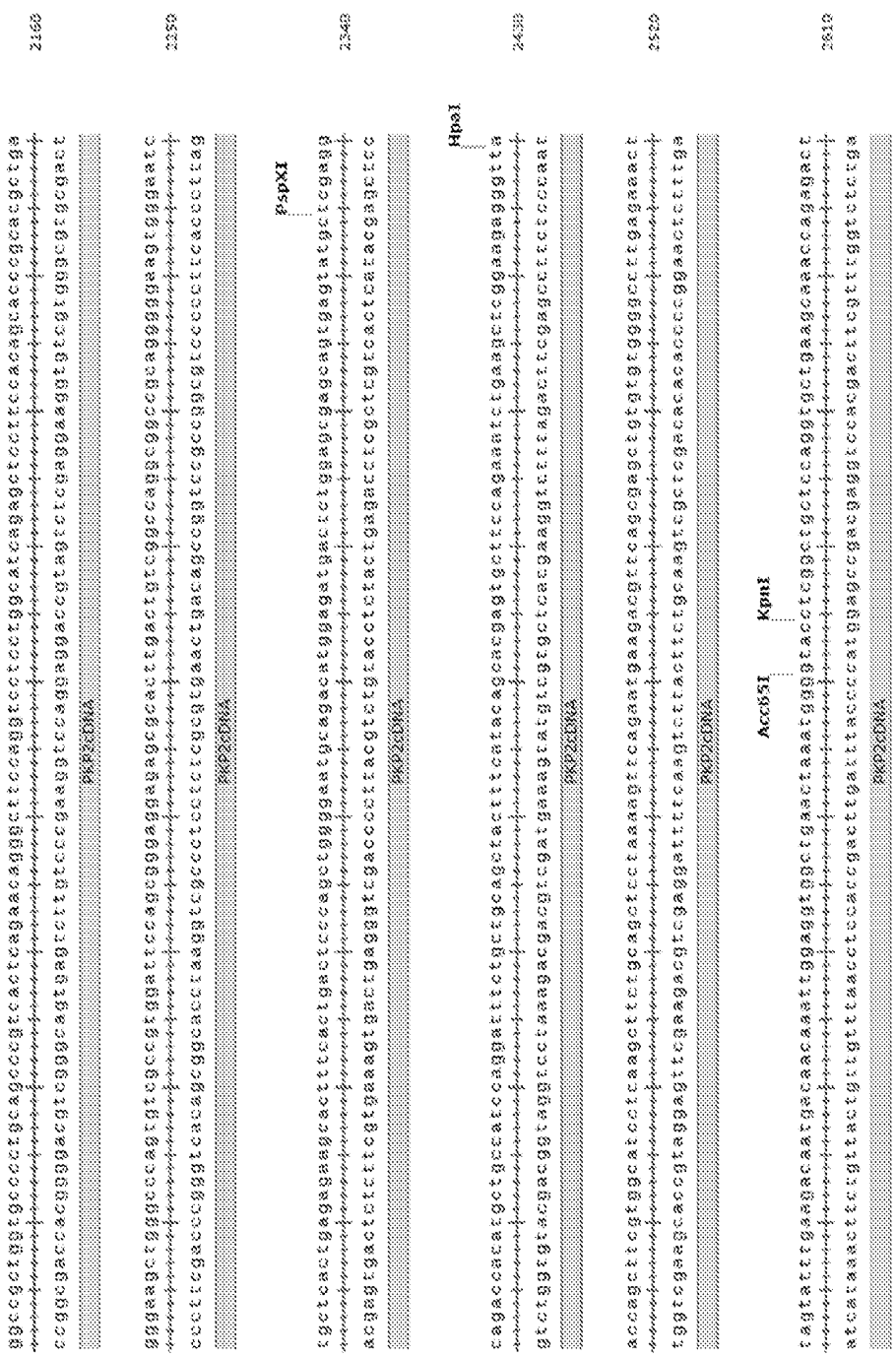

2160
2250
2340
2430
2520
2610
PspXI
HpaI
Acc65I
KpnI
PKP2cDNA

FIG. 4B – cont.

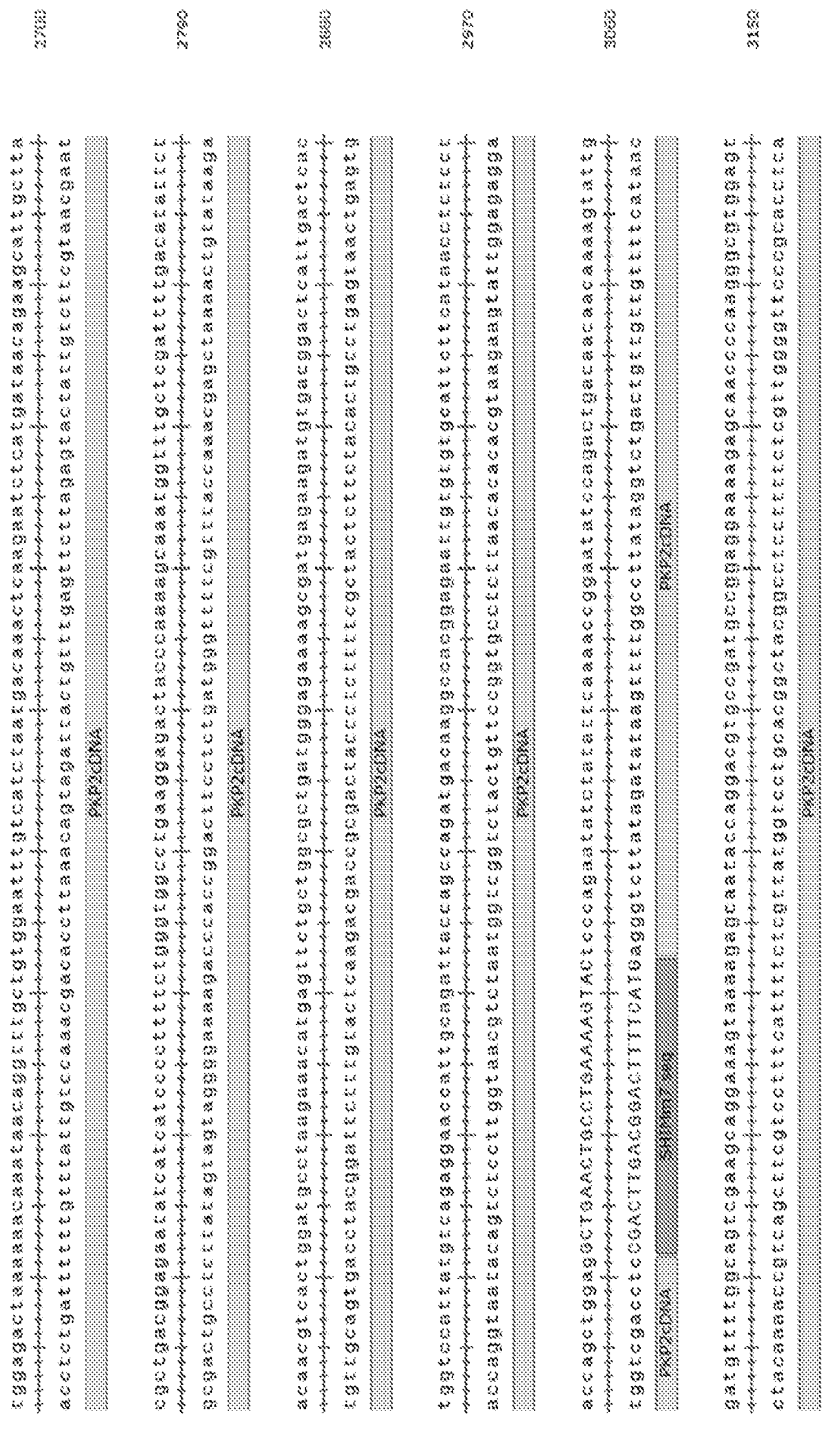


FIG. 4B – cont.

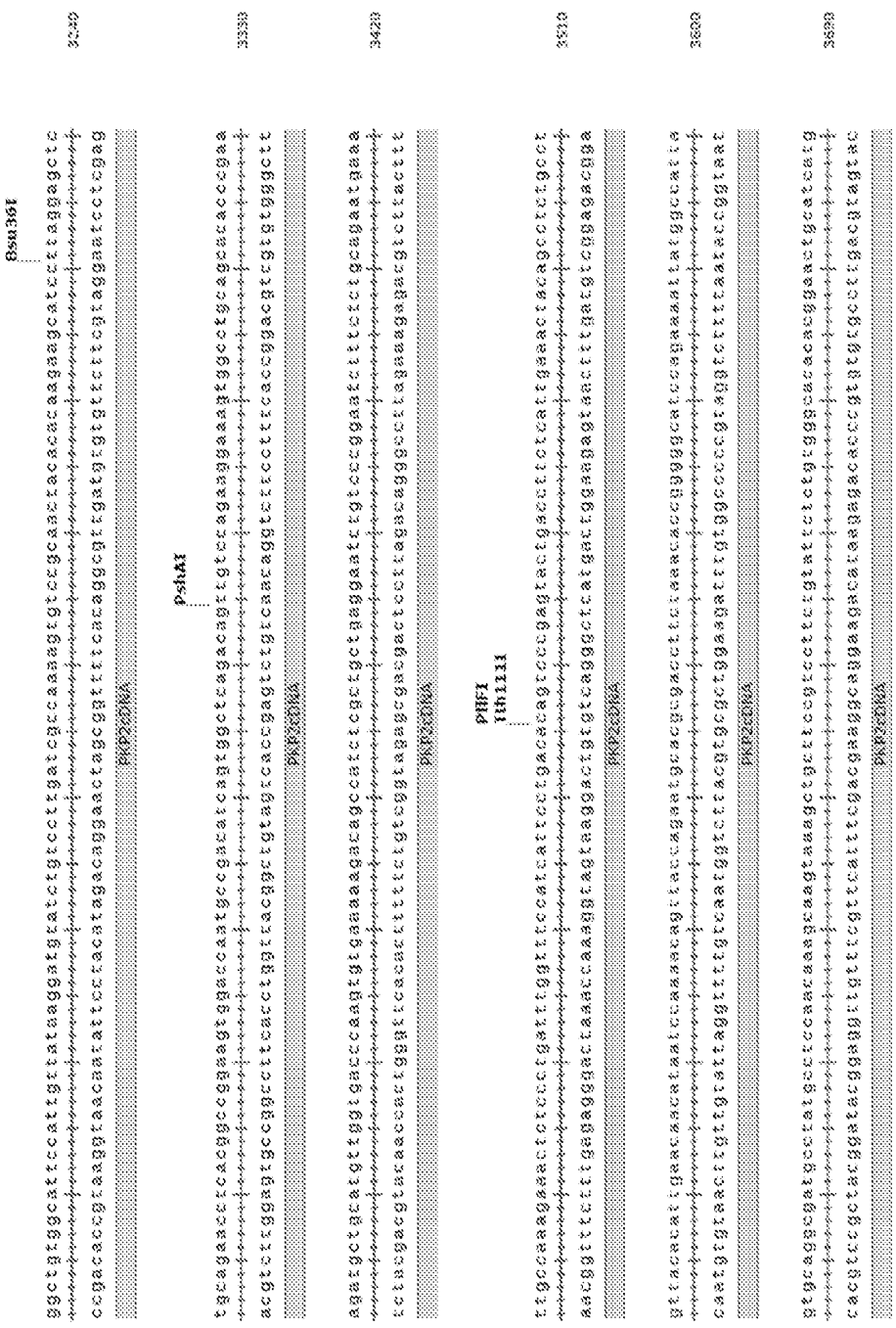


FIG. 4B – cont.

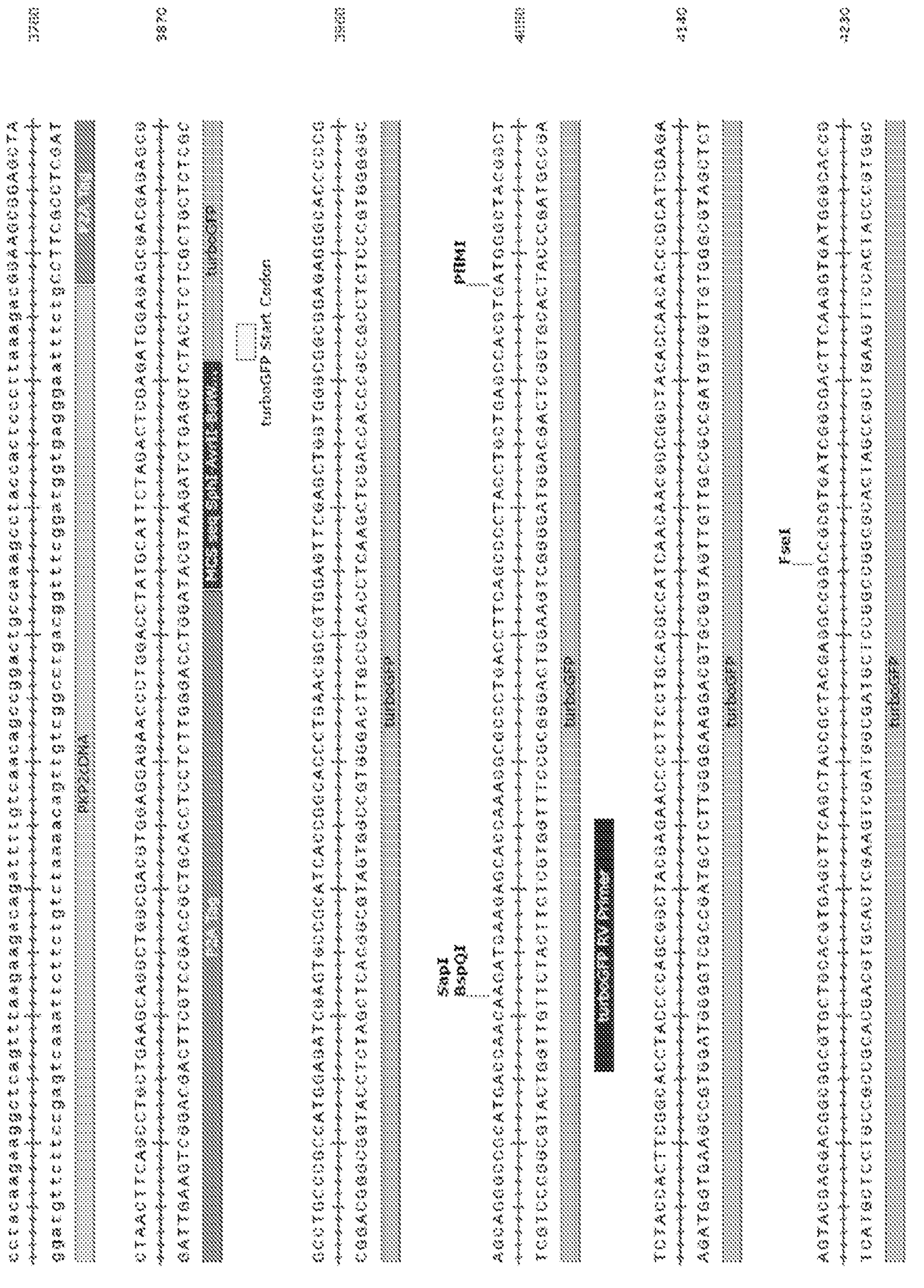

SapI
BspQI
PflMI
FseI
turboGFP
turboGFP Start Codon
turboGFP RV Primer

FIG. 4B – cont.

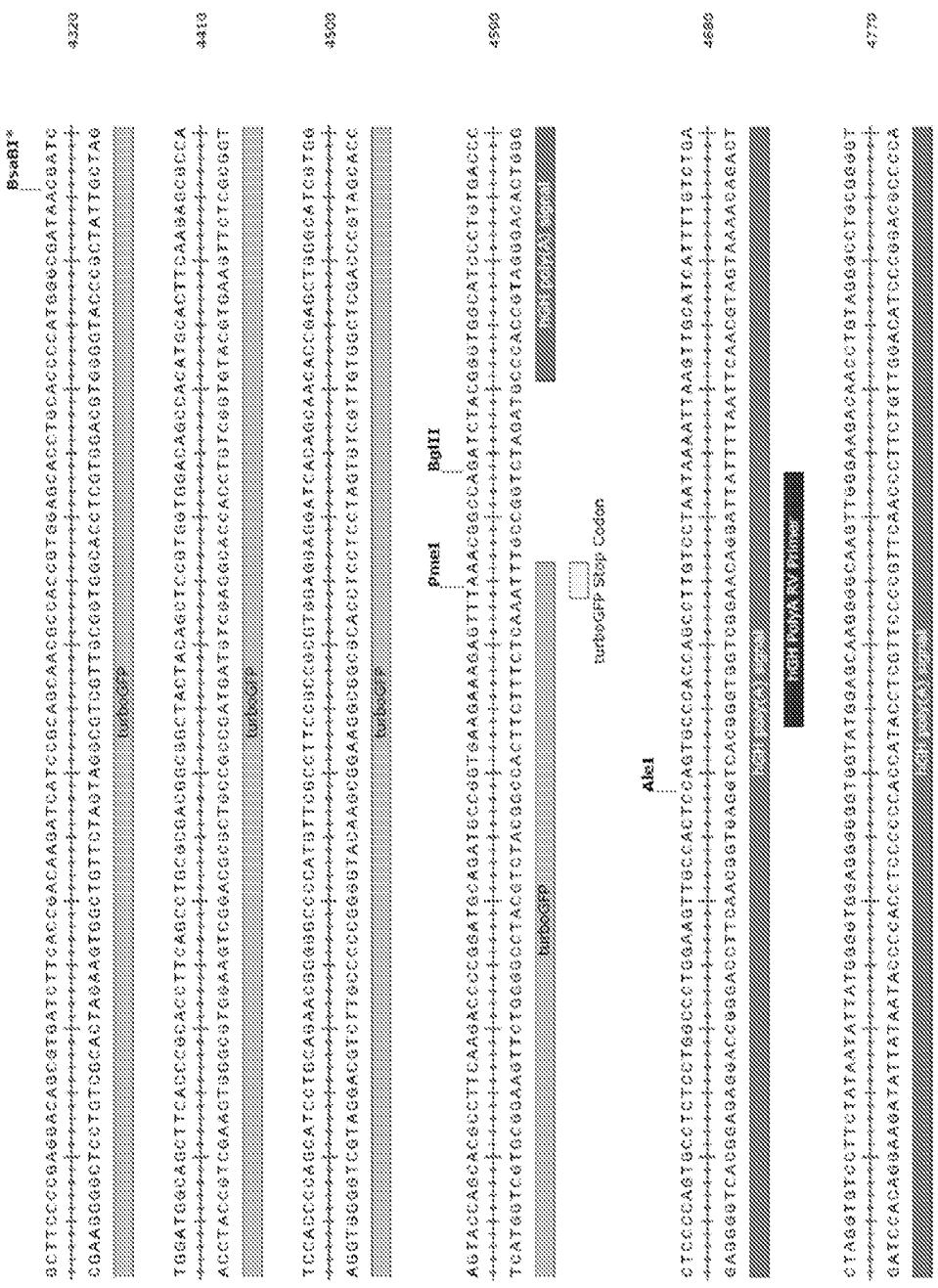

BsaBI*
4320
4410
4500
4590
4680
4770
turboGFP
PmeI
BglII
turboGFP Stop Codon
AleI

FIG. 4B – cont.

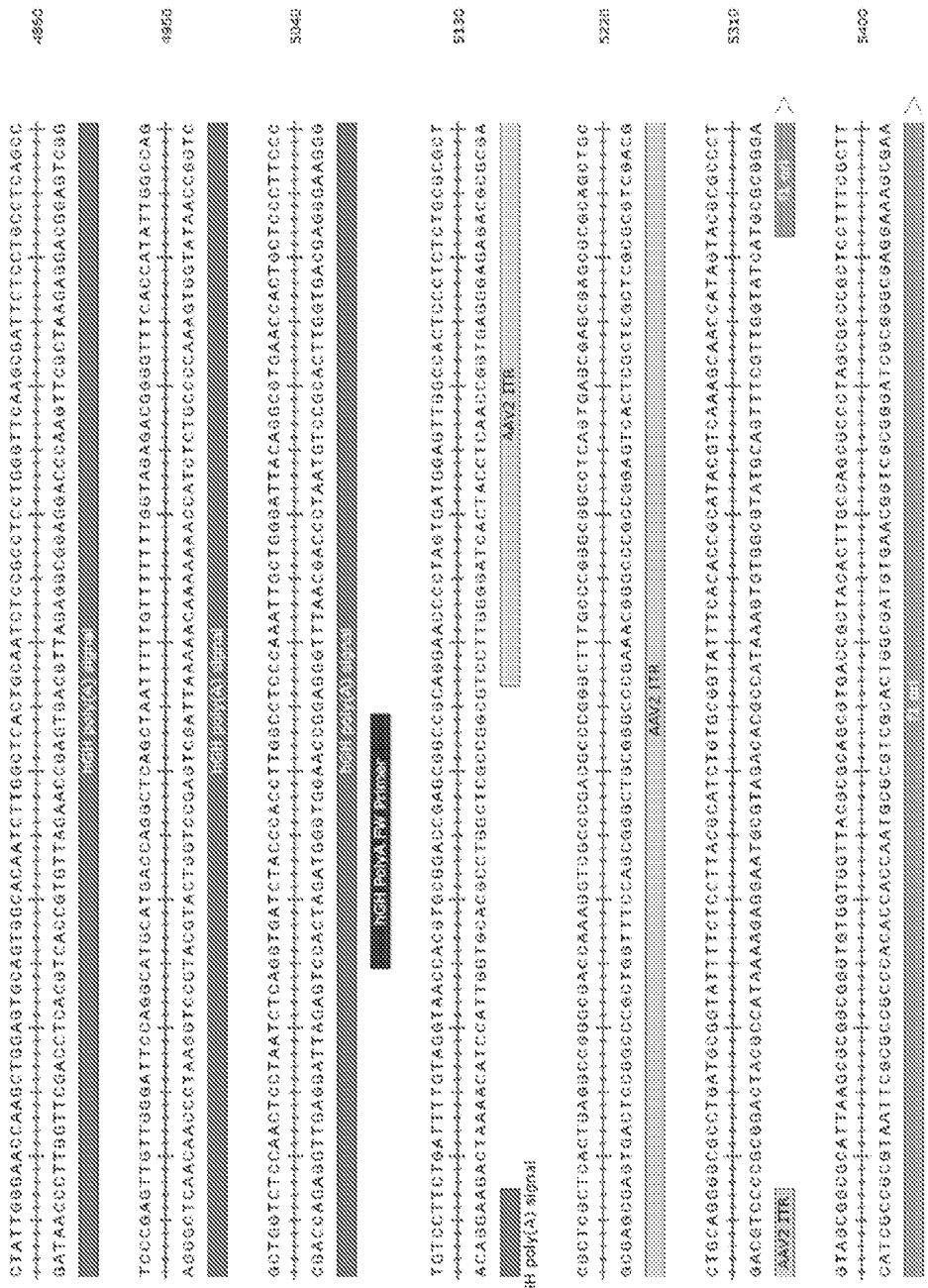

4860
4920
4980
5040
5100
5160
5220
5280
5340
5400
hGH poly(A) signal
AAV2 ITR
AAV2 ITR

FIG. 4B – cont.



FIG. 4B – cont.

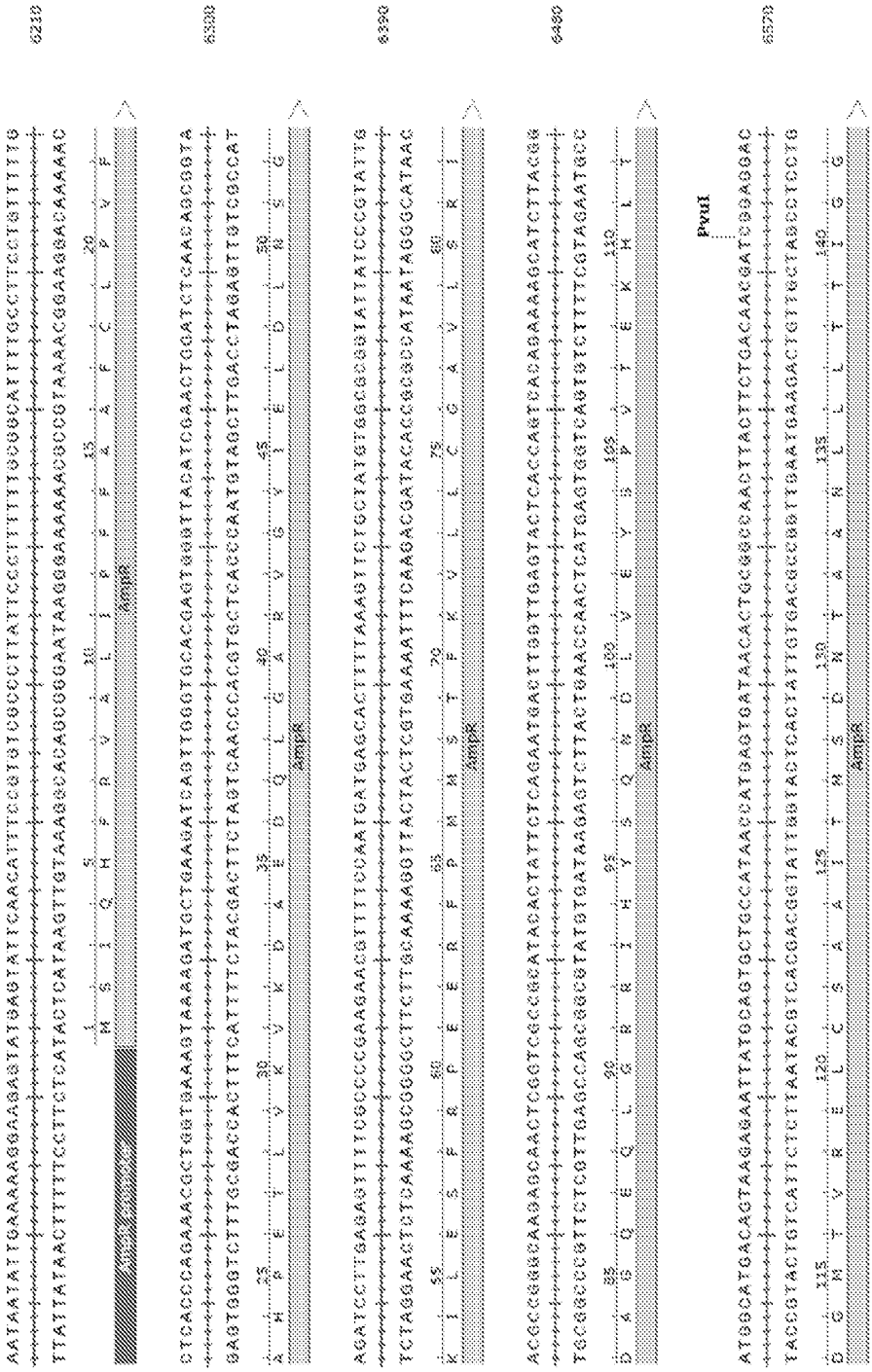


FIG. 4B – cont.

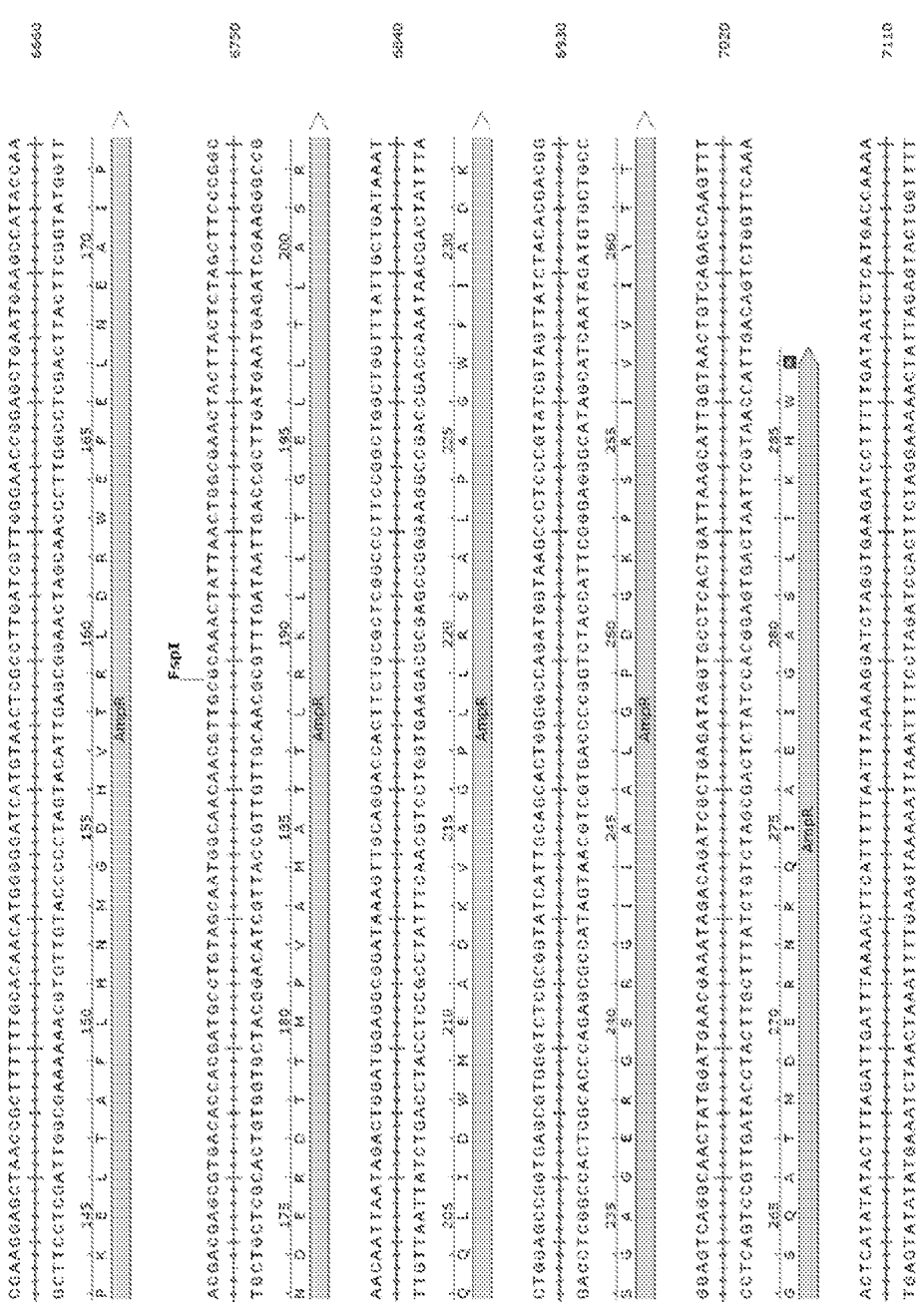


FIG. 4B – cont.

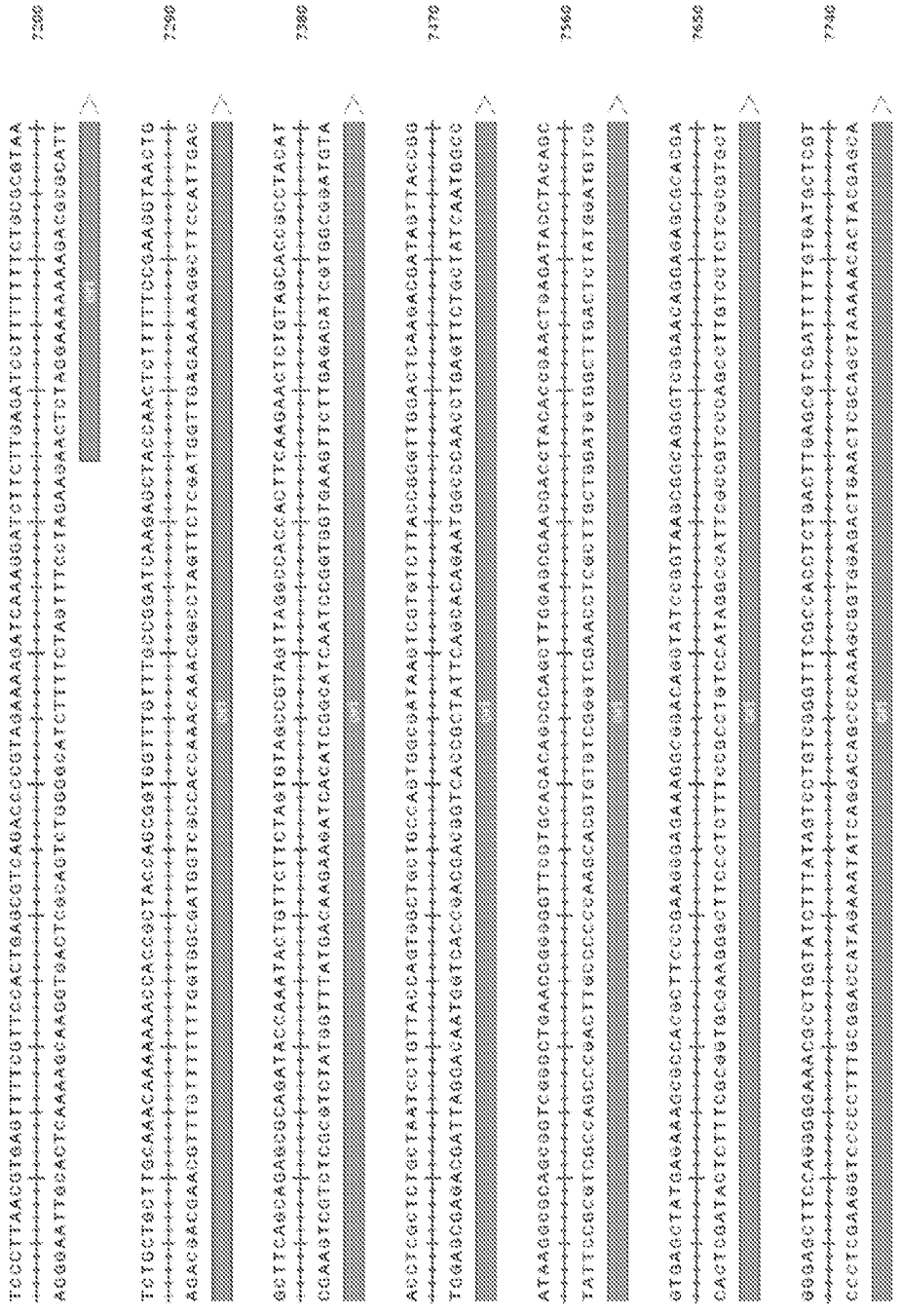


FIG. 4B – cont.

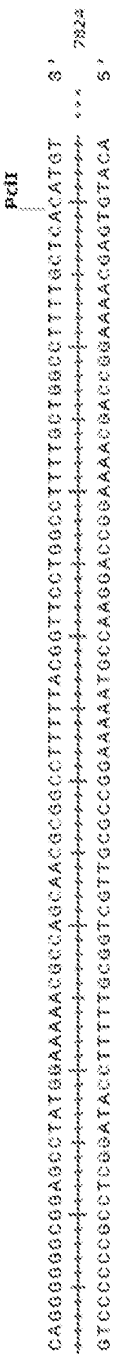

PciI
CAGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTCACATGT 3'
GTCCCCCGCCTCGGATACCTTTTTGCGGTCGTTGCGCCGGAAAAATGCCAAGGACCGGAAAACGACCGGAAAACGAGTGTACA 5'
7824

FIG. 4B – cont.

PKP2 (NM_001005242.3)
sh#36: GCAGAGCTCCCAGAGAAATAT (SEQ ID NO:52)
shIMM: GCTGAACTGCCTGAAAAGTAC (SEQ ID NO:990)

Nucleic Acid (wt)

ATGGCAGCCCCCGGCGCCCCAGCTGAGTACGGCTACATCCGGACCGTCCTGGGCCAGCAGATCCTGGACAACTGGACAGCTCCAGCCTGGCGCTGCCCTCCG
AGGCCAAGCTGAAGCTGGCGGGGAGCAGCGGCCGCGGCCGGCCAGACAGTCAAGAGCCTGCGGATCCAGGAGCAGGTGCAGCAGACCCTCGCCCGGAAGGGCCG
CAGCTCCGTGGGCAACGGAAAATCTTCACCGAACCAGCAGTGTTCCTGAGTATGTCTACAACCTACACTTGGTTGAAAATGATTTGTTGGAGGCCGTTCCCCT
GTTCCTAAAACCTATGACATGCTAAAGGCTGGCACAACTGCCACTTATGAAGGTCGCTGGGGAAGAGGAACAGCACAGTACAGCTCCCAGAAGTCCGTGGAAG
AAAGGTCCTTGAGGCATCCTCTGAGGAGACTGGAGATTTCTCCTGACAGCAGCCCGGAGAGGGCTCACTACACGCACAGCGATTACCAGTACAGCCAGAGAAG
CCAGGCTGGGCACACCCTGCACCACCAAGAAAGCAGGCGGGCCGCCCTCCTAGTGCCACCGAGATATGCTCGTTCCGAGATCGTGGGGTCAGCCGTGCTGGC
ACCACAAGCAGGCAGCGCCACTTTGACACATACCACAGACAGTACCAGCATGGCTCTGTTAGCGACACCGTTTTTGACAGCATCCCTGCCAACCCGGCCCTGC
TCACGTACCCCAGGCCAGGGACCAGCCGCAGCATGGGCAACCTCTTGGAGAAGGAGAACTACCTGACGGCAGGGCTCACTGTCGGGCAGGTCAGGCCGCTGGT
GCCCCTGCAGCCCGTCACTCAGAACAGGGCTTCCAGGTCCTCCTGGCATCAGAGCTCCTTCCACAGCACCCGCACGCTGAGGGAAGCTGGGCCCAGTGTCGCC
GTGGATTCCAGCGGGAGGAGAGCGCACTTGACTGTCGGCCAGGCGGCCGCAGGGGGAAGTGGGAATCTGCTCACTGAGAGAAGCACTTTCACTGACTCCCAGC
AGCAAACCAGAGACTTGGAGACTAAAAAACAAATAACAGGTTTGCTGTGGAATTTGTCATCTAATGACAAACTCAAGAATCTCATGATAACAGAAGCATTGCT
TGGGGAATGCAGACATGGAGATGACTCTGGAGCGAGTATGCTCGAGGCAGACCACATGCTGCCATCCAGGATTTCTGCTGCAGCTACTTTCATACA
TACGCTGACGGAGAATATCATCATCCCCTTTTCTGGGTGGCCTGAAGGAGACTACCCAAAAGCAAATGGTTTGCTCGATTTTGACATATTCTACAACGTCACT
GCACGAGTGCTTCCAGAAATCTGAAGCTCGGAAGAGGGTTAACCAGCTTCGTGGCATCCTCAAGCTTCTGCAGCTCCTAAAAGTTCAGAATGAAGACGTTCAG
GGATGCCTAAGAAACATGAGTTCTGCTGGCGCTGATGGGAGAAAAGCGATGAGAAGATGTGACGGACTCATTGACTCACTGGTCCATTATGTCAGAGGAACCA
TTGCAGATTACCAGCCAGATGACAAGGCCACGGAGAATTGTGTGTGCATTCTTCATAACCTCTCCTACCAGCTGGAGGCAGGAAAGTAAAAGAGCAATACCAGGACGTG
GAATATCTATATTCAAAACCGGAATATCCAGACTGACAACAACAAAAGTATTGGATGTTTTGGCAGTCGAAGCAGGAAAGTAAAAGAGCAATACCAGGACGTG
CCGATGCCGGAGGAAAAGAGCAACCCCAAGGGCGTGGAGTGGCTGTGCATTCCATTGTTATAAGGATGTATCTGTCCTTGATCGCCAAAAGTGTCCGCAACT
ACACACAAGAAGCATCCTTAGGAGCTCTGCAGAACCTCACGGCCGGAAGTGGACCAATGCCGACATCAGTGGCTCAGACAGTTGTCCAGAAGGAAAGTGGCCT
GCAGCACACCCGAAAGATGCTGCATGTTGGTGACCCAAGTGTGAAAAAGACAGCCATCTCGCTGCTGAGGAATCTGTCCCGGAATCTTTCTCTGCAGAATGAA
ATTGCCAAAGAAACTCTCCCTGATTTGGTTTCCATCATTCCTGACACAGTCCCGAGTACTGACCTTCTCATTGAAACTACAGCCTCTGCCTGTTACACATTGA
ACAACATAATCCAAAACAGTTACCAGAATGCACGCGACCTTCTAAACACCGGGGGCATCCAGAAAATTATGGCCATTAGTGCAGGCGATGCCTATGCCTCCAA
CAAAGCAAGTAAAGCTGCTTCCGTCCTTCTGTATTCTCTGTGGGCACACACGGAACTGCATCATGCCTACAAGAAGGCTCAGTTTAAGAAGACAGATTTTGTC
AACAGCCGGACTGCCAAAGCCTACCACTCCCTTAAAGACTGA (SEQ ID NO:1038)

FIG. 4C

Nucleic Acid (shIMM)

ATGGCAGCCCCCGGCGCCCCAGCTGAGTACGGCTACATCCGGACCGTCCTGGGCCAGCAGATCCTGGACAACTGGACAGCTCCAGCCTGGCGCTGCCCTCCGA
GGCCAAGCTGAAGCTGGCGGGCGAGCAGCCGGCCGGCCAGACAGTCAAGAGCCTGCGGATCCAGGAGCAGGTGCAGCAGACCCTCCGCCCGGAAGGGCCGCA
GCTCCGTGGGCAACGGAAATCTTCACCGAACCAGCAGTATGTTCCTGAGTATGTCTACAACCTACACTTGGTTGAAAATGATTTTGTTGGAGGCCGTTCCCCTGTT
CCTAAAACCTATGACATGCTAAAGGCTGGCACACAACTGCCACTTATGAAGGTCGCTGGGGAAGAGGAACAGCACACAGTACAGCTCCCAGAAGTCCGTGGAAGAAAG
GTCCTTGAGGCATCCTCTGAGGAGATTTCTCCTGACACAGCAGCCCGGAGAGGGCTCACTACACGCACACAGCGATTACCAGTACACAGCCAGAGAAGCCAGG
CTGGGCACACCCTGCACCACCAAGAAAGCAGGCGGCGCCTCCTAGTGCTCGTTCCGAGATCGTGGGGGTCAGCCGTGCTGGCACCACA
AGCAGGCAGCGCCACTTTGACACATACCACAGACAGTACCATGGCTCTGTTAGCGACACCGTTTTTGACAGCATCCCTGCCAACCCGGCCCTGCTCACGTA
CCCCAGGCCAGGGACCAGCCGCAGCATGGGCAACCTCTTGGAGAAGGAGAACTACCTGACGGCAGGGCTCACTGTCGGGCAGGTCAGGCCGCTGGTGCCCCTGC
AGCCCGTCACTCAGAACAGGGCTTCCAGGTCCTCCTGGCATCAGAGCTCCTTCCACAGCACCCGCACGCTGAGGGAAGCTGGGCCCAGTGTCGCCGTGGATTCC
AGCGGGAGGAGAGCGCACTTGACTGTCGGCCAGGCGGCCGCAGGGGGAAGTGGGAATCTGCTCACTGAGAGAAGCACTTTCACTGACTCCCAGCTGGGGAATGC
AGACATGGAGATGACTCTGGAGCGAGCAGTGAGTATGCTCGAGGCAGACCACATGCTGCCATCCAGGATTTCTGCTGCAGCTACTTTCATACAGCACGAGTGCT
TCCAGAAATCTGAAGCTCGGAAGAGGGTTAACCAGCTTCGTGGCATCCTCAAGCTTCTGCAGCTCCTAAAAGTTCAGAATGAAGACGTTCAGCGAGCTGTGTGT
GGGGCCTTGAGAAACTTAGTATTTGAAGACAATGACAACAAATTGGAGGTGGCTGAACTAAATGGGGTACCTCGGCTGCTCCAGGTGCTGAAGCAAACCAGAGA
CTTGGAGACTAAAAAACAAATAACAGGTTTGCTGTGGAATTTGTCATCTAATGACAAACTCAAGAATCTCATGATAACAGAAGCATTGCTTACGCTGACGGAGA
ATATCATCATCCCCTTTTCTGGGTGGCCTGAAGGAGACTACCCAAAAGCAAATGGTTTGCTCGATTTTGACATATTCTACAACGTCACTGGATGCCTAAGAAAC
ATGAGTTCTGCTGGCGCTGATGGGAGAAAAGCGATGAGAAGATGTGACGGACTCATTGACTCACTGGTCCATTATGTCAGAGGAAAGTGGCCTGCAGCACACCCG
AGATGACAAGGCCACGGAGAATTGTGTGCATTCTTCTTCATAACCTCTCCTACCAGCTGGAGGCTGAACTGCCTGAAAAGTACTCCCAGAATATCTATATTCAAA
ACCGGAATATCCAGACTGACAACAACAAAGTATTGGATGTTTTGGCAGTCGAAGCAGGAAAGTAAAAGAGCAATACCAGGACGTGCCGATGCCGGAGGAAAAG
AGCAACCCCAAGGGCGTGGAGTGGCATTCCATTGTTATAAGGATGTATCTGTCCTTGATCGCCAAAAGTGTCCGCAACTACACACACAAGAAGCATCCTT
AGGAGCTCTGCAGAACCTCACGGCCGGAAGTGGACCAATGCCGACATCAGTGGCTCAGACAGTTGTCCAGAGGAAAGTGGCCTGCAGCACACCCGAAAGATGC
TGCATGTTGGTGACCCAAGTGTGAAAAAGACAGCCATCTCGCTGCTGAGGAATCTGTCCCGGAATCTTTCTCTGCAGAATGAAATTGCCAAAGAAACTCTCCCT
GATTTGGTTTCCATCATTCCTGACACAGTCCCGAGTACTGACCTTCTCATTGAAACTACAGCCTCTGCCTGTTACACATTGAACACAACATAATCCAAAACAGTTA
CCAGAATGCACGCGACCTTCTAAACACCGGGGGCATCCAGAAAATTATGGCCATTAGTGCAGGCGATGCCTATGCCTCCAACAAAGCAAGTAAAGCTGCTTCCG
TCCTTCTGTATTCTCTGTGGGCACACACGGAACTGCATCATGCCTACAAGAAGGCTCAGTTTAAGAAGACAGATTTTGTCAACAGCCGGACTGCCAAAGCCTAC
CACTCCCTTAAAGACTGA (SEQ ID NO:1039)

Amino Acid

MAAPGAPAEYGYIRTVLGQQILGQLDSSSLALPSEAKLKLAGSSGRGGQTVKSLRIQEQVQQTLARKGRSSVGNGNLHRTSSVPEYVYNLHLVENDFVGGRSPV
PKTYDMLKAGTTATYEGRWGRGTAQYSSQKSVEERSLRHPLRRLEISPDSSPERAHYTHSDYQYSQRSQAGHTLHHQESRRAALLVPPRYARSEIVGVSRAGTT
SRQRHFDTYHRQYQHGSVSDTVFDSIPANPALLTYPRPGTSRSMGNLLEKENYLTAGLTVGQVRPLVPLQPVTQNRASRSSWHQSSFHSTRTLREAGPSVAVDS
SGRRAHLTVGQAAAGGSGNLLTERSTFTDSQLGNADMEMTLERAVSMLEADHMLPSRISAAATFIQHECFQKSEARKRVNQLRGILKLLQLLKVQNEDVQRAVC
GALRNLVFEDNDNKLEVAELNGVPRLLQVLKQTRDLETKKQITGLLWNLSSNDKLKNLMITEALLTLTENIIIPFSGWPEGDYPKANGLLDFDIFYNVTGCLRN
MSSAGADGRKAMRRCDGLIDSLVHYVRGTIADYQPDDKATENCVCILHNLSYQLEAELPEKYSQNIYIQNRNIQTDNNKSIGCFGSRSRKVKEQYQDVPMPEEK
SNPKGVEWLWHSIVIRMYLSLIAKSVRNYTQEASLGALQNLTAGSGPMPTSVAQTVVQKESGLQHTRKMLHVGDPSVKKTAISLLRNLSRNLSLQNEIAKETLP
DLVSIIPDTVPSTDLLIETTASACYTLNNIIQNSYQNARDLLNTGGIQKIMAISAGDAYASNKASKAASVLLYSLWAHTELHHAYKKAQFKKTDFVNSRTAKAY
HSLKD (SEQ ID NO:1040)

FIG. 4C – cont.

FIG. 5A

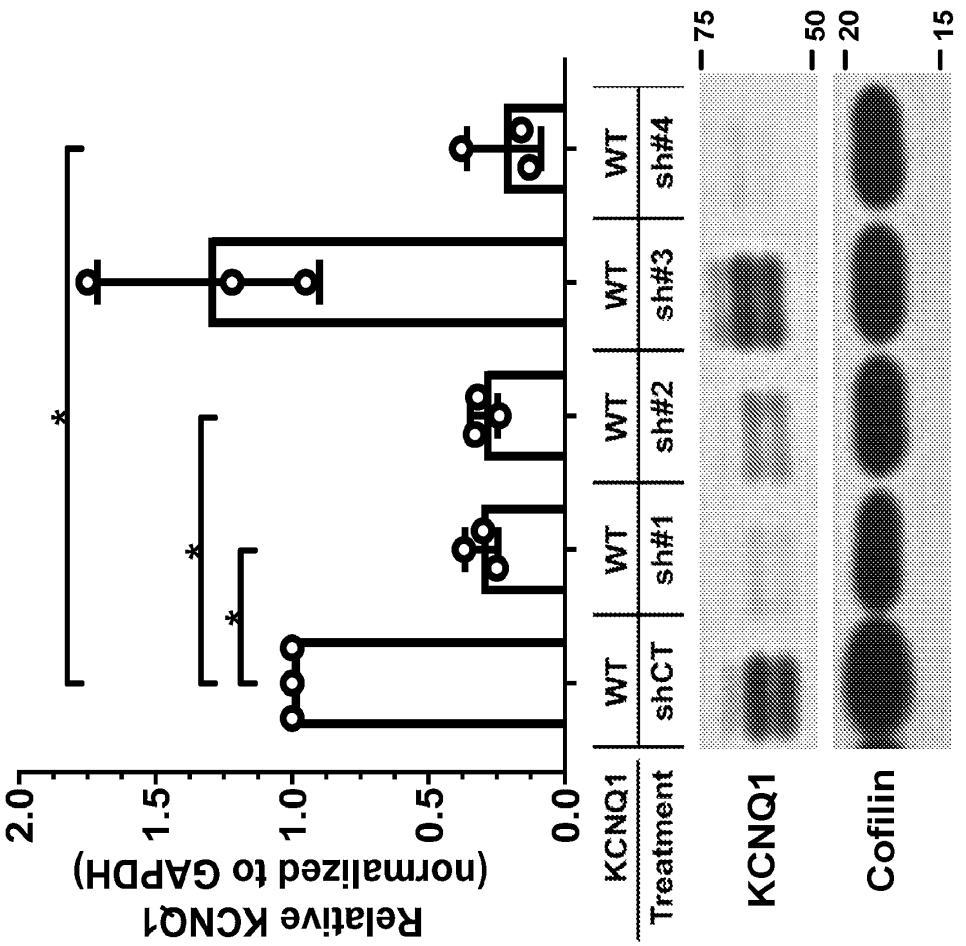

Relative KCNQ1
(normalized to GAPDH)
2.0
1.5
1.0
0.5
0.0
n.s. (p's>0.98)
*
*
*
KCNQ1
Treatment
WT
shCT
WT
sh#1
WT
sh#2
WT
sh#3
WT
sh#4
75
50
20
15
KCNQ1
Cofilin

FIG. 5B

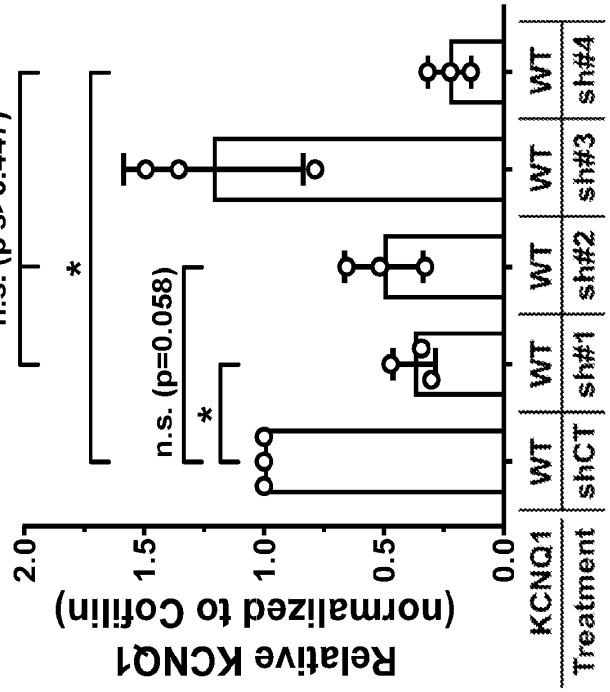

Relative KCNQ1
(normalized to Cofilin)
2.0
1.5
1.0
0.5
0.0
n.s. (p's>0.447)
*
n.s. (p=0.058)
*
KCNQ1
Treatment
WT
shCT
WT
sh#1
WT
sh#2
WT
sh#3
WT
sh#4

FIG. 5C

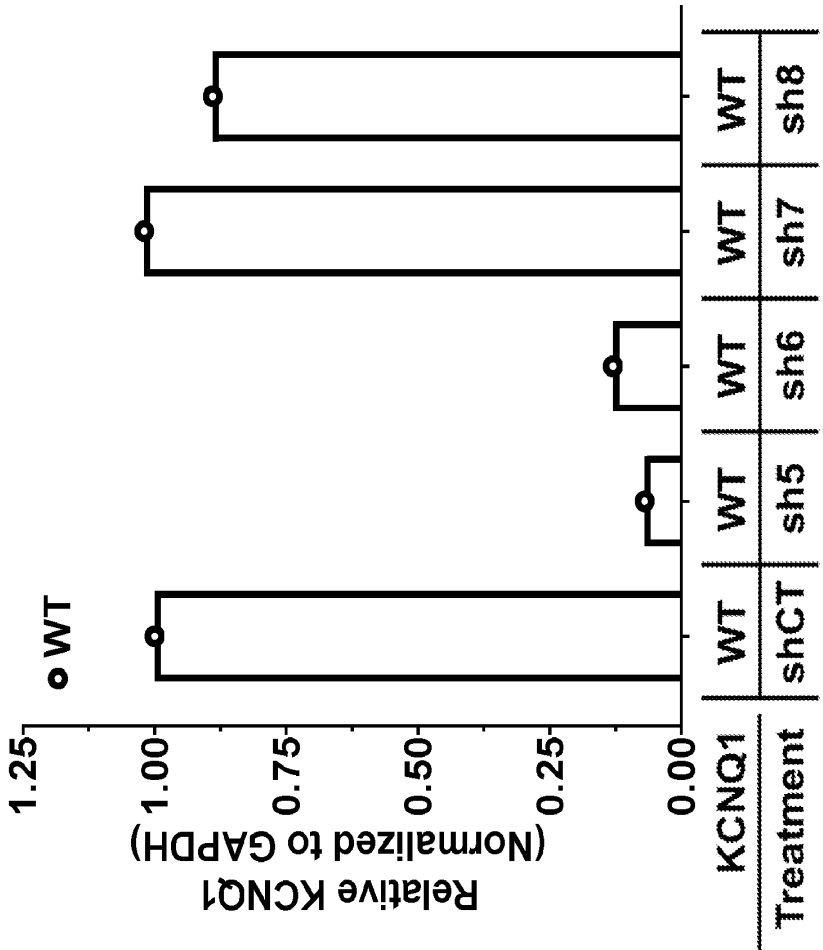

Relative KCNQ1
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
KCNQ1
Treatment
WT
shCT
WT
sh5
WT
sh6
WT
sh7
WT
sh8

FIG. 6A

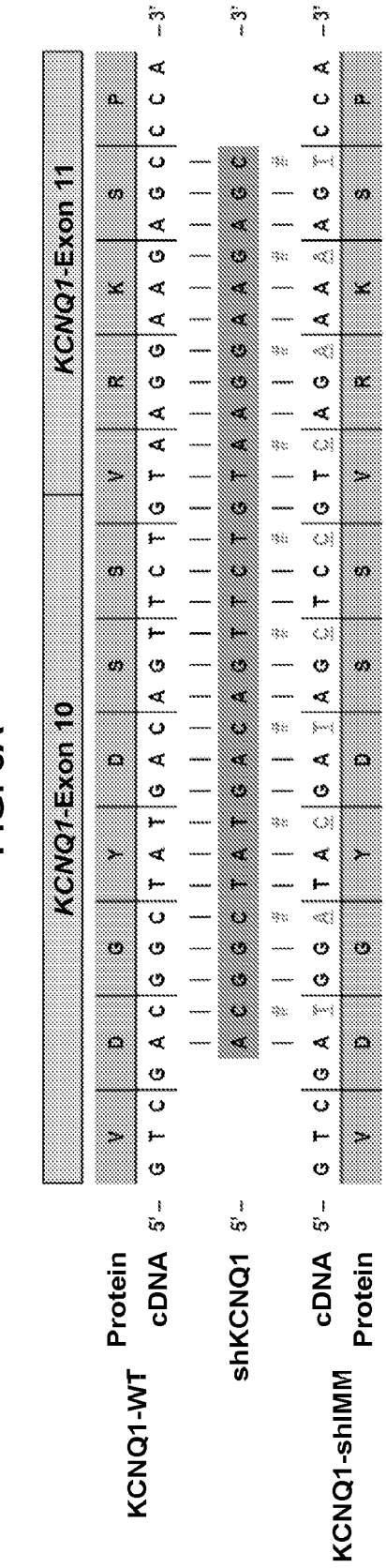

KCNQ1-Exon 10
KCNQ1-Exon 11
KCNQ1-WT
Protein
cDNA
5'–
–3'
shKCNQ1
5'–
–3'
KCNQ1-shIMM
cDNA
5'–
–3'
Protein

FIG. 6B

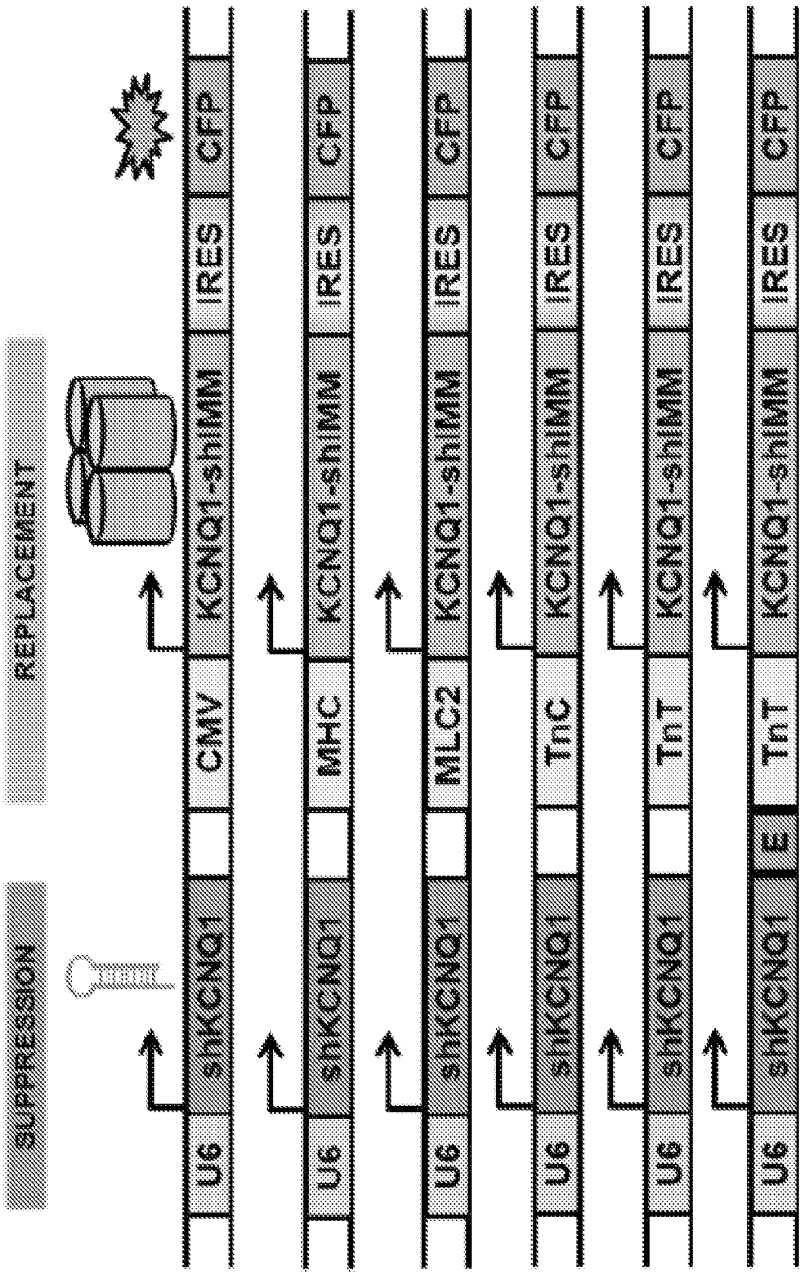

SUPPRESSION
REPLACEMENT
U6
shKCNQ1
CMV
KCNQ1-shIMM
IRES
CFP
U6
shKCNQ1
MHC
KCNQ1-shIMM
IRES
CFP
U6
shKCNQ1
MLC2
KCNQ1-shIMM
IRES
CFP
U6
shKCNQ1
TnC
KCNQ1-shIMM
IRES
CFP
U6
shKCNQ1
TnT
KCNQ1-shIMM
IRES
CFP
U6
shKCNQ1
E
TnT
KCNQ1-shIMM
IRES
CFP

FIG. 7A

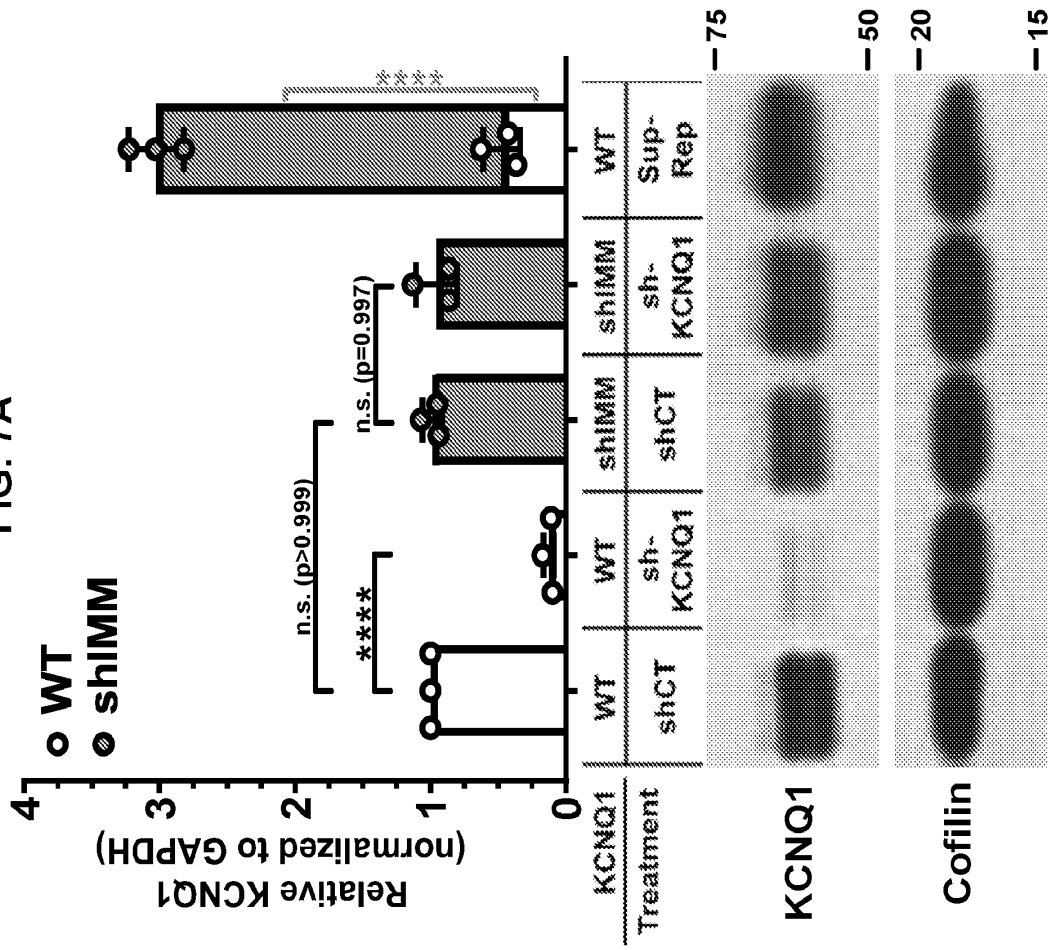

WT
shIMM
Relative KCNQ1 (normalized to GAPDH)
0
1
2
3
4
****
n.s. (p>0.999)
n.s. (p=0.997)
****
KCNQ1
Treatment
WT
shCT
WT
sh-KCNQ1
shIMM
shCT
shIMM
sh-KCNQ1
WT
Sup-Rep
75
50
20
15
KCNQ1
Cofilin

FIG. 7B

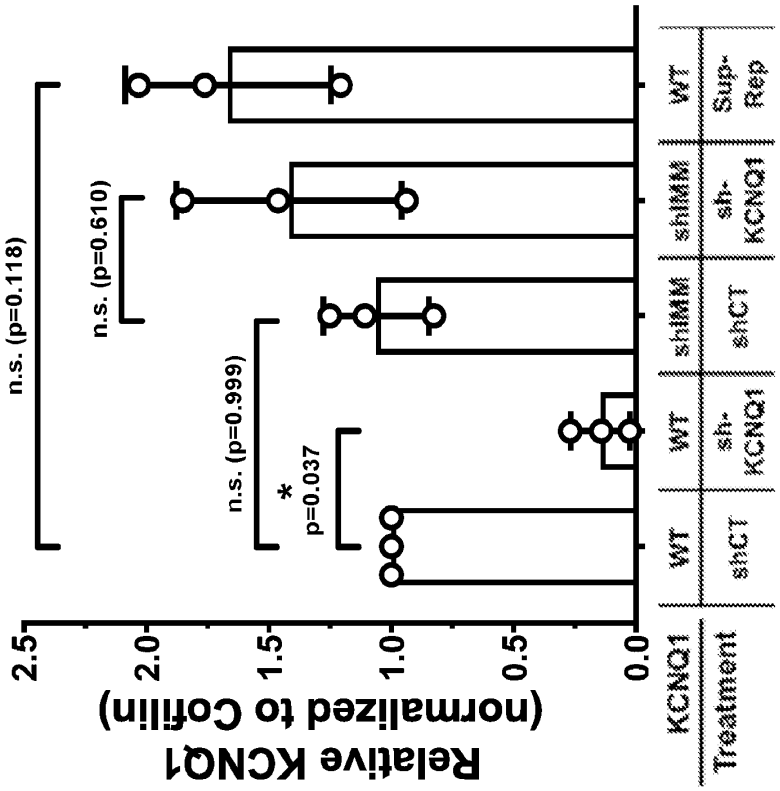

Relative KCNQ1
(normalized to Cofilin)
2.5
2.0
1.5
1.0
0.5
0.0
n.s. (p=0.118)
n.s. (p=0.610)
n.s. (p=0.999)
* p=0.037
KCNQ1
Treatment
WT
shCT
WT
sh-KCNQ1
shMM
shCT
shMM
sh-KCNQ1
WT
Sup-Rep

FIG. 8

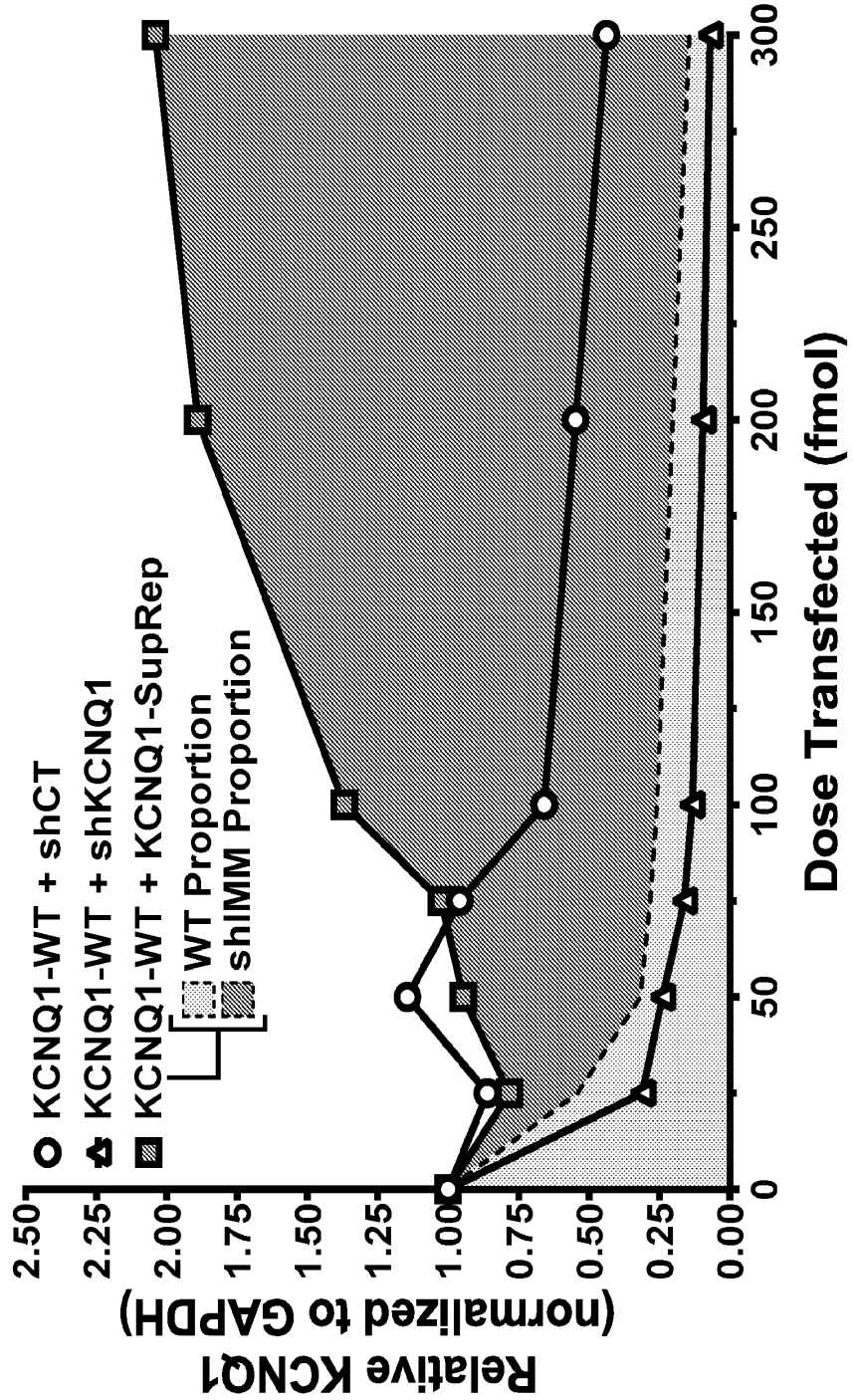

Relative KCNQ1 (normalized to GAPDH)
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
250
300
Dose Transfected (fmol)
KCNQ1-WT + shCT
KCNQ1-WT + shKCNQ1
KCNQ1-WT + KCNQ1-SupRep
WT Proportion
shIMM Proportion

FIG. 9

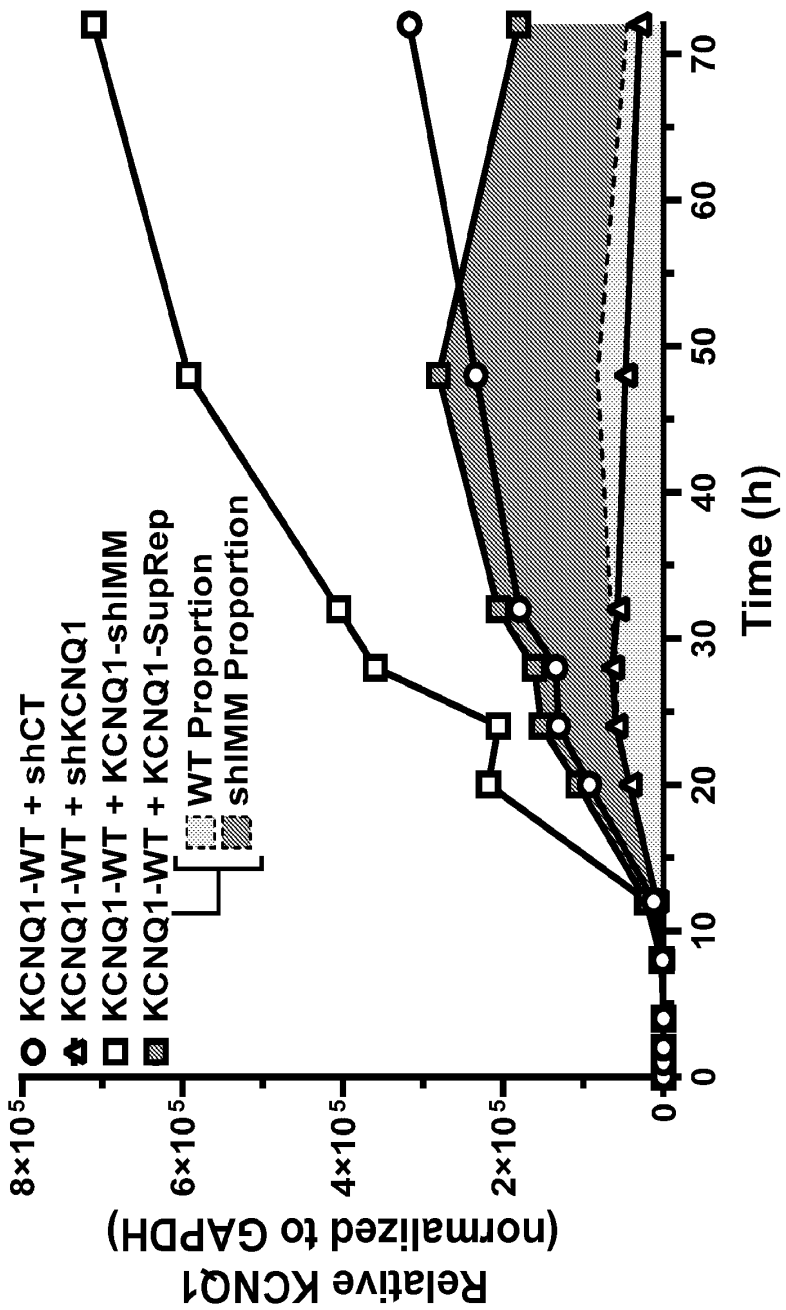
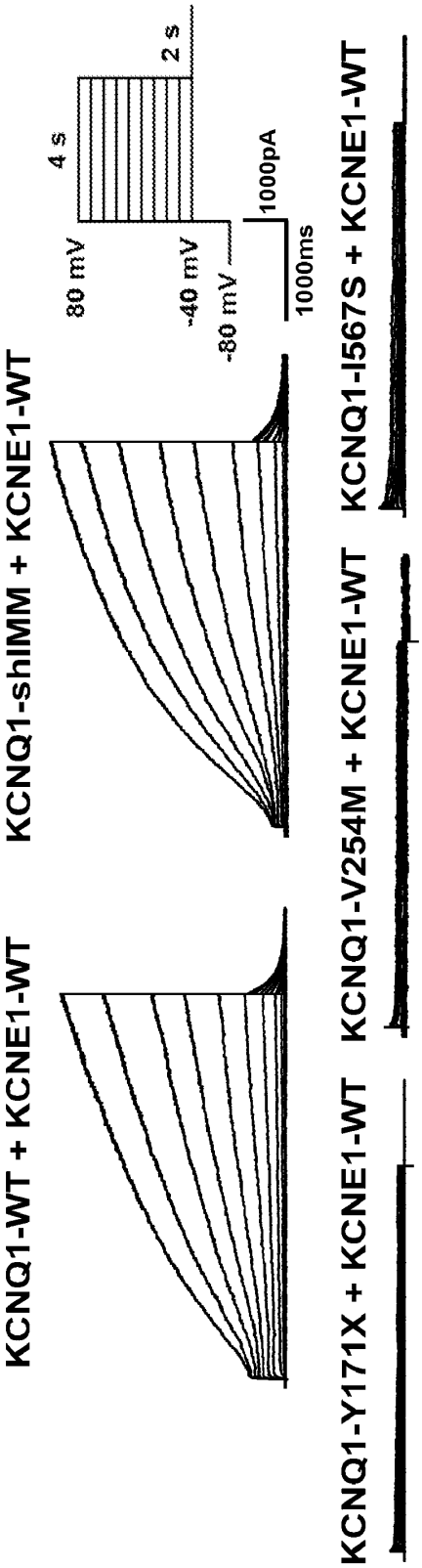

KCNQ1-WT + shCT
KCNQ1-WT + shKCNQ1
KCNQ1-WT + KCNQ1-shIMM
KCNQ1-WT + KCNQ1-SupRep
WT Proportion
shIMM Proportion
Relative KCNQ1 (normalized to GAPDH)
8×10^5
6×10^5
4×10^5
2×10^5
0
0
10
20
30
40
50
60
70
Time (h)

FIG. 10A


KCNQ1-WT + KCNE1-WT
KCNQ1-shIMM + KCNE1-WT
KCNQ1-Y171X + KCNE1-WT
KCNQ1-V254M + KCNE1-WT
KCNQ1-I567S + KCNE1-WT
80 mV
4 s
2 s
-40 mV
-80 mV
1000pA
1000ms


Peak Current Density (pA/pF)
1000
800
600
400
200
0
-40
-20
0
20
40
60
80
100
Voltage (mV)
WT + KCNE1 (n=14)
shIMM + KCNE1 (n=18)
Y171X + KCNE1 (n=19)
V254M + KCNE1 (n=12)
I567S + KCNE1 (n=11)

FIG. 10B

FIG. 10C

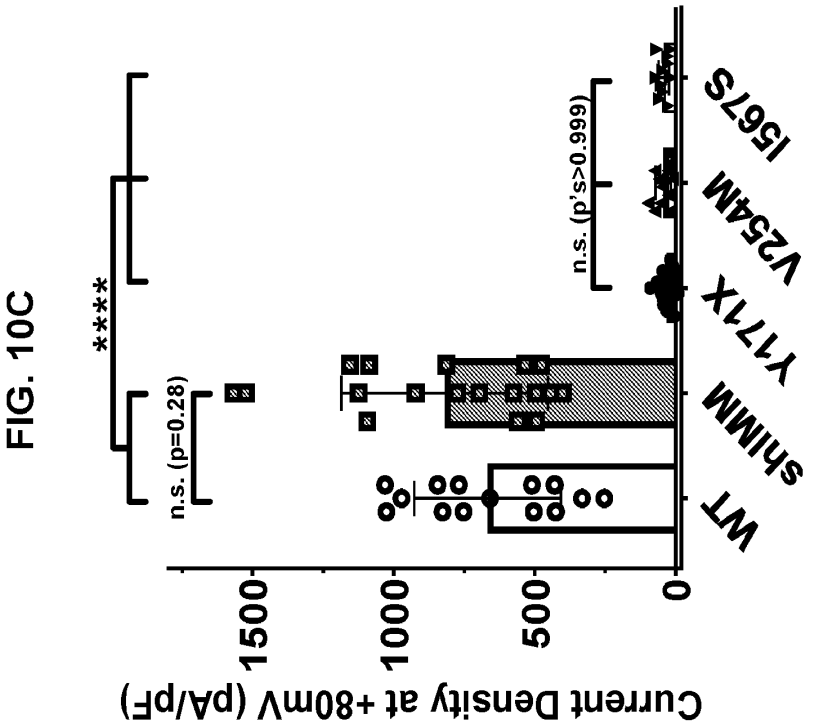

Current Density at +80mV (pA/pF)
1500
1000
500
0
WT
shIMM
Y174X
V254M
I567S
n.s. (p=0.28)
****
n.s. (p's>0.999)

FIG. 11

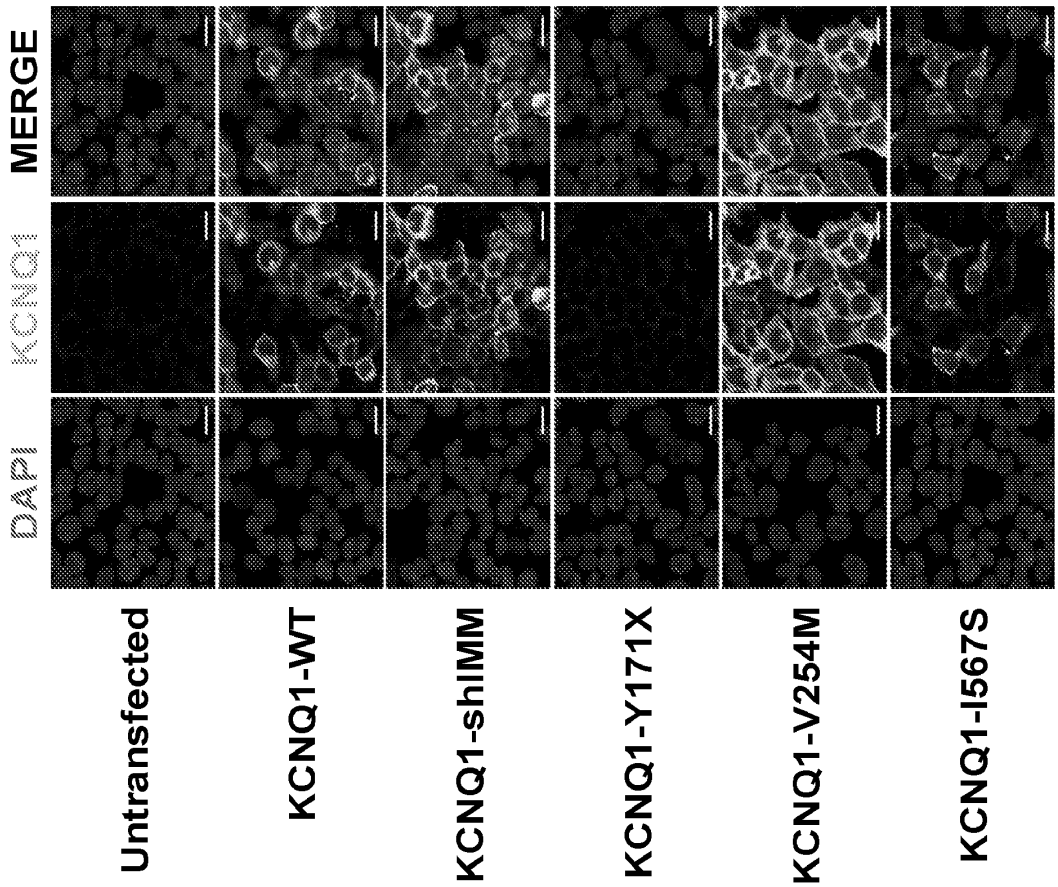

DAPI
KCNQ1
MERGE
Untransfected
KCNQ1-WT
KCNQ1-shIMM
KCNQ1-Y171X
KCNQ1-V254M
KCNQ1-I567S

FIG. 12

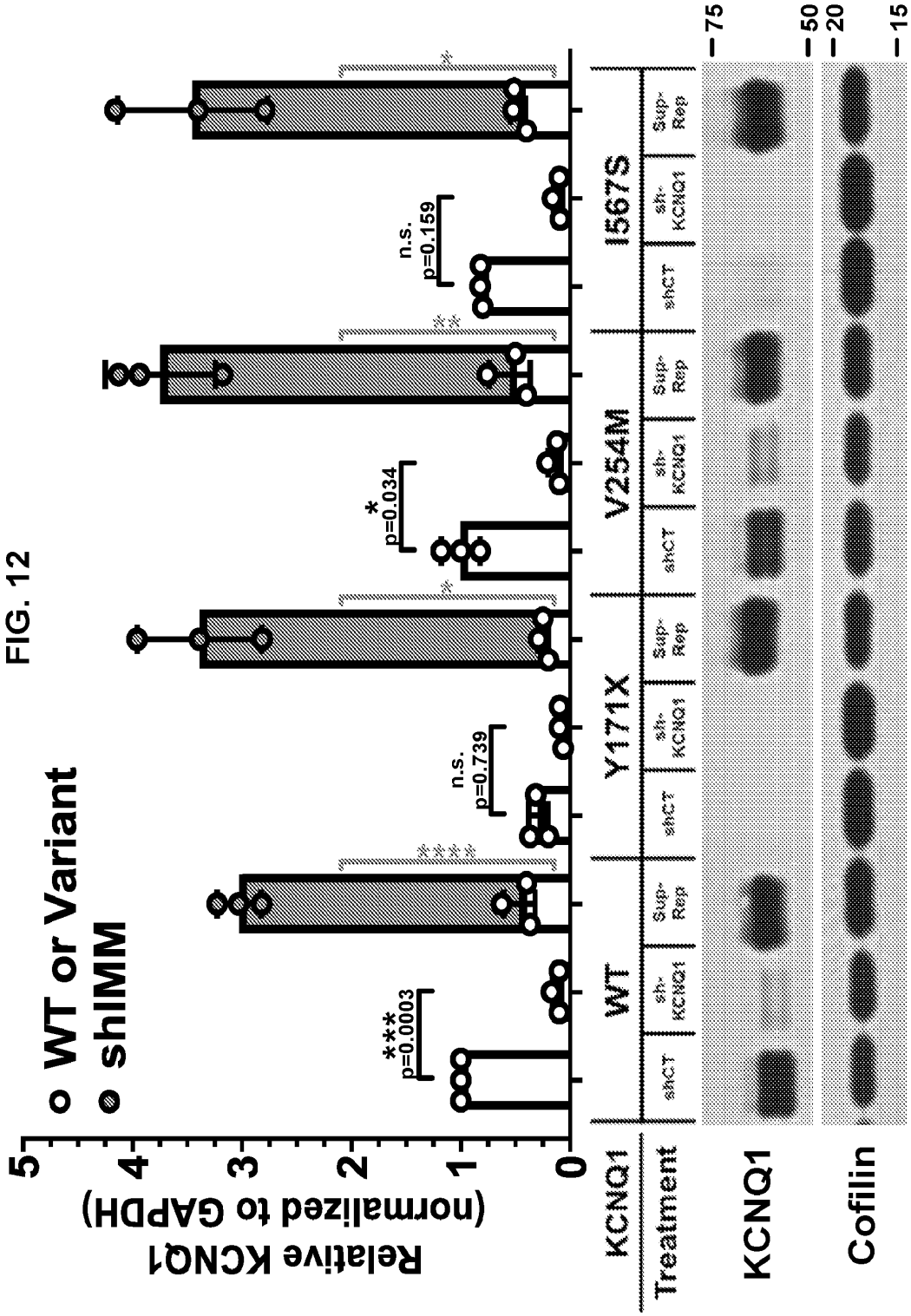

o WT or Variant
● shIMM
Relative KCNQ1 (normalized to GAPDH)
5
4
3
2
1
0
***
p=0.0003
****
n.s.
p=0.739
*
p=0.034
**
n.s.
p=0.159
*
KCNQ1
WT
Y171X
V254M
I567S
Treatment
shCT
sh-KCNQ1
Sup-Rep
KCNQ1
Cofilin
75
50
20
15

FIG. 13A

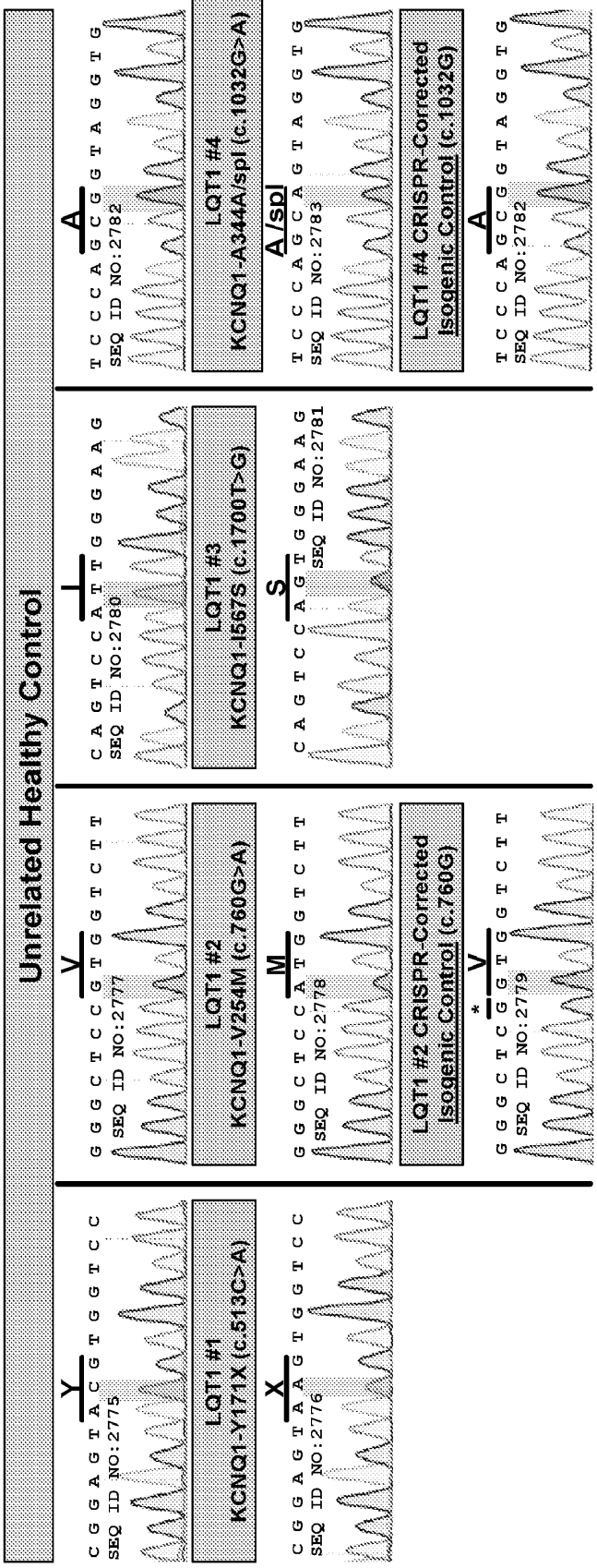
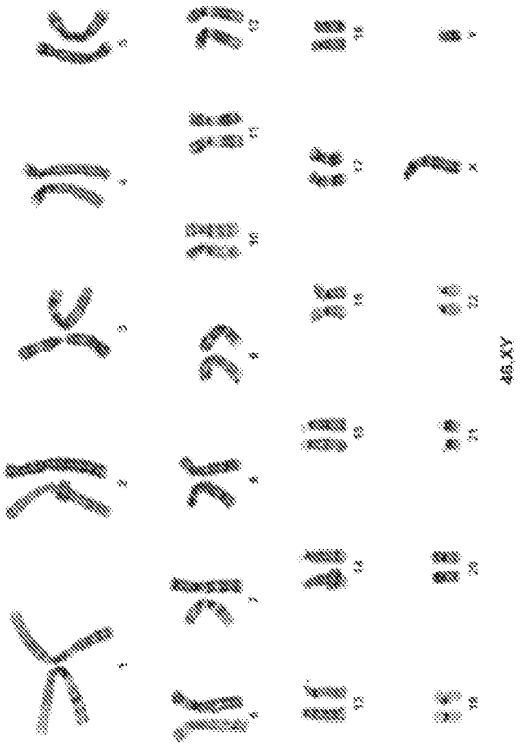

Unrelated Healthy Control
SEQ ID NO:2775
SEQ ID NO:2780
SEQ ID NO:2777
SEQ ID NO:2783
LQT1 #1
KCNQ1-Y171X (c.513C>A)
LQT1 #3
KCNQ1-I567S (c.1700T>G)
LQT1 #2
KCNQ1-V254M (c.760G>A)
LQT1 #4
KCNQ1-A344A/spl (c.1032G>A)
LQT1 #2 CRISPR-Corrected
Isogenic Control (c.760G)
LQT1 #4 CRISPR-Corrected
Isogenic Control (c.1032G)

FIG. 13C



FIG. 13B



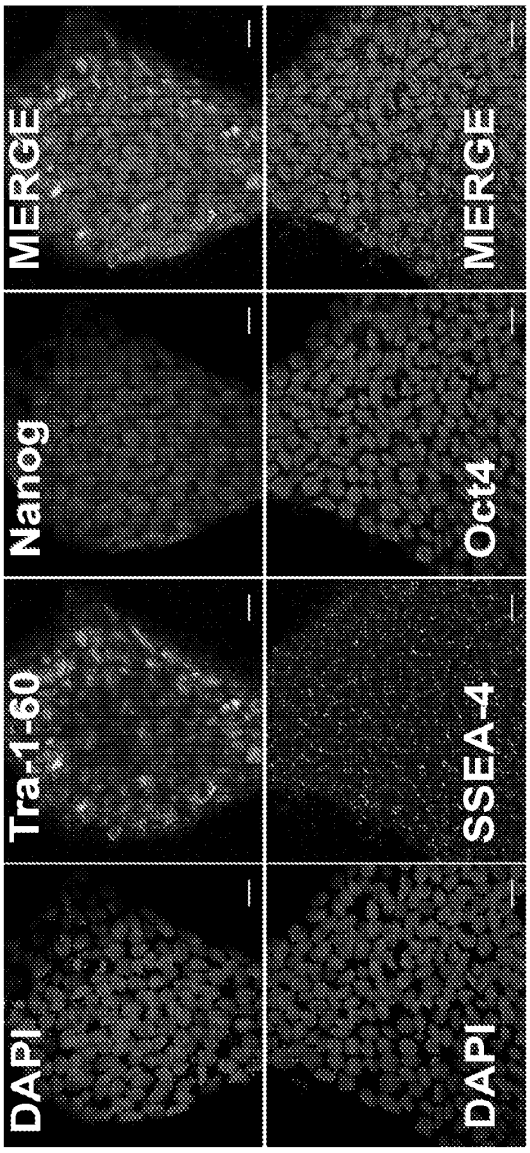


FIG. 13D

FIG. 14

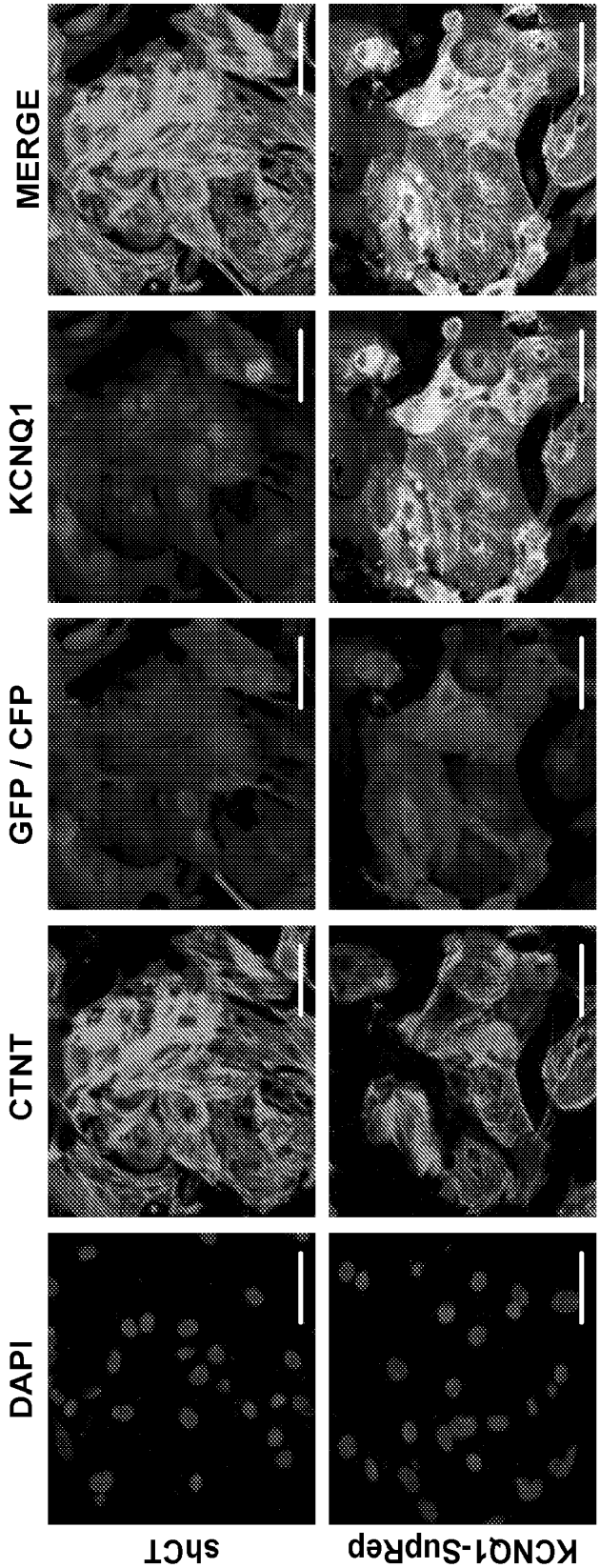




FIG. 15A

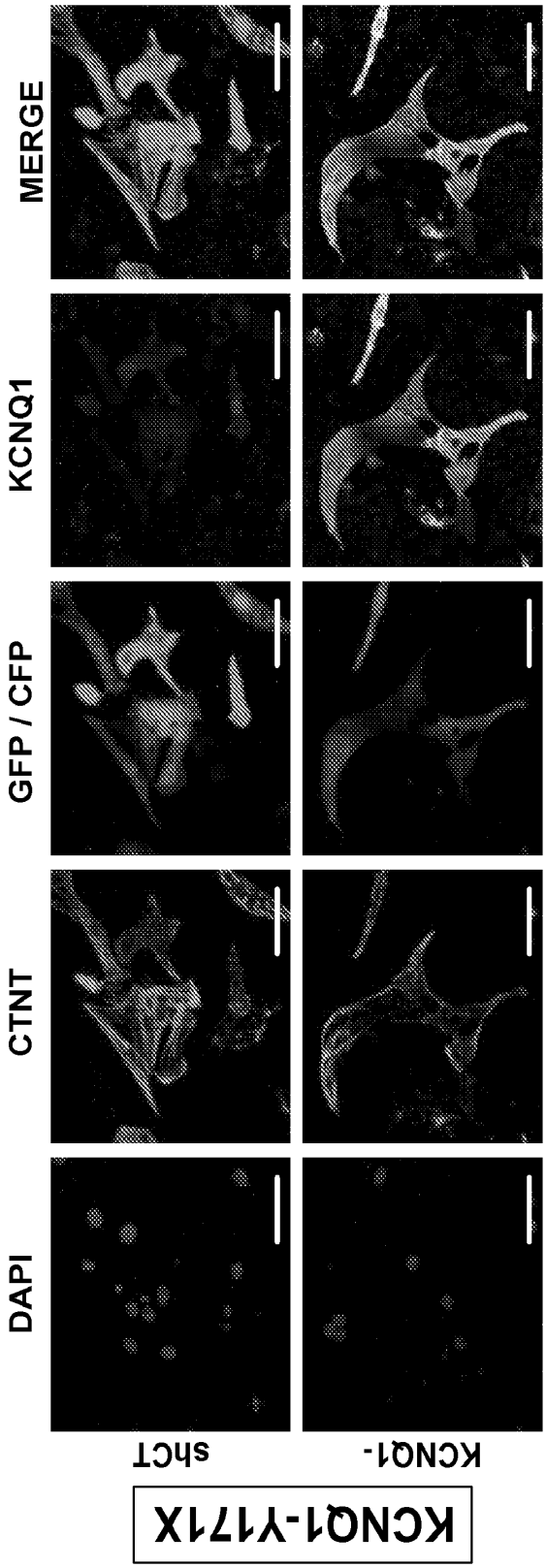


FIG. 15B

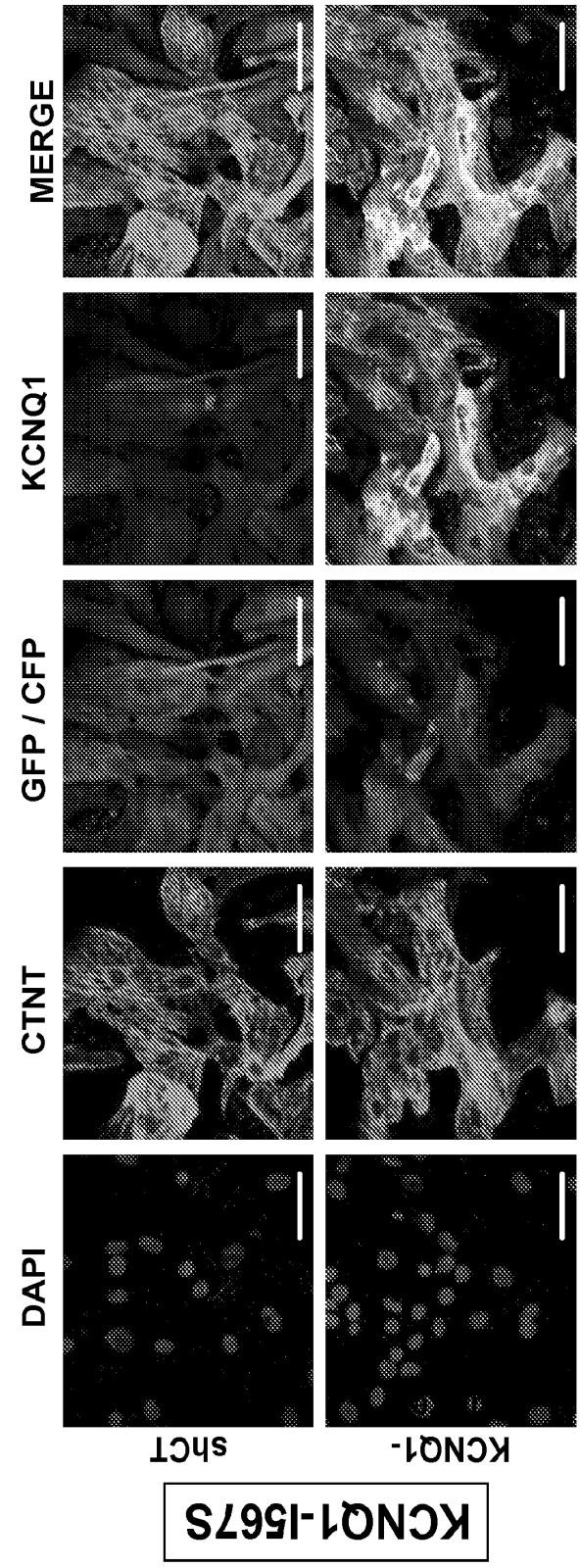


FIG. 15C

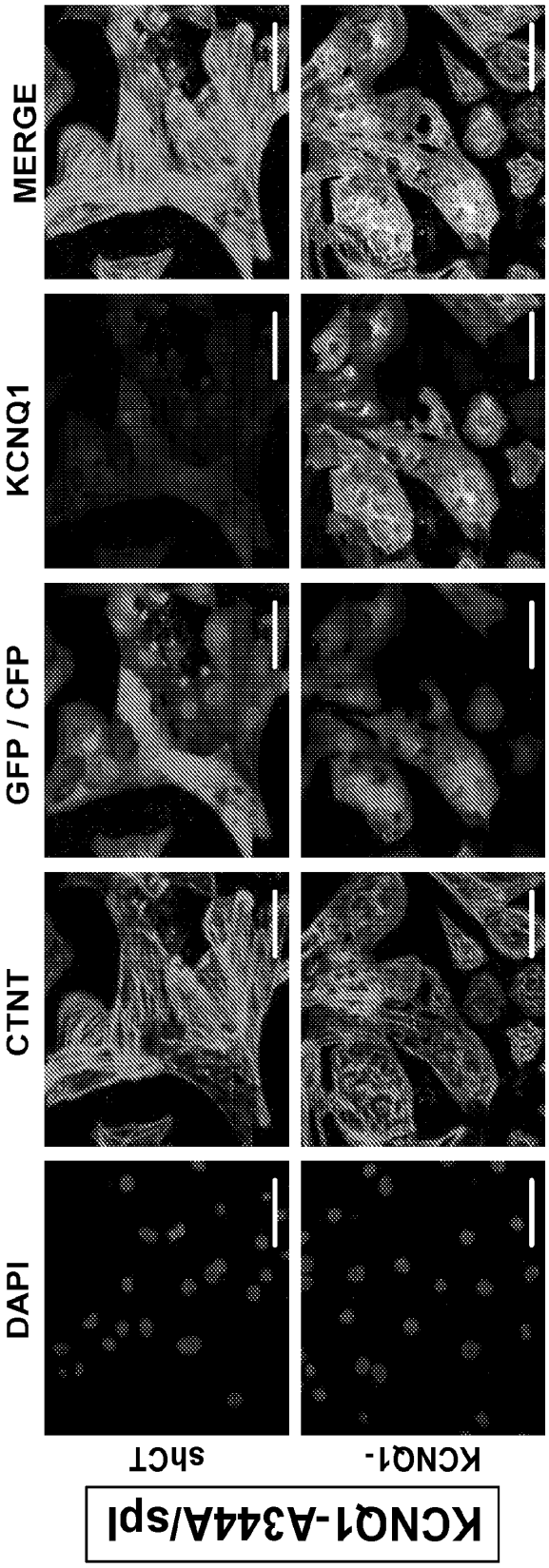


FIG. 15D

FIG. 16A

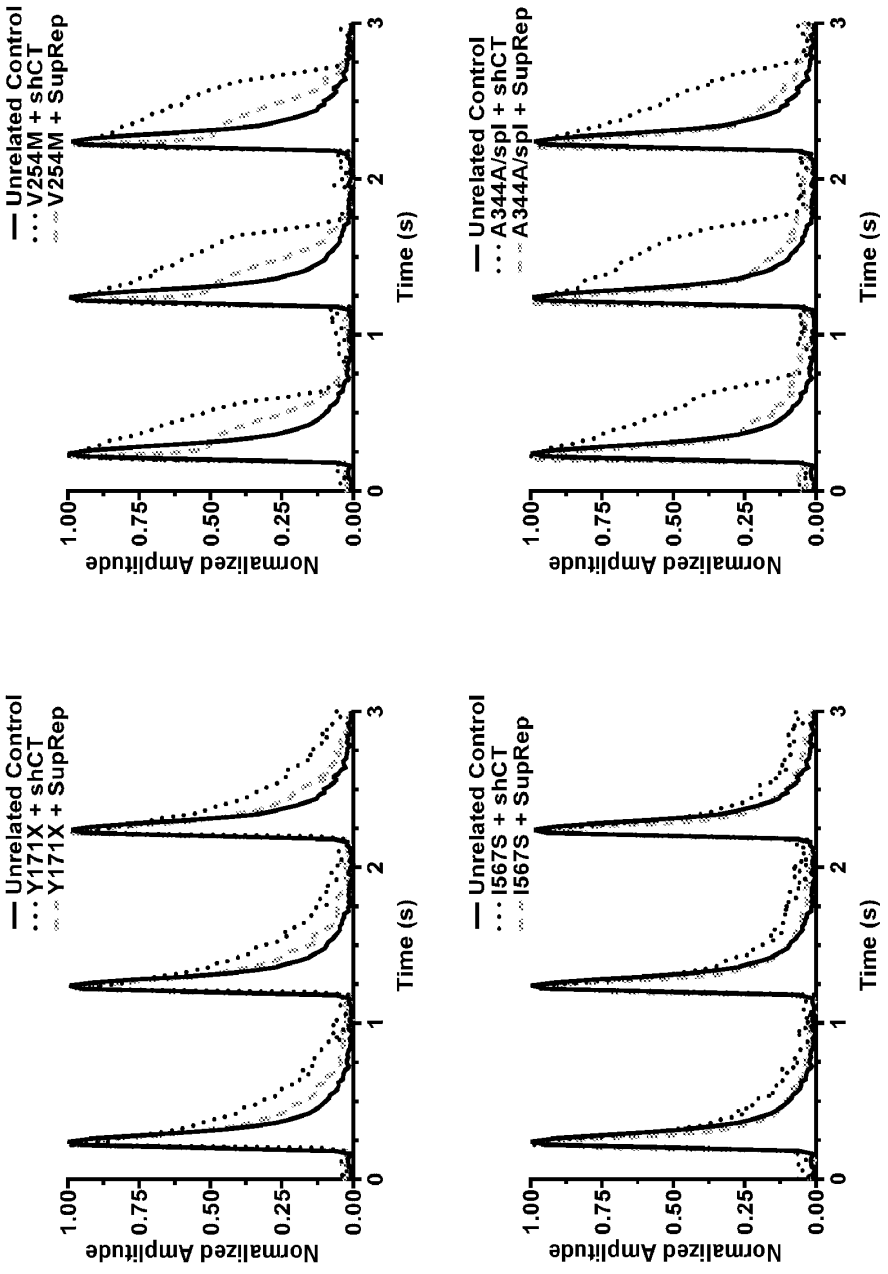

Unrelated Control
Y171X + shCT
Y171X + SupRep
V254M + shCT
V254M + SupRep
I567S + shCT
I567S + SupRep
A344A/spl + shCT
A344A/spl + SupRep
Normalized Amplitude
Time (s)
0.00
0.25
0.50
0.75
1.00
0
1
2
3

FIG. 16B

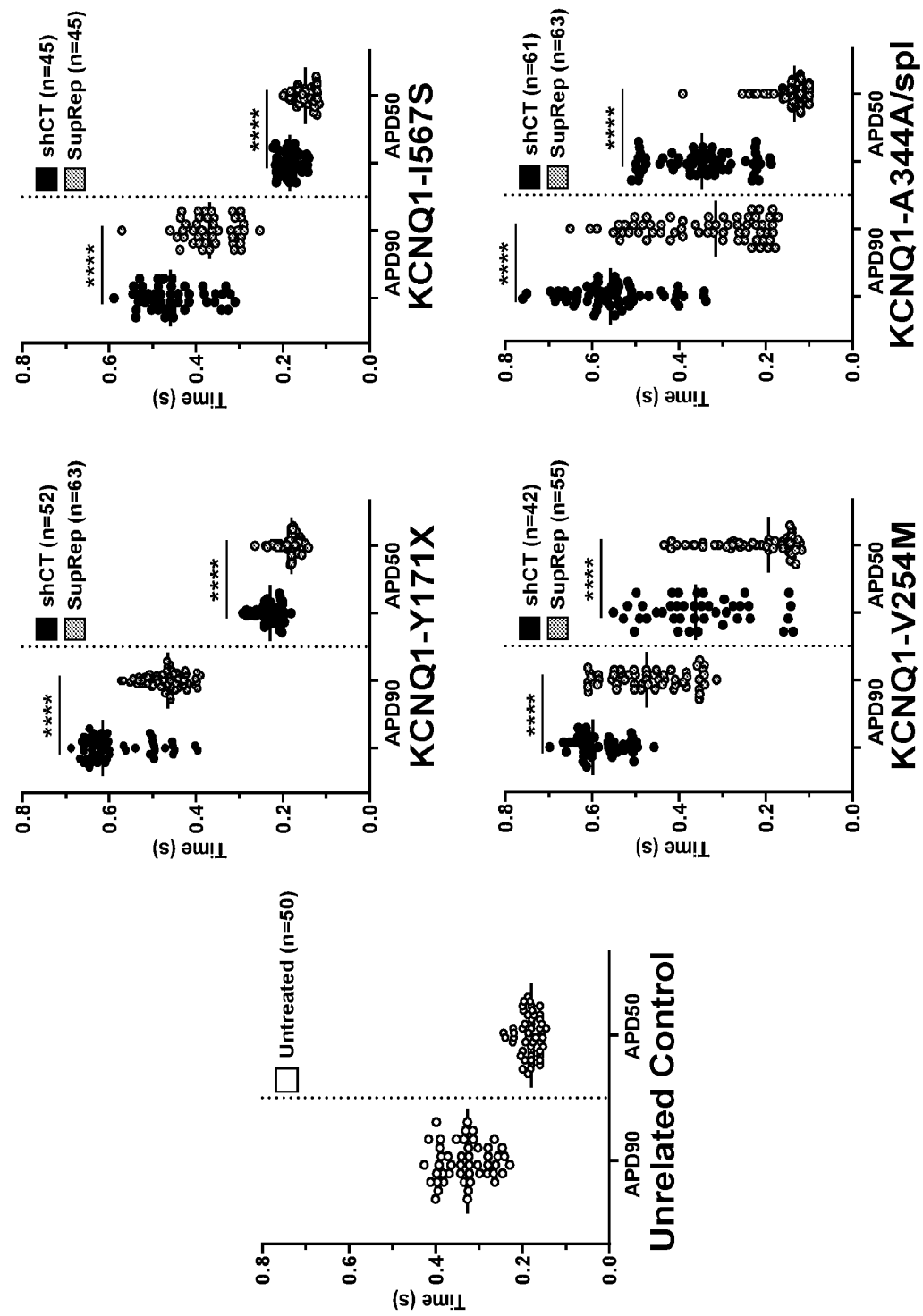
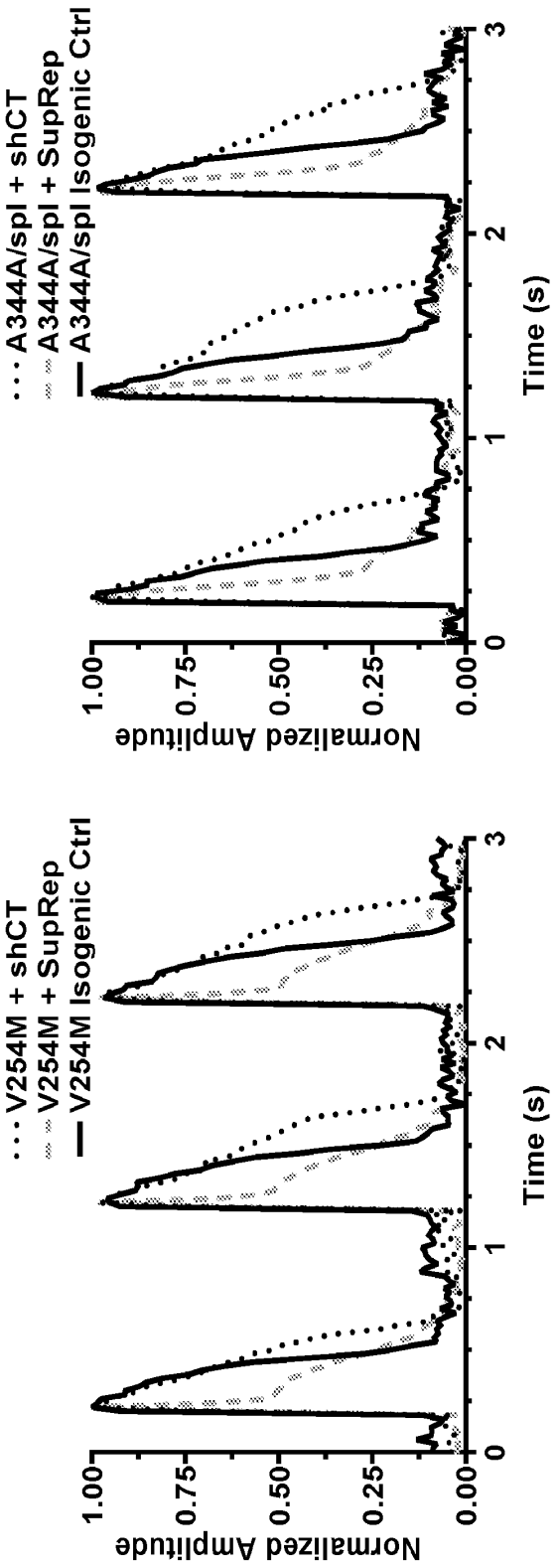

shCT (n=45)
SupRep (n=45)
KCNQ1-I567S
shCT (n=52)
SupRep (n=63)
KCNQ1-Y171X
shCT (n=61)
SupRep (n=63)
KCNQ1-A344A/spl
shCT (n=42)
SupRep (n=55)
KCNQ1-V254M
Untreated (n=50)
Unrelated Control
APD90
APD50
Time (s)
****

FIG. 17A


A344A/spl + shCT
A344A/spl + SupRep
A344A/spl Isogenic Ctrl
Normalized Amplitude
1.00
0.75
0.50
0.25
0.00
0
1
2
3
Time (s)
V254M + shCT
V254M + SupRep
V254M Isogenic Ctrl
Normalized Amplitude
1.00
0.75
0.50
0.25
0.00
0
1
2
3
Time (s)

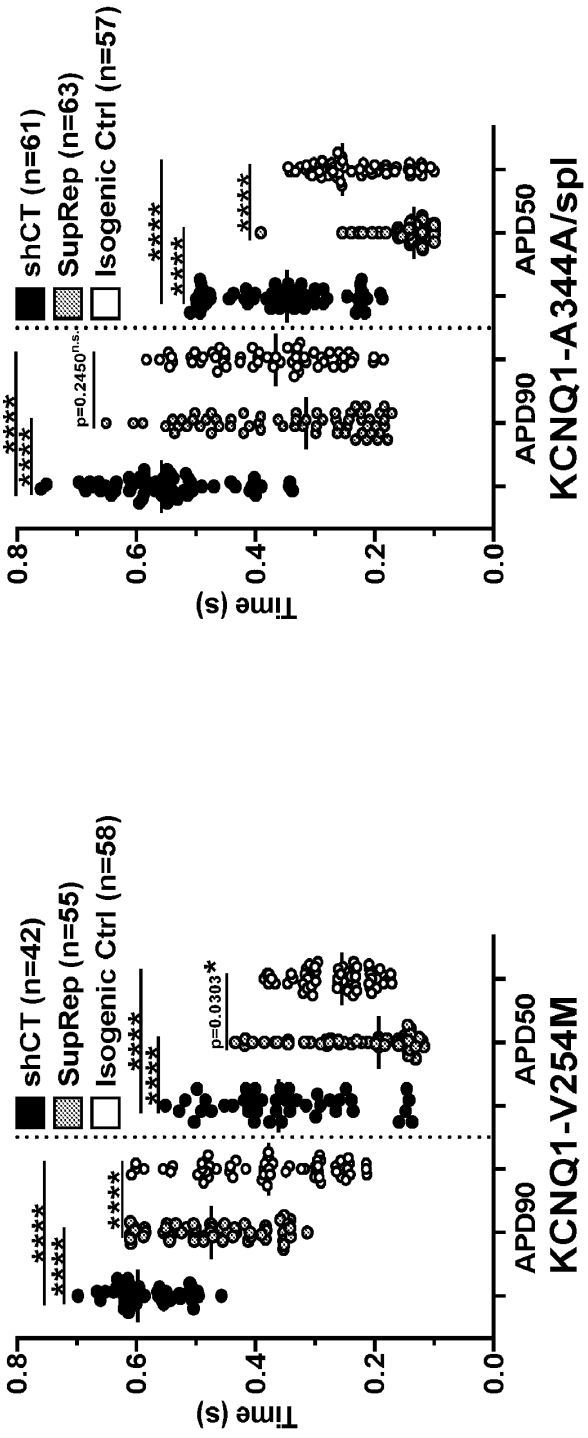

shCT (n=61)
SupRep (n=63)
Isogenic Ctrl (n=57)
p=0.2450n.s.
****
****
****
****
Time (s)
0.8
0.6
0.4
0.2
0.0
APD90
APD50
KCNQ1-A344A/spl
shCT (n=42)
SupRep (n=55)
Isogenic Ctrl (n=58)
p=0.0303*
****
****
****
****
Time (s)
0.8
0.6
0.4
0.2
0.0
APD90
APD50
KCNQ1-V254M

FIG. 17B

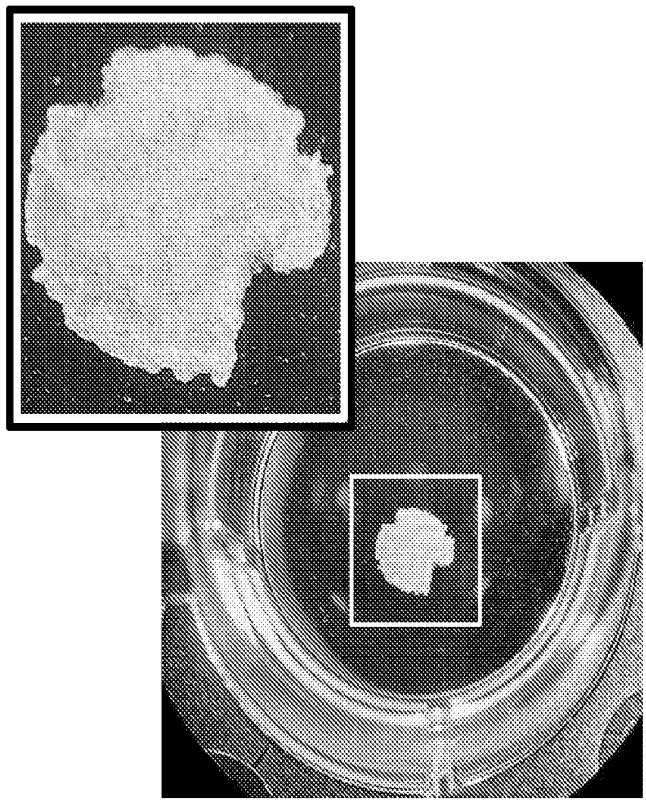


FIG. 18A

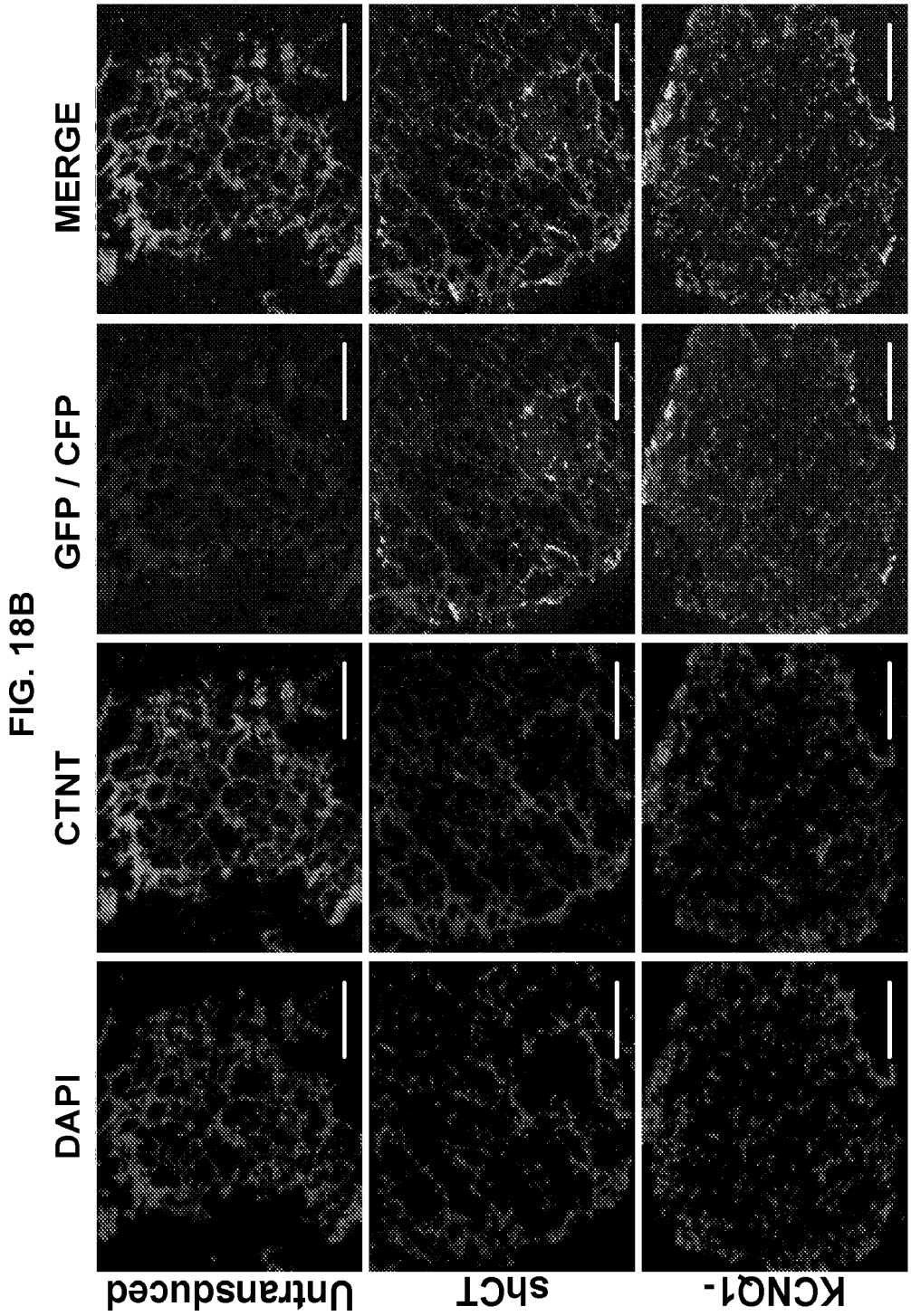


FIG. 18B

FIG. 18D

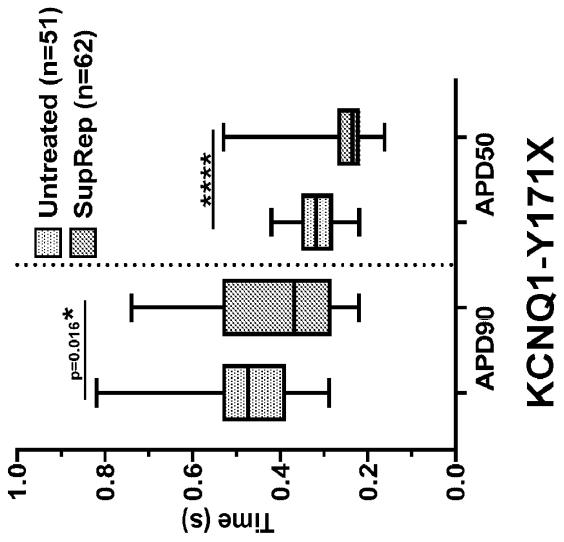


FIG. 18C

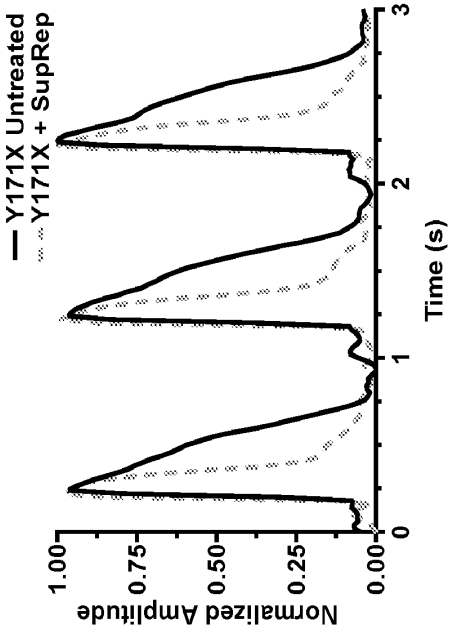


FIG. 19A

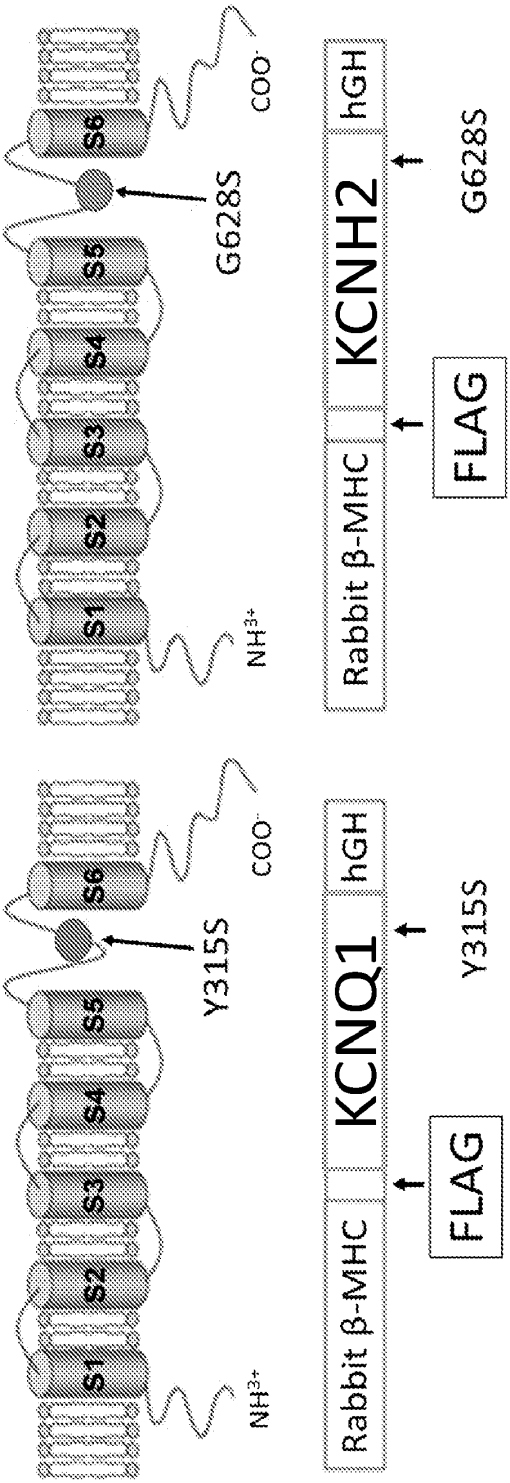

S1
S2
S3
S4
S5
S6
NH3+
COO-
Y315S
G628S
Rabbit β-MHC
FLAG
KCNQ1
KCNH2
hGH

FIG. 19B

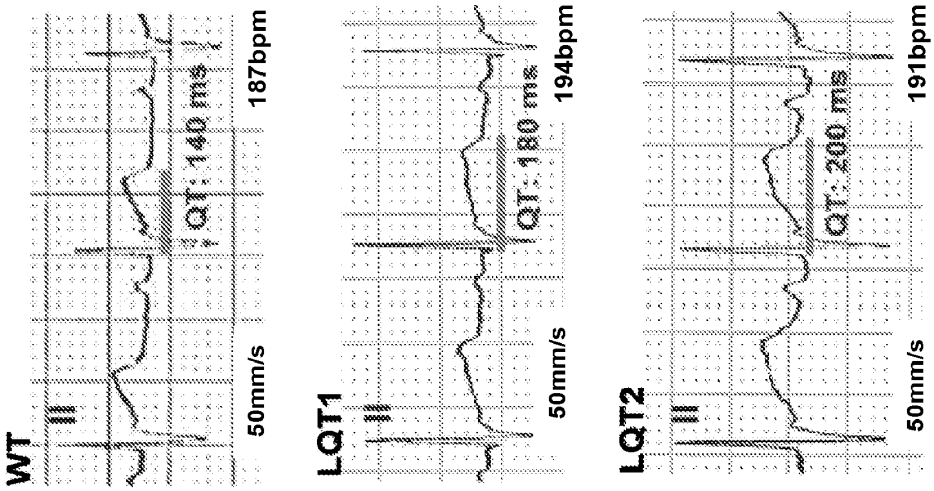
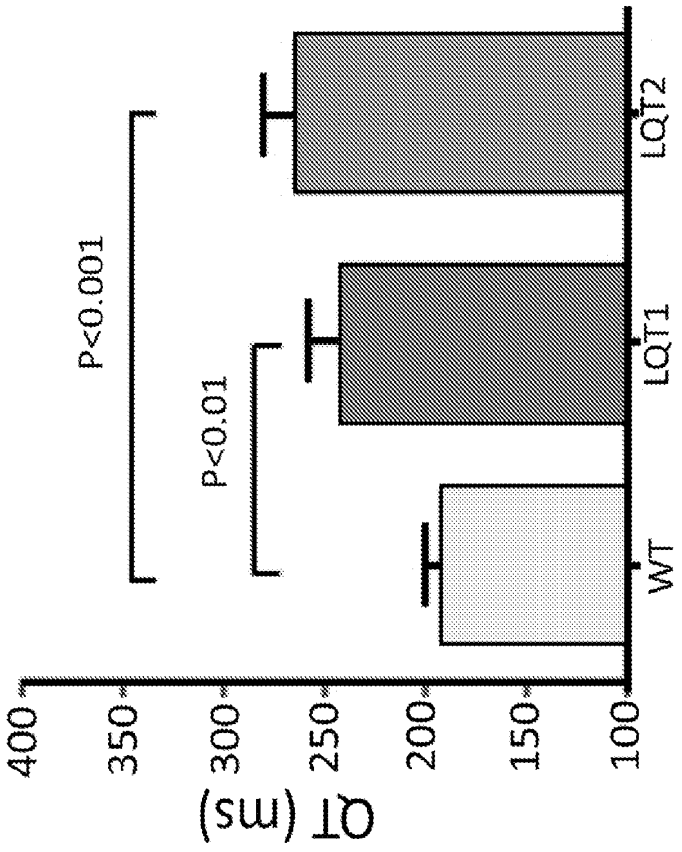

WT
II
QT: 140 ms
187bpm
50mm/s
LQT1
II
QT: 180 ms
194bpm
50mm/s
LQT2
II
QT: 200 ms
191bpm
50mm/s

FIG. 19C



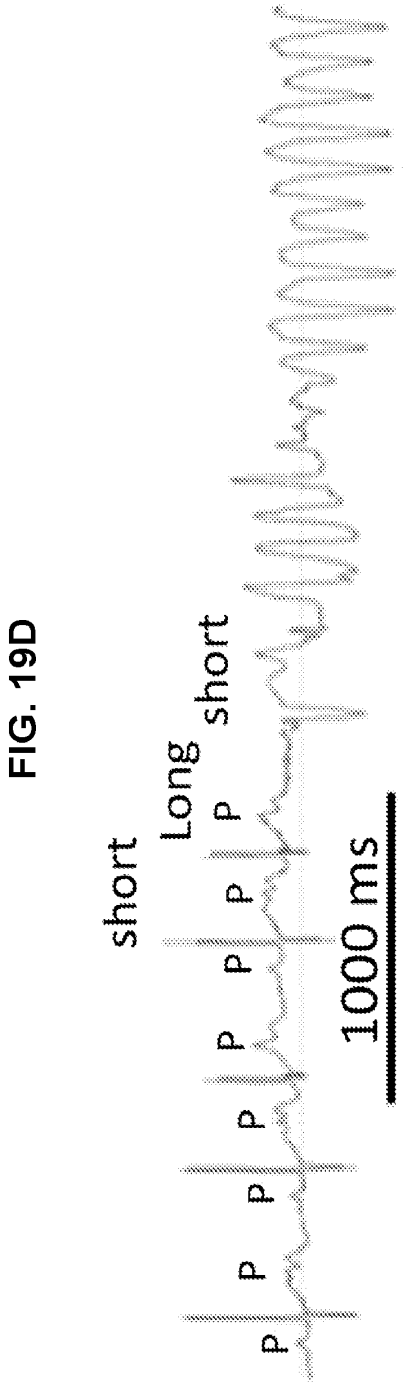


FIG. 19D

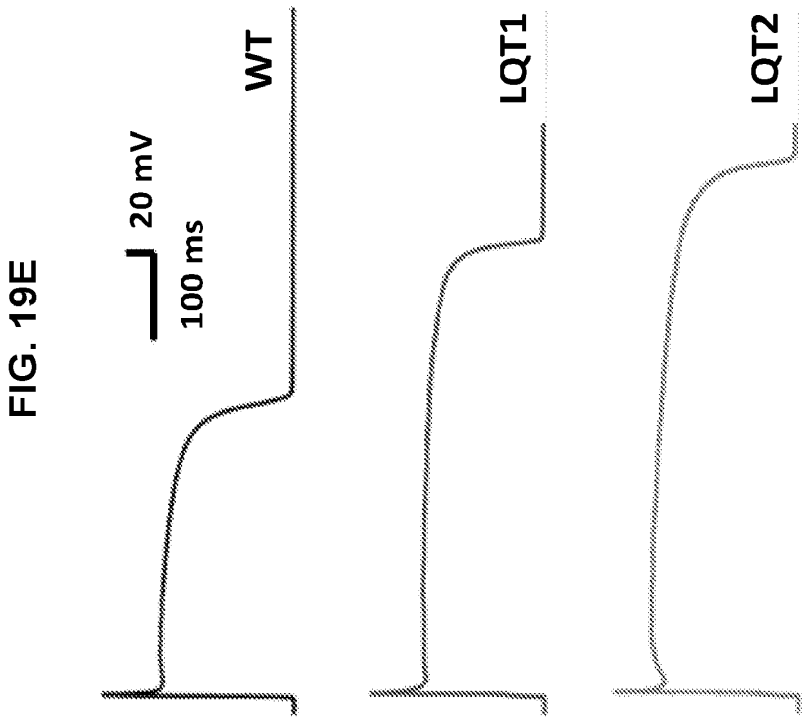
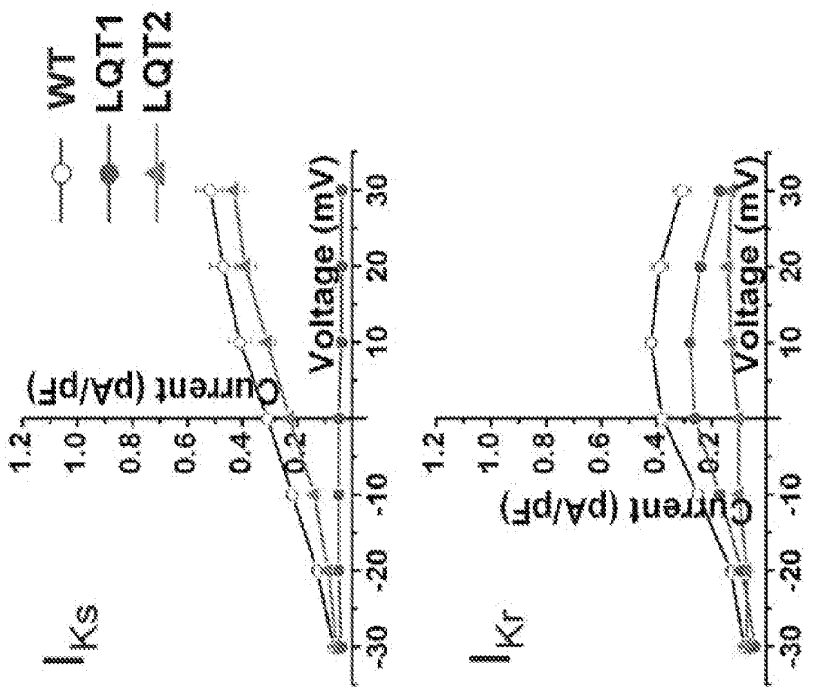


FIG. 19E

FIG. 19F


WT
LQT1
LQT2
Current (pA/pF)
Voltage (mV)
1.2
1.0
0.8
0.6
0.4
0.2
30
20
10
-10
-20
-30
I_{Ks}
I_{Kr}

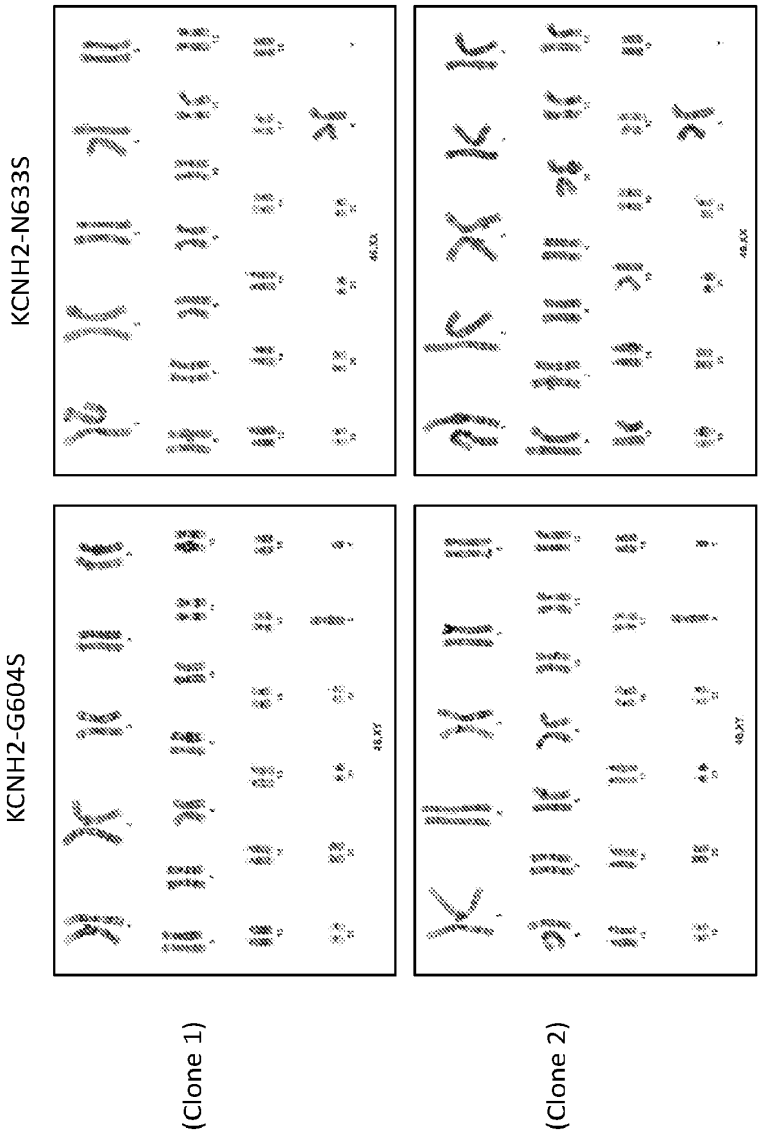


FIG. 20A

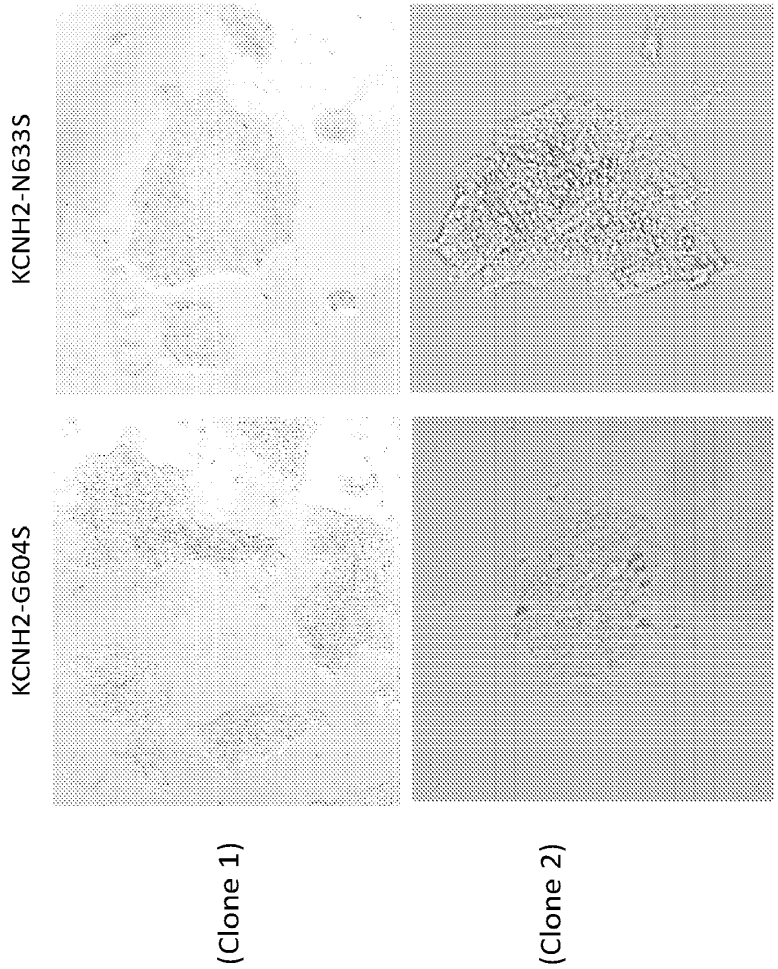


FIG. 20B

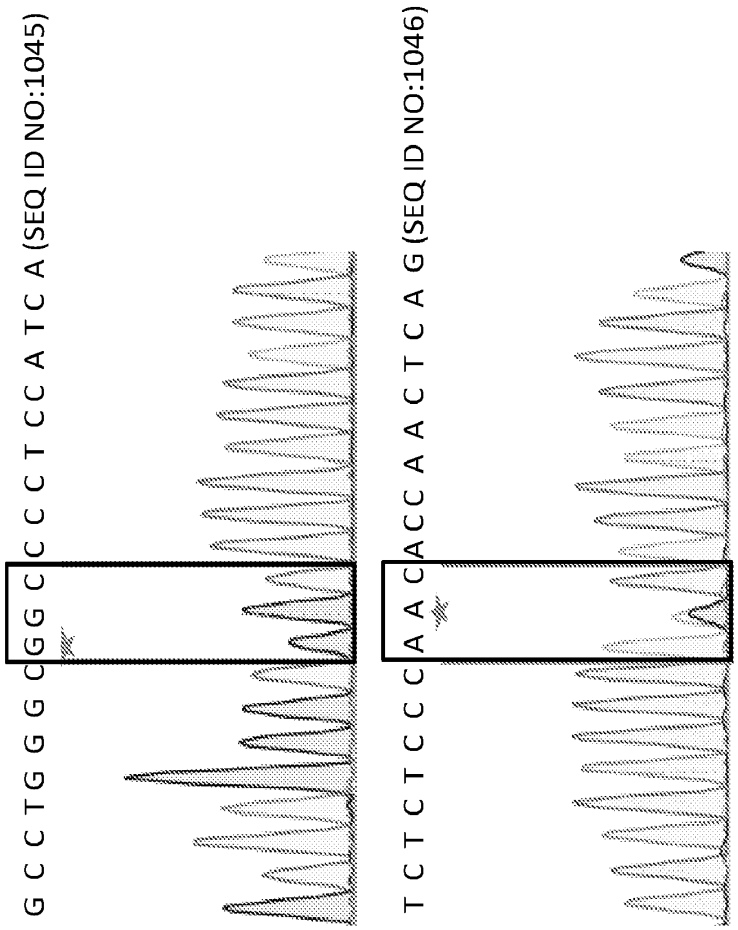

G C C T G G G C G G C C C C T C C A T C A (SEQ ID NO:1045)
T C T C T C C C A A C C A A C T C A G (SEQ ID NO:1046)
p.G604S
p.N633S

FIG. 20C

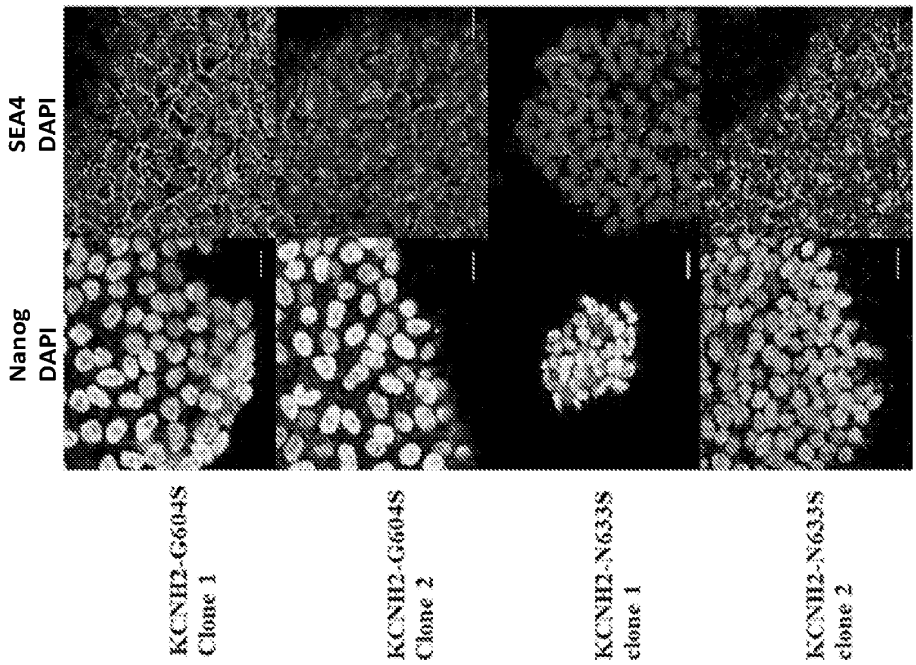

SEA4
DAPI
Nanog
DAPI
KCNH2-G604S
Clone 1
KCNH2-G604S
Clone 2
KCNH2-N633S
clone 1
KCNH2-N633S
clone 2

FIG. 21

FIG. 22

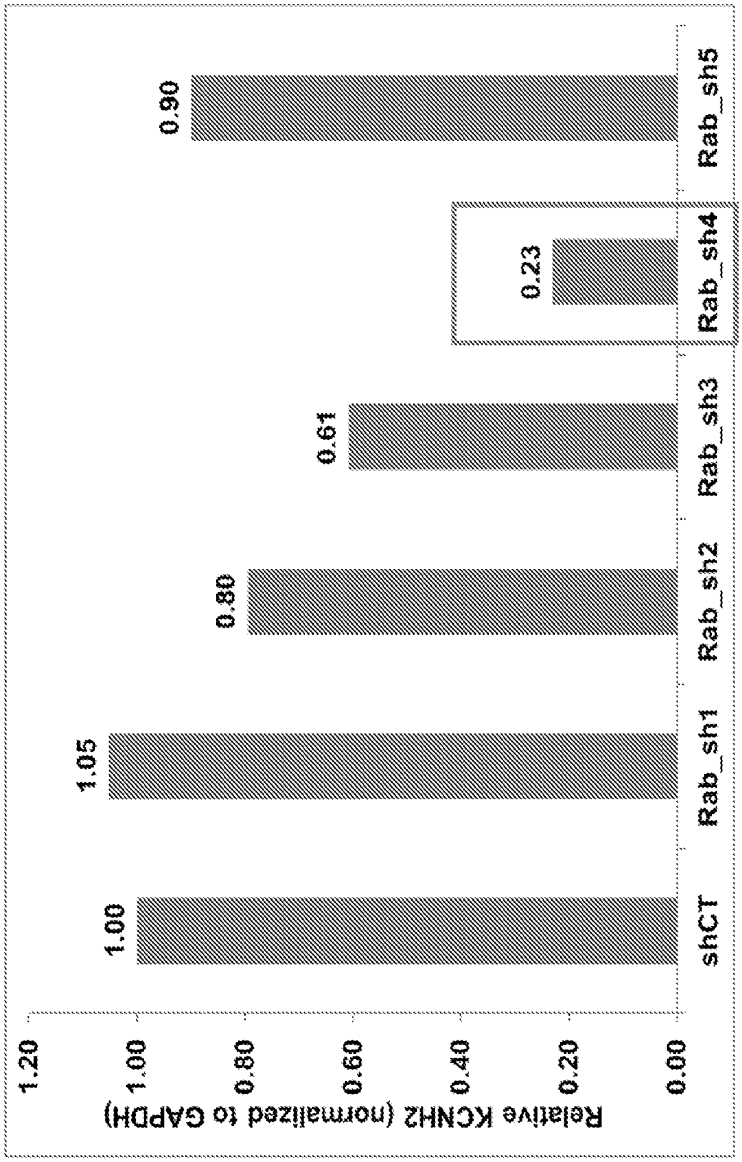

Relative KCNH2 (normalized to GAPDH)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.00
1.05
0.80
0.61
0.23
0.90
shCT
Rab_sh1
Rab_sh2
Rab_sh3
Rab_sh4
Rab_sh5

FIG. 23

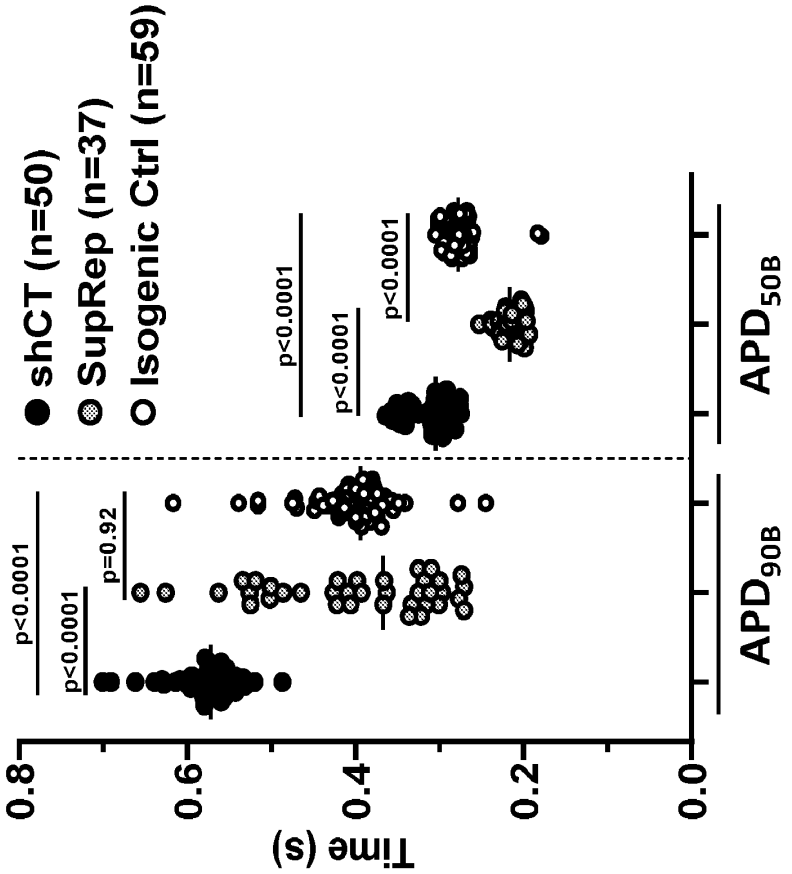

shCT (n=50)
SupRep (n=37)
Isogenic Ctrl (n=59)
p<0.0001
p<0.0001
p=0.92
p<0.0001
p<0.0001
p<0.0001
Time (s)
0.8
0.6
0.4
0.2
0.0
APD90B
APD50B

FIG. 24

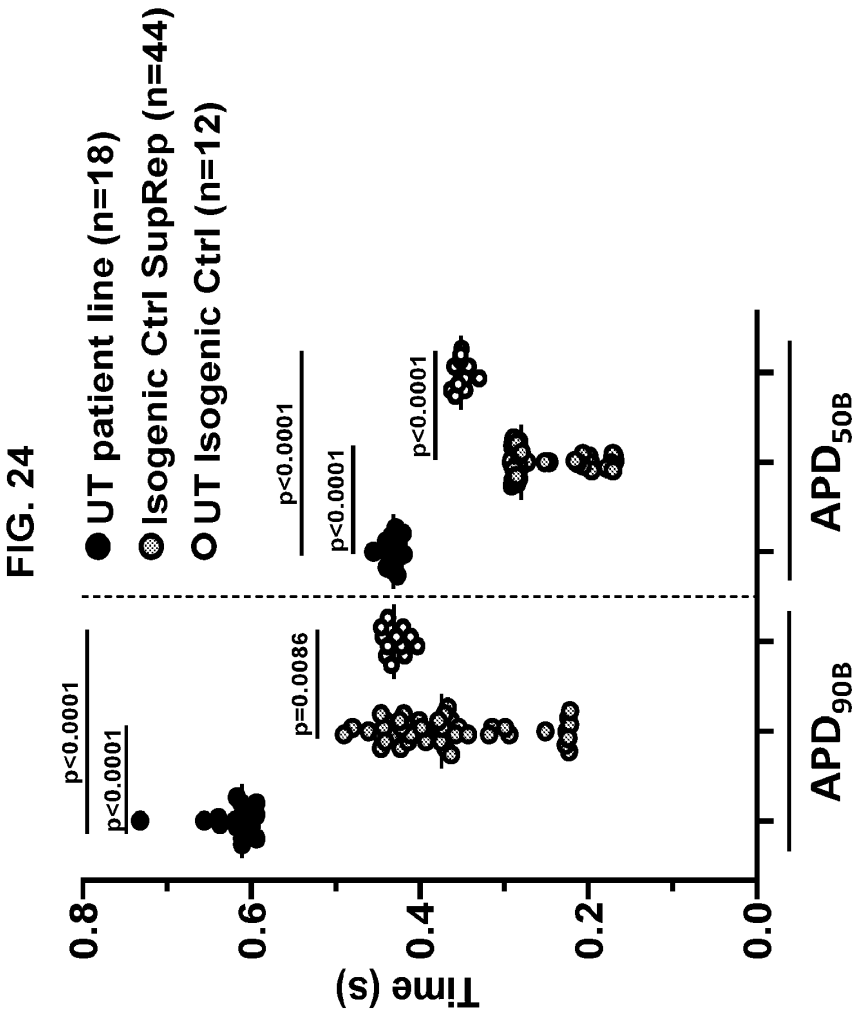
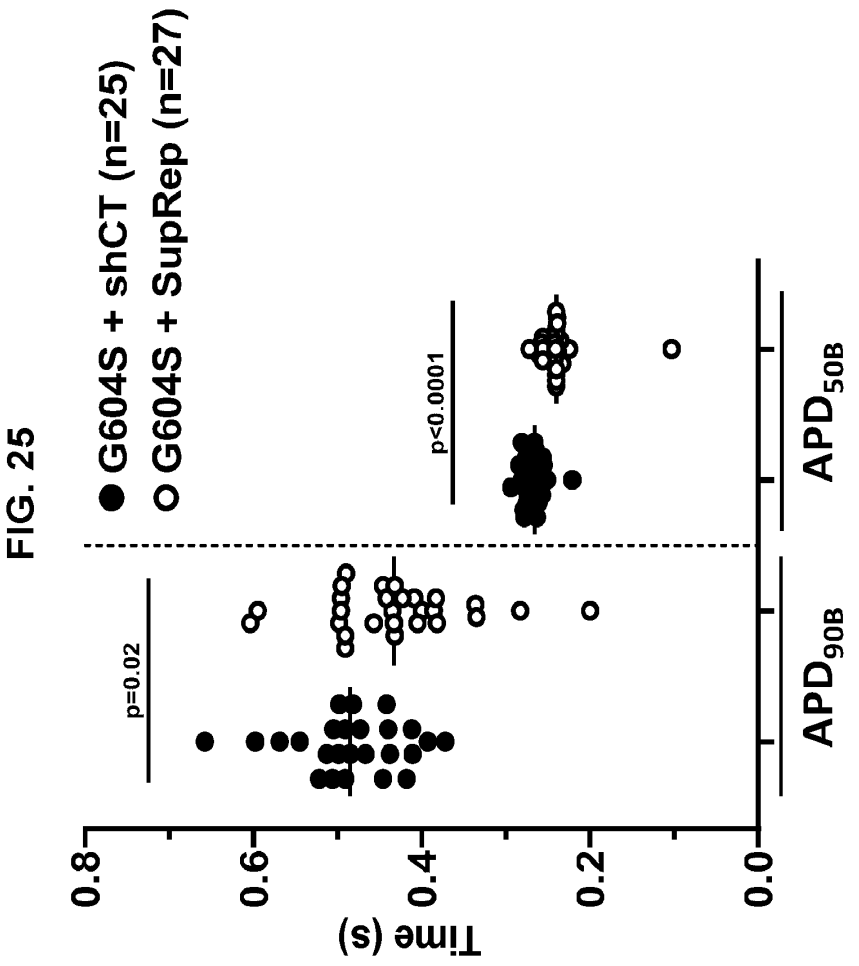

UT patient line (n=18)
Isogenic Ctrl SupRep (n=44)
UT Isogenic Ctrl (n=12)
p<0.0001
p<0.0001
p=0.0086
p<0.0001
p<0.0001
p<0.0001
Time (s)
0.8
0.6
0.4
0.2
0.0
APD90B
APD50B

FIG. 25


● G604S + shCT (n=25)
○ G604S + SupRep (n=27)
p=0.02
p<0.0001
APD90B
APD50B
Time (s)
0.8
0.6
0.4
0.2
0.0

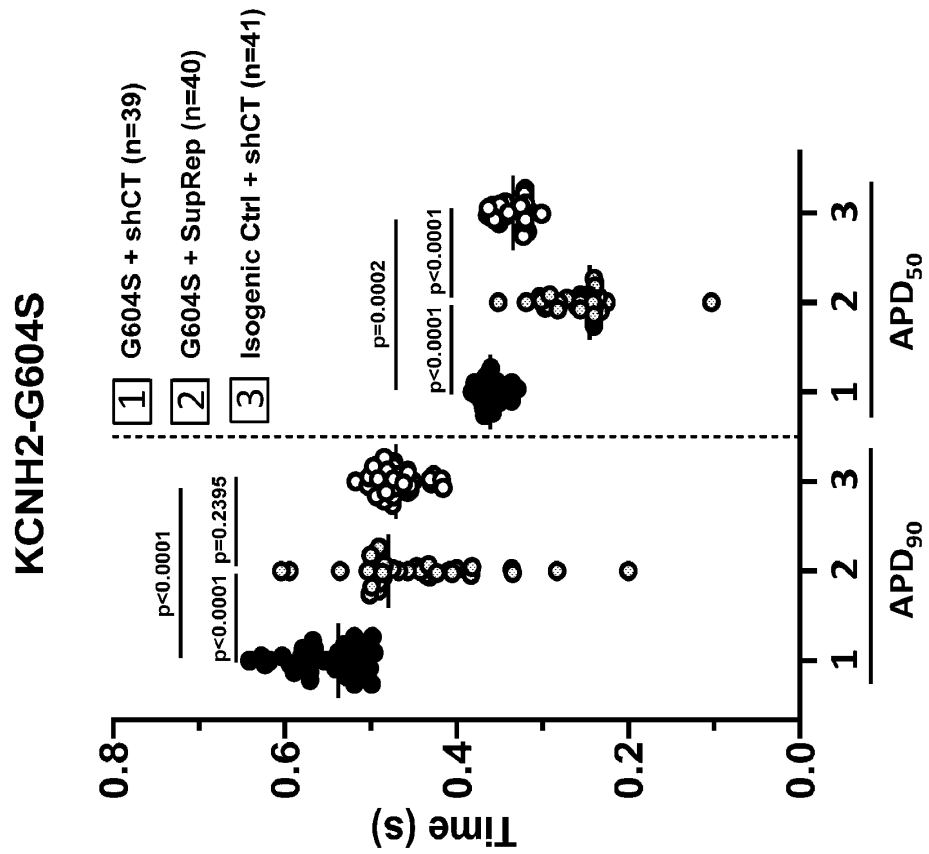

KCNH2-G604S
1 G604S + shCT (n=39)
2 G604S + SupRep (n=40)
3 Isogenic Ctrl + shCT (n=41)
p<0.0001
p<0.0001
p=0.2395
p=0.0002
p<0.0001
p<0.0001
Time (s)
0.8
0.6
0.4
0.2
0.0
1
2
3
APD90
1
2
3
APD50

FIG. 26

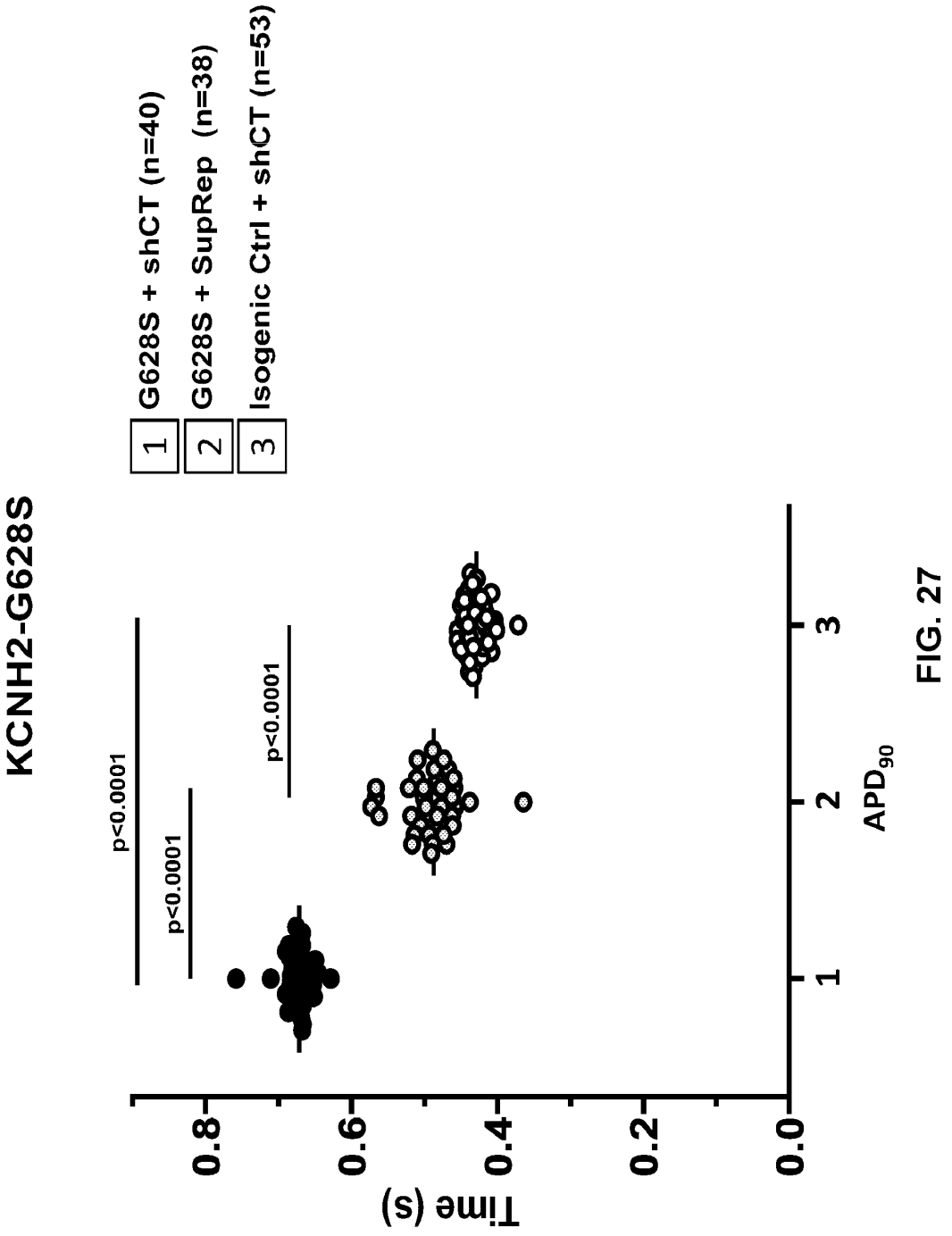

KCNH2-G628S
1 G628S + shCT (n=40)
2 G628S + SupRep (n=38)
3 Isogenic Ctrl + shCT (n=53)
p<0.0001
p<0.0001
p<0.0001
Time (s)
0.0
0.2
0.4
0.6
0.8
1
2
3
APD90

FIG. 27

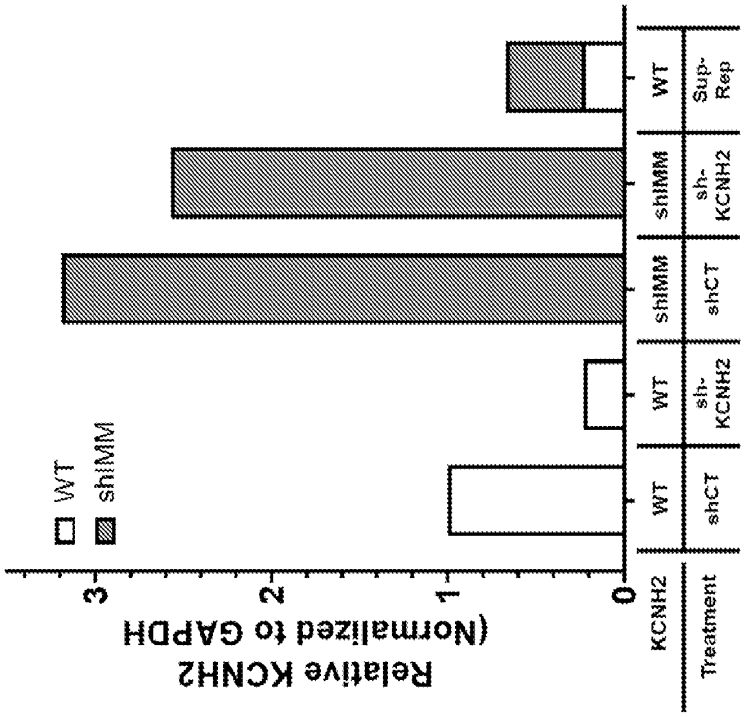


FIG. 28A

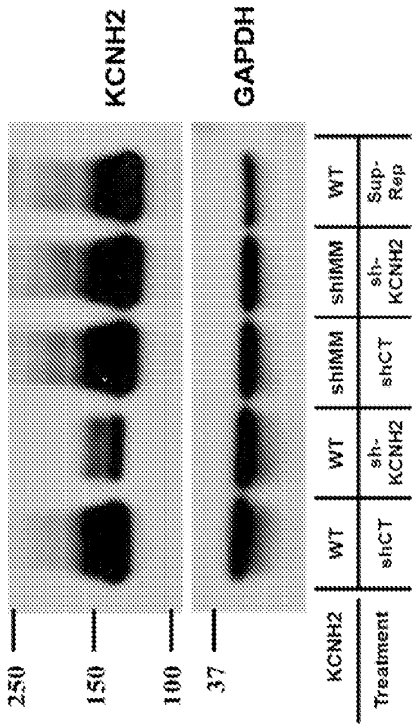


FIG. 28B

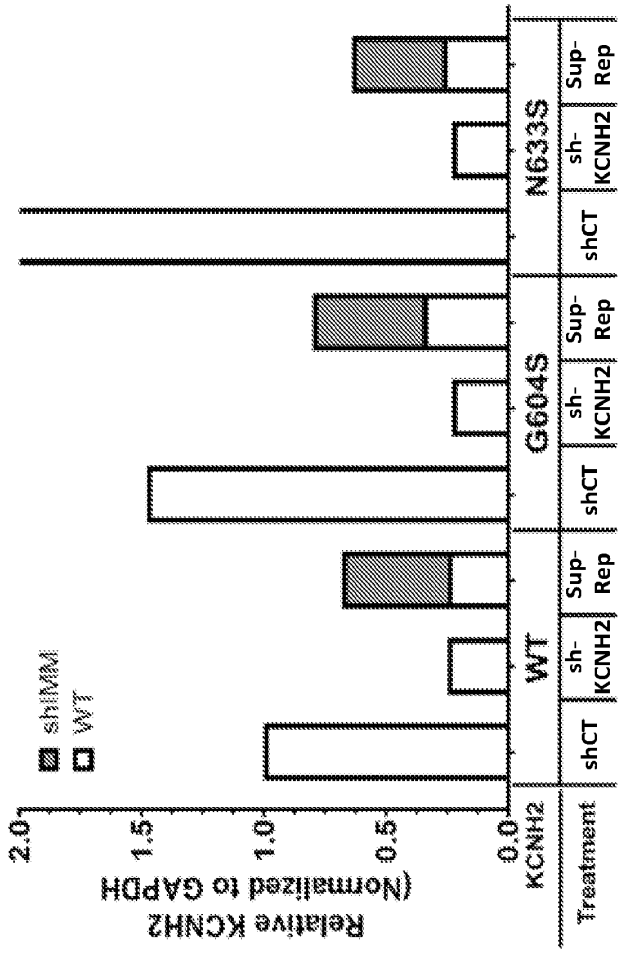

Relative KCNH2
(Normalized to GAPDH
2.0
1.5
1.0
0.5
0.0
shMM
WT
KCNH2
Treatment
shCT
sh-KCNH2
Sup-Rep
WT
G604S
N633S

FIG. 29A

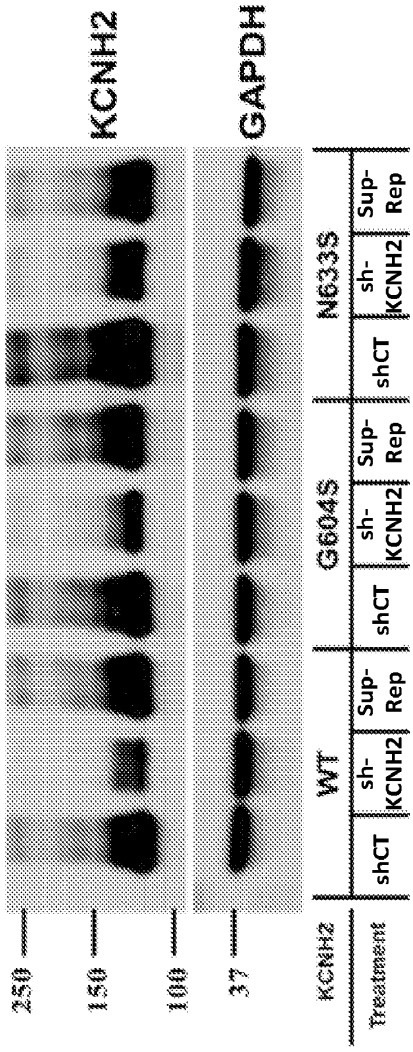

KCNH2
GAPDH
250
150
100
37
KCNH2
Treatment
WT
G604S
N633S
shCT
sh-KCNH2
Sup-Rep

FIG. 29B

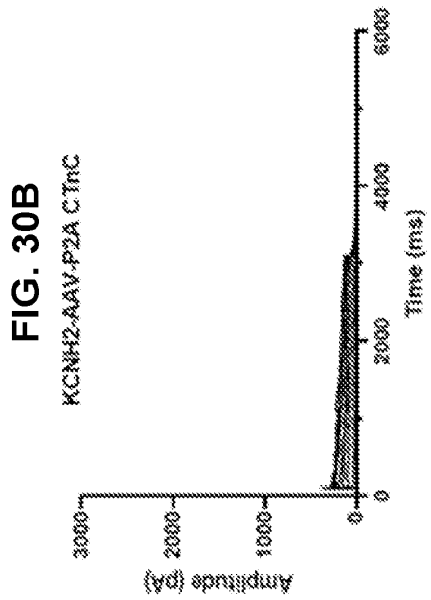


FIG. 30B

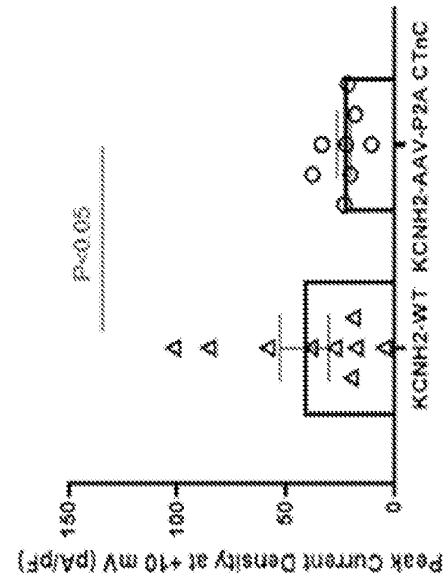


FIG. 30D

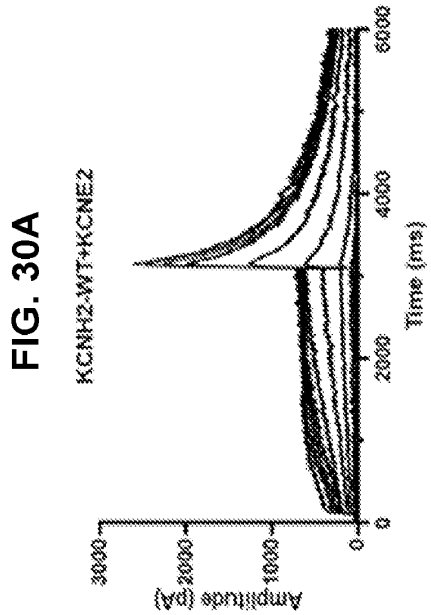


FIG. 30A

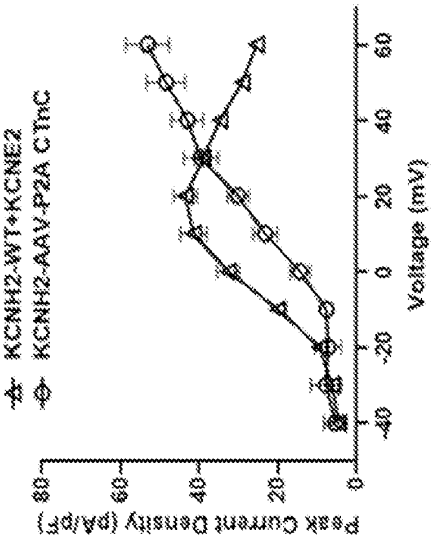


FIG. 30C

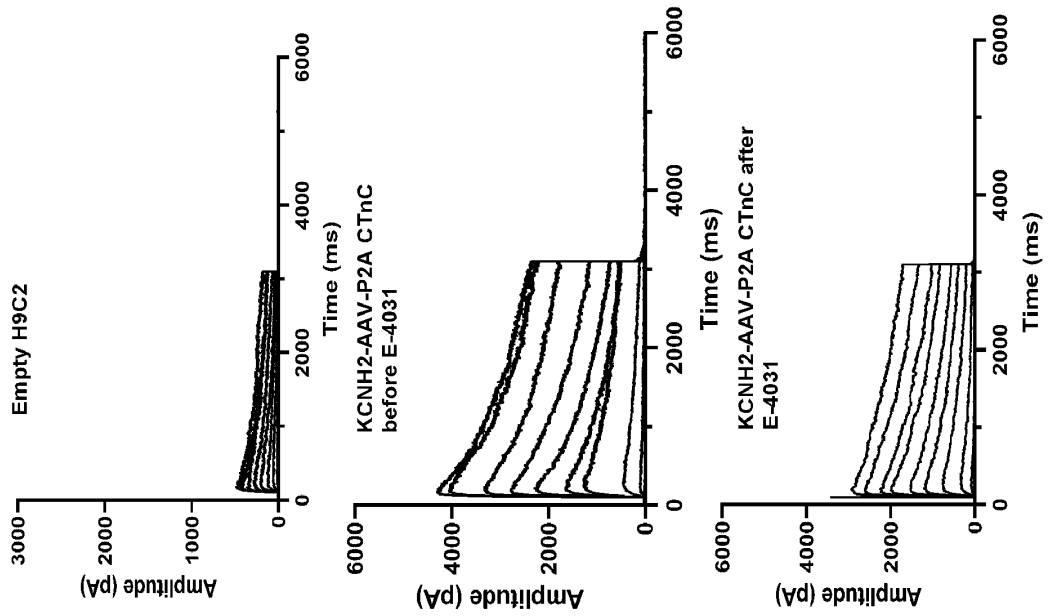

Empty H9C2
KCNH2-AAV-P2A CTnC before E-4031
KCNH2-AAV-P2A CTnC after E-4031
Amplitude (pA)
Time (ms)
0
1000
2000
3000
4000
6000

FIG. 31A

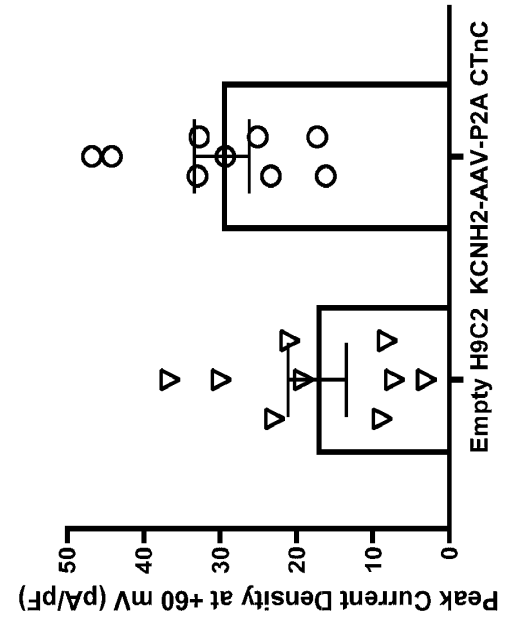


FIG. 31C

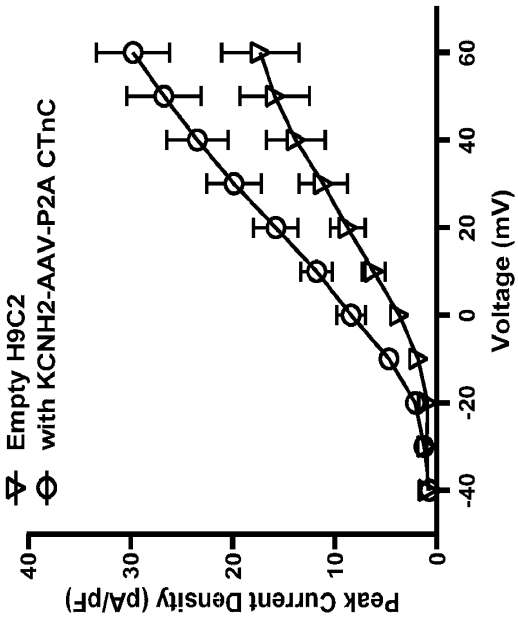


FIG. 31B

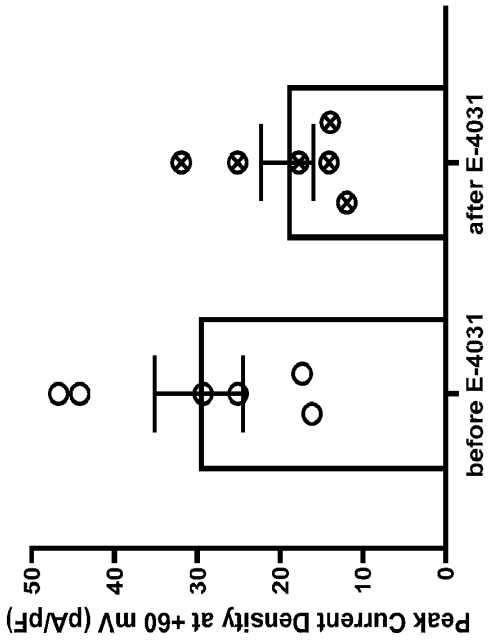


FIG. 31E

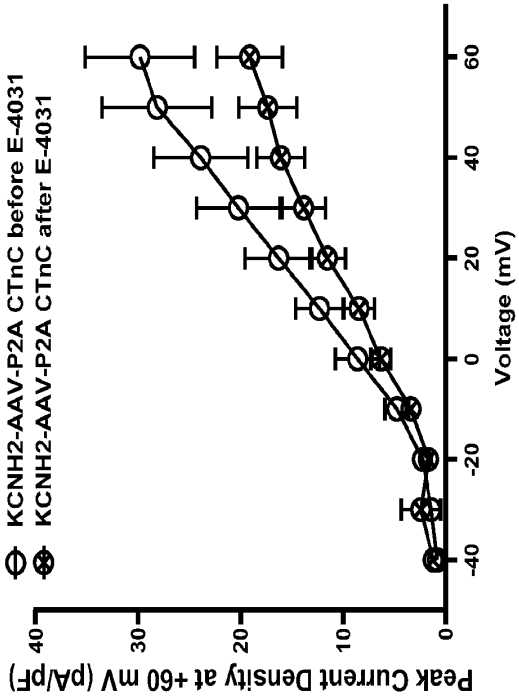


FIG. 31D

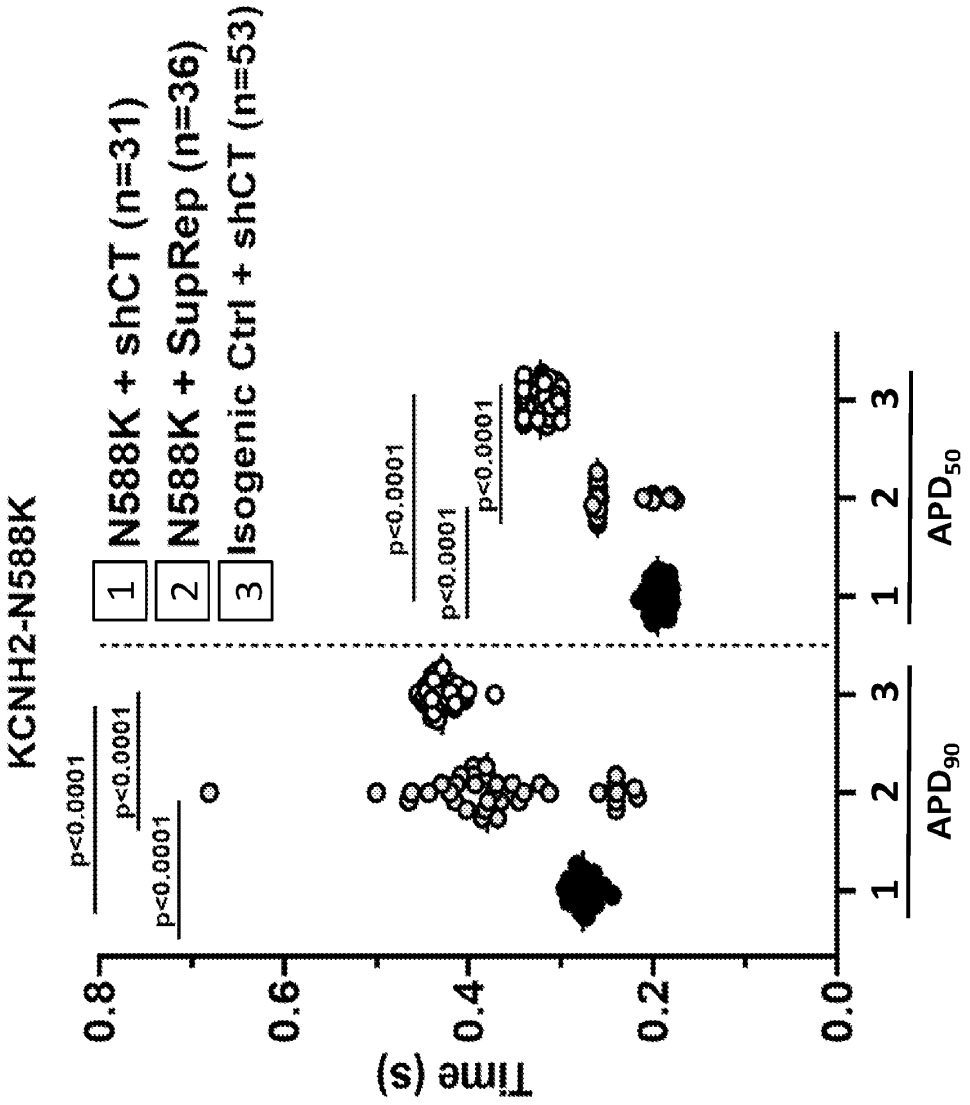

KCNH2-N588K
1 N588K + shCT (n=31)
2 N588K + SupRep (n=36)
3 Isogenic Ctrl + shCT (n=53)
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p<0.0001
Time (s)
0.0
0.2
0.4
0.6
0.8
1 2 3
APD90
1 2 3
APD50

FIG. 32

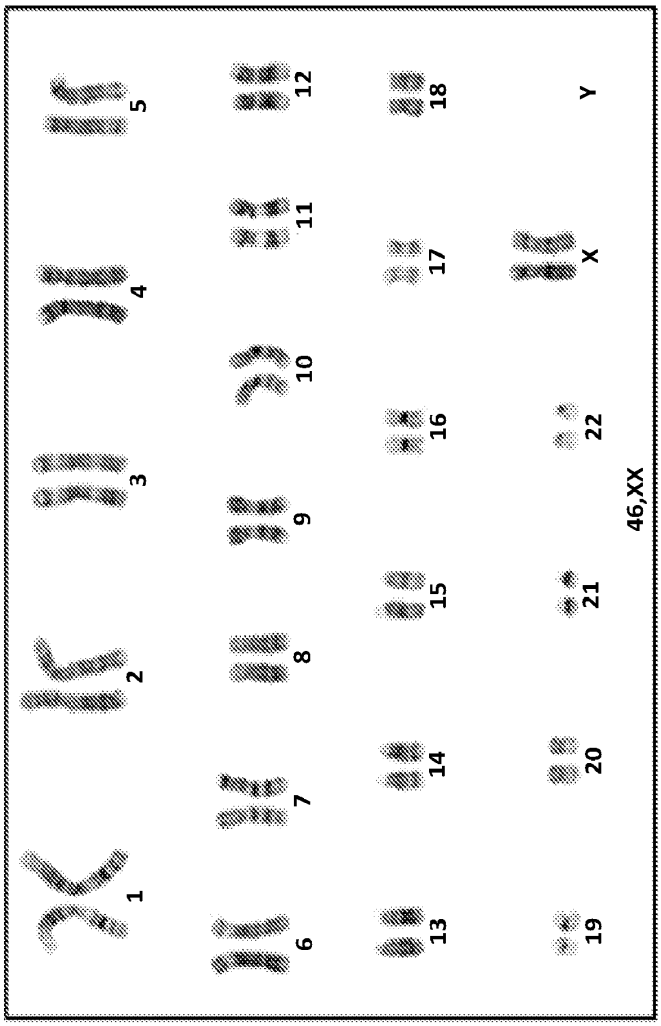


FIG. 33C

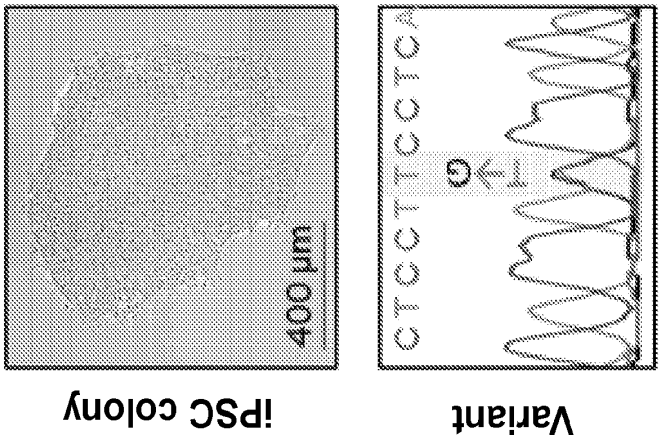


FIG. 33B

FIG. 33A

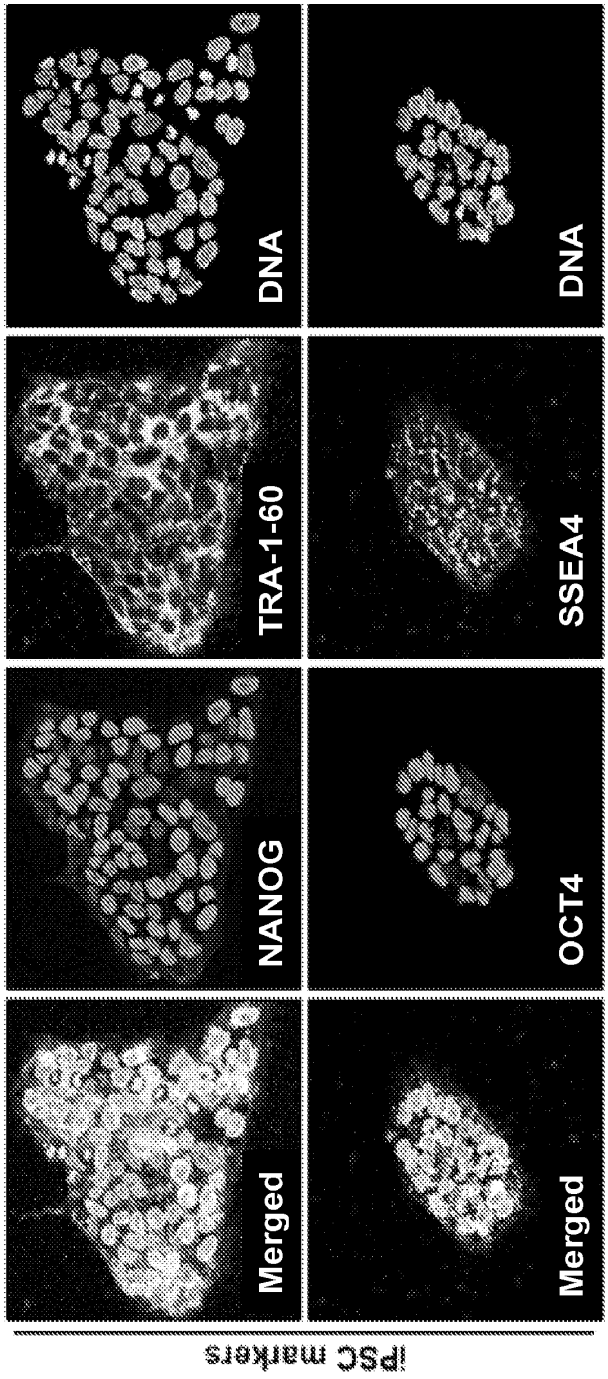
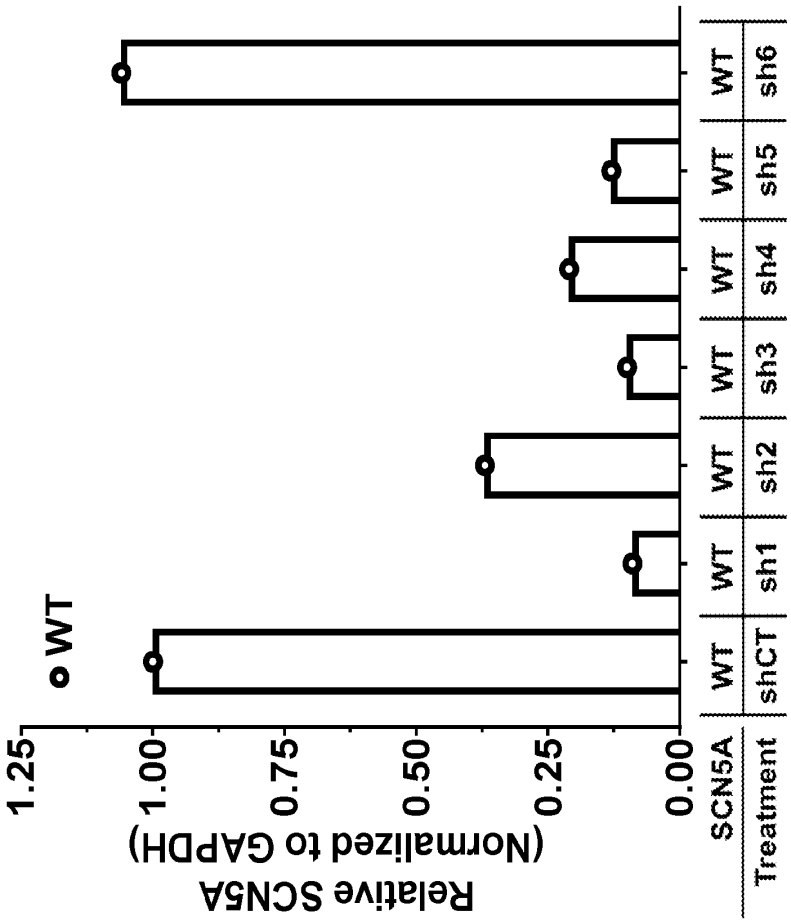


FIG. 33D

FIG. 34


WT
Relative SCN5A
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
SCN5A
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

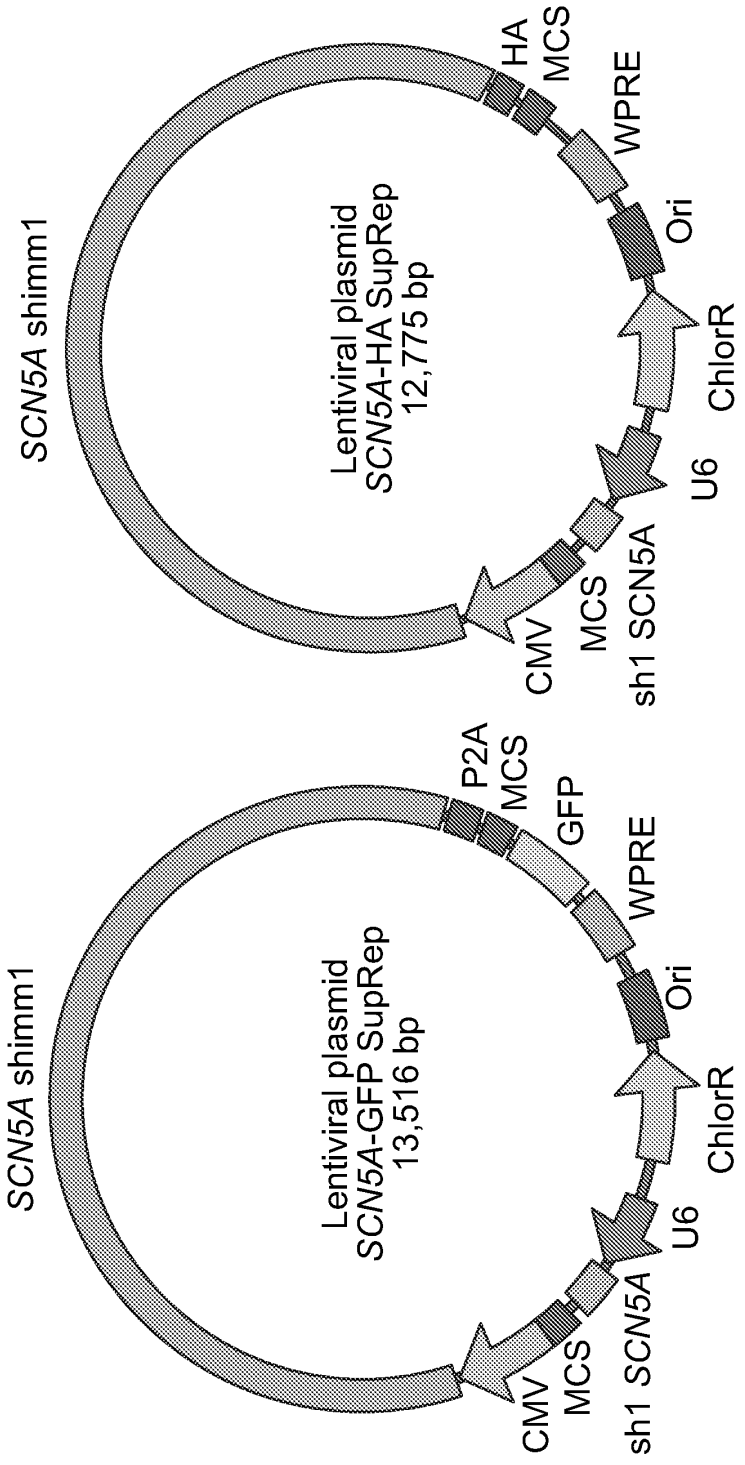

SCN5A shimm1
Lentiviral plasmid
SCN5A-HA SupRep
12,775 bp
HA
MCS
WPRE
Ori
ChlorR
U6
sh1 SCN5A
MCS
CMV
SCN5A shimm1
Lentiviral plasmid
SCN5A-GFP SupRep
13,516 bp
P2A
MCS
GFP
WPRE
Ori
ChlorR
U6
sh1 SCN5A
MCS
CMV

FIG. 35

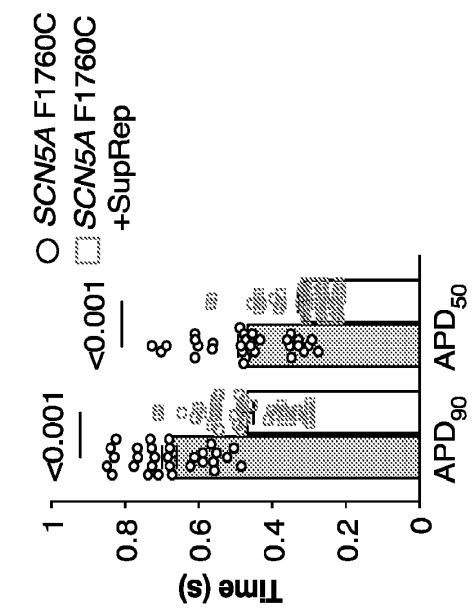


FIG. 36A

FIG. 36B

FIG. 37

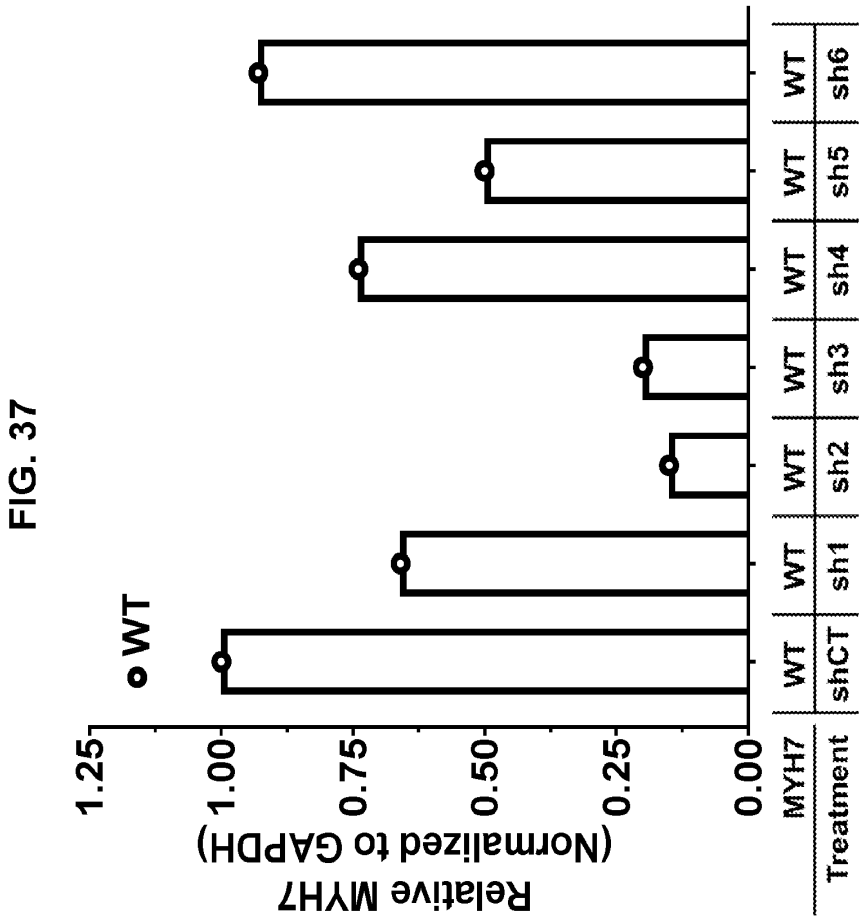
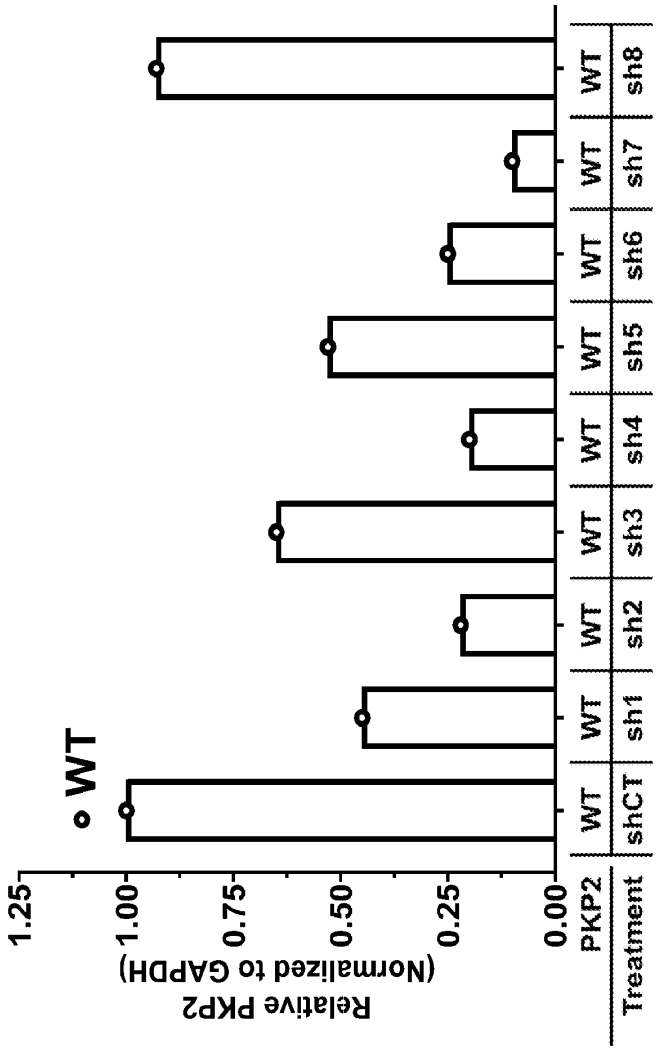

WT
Relative MYH7
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
MYH7
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

FIG. 38


Relative PKP2
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
Treatment
PKP2
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6
WT
sh7
WT
sh8

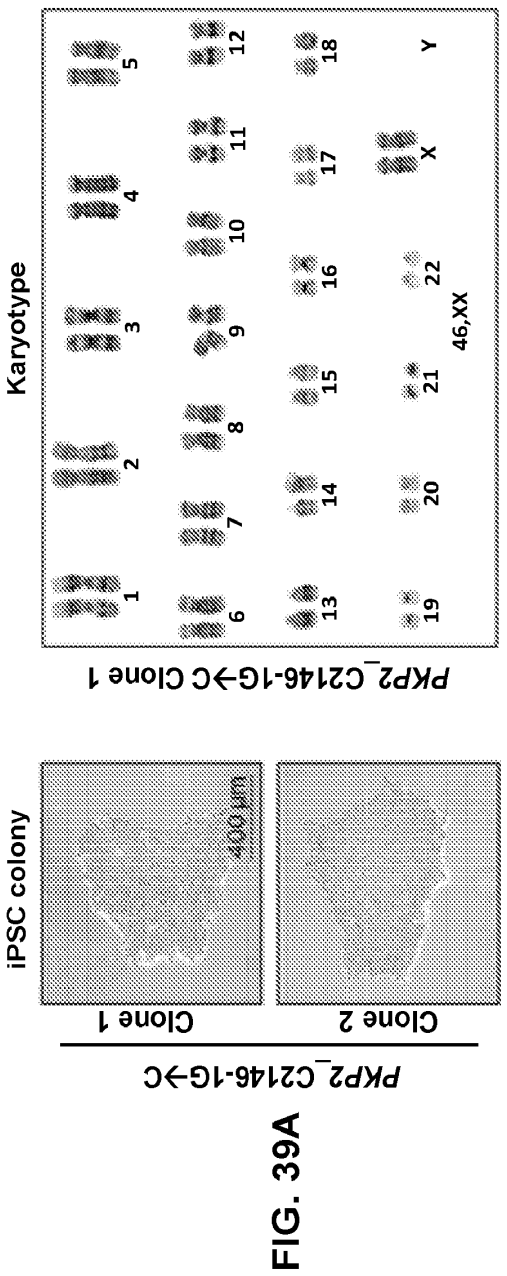


FIG. 39A

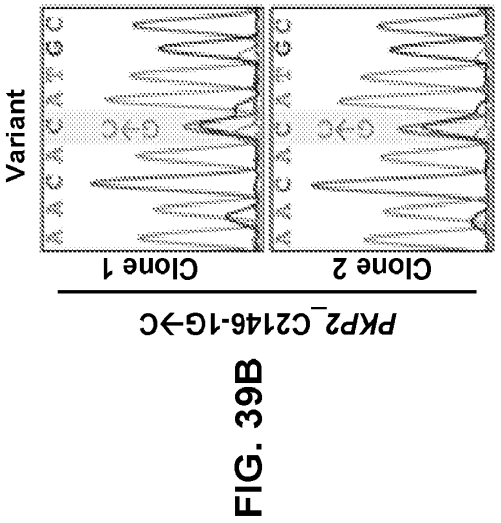


FIG. 39B

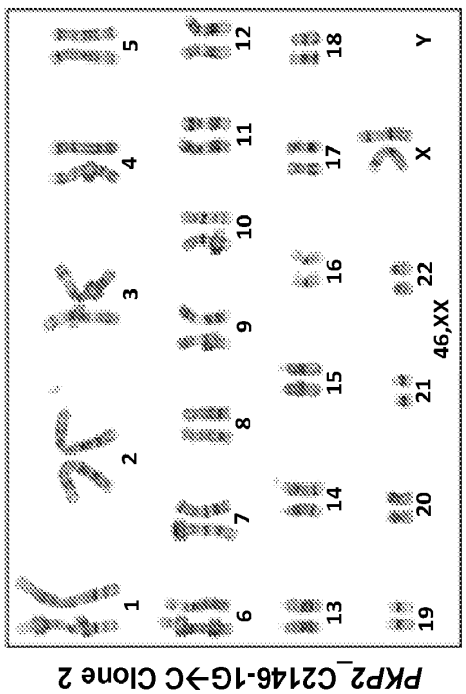


FIG. 39C

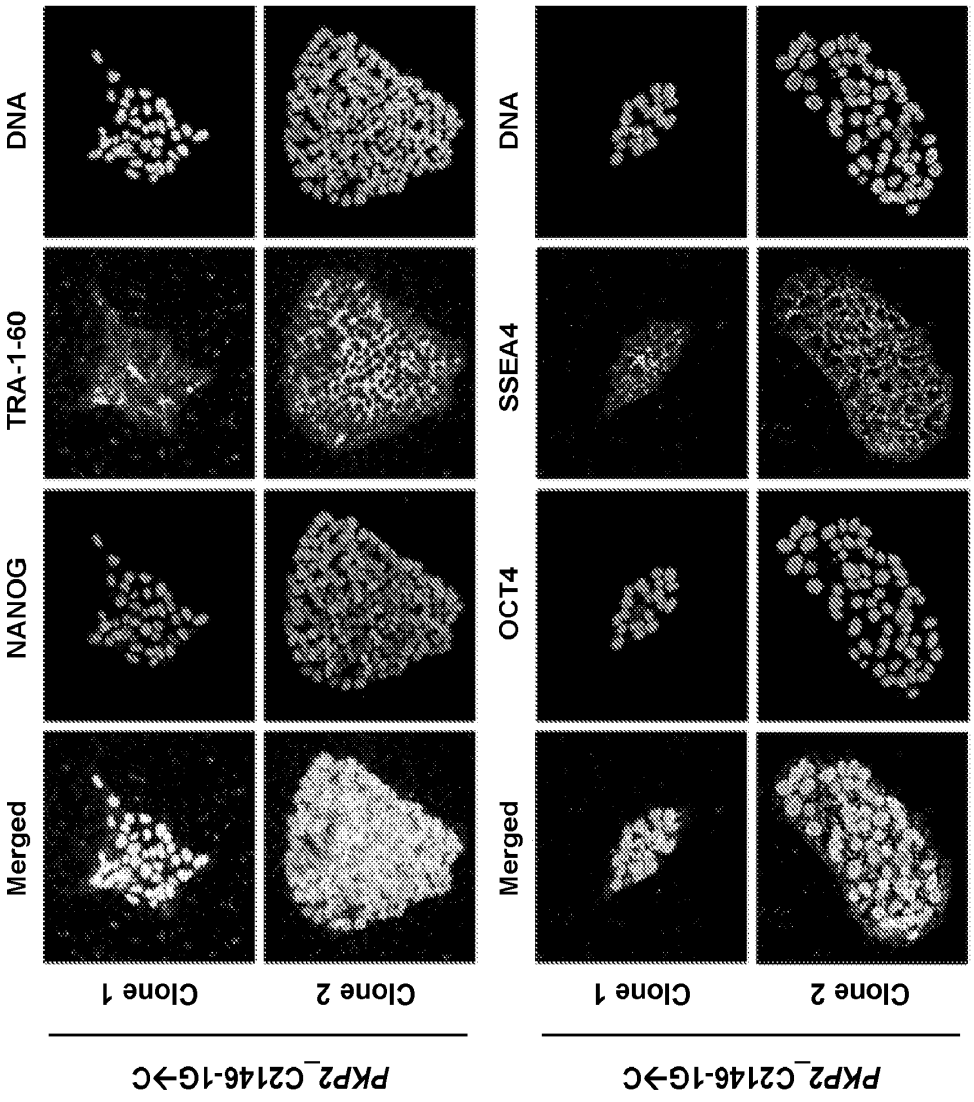

PKP2_C2146-1G→C
PKP2_C2146-1G→C
Clone 1
Clone 2
Clone 1
Clone 2
DNA
TRA-1-60
NANOG
Merged
DNA
SSEA4
OCT4
Merged

FIG. 39D

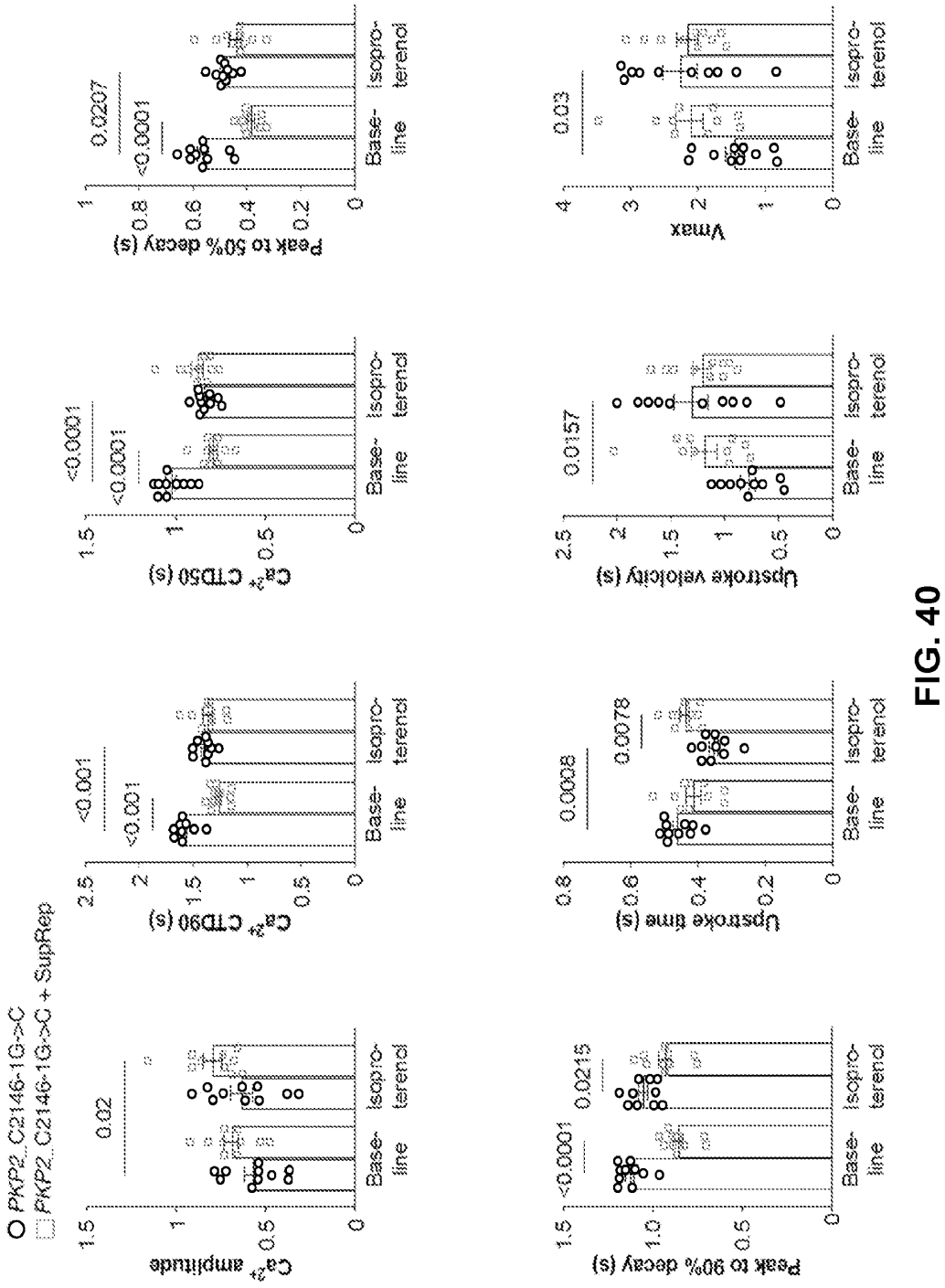

PKP2_C2146-1G->C
PKP2_C2146-1G->C + SupRep
Ca2+ amplitude
Ca2+ CTD90 (s)
Ca2+ CTD50 (s)
Peak to 50% decay (s)
Peak to 90% decay (s)
Upstroke time (s)
Upstroke velocity (s)
Vmax
0.02
<0.001
<0.0001
<0.0001
0.0207
<0.0001
0.0215
0.0008
0.0078
0.0157
0.03
Base-line
Isoproterenol

FIG. 40

FIG. 41

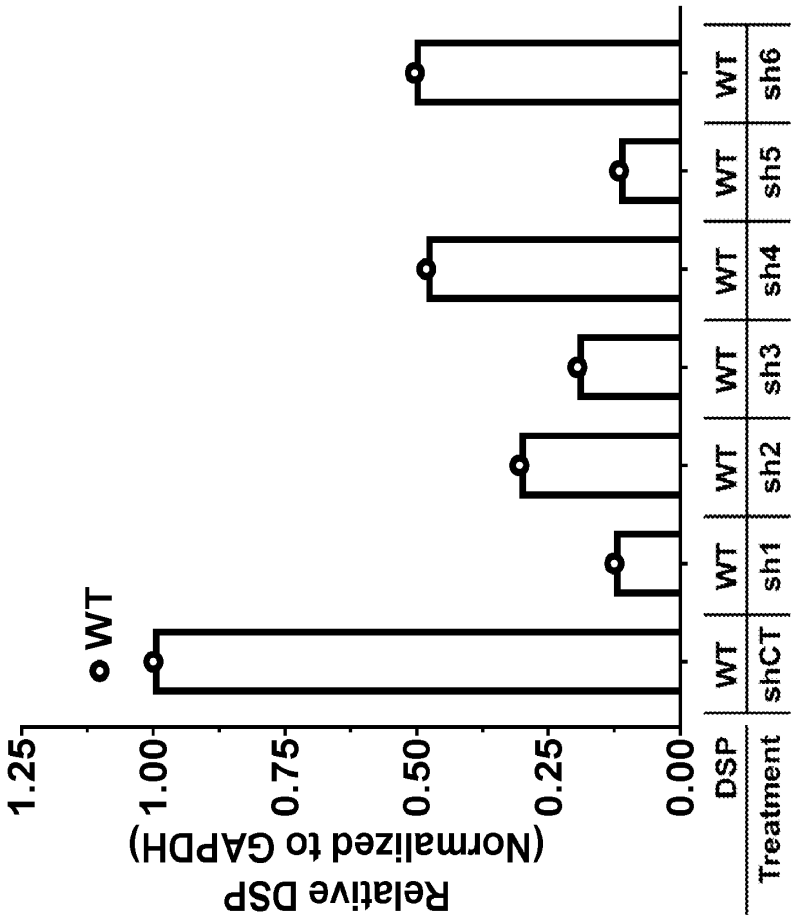

Relative DSP
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
DSP
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

FIG. 42

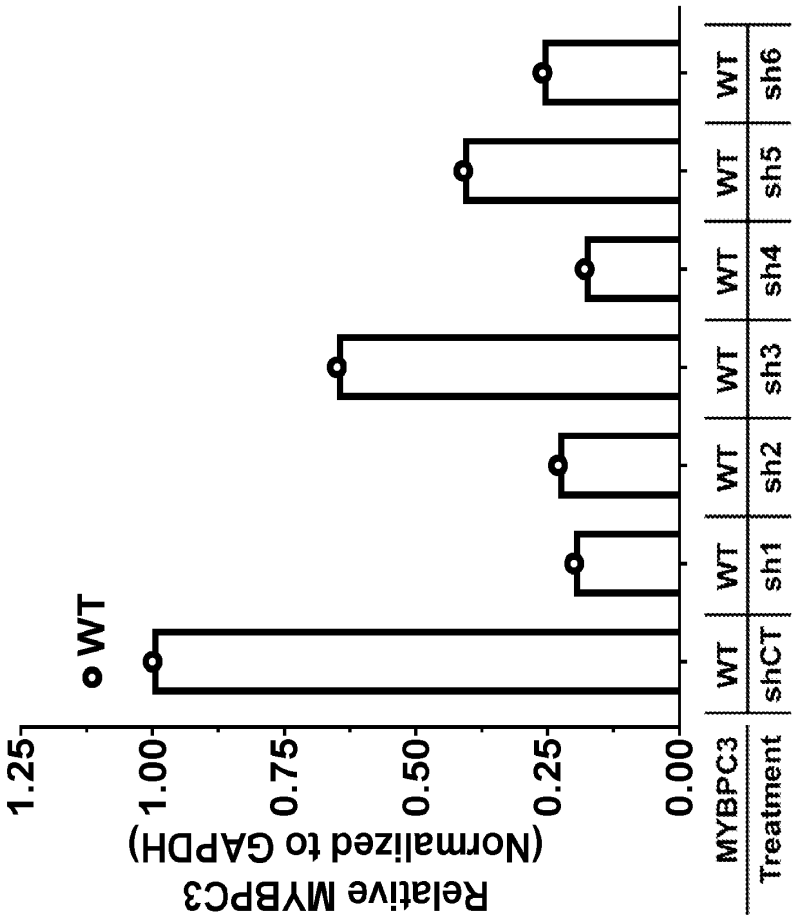

Relative MYBPC3
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
MYBPC3
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

FIG. 43

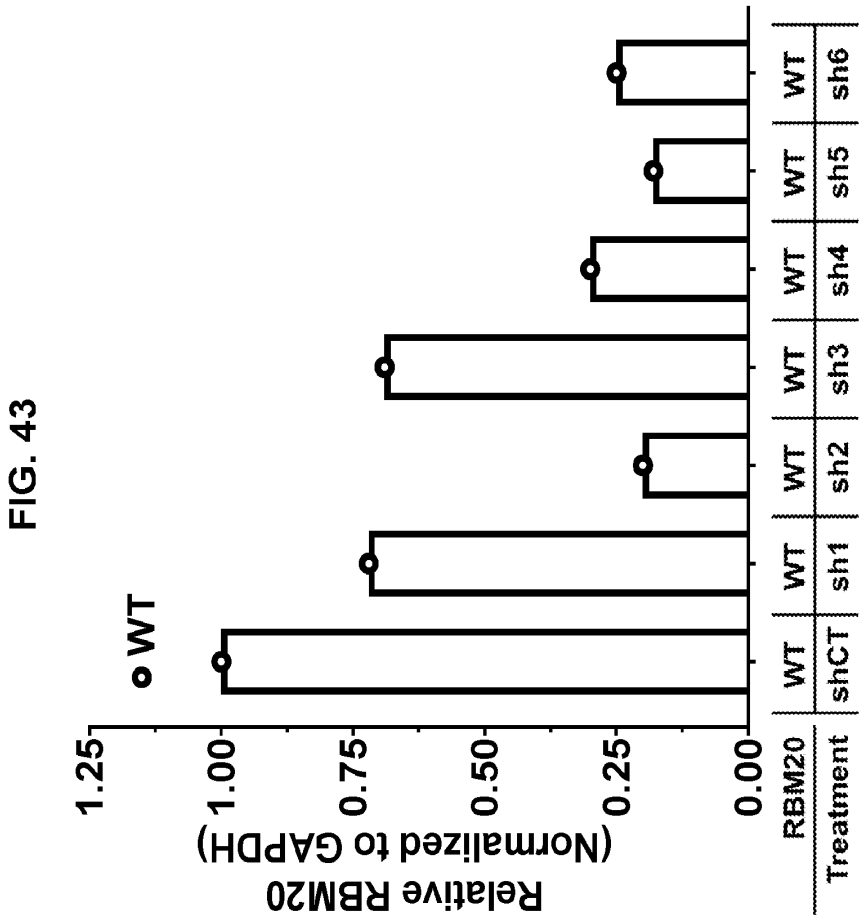
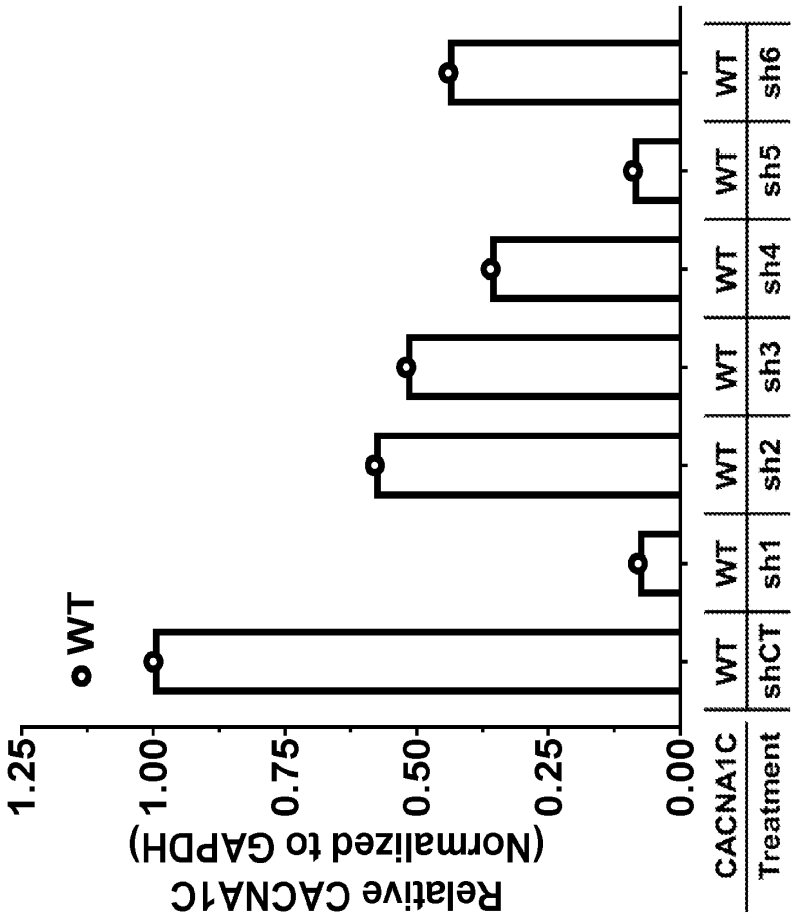

WT
Relative RBM20
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
RBM20
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

FIG. 44


Relative CACNA1C
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
CACNA1C
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

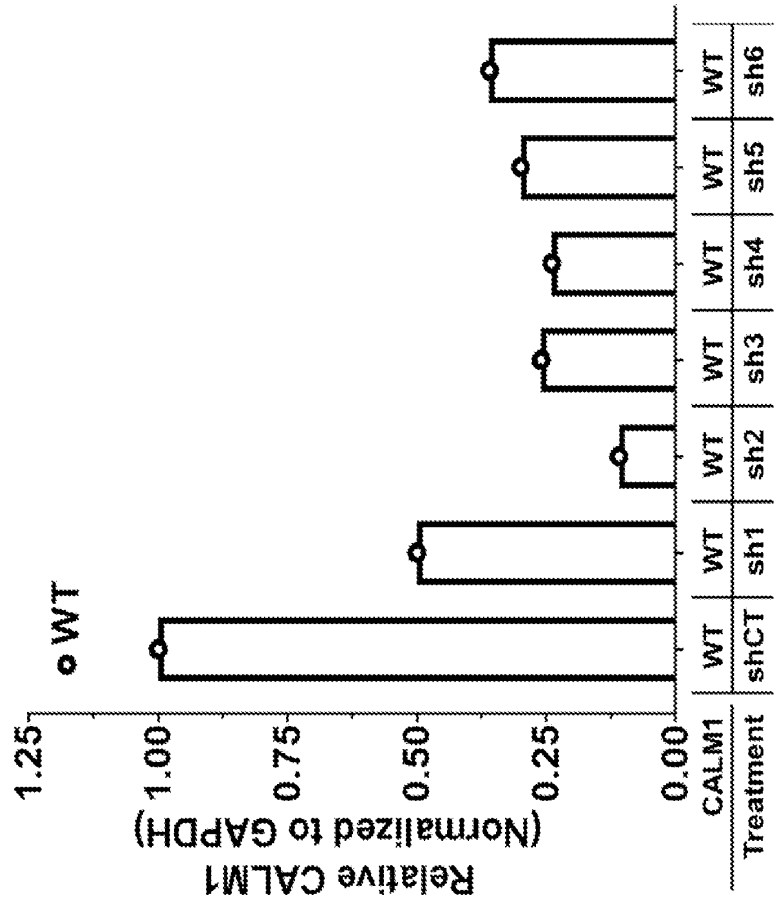

Relative CALM1
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
CALM1
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

FIG. 45

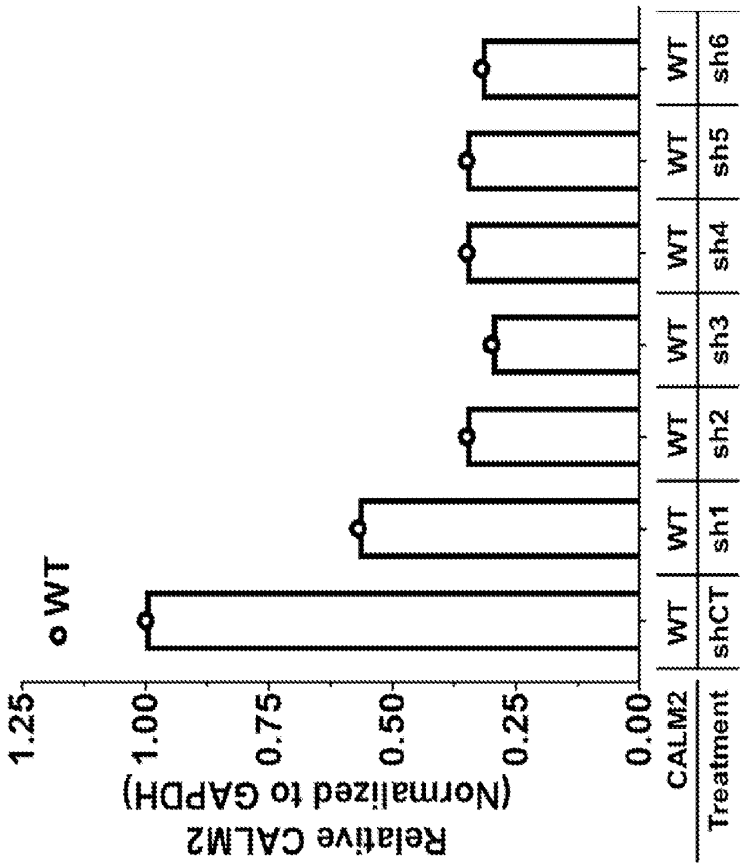

Relative CALM2
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
CALM2
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

FIG. 46

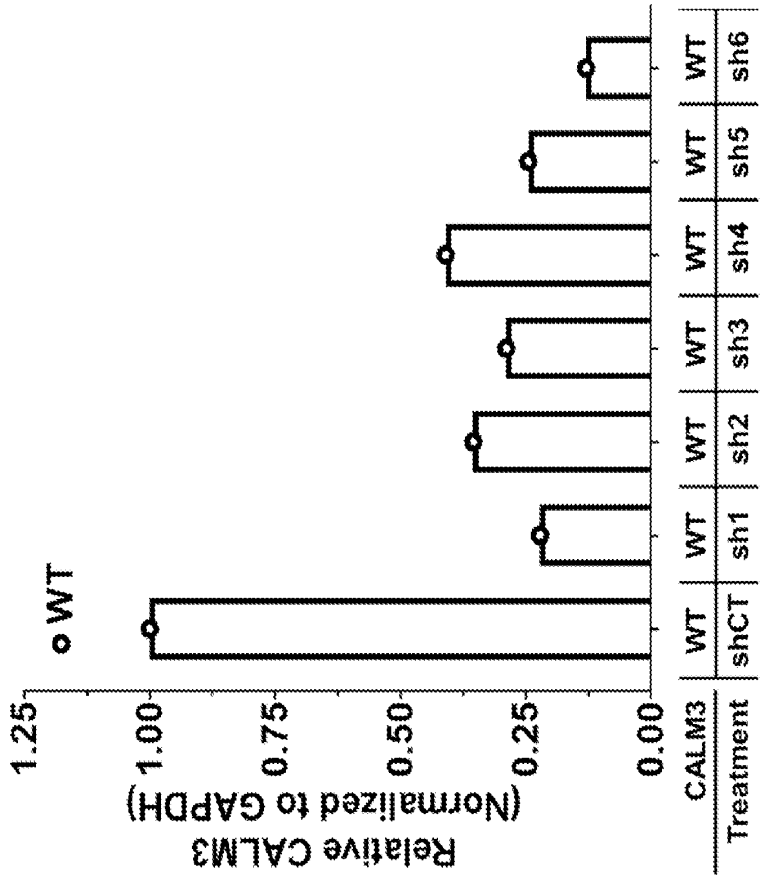

o WT
1.25
1.00
0.75
0.50
0.25
0.00
Relative CALM3
(Normalized to GAPDH)
CALM3
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

FIG. 47

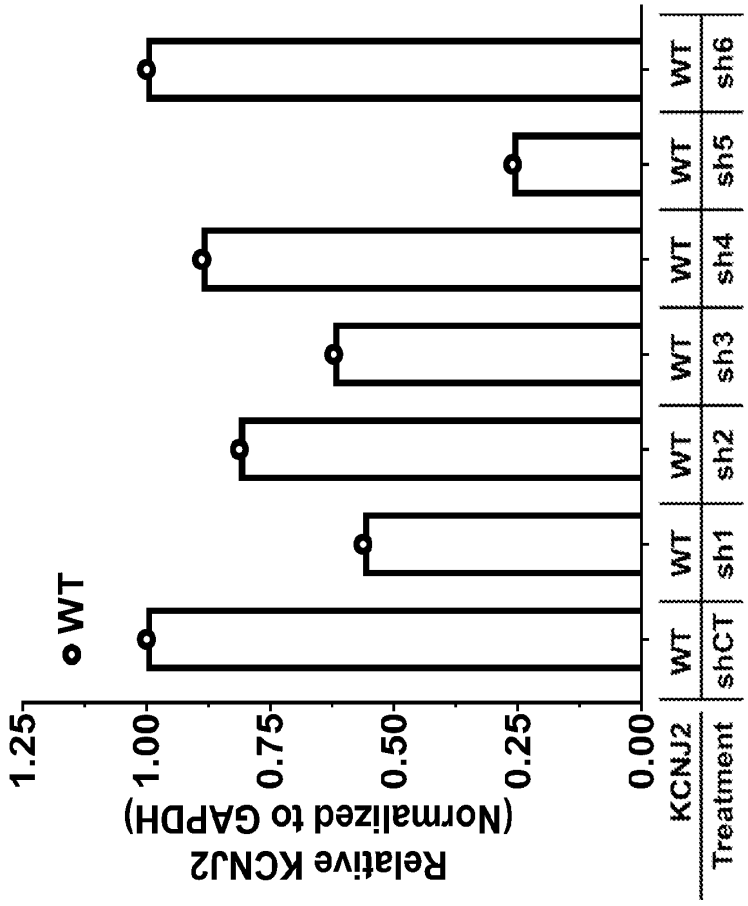

Relative KCNJ2
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
KCNJ2
Treatment
shCT
sh1
sh2
sh3
sh4
sh5
sh6
WT

FIG. 48

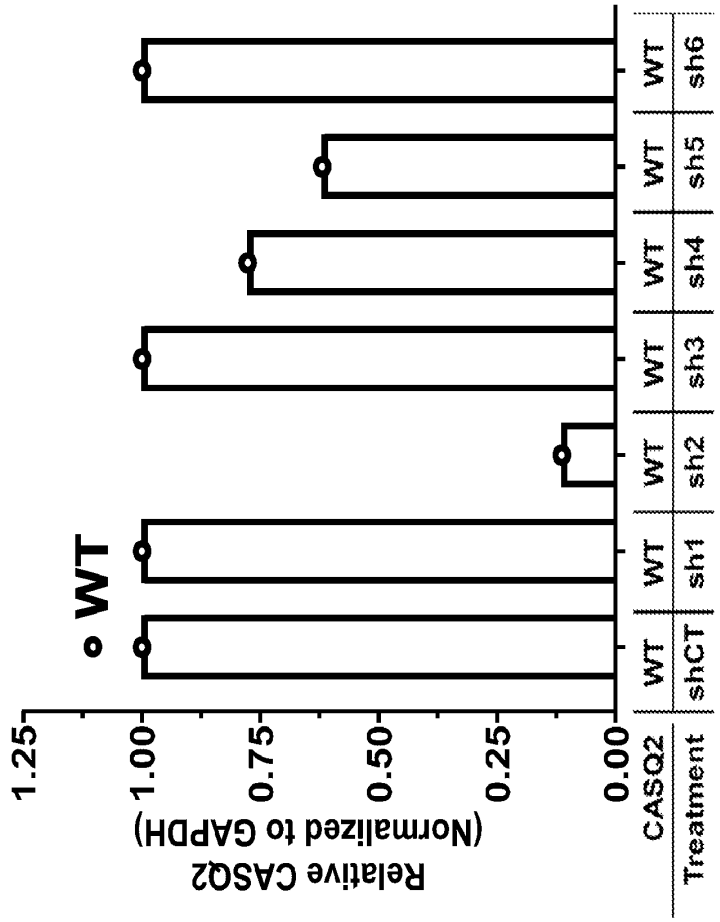

Relative CASQ2
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
Treatment
CASQ2
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

FIG. 49

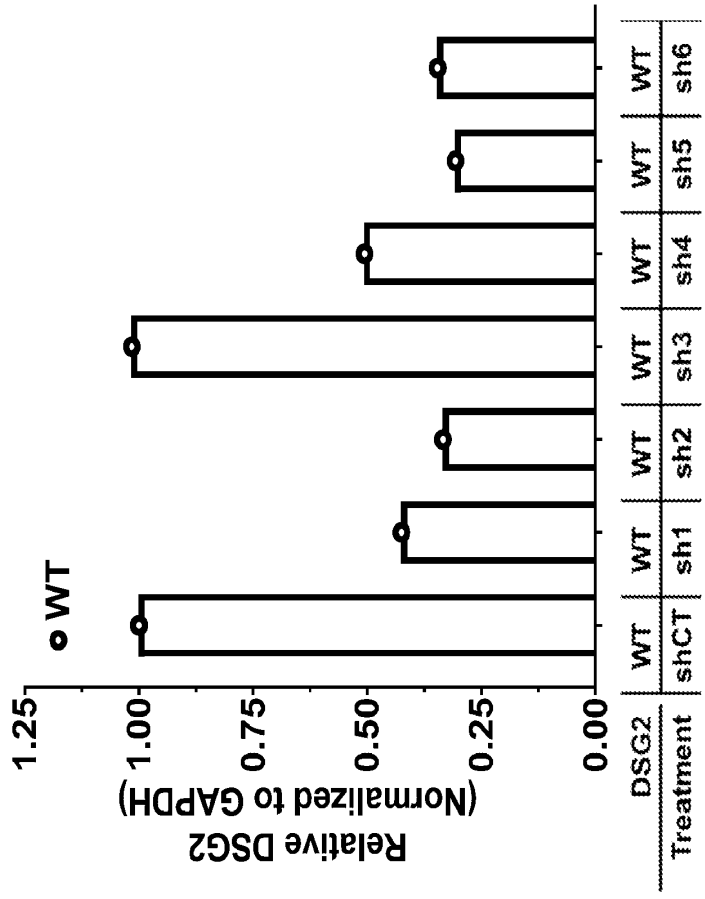

Relative DSG2
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
DSG2
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

FIG. 50

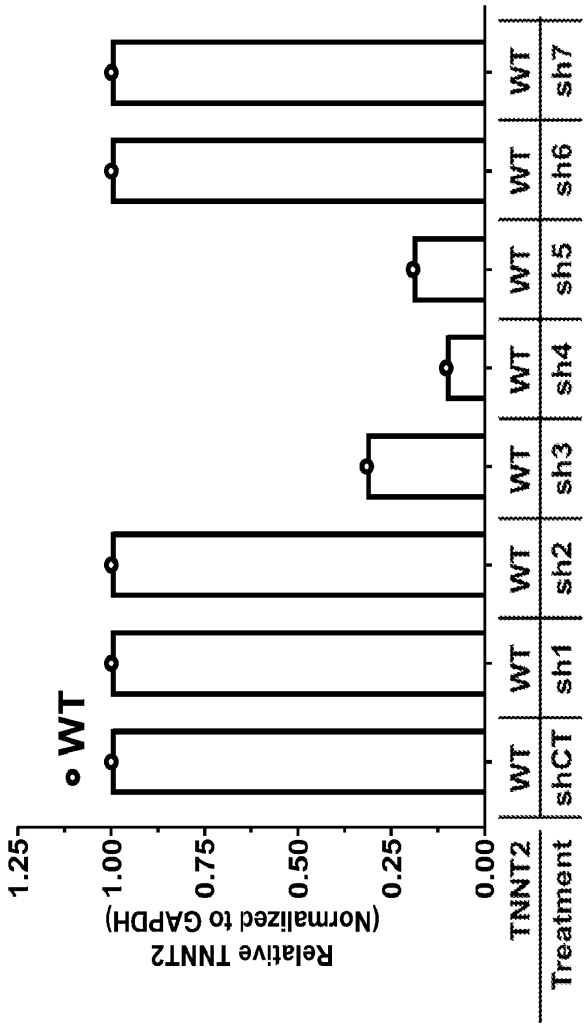

Relative TNNT2
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
TNNT2
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6
WT
sh7

FIG. 51

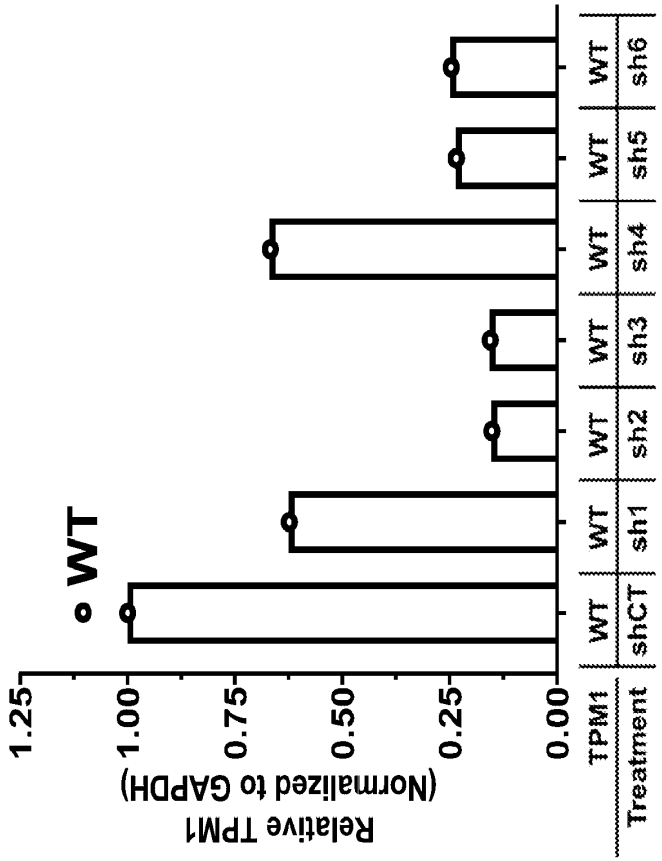

Relative TPM1
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
TPM1
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

FIG. 52

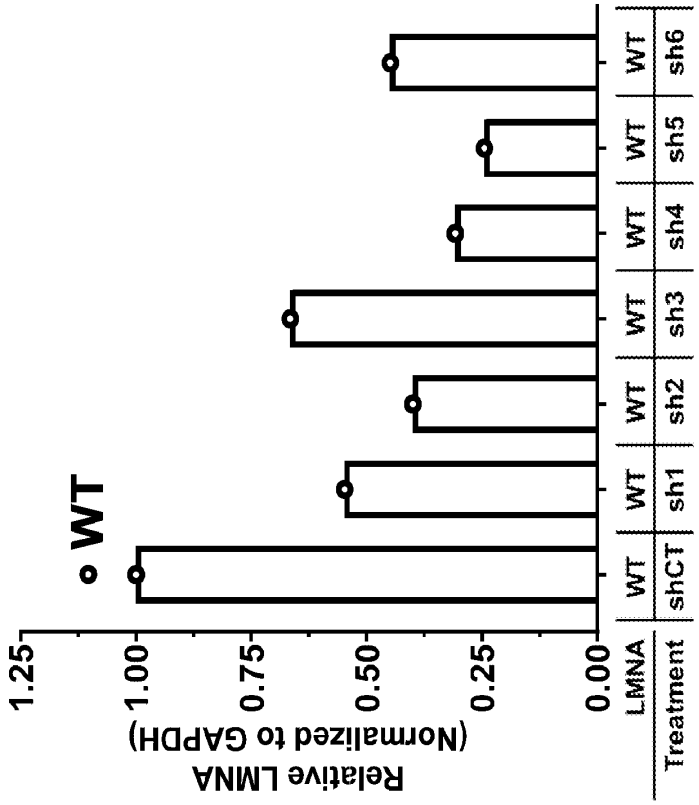

Relative LMNA
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
Treatment
shCT
sh1
sh2
sh3
sh4
sh5
sh6
LMNA
WT

FIG. 53

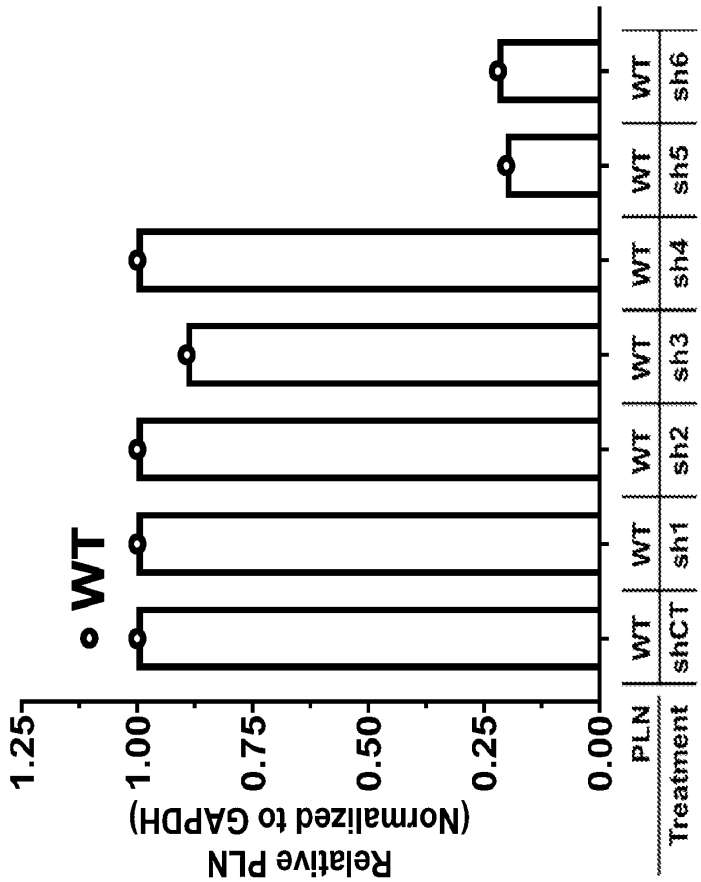

Relative PLN
(Normalized to GAPDH)
1.25
1.00
0.75
0.50
0.25
0.00
WT
PLN
Treatment
WT
shCT
WT
sh1
WT
sh2
WT
sh3
WT
sh4
WT
sh5
WT
sh6

FIG. 54